SPECIES

The status of superb poppy, *meconopsis superba* in Jigme khesar strict nature reserve, its natural habitat

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ABSTRACT

The Jigme Khesar Strict Nature Reserve (JKSNR) in Bhutan, which is the country's only "Strict Nature Reserve" category of protected area, is home to the endangered *Meconopsis superba*, also known as *Papaver superbum*, a beautiful Superb Poppy that is endemic to Haa district and therefore of great importance. In this study in the Reserve, *M. superba* was found to occur between elevations of 3903masl to 4366masl, primarily in wet rocky outcrops and moist stream beds in 32 out of 33 surveyed plots, 10 x 10 m. Preliminary threat research revealed grazing by locals in most of the plots (80%), while fire (30%) and trampling (50%) were also recorded. However, there were no attempts to harvest the species by locals, contrary to expectations. The mean height of full-grown *M. superba* individuals was approximately 123.15cm, with most individuals (97.7%) being healthy and in the flowering stage (51.5%). Profuse regeneration was found on east and southeast facing slopes (100° to 250° aspect). We identified 77 species of shrubs and 55 species of herbs. *Rhododendron* species were the most frequently observed, while in the herbaceous layer, *Potentilla* species were the most commonly counted. While there were no immediate threats to *M. superba* or its shrub and herb associates, anthropocentric activities were visibly present in the study area. The study recommends ongoing protection efforts in the Reserve, with a special focus on fire incidence as regenerations were reduced in fire burnt areas across the survey plots. Regular monitoring and habitat assessment are suggested as important conservation measures to ensure the perpetuity of this species.

Keywords: Distribution, disturbances, diversity, herbs, *Meconopsis superba*, shrubs

1. INTRODUCTION

Bhutan, a small Himalayan Kingdom squeezed in between two giants, China in the north and India in most of its south, east and west is home to ca. 15 species of *Meconopsis* (Long, 1984; Iwashina et al., 2019) out of which as many as eight are found in Haa district (Table 1) more than one third of which are declared as Jigme

DISCOVERY SCIENTIFIC SOCIETY

Khesar Strict Nature Reserve (JKSNR) (Figure 1). JKSNR stands out as the sole strict nature reserve in the country, with all other protected areas being designated as either a National Park or Wildlife Sanctuary. Covering an area of 609.5 square kilometres, the Reserve boasts a rich collection of rare, endemic and high-value medicinal plant species, globally endangered animals, as well as awe-inspiring landscapes and a distinctive culture.

Table 1 List of *Meconopsis* species found in Bhutan with locality information

Sl. No.	Species	Author	Distribution/places grown
1	*Meconopsis bella*	Prain	Paro
2	*Meconopsis bella merakensis*	T. Yoshida, R. Yangzom & D. G. Long	Merak in Tashigang
3	*Meconopsis bhutanica*	T. Yoshida & Grey-Wilson	Paro (Tshophutsho)
4	*Meconopsis elongate*	T. Yoshida, R. Yangzom & D. G. Long	Haa, Paro, Thimphu
5	*Meconopsis gakyidiana*	T. Yoshida, R. Yangzom & D. G. Long	Sakteng
6	*Meconopsis grandiflora*	Prain	Haa, Paro, Thimphu
7	*Meconopsis horridula*	Hook. f. & Thoms.	Haa, Paro, Thimphu
8	*Meconopsis horridula drukyulensis*	Grey-Wilson	Paro
9	*Meconopsis ludlowii*	Grey-Wilson	Multiple places
10	*Meconopsis paniculata*	(D. Don) Prain	Haa, Paro Thimphu
11	*Meconopsis polygonoides*	Prain	Paro and Haa
12	*Meconopsis primulina*	Prain	Haa, Paro, Thimphu
13	*Meconopsis sherriffii*	G. Taylor	Thimphu, Bumthang, Paro
14	*Meconopsis simplicifolia grandiflora*	(D. Don) Walp.	Haa, Paro, Thimphu, Wangdue, Bumthang
15	*Meconopsis sinuate*	Prain	Paro
16	*Meconopsis bella subintegrifolia*	Grey-Wilson	Multiple places
17	*Meconopsis superba*	Ex. Prain	Haa
18	*Meconopsis wallichii fusco-purpurea*	Hook.	Haa, Paro and Chukha

The Reserve also boasts the most extensive collection of unspoiled temperate and alpine ecosystems in Bhutan (TSNRMP, 2012). It is also the only protected area in Bhutan that is included in the trans-boundary conservation landscape of Kanchenjunga, forming a sacred part of the Himalayan landscape of the Eastern Himalayas (Worboys et al., 2010), popularly referred to as the "snow leopard landscape" and is a part of the Himalaya Biodiversity hotspots (Mittermeier et al., 2004). Moreover, the Reserve is an inherent constituent of two of the WWF Global 200 ecoregions (Olson and Dinerstein, 2002), despite not being intentionally included as such.

The floral diversity of JKSNR is still being documented and until 2019, the Reserve documented a total of 427 identified species, four of which are listed in Schedule I of the Forest and Nature Conservation Act of Bhutan, (1995), including *Lloydia yunnanensis, Meconopsis superba, Panax-pseudoginseng* and *Taxus baccata*. Furthermore, other notable floral species of considerable significance include *Bhutantherra himalayana*, *Bryocarpum himalaicum* and *Viola bhutanica*, which are protected under the CITES Appendix II species group.

The selection of *M. superba* for this study is justified by its beauty (Grey-Wilson, 2017), historical significance (being first collected from Ho Ko Chu in 1884), its IUCN status as endangered (Bhutan Endemic Flowering Plants Workshop, 2017) and its endemism to Haa (Debnath and Nayar, 1986; Gyeltshen et al., 2020; NBC, 2014; Yoshida et al., 2016). The conservation of this crucial species falls under the responsibility of the country and can only be achieved through a thorough understanding of its status in its natural habitat. Thus, the objective of our study was to investigate the occurrence, abundance, health and seasonal patterns of the Superb Poppy (*M. superba*) and its associate shrubs and herbs in JKSNR, which are known to be its natural habitat. We posited that

this endangered species may face threats from activities such as grazing, forest fires, trampling and harvesting by local communities.

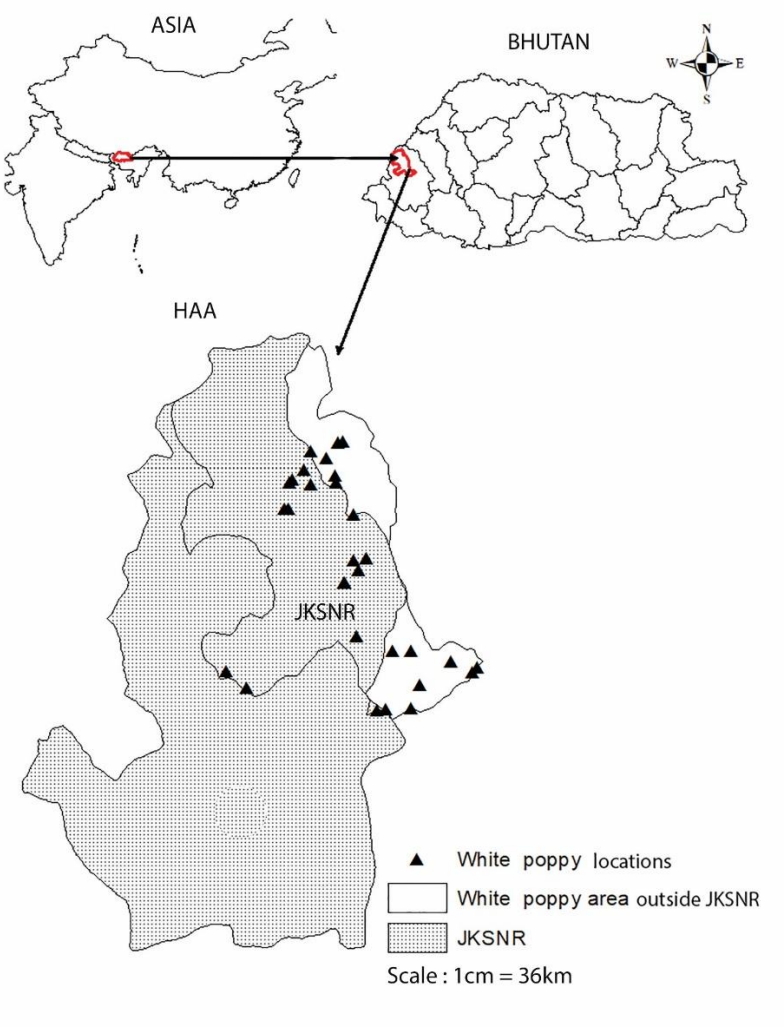

Figure 1 Location of Jigme Khesar Strict Nature Reserve (JKSNR), Haa, Bhutan

2. MATERIALS AND METHOD

We selected a total of 33 plots in 21 localities for our study based on initial data shared by local yak herders regarding the presence of the species in their grazing areas. To ensure consistent coverage of the *M. superba* habitat within the Reserve, we identified the lowest location where the species has been historically observed and divided the area into grids extending to the highest historically observed location. We allotted more plots to comparatively larger habitat like Lamsa (n=5), Lamtsatsho (n=4), Labana (n=3), two each for Laja, Kaptongtsho and Jara and one for rest of the localities. We predetermined the coordinates of our plots and used handheld Etrex Garmin GPS to locate them during the survey that occurred from June 17 to July 20, 2015, with funding support from Bhutan Trust Fund for Environmental Conservation (BTFEC). A total of ten foresters were involved in collecting the data.

Disturbance detection

After identifying the plot, we marked a circular area of 50 meters using a measuring tape. Within this circular plot, we recorded any threats that the *M. superba* species faced, such as *grazing*, *fire*, *trampling* and *harvesting* by locals. We assigned a score of '0' when these threats were absent and '1' when they were present. We defined *grazing* as the presence of yaks, sheep, horses and other wild herbivores on the plot. Evidence of grazing includes hoof tracks, dung, bite marks on grasses and other palatable plant species within the plot. *Fire* disturbance was considered to have occurred when visible carbonized ground surfaces, burnt stumps of woody shrubs and burnt marks on stems of species were observed within the plots. *Trampling* was noted when surveyors observed *M.*

superba being run over by ungulate hoofs or being grounded by animals or humans. *Harvesting* was documented when surveyors observed *M. superba* stumps with flowers that had clearly been plucked by human hands.

Matured *M. superba* count

Additionally, we placed a square plot of 10 meters by 10 meters within the circular area to record the coordinates, elevation (in meters above sea level), slope (expressed as a percentage) and aspect (ranging from 0 to 360 degrees). We also counted and recorded the heights of the fully grown *M. superba* and those in the process of regeneration, along with any associated shrubs. To document the herb associates of *M. superba*, we established another square plot measuring 1 meter by 1 meter within the 10-meter by 10-meter square plot.

***M. superba* regeneration assessment**

To assess the regeneration status of *M. superba*, we defined "regeneration" as "plants below 30cm in height without a stem but with decorative rosette of blue-grey leaves" (Figure 9) and tallied all of them in all plots. We made note of whether the regenerating saplings had been browsed or not; the microsite where they were found; their height and their overall vitality. To detect the correct habitat of regenerating *M. superba*, we categorized the microsites on which they were found growing as follows: '1' for moss, '2' for mineral soil, '3' for nurse log, '4' for litter, '5' for organic soil, '6' for boulder and '7' for woody debris. We used the term "vitality" to describe the condition of the *M. superba* individuals, including the new recruits (regeneration), as well as other herb and shrub associates in the respective plots. We scored individuals as '0' if they were dead, '1' if they were perishing, '2' if they were weak and fragile and '3' if they were healthy.

We used a similar scoring scheme as above to determine the percentage coverage of individual herb species in the 1m-by-1m plot. A score of '1' was given if the herb species covered less than 1% of the plot, '2' if the species was frequently observed but still totaled less than 1%, '3' if it covered 2 to 10%, '4' if it covered 10 to 20%, '5' if it covered 20 to 30%, '6' if it covered 30 to 50%, '7' if it covered 50 to 80% and '8' if it covered 80 to 100%. In addition, we recorded the phenology of *M. superba*, shrubs and herbs in all plots. We assigned a score of '1' to species in the flowering stage, '2' to those in the flower bud stage, '3' to those in the fruiting stage, '4' to those with leaves and '5' to those without leaves.

Data analysis

We used R (Version 4.2.2) and latest version of the data storage MS Excel to analyze data to produce appropriate graphs and other results. To make map, we used Arc GIS version (10.5).

3. RESULTS AND DISCUSSION

Distribution

We documented the presence of *M. superba* in 31 out of 33 total plots and 19 out of 21 localities. The species was not found in Zeprotogang and Woshenhadum (Figure 2). Based on our field data, it appears that *M. superba* has a restricted distribution in Haa district, with the majority of occurrences observed in JKSNR. Interestingly, there have been recent sightings of the species by fellow field foresters in Paro Forest Division, which is also part of Haa district and shares a boundary with JKSNR (Figure 1).

Disturbances

In 27 out of the 33 total plots, *grazing* was observed, whereas only 10 plots recorded instances of *fire*. As anticipated, *trampling* of *M. superba* caused by grazing yaks and others occurred in 15 of the 33 total plots while no evidence of *harvesting* activities was found (Figure 3).

Grazing

Grazing in the Reserve was obvious because of the tradition of maintaining yaks (Figure 4) by the people of Haa for their livelihood. Our research has identified grazing in 27 out of the 33 plots surveyed, indicating that all the areas where *M. superba* grows are subject to some form of grazing. Four plots out of five in Lamsa and the plot in Woshenhadum was not grazed at all. Generally, grazing is known to disturb habitats and degrade the quality of the area for both plants and animals. However, in recent years, we, as local foresters, have observed a reduction in the number of yaks and herders across the high mountains of JKSNR. This decline can be attributed to younger generations in Haa embracing urban lifestyles, which has resulted in families reducing their yak numbers due to challenges in keeping their children involved in yak husbandry, unlike a few decades ago.

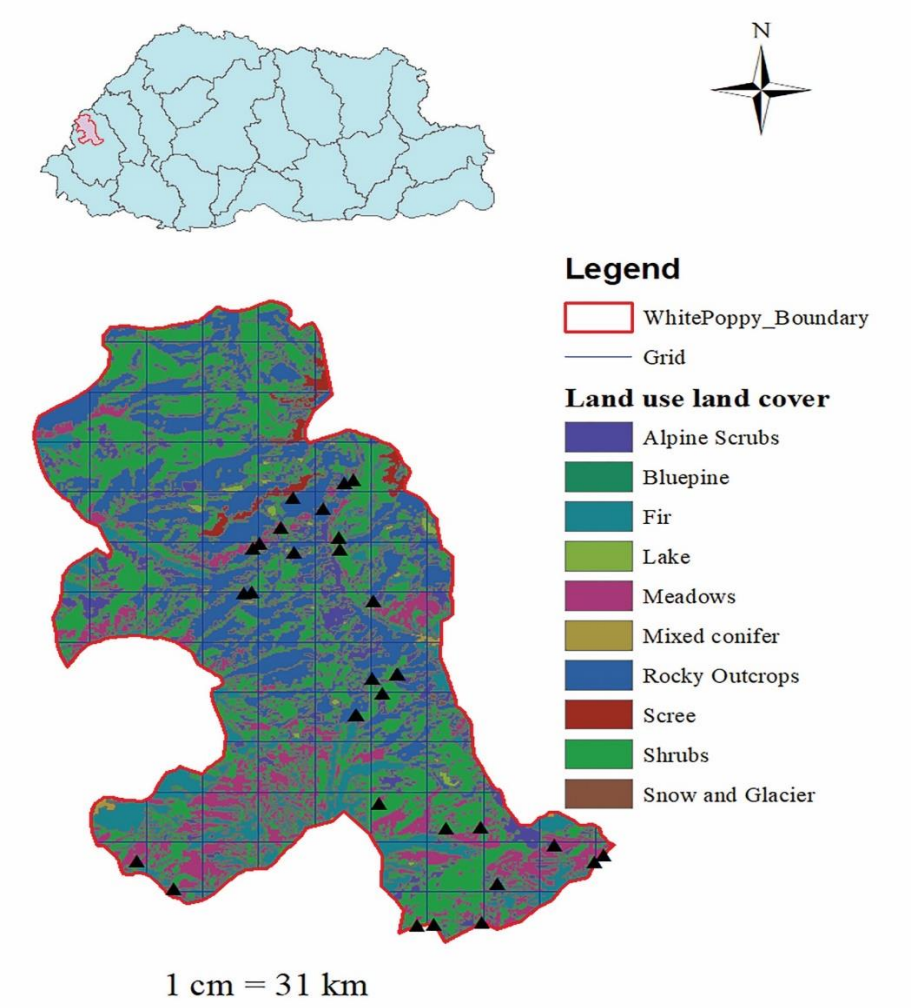

Figure 2 Land use map of JKSNR with location of White poppy *M. superba*

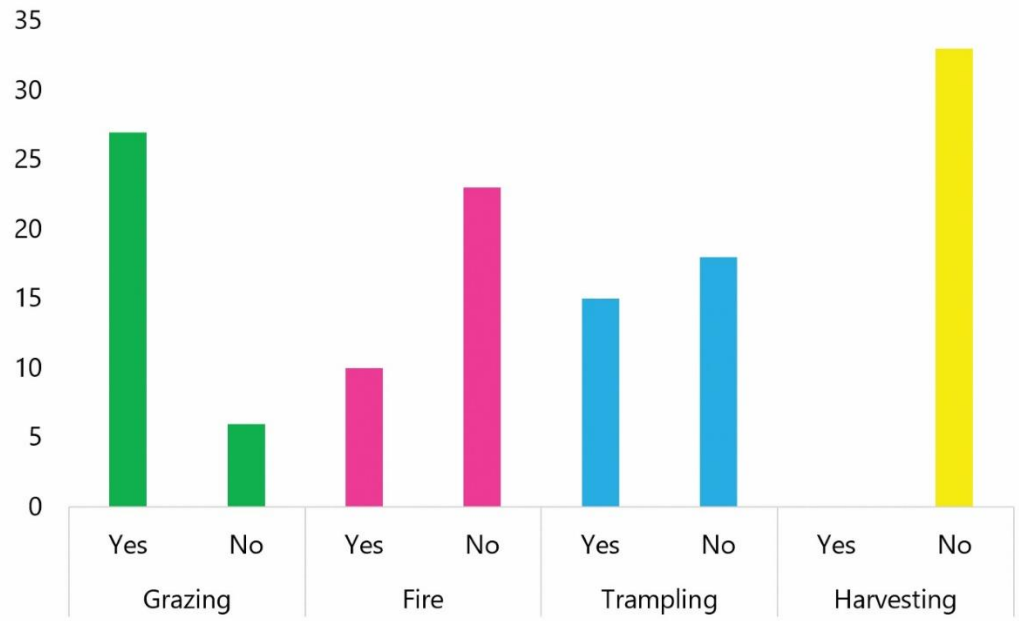

Figure 3 Disturbances by number of plots

Indeed, our research does not provide conclusive evidence on whether *M. superba* thrives better with high or low levels of grazing. However, the findings from this study can serve as a valuable baseline for future research to compare and measure the differences between periods of high and low yak populations. By collecting data on *M. superba* abundance and other ecological parameters during periods of varying grazing pressure, we can gain insights into the species' response to grazing and better understand its ecology and conservation needs in the context of changing yak populations. This can aid in developing effective management strategies for the conservation of *M. superba* and its habitat in Bhutan.

Figure 4 White yak at Yaktola, above Chaladophu on the way to Tshonathangkha inside the *M. superba* habitat. The domestic yaks are free range grazers in JKSNR (Photo: Sangay Gyaltshen)

Fire

We observed fire incidents in 10 out of 33 plots, including Tsang top, Jadoteng, Tshocheykha, Ngaeto Tshotop, Yakuna, Jula, Jichula, as well as one out of three plots in Labana and both plots in Jaara were affected by recent fires. However, our analysis did not reveal significant differences in the number of mature *M. superba* or regenerations, shrub and herb associates between the fire-affected plots and those that were not affected ($p>0.05$). Nevertheless, we noticed a higher count of regenerations (n=353) in plots that were not impacted by fire compared to those that were affected (n=242), which could be attributed to the smaller number of plots that were affected by fire (n=10) in contrast to those that were not affected (n=23). This suggests that fire may have had a detrimental effect on the regeneration of *M. superba*, as evidenced by the lower number of regenerations in the fire-affected plots. Further investigation and analysis are needed to better understand the specific impacts of fire on *M. superba* regeneration in the study area, as well as the underlying factors influencing these observed patterns.

Trampling

Trampling on *M. superba* occurred in 15 of the 33 plots that included Chaladophu, Pempo Jangsa, Zeprotogang, Jadoteng, Ngaeto Tshotop, Yakuna, Damthang (Tshona), one of the three plots in Labana, two plots each in Lajab and Kabtongtsho, three of the four plots in Lamtsatsho. We counted 373 regenerations in the plots that were not affected by trampling while there were 222 regenerations in the plots affected by trampling which indicated the negative impact of trampling on the species. Otherwise, there were no significant differences in shrubs and herb contents of the plots across all the locations.

Harvesting

M. superba is commonly cultivated in captivity for seed production, which is used for growing and marketing the magnificent flowers. However, during our survey in the study area, we did not observe any evidence of harvesting of *M. superba.* It is possible that Superb Poppy growers could *potentially* benefit from wild *M. superba* seeds if their cultivated seeds fail, but proper material transfer procedures should be followed to ensure responsible and sustainable collection of wild seeds, in accordance with conservation guidelines and regulations. Careful management and conservation of *M. superba* populations and their genetic resources are important to ensure the long-term sustainability of this species and its habitat. Since the locals are not harvesting the plant, it is safe to conclude that they are not under any cultivation threat.

Quantity, height, health and phenology of matured *M. superba*

The count of mature *M. superba* (Figure 5) individuals totaled 198, distributed across 21 different locations encompassing 32 plots. It's worth noting that only one site, Woshenhadum (Figure 6), did not have any matured *M. superba*. The highest number of *M. superba* was recorded in Lamsa (plot n=5) with 28 individuals, followed by Labana (n=23), Kabtongtsho and Lajab (n=18 each), Hala (n=15), Jara (n=13), Lamtsatsho (n=12), Tshocheykha (n=11), Jadoteng (n=9), Damthang (Tshona), Shokopadey and Yakuna (n=7 each), Chaladophu and Tsangtop (n=6 each), while Zeprotogang (n=5), Jula (n=4), Tamzhi (n=3) and Jichula (n=2) had the lowest numbers of *M. superba* individuals.

Figure 5 The pink and white flowers of matured *M. superba* from JKSNR (Photo: Gyeltshen Dorji)

Height

All 198 *M. superba* in the survey plots were measured for their height and the mean height was calculated for all 21 localities. The tallest group of *M. superba* was observed in Chaladophu (Figure 7), with a mean height of 168.33cm measured from a plot containing six individuals, followed by Lamtsatsho with a mean height of 164.83cm (Table 2). The population of *M. superba* in the southeast and south-facing area of Chaladophu (Table 2) was the healthiest. Conversely, the abandoned grazing site of Woshenhadum did not have any matured individuals and poorly grown individuals were found at Tsangtop (with a mean height of 50.33cm) and Tshocheykha, where the average height of 11 individuals from a single plot was only 85cm (Table 2).

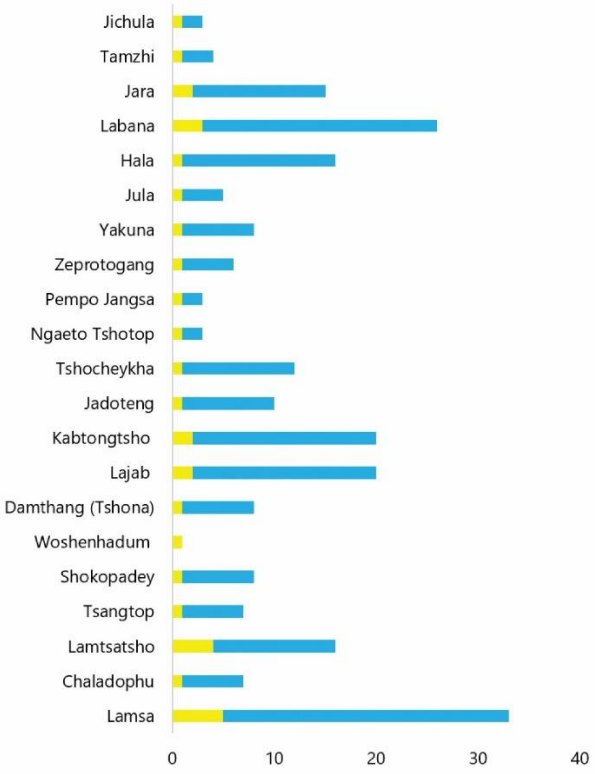

Figure 6 The number of plots in each locality (yellow bars) with number of *M. superba* (sky-blue bars)

Table 2 The average height of matured *M. superba* in different localities

Location	Number of Plots	Number_Ms	Mean_Height	Aspect (0°-360°)
Lamsa	5	28	146.57	140
Chaladophu	1	6	168.33	130
Lamtsatsho	4	12	164.83	130
Tsangtop	1	6	50.33	230
Shokopadey	1	7	128.57	185
Woshenhadum	1	0	0	175
Damthang (Tshona)	1	7	128.57	90
Lajab	2	18	109.44	220
Kabtongtsho	2	18	137.78	220
Jadoteng	1	9	102.22	120
Tshocheykha	1	11	85	225
Ngaeto Tshotop	1	2	100	270
Pempo Jangsa	1	2	105	235
Zeprotogang	1	5	127	180
Yakuna	1	7	89.85	225
Jula	1	4	117.5	160
Hala	1	15	124.33	135
Labana	3	23	130.04	150
Jara	2	13	98.84	180
Tamzhi	1	3	133.33	170
Jichula	1	2	100	215

Figure 7 Chaladophu valley, where tallest *M. superba* were recorded (Photo: Sangay Gyaltshen)

Out of the 198 individual *M. superba* evaluated for vitality in the survey areas, only two were scored with a value of '1,' indicating they were dying, and three were found to be weak with a score of '2'. As a result, a vast majority of the assessed individuals, accounting for 97.7%, were determined to be very healthy. The study's assessment of the phenology of *M. superba* indicated that the majority of plants were in the flowering stage (n=102), while some were in the flower bud stage (n=41) and fruit stage (n=45). At least ten plants were in the leaves stage (Figure 8). Based on the phenological observations, it was noted that *M. superba* predominantly flowers during the months of June and July in its native habitats.

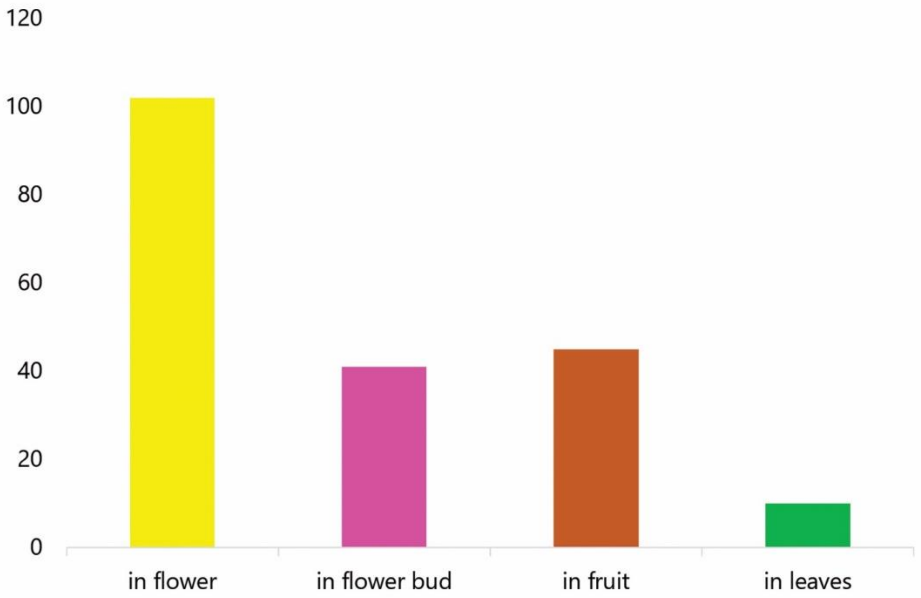

Figure 8 Phenology of 198 *M. superba* individuals

Quantity, health and phenology of *Meconopsis superba* regenerations

A total of 595 regenerations (Figure 9), were counted within all areas of the 10m- x -10m plot. Labana had the highest number of regenerations (n=96), followed by Kabtongtsho (n=73), Hala (n=55), Lamsa and Jara (n=50 each), Jadoteng (n=48), Tshocheykha (n=43), Lamtsatsho and Jula (n=35 each), Lajab and Tsangtop (n=25 each) and Shokopadey (n=14), respectively (Figure 10), while the remaining areas had ten or fewer regenerations.

Figure 9 *M. superba* regeneration at Chaladophu plot (Photo: Sangay Gyaltshen)

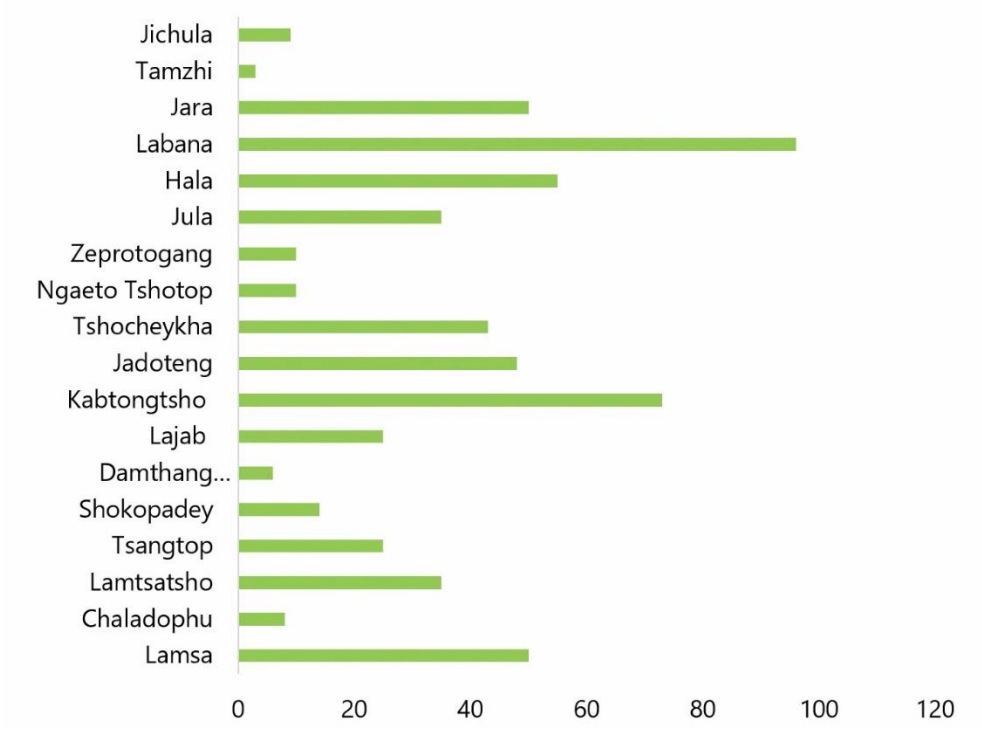

Figure 10 Number of *M. superba* regeneration in each locality

We assessed the health of all 595 individual *M. superba* regenerations using vitality score as the scale of measurement and found that 592 out of 595 saplings were healthy. Only three saplings, which were found in the Tamzhi area, were considered weak, with rosette leaf height of 10cm. We also observed evidence of browsing on the *M. superba* by animals in at least 10 out of the 31 plots that had regeneration. We found most of the regenerations grown on the soils attached to boulders (67%) followed by ground surfaces with woody debris (18%), mosses (9%) and mineral soil surface and organic soil surface sharing 3% each (Figure 11)

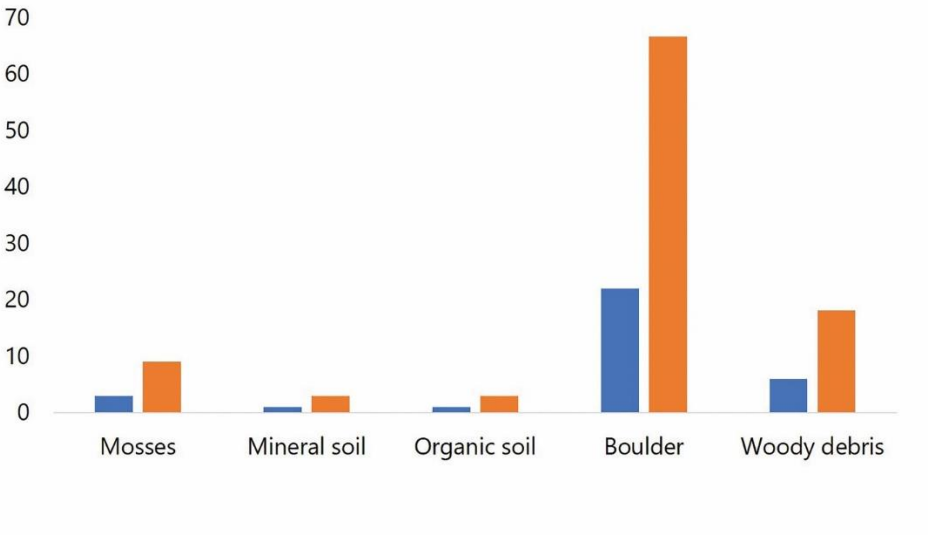

Figure 11 Number (blue bar) and percent (red bar) of regenerations found in different microsites

Shrub associates of *M. superba*

We counted 1489 individual shrub species across 33 plots in 21 localities and identified 77 different species. The dwarf *Rhododendron* species (n=327) like *R. aeruginosum* (35), *R. anthopogon* (39), *R. lepidotum* (174), *R. lowndesii* (3), *R. setosum* (57) and an unidentified *Rhododendron* sp. (19) dominated the survey sites as *M. superba*'s topmost associate in the entire habitat. Fern species (n=310), *Berberis* sp. (n=296), *Rosa* sp. (n=125), *Juniperus* sp. (n=80), *Potentila* sp. (n=50), *Rhodiola* sp. (n=37), *Clematis* sp. (n=32), *Cotoneaster* sp. (n=30) and other high-altitude shrubs occupied the plots. At least 168 species of shrubs found in the plots could not be identified (Figure 12).

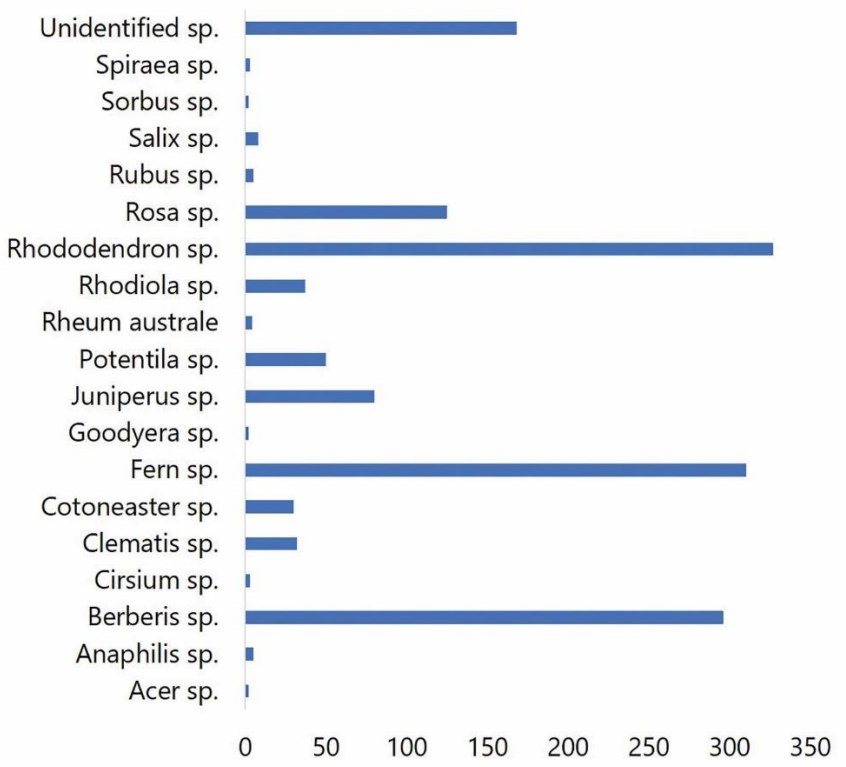

Figure 12 Number of different types of shrubs found in the *M. superba* growing areas

Areas wise shrub diversity and density

The diversity and density of shrub species varied across different plots and localities. Lamsa had the highest density of shrubs (n=256) and was also the most diverse with eight different species, followed by Labana with seven species (n=255), Jadoteng with

seven species (n=157) and Lamtsatsho with seven species (n=109). Kabtongtsho had high shrub density (n=173) but low diversity with only three species, while Jichula had high density (n=173) but only two species. Pempo Jangsa had 80 individual plants of three species. Damthang (Tshona) was diverse with seven species, but thinly distributed (n=20) compared to Woshenhadum, which had only four species and 11 individual shrubs (Figure 13).

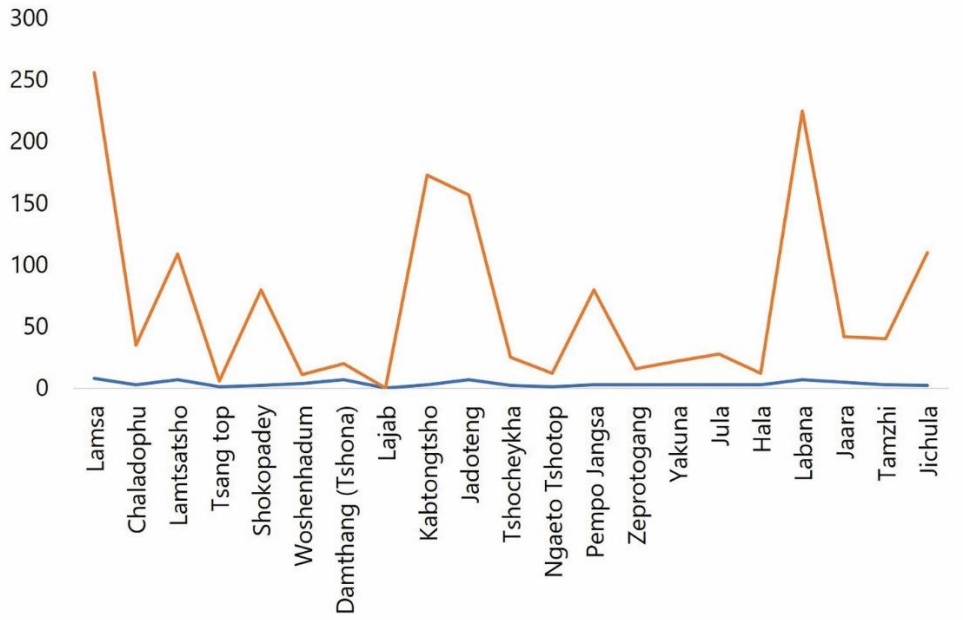

Figure 13 Density (orange line) and diversity (blue line) of shrub species in the survey areas

Shrub phenology

Most of the shrubs were "in leaves" (n=44) during the survey followed by those "in flower" (n=34). A very small number were in other stages such "in flower bud" (n=7), "in fruit" (n=3), "dying" (n=3) and "leafless" (n=2) (Figure 14).

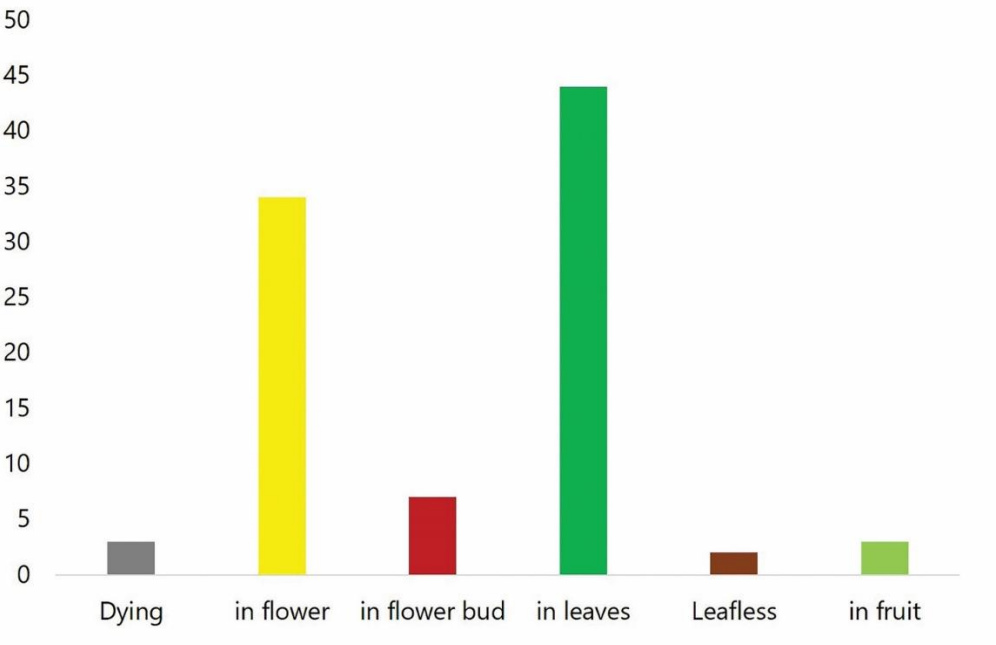

Figure 14 Phenology of shrubs across the survey area

Herb associates of *M. superba*

We counted 178 individual herbs in the 1m-by-1m plot in 33 plots across 21 sites and identified 55 species (including unidentified mosses as single species) many of which were up to the genus level. The most commonly found herb was *Potentila* sp. (n=20) followed by *Bistorta* sp. (n=12), ferns (n=11), *Pedicularis* sp. (n=10), *Rheum australe* (n=10), eight different species of grass, seven each of different species of genus *Aconitum*, *Aconogonum*, *Anaphalis* and *Senecio*; six each of different species of genus *Carex*, *Nardostachys* and *Rhodiola*. Other species of prominence found in the Reserve's survey area are *Cersium* (n=4), *Primula sikkimensis* (n=4), three each of *Anemone biflora*, *Meconopsis simplicfolia*, other *Primula* sp. and *Rheum nobile.* We counted two each of *Arisaema* sp. *Megacodon* sp., *Morina* sp., *Polygonum* sp., *Rheum acuminatum* and mosses. We also counted single records of many other species as listed (Figure 15).

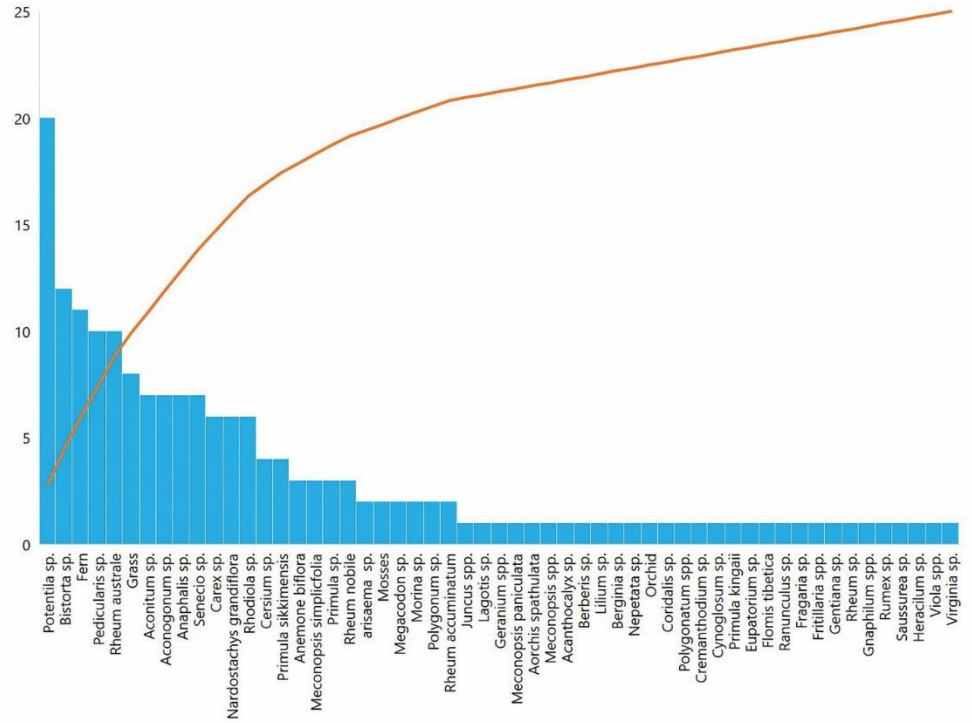

Figure 15 Herb species by number of species encountered in the entire survey plots

We looked at the diversity and density of herb species inside different localities. Lamsa followed by Lamtsatsho and Labana respectively happened to have dense herb population as well as diversity, although Lajab and Kabtongtsho are not behind Lanana in terms of both diversity and density (Figure 16). Otherwise, all the areas surveyed have some species of herbs in their vicinity.

Herb phenology

We evaluated the phenological state (Figure 17) of the herbs in the plots and found most of them, "in leaves" stage (n=102), followed by "in flower" (n=55), "in flower bud" (n=8), "dying" (n=8) and "in fruit" (n=5).

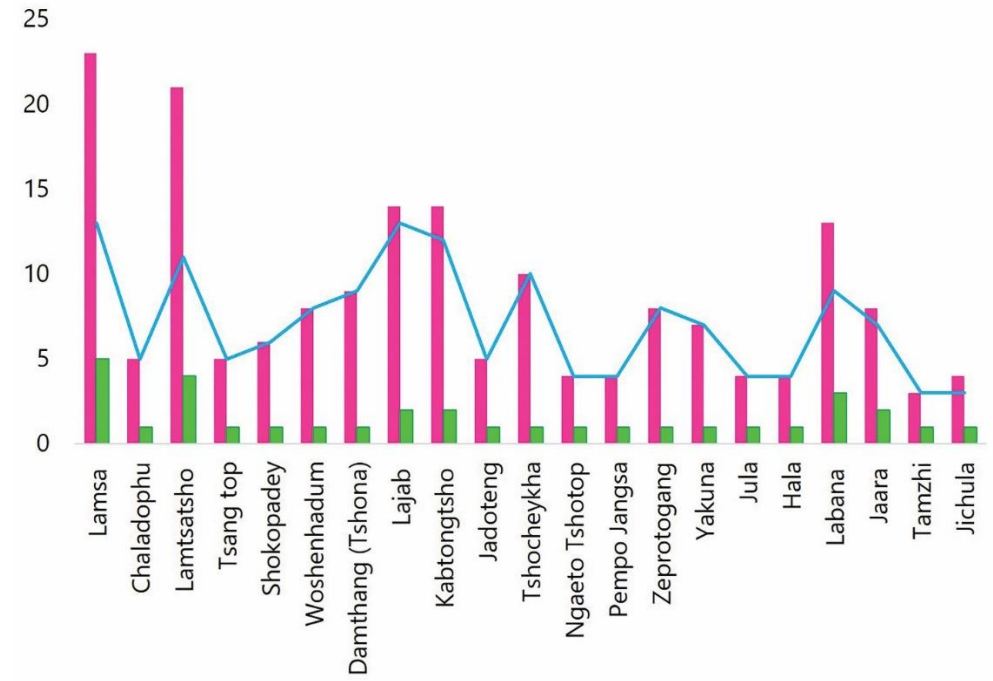

Figure 16 Herb diversity (sky-blue line), density (pink bar) and number of plots (green bar) in each locality

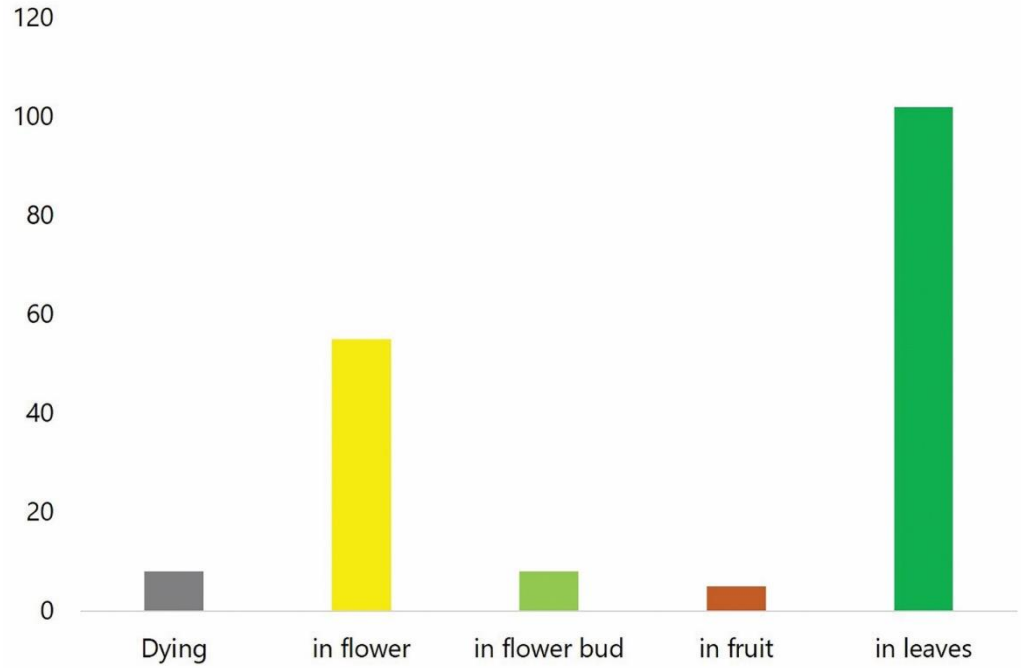

Figure 17 Phenology of herb species of the entire survey plots

4. CONCLUSION

Based on our research findings, we can conclude that *M. superba*, an endangered and endemic species, has a sparse distribution within JKSNR and nearby areas within Haa district. Despite some anthropocentric disturbances, like grazing, fire and trampling in most of the areas, *M. superba* is still thriving in its natural habitat. This suggests that efforts to conserve and protect *M. superba* within JKSNR and nearby areas have been effective in maintaining its population despite human activities, a positive impact of the protective area. However, further studies and conservation efforts may be needed to address any ongoing threats to the species and ensure its long-term survival.

Based on our assessment of 198 matured *M. superba* plants in 32 plots, we observed variations in population quantity among different areas. Some areas, such as Lamsa and Labana, had a good population of *M. superba* plants, while other areas like Tamzhi

and Jichula had fewer plants. Among the areas surveyed, Chaladophu and Lamtsatsho were identified as two of the best areas for *M. superba* growth, located in the southeast and south-facing landscapes.

Further vitality tests conducted on the assessed individuals revealed that 97.7% of the plants were healthy, indicating that the species is thriving in the assessed areas. Additionally, most of the plants were in the flowering stage of their phenology, suggesting that *M. superba* tends to flower in June and July. This information provides important insights into the species' reproductive behavior and phenological patterns, which can be crucial for its conservation and management.

Overall, our assessment indicates that while some areas within the study area have a good population of healthy *M. superba* plants, other areas may require further attention in terms of conservation efforts. The identification of favorable growing areas and the understanding of the species' phenology can contribute to the development of effective conservation strategies to ensure the continued survival of *M. superba* in its natural habitat.

By way of regeneration, our research revealed that *M. superba* showed good regeneration in several plots, particularly in Labana, Kabtongsho, Hala, Lamsa, Jara, Jadoteng, Tshocheykha, Lamtsatsho and Jula, compared to other plots, including Chaladophu which had the tallest plants during the survey. Out of the total 595 *M. superba* plants assessed, 592 were healthy, with only three weak plants identified from the Tamzhi plots. We also observed browsing on the regenerations in 10 out of 31 plots where regenerations were found, indicating potential herbivory impacts on the species' regeneration success. Furthermore, we found that regenerations were predominantly located on soils attached to boulders and on woody debris, suggesting that these microhabitats may play a role in supporting the regeneration of *M. superba*.

These findings provide valuable insights into the regeneration dynamics of *M. superba* in different plots, highlighting areas where regeneration appears to be more successful and identifying potential threats such as browsing by herbivores. Understanding the factors influencing regeneration success is crucial for the conservation and management of *M. superba* and further research and conservation efforts may be needed to ensure its sustained regeneration and population growth in the study area.

With regard to shrub associates of *M. superba*, our research identified a total of 77 different species of shrubs, with a combined total of 1489 individuals in the study plots. These included species such as *Rhododendrons*, Ferns, *Berberis*, *Rosa*, *Juniperus*, *Potentila*, *Rhodiola*, *Clematis*, *Cotoneaster*, as well as other unidentified species that were observed in the survey plots and the surrounding landscape. Among the plots, Lamsa had the highest shrub diversity, followed by Labana, Jadoteng and Lamtsatsho, respectively. This indicates that these areas have a higher abundance and variety of shrub species associated with *M. superba*, which may contribute to the overall ecological dynamics of the study area. In terms of phenology, most of the observed shrubs were in the "in leaves" stage during the survey, indicating that they were actively growing and leafed out at the time of assessment. This information provides insights into the phenological patterns of the shrub associates of *M. superba*, which can have important implications for understanding their ecological interactions and the overall functioning of the ecosystem.

By way of herb associates of *M. superba*, our research recorded a total of 178 individual herbs belonging to 55 species (including unidentified mosses as a single species) in 33 plots. The most abundant herb species, in terms of maximum count, were different species of *Potentila*, *Bistorta*, ferns, *Pedicularis*, *Rheum*, grasses, *Aconitum, Aconogonum, Anaphalis, Senecio, Carex, Nardostachys, Rhodiola, Cersium, Primula, Anemone, Meconopsis simplicifolia, Arisaema, Megacodon, Morina, Polygonum* and some mosses. Among the plots, herbs were found to be dense in Lamsa, followed by Lamtsatsho and Labana, respectively. This indicates that these areas have higher herb abundance and diversity compared to other plots, which may be influenced by various ecological factors such as microclimate, soil conditions and habitat characteristics. Phenology wise, the majority of the observed herbs were in the "in leaves" stage during the survey, indicating active growth and leafing out.

The diverse shrub and herb communities associated with *M. superba*, along with the variation in phenology among different species, highlights the complex ecological relationships in the study area. Further research on the dynamics of shrub and herb associates and their interactions with *M. superba* can contribute to a more comprehensive understanding of the species' habitat requirements and inform conservation strategies aimed at preserving the entire ecosystem.

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Author contribution

Jigme Tshelthrim Wangyal planned and designed research, analysed data, wrote the manuscript and prepared the figures. Gyeltshe Dorji prepared maps with edits from Jigme Tshelthrim Wangyal while Gyeltshen Dorji, Bhakta Bdr Ghalley and Ugyen Tshering helped in planning and designing research. Gyeltshen Dorji, Bhakta Bdr Ghalley, Ugyen Tshering, Dechen Wangda, Sangay Gyaltshen, Kinley Tenzin and Wangchuk conducted fieldwork.

Informed consent

Not applicable.

Ethical approval

The ethical guidelines are followed in the study for species observation & identification.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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