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Species diversity of ectomycorrhizal fungi associated with temperate forest of Western Himalaya: a preliminary assessment

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An attempt has been made to give an assessment of the species diversity of epigeous ectomycorrhizal fungi of the temperate forests of Western Himalaya, based on studies carried out in the region. The main hosts were oaks (primarily *Quercus leucotrichophora* and *Q. floribunda*), pines (*Pinus roxburghii* and *P. wallichiana*) and deodar (*Cedrus deodara*). The species richness of ectomycorrhizal fungi was 43 in oak forests and 55 in conifer forests, which is close to midpoint values on the range derived from the literature for similar forest types. The major genera in terms of species were *Amanita* (15 sp.), *Russula* (13 sp.), *Boletus* (12 sp.), *Lactarius* (9 sp.), *Hygrophorus* (4 sp.) and *Cortinarius* (4 sp.). Some of these genera showed clear-cut host specificity – *Amanita* was primarily associated with conifers and *Russula* and *Boletus* with oaks. All these forests with the dominance of ectomycorrhizal hosts, had low tree species diversity.

ECTOMYCORRHIZAL fungi can account for 25% or more of the root mass of forests, thus representing a major below-ground structural component of the forest ecosystem. However, it remains the least known component from the standpoint of species diversity. Most of the past research on mycorrhizae was centred on plant–fungus interactions; their role on community ecosystem development, though important, remains poorly understood¹. Interest in the diversity of macro fungi has grown in recent years, but it largely concerns species number at the global level². We need to know about diversity of these fungi at a community or local level to develop management plans³ and to understand the pattern of diversity in relation to environmental changes. According to an estimate², less than a dozen published studies have measured species richness of epigeous macro fungi at the community scale

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in North America, and there are fewer from Europe and other continents. Some of the important studies pertaining to species richness of ectomycorrhizal fungi of forests in North America include spruce and hardwood forest of West Virginia⁴, and loblolly pine⁵ and alder forest in Alaska⁶. Clearly, this shortlist of investigation indicates that local or community level fungal diversity is poorly known, compared to many other groups of organisms and this also applies to India.

It may be pointed out that the species richness of mycorrhizal fungi does not follow the pattern of increasing species richness from the poles to the tropics, which is applicable to plants and many other organisms⁷. This is largely because fungal species richness varies among sites or forest stands and communities, regardless of latitude⁸. Thus, in the case of mycorrhizal diversity, it is important to know how it is associated with the dominant plant species and the communities they form.

The present study gives a preliminary account of species richness of fruiting fungi with emphasis on those forming ectomycorrhizal association with trees of the Western Himalayan temperate forest. This is based on studies carried out by various investigators during the last 100 years. The main objective is to give an estimate of species richness of ectomycorrhizal fungi at a scale of forest community. Giving an account of host range of ectomycorrhizal fungi is a secondary objective. The major communities are oak and mixed conifer forests.

We collected information from various published reports⁹⁻¹². The forest of the Western Himalayan region is dominated by ectomycorrhizal tree species only at the altitudinal range of 3000 m, starting from the foothills to the timberline. Limitations of the estimates include the fragmentary nature of data and consideration of only the species associated with dominant trees. We undertook this compilation work to synthesize existing information on species diversity of ectomycorrhizal fungi, so as to provide a ground for further work on alpha diversity (within community diversity) of these fungi and the communities that they form. Moreover, with ongoing forest degradation in the Himalaya, we may lose many species without knowing them. It is important to have some database on ectomycorrhizal and other fruiting fungi before their diversity is depleted as a result of anthropogenic pressure and climatic change.

In the Western Himalayan region, forests occur from the foothills to about 3500 m altitude and all dominant tree species have ectomycorrhizal association. The dominant tree species are *Shorea robusta* (Sal) in the foothills, *Pinus roxburghii* (pine) from 1000 to 1800 m, *Quercus* sp. (oak) from 1000 to 3000 m, *Cedrus deodara* (deodar) from 1800 to 2200 m, *Abies pindrow* (silver fir) from 2400 to 3000 m, *Betula utilis* (birch) from 2800 to 3500 m altitude¹⁰.

The temperate forest zone has two major forest types, viz. oak forest and mixed conifer forest. The common oak

studied for fruiting fungi by various investigators were *Quercus floribunda* and *Q. leucotrichophora*. The conifer forests generally consisted of pines (*P. roxburghii* and *P. wallichiana*), deodar (*C. deodara*) and fir (*A. pindrow*). In both these forests, generally 5–8 tree species occur¹³. All these forests tend to show a strong domination of one or two species, but occasionally they are interspersed with arbuscular mycorrhizal tree species like ash (*Fraxinus micrantha*) and maple (*Acer* sp.). The region has monsoon rainfall pattern. The annual precipitation is generally between 100 and 250 cm. In the foothills, the mean annual temperature is about 23°C, which declines at the rate of 0.46°C/100 m rise in altitude.

In ecosystem functioning, these forests resemble tropical forests more than temperate ones¹⁴. For example, decomposition of organic matter is rapid and forest floor litter mass and soil organic contents are low.

We have tabulated all the mycorrhizal genera and species found in the Western Himalaya, particularly Himachal Pradesh, with their specific host species by conducting an extensive search of contents of the published papers related to macro fungi of the area. The species surveyed were divided into two groups, viz. epigeous ectomycorrhizal and non-mycorrhizal. The epigeous ectomycorrhizal species (hereafter referred to as ectomycorrhizal) were subdivided into different groups depending upon their specific host species. The total number of species associated with each host species was estimated.

Following Molina *et al.*¹, all the ectomycorrhizal fungal species were categorized into three groups depending upon their host range, viz. narrow, intermediate and broad. The narrow host range included fungi forming ectomycorrhizal association with only one genus of the host family. The fungi in the intermediate host range category were limited to a single family of host plants, while those belonging to the broad range formed mycorrhizal association with diverse host plants typically crossing between plant families, order and even classes.

Across the oak and conifer forests of the Western Himalaya, the total number of epigeous fruiting fungi recorded was 298. Among these, ectomycorrhizal and non-

Table 1. Important ectomycorrhizal fungi of Western Himalaya and their association with oaks and conifers

Genus	Total mycorrhizal species	Per cent association with	
		Oaks	Conifers
<i>Amanita</i>	15	20	80
<i>Russula</i>	13	80	20
<i>Boletus</i>	12	83.3	16.6
<i>Lactarius</i>	9	45.5	55.5
<i>Suillus</i>	7	–	100
<i>Hygrophorus</i>	4	25	75
<i>Cortinarius*</i>	4	–	75

*Twenty-five per cent of the remaining species of *Cortinarius* are associated with *Betula*.

ectomycorrhizal, soil-inhabiting macro fungi were 101 and 197 respectively. The ratio between them was about 1:2 (33.9% mycorrhizal and 66.1% non-mycorrhizal). The total number of ectomycorrhizal species was 43 in oak forest and 55 in conifer forest. The major ectomycorrhizal fungal genera in terms of species number were *Amanita*, *Russula*, *Boletus*, *Lactarius*, *Suillus*, *Hygrophorus*, *Cortinarius* and *Lecinum* (Table 1). *Amanita* was predominantly mycorrhizal with pines, and *Boletus* and *Russula* with oaks. *Suillus* showed extreme host specificity, as all seven species were associated with pine. *Lactarius* indicated a wider host range. Out of nine species of this genus, five were associated with oaks and four with conifers. *Cortinarius* and *Hygrophorus* were also found to be

mainly associated with the roots of conifers. However, one species each was mycorrhizal with other trees, viz. *Betula* and *Rhododendron* respectively (Table 1). When both ectomycorrhizal and non-ectomycorrhizal fungi are considered, the following genera are most important in terms of species number: *Boletus* – 41 sp. (12 mycorrhizal, 29 non-mycorrhizal), *Russula* – 34 sp. (13 mycorrhizal, 21 non-mycorrhizal), *Lactarius* – 24 sp. (9 mycorrhizal, 15 non-mycorrhizal), *Amanita* – 23 sp. (15 mycorrhizal, 8 non-mycorrhizal), *Coprinus* – 13 sp. (all non-mycorrhizal), *Agaricus* – 12 sp. (2 mycorrhizal, 10 non-mycorrhizal), *Cortinarius* – 10 sp. (5 mycorrhizal, 5 non-mycorrhizal) and *Inocybe* – 9 sp. (2 mycorrhizal, 7 non-mycorrhizal).

Table 2. Number of ectomycorrhizal fungal species with varying range of host species

Category of host range		
Narrow (one host genus)		
Genus	No. of species	Major host species
<i>Boletus</i>	11	<i>Quercus</i> (Fagaceae), <i>Cedrus</i> (Pinaceae)
<i>Amanita</i>	10	<i>Pinus</i> (Pinaceae), <i>Quercus</i> (Fagaceae), <i>Cedrus</i> (Pinaceae)
<i>Russula</i>	10	<i>Pinus</i> (Pinaceae), <i>Quercus</i> (Fagaceae), <i>Cedrus</i> (Pinaceae)
<i>Lecinum</i>	6	<i>Quercus</i> (Fagaceae), <i>Betula</i> (Betulaceae)
<i>Lactarius</i>	6	<i>Abies</i> (Pinaceae), <i>Quercus</i> (Fagaceae)
<i>Suillus</i>	5	<i>Pinus</i> (Pinaceae)
<i>Suillus</i>	5	<i>Pinus</i> (Pinaceae)
<i>Hygrophorus</i>	4	<i>Picea</i> (Pinaceae), <i>Rhododendron</i>
<i>Hygrophorus</i>	4	<i>Picea</i> (Pinaceae), <i>Rhododendron</i>
<i>Cortinarius</i>	4	<i>Picea</i> (Pinaceae), <i>Salix</i> (Salicaceae)
<i>Clitocybe</i>	3	<i>Pinus</i> (Pinaceae), <i>Quercus</i>
Genus having < 3 species.		
<i>Strobilomyces-Quercus</i> (Fagaceae); <i>Laccaria-Pinus</i> (Pinaceae); <i>Lapista-Quercus</i> (Fagaceae); <i>Leucopaxillus-Picea</i> (Pinaceae); <i>Oudemansiella-Quercus</i> (Fagaceae); <i>Tricholoma-Cedrus</i> (Pinaceae) and <i>Betula</i> (Betulaceae); <i>Agaricus-Quercus</i> (Fagaceae); <i>Cystoderma-Cedrus</i> (Pinaceae); <i>Lepiota-Cedrus</i> (Pinaceae); <i>Gomphus-Picea</i> (Pinaceae) and <i>Cedrus</i> (Pinaceae); <i>Hygrocybe-Quercus</i> (Fagaceae) and <i>Cedrus</i> (Pinaceae); <i>Inocybe-Salix</i> (Salicaceae) and <i>Pinus</i> (Pinaceae).		
Intermediate (one host family)		
Genus	No. of species	Major host family
<i>Lactarius</i>	3	Pinaceae
<i>Amanita</i>	2	Pinaceae
<i>Hebeloma</i>	2	Pinaceae
<i>Suillus</i>	1	Pinaceae
<i>Volvariella</i>	1	Pinaceae
<i>Inocybe</i>	1	Pinaceae
<i>Cortinarius</i>	1	Pinaceae
<i>Galarina</i>	1	Pinaceae
<i>Tubaria</i>	1	Pinaceae
<i>Lacrymaria</i>	1	Pinaceae
Broad (little host restriction)		
Genus	No. of species	
<i>Russula</i>	3	
<i>Amanita</i>	2	
<i>Boletus</i>	1	
<i>Lactarius</i>	1	
<i>Agaricus</i>	1	

In the study forest of the Western Himalaya, 21 genera (with 79 sp.) existed under narrow host range, while 10 genera (with 14 sp.) fell under intermediate range. Only 5 genera showed little host restriction and belonged to the broad host range category (Table 2).

Among the different types of mycorrhizae, ectomycorrhizae have clearly more species globally (more than 5000 in the world), than arbuscular and other types of mycorrhizae. Information about species richness of these fungi at the community level is scanty². In hardwood and conifer forests of North America and other temperate regions, species richness^{2,7} of ectomycorrhizal fungi is reported to range between 18 and 114. Our values of species richness are close to the midpoint of this range, i.e. 43 in oak forest and 54 in conifer forest. These values are similar to those reported from other Himalayan regions, viz. Kashmir and Nepal conifer forests (53 and 52 species respectively)^{12,15}. Based on an intensive study of oak forests of Kumaun Himalaya, Pande, Veena (unpublished data) recorded 54 species of ectomycorrhizae. It seems that 40–50 species are fairly representative numbers for ectomycorrhizal species of Himalayan forests.

A comparison of the number of genera of ectomycorrhizal fungi across different forest types, viz. Australia, California, Italy and Himalaya indicates that the numbers are fairly similar for widely differing forest communities of the world (18–27). As expected, our forests are similar in composition of genera with those at Nepal (80% community coefficient). Interestingly, there is considerable similarity between our forests and those of Mediterranean woodlands (37–58%; Table 3). These values are high, though there is little similarity in tree species composition of these forests.

In terms of species number, the important ectomycorrhizal genera in the world as listed by Molina *et al.*¹ in decreasing order are, *Cortinarius*, *Russula*, *Hygrophorus*, *Inocybe*, *Amanita*, *Lactarius*, *Entoloma* and *Boletus* (Table 4). The same in our study are *Amanita*, *Russula*, *Boletus*, *Lactarius*, *Hygrophorus* and *Cortinarius*. It is interesting that two genera, viz. *Leccinum* (with 6 sp.) and *Suillus* (with 7 sp.) which are important in our study area, do not find a place among the top ten ectomycorrhizal genera of the world.

Species of most of the important genera showed host specificity, majority of the species being associated either

with oaks or conifers. In this respect, they resemble the pattern described in the global summary of ectomycorrhiza¹ (Table 1). Molina *et al.*¹ emphasized that ectomycorrhizae generally have intermediate-to-broad host range. Majority of ectomycorrhizae in the Western Himalaya have narrow host range (Table 2). However, this needs to be taken with caution as it may simply reflect that the entire host range of these mycorrhizae was not investigated.

Several ectomycorrhizal genera had more than five species in a forest type, which is generally not found in other groups of organisms. Our observations indicate that an ectomycorrhizal genus has several species in a forest. For example, 12 species of *Amanita* occurred in conifer forests, whereas *Russula* and *Boletus* had 12 and 10 species respectively, in oak forests.

Most of the Western Himalayan forests have ectomycorrhizal covering at the altitudinal range of over 3000 m and all of them are species-poor – tree species seldom exceeding 10 within a stand¹³. Allen *et al.*⁷ made an interesting observation that forests having trees with ectomycorrhizal association invariably have low plant species diversity.

Understanding the factors that control diversity is high on the agenda for ecologists and conservationists but the focus is generally on the above-ground processes. What is happening beneath the soil is ignored¹⁶. Recently, Rajaniemi *et al.*¹⁷ have experimentally shown that the reduction in plant diversity in old field grassland habitat largely occurred due to the effect of roots. They stressed that roots of some species were more effective in tapping soil nutrients than others, thus achieving dominance. The effect of below-ground processes on the above-ground species dominance or species diversity is likely to be modified by mycorrhizal activity and fungal symbionts¹⁶. It seems that the ectomycorrhizae, by enabling the host to effectively tap resources through a prolific mycelial network, promote exclusion of other species having arbuscular or other forms of mycorrhizae. Formation of single species stands in the Western Himalayan region by ectomycorrhizal host encompasses widely different trees species such as tropical sal (*S. robusta*), sub-tropical and temperate pines (*P. roxburghii* and *P. wallichiana*) and deodar (*C. deodara*), oaks of different climatic belts (*Quercus floribunda*, *Q. leucotrichophora* and *Q. semicarpifolia*) and birch (*B. utilis*).

Table 3. Comparison of genera of ectomycorrhizal fungi across three Mediterranean wood-land communities, Nepal forests and the study forest. Calculations based on data from Allen *et al.*⁷, Lakhanpal¹¹ and Adhikari¹⁵

	California (C)	Italy (I)	Australia (A)	Western Himalaya (WH)	Nepal (N)
Genus number	21	18	24	27	23
Community coefficient (%)	CI = 41 CA = 44 CWH = 37 CN = 40	IA = 57 IWH = 48 IN = 58	AWH = 39 AN = 46	WHN = 80	

Table 4. Important genera (in terms of number of species) of ectomycorrhizae in the world (based on the estimates of Molina *et al.*¹) and their species number in the present study

Genus (with family in parenthesis)	Species number		
	World	Present study	Predominantly associated with
<i>Cortinarius</i> (Cortinariaceae)	900	4	Conifers
<i>Russula</i> (Russulaceae)	500	13	Oaks
<i>Hygrophorus</i> (Hygrophoraceae)	250	4	Conifers
<i>Inocybe</i> (Cortinariaceae)	210	2	Conifers
<i>Amanita</i> (Amanitaceae)	200	15	Conifers
<i>Lactarius</i> (Russulaceae)	200	9	Both conifers and oaks
<i>Entoloma</i> (Entolomataceae)	160	0	–
<i>Boletus</i> (Bolataceae)	150	12	Oaks
<i>Tricholoma</i> (Tricholomaceae)	150	2	Conifers
<i>Hebeloma</i> (Cortinariaceae)	120	0	–

This study on species diversity of ectomycorrhizal fungi mainly concerns oak and conifer forests of the Western Himalaya. These forest communities are part of a wide altitudinal transect largely dominated by trees having ectomycorrhizal fungi. This feature warrants attention because the transect includes a range of over 15°C mean annual temperature and widely different conditions of topography, soil and precipitation. The diversity values of ectomycorrhizal fungi may not represent the actual picture as not all studies are based on thorough sampling; nevertheless, they give an approximate estimate of ectomycorrhizal diversity which falls within the range described for similar forests elsewhere, thus lending support to certain generalizations relating to ectomycorrhizal diversity at the forest community level.

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Somatic embryogenesis and plantlet regeneration from leaf and inflorescence explants of arecanut (*Areca catechu* L.)

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A protocol for arecanut tissue culture was evolved and observed to be repeatable. It was first standardized with leaf explants excised from one-year-old seedlings and later modified for immature inflorescence sampled from adult palms. The protocol was also tested with different arecanut varieties. The basal medium used was MS. Picloram was found to be the most suitable callogenetic agent for both types of explants as well as for the varieties tried. Serial transfer of explants from high to low auxin concentration was essential for sustained growth of callus and somatic embryo induction. Somatic embryogenesis was achieved in hormone-free MS medium. Somatic embryos were germinated in MS medium supplemented with cytokinin; 20 µM BA was found to be the best. No variation was noticed for callus initiation, somatic embryogenesis and plantlet development in different varieties except for the period of culturing. To achieve rapid growth and development of germinated somatic embryos, MS liquid medium supplemented with 5 µM BA was used. Plantlets with 2–4 leaves and good root system were veined using sand : soil (5 : 1) potting mixture.

ARECA catechu L. is an unbranched, erect, medium-sized monoecious palm growing in hot, humid tropical regions¹. Apart from its popularity as a masticatory nut, indigenous communities traditionally use it in religious and social functions and it is an ingredient in traditional medicines¹. Tannin extracted from tender arecanut is considered to be an excellent source of natural dye, tanning agent and ad-

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