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Morpho-anatomical Details of One Year Old Mycorrhizal Roots of Sal Seedlings Formed After Inoculation with Indigenous Species of *Russula* and *Lactarius* from Indian Shivaliks

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ABSTRACT

This paper describes the morpho-anatomical details of 1 year old mycorrhizal roots of sal seedlings formed after inoculation between the dipterocarp tree *Shorea robusta* Gaertn. and ectomycorrhizal mushrooms, *Russula kanadii* Dutta & Acharya, *R. cyanoxantha* (Schaeff.) Fr. and *Lactarius shiwalikensis* Kumar & Atri. The sporophore and pure culture of each of these mushrooms were identified using morphological characters and phylogenetic analysis of the internal transcribed spacer region of nuclear ribosomal DNA (ITS). The inocula were prepared using wheat grains for inoculating the germinating *Shorea* seeds for establishing the mycorrhizal association under aseptic conditions. After 3 months following inoculation, the mushroom mycelia of the 3 fungal isolates were observed colonizing the roots of *Shorea robusta* seedlings. The samples of mycorrhizal roots of the seedlings were collected after 1 year of growth and subsequently, each one of these were studied for their morphology, appearance and structure of mantle and Hartig net.

Keywords: Sal forests, Himalayas, Artificial synthesis, Ectomycorrhiza, *Shorea robusta*.

INTRODUCTION

In temperate and tropical forest ecosystems, most tree species are reported to from ectomycorrhizal (EcM) association with diverse species of fungi belonging to Phylum *Ascomycota* and *Basidiomycota* (Kumar and Atri, 2018). These fungi are reported to play a crucial role in the establishment and growth of forest trees by enhancing their nutrient and water acquisition (Smith and Read, 2008; Kumar and Atri, 2023), and imparting tolerance/resistance against biotic and abiotic stresses (Xu *et al*., 2016; Sebastiana *et al*., 2018; Guo, 2018; Luo *et al*., 2014). In spite of the great diversity and distribution of EcM fungi in the temperate and boreal ecosystem, diversity and distribution of tropical EcM fungi is still poorly known. In India, large area of tropical and subtropical ecosystems is mainly dominated by ectomycorrhizal trees. For instance, the tropical moist deciduous forests of India are largely dominated by economically important dipterocarp tree *Shorea robusta* Gaertn. (sal) which represents a major source of commercial timber (Singh and Singh, 1992). Plants of *S. robusta* are reported to form obligatory ectomycorrhizal association with number of fungi (Natarajan *et al*., 2005; Kumar and Atri, 2018). Several species of Basidiomycetous fungi have been reported from sal forests as EcM associates of *Shorea robusta* roots based on sporophore surveys and putative characterisation of ectomycorrhizal roots (Pyasi *et al*., 2011, 2013; Tapwal *et al*., 2013, 2015; Kumar and Atri, 2019, 2020a, 2021b). The roots of *Shorea robusta* are reported to be forming putative association with the species of *Amanita, Asproinocybe, Boletus, Inocybe, Pulveroboletus, Russula, Lycoperdon, Scleroderma, Lactifluus* and *Lactarius* in natural condition (Bakshi 1974; Tapwal *et al*. 2013; Kumar and Atri 2016, 2019, 2020a, 2021a, 2021b). However, 3 mushrooms, namely *Russula michiganensis* Shaffer., *R. amoena* Quél. and *Lycoperdon compactum* G. Cunn. were clearly confirmed as EcM associates of *Shorea robusta* by synthesizing EcM under field experiment (Pyasi *et al*., 2013; Tapwal *et al*., 2015). The present study investigates the mycorrhizal development of *Shorea robusta* seedlings inoculated with 3 fungal species, namely *Russula kanadii* Dutta & Acharya, *R. cyanoxantha* (Schaeff.) Fr. and *Lactarius shiwalikensis* Kumar & Atri. The aim is to explore their role in seedling growth and establishment under field conditions, addressing the lack of data on mycorrhization in sal seedlings. In this paper, the morpho-anatomical details of 1 year old mycorrhizal roots of sal seedlings formed after inoculation has been presented.

MATERIALS AND METHODS

Material collection, culturing and mass inoculums production of EcM fungi

Sporocarps of most common putative mycorrhizal mushroom associates of Sal were collected from the Sirmour district of Himachal Pradesh and Dehradun district of Uttarakhand, India. The macroscopic and microscopic details were worked out as per standard methodology (Singer, 1986; Atri *et al*., 2017) for identification. Fresh and tender sporophores of putative EcM agarics were used for raising the pure culture. The healthy sterilized mushroom tissue slices were inoculated into Petri plates and test tube slants containing sterilized pre-prepared Modified Melin-Norkrans nutrient media (Marx, 1969). The inoculated Petri plates and test tubes were then incubated in BOD incubator at 26°C and observed regularly for the appearance of mycelial growth. Repeated sub-culturing was done to get pure cultures. The mycelial inocula of EcM symbionts were prepared according to the method of Kannan and Natarajan (1988).

Molecular analysis

DNA was extracted from 15 days old mycelial culture using DNeasy plant mini kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. For identification of culture, Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA was amplified using fungal specific primer (White *et al*., 1990). Amplification was performed with DNA thermal cycler (BIORAD T100 TM). The final PCR product was separated by electrophoresis in 1% agarose gel with ethidium bromide in TAE (Tris- Acetic acid - EDTA) running buffer and then purified using a QIA quick gel extraction kit (Qiagen). Sequencing reactions were performed on both forward and reverse strands of purified product by Sanger sequencing on an AB13730X1 DNA analyser (applied biosystems, Foster city, California) using the same primers. Fungal taxa were identified by running BLAST queries in GenBank database (www.ncbi.nlm.nih.gov.). The generated sequences were deposited to GenBank for accession numbers.

Artificial synthesis and characterization of mycorrhiza

For artificial inoculation, soil was collected from the natural Sal growing area in Shivalik range. Soil was cleaned, sieved and then autoclaved so as to remove any pathogens/microbes from it. Seeds were collected in the month of June from Asharodi range of Sal forest of Dehradun, Uttrakhand, India. The polybags (20 x 21 cm^2) were filled with autoclaved soil (2 kg/bag) for each mycorrhizal inoculum which were subsequently inoculated with inocula of *Russula kanadii*, *Russula cyanoxantha* and *Lactarius shiwalikensis* (5 g/ bag) under single sal seed sown in each bag. For morphological characterization, developed ectomycorrhizae were observed under a stereomicroscope (Magnus MSZ-TR), photographed and described by careful examination following Agerer (1987- 2012) and Agerer and Rambold (2004 - 2024). All the root tips were examined for the morphological characters such as shape, size, colour, texture, presence/absence of rhizomorphs and extra-radical mycelium. The confirmation of ectomycorrhizal colonization was done by examining the cross sections and longitudinal sections of EcM roots (cut manually) under a compound microscope and photographed under digital microscope (Leica DM4000 B LED) so as to study the architecture of the mantle and the Hartig net. The colour terminology used is that of Kornerup and Wanscher (1978). Chemical colour reactions were performed on the mantle layer using Cotton Blue, Melzer's Reagent, Sulphovanillin, ferrous sulphate, potassium hydroxide and ethanol.

RESULTS

Taxonomic and molecular analysis

Morphologically 2 species of *Russula* were identified as *Russula kanadii* (Dutta *et al*., 2015) and *R. cyanoxantha* (Romagnesi, 1967), whereas *Lactarius* species was describes as *Lactarius shiwalikensis*, which was new to science. To validate the taxonomic result, pure culture of both *Russula* species and sporocarp tissue and pure culture of *Lactarius* species were further subjected to molecular study for identification by sequencing the Internal transcribe spacer (ITS) region of their genomic DNA. The ITS sequences generated were compared with the available sequences in the GenBank using the nucleotide BLAST search algorithm. Sequence reads were submitted to GenBank (www.ncbi.nlm.nih.gov.) with accession numbers KY931026, KY931025 for *Russula kanadii* and *R. cyanoxantha*, respectively, and MH635422 (sporophore) and MW466549 (Pure culture) for *Lactarius shiwalikensis*. The dried materials of these 3 mushroom species were

deposited in the Herbarium of Department of Botany, Punjabi University, Patiala under PUN numbers 9140, 9119 and, 9172, respectively.

1. Description of ectomycorrhizae: *Russula kanadii* Dutta & Acharya + *Shorea robusta* Gaertn. Figure 1 and 2

Morphological characters: Mycorrhizal system monopodial pinnate dichotomous-like, occasionally simple, with zero to one order of ramification, 2.0-5.5 mm long; main axes 0.2-0.5 mm in diameter. Unramified ends dense, slightly bent to straight, not inflated, cylindrical, 1-2 mm in length and 0.2-0.5 mm in diameter, tips rounded. Surface of unramified ends rough; densely short spiny, mostly with soil particles, mycorrhizae white to grayish brown, unchanging, not secreting latex or any other fluid when injured; mantle not transparent, hydrophobicity absent, tip shows the same colour as rest of the mycorrhizae. Emanating hyphae observed, concentrated mostly distally. Cystidia present, arising from the bulbous base. Sclerotia not observed.

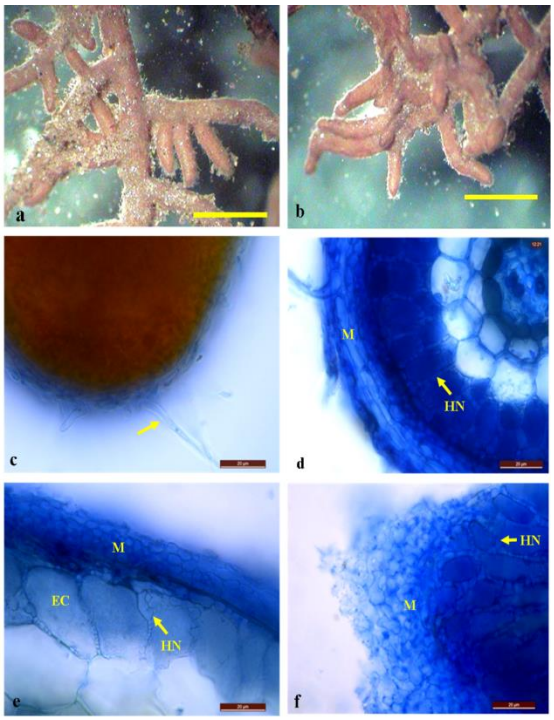

Figure 1: *Russula kanadii* + *Shorea robusta*: a-b, Mycorrhizal system; c, Surface view of unramified end showing cystidia (arrow) on outer mantle; d, Cross section of ectomycorrhizal root showing mantle (M); e, Longitudinal section of ectomycorrhizae showing mantle (M) and radially elongated epidermal cell (EC) with Hartig net (HN); f, Longitudinal section of root tip showing plectenchymatous mantle (M). Scale bar: a-b=1 mm.

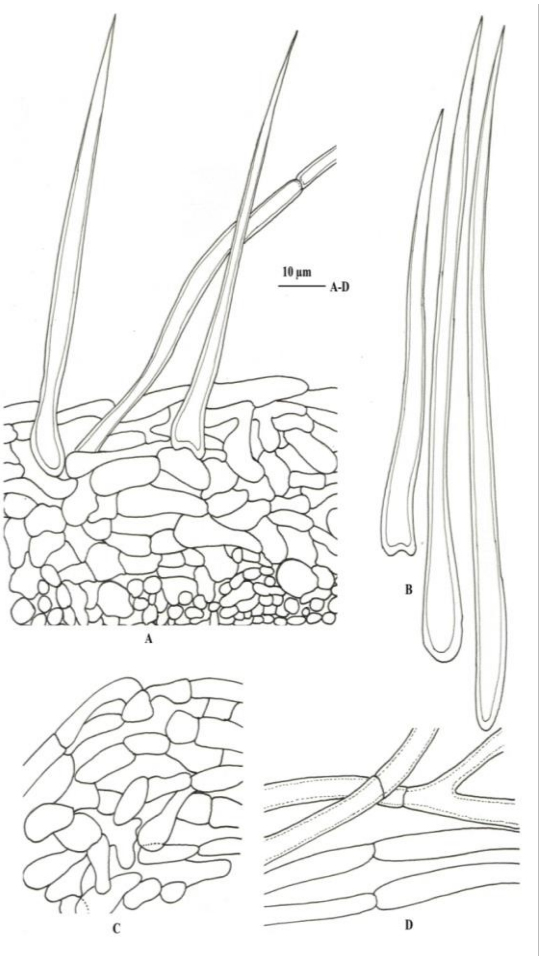

Figure 2: *Russula kanadii* + *Shorea robusta*: A, Mantle; B, Cystidial elements; C, Root tip mantle; D, Emanating hyphae.

Anatomical characters of mantle in plan view: Mantle thickness 29-32 (36) µm, distinct, hyphae of both mantle layers colorless, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 24-29 µm, plectenchymatous, compactly arranged, representing type D pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024); hyphal cells 8-22 µm tangentially and 4.0-8.0 µm radially, compactly arranged, smooth, hyaline, septate, thin walled (0.5 µm), not constricted at septa, clampless; septa as thick as hyphal wall. Inner mantle layer 5.0-7.0 µm, composed of interlocking irregular pseudoparenchyma representing type K pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024), mantle layer transitional type in some parts, clear hyphal arrangement is observed, hyphal cells colourless, hyaline, thin walled, variable in shape measuring 3.0-6.5 µm tangentially and 3.0-6.5 µm radially, diameter of hyphal cells decreases from outer to inner side.

Anatomical characters of emanating elements: Emanating hyphae 2.5-6.5 µm, thick walled (1 µm), septate, not constricted at the septa, without clamps, septa as thick as hyphal wall (up to 1 µm). Cystidia 89-146 × 3-6 µm, present on the outer mantle layer, most distinct and often frequent exhibiting type1pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024), subcylindrical to awl-shaped with almost acute apex and swollen or rounded base (Agerer, 1987-2012; Agerer and Rambold, 2004-2024), hyaline, smooth, thick walled (up to 1 µm), aseptate without clamps.

Anatomical characters in longitudinal section: Mantle 24-37 µm, differentiated into outer and inner mantle layer. Outer mantle layer 24-30 µm, more or less plectenchymatous, compact, representing type D pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024), hyphal cells 5-20 µm tangentially and 5-8 µm radially, without any content and clamp connection. Inner mantle layer 5-7 µm, pseudoparenchymatous; hyphal cells 3.0-6.5 µm tangentially and 3-5 µm radially. Hartig net one cell deep, palmetti type with one row of 3-7 µm radially and 3.5 µm tangentially, roundish to cylindrical hyphal cells and is restricted to the anticlinal walls of the cortex cells (paraepidermal). Root tip mantle up to 81.5 µm different from rest of the mantle, more or less plectenchymatous, having 3.3-4.0 µm interwoven, septate, hyaline hyphal cells, hyphae rather irregularly arranged and no special pattern discernible representing type B (Agerer, 1987-2012; Agerer and Rambold, 2004-2024). Hartig net also paraepidermal at very root tip with one row of roundish cells measuring 3.3-9.8 µm tangentially 3.3-4.9 µm radially. Hartig net cells have larger diameter at root tip as compared to rest of the Hartig net. Epidermal cells radially elongated to increase the area available for the Hartig net, 29.0-40.7 × 8.0-9.8 µm, tangentially oval to elliptic or cylindrical, and oriented obliquely. Tannin cells not observed.

Colour reactions with different reagents: $FeSO_4$: n. r. (no reaction); Sulphovanillin: brown; Ethanol (70%): n. r.; KOH (10%): n. r.; Acetic acid: n. r.; Melzer: light yellow; Cotton blue: hyphae dark blue.

2. Description of ectomycorrhizae: *Russula cyanoxantha* (Schaff.) Fr. + *Shorea robusta* Gaertn. Figure 3 and 4

Morphological characters: Mycorrhizal system mostly simple to monopodial pinnate, up to 7 mm long; main axes 0.2-0.4 mm in diameter. Unramified ends shiny, straight, rarely bent, cylindrical, 0.8-3.0 mm in length and 0.2-0.4 mm in diameter, tips rounded. Surface of unramified ends not smooth, densely short-spiny, at some places covered with soil particle, white to silvery brown, unchanging, not secreting latex or any other fluid when injured; mantle not transparent, mantle hydrophobicity absent; tip shows the same colour as rest of the mycorrhiza. Rhizomorphs not observed. Emanating hyphae frequent, not specifically distributed. Cystidia present. Sclerotia not observed.

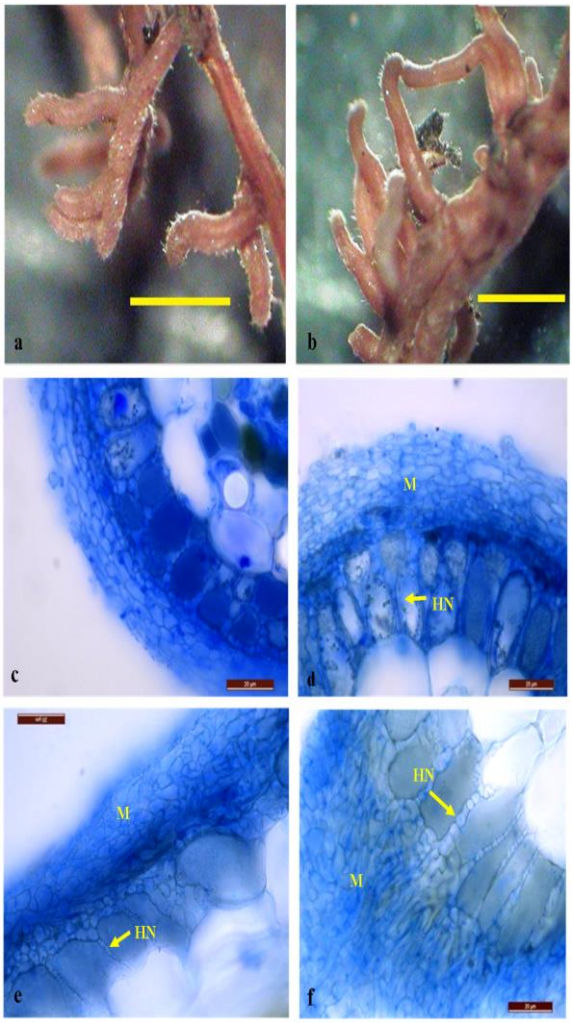

Figure 3: *Russula cyanoxantha* + *Shorea robusta*: a-b, Mycorrhizal system; c-d, Cross section of ectomycorrhizal root showing mantle (M) and Hartig net (HN); e, Longitudinal section of ectomycorrhizae showing mantle (M) and radially elongated epidermal cell with Hartig net (HN); f, Longitudinal section of root tip showing plectenchymatous mantle (M) and Hartig net (HN). Scale bar a-b=1 mm.

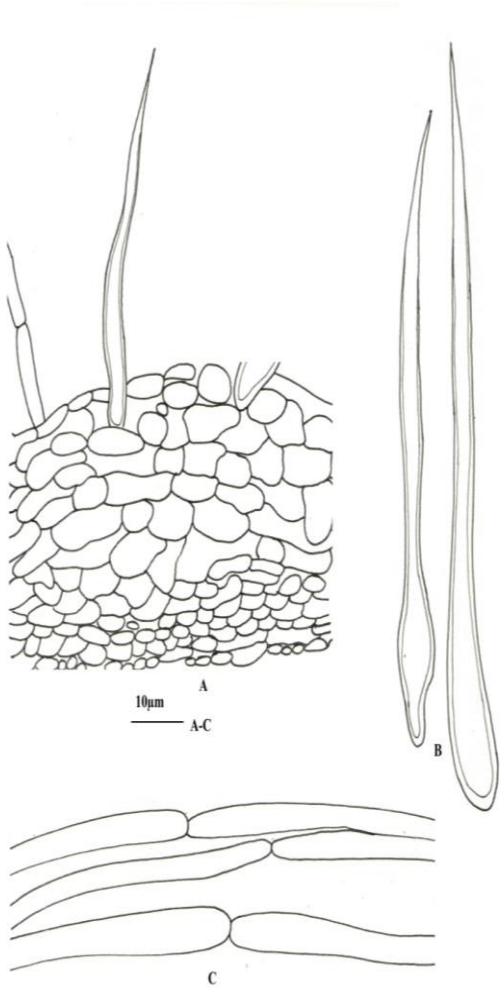

Figure 4: *Russula cyanoxantha* + *Shorea robusta*: A, mantle; B, Cystidial elements; C, Emanating hyphae.

Anatomical characters of mantle in plan view: Mantle thickness 27-35 (41) µm, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 21-25 µm, plectenchymatous, compactly arranged, representing type D pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024); hyphal cells 5-16 µm tangentially and 3-9 µm radially, compactly arranged, smooth, hyaline, septate, thin walled (0.5 µm), not constricted at septa, clampless, septa as thick as hyphal wall. Inner mantle layer 8-11 µm, pseudoparenchymatous representing type K pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2021), hyphal cells colourless, hyaline, thin walled, variable in shape measuring 3-10 µm tangentially and 3.0-6.5 µm radially.

Anatomical characters of emanating elements: Rhizomorphs absent. Emanating hyphae 3-5 µm, thin walled (0.8 µm), septate, not constricted at the septa, without clamps, septa as thick as hyphal wall (up to 0.8 µm). Cystidia 52-114 × 3.0-6.5 µm, present on the outer mantle layer, the most distinct and often infrequent and exhibiting type1 pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024), awl-shaped with almost acute apex and swollen or bean shaped base showing type A pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024), hyaline, smooth, thick walled (up to 1.5 µm), aseptate without clamps.

Anatomical characters in longitudinal section: Mantle 28-32 (40) µm, differentiated into outer and inner mantle layer. Outer mantle layer 23-25 µm, more or less plectenchymatous, compact, representing type D pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024), hyphal cells 5-16 µm tangentially and 3-8 µm radially, without any content and clamp connections. Inner mantle layer 5-7 µm, pseudoparenchymatous; hyphal cells 3-6 µm tangentially and 3-5 µm radially. Hartig net one cell deep, the Hartig net palmetti type with one row of 3-7 µm radially and 1.6-3.5 µm tangentially, roundish to cylindrical hyphal cells and is restricted to the anticlinal walls of the cortex cells (paraepidermal). Root tip mantle up to 52 µm different from rest of the mantle, more or less plectenchymatous, having 3-4 µm interwoven, septate, hyaline hyphal cells, hyphae rather irregularly arranged and no special pattern discernible representing type B pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024). Hartig net also paraepidermal at very root tip with one row of roundish cells measuring 3-9 µm tangentially 3-5 µm radially. Epidermal cells become radially elongated, 16-23 × 9-16 µm, tangentially oval to elliptic or cylindrical, and oriented obliquely. Tannin cells not observed.

Colour reactions with different reagents: $FeSO_4$: n. r. (no reaction); Sulphovanillin: brown; Ethanol (70%): n. r.; KOH (10%): n. r.; Acetic acid: n. r.; Melzer: light yellow; Cotton blue: hyphal cell wall dark blue.

3. Description of ectomycorrhizae: *Lactarius shiwalikensis* Kumar & Atri + *Shorea robusta* Gaertn. Figure 5 and 6

Morphological characters: Mycorrhizal system mostly simple, if ramified then monopodial-pinnate with 0-1 order of ramification, up to 4 mm long. Main axes 0.2-0.3 mm in diameter. Unramified ends slightly bent to straight, 0.7-2.5 mm in length and 0.1-0.2 mm in diameter; tips

rounded. Surface of unramified ends smooth, with no soil particles attached, reddish brown (8E8) to light brown (5D8), including the tip colour. Rhizomorphs present, scanty, connected to mantle at restricted point. Extraradical hyphae present on the surface. Cystidia not reported. Sclerotia lacking.

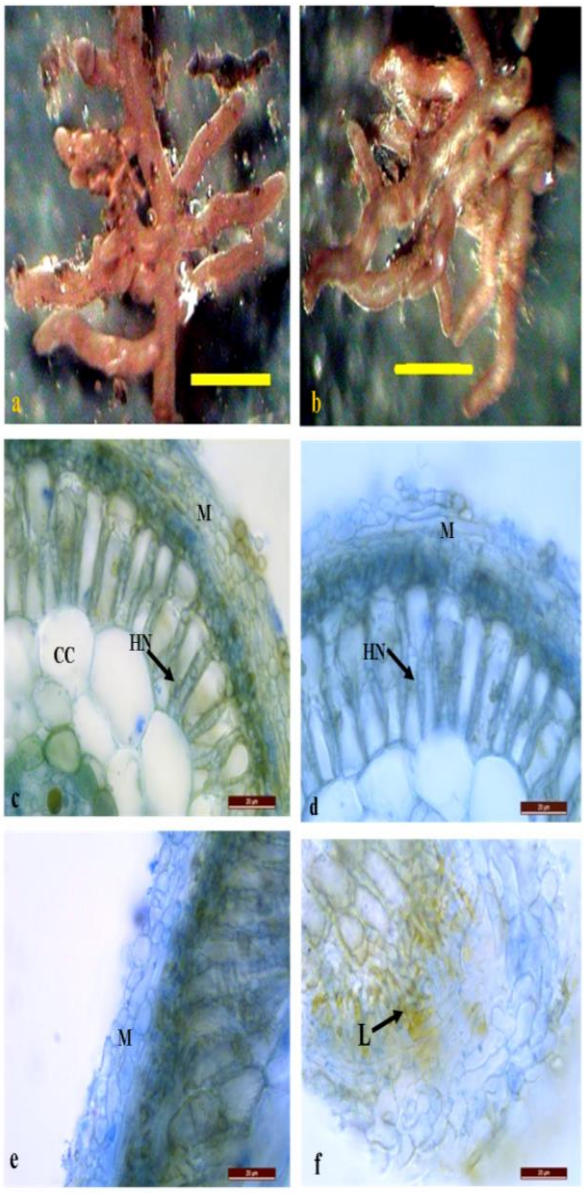

Figure 5: *Lactarius shiwalikensis* + *Shorea robusta*: a-b, Mycorrhizal system; c-d, Cross section of ectomycorrhizal root showing mantle (M) and Hartig net (HN); e, Longitudinal section of ectomycorrhizae showing mantle (M) and radially elongated epidermal cell with Hartig net (HN); f, Longitudinal section of root tip showing plectenchymatous outer mantle with leticifers (L). Scale bar a-b=1 mm.

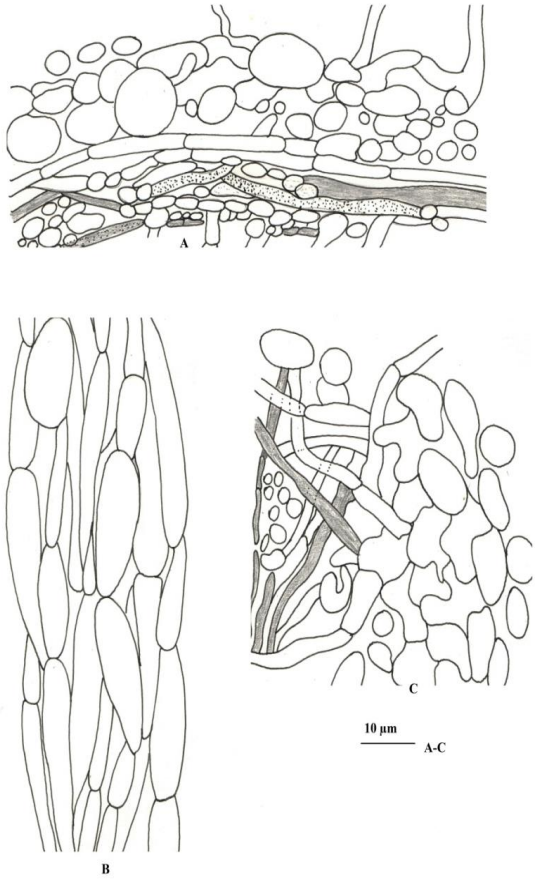

Figure 6: *Lactarius shiwalikensis* + *Shorea robusta*: A, Mantle; B, Rhizomorphs; C, Root tip mantle.

Anatomical characters of mantle in plan view: Mantle thickness 32.6-39.0 µm, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 18-24 µm, pseudoparenchymatous, with no hyphal net on the surface, representing type K (Agerer, 1987-2012; Agerer and Rambold, 2004-2024); hyphal cells 5.0-24.5 µm tangentially and 5-13 µm radially in diameter, irregularly shaped polygonal to rounded, colourless, smooth, septate, thin walled (0.5 µm), clampless, septa as thick as hyphal wall; laticifers lacking in the outer mantle layer. Inner mantle layer 10-16 µm, plectenchymatous and heterogenous, in some parts bundles of hyphae growing in parallel manner, representing type F pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024); hyphae 1.6-3.0 µm, thin walled (0.5 µm), septate, septa as thick as hyphal wall, not constricted; hyphal cells rounded to oval, 1.6-8.0 µm tangentially and 1.6-3.0 µm radially; laticifers with dark brown granular content present in the inner mantle layer, scanty and quite thick, septate, thick walled, not constricted; clamps not observed.

Anatomical characters of emanating elements: Rhizomorph present, white to light brown, covered with soil particle at some places; 12-24 µm thick, rough, hyphae 5-8 µm, margin smooth with very scanty emanating hyphae, denser arrangement with inflated hyphal cells (type E), some hyphae with prominent ampullate protrusions located medially or distally, hyphae much constricted at the septa, peripheral hyphae compact and curled to twisted in others, all the hyphae clampless and thin walled, nodia not observed. Emanating hyphae 1.6-4.0 µm, septate, colourless, hyaline, smooth, thin walled and form irregular interwoven network over the surface, septa as thick as hyphal wall, constriction at the septa common. Cystidia absent.

Anatomical characters in longitudinal section: Mantle 24-30 µm thick, outer mantle layer 13-19 µm, pseudoparenchymatous, representing type K pattern (Agerer, 1987-2012; Agerer and Rambold, 2004-2024); hyphae 1.6-3.3 µm in diameter, hyphal cells 1.6-4.0 µm tangentially and 1.6-5.0 µm radially, roundish or more elongated. Inner mantle layer 9.8-16.0 µm, heterogenous or more or less plectenchymatous. Laticifers present in the inner mantle layer, 2-4 µm, septate, thin walled (0.8 µm), not constricted. Clamps not observed. Root tip mantle distinct from rest of the mantle, 48-58 µm, more or less plectenchymatous, compact, single element difficult to measure; hyphae rather irregularly arranged representing type B pattern and no special pattern is discernible (Agerer, 1987-2012; Agerer and Rambold, 2004-2024). Hartig net paraepidermal at the tip with one row of roundish to elongated cells measuring 5-11 µm tangentially and 1.6-3.0 µm radially, thin walled.

Colour reactions with different reagents: $FeSO_4$: n. r. (no reaction); Ethanol (70%): n. r.; KOH (10%): n. r.; Sulphovanillin: Laticifers turn dark blue; Acetic acid: n.r.; Cotton blue: walls blue; Melzer: dark brown.

DISCUSSION

The mere occurrence of fruiting body in vicinity with host plant could not be taken as a proof for mycorrhizal association and only synthesis experiments under controlled condition can furnish the conclusive proof (Melin, 1936; Kumar and Atri, 2023). Mycorrhizal synthesis experiments are reported to be very useful so as to determine the potential fungus - plant host compatibility (Àgueda *et al*., 2008). In the recent years, the study undertaken by Kumar and Atri (2023) on selection of efficient ectomycorrhizal fungi for improved growth, biomass and nutrient uptake of *Shorea robusta* seedlings clearly confirms the compatibility of *S. robusta* with *Russula kanadii*, *Russula cyanoxantha* and *Lactarius shiwalikensis*. The mycorrhizal relationship of these 3 species was established with *Shorea robusta* for the first time (Kumar and Atri, 2023). Earlier *Russula michiganesis*, *Russula amoena* and *Lycoperdon compactum* was investigated for the establishment of mycorrhiza with *Shorea robusta* by Pyasi *et al*. (2013) and Tapwal *et al*. (2015).

Mycorrhizas synthesized artificially were examined microscopically so as to determine whether interspecific characteristics of Sal EcM corresponded to a particular mycorrhizal morphotype. As mycorrhizal morphology within a species/isolate may vary with substrate and other external environmental conditions, the descriptions reported were based exclusively on mycorrhizas developed under controlled condition. The inner tissues of ectomycorrhizas, i.e. the Hartig net structure, are important for the Intercellular symbiosis of the mycorrhiza. In particular, it is the key characteristic determining plant-fungus specificity (Kumar and Atri, 2018). In the present study, mycorrhizas formed under controlled condition had the characteristics of their respective fungal genera. *Shorea robusta* seedlings inoculated with inocula of these 3 EcM mushrooms resulted in well-developed fungal sheath and Hartig net mycelium colonizing the intercellular spaces, features characteristic of ectomycorrhizas. All the 3 artificially synthesized EcM share some macroscopic characteristics, but they are clearly different on some other features. EcM roots associated with *Russula kanadii* were white to grayish brown while those with *Russula cyanoxantha* were white to silvery brown. EcM roots associated with *Lactarius shiwalikensis* were red to reddish brown, similar to the colour of the sporophore. Mycorrhizal system was mostly monopodial pinnate in all the artificially synthesized EcM. Surface of unramified end was not smooth, but densely short-spiny in both *Russula* species, while it was smooth to cottony in *Lactarius* EcM. In all the 3 EcM, fungal hyphae were noticed neatly penetrating the intercellular spaces in epidermal layer, forming para-epidermal Hartig net, a typical sign of angiosperm type of EcM associations. The morpho-anatomical characters of *Russula cyanoxantha* EcM

documented in the present study were almost similar to those obtained under natural condition with few differences in size of mycorrhizal system and thickness of mantle layer (Kumar and Atri, 2020b). In artificially synthesized ECM of *R. cyanoxantha*, silvery brown mycorrhizal system were not recorded as observed in natural EcM and surface of unramified end was loosely short spiny. EcM of *Lactarius shiwalikensis* had well-developed fungal sheaths and extraradical mycelium, including rhizomorphs. They were thus comparable to naturally formed mycorrhizas reported from other plants (Agerer, 1995; Agerer, 1987-2012). *Lactarius shiwalikensis* mycorrhizas were opaque and matte due to well-developed gelatinous material, similar to that reported for other *Lactarius* spp. EcM (Agerer, 1995). Latex and any other secretion was altogether absent from both the EcMs of *Russula*. Ectomycorrhizae of both *Russula* species examined in the present samples are well characterized by the presence of plectenchymatous outer mantle layer with numerous cystidia which is quite common in the EcM association of various *Russula* species with *Shorea leprosula* (Lee *et al*., 1997) and *S. robusta* (Bakshi, 1974; Kumar and Atri, 2016; 2019). Present study describes for the first time the ectomycorrhizae synthesized between *Lactarius shiwalikensis* and the dipterocarp species *Shorea robusta*. To date, no description of ectomycorrhizae formed by *S. robusta* in association with *Lactarius* sp. is available. Most of the features of EcM synthesized presently resembles to those described for mycorrhiza formed by *Lactarius cistophilus* Bon &Trimbach with *Cistus* sp. (Comandini, 2006) from Sardinia, Italy, however emanating hyphae and rhizomorphs were observed in the present collection which were not reported in EcM of *Lactarius cistophilus*. The *Lactarius* mycorrhiza described here agrees in general with the description of *Lactarius*-type mycorrhiza pointed by Hutchinson (1999), in particular, the presence of laticiferous hyphae in the inner layer of the mantle. These anatomical features are similar to those in other *Lactarius* species such as *L. deliciosus* (L.:Fr.) SF Gray (Kernaghan *et al*., 1997) and *L. deterrimus* Gröger (Agerer, 1986). Similarly, the colour between fruit bodies and mycorrhizal system seems to be a consistent feature in *Lactarius* mycorrhiza, as observed in the present study.

CONCLUSION

The results of the present investigations undertaken clearly suggest that *Russula kanadii*, *Russula cyanoxantha* and *Lactarius shiwalikensis* are the EcM associates of *Shorea robusta*. For optimising the production of *Shorea robusta* in nursery, inoculation of the Sal seedlings with the mycelia of these tested mushrooms can be safely recommended. In addition, the use of these selected EcM fungi can be an effective and more environment friendly approach to Sal nursery growers.

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