Research Article

Developing a New Technique for Rapid Seed Extraction from the Cones of Himalayan Conifer (*Pinus Wallichiana* A.B. Jackson)

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Abstract

The conventional practice of seed extraction from cones of *Pinus wallichiana* consist of drying the cones in the sun. This process takes about four weeks. A reliable technique for quick seed extraction was developed by employing different seed extraction methods. Among the seed extraction methods, hundred per cent of seeds were extracted on 8th day (T_4) when the cones were dipped in water for 10 minutes and oven drying at 30^{o}C followed by T_3 i.e. dipping cones in water for 10 minutes and oven drying at 20^{o}C, which took nine days for complete extraction of seeds. Oven drying of cones at 30^{o}C also took nine days for complete extraction of seeds as compared to 29 days in traditional drying of cones in sun. There was no negative effect on germination percent of extracted seeds by different methods, highest germination percentage of 71.8 % was recorded when seeds were extracted by traditional method of sun drying followed by T_3: dipping of cones in water for 10 minutes and oven drying at 20^{o}C (70.25%) and T_1: drying of cones at 20^{o}C (69.75%) respectively. Highest percentage of (12.51%) seeds extracted on daily basis was recorded when cones were dipped in water for 10 minutes followed by drying at 30^{o}C.

Keywords: Blue pine, Cones, Extraction, Germination, *Pinus wallichiana*, Seed.

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Introduction

The blue pine (*Pinus wallichiana* A.B. Jackson) is a tall evergreen tree with spreading or drooping branches, found in the Himalayas from Kashmir to Bhutan at altitudes ranging from 1,800 to 3,700 m [1, 2]. In the western Himalayas the tree is gregarious at an altitudinal ranges between 1,800 and 2,500m, often forming extensive pure crops, owing to its capacity to come up in dense even aged masses [3]. The bark is smooth and resinous in young stem, turning grey and corky with shallow fissures with maturity. The blue pine timber is next to deodar in commercial importance. The sapwood is whitish, the heartwood light pink to red with darker striations, resinous, straight and fairly even-grained, medium fine textured, soft and moderately heavy [4]. The timber finds many uses; it is used for internal fittings of residential houses, such as planking, door and window frames, panels and furniture [5]. The wood after treatment is commonly used for making packing cases, camp furniture, drawing boards, fermentation vats and lorry bodies and railway sleepers [6]. Wood is also utilized for making cheap pencils, battery separators, violins and match-boxes, the stumps are used for burning as torches. Wood is a good fuel and yields excellent charcoal, with calorific value 4,995 cal [7]. The species produce good seed crop after every 4-5 year. The cones should be collected at proper time otherwise they burst on the trees and only empty raches/cones left on the tress [8]. The seeds extraction from cones of many species requires drying either natural or artificial heat sources and number of methods was employed for seeds extraction from such cones *viz*., Oven drying, Sun drying and drying of fruits/cones with artificial heat [9]. However, the drying of fruits/cones in heated kilns may be necessary for cool moist climate species where the climate in not suited for air drying [10]. It may also be necessary for a few refractory species which will not respond to sun drying even in a dry climate [11]. It is used most often for the cones of coniferous species [12]. The main difficulty in drying cones by natural method is the possibility of losing the viability seeds due to moisture lose and temperature variation [13]. However some species like *Casuarinas* may be readily extracted seeds by sun drying [14]. But some conifers have serotinous cones which require the high temperature of kiln (or in nature, bush fire) before they open [15].

In general, serotinous cones and fruits are morphologically dehiscent but their dehiscence requires an exceptionally high temperature in nature encountered during forest fires. In serotinous species cone scales remain closed due to sealing with resin. During exposure to high temperature the resin melts and cones open. Many

serotinous cones and fruits open upon exposure to temperature of at least 70°C to 80°C for several hours [16, 17] kiln opening of large quantities of cones [18, 19]. The *Pinus wallichiana* produce serotinous cones and requires well organized seed extraction operation/methods for complete extraction without losing the viability of seeds. Thus it is one of commercial issue in *Pinus wallichiana* seed production is the effectiveness of seed extraction from the cones and its effect on seed quality parameters. It is therefore, imperative to develop reliable techniques for quick and easy extraction of seeds from the cones of *Pinus wallichiana* without compromising the seeds value.

Material and Methods

The present study was conducted in the laboratory of Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Shalimar, Srinagar, Jammu and Kashmir. In this experiment the mature cones were collected during second fortnight of September from phenotypically superior trees of *Pinus wallichiana* from different sites and altitudes. The cones were packed in gunny bags and were bought to the laboratory. The seed extraction method [20] in serotinous cones of *Pinus roxburghii* were modified for this species as T_1: (Continuous heating at 20°C for 3, 6, 9 and 12 hours followed by shaking for 15 seconds), T_2: (Continuous heating at 30°C for 3, 6, 9 and 12 hours followed by shaking for 15 seconds), T_3: (Dipping in water for 10 minutes followed by drying at 20°C for 3, 6, 9, 12 hours followed by shaking for 15 seconds), T_4: (Dipping in water for 10 minutes followed by drying at 30°C for 3, 6, 9, 12 hours followed by shaking for 15 seconds) and T_5: (Drying in the sun during day followed by shaking for 15 seconds -control). The experiment was replicated four times with twenty cones per replications per treatment. The heating of the cones were done by rotator fan oven with constant temperature as per the methodology in all treatments except control. Artificial barrier was created between cones in every tray to prevent movement of seeds from one cone to another. Shaking of cones was done by hand for about 15 seconds every day on each treatment to release free seeds. Number of seeds extracted on every day was recorded till all the seeds were removed from the cones. The process of heating, shaking and wetting (wherever applicable) continued till all the seeds removed from cones.

The viability was determined by immersing seeds in 0.5% aqueous solution of 2,3,5, triphenyltetrazolium chloride (Tz). The moisture content was determined by toluene distillation method given by International Seed Testing Association (2011). To examine viability and moisture content 30 seeds per replication (in triplicate) were used. For germination test, seeds were disinfected with 0.04% $HgCl_2$ (1 min), washed thoroughly with double distilled water (DW) and placed in Petri dishes, lined with Qualigens (615 A) filter paper (3 replications/treatment, 50 seeds/replication). The Petri dishes were placed inside germinator (25.0±1.0ºC) in complete randomized design and monitored daily. The filter papers were moistened daily using DW. Seeds were considered germinated upon the radical emergence reached twice the seed size. The data were recorded daily upto 28 days to calculate germination per cent and other parameters (AOSA, 1981-1984). Various parameters of seed germination, *viz*., germination value (GV), germination energy (GE) and germination value index (GVI) were calculated. The GV which is an index combining speed and completeness of seed germination was calculated as GV=PV×MDG [21], where, PV=Peak value of germination and MDG=Mean daily germination or final daily germination speed and germination energy (GE) was calculated on the basis of the percentage of the total number of seeds that had germinated when the germination reached its peak generally taken as the highest number of germination in 24 hours period. While as the germination value/energy index (GVI) can be computed using the formula of Grouse and Zimmer [22] with some modifications.

$$GEI = A_1 + (A_1 + A_2) + A_1 + A_2 + A_3) + \ldots (A_1 + A_2 + A_3 \ldots.An)\ X \quad \frac{100}{N+ n}$$

Where, A_1, A_2, A_3…… An, are the numbers of seeds newly germinated on n^{th} day respectively, N is the total number of seeds used for experiment and n is the number of days of observation. The data were analyzed statistically using MS Excel 2000. The two years' results were subjected to Analysis of Variance (ANOVA) technique [23]. To determine significant difference among mean values of the various treatments, the Duncan Test (P<0.05) was used for cumulative quantity of seeds extracted on different days in each method.

Results and Discussion

The phenological observations revealed that leaf initiation of *Pinus wallichiana* starts in April at lower altitudes and in May at higher altitudes and leaf fall occurs throughout the year under temperate conditions of Kashmir valley in the

species. The average height (28.31m), DBH (42.89cm), crown depth (20.91m), crown spread (3.32m), basal area (1610 cm^2), needle length (13.31cm) and 5 needles in spur were also recorded during investigation. The mature cones (**Figure 1**) recorded average length (15.15cm), diameter (4.72mm), weight (88.69g), specific gravity (0.90cc), number of seeds/cone (101.29) and number of scales/cone (114.11) at maturity during 2nd fortnight of October. The freshly collected seeds showed 95.0 % viability and 17.61 % moisture content under laboratory conditions [24].

Figure 1 Mature cone

The results revealed (**Table 1**) that that maximum cumulative number of 39.46 per cent of seeds were extracted when the cones were dipped in water for 10 minutes followed by drying at 30°C (T_4) which was closely followed by T_3 (30.10%). The significantly least value 1.98 per cent was recorded when cones were dried in sun T_5 (control). However, the maximum cumulative number of seeds 82.34 per cent was extracted in 11 days period (D_{11}.) This was followed by 78.52 on D_{10}. The significantly least value of 0.00 per cent and 2.16 per cent were recorded on D_1 and D_4, respectively. The result pertaining to interaction effect (TxD) on seed extraction methods presented in Table 1 reveals that 100 per cent seeds were extracted (dipping in water for 10 minutes followed by drying at 30°C) when cones were treated for 08 days period (T_4D_8) (**Figures 2** and **3**).

Table 1 Effect of different seed extraction methods on cumulative seed number extracted (% basis) on various days in *Pinus wallichiana* (pooled over data 2011-2012)

Treatment	**Days**										
	D_1	**D_2**	**D_3**	**D_4**	**D_5**	**D_6**	**D_7**	**D_8**	**D_9**	**D_{10}**	**D_{11}**
T_1 Heating	0.00	0.00	0.00	0.00	0.00	4.98	12.77	42.47	72.29	86.72	100.00
T_2	0.00	0.00	0.00	0.06	13.38	26.49	39.30	78.95	100.00	-	-
T_3	0.00	0.00	0.00	2.46	25.44	44.01	72.90	96.01	100.00	-	--
T_4	0.00	0.00	0.00	8.28	39.85	76.59	90.96	100.00	-	--	-
*T_5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.52	3.68	5.88	11.72
Means	**0.00**	**0.00**	**0.00**	**2.16**	**15.73**	**30.41**	**43.19**	**63.59**	**55.19**	**18.52**	**22.34**
	SE(m)	**C.D. (P≤0.05)**									
Treatment	**0.18**	**0.51**									
Days	**0.27**	**0.76**									
Treatment × days	**0.61**	**1.70**									

*Note: Drying in the sun required 29 sunny days for complete seed extraction

The pooled data showed that no seed could be extracted during the first seven days by the traditional method of drying cones in the sun (T_5D_8). Continuous drying of cones under controlled conditions at a temperature of 20°C (T_1) and 30°C (T_2) in the oven required 11 days (D_{11}) and 9 days (D_9) period, respectively for extraction of all seeds from the cones. The treatment combination T_3D_9 and T_4D_8 are significantly (P<0.05) superior to the treatment combination of T_1D_{11} and T_2D_9 as former treatment combinations required slightly lesser number of days for seed extraction. The cones of *Pinus wallichiana* are serotinous in nature which requires high temperature for their morphologically dehiscence. The sealing of hard scales in cone of blue pine is due to presence of resin and the exposure to high temperature results in melting of resin and cones open for dehiscence of seeds. Cone drying by artificial heat should

be carried out in such a way as to obtain drying in the shortest possible time without damaging the viability of the seed. The results are in consonance with those of Kumar *et al.* [20] who reported that wetting of cones for 10 minutes by submerging them in water followed by drying at 60^0C in 24 hour cycle was a safe procedure for quick extraction of seed from cones of *Pinus roxburghii.*

Figure 2 Freshly collected seeds

Figure 3 Empty cone after seed extraction

The results are also supported by Aldhous [25] that safe temperature for all cones is about 30^0C, rising to 60^0C when the moisture content of the cone is below 10 per cent for seed extraction. The deleterious effect of high temperature on seed values have also been reported by various researchers [26]. The control treatment i.e. sun drying regime which takes approximately 29 sunny days for complete extraction of seeds. The results regarding the rate of seed extraction presented in **Table 2** showed the trend of $T_4 < T_3 < T_2 < T_1 < T_5$ for days taken in seed extraction. The maximum rate of seed extraction 12.51 per cent/day was recorded when the cones were dipped in water for 10 minutes followed by drying at 30^oC (T_4), which is closely followed by T_3 and T_2 having 11.12 and 11.11 per cent rate of seed extraction respectively. However, the minimum rate of seed extraction of 1.08 per cent was observed when the cones were dried in the sun (control T_5). The study is in accordance [27] that moisture of less than 50 per cent and seed extraction temperature of 35^oC was recommended in *Pinus strobes* cones.

Table 2 Effect of seed extraction methods on the rate of seed extraction in *Pinus wallichiana*

Treatment	Rate of seed extraction (%)/day
T_1	9.08±0.005
T_2	11.11±0.005
T_3	11.12±0.004
T_4	12.51±0.004
T_5	1.08±0.005
C.D.($P \leq 0.05$)	0.06

The present investigation on Blue pine indicated that seed extraction methods exert significant ($P<0.05$) influence on number of seed extracted on cumulative basis and on its germinability. The results (**Table 3**) showed that moisture lose of 3.35% against the control and treatment T_1 (14.80%), T_2 (13.56%), T_3 (14.68%) and T_4 (13.43%) are significantly ($p<0.05$) at par to each other. Similarly, Lodgepole pine required 15.5-18.0% moisture to release seeds from cones [26]. The similar trend shown by viability (**Figures 4** and **5**) with maximum at T_3 and T_4 (90%) followed significantly by T_2 (88.50%) and T_1 (88.34%) in the species. However, the results regarding the affect of seed extraction on germinability (**Figure 6**) under laboratory condition revealed that T_4 showed maximum germination (70.80%), germination value (35.19), germination energy (75.60) and germination value index (60.12) significantly ($p<0.05$) followed by T_3 treatment. The present study of Dipping in water for 10 minutes followed by drying at 30^oC for 23:50 hours followed by shaking for 15 seconds was effective in improve ultimate seed extraction. However, it was also effective in terms of germination and other parameters. The minimum values of these attributes were recorded at T_5 in the species. The moistening cones prior to heat treatment to assist resin bond breakage and cone scale refluxing and seed releases when heat is applied has been considerably recorded by other workers also [12, 28].

Table 3 Effect of seed extraction methods on moisture and germinability of *Pinus wallichiana* seeds

Treatment	Moisture per cent	Viability per cent	Germination per cent	Germination value	Germination energy	Germination value index
T_1	14.80	88.34	69.75	26.80	73.20	56.60
T_2	13.56	88.50	68.50	26.72	73.30	56.60
T_3	14.68	90.00	70.25	34.90	74.95	59.73
T_4	13.43	90.00	71.80	35.19	75.60	60.10
T_5	16.78	75.34	68.50	21.57	70.12	52.18
C.D. (P≤0.05)	1.72	3.32	2.27	1.25	0.80	0.90

Figure 4 Viable seeds

Figure 5 Floating test in seeds

Figure 6 Germination in seeds

Conclusion

The traditional practice of extracting seeds from cones of *Pinus wallichiana* consists of sun drying and takes about four weeks of sunny days. A new technique (soaking of cones in water for 10 minutes followed by drying at 30^{o}C) has been developed which achieves complete seed extraction only in eight days. The technique showed no adverse effect on seeds quality and germinability parameters of the species.

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