Short Communication ________________________________ *Chapter – 2*

CAPSULE MATURATION TIMING AND SEED GERMINATION IN *RHODODENDRON ARBOREUM* SMITH AT SUB-ALPINE REGION OF WESTERN HIMALAYA, UTTARAKHAND

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Abstract

The genera of Rhododendron belong to the Ericaceae family and are found at an altitudinal range of 1500–3500 m in the Himalayan region.*Rhododendrons* are the ecologically and economically important group of plants of Himalayan ecosystems. Due to human interference and natural calamities, the natural populations of *Rhododendrons* are gradually diminishing. *Rhododendrons* are one of the most exploited species due to its multifarious nature. The regeneration of various such multifarious species is very poor in nature due to their over exploitation and other climatic causes. The present study reports the capsule maturation time and seed germination status of *R. arboreum* in the high altitudes areas. The study site was located at 30°11´N and 79°39´E between 3233 and 3446m elevation in the western Himalaya. Physical parameters, capsule size, number of capsules, weight and mass of capsule were taken and germination was carried out in a dual chamber seed germinator for each collection date in laboratory. The tree density of *R. arboreum* was 80 ha^{-1}. The mean capsule size during collection ranged from 76.81 to 236.82 mm^{2},the mass per 100 capsules during study varied between 21.23and 33.43g. The weight, number and mass of 100 capsules was 36.33 g, 288.33 and 33.43 g at the time of maximum germination. Maximum germination 40.00% occurred at 25.90% moisture content. Capsule colour change, decline moisture content and change physical parameter is a reliable indicator of maturity.The capsule maturation timing of treeline *R. arboreum* was two or three weeks delayed as compare to those grow at sub-tropical and temperate region.

Keywords: *R. arboreum,* Maturity, Moisture Content, Germination, Treeline

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Introduction

The genus *Rhododendron* belong to the heater familyEricaceae, is an ecologically and economically important group of plants that occur from sub-tropical to alpine forests. It distributed throughout the world stretching from the highlands of Nepal, India and China and Malaysia (Chamberlain et al.1996) and are best suited to elevation between 2000-4000m that extends from eastern Nepal to Yunnan (de Milleville, 2002). *Rhododendron*s are mainly found at higher altitude, from dominating species all along the cool regions and generally, prefer to grow in regions of high rainfall, high humidity, and a temperate climate (Iqbal and Negi, 2017). The genus represented by 87 species, 12 subspecies and 8 varieties in Indian Himalayan Region (IHR) (Chandra and Srivastava, 2010). *R. arboreum* Smith is a common tree in western Himalaya, occurring chiefly at 2500-2800m in association with *Quercus leucotricophora*and *Lyonia ovalifolia*, and at the lower elevations with *Pinus roxburghii*, but ascending to 3400m or even higher (Iqbal and Negi, 2017). *R. arboreum* holds the Guinness Record for World Largest *Rhododendron* and is widely popular for its medicinal benefits and economic value (Srivastava, 2012). In Uttarakhand, it is widely popular for processed juice of its flowers which have gained market popularity as Rhodojuice/Sharbat. Due to human interference natural calamities, the natural populations of *Rhododendrons* in Uttarakhand and in the entire Himalaya are gradually diminishing. The major threats to *Rhododendrons* are deforestation, heavy grazing, unsustainable extraction for firewood, natural calamities like and incense by local people.

The timing of seed germination plays critical roles in the survival and persistence of plants in natural ecosystems, because high seedling mortality is a major limitation in the treeline areas (Lett and Dorrepaal, 2018). Seed maturation in many species is often accompanied with recognizable changes in size, colour, taste, odour and texture of the fruit and seed (Tewari and Tewari, 2019). Change in fruit/seed colour play a significant role to identify physiological maturity of tree seed in various species (Tewari et al. 2019). Seed maturation studies help in fixation of optimum stage of maturity that will yield abundant seeds higher germination and vigor (Tewari et al. 2016, 2017; Tamta and Singh, 2018). *Rhododendrons* are one of the most exploited speciesdue to its multifarious nature (Chandra and Srivastava, 2010). Theregeneration of various such multifarious species is very poorin nature due to their over exploitation (Tewari, 2005) and other climatic causes. To synchronize artificial regenerationof species the exact knowledge of the time of capsule maturation is essential for the collection of mature and viableseeds just before the opening of capsules to avoid seed dispersal as seeds are very minute (Tewari and Tewari, 2019). The aim of the present study was to assess the time of capsule maturation and seed germination of *R. arboreum* in treeline areas of western Himalaya of Uttarakhand.

Material and Methods

Study Site

The studied treeline site situated at 30°11'N and 79°39´E between 3233 and 3446msl in the sub-alpine zone of western Himalaya, Uttarakhand. Soil is sandy loam in texture and generally acidic with pH value 4 to 5 (Singh et al. 2019). The climate of the study area is characterized by short cool summers and long severe winters. The mean annual temperature of the sites varied from -8.0 and 24.8°C. Anthropogenic disturbance in mainly occurs in the form of migratory grazing and tourist activities (Singh et al. 2019).

Phytosociological Analysis

The phytosociological analysis of treeline tree species was done by placing 40 quadrats of 10 x 10m in the study site. The size and number of the samples were determined following Saxena and Singh (1982) and Singh et al*.* (2014). The importance value index was determined as the sum of the relative frequency, relative density and relative dominance (Ambasht and Ambasht, 2002).

Maturity Indices

Twenty-five individuals were select which had a well-developed crown with sufficient number of capsule and free from any disease. Capsule collection of *R. arboreum* was started from Ist week of August up to the availability of capsule till IIIrd week of December. The capsule collection was made directly from the tree and then seed germinated in the laboratory. For capsule physical parameters three replicate of 100 capsule each were taken and the different parameters includes capsule size (mm^2) (length ×width), number of capsules per 100g and weight and mass of 100 capsule. For weight parameters digital electronic balance and for size electronic digital vernier calliper was used. For calculating moisture content of capsule three replicates of 25 capsule were taken for each collection date. The moisture content was expressed on fresh weight basis in which capsule were dried at 103±2°C for 16±1 hour and then reweighed following (ISTA, 1981).Germination was carried out in a dual chamber seed germinator for each collection date. For germination 4 replicates of 1gm seeds each were used (Tewari and Tewari, 2019). The germination of seeds was carried out at 25±1ºC on top of the paper in seed germinator. Daily observation was taken and germination was counted when visible protrusion of radical (1mm) occurred. Water was added as required during the experiment. After completion of experiment germination percent was calculated following Paul (1972).

Statistical Test

The data were subjected to analysis of variance with a 95% confidence level using SPSS version 2016. Correlation coefficient was used for expressing relationship between different variables.

Results

Tree Layer Analysis

At studied tree line site the total tree density was 710 ha^{-1} and total basal area 75.42 m^2 ha^{-1}. The tree and seedling density of *R. arboreum* was 80ind. $ha^{-1}$and sapling density was 60 ind. ha^{-1}. The total basal area of *R. arboreum*was 5.96 m^2 ha^{-1} and the IVI was 32.09. The density of associated species was between 50 and 190 ind. ha^{-1} and total basal area ranged from 1.88 to 27.98 m^2 ha^{-1}.

Capsule Characteristics

The capsule colour of *R. arboreum* was light green during the first collection and black at final collection in both the years. The mean capsule size during first collection was 76.81±0.31 mm^2 in Yr1 and 78.47±1.18 mm^2 in Yr2 which gradually increased with each collection date. The maximum capsule size was 236.82±1.04 mm^2 in Yr1 and 226.60±1.95 mm^2 in Yr2 during the seventh and eighth collection respectively, after that the capsule size started declining and reached to 208.26±1.95 mm^2 in Yr1 and 199.52±1.85 mm^2 in Yr2 at final collection. The change was 131.45 mm^2 in Yr1 and 121.05 mm^2 in Yr2across the dates (Table 1).The mean weight of 100 capsules during first and last collection ranged from 30.33±1.20 to 32.33±0.33 g across the years. It was maximum 40.00±0.20 g during the eighth collection in Yr1 and 39.33±0.33 g during the fourth collection in Yr2. Similarly, the number of capsulesper 100 g varied between 317.67±1.21 and 328.33±1.77 across the years. The minimum number of capsules per 100 g was 249.67±0.88 during the eighth collection in Yr1 and 253.33±1.76 during the fourth collection in Yr2 (Table 1). The mass of 100 capsulesduring first collection was 21.23±0.84 g in Yr1 and 21.70±0.40 in Yr2 which gradually increased up to the 33.43±0.81 g in Yr1 and 32.20±0.53 g in Yr2 during the ninth collection, after that the capsule mass started declining and at final collection was 29.75±0.31 g in Yr1 and 29.13±0.31 g in Yr2. The maximum changes in capsule mass per 100 capsulesduring collection was12.20 g in Yr1and 10.50 g in Yr2(Table 1).

Germination

The seeds of *R. arboreum* failed to germinate for first five collections in both the years. During sixth collection the mean germination was 11.67±0.67% in Yr1 and 12.67±1.20% in Yr2 which continuously increased with each collection date. The maximum germination was 40.00±0.78% in Yr1 and 39.00±0.48% in Yr2 during the ninth collection, after that the germination started declining and reached to 38.67±1.20% in Yr1 and 36.67±0.88% in Yr2 at the final collection. The moisture content across all collection dates varied from 20.50±1.54 to 72.06±2.76% in Yr1 and 19.66±1.89 to 71.74±1.43% in Yr2. The maximum germination occurred when the moisture content was 25.90±1.99% in Yr1 and 22.96±0.58% in Yr2 (Fig. 1). *R. arboreum* attained maturity in the first week of December.

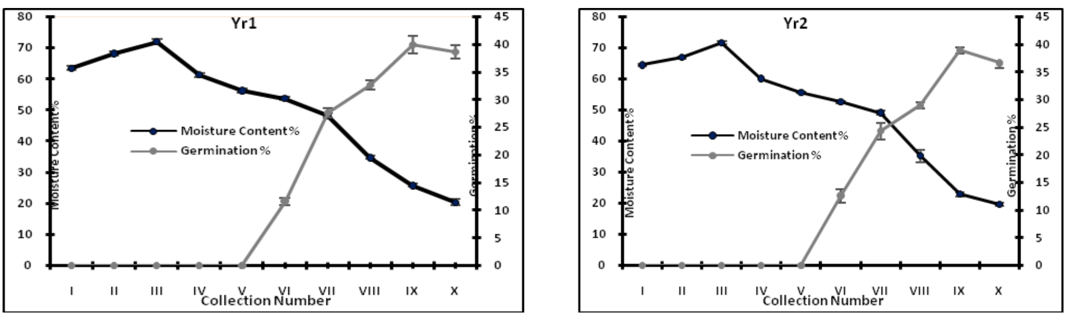

Fig.1. Relationship between moisture content of capsules and germination of seeds of *R. arboreum* over the collection dates in Yr1 and Yr2

Table 1. Variation in Physical Attributes of *R. Arboreum*capsules over the collection period in Yr1 and Yr2

Collection number	Collection date	Capsule colour	Capsule size (mm^2)		Weight of 100 capsules (g)		Number of capsules/100 (g)		Mass of 100 capsules (g)	
			Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2
Ist	Ist week of August	LG	76.81±0.31	78.47±1.18	30.33±1.20	31.00±0.58	326.67±1.88	328.33±1.77	21.23±0.84	21.70±0.40
IInd	IIIrd week of August	LG	136.89±0.23	138.23±1.11	34.67±0.88	35.33±0.33	299.33±2.96	299.00±2.08	24.27±0.62	24.73±0.23
IIIrd	Ist week of September	LG	162.22±1.04	160.40±1.31	36.67±0.67	37.00±0.58	286.00±1.66	286.00±2.00	25.67±0.47	25.90±0.40
IVth	IIIrd week of September	G	176.79±0.58	178.36±1.22	38.67±0.33	39.33±0.33	267.33±1.45	253.33±1.76	28.61±0.25	29.11±0.25
Vth	Ist week of October	DG	209.25±0.57	213.95±0.95	39.00±0.00	38.67±0.33	258.67±0.67	255.33±1.45	28.86±0.00	28.61±0.25
VIth	IIIrd week of October	DG	217.94±0.98	220.41±1.06	39.33±0.33	38.67±0.33	253.67±1.86	259.00±2.31	30.68±0.26	30.16±0.26
VIIth	Ist week of November	BG	236.82±1.04	221.19±1.21	39.67±0.33	37.33±0.88	251.67±1.67	262.00±2.51	30.94±0.26	29.12±0.69
VIIIth	IIIrd week of November	BG	230.38±0.21	226.60±1.95	40.00±0.20	38.67±0.67	249.67±0.88	260.67±1.61	33.20±0.00	32.09±0.55
IXth	Ist week of December	BR	224.24±0.21	199.75±1.49	36.33±0.88	35.00±0.58	288.33±1.37	285.00±2.89	33.43±0.81	32.20±0.53
Xth	IIIrd week of December	BL	208.26±1.95	199.52±1.85	32.33±0.33	31.67±0.33	317.67±1.21	325.00±1.04	29.75±0.31	29.13±0.31

Note: LG = Light Green, **G** = Green, **DG** = Dark Green, **BG** = Brownish Green, **BR** = Brown and **BL** = Black, **YR** = Year

ANOVA showed that the capsule size, weight of 100 capsules, number of capsules per 100 g, mass of 100 capsules, moisture content and germination varied significantly across the dates ($p<0.01$), but not with the two years of study. The interactions between years × dates varied significantly ($p<0.01$ and $p<0.05$).

A significant positive correlation was found between capsule size and weight of 100 capsules, capsule size and mass of 100 capsulesat 0.05% significant level ($p<0.05$) (Table 2).A significant negative correlation was observed between capsule size and moisture content, weight of 100 capsules and germination, mass of 100 capsules and moisture content and moisture content and germination at 0.05% significant level ($p<0.05$) (Table 2).

Table 2. Correlations between different capsule parameters and seed germination of *R. Arboretum*

CORRELATIONS						
Parameters	Capsule size	Weight of 100 capsules	Number of capsules/ 100 (g)	Mass of 100 capsules	Moisture content	Germination
Capsule size	1	**0.645****	-0.675**	**0.904****	-0.530**	**0.600****
Weight of 100 capsules		1	-0.966**	**0.615****	**0.169****	-0.053**
Number of capsules/100			1	-0.621**	-0.128**	**0.013****
Mass of 100 capsules				1	-0.695**	**0.714****
Moisture content					1	-0.932**
Germination						1

**Correlation is significant at the 0.05 level ($p<0.05$) (2-tailed)

Discussion

Maturity is the critical and the most important factor that determines the size and the quality of the seed. The stage and time of maturity ofseeds is essential for collection of abundantquantity of healthy and vigorous seeds for future multiplication of species. These characters could be achieved with the seed if it is harvested at optimum stage, which is normally designated as the physiological maturity of the seed (Mittal et al, 2017). The change in capsule colour from light green to black and decline in moisture content from 72 to 20% appear to be good indicators of seed maturity in *R. arboreum*. During initial collection the moisture content of capsule was very high as the capsule matured the moisture content declined continuously. *R. arboreum* produce very minute seed, small-seed species which usually present high mortality in the transition between seed germination and seedling emergence and tend to exhibit lower values of survival (Singh, 2019). In the present study the maximum germination of *R. arboreum* seed occurs at 22.95 to 25.90% moisture content. Tewari and Tewari (2019) reported the maximum germination in *R. arboreum* when the moisture content was 30.11% and Singh (2019) reported at 22 to 25% at treeline region and Mittal (2018) reported at 34 to 37% in Central Himalayan region. Negative correlation existed between germination and seed moisture content.

Various physical parameter such as, size, weight, number and mass of fruit have also been associated with maturation time. Generally, the fruit/seed attain maximum weight or mass at the time of maturation. At the time of maximum germination, the capsule size was 199 to 224 mm^2 and weight of 100 capsules was 35 to 36g. Mittal (2018) reported the weight of 100 capsules was 33 to 40g and capsule size was 208 to 240 mm^2 in sub-tropical region of Central Himalaya. Warming increased seed mass in plants and higher mass increased the seedling survival. In the present study the highest germination of seed was found when the mass of 100 capsule was 32 and 33g. Change in capsule colour, decline in fresh capsule moisture content and change physical parameters from maturing seeds is closely related to seed maturity.

Altitudinal variation affects the timing, duration and synchronization of various vegetative as well as reproductive phenophases of plants (Singh and Mittal, 2019). As a study by Singh (2014) and Singh et al (2015) in *R. arboreum*all the phenological events was earlier at lower elevation and delayed at higher elevations. The capsule maturation timing of treeline *R. arboreum*was two or three weeks delayed as compare to those grow at sub-tropical and temperate region this could be due to the variation in climate conditions such as temperature, rainfall, snow cover intensity of light and soil moisture of sub-alpine and alpine region. Warming affect the bud bursting and flowering time of *Rhododendrons*, and early flowering affect the capsule maturity time. Ranjitkar et al. (2012) and Tewari and Tewari (2019) also observed similar trend ofcapsule maturation in *R. arboreum*.

Conclusion

To summarise it is apparent from the present study that the capsule maturation period was early at sub-tropical *R. arboreum*as compare to alpine region. Changes in temperature affected capsule maturation of *R. arboreum*to a greater degree at higher elevations. There could be many other factors such as water stress, rainfall pattern, soil moisture, snow, wind speed, nutrients and oxygen levels that would be useful to better understand spatial patterns in the sensitivity of capsule maturation to temperature. The natural regeneration of this species was poorin such a case exact knowledge of the time of capsule maturation is essential for the collection of mature and viable seed/capsule for future multiplication.

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