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. It is ectomycorrhizal fungi with high ecological and economic value requiring an important ecological niche and symbiotic tree associates. 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 with plot size of 20m X 20m, 5m X 5m and 2m X 2m for trees, shrubs and herbs respectively. PAST (Paleontological Statistics) 4.10 software was used for diversity analysis.A total of 58 species under 44 genera belonging to 26 families were recorded in the natural habitat of matsutake. The study revealed that T. matsutake is associated with Quercus semecarpifolia Sm., Pinus wallichiana A.B. Jack., Rhododendron spp., and Pieris formosa (Wall.) D. Don. J-shaped distribution curve of DBH and height class with fair regeneration status is obtained for associated species in the habitat.The Menhinick's species richness of (2.26, 2) for both North facing habitat (NFH) and Southfacing habitats (SFH) indicated high species richness. Moderate and low diversity is indicated with Simpsons index 0.94 (NFH)and 0.76 (SFH), Shannon index 3.06 (NFH) and 2.49 (SFH) with moderately even distribution 0.74(NFH) and 0.73 (SFH) of species in thehabitats. Sorensens similarity index of 0.9 indicated highly similar species composition between two habitats. This research provides current 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 Northwest. It is also found associated with hardwoods such as Tanoak (*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. matsutak*e 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

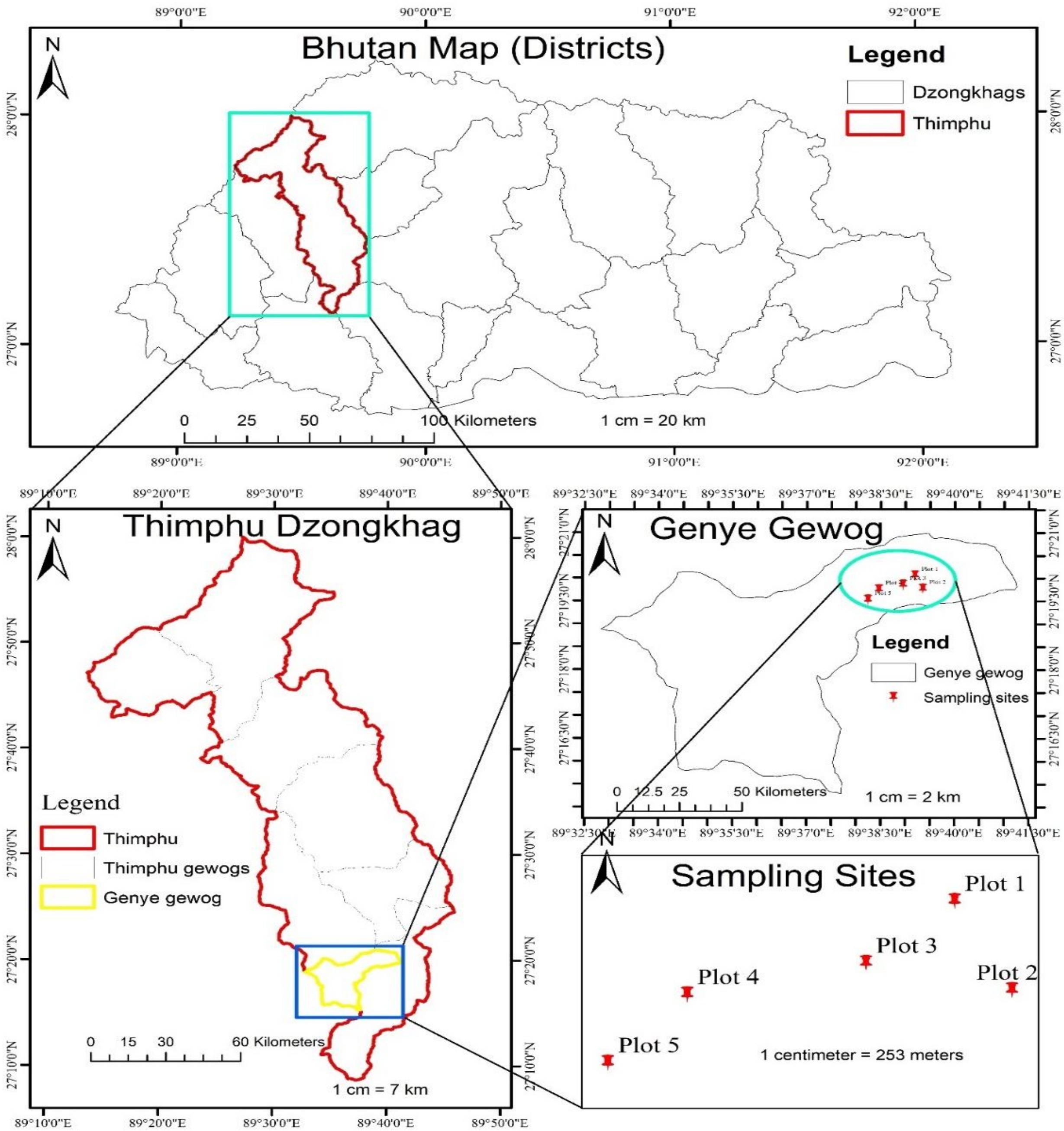


Figure 2: Study area; Habitat of Tricholoma matsutake, Genekha, Thimphu, Bhutan

b. Sampling and Data collection

*Tricholoma matsutake*habitat 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., 2022) 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) = $\frac{\text{No. of quadrats in which the species occurred}}{\text{Total no. quadrat studied}} x\, 100$ (Eq. 1)

Relative frequency (RF) = $\frac{\text{Frequency of species}}{\text{Total frequency of all species}} \text{X } 100$ (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) = $\frac{\text{Density of a species}}{\text{Total Density of all the species}} \text{ X } 100$(Eq. 5)

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

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

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

Abundance (A) = $\frac{\text{Total number of individuals of a species present in all plots}}{\text{Total number of plots of specis occurence}}$ (Eq. 9)

Relative Abundance (RA)= $\frac{\text{Abundance of a species}}{\text{Sum of abundance of all species}} \text{x } 100$ (Eq.10)

Importance Value Index (IVI) = Relative Frequency (RF) + Relative Density (RD) + Relative Abundance (RA). (Eq.11)

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).

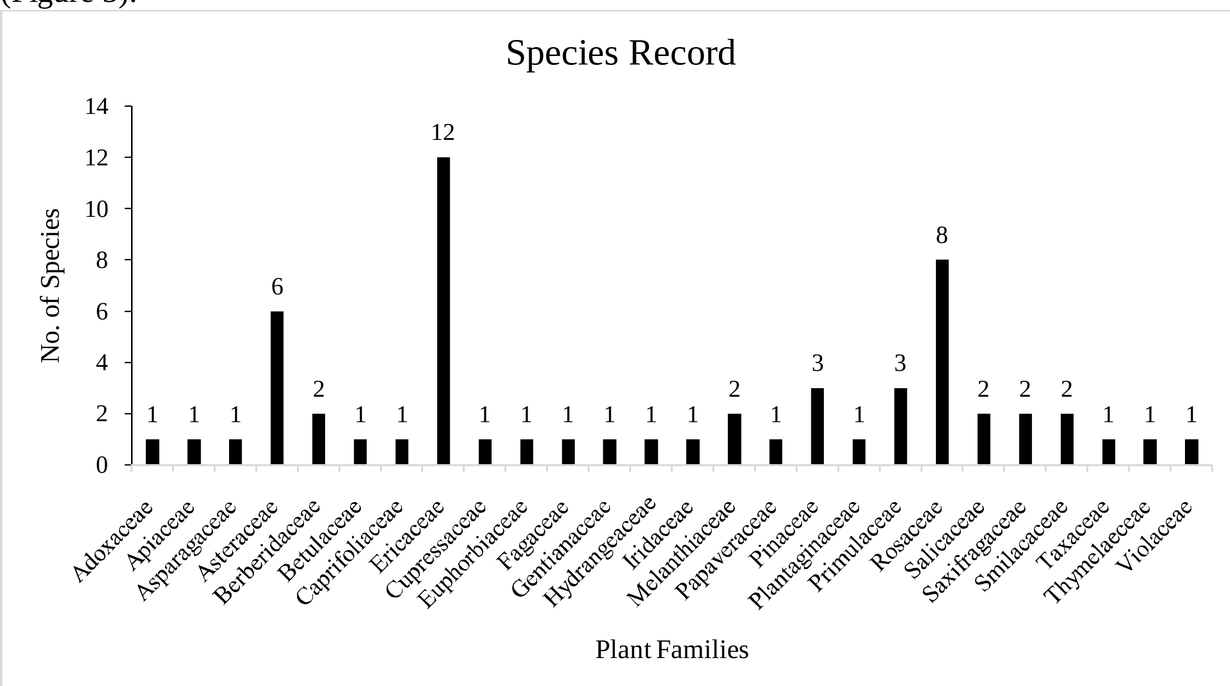


Figure 3: Plant species recorded from different family.

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.

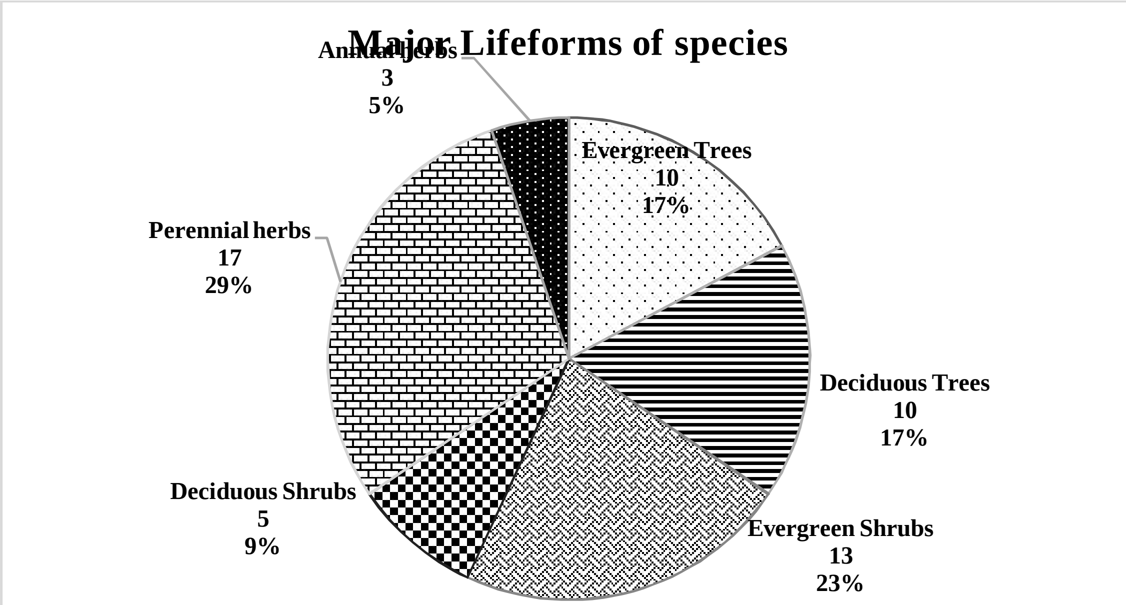


Figure 4: Major life forms of species.

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).

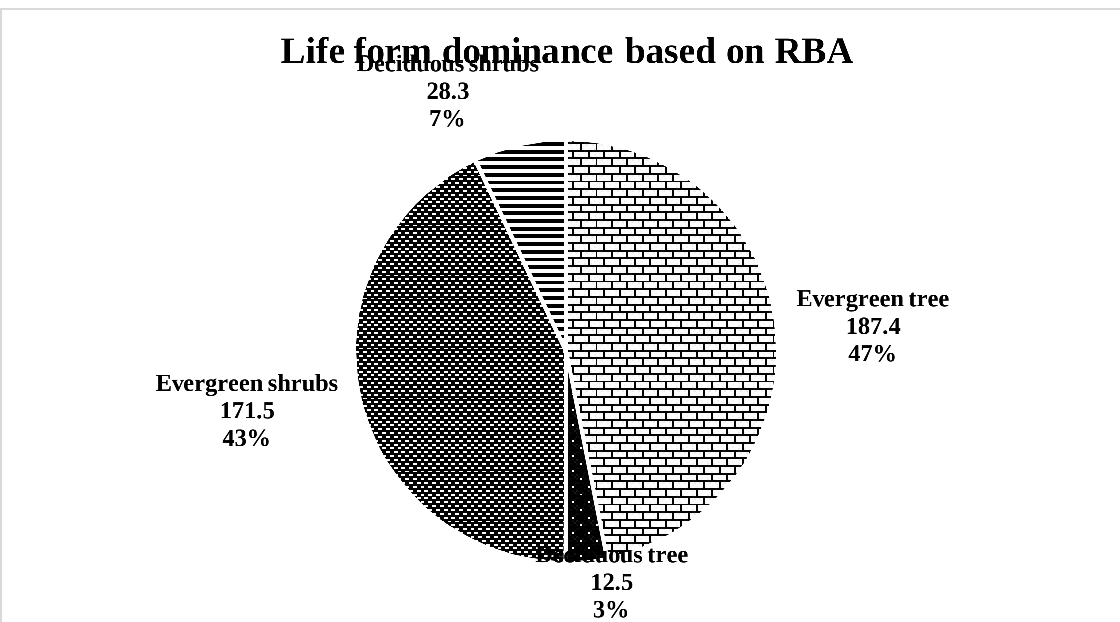


Figure 5: Life form dominance based on RBA.

*Tricholoma matsutake*is 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 lepidotum*Wall., 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 *Tricholoma*genus 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*= ± 25.2). 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*=± 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 6.

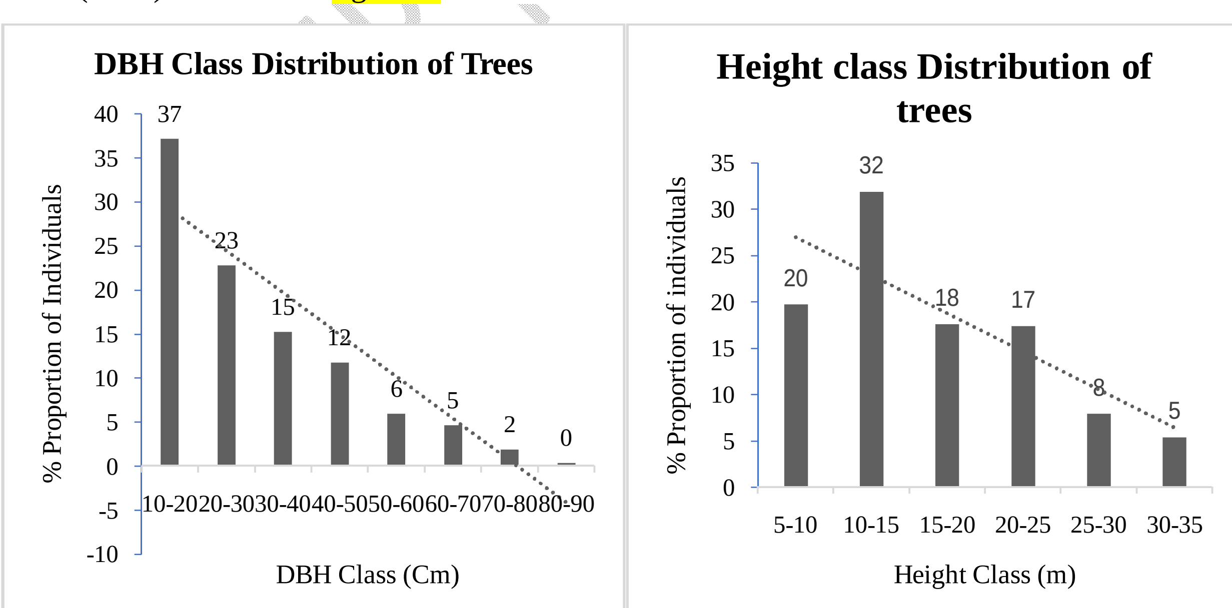


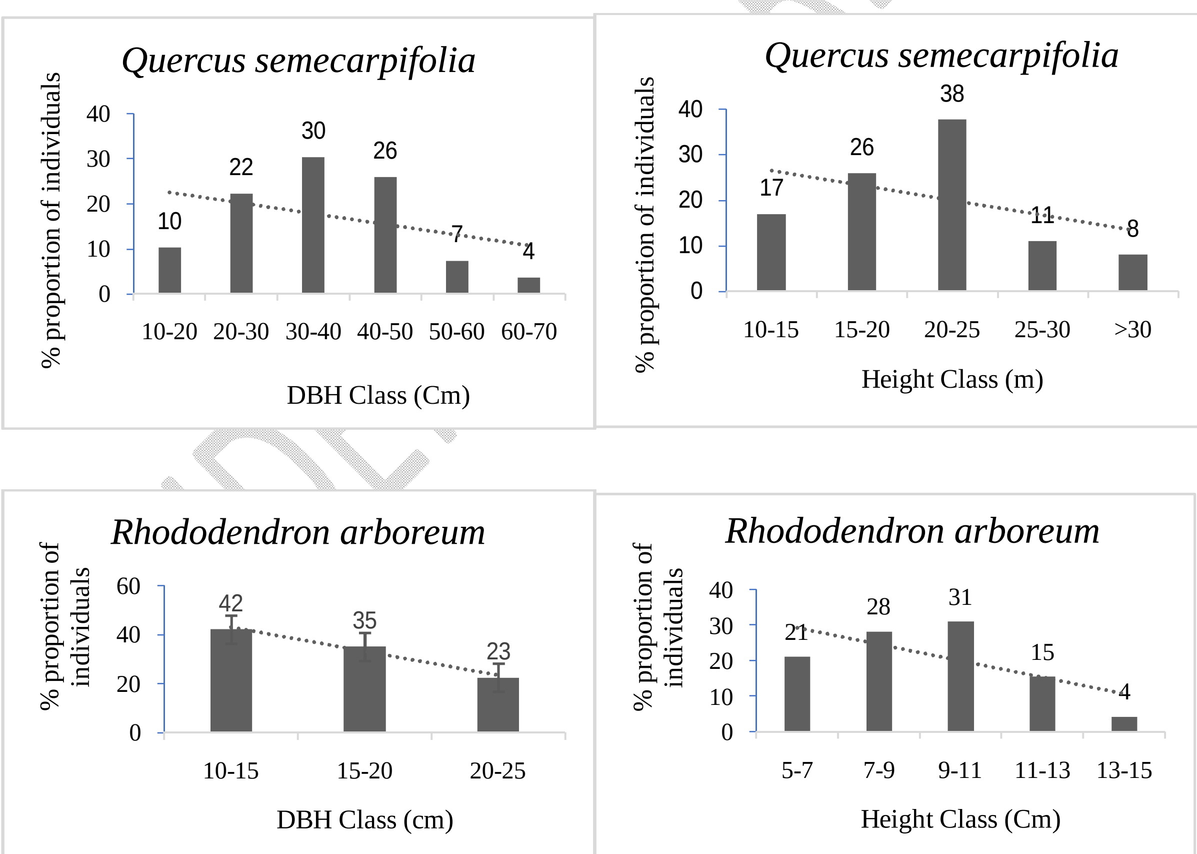
Figure 6: DBH and Height class distribution of tree species.

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



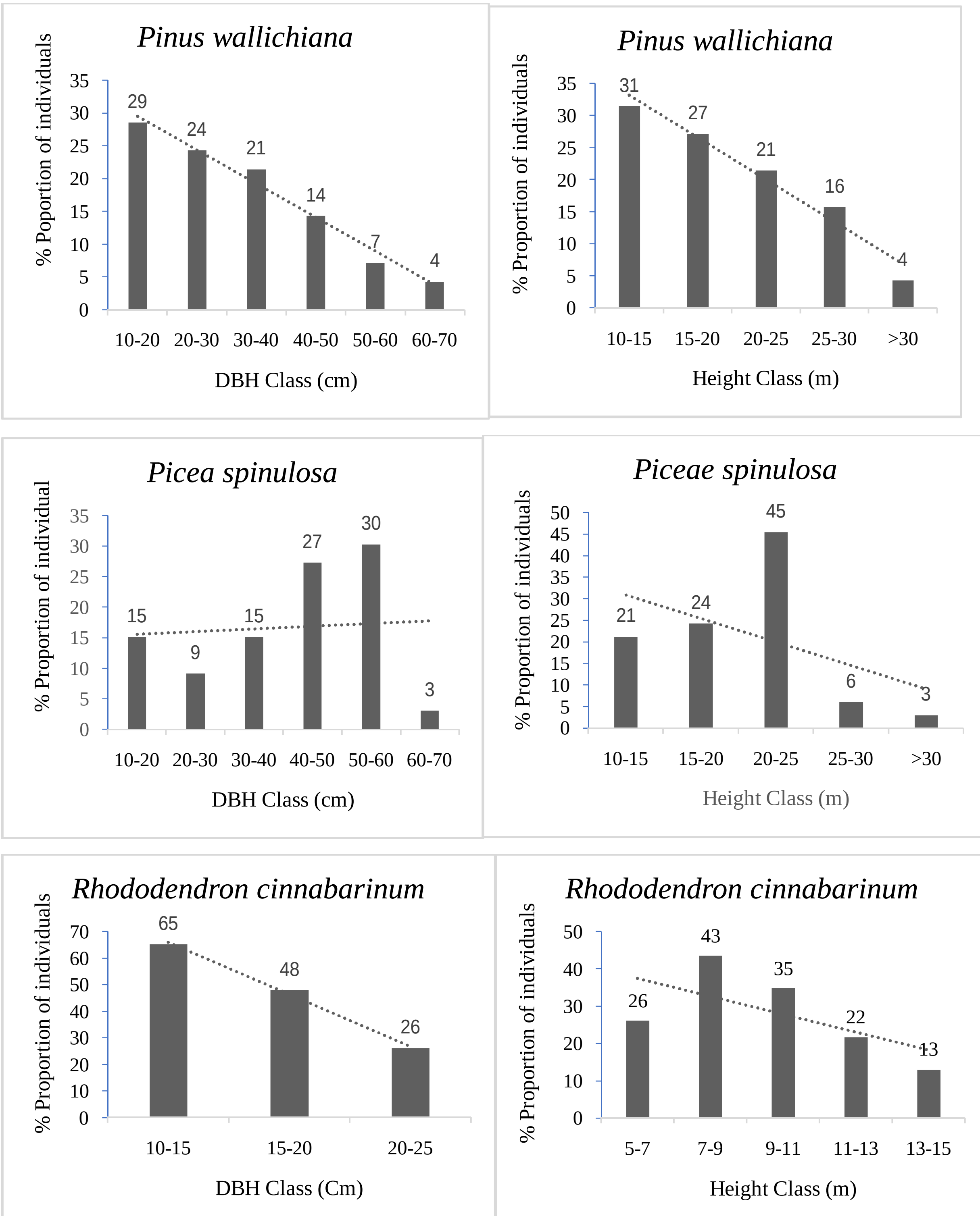


Figure 71:Patterns of frequency distribution of five dominant tree species over DBH and height classes in the *Tricholoma matsutake* habitat.

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 *Dichrocephalabenthamii*C.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 ≤ 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.

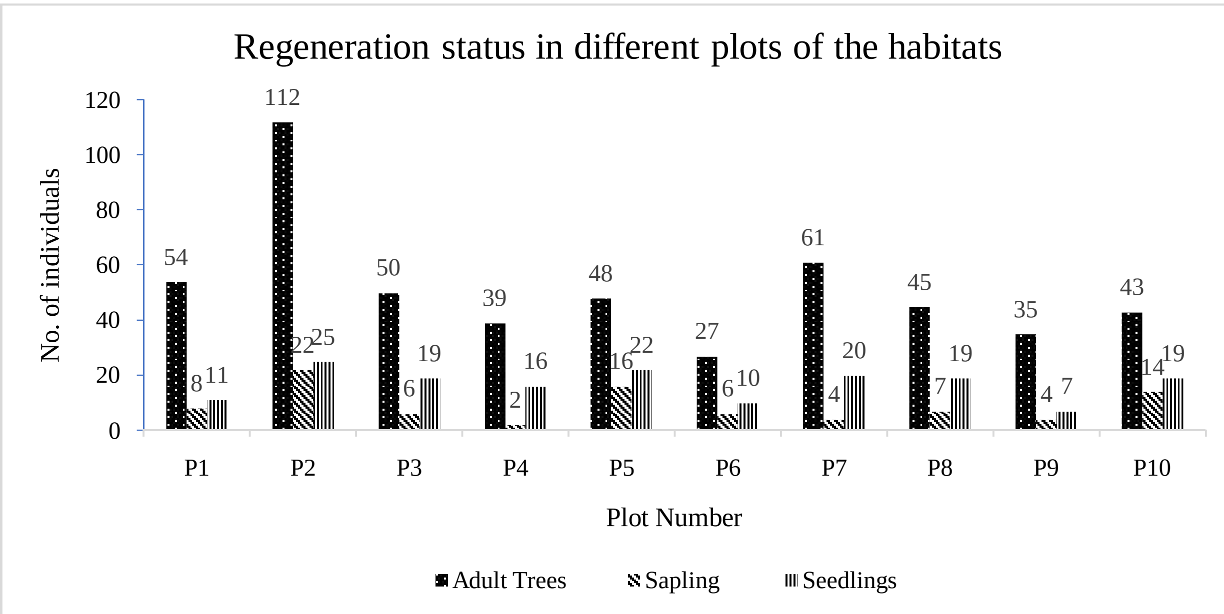


Figure 8: Regeneration status in different sample plots in habitats.

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*= ± 23.36) as shown infigure 8.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*= ± 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*= ± 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

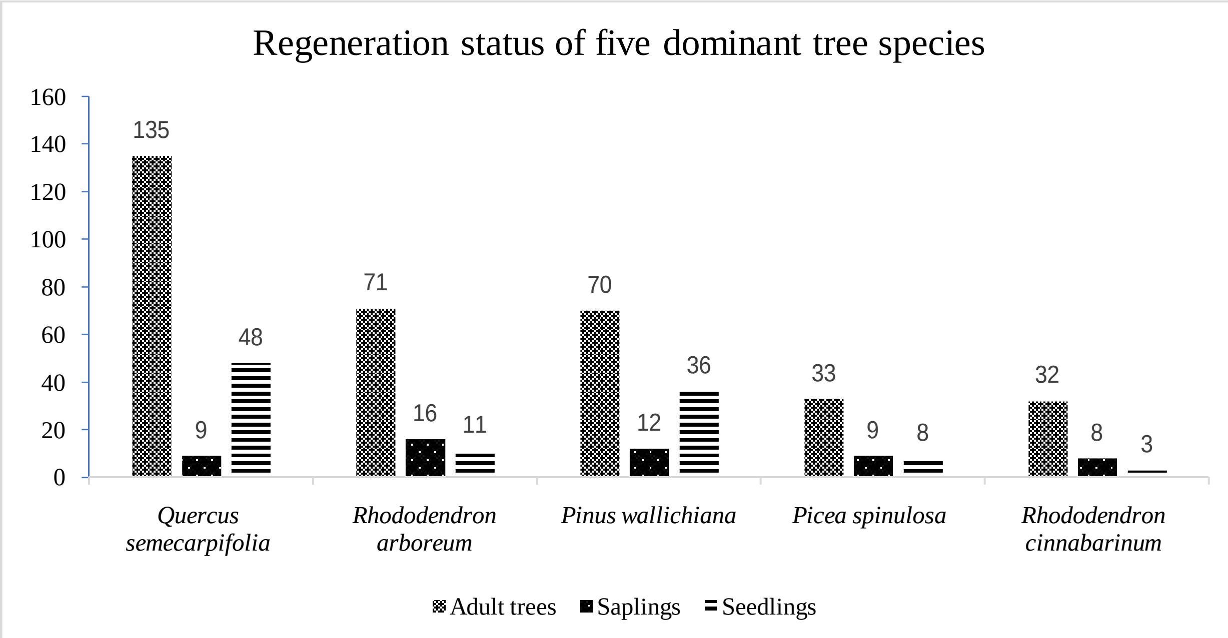


Figure 9: Regeneration status of five dominant tree species.

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.

The current regeneration assessment of the tree associates in matsutake habitat showed only fair regeneration with varying regeneration status as shown in figure 9. This may be due to the noticeable habitat destruction observed in area such as logging and timber extraction, collection of leaf litter and open grazing of cattle in the habitat. Habitat protectionof matsutake and improving the regeneration of associate tree species is crucial for its sustainability. Thus, practiced of not allowing leaf litter collection, controlled grazing and no permit for timber extraction from the core matsutake habitat can help in regeneration of associate tree species of matsutake.

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**			
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 ($3 \geq H > 4$) and high if ($H \geq 4$) (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. Reducing the habitat destruction activities that impact the regeneration of matsutake associated tree species and floral composition can maintain the sustainability of matsutake in Genekha. Therefore, no leaf litter collection and alternative timber extraction or logging sites and cattle grazing area must be allocated to protect and conserve the *Tricholomamatsuatake* habitat in Genekha, Thimphu. However, further longitudinal studies are recommended to capture a more comprehensive picture of floristic diversity and habitat dynamics.In addition, soil mycoflora analysis and ectomycorrhizalstudy with floral associates of *Tricholoma matsutake* will further enhance the habitat ecology *Tricholoma matsutake.*

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Disclaimer (Artificial intelligence)

Option 1:

We hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Table 2: Floristic composition of Tricholoma matsutake habitat.

Sl. No	Tree Species	Family	Life form	IUCN Status
1	*Abies densa*Griff.	Pinaceae	Evergreen	LC
2	*Acer champbellii*Hook.f. & Thomson ex Hiern	Sapindaceae	Deciduous	LC
3	*Betula utilitis*D. Don	Betulaceae	Deciduous	LC
4	*Hydrangea* sp.	Hydrangeaceae	Deciduous	
5	*Juniperus recurva*Buch.-Ham. ex D.Don	Cupressaceae	Evergreen	LC
6	*Lyonia villosa*(Wall. ex C.B. Clarke) Hand.-Mazz	Ericaceae	Deciduous	
7	*Malus baccata* (L.) Borkh	Rosaceae	Deciduous	
8	*Picea spinulosa* (Griff.) A. Henry	Pinaceae	Evergreen	LC
9	*Pinus wallichiana*A.B. Jacks	Pinaceae	Evergreen	LC
10	*Populus ciliata* Wall. Ex Royle	Salicaceae	Deciduous	LC
11	*Prunus rufa*Wall. ex Hook.f.	Rosaceae	Deciduous	
12	*Quercus semecarpifolia* Sm.	Fagaceae	Evergreen	LC
13	*Rhododendron arboreum*Sm.	Ericaceae	Evergreen	LC
14	*Rhododendron barbatum*Wall. ex G. Don	Ericaceae	Evergreen	
15	*Rhododendron campylocarpum*Hook.f.	Ericaceae	Evergreen	
16	*Rhododendron cinnabarinum*Hook.f.	Ericaceae	Evergreen	
17	*Salix sikkimensis* Andersson	Salicaceae	Deciduous	LC
18	*Sorbus rufopilosa*C.K. Schneid.	Rosaceae	Deciduous	NE
19	*Taxus wallichiana*Zucc.	Taxaceae	Evergreen	EN
20	*Viburnum cotinifolium*D.Don	Adoxaceae	Deciduous	

	Shrub species			
1	*Berberis aristata*DC.	Berberidaceae	Deciduous	LC
2	*Berberis hookeri* Lem.	Berberidaceae	Deciduous	NE
3	*Chimaphila japonica*Miq.	Ericaceae	Evergreen	NE
4	*Cotoneaster horizontalis*Decne.	Rosaceae	Deciduous	NE
5	*Cotoneaster sherriffii* Klotz	Rosaceae	Evergreen	
6	*Daphne bholua* Buch.-ex D.Don	Thymelaeceae	Evergreen	
7	*Gaultheria nummularioides*D.Don	Ericaceae	Evergreen	
8	*Lonicera obovata*Royle	Caprifoliaceae	Deciduous	
9	*Pieris formosa* (Wall.) D. Don	Ericaceae	Evergreen	LC

10	*Rhododendron lepidotum*Wall.	Ericaceae	Evergreen	
11	*Rhododendron pendulum* Hook.f.	Ericaceae	Evergreen	
12	*Rhododendron thomsonii*Hook.f.	Ericaceae	Evergreen	
13	*Rhododendron wallichii*Hook.f.	Ericaceae	Evergreen	
14	*Rubus nepalensis* (Hook.f.) Kuntze	Rosaceae	Evergreen	
15	*Rosea sericeae*Lindl.	Rosaceae	Deciduous	
16	*Smilax munita* S. C. Chen	Smilacaceae	Evergreen	
17	*Smilex* sp.	Smilacaceae	Evergreen	
18	*Spiraea bella*Sims	Rosaceae	Evergreen	LC
	Herb species			
1	*Ainsliaea aptera* DC.	Asteraceae	Perennial	
2	*Anaphalis busua*(Buch.-Ham.) DC.	Asteraceae	Perennial	
3	*Anaphalis triplenervis* (Sims) C.B. Clarke	Asteraceae	Perennial	
4	*Astilbe rivularies*Buch.-Ham.exD.Don	Saxifragaceae	Perennial	
5	*Corydalis leptocarpa*Hook.f. & Thomson	Papaveraceae	Annual	NE
6	*Dichrocephalabenthamii C.B. Clarke*	Asteraceae	Annual	
7	*Dichrocephalachrysanthemifolia (Blume) DC.*	Asteraceae	Annual	
8	*Chrysospleniumnepalensis D. Don*	Saxifragaceae	Perennial	
9	*Euphorbia griffithii*Hook.f.	Euphorbiaceae	Perennial	
10	*Gentiana capitata*Buch.-Ham. 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
17	*Selinumwallichianum*(DC.) Raizada & H. O Saxena	Apiaceae	Perennial	
18	*Senecio laetus*Edgew.	Asteraceae	Perennial	NE
19	*Trillimgovanianum*Wall. ex D. Don	Melanthiaceae	Perennial	EN
20	*Viola betonicifolia*Sm.	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 champbellii*Hook.f. & Thomson ex Hiern	5.08	1.98	0.52	7.6
*Betula utilitis*D. 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) Hand.-Mazz.	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 rufa*Wall. ex Hook.f.	6.78	2.97	1.04	10.8
Quercus semecarpifolia Sm.	8.47	23.76	24.17	56.4
*Rhododendron arboreum*Sm.	8.47	15.18	6.63	30.3
*Rhododendron barbatum*Wall. ex G. Don	5.08	3.96	1.35	10.4
*Rhododendron campylocarpum*Hook.f.	3.39	3.96	1.01	8.4
*Rhododendron cinnabarinum*Hook.f.	6.78	6.93	1.81	15.5
Salix sikkimensis Andersson	3.39	0.66	0.17	4.2
*Sorbus rufopilosa*C.K. Schneid.	5.08	0.99	0.25	6.3
*Taxus wallichiana*Zucc.	3.39	2.31	2.73	8.4
*Viburnum cotinifolium*D.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 aristata*DC.	3.85	1.82	1.58	7.24
Berberis hookeri Lem.	3.85	2.08	1.87	7.79
*Chimaphila japonica*Miq.	7.69	7.27	0.43	15.4
*Cotoneaster horizontalis*Decne.	7.69	7.01	9.33	24.03
Cotoneaster sherriffii Klotz	7.69	6.49	5.02	19.21
Daphne bholua Buch.-ex D.Don	3.85	2.34	4.02	10.2

*Gaultheria nummularioides*D.Don	7.69	4.42	4.88	16.99
*Lonicera obovata*Royle	5.77	2.86	4.02	12.64
Pieris formosa (Wall.) D. Don	9.62	18.44	53.23	81.29
*Rhododendron lepidotum*Wall.	9.62	15.84	6.31	31.77
*Rhododendron thomsonii*Hook.f.	1.92	0.52	1.15	3.59
Rubus nepalensis (Hook.f.) Kuntze	9.62	22.86	4.73	37.21
*Rosea sericeae*Lindl.	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 bella*Sims	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 rivularies*Buch.-Ham.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 griffithii*Hook.f.	6.25	4.8	5.1	16.1
*Gentiana capitata*Buch.-Ham. 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 gracilipes*W. G. Craib	6.25	5.4	5.7	17.3
Selinumwallichianum(DC.) Raizada & H. O Saxena	9.83	6.5	4.7	20.6
*Trillimgovanianum*Wall. ex D. Don	3.13	1.2	2.6	6.9
*Viola betonicifolia*Sm.	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 densa*Griff.	2.22	4.78	4.28	11.29
*Acer champbellii*Hook.f. & Thomson ex Hiern	2.22	0.48	0.39	3.09
*Betula utilitis*D. Don	4.44	2.39	0.74	7.57
*Juniperus recurva*Buch.-Ham. ex D.Don	2.22	1.44	0.01	3.66
Lyonia villosa(Wall. ex C.B. Clarke) Hand.-Mazz	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 wallichiana*A.B. Jacks	11.11	16.75	11.87	39.73
Populus ciliata Wall. Ex Royle	4.44	3.35	3.17	10.96
*Prunus rufa*Wall. ex Hook.f.	8.89	3.35	1.26	13.5
Quercus semecarpifolia Sm.	11.11	31.1	46.07	88.29
*Rhododendron arboreum*Sm.	8.89	11.96	3.61	24.46
*Rhododendron barbatum*Wall. ex G. Don	4.44	4.31	1.52	10.27
*Rhododendron campylocarpum*Hook.f.	2.22	0.96	0.003	3.18
*Rhododendron cinnabarinum*Hook.f.	8.89	5.26	1.717	15.87
*Sorbus rufopilosa*C.K. Schneid.	4.44	1.44	0.002	5.88
*Taxus wallichiana*Zucc.	4.44	1.91	4.836	11.19
*Viburnum cotinifolium*D.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 aristata*DC.	9.091	3.583	0.999	13.67
Berberis hookeri Lem.	1.818	0.977	1.148	3.94
*Chimaphila japonica*Miq.	3.636	2.932	0.423	6.99
*Cotoneaster horizontalis*Decne.	3.636	2.932	0.611	7.18
Cotoneaster sherriffii Klotz	9.091	6.189	0.66	15.94
Daphne bholua Buch.-ex D.Don	5.455	2.606	3.755	11.82
*Gaultheria nummularioides*D.Don	9.091	9.772	1.361	20.22
*Lonicera obovata*Royle	5.455	1.629	0.537	7.62
Pieris formosa (Wall.) D. Don	9.091	20.195	70.286	99.57
*Rhododendron lepidotum*Wall.	3.636	6.189	1.181	11.01
Rhododendron pendulum Hook.f.	3.633	2.932	1.132	7.7
*Rhododendron thomsonii*Hook.f.	3.636	4.235	1.325	9.2
*Rhododendron wallichii*Hook.f.	3.636	2.932	1.514	8.08
*Rosea sericeae*Lindl.	7.273	1.954	5.437	14.66
Rubus nepalensis (Hook.f.) Kuntze	9.091	22.15	6.672	37.91
Smilax munita S. C. Chen	9.091	7.166	1.707	17.96
Smilex sp.	1.818	0.977	1.073	3.87
*Spiraea bella*Sims	1.818	0.651	0.18	2.65
Total	**100**	**100**	**100**	**300**

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(Buch.-Ham.) DC.	6.12	4.688	4.079	14.89
Anaphalis triplenervis (Sims) C.B. Clarke	10.2	18.229	9.517	37.95
*Astilbe rivularies*Buch.-Ham.exD.Don	2.04	1.563	4.079	7.68
*Corydalis leptocarpa*Hook.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
*Chrysospleniumnepalensis*D. Don	2.04	1.563	4.079	7.68
*Euphorbia griffithii*Hook.f.	6.12	7.813	6.798	20.73
*Gentiana capitata*Buch.-Ham. 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 laetus*Edgew.	4.08	2.604	3.399	10.08
*Trillimgovanianum*Wall. ex D. Don	2.04	1.563	4.079	7.68
*Viola betonicifolia*Sm.	8.16	6.25	4.079	18.49
Total	**100**	**100**	**100**	**300**