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Research Article

Ectomycorrhizal biology of *Shorea robusta* roots in association with Russula Pers. from foothills of Himalayas, India

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Abstract: In this article, morphoanatomical details of mycorrhizal roots of *Shorea robusta* associated with three *Russula* species viz. *Russula chlorinosma* Burl., *Russula azurea* Bres. and *Russula cyanoxantha* (Schaff.) Fr. were investigated for the first time. Studies were conducted in the Sal forests of Shivalik mountain range of Himalayas at North West India where *Shorea robusta* solely dominates. Ectomycorrhizal (EcM) studies viz, sporophore association with *S.robusta* were done according to the standard methodology. In our observation, EcM colonized roots are distinguished by differences in the shape and color of mycorrhizal system, surface texture, size and shape of cystidia, cell shape of mantle, as well as the differing chemical reactions and the roots of *S. robusta* are much shorter than non-mycorrhizal roots which are found to be variously branched. Illustration with micro photographs has been provided for this study to facilitate Ectomycorrhizal studies.

Keywords: *Shorea robusta*; *Russula spp.;* Ectomycorrhiza

Introduction

Shorea robusta Gaertn. (Dipterocarpaceae) which is commonly known as Sal, is a major constituent of moist deciduous broad-leaved tropical forests in India. Sal forest ecosystem has a wide distribution in India and spans across 10 million hectare through a number of states including Assam, West Bengal, Odisha, Jharkhand, Madhya Pradesh, Haryana, Himachal Pradesh and Uttrakhand (Singh and Singh 1992). Ectomycorrhizal (EcM) fungi are considered as key ecological factors in governing and maintaining the terrestrial ecosystem (Wang *et al.* 2017). Despite the great bio-geographic significance of tropical dipterocarp forest, it remains poorly documented in terms of EcM diversity (Alexander and Selosse 2009, Kumar and Atri 2019). The Sal roots have been reported to be putatively associated with the species of various fungal genera. Based on sporocarps surveys, fruiting body of *Russula, Boletus, Agaricus, Amanita, Lactarius, Laccaria, Pisolithus, Suillus,* and *Cantharellus* were found growing under Sal tree (Natarajan *et al.* 2005, Tapwal *et al.* 2013, Kumar and Atri 2016, 2019).

Russula Pers. (Russulaceae; Basidiomycota) is one of the dominant EcM genera, represented by ca. 158 taxa from India (Sharma *et al.* 2017). *Russula* is reported to be one of the highly diverse EcM group in Agaricomycetes associated with the roots of higher plants and play a important role in maintaining forest ecosystems and diversity (Avis 2012, Corrales *et al.* 2016 Uesugi *et al.* 2016). It is reported that *Russula* spp. can form EcM with many temperate and tropical plant families, including Leguminosae, Fagaceae, Cistaceae Dipterocarpaceae, Salicaceae, Betulaceae, Nothofagaceae Myrtaceae and Pinaceae (Tedersoo *et al.* 2010,

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Wang *et al.* 2017). To date, approximately 1100 *Russula* species have been reported worldwide (Kirk 2014), and distributed across a wide range of habitats from the tropics to arctic zones (Riviére *et al.* 2007, Ba *et al.* 2012, Atri *et al.* 2016). Thus *Russula* are important members of many well studied EcM fungal communities world-wide, indicating that this genus likely plays a significant role in sustaining the forest eco-system (Lee *et al.* 1997, Smith *et al.* 2011).

In India, however, information on native EcM fungi has been based on the occurence of fungi fruiting under putative EcM trees without confirming the actual EcM association below ground. Hence, most native EcM hosts and their EcM fungal assemblages have not been studied and described in India so far. However, we also lack information on characterization of EcM roots, with only few available descriptions (Mohan *et al.* 1993a, b, c, Kumar and Atri 2016, 2019). Of the total EcM roots descriptions published so far only 13 descriptions are available for different species of *Shorea* in Dipterocarpaceae (Agerer and Rambold 2004-2020, Roman *et al.* 2005, Rinaldi *et al.* 2008) with only 5 descriptions available for *Shorea robusta* (Kumar and Atri, 2016 & 2019). Hence, the present study was taken up to characterize and identify more EcM roots of *Shorea robusta*.

In the present study sporocarps and their EcM colonized roots were collected by tracing the hyphal or rhizomorphs connections in association with *Shorea robusta* from pure Sal forests. Tracing the mycelial or rhizomorphs connections in association with fruit body and EcM colonized roots is still the most reliable way of assessing the EcM status in the field (Agerer 1986, 2006). The macroscopic and microscopic details of each investigated taxa was worked out and identified up to species level. The aim of this paper is to characterize ectomycorrhizae of *Russula chlorinosma* Burl., *Russula azurea* Bres. and *Russula cyanoxantha* (Schaff.) Fr. collected in their natural habitat associated with the roots of *Shorea robusta*.

Materials and Methods

Study area

Area selected for present investigation is Sal forests of Shivalik mountain range of North West India, which are the geologically lowest and youngest mountain range of Himalaya. Dipterocarp tree *Shorea robusta* purely dominates the Sal forests. The study area is located between 29^0 58/ – 31^0 2/ -Northern latitude 77^0 34/ – 78^0 18/ -Eastern longitudes. The average elevation of the area is 400-1500 m and vegetation of the area is typical of tropical moist deciduous forests (Champion and Seth 1968).

Sampling, identification and characterization of sporocarps:

EcM root tips and all epigeous sporocarps of putative EcM genera were collected from different sites in pure Sal forests, ca.700 m above sea level during the rainy season (July to October) in 2013 and 2015. The *Russula chlorinosma, Russula azurea* and *Russula cyanoxantha* sporocarps and their EcM colonized roots were collected by tracing the hyphal connections between *Shorea robusta* roots and sporocarps. Morphological characters of each specimen were noted on the field key (Atri *et al.* 2005). Sporocarps were air dried at 40-45^{0}C in a drier specially designed for drying mushroom specimens (Atri *et al.* 2005) and finally packed in a cellophane packet for permanent preservation in Herbarium. The macroscopic and microscopic details of each investigated taxa was worked out as per standard methodology (Singer 1986, Atri and Saini 2000) and identified up to species level using standard literature (Burlingham 1924, Romagnesi 1967).

Sampling, identification and characterization of EcM roots:

Mycorrhizal roots underneath sporocarps were collected and wrapped in polythene bags and

brought to the laboratory for further analysis. The collected roots were first gently washed with flowing tap water on a 250 µm mesh to remove soil and attached debris. Before morphological characterization EcM roots were submerged in distilled water in a Petri dish. Morphological characterization of EcM roots was performed under a stereo-microscope (Magnus MSZ-TR), photographed and descrybed by careful examination following Agerer (1987-2012) and Agerer and Rambold (2004-2020) and compared to other available descryptions of *Russula* ectomycorrhizae. The mycorrhizal roots were fixed in FAA [5 ml formalin (37%) + 5 ml acetic acid (100%) + 90 ml alcohol (50%)] for anatomical characterization.

EcM colonization was confirmed by preparation of cotton blue stained semi-thin sections of EcM roots, and microscopic examination by checking for the presence of mantle and Hartig net. The cross section and longitudinal section of EcM roots were examined and drawn under a compound microscope and photographed under digital microscope (Leica DM4000 B LED) for the presence of the mantle, Hartig net, hyphal and rhizomorphs characteristics. The color terminology used is that of Kornerup and Wanscher (1978). Micro chemical reactions on EcM roots were performed using $FeSO_4$, Sulphovanillin, Ethanol, KOH, Melzer and Cotton blue.

Results

Description of ectomycorrhizal roots

Russula chlorinosma* + *Shorea robusta

Morphological characters:

Mycorrhizal system or roots irregularly pinnate to simple with zero to one order of ramification, 2.5-5.0 mm long; main axes 0.2-0.3 mm in diameter. Unramified ends sinuous to slightly bent, cylindrical, 1-4 mm in length and 0.1-0.3 mm in diameter, tips rounded not swollen. Surface of unramified ends stringy to cottony, occasionally with soil particles, younger mycorrhizal roots white with grayish brown patches and older grayish brown (5D3), unchanging, not secreting latex or any other fluid when injured; mantle not transparent; mantle hydrophobicity absent, tip shows the same color as rest of the mycorrhizal roots. Emanating hyphae rarely observed. Cysti-dia present. Sclerotia not observed.

Anatomical characters of mantle in plan view: Mantle thickness (57) 62-73 (81) µm, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer (32) 40-57 (65) µm, loosely plectenchymatous, arranged net like, with almost parallel hyphae, representing type D pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020); hyphal cells 3-5 µm, compactly arranged, smooth, hyaline, filled with oily droplets as observed in hyphae of the sporophore, that do not stain in sulpho-vanillin, septate, thin walled (0.5 µm), constricted at septa, clamp-less; septa as thick as hyphal wall, anastomoses common. Inner mantle layer (16) 19.6-21.0 (24.5) µm, pseudoparenchymatous representing type K pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020), hyphal cells colourless, hyaline, thin walled, 3.0-4.5 µm broad.

Anatomical characters of emanating elements: Rhizomorphs not observed. Emanating hyphae originating from mantle 3-4 µm broad, thin walled (0.8 µm), abundantly or sparsely filled with oily droplets, septate, not constricted at the septa, without clamp, septa as thick as hyphal wall (up to 1 µm). Cystidia 16-50 × 3-5 µm, present on the outer mantle layer, the most distinct and often infrequent and type1 pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020), sub-cylindrical to obclavate and clavate to flask-shaped with acute to rounded apex and swollen or rounded base, exhibiting type B and O pattern (Agerer 1987-2012, Agerer and Rambold, 2004-2020), hyaline, smooth, thick-

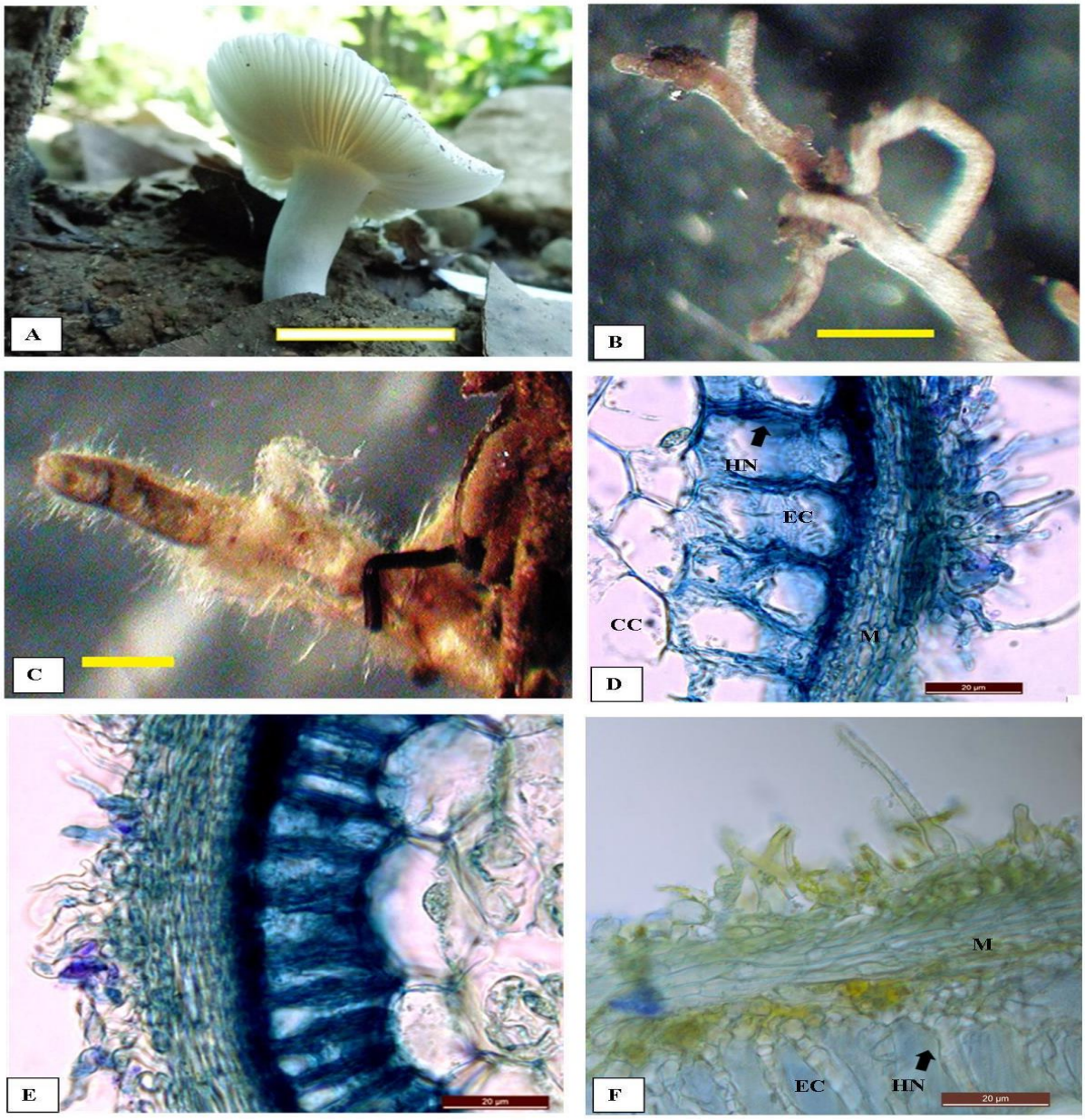

Figure 1. ***Russula chlorinosma* + *Shorea robusta*:** a. Sporophore in association with *Shorea robusta* root, b-c. Mycorrhizal roots d-e. Cross section of ectomycorrhizal root showing mantle (M) and Hartig net (HN), f. Longitudinal section of ectomycorrhizal root showing mantle and radially elongated epidermal cell (EC) with Hartig net. Scale bar a= 3 cm, b-c=1 mm

walled (up to 1.5 µm), aseptate without clamps. Similar cystidia were observed on pileipellis of the sporophores.

Anatomical characters in longitudinal section: Mantle 57-73 µm, differentiated into outer and inner mantle layer. Outer mantle layer 32-57 µm, plectenchymatous, compact, representing type D pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020), hyphal cells 3-4 µm broad, without any content and clamp connections. Inner mantle layer 16-22 µm, pseudoparenchymatous; hyphal cells 3-4 µm broad. Hartig net one cell deep, palmetti type with one row of 1.6-3.5 µm broad cylindrical hyphal cells and is restricted to the anticlinal walls of the cortex cells (paraepidermal).

Root tip mantle up to 195.6 µm, different from rest of the mantle, plectenchymatous, having 3.0-5.7 µm interwoven, septate, hyaline hyphal cells,broader than rest of the mantle, hyphae rather irregularly arranged branched and no special pattern discernible representing type B pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020); anastomoses common, open forming H shaped bridge. Hartig net is also paraepidermal at the very root tip with one row of cylindrical hyphal cells. Epidermal cells become radially elongated to increase the area available for the Hartig net, 32.6-40.7 × 13.0-19.6 µm, tangentially oval to elliptic or cylindrical, and oriented obliquely. Tannin cells not observed.

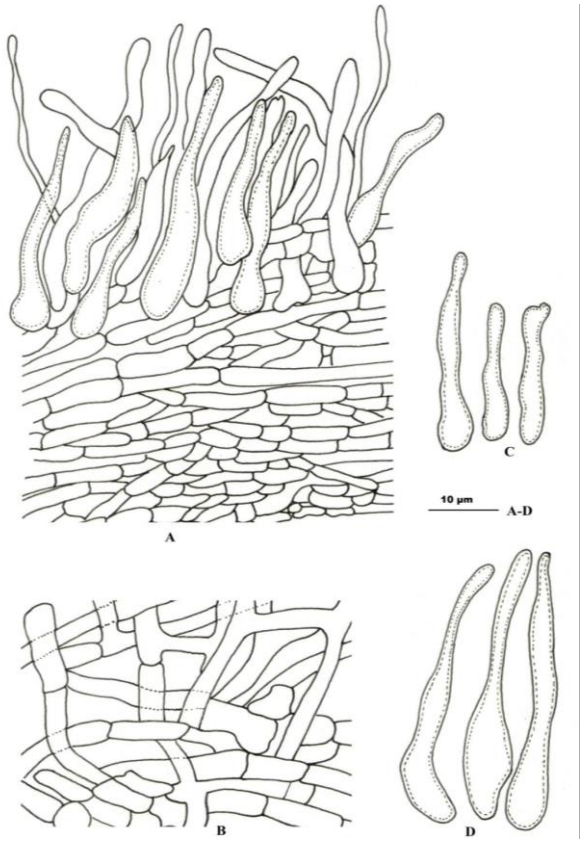

Figure 2. *Russula chlorinosma* + *Shorea robusta*: A. mantle, B. Root tip mantle, C-D. Cystidial elements.

Color reactions with different reagents: $FeSO_4$: brown; Cotton blue: walls blue; Sulphovanillin: brown; Acetic acid: n.r.; Ethanol (70%): n. r.; KOH (10%): n. r.; Melzer: light yellow.

Russula azurea* + *Shorea robusta

Morphological characters: Mycorrhizal roots irregularly pinnate to coralloid with zero to one order of ramification, 2-6 mm long; main axes 0.2-0.4 mm in diameter. Unramified ends abundant, sinuous to slightly bent, cylindrical, 1-4 mm in length and 0.1-0.4 mm in diameter, tips rounded not swollen. Surface of unramified ends shiny, smooth, occasionally with soil particles, younger mycorrhizal roots purple to grayish purple and older reddish brown (5D8) to purplish brown, grayish orange (6B3) to often with violet brown (10E4) to grayish violet similar to color of sporophore cap, unchanging, not secreting latex or any other fluid when injured: mantle not transparent; mantle hydrophobicity present, tip dark brown in color. Rhizomorps present. Emanating hyphae present, not frequent. Cystidia present. Sclerotia not observed.

Anatomical characters of mantle in plan view: Mantle thickness 65-97 µm, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 32-65 µm, plectenchymatous, with cystidial elements on the surface, slightly gelatinized, compactly arranged, representing type C pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020); hyphal cells 2.5-4.0 µm broad, compactly arranged, smooth, granulated, memberaneously pigmented, light brown, septate, thin walled (0.8 µm), slightly constricted at the septa, clamp-less; septa as thick as hyphal wall. Inner mantle layer 16-25 µm, pseudoparenchymatous, with oval to epidermoid cells representing type M arrangement (Agerer 1987-2012, Agerer and Rambold 2004 -2020), hyphal cells colourless, hyaline, thin walled (0.8 µm), 3.0-4.5 µm broad, tangentially-oval, elliptic to cylindrical and oriented parallel to the root axis.

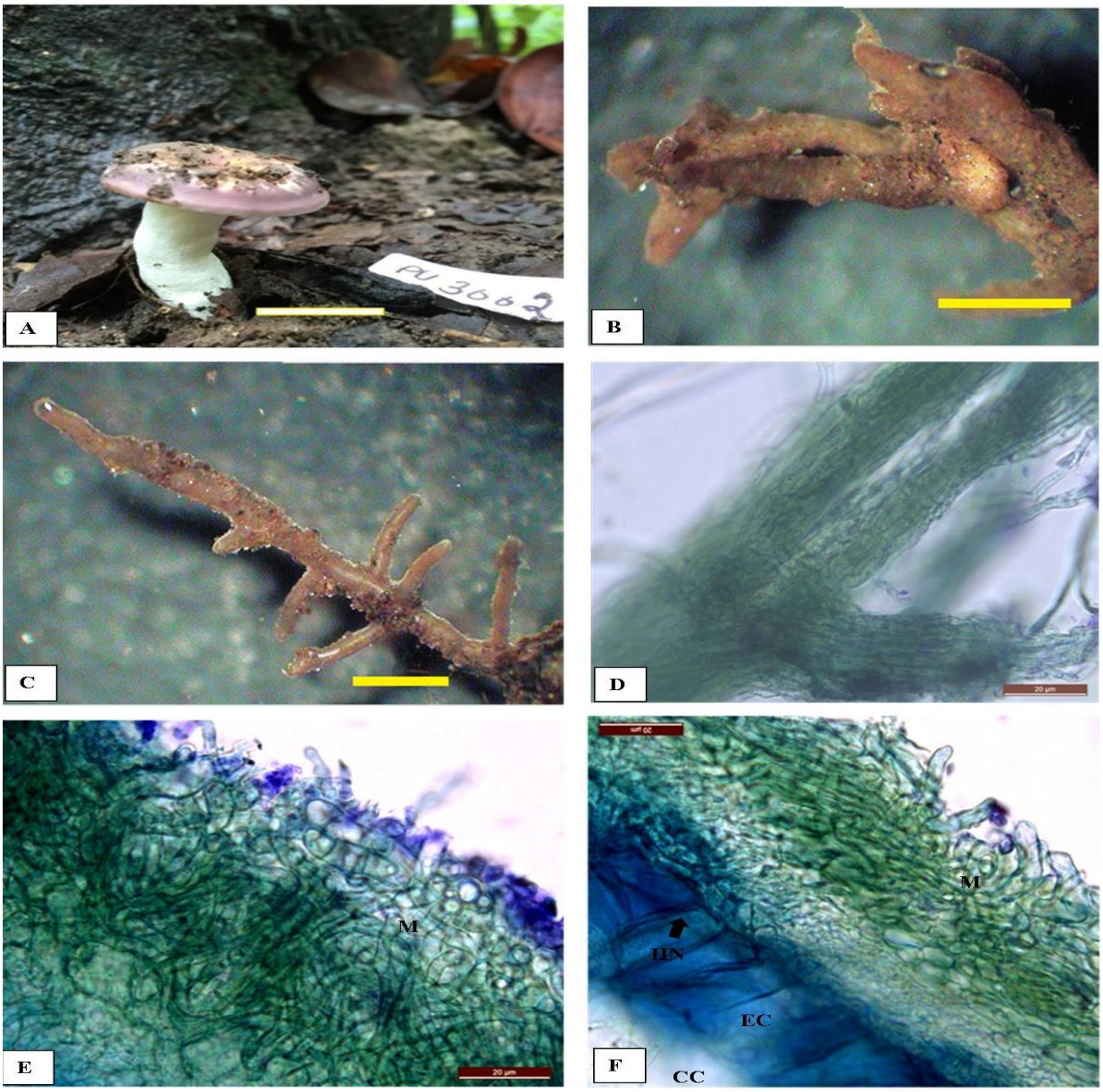

Figure 3. *Russula azurea* + *Shorea robusta*: a. Sporophore in association with *Shorea robusta* root, b. Mycorrhizal root, c. Surface view of unramified end showing rhizomorphs in connection with mantle, d. Cross section of ectomycorrhizal root showing mantle (M) and Hartig net (HN), e. Longitudinal section of ectomycorrhizal root showing mantle and radially elongated epidermal cell (EC) with Hartig net, f. Longitudinal section of root tip showing plectenchymatous mantle. Scale bar a= 3 cm, b=1 mm.

Anatomical characters of emanating elements: Rhizomorphs present, 40-80 µm, milky-white, composed of loosely woven, undifferentiated hyphae of equal diameter, frequent, flat, oblique and with rough surface having extra-radical hyphae emanating from the surface, at some places covered with soil particle; hyphae 1.6-4.0 µm broad, thick-walled (up to 1 µm), septate, constricted at the septa, without clamp, septa as thick as hyphal wall, central hyphae almost parallel, sometime bent and curled to twisted; peripheral hyphae intermingled. Anastomoses between hyphae are not observed, nodia and internodia present, ramification with one or two branches at nodia. Emanating hyphae originating from rhizomorphs, 3-5 µm broad, frequent, branched to monopodial, thin walled (0.8 µm), granulated, septate, without clamp,

constricted at the septa. Emanating hyphae 3-4 µm broad, thin-walled (0.8 µm), septate, not constricted at septa, without clamp, septa as thick as hyphal wall (up to 1 µm). Cystidia 16-40 × 3-5 µm, present on the outer mantle layer, the most distinct and often infrequent and representing type1 pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020), sub cylindrical to clavate with rounded apex, slightly tapering, often rather similar to the ends of normal hyphae showing type O (Agerer 1987-2012, Agerer and Rambold 2004-2020), slightly gran-ulated to non-granulated, smooth, thick-walled (up to 1.5 µm), aseptate to septate without clamps.

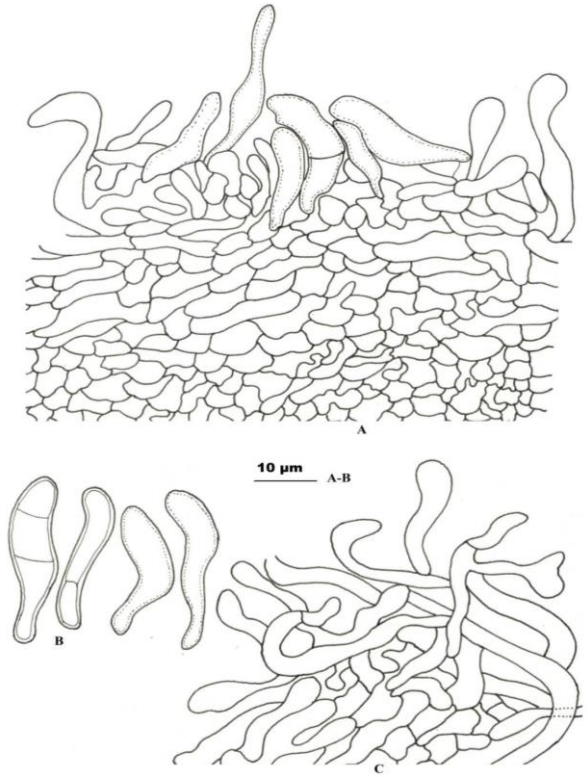

Figure 4. *Russula azurea* + *Shorea robusta*: A. mantle, B. Cystidial elements, C. Root tip mantle.

Anatomical characters in longitudinal section: Mantle 56-73 µm, differentiated into outer and inner mantle layer. Outer mantle layer 34-57 µm, plectenchymatous, compact, slightly gelatinized, representing type D pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020), hyphal cells 3-4 µm broad, without any content and clamp connection. Inner mantle layer 16-22 µm, pseudoparenchymatous; hyphal cells 3-4 µm broad. Hartig net one cell deep, palmetti type with one row of 1.6-3.5 µm broad cylindrical hyphal cells and is restricted to the anticlinal walls of the cortex cells (paraepidermal). Root tip mantle 114-203 µm similar to rest of the mantle, plectenchymatous, having 3.0-5.7 µm interwoven, septate, hyphal cells, broader than rest of the mantle, hyphae rather irregularly arranged and no special pattern discernible representing type B pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020). Hartig net is also paraepidermal at very root tip with one row of cylindrical hyphal cells. Epidermal cells become radially elongated to increase the area available for the Hartig net, 40-46 × 13-20 µm, tangentially oval to elliptic or cylindrical, and oriented obliquely. Tannin cells not observed.

Color reactions with different reagents: $FeSO_4$: n. r. brown; Sulphovanillin: pale brown; Ethanol (70%): n. r.; Acetic acid: n.r.; KOH (10%): olive; Melzer: light yellow; Cotton blue: wall dark blue.

Russula cyanoxantha* + *Shorea robusta

Morphological characters: Mycorrhizal system 3-9 mm long, mostly simple, if ramified then simple monopodial pinnate with 0-1 orders of ramification. Main axes 0.2-0.4 mm in diameter. Unramified ends slightly bent, cylindrical, 0.8-1.8 mm in length and 0.2-0.3 (0.4) mm in diameter, tips rounded. Surface of unramified ends are not smooth, loosely short spiny, light brown unchanging, not secreting latex or any other fluid when injured; mantle not transparent, mantle hydrophobicity absent; tip shows the same color as rest of the mycorrhizal roots. Rhi-zomorphs not observed. Emanating hyphae frequent. Cystidia present. Sclerotia not observed

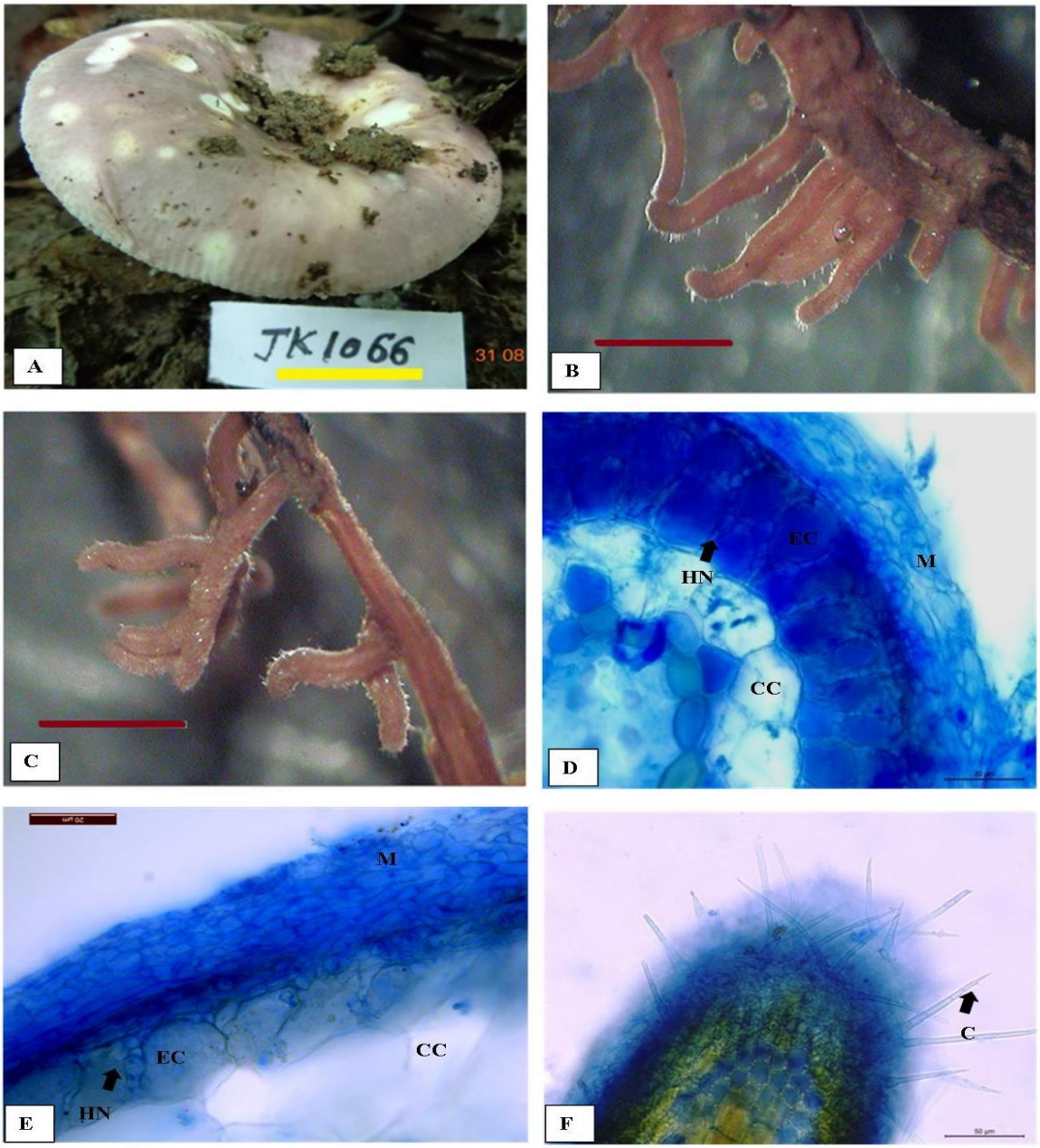

Figure 5. ***Russula cyanoxantha* + *Shorea robusta*:** a. Sporophore in association with *Shorea robusta* root, b-c. Mycorrhizal root, d. Cross section of ectomycorrhizal root showing mantle (M) and Hartig net (HN), e. Longitudinal section of ectomycorrhizal root showing mantle and radially elongated epidermal cell (EC) with Hartig net, f. Longitudinal section of root tip showing cystidia (C). Scale bar a= 3 cm, b-c=1 mm.

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, repres-enting type D organization (Agerer 1987-2012, Agerer and Rambold 2004-2020); hyphal cells 5-16 µm tangentially and 3-9 µm radially, compactly arranged, smooth, hyaline, septate, thin-walled (0.5 µm), slightly constricted at septa, clamp-less; septa as thick as hyphal wall. Inner mantle layer 8-11 µm, pseudoparenchymatous repress-enting type K pattern (Agerer 1987-2012, Agerer and Rambold, 2004-2020), hyphal cells colourless, hyaline, thin walled, variable in shape measuring 3-10 µm tangentially and 3.0-6.5 µm radially.

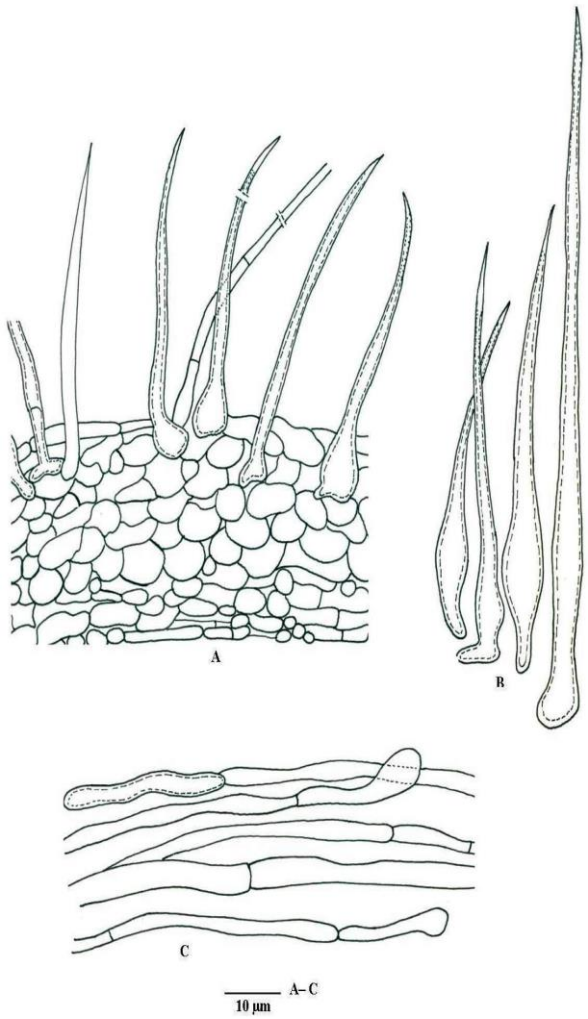

Figure 6. ***Russula cyanoxantha*** **+** ***Shorea robusta***: A. mantle, B. Cystidia, C. Emanating hyphae.

Anatomical characters of emanating elements: Rhizomorphs absent. Emanating hyphae 3-5 µm, thin walled (up to 0.8 µm), septate, not constricted at the septa, without clamp, septa as thick as hyphal wall (up to 0.8 µm). Cystidia 52-106 (114) × 3.0-6.5 µm, present on the outer mantle layer, at root tip cystidia are also present on the inner mantle layer, the most distinct and often infrequent and depicting type1 pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020), awl-shaped with almost acute apex and swollen or bean shaped base showing type A pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020), 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 arrangement (Agerer 1987-2012, Agerer and Rambold 2004-2020), hyphal cells 5-16 µm tangentially and 3-8 µm radially, cylindrical to inflated, without any content and clamp connection. Inner mantle layer 5-7 µm, pseudoparenchymatous; hyphal cells 3-6 µm tangentially and 3-5 µm radially, some hyphal cells epimemberaneously yellow-ish, hyphal wall pale blue. Hartig net one cell deep, palmetti type with one row of 3-7 µm radially and 3-5 tangentially roundish to cylin-drical hyphal cells and is restricted to the anticlinal walls of the cortex cells (para-epider-mal). Root tip mantle up to 52 µm, different from rest of the mantle, more or less pseudo-parenchyma-tous, having 3-4 µm interwoven septate hyaline hyphal cells, hyphae rather ire-gularly arranged, no special pattern discernible, representing type B pattern (Agerer 1987-2012, Agerer and Rambold 2004-2020). Hartig net is also para-epidermal at the very root tip with one row of roundish cells measuring 3-9 µm tangentially 3-5 µm radially. Epidermal cells become radially elongated to increase the area available for the Hartig net, 16-23 × 9-16 µm, tangentially oval to elliptic or cylindrical, and oriented obliquely. Tannin cells not observed.

Color reactions with different reagents: $FeSO_4$: n. r. (no reaction); Cotton blue: walls blue; Sulphovanillin: brown, content of cystidia dark brown; Ethanol (70%): n. r.; KOH (10%): n. r.; Melzer: light yellow.

Discussion

In this study when compared, Mycorrhizal roots of *Russula chlorinosma* are mainly characterized by irregularly pinnate to simple mycorrhizal system, stringy to cottony, white to grayish brown surface, while *Russula azurea*

have irregularly pinnate to coralloid, smooth, purple to grayish purple mycorrhizal system, whereas it is mostly simple, to monopodial pinnate with loosely short-spiny and light brown surface in case of *Russula cyanoxantha*. Size of cystidial elements are much larger in mycorrhizal roots of *Russula cyanoxantha* (52-114 × 3.0-6.5 µm) as compared to *Russula chlorinosma* (16-50 × 3-5 µm) and *R. azurea* (16-40 × 3-5 µm). In *Russula chlorinosma* cystidia are sub-cylindrical to obclavate and clavate to flask-shaped with acute to rounded apex and it was awl-shaped in *Russula cyanoxantha* with almost acute apex and swollen or bean shaped base, whereas it is sub-cylindrical to clavate with rounded apex, often similar to the ends of normal hyphae in *R. azurea.*

Family *Russulaceae* show a great diversity in tropical forest ecosystems, and are among the commonest EcM families (Malysheva *et al.* 2016, Riviére *et al.* 2007, Reis *et al.* 2018). Smith *et al.* (2011) reported that only the /russulalactarius lineage is more diverse in tropical than in temperate habitats. The fungal genus *Russula* is reported to be one of the highly diverse EcM group and play a critical role in maintaining forest ecosystems and biodiversity (Henkel *et al.* 2011, Avis 2012, Corrales *et al.* 2016). From dipterocarp forest number of Basidiomycete fungi including *Russula adusta, R. cinerella, R. congoana, R. delicula, R. michiganensis, R. amoena, R. delica, Amanita hemibapha, A. verna, Astraeus hygrometricus, Boletus edulis, B. fallax, Geastrum fimbriatum, G. triplex, Lycoperdon compactum, Scleroderma bovista, S. geaster, S. verrucosum* and *Agaricus trisulphuratus* are reported as EcM associates of *Shorea robusta* roots based on sporophore surveys (Pyasi *et al.* 2011, 2013, Tapwal *et al.* 2013, 2015). Out of these only *Russula michiganensis*, *R. amoena* and *Lycoperdon compactum* confirmed as EcM associates of *Shorea robusta* by synthesizing EcM in field experiment and rest is based on unsubstantiated observations. However, *Russula feugiana*, *R. cremeoavallanea, R. romagnesiana*, *R. nigricans* and *Lactifluus volemus* var. *volemus,* were also confirmed as EcM associates of Sal roots by observing the direct hyphal connection between Sal roots and mushrooms besides examining the morphoanatomical details of these roots (Kumar and Atri 2016, 2019). So very little is known about EcM diversity of *Shorea robusta* from Indian tropics.

In recent years, to add more to EcM diversity of *Shorea robusta* we have been carrying out a study on the diversity, ecology, and EcM biology of mushroom species occurring in direct association with *Shorea robusta* roots from North West India. In this present study, it is for the first time that *Russula chlorinosma, Russula azurea* and *Russula cyanoxantha* were recorded to form mycorrhizal association with Sal roots. All the mycorrhizal roots examined were EcM, because they showed both well-developed fungal sheaths and Hartig nets. The intimacy and the type of association were confirmed by observing the direct hyphal or rhizomorphs connection bet-ween *Shorea robusta* roots and mushrooms besides examining the morphoanatomical det-ails of these roots.

Russula chlorinosma was for the first time recorded from North America from mixed forest (Burlingham 1924). Romagnesi (1967) found *R. azurea* growing in shady broad-leaved woods on calcareous ground from Europe and Rayner (1970) found this species growing under conifers, especially spruce trees.

Mycorrhizal roots of *Russula cyanoxantha* are mostly simple, if ramified then monopodial pinnate with loosely short-spiny, light brown surface. Cystidia awl shaped with an almost acute apex and swollen or bean shaped base. *Russula cyanoxantha* was described as a putative EcM associate of *Shorea leprosula* from Pasoh, Malaysia (Lee *et al.* 1997). Morphoanatomical

study on EcM of *Russula cyanoxantha* in association with *Fagus sylvatica* L. roots was investigated by Beenken (2001). Morphological description given by Beenken (2001) was similar to the present study but differences were there in anatomical study. In the present study inner mantle layer is pseudoparenchymatous whereas it was reported to be plectenchymatous in association with *Fagus sylvatica*. Outer mantle layer was gelatinized and had variously shaped, infrequent, cylindrical, fusiform to bottle-shaped, non-capitate, knobbed cystidia in EcM roots of *Fagus sylvatica* while there is only awl shaped cystidia on outer mantle in association with *Shorea robusta* roots. The most of the features of EcM roots resembles to those described for mycorrhiza formed by unknown species of *Russula* with *Shorea leprosula* from Pasoh, Malaysia (Lee *et al.* 1997). Pešková *et al.* (2013) reported it fruiting under oak tree from Drevíc, Czech Republic. The morphoanatomical details of EcM roots of *Shorea robusta* in association with *Russula chlorinosma, Russula azurea* and *Russula cyanoxantha* has been described for the first time.

Presently collected mycorrhizal roots of *Russula cyanoxantha* are close to *Russula cremeoavallanea* and *Russula romagnesiana*. *Russula cremeoavallanea* differ from *Russula cyanoxantha* in having monopodial pinnate to irregularly pinnate and grayish brown to reddish brown, mycorrhizal roots while it was monopodial pinnate and white silvery to brown in case of *Russula romagnesiana*. Outer mantle layer is plectenchymatous, compactly arranged and inner mantle layer is pseudoparenchymatous in all the presently examined collection as observed in case of *Russula cremeoavallanea* and *R. romagnesiana* (Kumar and Atri 2019). The pseudoparenchymatous or epidermoid mantle, simple septate hyphae and cystidia are reported to be the common features of EcM formed by plant roots with various species of *Russula* (Agerer 1986). EcM of *Russula chlori-nosma, R. azurea* and *R. cyanoxantha* also differ in case of cystidial shape and size on the outer mantle surface. Cystidia are subcylindrical to obclavate and clavate to flask-shaped with acute to rounded apex and swollen or rounded base, exhibiting type B and O pattern (Agerer and Rambold 2004-2020) in case of *Russula chlorinosma* observed in case of mycorrhizal roots of *Shorea robusta* in association with *R. feugiana* (Kumar and Atri 2016). Cystidia in case of *Russula azurea* were also subcylindrical to clavate with rounded apex, slightly tapering, often rather similar to the ends of normal hyphae showing type O (Agerer and Rambold 2004-2020). Cystidia were awl-shaped with almost acute apex and swollen or bean shaped base showing type A pattern (Agerer and Rambold 2004-2020) in case of *Russula cyanoxantha*. Similar cystidial details were observed by Beenken (2001) in case of *R. cyanoxantha* in association with *Fagus sylvatica* L. from Europe. Rhizomorphs were observed in case of *Russula azurea* but these were not there in case of *Russula chlorinosma* and *R. cyanoxantha.* Hyphal morphology in carpophores and their respective EcM roots in all the studied samples were exactly alike. Hartig net is para-epidermal in all the EcM roots observed which is a typical character of angiosperm plants and epidermal cells of mycorrhizal roots become radially much elongated as compared to non-mycorrhizal roots which helps to increase the area available for the exchange of nutrients in the Hartig net region.

Conclusion

As all the three *Russula* species were found in direct organic connection with *Shorea robusta* roots and there is similarity in hyphal features of sporophores and the mantle, hence all the presently investigated species viz. *Russula chlorineosma R. azurea* and *Russula cyanoxantha* are EcM associates of *Shorea robusta*. The present

study enhances our knowledge of *Shorea* mycorrhizal biology, Moreover, close examin-ation of EcM features revealed differences amongst different *Russula* species EcM. In the future, it would be interesting to test the proposed EcM associates for synthesis of EcM in nursery for better survival, growth and establis-hment of *Shorea robusta* seedlings which hardly survive without its EcM associates.

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