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Seed Germiability, viability and longevity of *Rhododendron arboreum* var. *arboreum* Sm.

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Abstract
The present study has been undertaken with the objective to assess the germiability, viability and longevity of *R. arboreum* seeds at laboratory conditions as these factors are very important to promote the process of natural regeneration of species in wild which ultimately responsible for the better survival and existence of species. The seeds for experiment were collected from Craignaino forest in Mashobara Range of Shimla forest division. Seed parameters like Germination per cent, germination energy and germination value were calculated to test the germiability from the freshly collected seeds continuously for the period of six years. Seed viability was assessed through germination method and TTZ (Tetrazolium salt) method. A storage trial was designed by using polythene bags and cloth bags as a medium of storage of seeds, to check the longevity of the seeds under three different temperature gradients (deep freezer, Multi cooling chamber and normal room temperature). The results are diverse, having common observation with higher viability in freshly collected seeds as compared to the previous years. The overall Results showed tendency of decline in germiability as well as loss in viability over a period of time. The deep freeze (-20°C) is the best suitable temperature gradient for the storage of *R. arboreum* seeds as the maximum viability of the seeds has been observed in this medium with the 50% decline in viability in the period of 3 years.

Keywords: germination energy, germination value, germiability, longevity, viability

Introduction

Rhododendron arboreum var. *arboreum* Sm. holds a prominent role due to its aesthetic, medicinal and ecological importance in the world. The species is a primitive group of flowering plant which has survived through millions of years in the northern hemisphere (Milleville, 2002) [35]. The genera comprise of more than 1200 species (Wang, 1985, Neary *et al*., 1984, Rotherham, 1983, Clinton, 1996, Clinton and Vose, 1996, Colak, 1997, Paul *et al*., 2005 and Pradhan, 2011) [59, 39, 49, 10, 42, 44]. The northern hemisphere has 850 species of the genus (Mabberley, 1997) [29]. The center of diversity in the Himalayas and Southeast Asia where they grow naturally and with less diversity in North America and Europe and elsewhere in Asia in moist frequently montane ecosystems (Gibbs *et al*., 2011) [19].

A total of 87 species, 12 subspecies, and 8 varieties of *Rhododendrons* are found in Indian Himalayan Region (IHR) in India. Among 87 species, *Rhododendron anthopogon*, R. *arboreum*, *R. barbatum*, *R. campanulatum*, *R. lepidotum* and *R. nivale* are found in Western Himalaya while rest are located in Eastern Himalaya. Among twelve Biogeographical zones of India (Rodger and Panwar, 1988) [47], the genus is present in five namely Trans-Himalaya, Western Himalaya, Eastern Himalaya, Northeastern India, and the Western Ghats. The Eastern Himalaya is most concentrated and 75 species occur in the state of Arunachal Pradesh itself and contribute 86% out of 87 species known so far (Sekar and Srivastava, 2010) [50] and the state has been placed second after Yunnan, China for the diversity of *Rhododendrons* in the world (Cox, 1990) [12]. The *Rhododendrons* are known for their grandeur beauty of different colours of the flowers. The species ranges from small creeping shrublet (*R. pumilum*) to big trees (*R. arboreum)* (Bhattacharyya, 2005) [6] but few are epiphytic (*R. vaccinioides*). *Rhododendrons* normally produce numerous amounts of seeds (Cox, 1990; Romancier, 1970) [12, 48] however; viable seeds are not always available on a yearly basis. They are extremely small and size can vary greatly among species (Arocha *et al*., 1999; Blazich *et al*., 1991; Glenn *et al*., 1998; Olson, 1974) [3, 7, 20, 40].

R. arboreum, locally known as 'Burans', is amongst highly valued wild edible tree growing in hills of HP state found distributed in Shimla, Solan, Kinnaur, Mandi, Kangra, Kullu, Sirmour and Chamba districts of Himachal Pradesh (Kharwal and Rawat, 2013) [25]. Keeping aside the aesthetic value of the species is a rich source of anti-oxidants, β-carotene, flavanoids, vitamins and essential minerals in its fruit extract (Caius *et al*., 2000) [8]. *R. arboreum* species plays a vital role in the treatment and prevention of atherosclerotic blockage in the blood vessels particularly in coronary and cerebral blood vessels (Hardman *et al.*, 1996; Longman, 1997) [21, 28]. *R. arboreum* is keystone species in the high-elevation portions of the state and plays a crucial role in maintaining the organization and diversity with ecological communities. Unfortunately, increasing anthropogenic activities and technological interventions in developmental activities have left the region no longer tolerent due to large scale land use change (Menon *et al*. 2010) [34]. With the constant increase of mitigation crisis, caused by the various environmental ailing factors like climate change and other anthropogenic activities *R. arboreum* is under vulnerable stage (Singh *et al*., 2003) [53]. According to the Red list of *Rhododendrons* 2 species has been extinct, 36 are critically endangered, 39 are endangered, 241 are vulnerable and 290 are near threatened. All the four species found in Himachal Pradesh has been put under least concern category (Gibbs *et al*, 2011) [19]. The species is now in definite danger of elimination unless immediate restoration measures are made (Kumar *et al*., 2004) [26].

The seed is a miniature plantlet and is one of the most important factors in determining the viability of future plant. A healthy and viable seeds is of utmost important for the preservation of its density and diversity (Ahmad, 2001) [1]. Viability of seed in most of the species is increased under low temperature and low moisture conditions (Roberts 1973; Ellis *et al.* 1985; Dickie *et al.* 1990) [46, 17, 16]. Seeds kept at higher temperatures and higher relative humidity may reduce the viability (Simic *et al*., 2006) [52]. The ability of seed to resist degradation changes by protection mechanisms decides the ageing of the seed and varies for species to species (Balesevic, 2001) [4]. Testing of various parameters which decides the vigour of the seeds becomes more significant if seed were stored under known or unknown storage conditions (Andric *et al.*, 2007; Mendes and Moraes 2009) [2, 33]. Sometime unfavorable storage conditions can aggravate significant variations in seed viability (Tatic *et al.*, 2008) [56]. For testing the seed viability several tests such as cold, accelerated aging and Hiltner can be applied (Milosevic and Malesevic, 2004) [36]. There are various factors that affect the quality of seed during storage *viz.* drying temperature, moisture content, storage temperature, oxygen supply, pest and diseases, packaging material and duration of storage (Muangkaeo *et al.*, 2005; Chattha *et al*., 2012; Jyoti and Malik, 2013) [37, 9, 24]. The most important among them are temperature and moisture content. (Pradhan and Badola, 2012) [43]. Decrease in storage temperature and seed moisture content has been shown to enhance seed storage time (Mbofung *et al*., 2013) [31]. To achieve suitable levels of germination and vigour, proper packaging is vital because improper packaging material will deteriorate the quality of seed during storage (Naguib *et al*., 2011) [38]. Poor storage conditions may leads to the occurrence of pests and disease, reduction in seed vigour and thus loss of germination (Vange *et al*., 2016) [58].

Germination energy, Germination value, Germination percent and energy period are the main indicators of the seed viability. Thus, the study on *R. arboreum* could be inspiring steps to observe the importance of the species and extends as viable example for treasuring natural resources of the state. Therefore, study has been undertaken with the objectives to access germiability, viability and longevity in freshly collected seeds in three different storage mediums at laboratory conditions supplement the research data in future regeneration propagation and plantation activities of the species.

Material and Method

Study Area

Himachal Pradesh extends between 30°22'40" N and 33°12'20" N latitude and 75°45'55" E and 79°04'20" E longitude is situated at an altitudinal range of 350 m - 6,975 m amsl, which covers an area of 55,673 km^2 which is about 1.69% of India's total geographical area. The state is compact in shape and almost wholly mountainous with altitude varying from 300m in plains of Kangra to nearly 7000m in Central Himalaya of Lahual and Spiti. The annual rainfall of Himachal Pradesh is 2909 to 3800 mm with maximum temperature ranging 42.2°C and minimum is -14°C. The highest amount of rainfall that has been recorded in Dharmasala is 3400 mm. District Shimla lies between longitude 77º00" and 78º.19" East and latitude 30º.45" and 31º.44" North, is surrounded by Mandi and Kullu in the North, Kinnaur in the East, Uttarakhand in the South-East, Solan to the South-West and Sirmaur in South, having a total geographical area of the district is 5,131 km^2 out of which 3,418 km^2 (46.46 percent) is forest area. Physiographically, the state can be divided into five zones - *viz.* (i) Wet Sub-temperate zone, (ii) Humid Sub-temperate zone, (iii) Dry temperate-alpine High lands, (iv)Humid Sub-tropical zone (v) Sub-Humid Sub-tropical zone. The elevation of the district which ranges from 300-6000 m. The climate is cool during winters (4ºC) and moderately warm during summer (31ºC). The average total precipitation is 1575 ml. Snowfall occurs in December but has shifted to January or early February every year for the last fifteen years. The main forests in and around the district are of Pine, Deodar, Oak and Rhododendron.

The study was conducted at seed laboratory of Himalayan Forest Research Institute, Panthaghati, Shimla. Rhododendron capsules were collected in the month of October every year *i.e.* 2014, 2015, 2016, 2017, 2018 and 2019 from Craignano forest, situated at height of 2276m amsl in the longitude 077º13'94.4'' and latitude 31º07'65.3'' of Mashobra range in Shimla district, Himachal Pradesh. The collected capsules were dried at normal room conditions and seeds were extracted. Following tests were conducted to assess the germination and viability of the seeds:

Viability Tests

Germination method: Germinability measures the capability of seeds to grow to its full potential under favourable conditions (Bewley and Black, 1978) [5]. To assess germination seeds were sown on simple whatman filter paper no. 1 kept in petriplate at normal room temperature. Hundred seeds were taken in four replicates. The final germination count of the seeds was taken to assess the germination per cent.

GP= [SG/TSS] X 100

Where, GP= Germination Percent, SG= Seeds Germinated, TSS= Total Seeds Sown. (Islam *et al.*, 2012; Wani and Singh, 2016) [23, 60].

Tetrazolium method: Seed vigour was tested with the tetrazolium test (Agrawal *et al*., 1973) [46]. Hundred seeds in four replicates were cut longitudinally half and stained with 2% tetrazolium solution for two hours. The stained seeds were seen under light microscope (Nikon SMZ 1500) and the red stained embryos were counted to calculate the staining per cent.

SP= [RSS/TSS] X 100

Where, SP= Staining Percent, RSS=Red Stained Seeds, TSS= Total Stained Seeds. (Deminicis *et al*, 2014; Senoz, 2012; Patil and Dadlani, 2009; Leadem, 1984; Copeland and McDonald, 2001 and McDonald and Wilson, 1979) [14, 51, 41, 27, 11, 32]

Germiability Tests

The study was conducted by using seed for six successive years collected in the month of October every year *i.e.* 2014, 2015, 2016, 2017, 2018 and 2019. To assess the germination seeds were sown on whatman paper kept in Petri plate moisten with distilled water at normal room temperature. For the seed germination studies, 100 seeds were taken in four replicates. Germination count of seeds was recorded daily till the final results.

Peak value: Peak value is calculated as the maximum mean daily germination (MDG) reached at any time during the period of test (Czabator, 1962) [13].

Energy period: Energy period is the period of daily seed germination which is taken into consideration for the deduction of germination energy.

Germination Energy: Speed with which the seeds germinate is expressed as a percentage of the seeds germinated in the specified energy period with respect to overall germination. For the calculation of germination energy, peak value must be taken in to the consideration and the daily count less than 25 percent of the peak value will not be considered in the energy period because this may leads to ambiguous calculation of the germination energy due to the inclusion of seeds with the low seed vigor (Willan, 1985) [61].

GE= [NSG/TS] X 100

Where, GE= Germination Energy, NSG= Number of seeds Germinated until daily germination falls less the 25 percent of the peak value, TS= Total Seeds

Germination Value: The Germination value is being calculated by following Czabator Method. (Czabator, 1962) [13]. Parameters like Daily germination, Daily Germination per cent *etc*. were recorded and thus germination value is deduced. (Masoodi *et al*., 2014; Fetouh and Hussan, 2014; Thomson and Kassaby, 1993; Hossain *et al*., 2005; Swaminathan and Revathy, 2013 and Razvi *et al*., 2012) [30, 18, 57, 22, 55, 45].

Czabator Method

GV= [Final MDG or DGS] X Peak Value of DG

Where, GV= Germination Value, MDG or DGS= Mean daily Germination or Daily Germination Speed is calculated through cumulative Germination per cent by number of days since sowing (Diavanshir and Pourbeik, 1976) [15].

Seed Longevity test (Storage trials)

The seeds collected in the year October, 2014 were packed in two types of storage mediums i.e. polythene bags (P.B.) and cloth bags (C.B.). These bags were stored at three different temperature gradients (Deep freezer, Multi cooling chamber and at Room Temperature). The viability of these stored seeds has been checked by using simple germination method at quarterly intervals for subsequent years *i.e.* 2014, 2015, 2016, 2017, 2018 and 2019 using four replicates of hundred seeds each.

Results and Discussion

Viability check

In order to check the viability two types of experiments were laid, first by assessing the germination directly on filter paper in petriplates and secondly by using Tetrazolium salt.

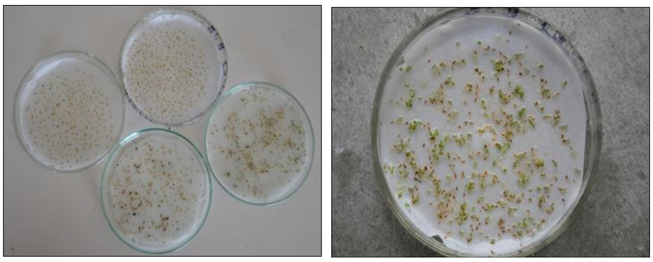

Fig 1&2: Viability Testing through Germination Method

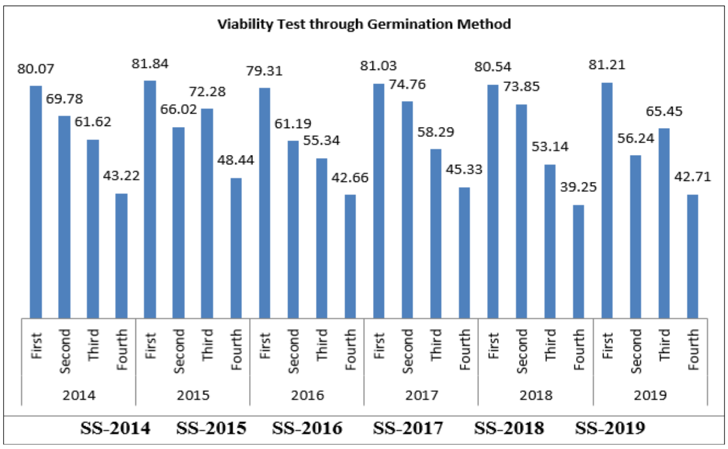

Fig 3: Graphical representation of the Quarter wise data of viability of freshly collected seeds tested through germination method

The freshly collected seed source of the year 2014, 2015, 2016, 2017, 2018 & 2019 after stratification and drying were tested for the viability in the four quarters of year 2015, 2016, 2017, 2018, 2019 & 2020. The results are diverse with common exception of viability being more in newly collected seeds decreases with the passage of time. The trend of decline in viability remained same in the successive years from I[st] quarter (Jan-March) to 4[th] quarter (October-December) i.e 80.07 % (1[st] Qtr) to 43.22 % (4[st] Qtr) of (SS-2014), 81.84 % (1[st] Qtr) to 48.44 % (4[st] Qtr) of (SS-2015) and 79.31% (1[st] Qtr) to 42.66 % (4[st] Qtr) of (SS-2016), 81.03% (1[st] Qtr) to 45.33 % (4[st] Qtr) of (SS-2017), 80.54% (1[st] Qtr) to 39.25 % (4[st] Qtr) of (SS-2018), 81.21% (1[st] Qtr) to 42.71 % (4[st] Qtr) of (SS-2019). (Fig. 3)

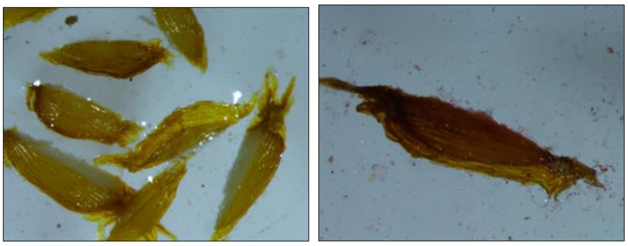

Fig 4&5: Viability Testing through Tetrazolium salt Method

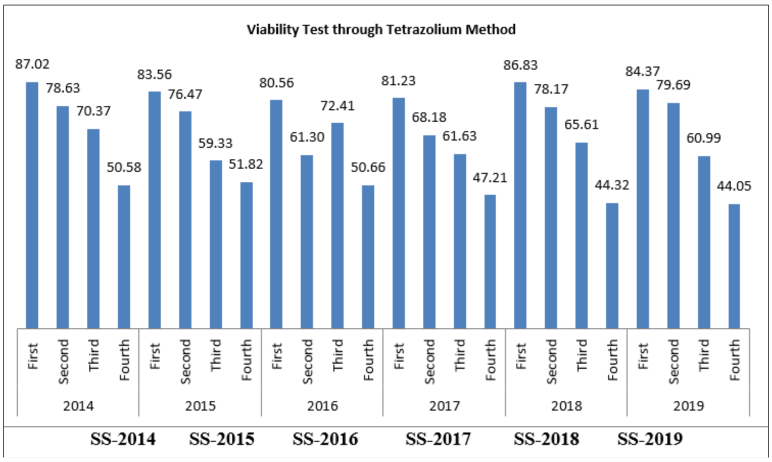

Fig 6: Graphical representation of the Quarter wise data of viability of freshly collected seeds tested through Tetrazolium salt method

In second method, the viability percentage recorded was low with similar trend of decline in viability. The trend of decline in viability remained same in the successive years from I[st] quarter (Jan-March) to 4[th] quarter (October-December) *i.e* 87.02 % (1[st] Qtr) to 50.58 % (4[st] Qtr) of (SS-2014), 83.56 % (1[st] Qtr) to 51.82 % (4[st] Qtr) of (SS-2015) and 80.56% (1[st] Qtr) to 50.66 % (4[st] Qtr) of (SS-2016), 81.23% (1[st] Qtr) to 47.21 % (4[st] Qtr) of (SS-2017), 86.83% (1[st] Qtr) to 44.32 % (4[st] Qtr) of (SS-2018), 84.37% (1[st] Qtr) to 44.05 % (4[st] Qtr) of (SS-2019). (Fig. 2)

Table 1: Germiabilty parameters of freshly collected Seeds for Six consecutive years

	Energy Period (Days)	Germination Percentage	Germination Energy (%)	Germination Value
SS-2014	22	80.50	71.25	8.15
SS-2015	20	81.25	74.25	9.46
SS-2016	24	79.75	74.75	7.15
SS-2017	21	81.25	75.75	8.62
SS-2018	24	80.50	73.25	6.85
SS-2019	21	81.00	72.00	8.28

The Energy period, Germination Per cent, Germination Energy and Value plays pivotal role in deciding the fate of the seeds and survival of the seedling in the near future, provided that all the conducive conditions are fulfilled. These all factors decide the quality of seeds. Above results shows that seed collected in the year, 2015 have more germiability i.e. in comparison to the rest of years. (Table 1)

Table 2: Calculation of Germinability parameters (Germination Percentage, Germination Energy and Germination Value) for the seed source of the year, 2014

Days	Daily count	Cumulative total	Cumulative Germination %	Mean Germination or Daily Germination Speed (Colum 4/1)	Σ DGS	Number of counts	Σ DGS/N(Colum7/6)
11	7	7	1.75	0.16	0.16	1	0.16
12	13	20	5.00	0.42	0.58	2	0.29
13	42	62	15.50	1.19	1.77	3	0.59
14	36	98	24.50	1.75	3.52	4	0.88
15	22	120	30.00	2.00	5.52	5	1.10
16	30	150	37.50	2.34	7.86	6	1.31
17	28	178	44.50	2.62	10.48	7	1.50
18	31	209	52.25	2.90	13.38	8	1.67
19	15	224	56.00	2.95	16.33	9	1.81
20	21	245	61.25	3.06	19.39	10	1.94
21	24	269	67.25	3.20	22.59	11	2.05

22	16	285	71.25	3.24	25.83	12	2.15
23	8	293	73.25	3.18	29.02	13	2.23
24	4	297	74.25	3.09	32.11	14	2.29
25	1	298	74.50	2.98	35.09	15	2.34
26	5	303	75.75	2.91	38.01	16	2.38
27	6	309	77.25	2.86	40.87	17	2.40
28	4	313	78.25	2.79	43.66	18	2.43
29	1	314	78.50	2.71	46.37	19	2.44
30	6	320	80.00	2.67	49.03	20	2.45
31	0	320	80.00	2.58	51.62	21	2.46
32	2	322	80.50	2.52	54.13	22	2.46

Table 3: Calculation of Germiability parameters (Germination Percentage, Germination Energy and Germination Value) for the seed source of the year, 2015

Days	Daily count	Cumulative total	Cumulative Germination %	Mean Germination or Daily Germination Speed (Colum 4/1)	Σ DGS	Number of counts	Σ DGS/N(Colum7/6)
10	2	2	0.50	0.05	0.05	1	0.05
11	18	20	5.00	0.45	0.50	2	0.25
12	56	76	19.00	1.58	2.09	3	0.70
13	39	115	28.75	2.21	4.30	4	1.07
14	29	144	36.00	2.57	6.87	5	1.37
15	36	180	45.00	3.00	9.87	6	1.65
16	35	215	53.75	3.36	13.23	7	1.89
17	21	236	59.00	3.47	16.70	8	2.09
18	25	261	65.25	3.63	20.33	9	2.26
19	17	278	69.50	3.66	23.98	10	2.40
20	19	297	74.25	3.71	27.70	11	2.52
21	8	305	76.25	3.63	31.33	12	2.61
22	3	308	77.00	3.50	34.83	13	2.68
23	4	312	78.00	3.39	38.22	14	2.73
24	5	317	79.25	3.30	41.52	15	2.77
25	0	317	79.25	3.17	44.69	16	2.79
26	2	319	79.75	3.07	47.76	17	2.81
27	0	319	79.75	2.95	50.71	18	2.82
28	2	321	80.25	2.87	53.58	19	2.82
29	0	321	80.25	2.77	56.34	20	2.82
30	1	322	80.50	2.68	59.03	21	2.81
31	2	324	81.00	2.61	61.64	22	2.80
32	2	326	81.50	2.55	64.19	23	2.79

Table 4: Calculation of Germiability parameters (Germination Percentage, Germination Energy and Germination Value) for the seed source of the year, 2016

Days	Daily count	Cumulative total	Cumulative germination %	Mean Germination or Daily Germination Speed (Colum 4/1)	Σ DGS	Number of counts	Σ DGS/N(Colum7/6)
13	18	18	4.50	0.35	0.35	1	0.35
14	36	54	13.50	0.96	1.31	2	0.66
15	28	82	20.50	1.37	2.68	3	0.89
16	34	116	29.00	1.81	4.49	4	1.12
17	31	147	36.75	2.16	6.65	5	1.33
18	32	179	44.75	2.49	9.14	6	1.52
19	21	200	50.00	2.63	11.77	7	1.68
20	26	226	56.50	2.83	14.59	8	1.82
21	32	258	64.50	3.07	17.67	9	1.96
22	18	276	69.00	3.14	20.80	10	2.08
23	12	288	72.00	3.13	23.93	11	2.18
24	11	299	74.75	3.11	27.05	12	2.25
25	3	302	75.50	3.02	30.07	13	2.31
26	4	306	76.50	2.94	33.01	14	2.36
27	3	309	77.25	2.86	35.87	15	2.39
28	0	309	77.25	2.76	38.63	16	2.41
29	3	312	78.00	2.69	41.32	17	2.43
30	0	312	78.00	2.60	43.92	18	2.44
31	2	314	78.50	2.53	46.45	19	2.44
32	0	314	78.50	2.45	48.90	20	2.45
33	1	315	78.75	2.39	51.29	21	2.44
34	2	317	79.25	2.33	53.62	22	2.44
35	2	319	79.75	2.28	55.90	23	2.43

Table 5: Calculation of Germiability parameters (Germination Percentage, Germination Energy and Germination Value) for the seed source of the year, 2017

Days	Daily count	Cumulative total	Cumulative Germination %	Mean Germination or Daily Germination Speed (Colum 4/1)	Σ DGS	Number of counts	Σ DGS/N(Colum7/6)
11	8	8	2.00	0.18	0.18	1	0.18
12	24	32	8.00	0.67	0.85	2	0.42
13	48	80	20.00	1.54	2.39	3	0.80
14	56	136	34.00	2.43	4.82	4	1.20
15	31	167	41.75	2.78	7.60	5	1.52
16	23	190	47.50	2.97	10.57	6	1.76
17	35	225	56.25	3.31	13.88	7	1.98
18	22	247	61.75	3.43	17.31	8	2.16
19	19	266	66.50	3.50	20.81	9	2.31
20	21	287	71.75	3.59	24.39	10	2.44
21	16	303	75.75	3.61	28.00	11	2.55
22	5	308	77.00	3.50	31.50	12	2.63
23	3	311	77.75	3.38	34.88	13	2.68
24	4	315	78.75	3.28	38.16	14	2.73
25	1	316	79.00	3.16	41.32	15	2.75
26	0	316	79.00	3.04	44.36	16	2.77
27	0	316	79.00	2.93	47.29	17	2.78
28	0	316	79.00	2.82	50.11	18	2.78
29	2	318	79.50	2.74	52.85	19	2.78
30	0	318	79.50	2.65	55.50	20	2.78
31	1	319	79.75	2.57	58.07	21	2.77
32	2	321	80.25	2.51	60.58	22	2.75
33	2	323	80.75	2.45	63.03	23	2.74
34	2	325	81.25	2.39	65.42	24	2.73

Table 6: Calculation of Germiability parameters (Germination Percentage, Germination Energy and Germination Value) for the seed source of the year, 2018

Days	Daily count	Cumulative total	Cumulative Germination %	Mean Germination or Daily Germination Speed (Colum 4/1)	Σ DGS	Number of counts	Σ DGS/N(Colum7/6)
14	13	13	3.25	0.23	0.23	1	0.23
15	12	25	6.25	0.42	0.65	2	0.32
16	34	59	14.75	0.92	1.57	3	0.52
17	42	101	25.25	1.49	3.06	4	0.76
18	47	148	37.00	2.06	5.11	5	1.02
19	23	171	42.75	2.25	7.36	6	1.23
20	35	206	51.50	2.58	9.94	7	1.42
21	29	235	58.75	2.80	12.73	8	1.59
22	24	259	64.75	2.94	15.68	9	1.74
23	23	282	70.50	3.07	18.74	10	1.87
24	11	293	73.25	3.05	21.79	11	1.98
25	6	299	74.75	2.99	24.78	12	2.07
26	4	303	75.75	2.91	27.70	13	2.13
27	1	304	76.00	2.81	30.51	14	2.18
28	3	307	76.75	2.74	33.25	15	2.22
29	0	307	76.75	2.65	35.90	16	2.24
30	4	311	77.75	2.59	38.49	17	2.26
31	2	313	78.25	2.52	41.02	18	2.28
32	0	313	78.25	2.45	43.46	19	2.29
33	4	317	79.25	2.40	45.86	20	2.29
34	1	318	79.50	2.34	48.20	21	2.30
35	2	320	80.00	2.29	50.49	22	2.29
36	2	322	80.50	2.24	52.72	23	2.29

Table 7: Calculation of Germiability parameters (Germination Percentage, Germination Energy and Germination Value) for the seed source of the year, 2019

Days	Daily count	Cumulative total	Cumulative Germination %	Mean Germination or Daily Germination Speed (Colum 4/1)	Σ DGS	Number of counts	Σ DGS/N(Colum7/6)
12	15	15	3.75	0.31	0.31	1	0.31
13	38	53	13.25	1.02	1.33	2	0.67
14	22	75	18.75	1.34	2.67	3	0.89
15	58	133	33.25	2.22	4.89	4	1.22
16	36	169	42.25	2.64	7.53	5	1.51
17	40	209	52.25	3.07	10.60	6	1.77
18	35	244	61.00	3.39	13.99	7	2.00

19	16	260	65.00	3.42	17.41	8	2.18
20	18	278	69.50	3.48	20.89	9	2.32
21	10	288	72.00	3.43	24.32	10	2.43
22	5	293	73.25	3.33	27.64	11	2.51
23	3	296	74.00	3.22	30.86	12	2.57
24	5	301	75.25	3.14	34.00	13	2.62
25	0	301	75.25	3.01	37.01	14	2.64
26	4	305	76.25	2.93	39.94	15	2.66
27	2	307	76.75	2.84	42.78	16	2.67
28	4	311	77.75	2.78	45.56	17	2.68
29	3	314	78.50	2.71	48.27	18	2.68
30	4	318	79.50	2.65	50.92	19	2.68
31	0	318	79.50	2.56	53.48	20	2.67
32	2	320	80.00	2.50	55.98	21	2.67
33	2	322	80.50	2.44	58.42	22	2.66
34	2	324	81.00	2.38	60.80	23	2.64

Longevity check

The stored seed samples of the year, 2014, 2015, 2016, 2017 & 2018 in DF, MCC & Room temperature conditions were used for this study. The seeds were stored in two storage mediums i.e. Polythene bag Cloth bag for the minimum period of one year. To check the longevity of stored seeds again viability of these seeds was assessed using the Germination method.

1. Results for S.S.-2014

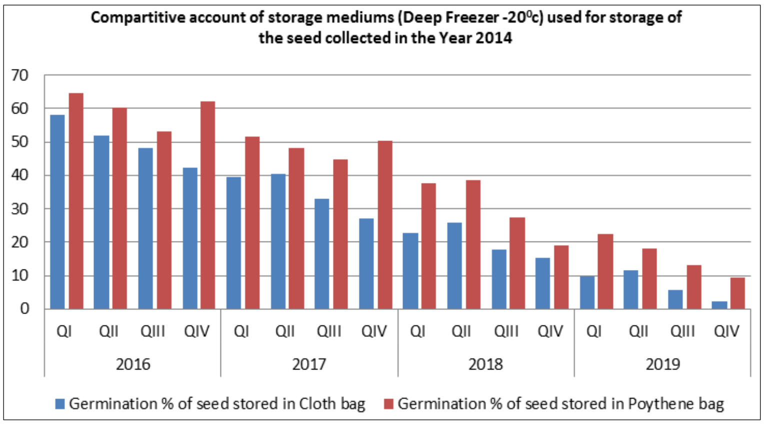

Fig 7: Graphical representation of the quarter wise data obtained for the viability of seeds stored in Deep freezer (-20^{0}C) by Germination test consequently for four years for SS-2014

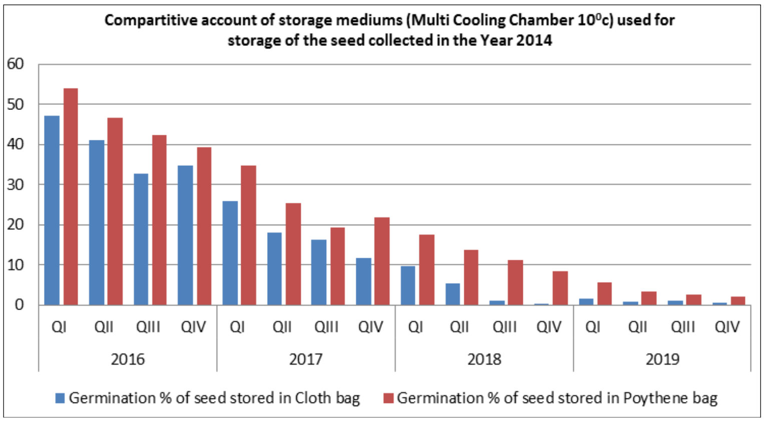

Fig 8: Graphical representation of the quarter wise data obtained for the viability of seeds stored in Multi cooling chamber (10^{0}C) by Germination test consequently for four years for SS-2014

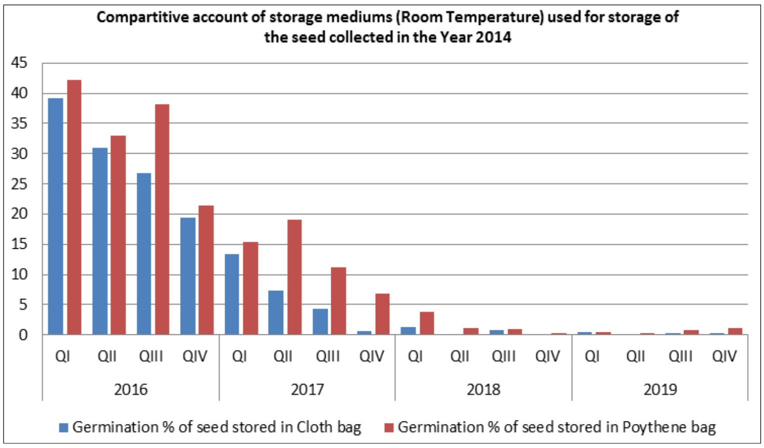

Fig 9: Graphical representation of the quarter wise data obtained for the viability of seeds stored at normal room temperature by Germination test consequently for four years for SS-2014

The results shows decline in the viability of seed i.e from 64.66 % to 9.42% stored in polythene bag and 58.25 % to 2.29% in cloth bag from 2014 to 2019 stored in Deep Freezer at -20^{0}C. Same declining trend of seed viability is observed in Multi cooling chamber (10^{0}C) i.e 54.04 % to 2.01% stored in polythene bag and 47.13% to 0.56% in cloth bag from 2014 to 2019. At room temperature 42.13% to 1.05% stored in polythene bag and 39.18% to 0.35% in cloth bag from 2014 to 2019. Overall results shows decline in the viability of stored seeds in all the three temperature conditions. It is also clear that the air tight polythene bags can be used for the long duration storage. The seeds stored in the Deep freezer (-20^{0}C) remain more viable than in Multi cooling chamber (10^{0}C) and room temperature conditions. (Fig. 7, 8 & 9)

2. Results for S.S.-2015

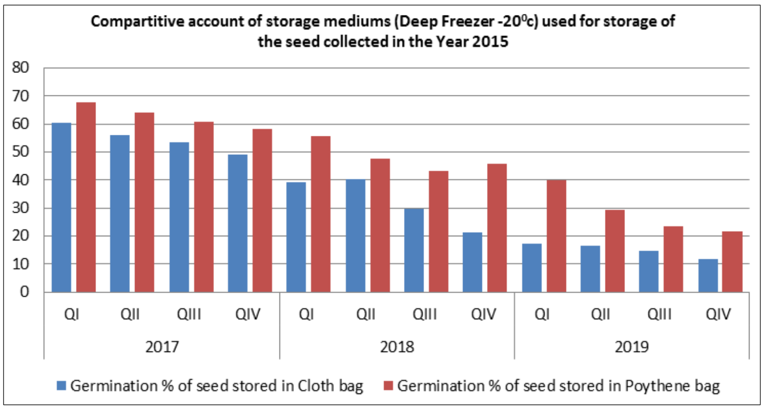

Fig 10: Graphical representation of the quarter wise data obtained for the viability of seeds stored in Deep freezer (-20^{0}C) by Germination test consequently for three years for SS-2015

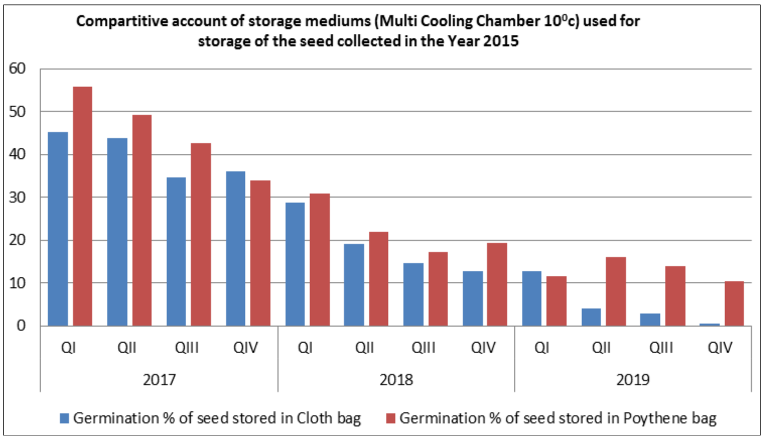

Fig 11: Graphical representation of the quarter wise data obtained for the viability of seeds stored in Multi cooling chamber (10^{0}C) by Germination test consequently for three years for SS-2015

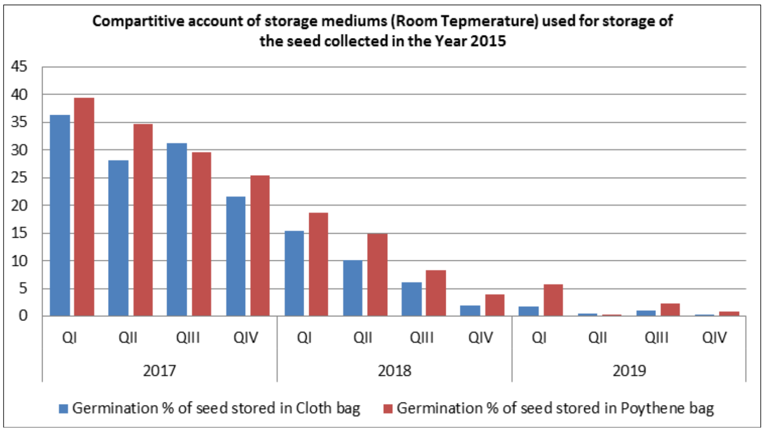

Fig 12: Graphical representation of the quarter wise data obtained for the viability of seeds stored at normal room temperature by Germination test consequently for three years for SS-2015

The results shows decline in the viability of seed i.e from 67.82 % to 21.42% stored in polythene bag and 60.44 % to 11.58% in cloth bag from 2015 to 2019 stored in Deep Freezer at -20^{0}C. Same declining trend of seed viability is observed in Multi cooling chamber (10^{0}C) i.e 55.83 % to 10.51% stored in polythene bag and 45.35% to 0.53% in cloth bag from 2015 to 2019. At room temperature 39.34% to 0.77% stored in polythene bag and 36.33% to 0.26% in cloth bag from 2015 to 2019. Overall results shows decline in the viability of stored seeds in all the three temperature conditions. It is also clear that the air tight polythene bags can be used for the long duration storage. The seeds stored in the Deep freezer (-20^{0}C) remain more viable than in Multi cooling chamber (10^{0}C) and room temperature conditions. (Fig. 10, 11 & 12)

3. Results for S.S.-2016

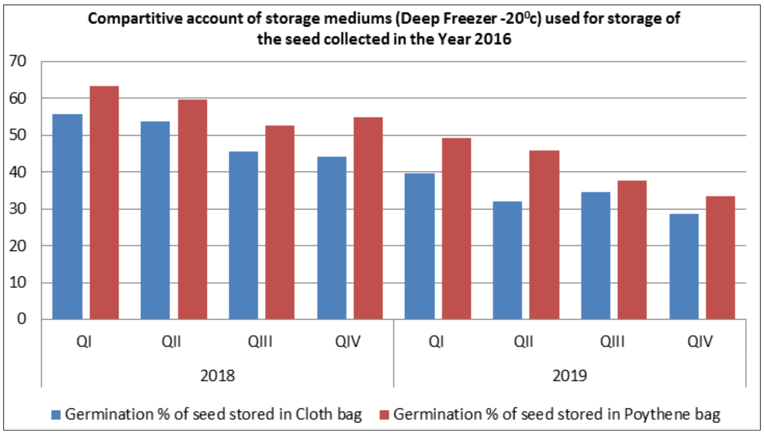

Fig 13: Graphical representation of the quarter wise data obtained for the viability of seeds stored in Deep freezer (-20^{0}C) by Germination test consequently for two years for SS-2016

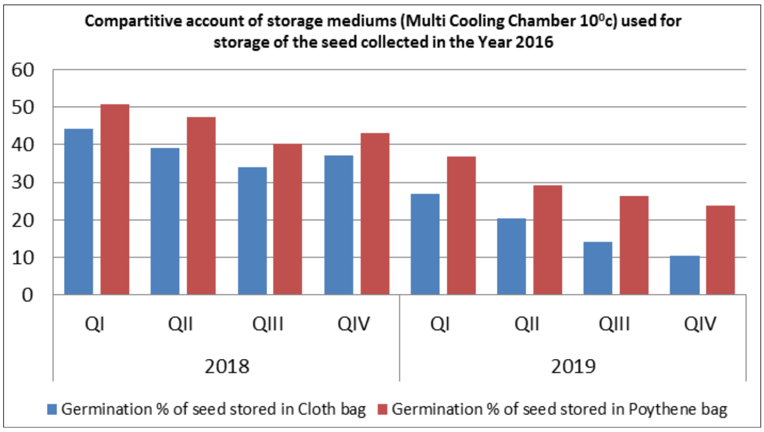

Fig 14: Graphical representation of the quarter wise data obtained for the viability of seeds stored in Multi cooling chamber (10^{0}C) by Germination test consequently for two years for SS-2016

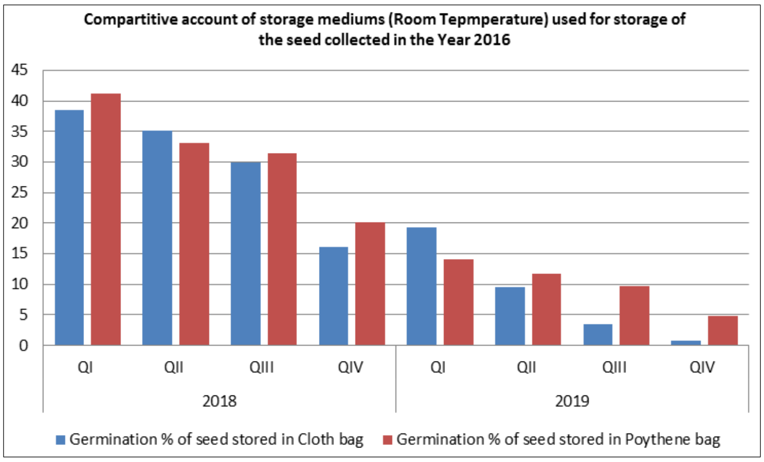

Fig 15: Graphical representation of the quarter wise data obtained for the viability of seeds stored at normal room temperature by Germination test consequently for two years for SS-2016

The results shows decline in the viability of seed i.e from 65.75 % to 54.20% stored in polythene bag and 61.80 % to 49.46% in cloth bag from 2016 to 2019 stored in Deep Freezer at -20^{0}C. Same declining trend of seed viability is observed in Multi cooling chamber (10^{0}C) i.e 53.13 % to 39.63% stored in polythene bag and 46.25% to 36.04% in cloth bag from 2016 to 2019. At room temperature 39.34% to 0.77% stored in polythene bag and 38.35% to 17.33% in cloth bag from 2016 to 2019. Overall results shows decline in the viability of stored seeds in all the three temperature conditions. It is also clear that the air tight polythene bags can be used for the long duration storage. The seeds stored in the Deep freezer (-20^{0}C) remain more viable than in Multi cooling chamber (10^{0}C) and room temperature conditions. (Fig. 13, 14 & 15)

4. Results for S.S.-2017

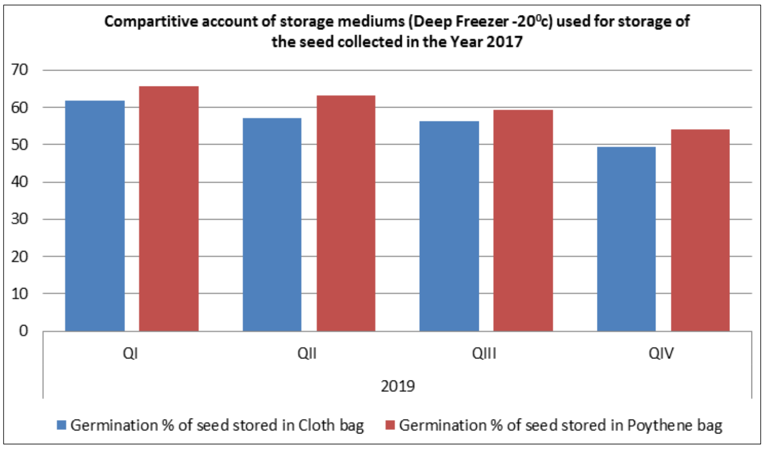

Fig 16: Graphical representation of the quarter wise data obtained for the viability of seeds stored in Deep freezer (-20^{0}C) by Germination test for one year for SS-2017

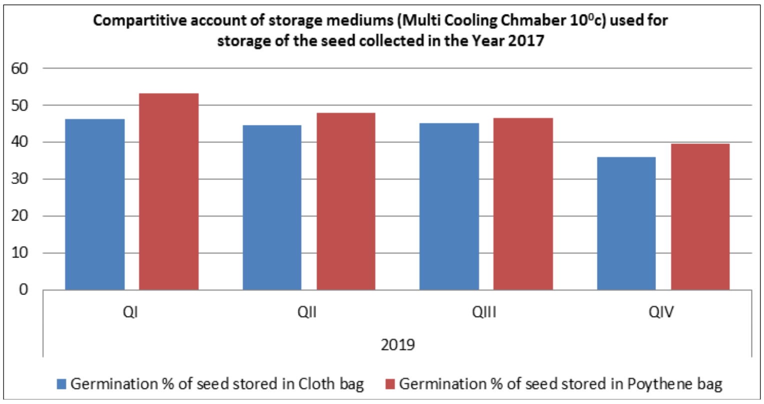

Fig 17: Graphical representation of the quarter wise data obtained for the viability of seeds stored in Multi cooling chamber (10^{0}C) by Germination test for one year for SS-2017

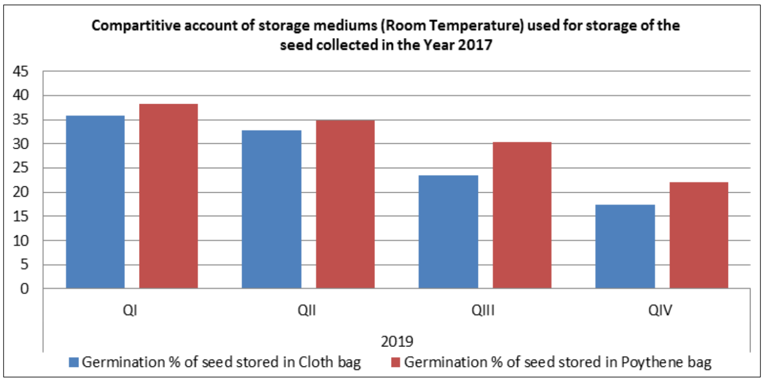

Fig 18: Graphical representation of the quarter wise data obtained for the viability of seeds stored at normal room temperature by Germination test for one year for SS-2017

The results shows decline in the viability of seed i.e from 64.66 % to 9.42% stored in polythene bag and 58.25 % to 2.29% in cloth bag from 2017 to 2019 stored in Deep Freezer at -20^{0}C. Same declining trend of seed viability is observed in Multi cooling chamber (10^{0}C) i.e 54.04 % to 2.01% stored in polythene bag and 47.13% to 0.56% in cloth bag from 2016 to 2018. At room temperature 42.13% to 1.05% stored in polythene bag and 39.18% to 0.35% in cloth bag from 2016 to 2018. Overall results shows decline in the viability of stored seeds in all the three temperature conditions. It is also clear that the air tight polythene bags can be used for the long duration storage. The seeds stored in the Deep freezer (-20^{0}C) remain more viable than in Multi cooling chamber (10^{0}C) and room temperature conditions. (Fig. 16, 17 & 18)

Conclusion
It is concluded from the present study that the month of October is the best suitable time for the collection of *R. arboreum* seeds as the germination/viability up to 80% was observed in the seeds collected during this month. Germination method is the best suitable method to study viability of the *R. arboreum* seeds as compared tetrazolium salt test. At normal conditions the germiability and viability is more prominent in freshly collected seeds which decreases with the passage of time from 1st quarter to the 4th quarter of a year as per studies conducted in the laboratory. The deep freezer (-20°C) is the best suitable temperature gradient for the storage of *R. arboreum* seeds as the maximum viability of the seeds has been observed in this medium with the approximately 50% decline in viability in the period of 3 years.

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