Eco-Habitat Assessment of One of the Most Expensive Edible Mushrooms (*Tricholoma matsutake*) in Genekha, Thimphu, Bhutan.

Abstract

Tricholoma matsutake (S. Ito & Imai) Singer is most expensive edible mushroom, naturally grown dispersed in temperate oak pine forested areas in Bhutan. The present study is an attempt to give an account on floristic composition and vegetation structure of Tricholoma matsutake habitat in Genekha, Thimphu. A total of 10 plots were enumerated and habitat parameters such as altitude, aspect, slope, and vegetation composition were enumerated using the quadrants method. The study recorded 58 species under 44 genera belonging to 26 families. The most species rich families are Ericaceae (12 species), followed by Rosaceae (8 species) and Asteraceae (6 species). The study revealed that Tricholoma matsutake is associated with Quercus semecarpifolia Sm., Pinus wallichiana A.B. Jack., Rhododendron spp., Pieris formosa (Wall.) D. Don and Anaphalis triplenervis (Sims) C.B. Clarke, with those species having maximum IVI in the habitat. Vegetation observed was mostly evergreen trees with 47 % (RBA=187.4) followed by evergreen shrubs with 43% (RBA= 171.5). J-shaped distribution curve of DBH and height class is obtained for the vegetation structure of the habitat. Regeneration survey revealed fair regeneration status in the habitat. The Menhinick's species richness index revealed high species richness (2.26, 2) for both habitats. Simpsons index 0.94 obtained, indicated high diversity for north facing habitat (NFH), and moderate diversity (0.76) for south facing habitat (SFH). Shannon index value indicated moderate diversity (3.06) in NFH, while low diversity (2.49) in SFH with moderately even distribution (0.74, 0.73) of species in the both the habitats. Sorensens similarity index of 0.9 indicated highly similar species composition between two habitats. This research provides floristic and vegetation structure of Tricholoma matsutake habitat, that will have high significance in habitat management and conservation.

Keywords: Floristic, Habitat, Importance Value Index, Regeneration, Species Diversity.

Introduction

Matsutake [*Tricholoma matsutake* (S. Ito et S. Imai) Singer] is a well-known ectomycorrhizal fungus highly prized for the medicinal values of its edible fruiting bodies (Aoki et al., 2022; Ji et al., 2022). It is considered a special traditional delicacy in Japan (Wang et al., 2017) and one of

the most widely known and expensive edible mycorrhizal mushroom in the world (Brandud & Bendiksen, 2014; Wang et al., 2017; Yamanaka et al., 2020). Matsutake is highly prized and valued due to its distinctive spicy aroma and taste (Miyauchi et al., 2020; Vaario et al., 2017; Winkler, 2009). It is used in traditional Chinese medicines (Liu et al., 2010) and has high nutrient content favoring great health benefits (Hou et al., 2013; Zhu et al., 2021). Matsutake is generally exported to Japan and the explorations of new matsutake producing areas continues in various countries across the Asia, Europe and America (Wang et al., 2017).

Matsutake comprises of several closely related species belonging to the *Tricholoma* genus growing under various conifer tree species and oak family (Pedersen, 2020). The Matsutake species are known from Eastern Asia, Himalaya, Northern Africa, some European countries, the Northwestern part of United States, and Canada (Brandrud & Bendiksen, 2014; Vaario et al., 2017). However, the true Matsutake (*Tricholoma matsutake*) only grows in few Asian countries like Japan, China, Tibet, Nepal, and Bhutan (Pedersen, 2020). In Bhutan, *T. matsutake* isabundantly found in the natural forest of Bumthang (Ura) and Thimphu (Genekha) and sporadically in forested areas in Paro, Gasa (Laya, Lunanageog), Wangdue, and Haa districts. Its presence has also been reported from Tashigang (Yangneer) eastern parts of country(Mata et al., 2010) as cited in Bhutan Biodiversity Portal. However, despite its global presence and cultural significance, comprehensive studies focusing on the habitat ecology of *T. matsutake* are relatively scarce. This research aims to fill that gap by assessing the eco-habitat of *T. matsutake* in Genekha gewog, Thimphu, Bhutan, including floristic composition and diversity, vegetation structure and regeneration status of associate tree species found in the habitat.

Numerous studies have explored the symbiotic relationship between T. matsutake and its host trees. Tricholoma matsutake forms a symbiotic relationship with the roots of a specific limited tree species. It is commonly associated with Japanese Red Pine (Pinus densifoliai) in Japan. It is found in coniferous forest made up of Douglas-fir (Pseudotsuga menziesii), Noble Fir (Abies procera), Shasta Red Fir (Abies magnifica), Sugar Pine (Pinus lambertiana), Ponderosa Pine (Pinus ponderosa) or Lodgepole Pine (Pinus contorta) in parts of North American Pacific is also found associated with hardwoods such (Notholiothocarpusdensiflorus) and Madrone (Arbutus menziesii) in California (Miyauchi et al., 2020). Fruiting bodies of Matsutake are generally found covered under the leave litters and debris on the forest floor. T. matsutake usually forms white, solid aggregates of mycelia and mycorrhizas called "Shiro" underneath the litter layers. It mainly lives as an ectomycorrhizal symbiont, but which can also feed as a saprotroph. This flexible trophic ecology of *T. matsutake* confers greater advantages to adapt in complex soil litter environment (Miyauchi et al., 2020).

Recent research has shown that *T. matsutake* has wide and scattered distribution in temperate and boreal forests of Eurasia and subtropical China, mainly associated with *Pinus*, *Picea*, *Tsuga*, *Abies* and fagaceous broadleaves (Vaario et al., 2017). In Bhutan, *T. matsutake* is collected from warm temperate oak-pine forests (Winkler, 2009) and grows scattered and gregarious mainly in oak and spruce forest (Mata et al., 2010) as cited in Bhutan Biodiversity Portal and mentioned in "Bhutan Standard, *Tricholoma matsutake*" (BSB, 2022).

In recent decades, human activities such as deforestation, climate change, and unsustainable forest management practices have contributed to the decline in *T. matsutake* populations in

certain regions. In Russia, the species population declined due to clear felling, habitat degradation and over harvesting. In Asia, population of *T. matsutake* declined due to severe pine forest die-back and afforestation (Brandrud & Bendiksen, 2014). In Japan, threats include deforestation and infestation by the pinewood nematode (*Bursaphelenchusxylophilus*) on the host plant *Pinus densiflora*(Miyauchi et al., 2020). Moreover, the population of *T. matsutake* has declined due to intensive harvest and non-scientific collection techniques(*Brandrud*, 2020; Miyauchi et al., 2020). Similarly, habitat destruction, waste disposal, non-scientific and over harvesting and limited effort from people and agencies to regulate combing of forest for mushroom by outsiders were reported to decline the Matsutake production in Bhutan(Wangdi, 2015).

T. matsutake is listed as vulnerable species under the IUCN Red List of Threatened Species in 2019 (Brandrud, 2020). And different efforts of conservation must consider both its ecological and economic value. Sustainable harvesting practices are essential to ensure that matsutake populations can recover from overharvesting, while forest management strategies should focus on maintaining the specific environmental conditions that support matsutake growth. Therefore, this study aims to provide a comprehensive ecological assessment of Tricholoma matsutake, focusing on its habitat structure, ecological interactions and forest habitat preferences in GenekhaGeowog, Thimphu, Bhutan. By integrating field observations with existing research, this paper seeks to contribute to the growing body of knowledge on the ecology of matsutake, emphasizing the need for sustainable management practices that consider both economic and ecological factors in Bhutan.

Methodology

a. Study site

Genekha gewogis located in Thimphu dzongkhag, about 32 km from the Dzongkhag headquarter. Gewog has an area of 60.925 square kilometers within the elevation of 2120-4240 meter above sea level. The percentage forest cover of Geney gewog is about 83.12 % of the total area. The forest of Geney gewog is habitat of one of the most expensive edible mushrooms (*Tricholoma matsutake*) locally known as *Sangya shamu*.



Figure 1: Habitat of *Tricholoma matsutake* in Genekha, Thimphu, Bhutan

Genekha has mixed conifer forest type with vegetation mainly composed of *Pinus wallichiana*, *Picea spinulosa*, *Quercus semecarpifolia*, *Rhododendron* spp., *Acer champbelii* and *Betula*

utilities. The habitat of *Tricholoma matsutake* ranges from the elevation of 3000m to 3400 meter above sea level. The habitats lie between lat. long. (27°19'40.21"N, 89°37'41.90"E) to (27°19'55.76"N, 89°39'24.66"E) in the forest of south facing slope and lat. Long. (27°17'33.79"N, 89°36'35.59"E) to (27°18'19.23"N, 89°39'16.46"E) in the forest of north facing slope

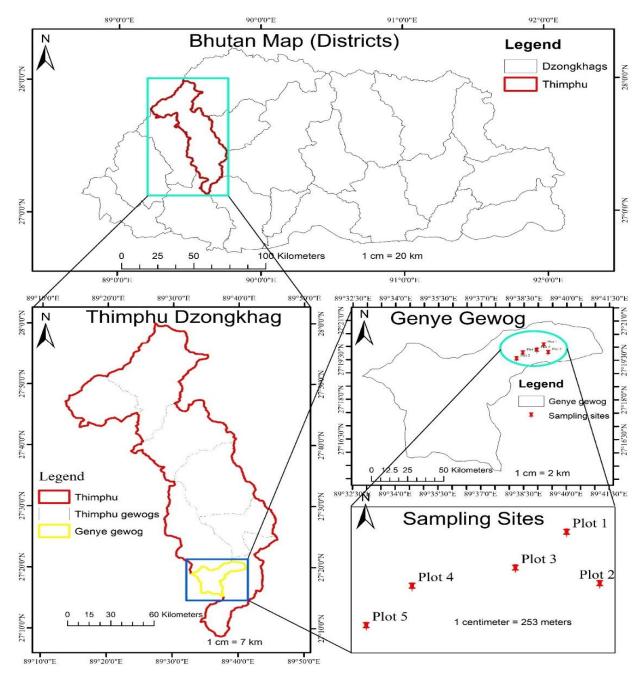


Figure 2: Study area; Habitat of Tricholoma matsutake, Genekha, Thimphu, Bhutan

b. Sampling and Data collection

Tricholoma matsutakehabitat is categorized into North Facing Habitat (NFH) and South Facing Habitat (SFH) based on the presence of *Tricholoma matsutake* in the area. Bhutan Flora Monitoring Protocol, 2020 (DoFPS, 2020) was adapted to develop sampling layout. Using QGIS fishnet, 100m x 100 m grid cells were laid across the habitat of *Tricholoma matsutake* area and 10 grids were randomly selected for conducting the field work. The habitat parameters such as altitude, aspect, slope, and vegetation compositions were recorded for each sampling plots. Within the sampling plots, vegetation survey and plant data were collected based on vegetation strata; trees, shrubs and herbs, similar to methods used by (Ghemiray, 2016; Rabten, 2016). The plot size of 20m x 20m for tree (Tshering Samdrup et al., 2020; Tshewang et al., 2022), 5m x 5m for shrubs (Rabten, 2016) and 2m x 2m for herbs (Ghemiray, 2016; Rabten, 2016; Tshering Samdrup et al., 2020; Tshewang et al., 2020) were sampled inside each selected plot.

c. Data Analysis

Paleontological Analysis Statistical Tool was employed to determine the floristic diversity of the habitats using different univariate indices such as Menhinick species richness index, Simpson dominance index, Shannon diversity index, Pielou evenness. Frequency, relative frequency, dominance and relative dominance, density, relative density and importance value index (IVI)species were calculated using the standard phytosociological methods (Curtis & McIntosh, 1950, 1951). IVIwas calculated as the sum of relative frequency, relative density and relative basal area for tree and shrub species, while species abundance and relative abundance was used to calculated IVI for herb species (Baudoin et al., 2020; Bhadra, 2017; Dash et al., 2020; Replan & Malaki, 2017).

% Frequency (F) =
$$\frac{\text{No. of quadrats in which the species occurred}}{\text{Total no. quadrat studied}} \times 100$$
 (Eq. 1)

Relative frequency (RF) =
$$\frac{\text{Frequency of species}}{\text{Total frequency of all species}} X 100$$
 (Eq. 2)

Density (**D**) =
$$\frac{\text{Total Number of individuals of a species}}{\text{Sampled Area in square meter (m2)}}$$
 (Eq. 3)

Density (**D**) =
$$\frac{\text{Total number of individuals of a species present in all plots}}{\text{Total number of plots studied}}$$
 (Eq.4)

Relative density (RD) =
$$\frac{\text{Density of a species}}{\text{Total Density of all the species}} \times 100(\text{Eq. 5})$$

Relative Basal Area (**RBA**) =
$$\frac{\text{Total Basal Area of species}}{\text{Total Basal Area of All Species}} \times 100(\text{Eq. 6})$$

Importance Value Index (IVI) = Relative Frequency (RF) + Relative Density (RD) + Relative Basal Area (RBA)(Eq. 7)

Basal Area (**BA**) =
$$\pi r^2$$
 or $\pi d^2 / 4$ (Eq.8)

Results and Discussion

1. Floristics Composition of the habitat

A total of 58 species under 44 genera; 20 trees, 18 shrubs and 20 herb species belonging to 26 families were recorded from the habitat of *Tricholoma matsutake* as shown in figure 3. and table 1. The most species rich families are Ericaceae (12 species), followed by Rosaceae (8 species) and Asteraceae (6 species). Pinaceae and Primulaceae is represented by 3 species each. Five families including Berberidaceae, Melanthiaceae, Salicaceae, Saxifragraceae and Smilacaeae arerepresented by 2 species and the rest of the families represented by only one species each (Figure 3).

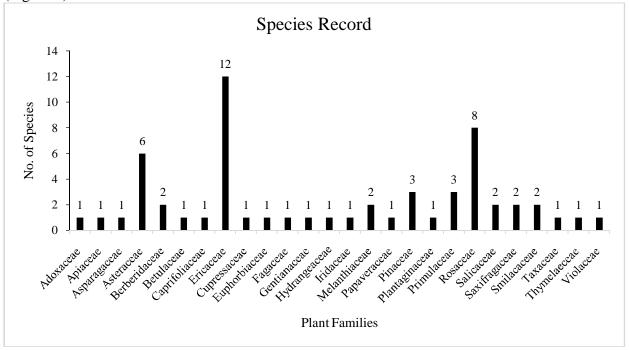


Figure 3: Plant species recorded from different family.

2. Life forms of Plant compositions in the habitat

The vegetation composition of the habitat includes 10 evergreen and 10 deciduous trees, 13 evergreen and 5 deciduous shrubs and 17 perennial and 3 annual herbs as shown in figure 4.

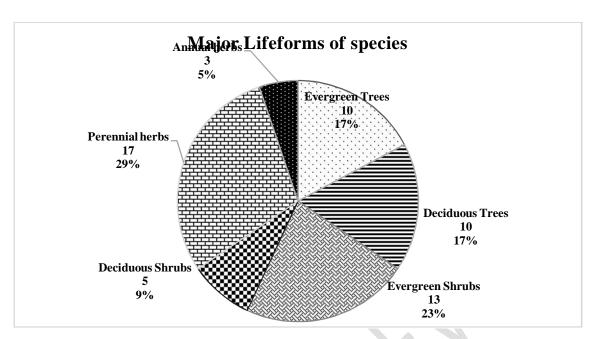


Figure 4: Major life forms of species.

The Habitat of *Tricholoma matsutake* was dominated by evergreen trees with 47 % (RBA=187.4) followed by evergreen shrubs with 43% (RBA= 171.5) and deciduous shrubs of 7% (RBA= 28.3). The lowest life form was observed with deciduous trees with 3% (RBA=12.5) (figure 5).

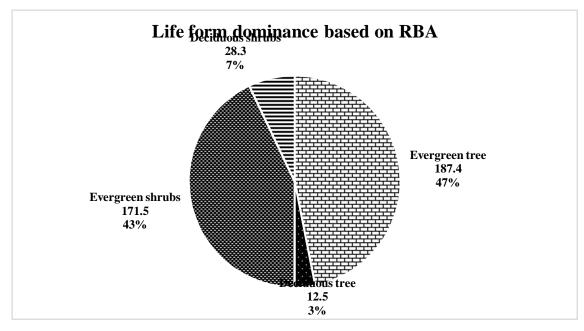


Figure 5: Life form dominance based on RBA.

Tricholoma matsutakeis floristically associated with tree species like Quercus semecarpifolia Sm., Pinus wallichiana A.B. Jack., Rhododendron spp. and shrub species such as Pieris formosa (Wall.) D. Don and Rhododendron lepidotumWall., and Gaultheria nummularoides D.Don. Ground vegetation of Tricholoma matsutake habitatscomprises of Anaphalis triplenervis (Sims)

C.B. Clarke, *Primula denticulata* Sm., *Dichrocephalachrysanthemifolia* (Blume) DC, and *Dichrocephalabenthamii* C.B. Clarke.

T. matsutake has wide and scattered distribution in temperate and boreal forests of Eurasia and subtropical China associated with tree species such as Pinus, Picea, Tsuga, Abies and fagaceous broadleaves (Vaario et al., 2017), in Quercus mongolica pure or mixed forests (Si et al., 2022) and also associated with Castanopsis and Quercus species (Pedersen, 2020). The related species of Tricholomagenus grows under various conifer tree species of Pinus, Abies, Picea, Larix, Cedrus, etc.) and trees of the oak family (Miyauchi et al., 2020). The similar floristic compositions were also found in the habitats of Tricholoma matsutake in Genekha.

3. Vegetation structure of Tricholoma matsutake Habitat

a. DBH and Height class distribution

The DBH of tree ranges from 10 cm to 95. 5 cm (M=25.2, $SD=\pm25.2$). The largest individual tree species was *Abies densa* Griff. with DBH of 95.5 cm, while smallest individual tree includes *Rhododendron arboreum* Sm., and *Rhododendron cinnabarinum*Hook.f. with DBH 10 cm. According to DBH class distribution of individual trees (figure 6), about 37 % (n=192) of the tree constitutes the DBH class 10-20 cm including maximum individual trees. It is followed by DBH Class 20-30 constituting 23 % (n=118), then DBH class 30-40 with 15 % (n=79) and subsequently followed by higher DBH class with gradual decrease in number of individual trees in the each DBH class. Hence the DBH class of 80-90 has minimum tree counts which constitutes only 0.4 % (n=2) as shown in figure 6.

The height of the tree species in the *Tricholoma matsutake* habitat ranges from 7 m to 35 m (M=21.030, SD= \pm 8.55). The maximum individuals 19.7 % (n=102 were found within height class of 5-10 m, followed by height class 10-15m, then by height class 20-25 with 17.6 % (n=9) and subsequently higher height class with gradual decrease in the number of individuals in respective height class. The height class of 30-35 has minimum tree counts constituting only 5.4% (n=28) as shown in figure 9.

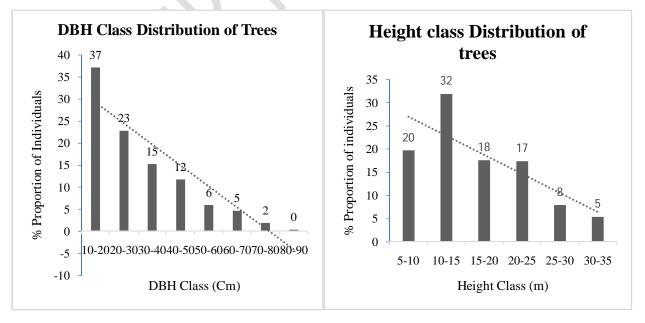


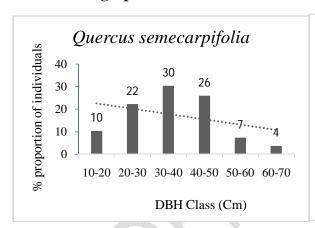
Figure 6: DBH and Height class distribution of tree species.

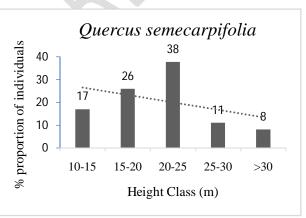
The DBH and height class distribution of the vegetation in *Tricholoma matsutake* habitat showed an inverted J-shaped distribution (figure 9) similar to findings of Sharma et al., (2023) in community forest, Nepal., Li et al., (2023) in forest of Tropic cancer, Chikanbanjar et al., (2020) in Panchase protected forest, Nepal., Nero et al., (2018) in the tree community, Ghana and Kunwar & Sharma, (2004) in community forest in Dolpa districts, mid-west Nepal.

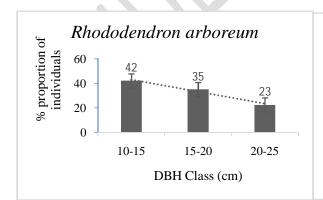
This is the general pattern of the normal population structure of the forest, with majority of tree species constituting lower DBH and height classes with gradual decrease towards both higher classes. The J-shaped distribution curve of DBH and height class depicts good reproduction and recruitment potentials of the vegetation in the *Tricholoma matsutake*habitat. It indicates a sustainable natural regeneration and successful recruitment(Chikanbanjar et al., 2020; Hossain et al., 2017; Li et al., 2023; P. Sharma et al., 2023).

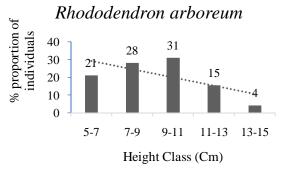
However, it does not represent the general trends of population dynamics and recruitment of an individual species. Therefore, analysis on population structures of five major tree species was provided in figure 7. for more realistic and specific information for future conservation measures.

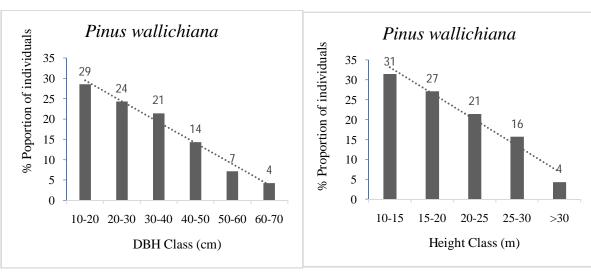
b. Demographic traits of five dominant tree species

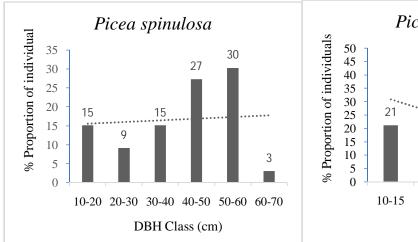


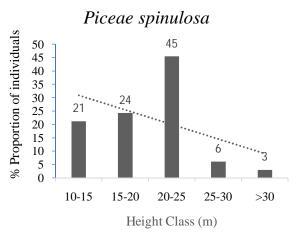


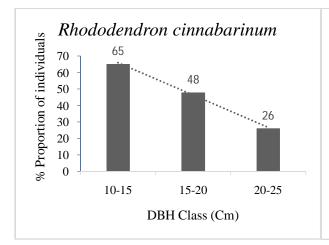












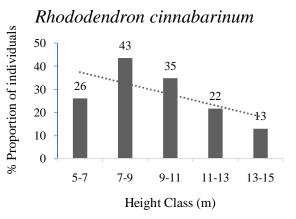


Figure 71:Patterns of frequency distribution of five dominant tree species over DBH and height classes in the *Tricholoma matsutake* habitat.

Diameter and height class distribution of five major dominant tree species of the habitat showed various patterns of population structure indicating divergent population dynamics among species as shown in figure 7.An inverted J-shaped distribution pattern was demonstrated by *Pinus wallichiana*A.B. Jacks, *Rhododendron arboreum*Sm. and *Rhododendron cinnabarinum*Hook.f. representing a relatively healthy regeneration of the species. On the other hand, other distribution patterns such as J shaped (*Picea spinulosa* (Griff.) A. Henry) and bell shaped (*Quercus semecarpifolia*Sm.) were also observed.

Pinus wallichiana A.B. Jacksresembled an inverted J-shaped curve indicating sustainable natural regeneration in line with studies conducted by Ghimire et al., (2011) and Måren et al., (2015) in trans-Himalayan dry valley of north-central Nepal. The weak regeneration of Quercus semecarpifolia Sm. is observed in the current study with bell shaped curve DBH and height distribution similar to findings of (Joshi, 2020) in forest below 2800 m elevations in Nepal. Differing inverted J-shaped curve was obtained for DBH distribution of Quercus semecarpifolia Sm. in old growth oak forest under Gidakom FMU- Bhutan(Tashi, 2004). In current study, Quercus semecarpifolia Sm. has maximum tree counts in DBH class of 30-50cm and height class of 20-25m, because of high stem density of individual trees in the habitat, preventing the increase in DBH and leading to the rapid vertical growth. Perhaps, bell shaped curve was obtained for the DBH and height distribution of Quercus semecarpifolia Sm.

4. Important value index (IVI) of plant species

The output of IVI analysis showed that *Quercus semecarpifolia* Sm. (56.4), *Rhododendron arboreum* Sm. (30.20), and *Pinus wallichiana* A.B Jacks (29.9) were the three most dominant tree species (Table 2). These species constituted 38.83 % of the total IVI of the tree species in north facing habitats. Correspondingly, *Quercus semicarpifolia*Sm (88.29), *Pinus wallichiana* A.B Jacks (39.73) and *Picea spinulosa* (Griff.) A. Henry (35.63) were the three most dominant tree species (Table 5), which constituted 54 % of IVI in the south facing habitat.

Kunwar & Sharma, (2004) reported *Pinus wallichiana* A.B Jacks and *Quercus semecarpifolia* Sm. having highest IVI and considered a dominant species in conifers and deciduous forest in Nepal. Similarly, Sharma et al., (2014) also recorded above mentioned species with highest IVI forming dominant species on ridge tops at upper Bhagirathi basin in Garhwal Himalaya. *Abies densa* Griff. has maximum value of RBA (43.16), and high IVI, (50.8), but is excluded from the dominant species as it has the least RF (1.69) and low RD (5.96).

The IVI analysis of the shrub showed that *Pieris formosa* (Wall.) D. Don (81.29), *Rubus nepalensis* (Hook.f.) Kuntze (37.1) and *Rhododendron lepidotum*Wall. (31.77) were the most dominant shrub species of the north facing habitat. Similarly, *Pieris formosa* (Wall.) D. Don (99.57), *Rubus nepalensis* (Hook.f.) Kuntze (37.91) and *Gaultheria nummularioides* D.Don (20.22) were the most dominant shrub species of the south facing habitat.

Anaphalis triplenervis(Sims) C.B. Clarke (45.4), Primula denticulata Sm. (38.8), and Dichrocephalachrysanthemifolia(Blume) DC. (30.8) as shown in (Table 6) were the most dominant herb species of the north facing habitat. Also, Anaphalis triplenervis(Sims) C.B. Clarke (37.95), Primula denticulata Sm. (22.68), and DichrocephalabenthamiiC.B. Clarke (21.7) were the most dominant herb species of the south facing habitat.

5. Natural Regeneration Status of Tree species in the Habitat

The regeneration status of tree species was determined based on the population sizes of adult trees, saplings and seedlings similar to (Aryal et al., 2021; Chikanbanjar et al., 2020; Malik et al., 2018; C. M. Sharma et al., 2018; Sunil & O., 2020). Accordingly, regeneration was categorized as Good: if seedlings > saplings > adults; Fair: if seedlings > saplings \leq adults; Poor: if there were saplings but no seedlings (though sapling may be less, more or equal to adults; No: if only adults were present, with no seedlings or saplings and New: if only saplings and/or seedlings were present, with no adults.

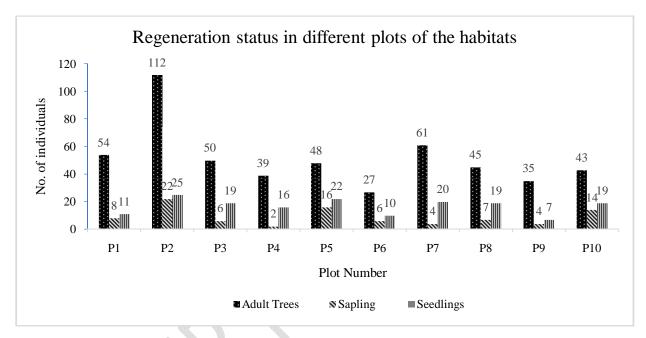


Figure 8: Regeneration status in different sample plots in habitats.

The number of adult tree species recorded from different plots ranges from 27 to 112 tree counts per plot with $(n=517, M=51.4, SD=\pm 23.36)$ as shown in figure 10. The highest tree count was recorded in plot 2 and lowest in plot 6. A total of 89 saplings were recorded from 10 plots, ranging from 2 to 22 counts with $(M=8.9, SD=\pm 6.36)$. The highest saplings were recorded from plot 5, while lowest counts from plot 4. Similarly, a total of 168 seedlings were recorded ranging from 7 to 25 seedlings with $(M=16.8, SD=\pm 1.81)$. The highest seedling counts was recorded from plot 2 and lowest from plot 9.

Regeneration survey showed that *Tricholomamtsutake*habitat of Genekha, indicated the fair regeneration status in accordance with various studies (Aryal et al., 2021; Chikanbanjar et al., 2020; Malik et al., 2018; C. M. Sharma et al., 2018; Sunil & O., 2020). Regeneration is said to be fair, if numbers of seedlings are more than saplings, though the saplings are equal or less than that of adults. Therefore, the natural regeneration status in the *Tricholoma matsutake* habitat is fair in all the sample plots.

6. Regeneration status of five dominant tree species

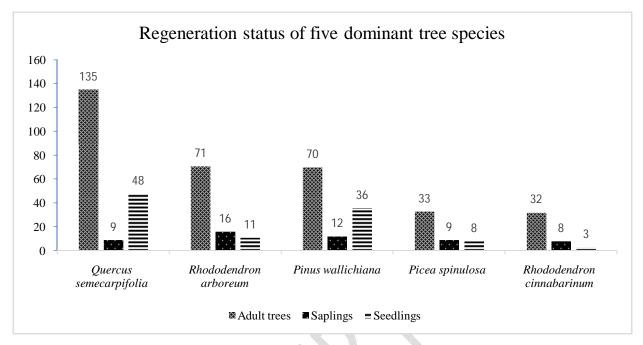


Figure 9: Regeneration status of five dominant tree species.

The natural regeneration status of the five dominant tree species indicated two types of natural regeneration status. *Quercus semecarpifolia* Sm., and *Pinus wallichiana* A.B Jacks exhibited fair regeneration withseedlings > saplings ≤ adults. Correspondingly, *Rhododendron arboreum* Sm., *Picea spinulosa* (Griff.) A. Henry and *Rhododendron cinnabarinum* Hook. f. indicated a poor regeneration; seedlings<saplings< adult trees. The similar regeneration status for *Quercus semecarpifolia* Sm., with abundant number of small seedlings with rare saplings were reported by Shrestha et al., (2004) in Shivapuri hill, Nepal.

7. Species diversity in the Tricholoma matsutake habitat

The total of 50 and 56 species were recorded from NFH and SFH respectively which is constituted by trees, shrubs and herbs, accounting a total species richness of 58 species in *Tricholoma matsutake* habitat (Table 1). However, species richness varies from season to season and is also affected by the area of the study site. In current study, only plant species that were found within the sampling plots were recorded and identified in order to attain a reliable estimates of species richness. Thus, it might have led to underestimation of species richness in the habitat.

Table 1: Diversity indices value of trees, shrubs and herbs in *Tricholoma matsutake* habitat.

Habitat	,	North Facing Habitat South Facing Habitat				tat		
Diversity indices	Trees	Shrubs	Herbs	Overall	Trees	Shrubs	Herbs	Overall
Taxa_S	19	16	15	50	18	18	20	56
Individuals	303	383	171	856	211	307	192	4532
Menhinicks	1.59	1.21	1.22	2.26	1.38	1.41	1.56	2.00
Simpson_1-D	0.85	0.85	0.82	0.94	0.78	0.85	0.86	0.76
Evenness_e^H/S	0.72	0.77	0.87	0.74	0.71	0.77	0.90	0.63
Shannon_H	2.13	2.07	1.8	3.06	1.82	2.12	2.13	2.49
Sorenson's					0.9			

Different diversity indices were estimated for trees, shrubs and herb species to describe the floristic composition of the habitat and to compare between NFH and SFH of the *Tricholoma matsutake*(Table 1). Menhinick's species richness index was 2.26 and 2.0 for NFH and SFH respectively, indicating high richness. However, species richness for tree, shrub and herb species in the habitat ranges from 1.2 to 1.59 indicating low species richness.

Simpson's index of 0.94 for NFH indicates higher diversity compared to SFH with index value of 0.76. Simpson's index of diversity (1- D) value ranges between 0 to 1. The index value closer to 1 represent higher diversity and the value closer to 0 represents low diversity (Simpson, 1949). Simpson's index has more weightage on abundant species, while addition of rare species has insignificant effect on the index value. Further, it has low sensitive to species richness. Possibly for this reason, the Simpson's diversity is higher for NFH compared to SFH with more species number. Correspondingly, the estimated Simpson's index for trees, shrubs and herbs ranges from 0.75 to 0.85 demonstrating high diversity, which contributed the overall high Simpson's diversity index in the habitat.

Pielou's uniformity index is 0.74 for NFH and 0.63 for SFH. Pielou index value ranges from 0 to 1, with the value closer to 1 indicating all species equally abundant and value closer to 0 indicates highly un even distribution (Pielou, 1966; Sharashy, 2022). Accordingly, the present study demonstrated that species distribution in the *Tricholoma matsutake* is moderately even in both north and south facing habitats.

In NFH, the distribution of the herb species (0.87) is more even compare to tree species (0.72) and shrub species (0.77). Similarly, the distribution of herbs species (0.90) is highly even compared to tress (0.71) and shrubs (0.77) in SFH. This un even distribution of species in the habitat is due to presence of few dominant tree species such as *Quercus semecarpifolia* Sm., *Rhododendron arboreum* Sm., and *Pinus wallichiana* A.B Jacks and dominant shrub species like *Pieris formosa* (Wall.) D. Don, and *Rubus nepalensis* (Hook.f.) Kuntze in the habitats.

The commonly used Shannon (H) diversity index was estimated to summarize, compare and describe the plant community of the *Tricholoma matsutake* habitat. Shannon diversity of 3.06 was obtained for NFH, which indicated moderate diversity. Correspondingly, Shannon diversity of 2.49 was obtained for SFH, which indicated low diversity. The diversity is low if (H < 3), moderate if $(3 \ge H > 4)$ and high if $(H \ge 4)$ (Sharashy, O, 2022; Ulfah et al., 2019). In present

study, the species diversity is higher in NFH compare to SFH of *Tricholoma matsutake* in Genekha, Thimphu.

The Sorensen similarity index (Sorensen, 1948) was estimated to determine the habitat similarity among the *Tricholoma matsutake* habitat in NFH and SFH. The Sorensen similarity index value ranges from 0 to 1, in which closer to 0 indicates complete dissimilarity, while closer 1 indicates complete similarity (Kanieski & Longhi, 2017). Accordingly, Sorensen index (0.9) obtained for the habitat indicated that the highly similar species composition between NFH and SFH. However, this does not provide the exact phytosociological similarity between the habitats, since species abundance is not considered in coefficients. All species present in the habitats have an equal weightage in the equation, whether rare or abundant (Ashtamoorthy, 2014; Kanieski & Longhi, 2017). Perhaps, different species composition with varying quantitative phytosociological can be observed in the habitats differing to respective seasons.

Conclusion

The study highlights the ecological significance of *Tricholoma matsutake* habitats in Genekha, Thimphu, Bhutan, emphasizing their rich floristic composition and diverse vegetation structure. With 58 identified plant species, predominantly from the Ericaceae and Rosaceae families, the habitat shows diverse phytosociological association of tree, shrub, and ground flora. The vegetation analysis reveals a favorable regeneration potential and moderate to high species diversity, although the findings are based on a single seasonal assessment. This research serves as a foundational step towards understanding and conserving the vital habitats of *Tricholoma matsutake*, addressing emerging threats, and supporting the livelihoods of local communities reliant on this important resource. Further longitudinal studies are recommended to capture a more comprehensive picture of floristic diversity and habitat dynamics.

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Table 2: Floristic composition of Tricholoma matsutake habitat.

Sl. No	Tree Species	Family	Life form	IUCN Status
1	Abies densaGriff.	Pinaceae	Evergreen	LC
2	Acer champbelliiHook.f. & Thomson ex Hiern	Sapindaceae	Deciduous	LC
3	Betula utilitisD. Don	Betulaceae	Deciduous	LC
4	Hydrangea sp.	Hydrangeaceae	Deciduous	
5	Juniperus recurvaBuchHam. ex D.Don	Cupressaceae	Evergreen	LC
6	Lyonia villosa(Wall. ex C.B. Clarke) HandMazz	Ericaceae	Deciduous	

7	Malus baccata (L.) Borkh	Rosaceae	Deciduous	
8	Picea spinulosa (Griff.) A. Henry	Pinaceae	Evergreen	LC
9	Pinus wallichianaA.B. Jacks	Pinaceae	Evergreen	LC
10	Populus ciliata Wall. Ex Royle	Salicaceae	Deciduous	LC
11	Prunus rufaWall. ex Hook.f.	Rosaceae	Deciduous	20
12	Quercus semecarpifolia Sm.	Fagaceae	Evergreen	LC
13	Rhododendron arboreumSm.	Ericaceae	Evergreen	LC
14	Rhododendron barbatumWall. ex G. Don	Ericaceae	Evergreen	
15	Rhododendron campylocarpumHook.f.	Ericaceae	Evergreen	
16	Rhododendron cinnabarinumHook.f.	Ericaceae	Evergreen	
17	Salix sikkimensis Andersson	Salicaceae	Deciduous	LC
18	Sorbus rufopilosaC.K. Schneid.	Rosaceae	Deciduous	NE
19	Taxus wallichianaZucc.	Taxaceae	Evergreen	EN
20	Viburnum cotinifoliumD.Don	Adoxaceae	Deciduous	
	Chamb on a			
1	Shrub spe		D :1	I. C.
1	Berberis aristataDC.	Berberidaceae	Deciduous	LC
2	Berberis hookeri Lem.	Berberidaceae	Deciduous	NE
3	Chimaphila japonicaMiq.	Ericaceae	Evergreen	NE
4	Cotoneaster horizontalis Decne.	Rosaceae	Deciduous	NE
5	Cotoneaster sherriffii Klotz	Rosaceae	Evergreen	
6	Daphne bholua Buchex D.Don	Thymelaeceae	Evergreen	
7	Gaultheria nummularioidesD.Don	Ericaceae	Evergreen	
8	Lonicera obovataRoyle	Caprifoliaceae	Deciduous	1.0
9	Pieris formosa (Wall.) D. Don	Ericaceae	Evergreen	LC
10	Rhododendron lepidotumWall.	Ericaceae	Evergreen	
11	Rhododendron pendulum Hook.f.	Ericaceae	Evergreen	
12	Rhododendron thomsoniiHook.f.	Ericaceae	Evergreen	
13	Rhododendron wallichiiHook.f.	Ericaceae	Evergreen	
14	Rubus nepalensis (Hook.f.) Kuntze	Rosaceae	Evergreen	
15	Rosea sericeaeLindl.	Rosaceae	Deciduous	
16	Smilax munita S. C. Chen	Smilacaceae	Evergreen	
17	Smilex sp.	Smilacaceae	Evergreen	I.C.
18	Spiraea bellaSims	Rosaceae	Evergreen	LC
	Herb spec	eies		
1	Ainsliaea aptera DC.	Asteraceae	Perennial	
2	Anaphalis busua(BuchHam.) DC.	Asteraceae	Perennial	
3	Anaphalis triplenervis (Sims) C.B. Clarke	Asteraceae	Perennial	
4	Astilbe rivulariesBuchHam.exD.Don	Saxifragaceae	Perennial	
5	Corydalis leptocarpaHook.f. & Thomson	Papaveraceae	Annual	NE
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6	Dichrocephalabenthamii C.B. Clarke	Asteraceae	Annual	
7	Dichrocephalachrysanthemifolia (Blume) DC.	Asteraceae	Annual	
8	Chrysospleniumnepalensis D. Don	Saxifragaceae	Perennial	
9	Euphorbia griffithiiHook.f.	Euphorbiaceae	Perennial	
10	Gentiana capitataBuchHam. ex D.Don	Gentianaceae	Perennial	
11	Hemiphragmaheterophyllum Wall.	Plantaginaceae	Perennial	
12	Iris tectorum Maxim.	Iridaceae	Perennial	
13	Maianthemum purpureum (Wall.) LaFrankia	Asparagaceae	Perennial	
14	Paris polyphylla sm.	Melanthiaceae	Perennial	VU
15	Primula denticulata Sm.	Primulaceae	Perennial	NE
16	Primula smithiana L.	Primulaceae	Perennial	NE
	Selinumwallichianum(DC.) Raizada & H. O			
17	Saxena	Apiaceae	Perennial	
18	Senecio laetusEdgew.	Asteraceae	Perennial	NE
19	TrillimgovanianumWall. ex D. Don	Melanthiaceae	Perennial	EN
20	Viola betonicifoliaSm.	Violaceae	Perennial	

Appendix Important Value Index of Plant Species Found in Tricholoma matsutake Habitat Table 3. IVI of tree species (NFH)

Tree Species	RF	RD	RBA	IVI
Abies densa Griff.	1.69	5.94	43.16	50.8
Acer champbelliiHook.f. & Thomson ex Hiern	5.08	1.98	0.52	7.6
Betula utilitisD. Don	6.78	3.63	1.39	11.8
Hydrangea sp.	3.39	0.66	0.15	4.2
Juniperus recurva Buch Ham. ex D.Don	3.39	1.65	0.39	5.4

Lyonia villosa(Wall. ex C.B. Clarke) HandMazz	6.78	6.6	1.42	14.8
Picea spinulosa (Griff.) A. Henry	8.47	5.61	3.38	17.5
Pinus wallichiana A.B. Jacks	8.47	11.55	9.85	29.9
Populus ciliata Wall. Ex Royle	3.39	0.99	0.44	4.8
Prunus rufaWall. ex Hook.f.	6.78	2.97	1.04	10.8
Quercus semecarpifolia Sm.	8.47	23.76	24.17	56.4
Rhododendron arboreumSm.	8.47	15.18	6.63	30.3
Rhododendron barbatumWall. ex G. Don	5.08	3.96	1.35	10.4
Rhododendron campylocarpumHook.f.	3.39	3.96	1.01	8.4
Rhododendron cinnabarinumHook.f.	6.78	6.93	1.81	15.5
Salix sikkimensis Andersson	3.39	0.66	0.17	4.2
Sorbus rufopilosaC.K. Schneid.	5.08	0.99	0.25	6.3
Taxus wallichianaZucc.	3.39	2.31	2.73	8.4
Viburnum cotinifoliumD.Don	1.69	0.66	0.15	2.5
Total	100	100	100	300

Table 4: IVI of Shrub species (NFH)

Shrub species	RF	RD	RBA IVI
Berberis aristataDC.	3.85	1.82	1.58 7.24
Berberis hookeri Lem.	3.85	2.08	1.87 7.79
Chimaphila japonicaMiq.	7.69	7.27	0.43 15.4
Cotoneaster horizontalisDecne.	7.69	7.01	9.33 24.03
Cotoneaster sherriffii Klotz	7.69	6.49	5.02 19.21
Daphne bholua Buchex D.Don	3.85	2.34	4.02 10.2
Gaultheria nummularioidesD.Don	7.69	4.42	4.88 16.99
Lonicera obovataRoyle	5.77	2.86	4.02 12.64
Pieris formosa (Wall.) D. Don	9.62	18.44	53.23 81.29
Rhododendron lepidotumWall.	9.62	15.84	6.31 31.77
Rhododendron thomsoniiHook.f.	1.92	0.52	1.15 3.59
Rubus nepalensis (Hook.f.) Kuntze	9.62	22.86	4.73 37.21
Rosea sericeaeLindl.	9.62	4.16	2.73 16.5
Smilax munita S. C. Chen	5.77	2.08	0.29 8.13
Smilex sp.	1.92	1.04	0.29 3.25
Spiraea bellaSims	3.85	0.78	0.14 4.77
Total	100	100	100 300

Table 5: IVI of herb species (NFH)

Herb species	RF	RD	RA	IVI
Ainsliaea aptera DC.	12.5	6.5	3.5	22.6
Anaphalis triplenervis (Sims) C.B. Clarke	12.5	21.4	11.5	45.4
Astilbe rivulariesBuchHam.exD.Don	6.25	3	3.2	12.4

Chrysospleniumnepalensis D. Don	3.13	4.8	10.2	18.1
Dichrocephalabenthamii C.B. Clarke	6.25	4.8	5.1	16.1
Dichrocephalachrysanthemifolia (Blume) DC.	9.38	12.5	8.9	30.8
Euphorbia griffithiiHook.f.	6.25	4.8	5.1	16.1
Gentiana capitataBuchHam. ex D.Don	6.25	3.6	3.8	13.6
Hemiphragmaheterophyllum Wall.	3.13	6	12.8	21.8
Paris polyphylla sm.	3.13	1.8	3.8	8.7
Primula denticulata Sm.	6.25	15.5	16.6	38.3
Primula gracilipesW. G. Craib	6.25	5.4	5.7	17.3
Selinumwallichianum(DC.) Raizada & H. O Saxena	9.83	6.5	4.7	20.6
TrillimgovanianumWall. ex D. Don	3.13	1.2	2.6	6.9
Viola betonicifoliaSm.	6.25	2.4	2.6	11.2
Total	100	100	100	300

Table 6: IVI of tree species (SFH)

Tree Species	RF	RD	RBA	IVI
Abies densaGriff.	2.22	4.78	4.28	11.29
Acer champbelliiHook.f. & Thomson ex Hiern	2.22	0.48	0.39	3.09
Betula utilitisD. Don	4.44	2.39	0.74	7.57
Juniperus recurvaBuchHam. ex D.Don	2.22	1.44	0.01	3.66
Lyonia villosa(Wall. ex C.B. Clarke) HandMazz	4.44	1.91	0.84	7.2
Malus baccata (L.) Borkh	2.22	0.48	0.27	2.97
Picea spinulosa (Griff.) A. Henry	8.89	7.66	19.09	35.63
Pinus wallichianaA.B. Jacks	11.11	16.75	11.87	39.73
Populus ciliata Wall. Ex Royle	4.44	3.35	3.17	10.96
Prunus rufaWall. ex Hook.f.	8.89	3.35	1.26	13.5
Quercus semecarpifolia Sm.	11.11	31.1	46.07	88.29
Rhododendron arboreumSm.	8.89	11.96	3.61	24.46
Rhododendron barbatumWall. ex G. Don	4.44	4.31	1.52	10.27
Rhododendron campylocarpumHook.f.	2.22	0.96	0.003	3.18
Rhododendron cinnabarinumHook.f.	8.89	5.26	1.717	15.87
Sorbus rufopilosaC.K. Schneid.	4.44	1.44	0.002	5.88
Taxus wallichianaZucc.	4.44	1.91	4.836	11.19
Viburnum cotinifoliumD.Don	4.44	0.48	0.339	5.26
Total	100	100	100	300

Table 7: IVI of shrub species (SFH)

Shrub species	RF	RD	RBA	IVI
Berberis aristataDC.	9.091	3.583	0.999	13.67
Berberis hookeri Lem.	1.818	0.977	1.148	3.94

Total	100	100	100	300
Spiraea bellaSims	1.818	0.651	0.18	2.65
Smilex sp.	1.818	0.977	1.073	3.87
Smilax munita S. C. Chen	9.091	7.166	1.707	17.96
Rubus nepalensis (Hook.f.) Kuntze	9.091	22.15	6.672	37.91
Rosea sericeaeLindl.	7.273	1.954	5.437	14.66
Rhododendron wallichiiHook.f.	3.636	2.932	1.514	8.08
Rhododendron thomsonii Hook.f.	3.636	4.235	1.325	9.2
Rhododendron pendulum Hook.f.	3.633	2.932	1.132	7.7
Rhododendron lepidotumWall.	3.636	6.189	1.181	11.01
Pieris formosa (Wall.) D. Don	9.091	20.195	70.286	99.57
Lonicera obovataRoyle	5.455	1.629	0.537	7.62
Gaultheria nummularioidesD.Don	9.091	9.772	1.361	20.22
Daphne bholua Buchex D.Don	5.455	2.606	3.755	11.82
Cotoneaster sherriffii Klotz	9.091	6.189	0.66	15.94
Cotoneaster horizontalisDecne.	3.636	2.932	0.611	7.18
Chimaphila japonicaMiq.	3.636	2.932	0.423	6.99

Table 8: IVI of herb species (SFH)

Herb species	RF	RD	RA IVI
Ainsliaea aptera DC.	10.2	7.292	3.807 21.3
Anaphalis busua(BuchHam.) DC.	6.12	4.688	4.079 14.89
Anaphalis triplenervis (Sims) C.B. Clarke	10.2	18.229	9.517 37.95
Astilbe rivulariesBuchHam.exD.Don	2.04	1.563	4.079 7.68
Corydalis leptocarpaHook.f. & Thomson	2.04	1.042	2.719 5.8
Dichrocephalabenthamii C.B. Clarke	6.12	8.333	7.251 21.71
Dichrocephalachrysanthemifolia(Blume) DC.	6.12	5.729	4.985 16.84
ChrysospleniumnepalensisD. Don	2.04	1.563	4.079 7.68
Euphorbia griffithiiHook.f.	6.12	7.813	6.798 20.73
Gentiana capitataBuchHam. ex D.Don	8.16	6.771	4.419 19.35
Hemiphragmaheterophyllum Wall.	6.12	7.292	6.345 19.76
Iris tectorum Maxim.	2.04	2.604	6.789 11.44
Maianthemum purpureum (Wall.) LaFrankia	2.04	1.563	4.079 7.68
Paris polyphylla sm.	2.04	2.04	5.438 9.56
Primula denticulata Sm.	6.12	8.854	7.705 22.68
Primula smithiana L.	2.04	1.563	4.079 7.68
Selinumwallichianum(DC.) Raizada & H. O Saxena	6.12	2.604	2.266 10.99
Senecio laetusEdgew.	4.08	2.604	3.399 10.08
TrillimgovanianumWall. ex D. Don	2.04	1.563	4.079 7.68
Viola betonicifoliaSm.	8.16	6.25	4.079 18.49
Total	100	100	100 300

