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Vegetational parameters and timing of capsule ripening of a high altitude Rhododendron (*R. campanulatum*) in Central Himalaya

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ABSTRACT

R.campanulatum forms krummholz close to the tree line in alpine areas. In the present study it is considered as a tree species and we concentrated our attention on capsule maturation timing and documentation of phenological events of this species which is expanding and regenerating at high altitudes. This species is relatively less studied in relation to its phenology, seed physical parameters and germination. The study was concentrated in a 5 ha area located between 3200 and 3450 m elevation in two consecutive years (2016-2017). The overhead forest canopy was dominated by *Quercus semecarpifolia* towards the upper side and by *Aesculus indica* on the lower side. In *R. campanulatum* flowering started from 2^{nd} and 3^{rd} week of April and maximum flowering was observed between 1^{st} and 4^{th} week of June. Capsule formation started from 3^{rd} and 4^{th} week of June to 4^{th} week of July and maturity of capsule was in the month of December to January. The colour of capsules was initially green and finally turned brown. The capsules of *R. campanulatum* were collected at ten days interval from the month of December up to the availability of capsules from selected sites. The number of seeds per capsule varied between 1012 and 1136. Germination was counted when visible radical had developed. The germination was maximum 42.5 ± 4.2 % at 26.3 ± 2 % moisture content. Fresh weight of capsule correlated positively with date of collection (r = 0.827; P<0.01) and germination (r = 0.627; P<0.01).

Key words : *Phenology, Moisture content, Germination, Capsule, Altitudes*

Introduction

The genus *Rhododendron* L belongs to family Ericaceae with over 900 species and numerous cultivars worldwide Davidian (1992). It is indigenous mainly to the Northern Hemisphere, with large concentrations in the mountain ranges of India, China, Tibet and upper Burma as well as to Japan and the Eastern United States. Plants commonly occur in regions with highly organic soils, high rainfall, high humidity and a temperate climate (Cox, 1990). *Rhododendron* species range from tiny, prostrate, alpine shrubs only 5 cm tall to trees with enormous leaves that reach heights of 24 m (Leach, 1961). Altitudinally, it occurs in vegetation zones that range from a few hundred meters to about 5500 m above sea level. In India, the species are mostly confined to the Himalayan region, particularly in Eastern Himalaya. A total of 87 species, 12 subspecies and 8 varieties of *Rhododendrons* have been recorded in IHR, among these 6 species and one subspecies is reported from Western Himalaya (Sekar *et al.,* 2010). *Rhododendrons* are one of the most exploited species due to its multifarious nature. (Chauhan *et al*., 2017);

Sekar *et al*. (2010). The regeneration of various such multifarious species is very poor in nature due to their over exploitation Tewari (2005) and other climatic causes. *Rhododendron campanulatum* D. Donforms under canopy forest between 2800 to 4000 m altitudes in oak dominated forest. It forms krummholz near the treeline and considered as a tree species in the present study Singh *et al*. (2018). It is found in the outer and inner ranges of the Alpine Himalayas in association with *Quercus semecarpifolia, Betula* species, *Aesculus indica*, and *Abies spectabilis*. Wood is moderately hard and used as an excellent fuel Joshi *et al*. (2012). While *R. arboretum* is well studied *R. campanulatum* is relatively less studied particularly in reference to climate change on phenology and capsule maturation timing. The phenological studies are important from the point of view of the conservation of the tree genetic resources and forestry management as well as for a better understanding of the ecological adaptations of the plant species and the community level interactions. Plant phenology, the series of phases in plant development throughout the year Lieth (1970), is an important morphological key to nutrient content (Robertson and Torrel, 1958), carbohydrate reserves (Coyne and Cook, 1970) and the growth of range species (Blaisdell, 1958). The phenological activities and several factors such as resource availability, pollination success, and predation of flowers, fruits and leaves, genetic makeup and age and size of plant affect seed production (Mishra *et al*., 2003). Seeds also play a vital role in natural and artificial regeneration. The collection of forest fruits and seeds are generally helped by reliable guidelines of maturity to allow the earliest possible collection. It is recognized by change in colour, size, odors and texture of fruit and seed Tewari (2005). The regeneration potential of vegetation largely depends upon the size of seed crop and seedling establishment (Zasda *et al.,* 1978). In the higher Himalayas due to harsh climatic condition and chilling weather in winter, the inhabitants mostly depend on fuel wood collected from *R. campanulatum*. Continued biotic stress of grazing and tree lopping, *R. campanulatum*, a nonpalatable species of wider niche width might dominate at the expense of herbs and other treeline species in future and may bring out compositional changes in treeline vegetation and carbon storage (Singh *et al*., 2018). The focus of the present study (2016 - 2017) was to gather baseline data on timing of phenological events and capsule maturation timing of this treeline species in a changing climatic scenario for future studies. Study on germination of seed of *R. campanulatum* need priority attention to include them in conservation and propagation programmes. The exact knowledge of the time of phenophases and capsule maturationis essential for the collection of mature and viable seeds during before the opening of capsules to avoid seed dispersal (very minute seeds). The data generated by the present study would be useful for baseline information, conservation and propagation for upcoming studies of this species which occurs near the treeline and is reported to be expanding into the adjoining alpine meadows due to global warming.

Study site

For the study 5 ha area between 30º, 03´ N latitude - 80º, 13´ E longitudes at North eastern aspect varying in elevation between 3200 and 3450 m was selected. Mean monthly maximum and minimum temperature ranged from 12 °C (January) to 26 °C (June) and -5.0 °C (Dec.-Jan.) to 12 °C (June) respectively. The mean annual total rainfall is 1959mm (more than half occurring during the rainy season about 400 mm in the month of August itself) Rawal *et al*. (1994).

Materials and Methods

Phytosociological analysis

The number and size of the quadrates were determined by the running mean method Bargali (2013). Thereby plots of 10 x 10 m along the altitudinal transact were randomly established. Trees were analysed in 10 x 10 m quadrates within each plot (Curtis and McIntosh, 1950). Circumference at breast height (cbh at 1.37 cm from the ground) of all trees was measured in each plot. Tree mean basal area of a species ($C^2/4ð$ where, C= sum of cbh value of all individuals of a tree species within each plot and ð = 3.14) was multiplied by its density value to calculate total basal area of a tree species. Importance Value Index (IVI) was calculated following Phillips (1959) as:

IVI = Relative frequency + relative density + relative dominance

Phenological analysis

For the phenological study, twenty trees of *R. campanulatum* of average size and diameter were marked at each selected site. The observations were made at weekly interval in two consecutive years (2016-2017). Every phenophase was considered to be at its peak when more than 70% of the marked trees showed the phenological event. The phenological observations were made for six major phenophases viz. leaf initiation, leaf drop, flowering initiation, complete flowering, capsule initiation and capsule ripening Ralhan (1985); Negi (1989).

Maturity Indices

Collection of capsules of *R. campanulatum* was started in the month of December at 10 days interval up to the availability of fruits, from all the sites, in two consecutive years (2016-2017). For maturity indices of both the species; capsule collection was made at weekly interval till the capsules were available. The capsules were directly collected from the selected trees at each collection date and seeds were extracted from them (Tewari *et al*., 2019).

The parameters for capsule were taken as weight of capsule and moisture content. All these parameters were taken for seeds also. Weight parameters were recorded with the digital electronic balance.

Initial moisture content of capsule was expressed on a fresh weight basis, at each collection date using three replicates of 25 capsules were oven dried at 103 ± 2° C for 16 ± 1 hr ISTA (1981) and then reweighed. Moisture content was calculated as-

$$\text{Moisture Content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

The petridishes and germination paper were sterilized at high temperature (130 °C) for 4 hr to make it free from fungal infection. For germination 3 replicates of 100g (because of minute seeds) seeds were carried out at 25 ± 1 °C on top of the paper in seed germinator for each collection date. Germination was counted when visible radical developed. Water was added as required during the experiment. After the completion of experiment germination percent was calculated following Paul (1972).

$$\text{Germination percent} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Results and Discussion

The mean tree height of selected *R.campanulatum* trees varied between 1.45 ± 0.3 m and 2.4 ± 0.05 m. and mean tree diameter at collar varied between 35.2 ± 0.7 cm and 35.6 ± 0.4 cm. (Table 1).

Phytosociological study

The density of *R. campanulatum* ranged between 150 – 190 trees/ha. The total basal area ranged between 2.21 - 2.43 m^2/ha. It is dominated as under canopy species (IVI ranged between 35.1- 42.4) whereas the density of *Aesculus indica* was 20 trees/ha. The total basal area observed 20.44 m^2 /ha. It is the canopy species of the study area with maximum IVI (48.8).

Phenological study

In *R. campanulatum* at selected site across both the years; leaf initiation started from 1st and 2nd week of May and continued upto 2nd week of June. Minimum leaf drop was observed during 2nd and 3rd week of December and maximum leaf drop was observed in 2nd and 3rd week of June. Flowering started from 3rd week of April and was complete in 1st week of June. Capsule formation started from 2nd and 3rd week of June and maturity of capsules/ seeds observed in the month of December to January (Table 2).

Maturity Indices

In *R. campanulatum*; the capsules were collected from all the selected trees, the capsule colour as initially green and finally turned brown over a 28 days period. The mean fresh weight of capsule attained a maximum range between (22.9 ± 2.5 g - 24.4 ± 2.6 g) at 3rd collection date. The number of seed per capsule varied between 1012 ± 0.2 and 1136 ± 0.12. (Table 3).

At 3rd collection mean germination of seeds

Table 1. Tree characteristics of *Rhododendron campanulatum* at selected sites at elevational range varied between 3200 – 3400m.

Species	Range of mean tree height (m)	Range of collar diameter (cm)	Associate species
Rhododendron campanulatum	1.45 ± 0.3 - 2.4 ± 0.05	35.2 ± 0.7 - 35.6 ± 0.4	*Aesculus indica, Quercus semecarpifolia, Betula alnoidis, R.arboreum Abies spectabilis*

reached maximum (31.5 ± 2.2 % -42.5 ± 4.2%) at (26.3 ± 2 % - 37.2 ± 1.4 %) moisture content of capsule (Table 3).

The analysis of variance (ANOVA) showed that the fresh weight of capsule varied significantly (P<0.01) across dates of collection and the moisture content of seed was significantly different across dates of collection (P<0.01). Fresh weight of capsule was correlated positively with date of collection (r = 0.827; P<0.01) and germination (r = 0.627; P<0.01). Similarly negative correlation was found between fresh weight and moisture content (r = 0.706; P<0.01).

There is relatively very little literature on the phenology and capsule/ seed maturation of *R. campanulatum*. It is an under canopy tree species under dominance of *Aesculus indica*, *Quercus semecarpifolia*, *Betula alnoidis*, *Rhododendron arboreum, Abies spectabilis* etc. Some of the species of family Ericaceae are a potential source of toxic diterpenes, named grayanotoxins. It causes livestock poisoning and food intoxication. The density of *R. campanulatum* was 190 trees/ha. Due to toxic nature of some *Rhododendron* species, animal avoid grazing them as it is non-palatable. This can be a major cause of dominance of this species in higher altitudes Singh (2018). A clear understanding of the structure and functioning of the ecosystem is affected when phenological information is combined with other biotic and abiotic data. Phenology is a dominant and often overlooked aspect of plant ecology, from the scale of individuals to whole ecosystems Bernier (1988). In the present study *R. Campanulatum* showed one month early flowering (April – June) in comparison to flowering time reported by Brandis (1990) which was May – June. Most of the researchers have reported that change in fruiting and flowering time is taking place due to climate change in *R. arboretum* Negi (1989); Verma (2012). The phenophases of plants such as vegetative bud break, flowering, fruiting and leaf drop are influenced by variations in temperature and rainfall changes over the years. The changes in phenological behaviour of species may be due to climate change since many species are highly sensitive even to smallest change in the long prevailing climate of any ecosystem. The knowledge of exact stage and time of maturity of capsules/seeds is essential for collection of abun-

Table 2. Periodicities of six phenophases in *R. campanulatum*at selected site at elevational ranging between 3200 – 3400m. in Year1 and Year2

Years	Leaf initiation	Leaf drop	Flowering initiation	Complete flowering	Capsule formation	Observation of Capsule maturation
Year 1	1st and 2nd week of May to 1st week of June	Minimum in 2nd and 3rd week of December and maximum in 1st and 3rd week of June.	3rd week of April	4thweek of May.	Started from 2nd week of June	December – January
Year 2	1st and 2nd week of May to 2nd week of June	Minimum in 2nd week of December and maximum in 2nd and 3rd week of June.	2nd and week of April	1stweek of June.	Started from 3rd week of June	December – January

Table 3. Variation in physical parameters of capsule and seeds of *R. campanulatum* over the collection period at 10 days interval in selected site at elevational range varied between 3200 – 3400m. The values are the mean of two years (±SE)

Collections of seed	Range of fresh weight of capsule (g)	Range of moisture content of capsule (%)	Range of mean Germination (%)
2/01/	10.1 ± 0.38- 10.75 ± 0.85	43.8 ± 2.1 - 49.5 ± 6.7	9.75 ± 0.38 – 10.11 ± 0.5
12/01	12.8 ± 1.45 – 18.5 ± 1.05	32.5 ± 2.7 - 42.8 ± 2.8	27.5 ± 1.5 – 33.4 ± 3.1
22/01	22.9 ± 2.5 – 24.4 ± 2.6	26.3 ± 2 - 37.2 ± 1.4	31.5 ± 2.2 – 42.5 ± 4.2
01/02	22.05 ± 1.6 - 24 ± 1.6	22.6 ± 2.4 - 28.3 ± 5.2	22.1 ± 2.7 – 32.85 ± 4.1
09/02	19.5 ± 1 - 21 ± 0.6	21.1 ± 1.5 - 22 ± 0	21 ± 0.6 - 22.5 ± 0.2

dant quantity of healthy and vigorous capsules/ seeds (Ahmad *et al*., 2014). The physical characters of fruits/seeds play a significant role in determining maturity of fruit/seeds.The physical parameters as moisture content and specific gravity are interrelated and more objective. Both have been reported as reliable maturity indices by numerous researchers (Tewari *et al*., 2011). Maturity of capsules/seeds was observed in the month of December/January in *R. campanulatum.* In July - August majority of trees or woody species were observed in the fruiting stage and seed dehiscence was common during October - November in various subalpine forests of western Himalaya (Bisht *et al*., 2014). Verma (2012) reported capsule ripening during December/January in Kumaun Himalaya in *R. arboreum*. The colour of capsules was initially green and finally turned brownin *R. campanulatum.* Upadhyay *et al*. (2006); Tewari *et al*. (2019) also found colour change to be one of the best criteria for determining maturity in *Bauhinia retusa* and *R. arboreum*. Decline in moisture content appears to be a good indicator of seed maturity in *R. campanulatum.* The moisture content of seeds declined as the fruits/capsules matured (Shah *et al*., 2010). Decline in moisture content indicates seed maturity in *R. arboretum* (Tewari *et al*., 2019). In *R. campanulatum* maximum germination occurred at 26 -37 % moisture content. Tewari *et al*. (2016) have reported maximum germination was observed at 30.11 ±0.57% moisture content in *P. cerasoides*. Shah *et al*. (2005) have been reported maximum germination at 23.4 – 36.1 % moisture content in *Pyracantha crenulata*. Mazeed *et al*. (2010) reported maximum germination at 58.37 % moisture content and 0.82 specific gravity found in *Aesculus indica*. Fresh weight of capsule was correlated positively with date of collection ($r = 0.827$; $P<0.01$) and germination ($r = 0.627$; $P<0.01$). Various researchers observed significant correlation between physical parameters and germination. Tewari (2011) found positive correlation between Seed size and germination in *P. cerasoides*. Shah (2005) also reported significant correlation between seed size and germination in *Myrica esculenta*. Negative correlation was found between fresh weight and moisture content ($r = 0.706$; $P<0.01$). Edward (1969) could not found any relationship between physical parameters and maturity.

It was concluded from the study that *R. campanulatum* dominates the undercanopy in the selected forest. It forms krummholz close to the upper limit of the treeline. There has been an advance in flowering time of the species by approximately one month which can seriously impact its seed ecology and germination. Already there are reports by several researchers that *R. campanulatum* is advancing into the alpine meadows. The warming appears to be favoring this high altitude species.

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Conflict of Interest

No conflict of interest

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