Int.J.Curr.Microbiol.App.Sci (2019) *8*(5): 2312-2323

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 05 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.805.273

Ectomycorrhizal Diversity in Zabarvan Forest Range of North Western Himalaya

P.A. Sheikh[1]*, Baby Summuna[1], G.H. Dar[1], Sajad-Un-Nabi[2] and Khurshid Ahmad Mir[3]

[1]Division of Plant Pathology, S.K. University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar-190 025 Jammu and Kashmir, India
[2]Central Institute of Temperate Horticulture, Indian council of Agriculture Research Srinagar-190007 Jammu and Kashmir, India
[3]Division of Vegetable science, S.K. University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar-190 025 Jammu and Kashmir, India

**Corresponding author*

Keywords

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

Article Info

Accepted:
18 April 2019
Available Online:
10 May 2019

A B S T R A C T

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.

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 km^2, 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 km^2 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).

The floristic composition of North West Himalayan mountains, have not extensively been surveyed and explored for the macrofungi emanating in different seasons, except for some widely used edible mushrooms (Kaul *et al.,* 1978; Walting and Abraham, 1992; Samant and Dhar, 1997). Hardly 250 macrofungi species have so far been reported from the J&K (Walting and Abraham, 1992; Beig *et al.,* 2008; Dar *et al.,* 2009; Sheikh *et al.,* 2014), mostly from Gulmarg and Pahalgam forests. The Zabarvan forest range which harbours mixed- and pure-conifer stands and stretches from Shankaracharia hills to Dachigham hills has not been extensively surveyed for mycotic flora so far. The baseline information on diversity in different vegetation types is essential for planning and managing ecosystem biodiversity (Engola *et al.,* 2007). The knowledge about biodiversity at community and species levels is important for monitoring the effectiveness and impact of natural and artificial disturbances (Packham *et al.,* 2002). Ectomycorhiza 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 km^2. The sites selected for macrofungal survey were Dachigam, Shalimar and Shankaracharia hills which cover an area of 141, 69 and 55 km^2, 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)

$$D = \frac{\Sigma n\,(n-1)}{N(N-1)}$$

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. sejenctum* and *Tricholoma* sp. were some of the species collectedonly 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 wallachiana*	Spring	Dachigam	2354
Astraeus hygometricus	Mycorrhizal with *P. wallachiana/ C. deodara*	Autumn	Shankaracharia Dachigam	2015/ 2189
Amanita ceciliae	Mycorrhizal with *P. wallachiana*	Spring	Dachigam	2458
Amanita excelsea	Mycorrhizal with *P. wallachiana*	Autumn	Dachigam	2369
Amanita inaurtia	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2487
Boletus subtomentosus	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam Shankaracharia	2095/2019
Boletus aereus	Mycorrhizal with *P. wallachiana/ C. deodara*	Autumn	Dachigam	2687
Boletus cavipes	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2596
Boletus gigas	Mycorrhizal with *P. wallachiana*	Autumn	Dachigam	2658
Cenoccoum geophyllum	Mycorrhizal with *P. wallachiana*	Autumn	Dachigam	2654
Chroomphogus tomentosus	Mycorrhizal with *C. deodara/ P. wallachiana*	Spring	Dachigam	2698
Chroomphogus vinicolor	Mycorrhizal with *P. wallachiana*	Spring	Dachigam	2586
Entoloma sinatum	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2478
Hebeloma	Mycorrhizal with	Autumn	Dachigam	2612

crustuliniforme	*P. wallachiana*			
Hebeloma cylindorosum	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 , Autumn	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
Macrolepoita 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

	C. deodara/ P. wallachiana			
Ramaria formosa	Mycorrhizal with *P. wallachiana*	Autumn	Dachigam	2104
Ramaria invalli	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2369
Ramaria kuenzii	Mycorrhizal with *C. deodara/ P. wallachiana*	Spring	Dachigam	2578
Rhizopogan roseulus	Mycorrhizal with *C. deodara/ P. wallachiana*	Spring	Dachigam	2598
Rhizopogan vinicolor	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2478
Rhizopogon vulgaris	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2659
Russula atropurpurea	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2548
Russula brevipes	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2487
Russula delica	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2587
Russula densifolia	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2547
Russula emetica	Mycorrhizal with *C. deodara*	Autumn	Dachigam	2014
Russula lilacea	Mycorrhizal with *P. wallachiana*	Autumn	Dachigam	2016
Russula lutea	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2314
Russula paludosa	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2366
Russula sanguinea	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2488
***Russula* sp.**	Mycorrhizal with *P. wallachiana*	Autumn	Dachigam	2115
Russula xerampelina	Mycorrhizal with *C. deodara*	Autumn	Dachigam	2136
Scleroderma verrucosum	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2147
Suillus cavipes	Mycorrhizal with *P. wallachiana*	Summer , Autumn	Dachigam	2132
Suillus granulates	Mycorrhizal with *C. deodara/ P. wallachiana*	Summer	Dachigam	2373
Suillus luteus	Mycorrhizal with *C. deodara/ P. wallachiana*	Summer	Dachigam	2347
Suillus placidus	Mycorrhizal with *C. deodara/ P. wallachiana*	Summer	Dachigam Shankaracharia	2365/2259
Thelephora terrestris	Mycorrhizal with	Autumn	Dachigam	2345/2236

	C. deodara/ P. wallachiana		Shankaracharia	
Tricholoma terreum	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam Shankaracharia	2317/2247
Tricholoma album	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2479
Tricholoma malvacereum	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2147
Tricholoma portentosum	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2689
Tricholoma scalpturattum	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2789
Tricholoma sejenctum	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2896
***Tricholoma* sp.**	Mycorrhizal with *C. deodara/ P. wallachiana*	Autumn	Dachigam	2687
***Tuber* sp.**	Mycorrhizal with *P. wallachiana*	Autumn	Shankaracharia	2,015

Table.2 Seasonal distribution of ectomycorrhizal species (No.) observed in Zabarvan forest range (2011-2013)

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

Fig.1 Zabarvan forest range in North Western Himalaya

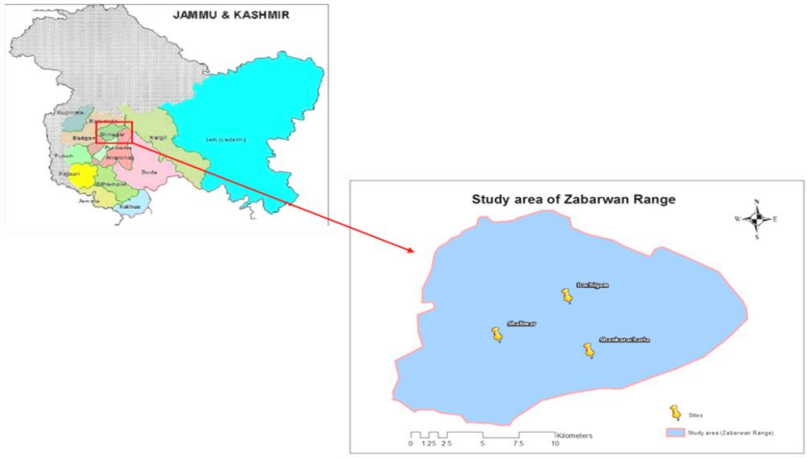

Table.3 Species- and family-wise distribution of ectomycorrhizal fungi observed in Zabarvan forest range

S. No.	Family	Species	Total
01	Agaricacea	*Agaricus xanthoderma, Lycoperdon pedicillatum, L. perlatum, L. pyriforme, L. saccatum*	5
02	Diplocystidiaceae	*Astraeus hygometricus*	1
03	Amanitaceae	*Amanita ceciliae, A. excelsea, A. inaurtia*	3
04	Boletaceae	*Boletus subtomentosus, B. aereus, B. cavipes, B. gigas*	4
06	Gloniaceae	*Cenoccoum geophyllum*	1
07	Gomphidiaceae	*Chroomphogus 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. delicious, 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	*Rhizopogan 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. malvacereum, T. portentosum, T. scalpturattum, 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
Spieces 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 mychorrhiza 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.

Acknowledgement

The authors are thankful to all the individuals directly and indirectly assisting in the sample collection and laboratory preparations throughout the study.

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How to cite this article:

Sheikh, P.A., Baby Summuna, G.H. Dar, Sajad-Un-Nabi and Khurshid Ahmad Mir. 2019. Ectomycorrhizal Diversity in Zabarvan Forest Range of North Western Himalaya. *Int.J.Curr.Microbiol.App.Sci.* 8(05): 2312-2323. doi: https://doi.org/10.20546/ijcmas.2019.805.273