**Original Research Article**

**Ectomycorrhizal Diversity in Zabarvan Forest Range of North Western Himalaya**

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**Abstract**

The aim of the present investigation was to prepare an inventory of ectomycorrhiza prevailing in Zabarvan forest range of Western Himalayas of Kashmir and assess their diversity index and species richness. Three forest sites viz., Dachigam, Shalimar and Shankaracharia hills were surveyed periodically for three consecutive years (2011-2013) for ectomycorrhiza. A total number of 67 species in 23 genera belonging to 21 families in 07 orders were recorded. The study revealed that mycorrhizal fungal species richness was more in autumn season (45 species) and less in summer (11 species). The Simpson diversity index of Dachigam, Shalimar and Shankaracharia hills was found to be 0.981, 0.910, 0.939 respectively, while Shannon’s diversity index of these sites was 4.03, 2.36, 2.903, respectively.

**Keywords**

Biodiversity index, Ectomycorrhiza, Kashmir Himalayas, Species Richness, Zabarvan forest

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**Introduction**

Biological diversity is a central determinant of ecosystem function and also a key contributor to the portfolio of services provided by ecosystems to humans (Carlson *et al.*, 2007). India, one among 12 mega diversity countries across the globe, possesses more than 8% of the world's total biodiversity and its bio-geographic ecosystems is classified into ten zones which include Trans-Himalaya and Himalaya zones (Rodgers *et al.*, 2002). Mapping biological diversity of a region is a major goal to the global conservation community (Gaston, 2000). Forest and tree cover of India constitutes nearly 789,164 km², which constitute 24% of geographical area of the country (FSI, 2013). The country’s rich vegetation and diversity is undoubtedly due to the immense variety of climatic and altitudinal variations (Reddy *et al.*, 2013). The state of Jammu and Kashmir
lies in the North Western Himalayan mountainous range between 32°17’ and 37°05’ North latitude and 72°31’ and 80°20’ East longitude with geographic area of 101,387 km² of which 19.95% area is covered by forests alone (ES, 2013-14). Of the total forest area in J&K state, 40.2% area lies in Kashmir valley alone which harbours rich floristic diversity.

As per the conservative estimates, about 1.5 million fungal species are present worldwide (Hawksworth, 2004) with one-third existing in India alone. Hardly 50% of these fungi have been identified and characterized so far (Manoharachary et al., 2005). Ectomycorrhizal plants, while taxonomically more rare, are common within boreal and temperate forests (e.g. Pinaceae, Fagaceae, Betulaceae, Nothofagaceae and others) (Tedersoo and Smith, 2013).

Ectomycorrhiza play important role in rendering the unavailable organic forms of soil nutrients available to the plants through various mechanisms including the production of extracellular enzymes (Read and Perez-Moreno, 2003; Aucina et al., 2007). Enormous interest has recently generated in the use of ectomycorrhiza as inocula for successful forest nursery raising especially in degraded and degenerated forests. The present study was therefore, aimed to assess the mycorrhizal diversity of Zabarvan forest range in Kashmir Himalayas, prepare inventory and assess their diversity index and species richness.

Materials and Methods

Collection site

The Zabarvan forest range (Fig. 1) lies in the north of Srinagar city of Jammu & Kashmir State which lies in the heart of North Western Himalaya. It lies at 34°02 and 34°08N latitude and 74°44 and 74°55 E longitude and covers an area of 265 km². The sites selected for macrofungal survey were Dachigam, Shalimar and Shankaracharia hills which cover an area of 141, 69 and 55 km², respectively, with altitude ranging from 1676-4267, 1624-3385 and 1585-3352 m masl, respectively. These forests are mostly dominated by conifers viz., cedar, pine, spruce, fir, etc. growing upto 3657 m masl and above this altitude lie meadows which bloom with rhododendrons, honey suckle and dwarf willows.

The vegetative cover existing in these forests provide best suited habitat for fungal flora. The area has temperate climate with average temperature of 13.5°C, the highest monthly average temperature of 17.3°C, 29.3°C and 25.5°C in March-April, June-July and September-October, respectively. The average rainfall is 710mm.
Sporocarp survey and identification

The survey for the collection of ectomycorrhizal was carried out at monthly interval from March onwards during the years 2011 and 2013 in each potential growing season viz., spring, summer and fall (autumn). The efforts were made to establish the relation/association of sporocarps with plant root by careful digging of soil and tracing their connection with the host plant roots as per the method of Young (1940) and Zak (1969). The epigeous ectomycorrhiza of each fungal species were collected and wrapped in thin aluminum foil paper placed in polybags separately, brought to the laboratory and analyzed for their identity. Photographs were taken using digital Sony camera DSC-RX100. The spore prints were taken on paper or glass slides to study the colour of spores, shape of gills and pores, and attachment of gills to the stipe (Kuo, 2001; Kuo, 2004). The colour terminology followed was that of Kornerup and Wanscher (1978). Melzer’s reagent was used to investigate amyloidity of pores and various other tissues. Cresyl blue solution was used to study the meta-chromatic reactions of spores. Specimens were identified on comparison with relevant literature (Kirk et al., 2001; de Roman et al., 2005; Agerer, 2006) and the information available at various web resources viz., Determination of Ectomycorrhiza (DEEMY), http://www.deemy.de; www.Mushroom Expert.com; mycokey, www.mycokey.com; Mycorrhiza literature exchange, http://mycorrhiza.ag.utk.edu, etc.. The sporocarps were preserved in the herbarium of Mycology and Forestry Section, Division of Plant Pathology, SKUAST-Kashmir, Shalimar, Srinagar (J&K).

Data analysis

Simpson’s diversity index was assessed as per Simpson (1949)

\[ D = \frac{\sum n (n - 1)}{N(N - 1)} \]

D = Simpson’s index, N = the total number of organisms of all species; n = the total number of organisms of a particular species

Shannon’s diversity index was estimated as per Margalef (2008).

\[ H = - \sum \left(\frac{n}{N}\right) \log_e \left(\frac{n}{N}\right) \]

Where, H = the diversity index, N = the total number of individuals of all species; n = the total number of individuals of the individual species

With the help of the values of diversity index, the evenness of ectomycorrhiza was calculated as per Pielou (1996)

\[ e = H / \log_e S \]

E = evenness, H = Shannon diversity index; S = the number of the species

Similarity of index was estimated as per Sorenson’s formula to assess the similarity in species occurrence (Odum, 1971). The similarity ranges from 0 to 1 (1 indicates very similar, 0 indicates no similarity)

\[ S = 2C / (A + B) \]

Wherein S is the degree of similarity, A and B are the number of the species at two different sites and C is number of species common to both collections.

Results and Discussion

During three year survey of Zabarvan forest hills in Kashmir, sixty seven ectomycorrhizal species were collected from different locations and seasons (Table 1). These
Macrofungi were collected at an altitudinal range of 1825 to 2896 m masl. The hill range has predominantly coniferous forest stands either pure or mixed with broad-leaved plant species which support rich macrofungal flora. Conifer habitat provides congenial conditions for growth and sporulation of diverse macrofungi. Vishwakarma (2010) and Vishwakarma et al., (2011) have also noticed the presence of diverse macrofungi in conifer forests in Western Himalayas of Himachal Pradesh (India). These ectomycorrhiza have been collected from other forest ranges in Kashmir and described (Cooke, 1870; Murrill, 1924; Batra and Batra, 1963; Kaul and Kachroo, 1974; Watling and Gregory, 1980).

During survey, the ectomycorrhiza production was observed to be 16% higher in year 2012 and 2013 than year 2011. Higher sporocarp production in 2012 and 2013 may be ascribed to the favourable agro-climatic conditions especially higher and timely precipitation and congenial temperature from April to October. The year 2011 with mean precipitation of 210 mm and mean temperature of 24.2°C was comparatively drier and slightly warmer than 2012 and 2013 which probably may have affected the species diversity and ectomycorrhiza production by individual ectomycorrhiza species. The findings are in agreement with Mihali (1995) who during two year study in 1992 and 1993 observed 83 macromycetous species that produced 817 fruiting bodies in beech stand at Jalna, Slovak Republic.

The seasonal distribution of ectomycorrhiza across the Zabarvan forest range varied significantly with maximum ectomycorrhizal species witnessed in Dachigam followed by Shankaracharia and least in Shalimar hills. These variations may be attributed to varied latitude, vegetation, topography, etc. and their effects on temperature and precipitation across the wide geographic distances or along the elevational gradients. These findings are in conformity with Wood-Eggenschwiler and Barlocher (1985) and Ohenoja (1993) (Table 2).

A total number of 67 species in 23 genera of 21 families belonging to 05 orders of Basidiomycotina and 02 orders of Ascomycotina were considered for the ecological studies. They were identified up to species level. Agaricales dominated by 39% (8 families and 26 species in 09 genera) 25% of Boletales (07 families, 08 genera and 17 species) 21% Russulales (01 family, 02 genera and 14 species) Gomphales (01 family, 01 genera and 06 species) Thelephorales (02 families, 02 genera and 02 species) Mytilinidales (01 family, 01 genera and 01 species) Pezizales (01 family 01 genera and 01 species). Out of 21 families, Russulaceae dominated by 21% this is followed by Tricholomataceae 11%, 9% Gomphaceae and 7% Boletaceae, Inocybaceae and Suillaceae each. A list of ectomycorrhiza species family wise has been provided in Table 3.

Ectomycorrhiza collected in three collection sites of Zabarvan forest range namely Dachigam, Shalimar and Shankaracharia were analyzed for ectomycorrhizal richness. The number of species collected area-wise showed maximum 65 species in Dachigam, 25 species in Shankaracharia and 11 species in Shalimar. Species diversity, richness and evenness of ectomycorrhiza in zabarvan range is shown in Table 4.

Out of 62 species collected from Dachigam, Agaricus xanthoderma, Boletus aereus, B. cavipes, B. gigas, Cenoccoum geophyllum, Chroomphogus tomentosus, C. vinicolor, Hebeloma crustuliniforme, H. cylindorosum, Hydnellum aurantiacum, Lycoperdon pedicillatum, Paxillus involutus, Pisolithus tinctorius, Ramaria aurea, R. flava, R. formosa, R. invalli, Rhizopogan roseulus, R.
vulgaris, Russula atropurpurea, R. brevipes, R. delica, R. densifolia, R. emetica, R. lilacea, R. lutea, R. paludosa, R. sanguine, R. xerampelina, Russula sp., Scleroderma verrucosum, Suillus cavipes, S. granulates, S. luteus, S. placidus, Tricholoma album, T. malvacereum, T. portentosum, T. sejunctum and Tricholoma sp. were some of the species collected only from this site alone. The Simpson and Shannon’s diversity index was observed to be 0.981 and 4.03, respectively, while evenness and species richness was 2.248 and 0.55, respectively. This high diversity of ectomycorrhizal diversity in Dachigam appears due to less human interference in this area as well as to the more availability of degradable materials. It was also noticed that huge plant litter accumulated in Dachigam forest floor may have helped to build up fertility and replenish the nutrients back into the soil.

Table.1 The ectomyorrhiza species collected from Zabarvan forest range in 2011, 2012 and 2013

| Ectomycorrhizal Species | Habitat                      | Season in which it noticed | Site of observation | Altitude at which collected (masl) |
|-------------------------|------------------------------|-----------------------------|---------------------|-----------------------------------|
| Agaricus xanthoderma    | Mycorrhizal with Cedrus deodara/ Pinus wallachian | Spring                      | Dachigam            | 2354                              |
| Amanita ceciliae        | Mycorrhizal with P. wallachian | Spring                      | Dachigam            | 2458                              |
| Amanita excelsa         | Mycorrhizal with P. wallachian | Autumn                      | Shankaracharia      | 2015/2189                         |
| Amanita inaurtia        | Mycorrhizal with P. wallachian | Autumn                      | Dachigam            | 2487                              |
| Boletus subtomentosus   | Mycorrhizal with P. wallachian | Autumn                      | Shankaracharia      | 2095/2019                         |
| Boletus aereus          | Mycorrhizal with P. wallachian | Autumn                      | Dachigam            | 2687                              |
| Boletus cavipes         | Mycorrhizal with P. wallachian | Autumn                      | Dachigam            | 2596                              |
| Boletus gigas           | Mycorrhizal with P. wallachian | Autumn                      | Dachigam            | 2658                              |
| Cenococcus geophyllum   | Mycorrhizal with P. wallachian | Autumn                      | Dachigam            | 2654                              |
| Chromophogus tomentosus | Mycorrhizal with P. wallachian | Spring                      | Dachigam            | 2698                              |
| Chromophogus vinicolor  | Mycorrhizal with P. wallachian | Spring                      | Dachigam            | 2586                              |
| Entoloma sinatum        | Mycorrhizal with P. wallachian | Autumn                      | Dachigam            | 2478                              |
| Hebeloma                | Mycorrhizal with P. wallachian | Autumn                      | Dachigam            | 2612                              |
| Species                      | Mycorrhizal with                          | Season     | Location        | Collection |
|------------------------------|------------------------------------------|------------|-----------------|------------|
| crustuliniforme              | P. wallachiana                           |            |                 |            |
| Hebeloma cylinderosum       | Mycorrhizal with C. deodara/ P. wallachiana | Autumn     | Dachigam        | 2489       |
| Hydnellum aurantiacum       | Mycorrhizal with C. deodara/ P. wallachiana | Spring     | Dachigam        | 2754       |
| Inocybe appendiculata       | Mycorrhizal with C. deodara/ P. wallachiana | Summer     | Dachigam/Shankaracharia | 2765/2345 |
| Inocybe fastigata           | Mycorrhizal with P. wallachiana          | Spring, Summer | Shankaracharia/Shalimar | 2142/1987 |
| Inocybe geophylla           | Mycorrhizal with C. deodara/ P. wallachiana | Spring     | Dachigam/Shankaracharia | 2698/2435 |
| Inocybe maculata            | Mycorrhizal with C. deodara/ P. wallachiana | Autumn     | Shalimar        | 2147       |
| Laccaria bicolor            | Mycorrhizal with C. deodara/ P. wallachiana | Summer     | Dachigam/Shankaracharia | 2578/2256 |
| Laccaria laccata            | Mycorrhizal with C. deodara              | Summer, Autumn | Dachigam/Shankaracharia | 2494/1998 |
| Lactarius controversus      | Mycorrhizal with P. wallachiana          | Autumn     | Shankaracharia/Shalimar | 1825/1894 |
| Lactarius delicious         | Mycorrhizal with C. deodara/ P. wallachiana | Autumn     | Dachigam/Shalimar | 2365/2134 |
| Lactarius pedicillatum      | Mycorrhizal with C. deodara/ P. wallachiana | Autumn     | Dachigam/Shankaracharia | 2398/2032 |
| Lycoperdon pedicillatum     | Mycorrhizal with C. deodara/ P. wallachiana | Autumn     | Dachigam/Shankaracharia | 2475/2258 |
| Lycoperdon perlatum         | Mycorrhizal with C. deodara/ P. wallachiana | Autumn     | Dachigam/Shankaracharia | 2457/2159 |
| Lycoperdon saccatum         | Mycorrhizal with C. deodara/ P. wallachiana | Autumn     | Dachigam/Shankaracharia | 2467/2164 |
| Lycoperdon pyriforme        | Mycorrhizal with C. deodara/ P. wallachiana | Summer     | Dachigam/Shalimar | 2787/2145 |
| Macrolepiota procera        | Mycorrhizal with P. wallachiana           | Autumn     | Dachigam/Shalimar | 2658/2147 |
| Macrolepiota puellaris      | Mycorrhizal with C. deodara/ P. wallachiana | Autumn     | Dachigam/Shalimar | 2475/2247 |
| Paxillus involutus          | Mycorrhizal with P. wallachiana           | Autumn     | Dachigam        | 2478       |
| Pisolithus tinctorius        | Mycorrhizal with P. wallachiana           | Summer     | Dachigam        | 2158       |
| Ramaria aurea               | Mycorrhizal with C. deodara/ P. wallachiana | Autumn     | Dachigam        | 2245       |
| Ramaria flaccid             | Mycorrhizal with P. wallachiana           | Autumn     | Dachigam/Shankaracharia | 2181/2097 |
| Ramaria flava               | Mycorrhizal with                           | Autumn     | Dachigam        | 2215       |
| Species                | Mycorrhizal with          | Season   | Location     | ID    |
|-----------------------|---------------------------|----------|--------------|-------|
| Ramaria formosa       | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2104  |
| Ramaria invalli       | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2369  |
| Ramaria kuenzii       | C. deodara/ P. wallachiana | Spring   | Dachigam     | 2578  |
| Rhizopogon roseulus   | C. deodara/ P. wallachiana | Spring   | Dachigam     | 2598  |
| Rhizopogon vinicolor  | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2478  |
| Rhizopogon vulgaris   | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2659  |
| Russula atropurpurea  | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2548  |
| Russula brevipes      | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2487  |
| Russula delica        | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2587  |
| Russula densifolia    | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2547  |
| Russula emetica       | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2014  |
| Russula lilacea       | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2016  |
| Russula lutea         | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2314  |
| Russula paludosa      | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2366  |
| Russula sanguinea     | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2488  |
| Russula sp.           | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2115  |
| Russula xerampelina   | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2136  |
| Scleroderma verrucosum| C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2147  |
| Suillus cavipes       | P. wallachiana             | Summer, Autumn | Dachigam | 2132  |
| Suillus granulates    | C. deodara/ P. wallachiana | Summer   | Dachigam     | 2373  |
| Suillus luteus        | C. deodara/ P. wallachiana | Summer   | Dachigam     | 2347  |
| Suillus placidus      | C. deodara/ P. wallachiana | Summer   | Dachigam Shankaracharia | 2365/2259 |
| Thelephora terrestris | C. deodara/ P. wallachiana | Autumn   | Dachigam     | 2345/2236 |
| Location          | Spring season | Summer season | Autumn season | Overall species noticed |
|-------------------|---------------|---------------|---------------|-------------------------|
| Dachigam         | 08            | 08            | 49            | 65                      |
| Shalimar         | 02            | 02            | 07            | 11                      |
| Shankaracharia   | 02            | 05            | 15            | 25                      |
| Total            | 09            | 11            | 45            | 67                      |

**Table.2** Seasonal distribution of ectomycorrhizal species (No.) observed in Zabarvan forest range (2011-2013)
Table 3 Species- and family-wise distribution of ectomycorrhizal fungi observed in Zabarvan forest range

| S. No. | Family               | Species                                                                 | Total |
|--------|----------------------|-------------------------------------------------------------------------|-------|
| 01     | Agaricaceae          | Agaricus xanthoderma, Lycoperdon pedicillatum, L. perlatum, L. pyriforme, L. saccatum | 5     |
| 02     | Diplocystidiaceae    | Astraeus hygometricus                                                   | 1     |
| 03     | Amanitaceae          | Amanita ceciliae, A. excelsa, A. inauriae                               | 3     |
| 04     | Boletaceae           | Boletus subtomentosus, B. aereus, B. cavipes, B. gigas                  | 4     |
| 06     | Gloniaceae           | Cenococcus geophyllum                                                   | 1     |
| 07     | Gomphidiaceae        | Chrooophagus tomentosus, C. vinicolor                                   | 2     |
| 08     | Entolomataceae       | Entoloma sinatum                                                        | 1     |
| 09     | Hymeogastraceae      | Hebeloma crustuliniforme, H. cylindorosum                               | 2     |
| 10     | Bankeraceae          | Hydnellum aurantiacum                                                   | 1     |
| 11     | Inocybaceae          | Inocybe appendiculata, I. fastigata, I. geophylla, I. maculate           | 4     |
| 12     | Hydnangiaceae        | Laccaria bicolor, Laccaria laccata                                      | 2     |
| 13     | Russulaceae          | Lactarius controversus, L. delicius, L. pedicillatum, Russula atropurpurea, R. brevipes, R. delica, R. densifolia, R. emetica, R. lilacea, R. lutea, R. paludosa, R. sanguinea, R. xerampelina, Russula sp. | 14    |
| 14     | Lepiotaceae          | Macrolepiota procera, M. puellaris                                     | 2     |
| 15     | Paxillaceae          | Paxillus involutus                                                      | 1     |
| 16     | Sclerodermataceae    | Pisolithus tinctorius, Scleroderma verrucosum                           | 2     |
| 18     | Gomphaceae           | Ramaria aurea, R. flaccid, R. flava, R. formosa, R. invalli, R. kuenzii | 6     |
| 19     | Rhizopogonaceae      | Rhizopogon roseulus, R. vinicolor, R. vulgaris                          | 3     |
| 20     | Suillaceae           | Suillus cavipes, S. granulates, S. luteus, S. placidus                   | 4     |
| 21     | Thelephoraceae       | Thelephora terrestris                                                    | 1     |
| 22     | Tricholomataceae     | Tricholoma terreum, T. album, T. malacereum, T. portentosum, T. sculpurattum, T. sejenctum, Tricholoma sp. | 7     |
| 23     | Tubaraceae           | Tuber sp.                                                               | 1     |

Table 4 Species diversity, richness and evenness of ectomycorrhiza in Zabarvan forest range of Kashmir

|                    | Dachigam | Shalimar | Shankaracharia |
|--------------------|----------|----------|----------------|
| No. of species     | 65       | 11       | 25             |
| Total No. of individuals | 1502     | 125      | 294            |
| Simpson diversity index (1-D) | 0.981    | 0.910    | 0.939          |
| Shannon diversity index (H) | 4.03     | 2.36     | 2.903          |
| Evenness (En)      | 2.248    | 2.26     | 2.195          |
| Species richness (s) | 0.55     | 0.19     | 0.40           |
Amongst the 21 species collected from Shankaracharia forest area, *Astraeus hygometricus* and *Tuber* sp. were two species collected only at this site. The Simpson and Shannon’s diversity index was 0.939 and 2.903, respectively, similarly evenness and species richness and was found to be 2.195 and 0.40 respectively, was found low compared to Dachigam forest range. This may due to the anthropogenic activity. Similarly, 11 species were collected from Shalimar site. The Simpson and Shannon’s diversity index was 0.91 and 2.36, respectively, while evenness and species richness and was found to be 2.26 and 0.19 respectively, was found lowest compared to Dachigam forest range and shankaracharia forest range. This may due to the interference of human activities and more so various tourist places have come up in this area. These results are in partial conformity with Bhatt (1986) and Adhikari (1999), in Dachigam, diversity index was high because it is legally protected and has less anthropogenic activity. Season-wise more diverse fungi were observed in Dachigam and low diversity index in Shalimar region.

It can be concluded Since the mycorrhiza play an important role to maintain the health of forests besides their medicinal importance and nutritional value in most of the cases, therefore it becomes quite necessary to explore, document and conserve this natural wealth.

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