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# Effect of different seed extraction methods for rapid extraction of seeds from the cones of Aleppo pine (*Pinus halepensis*) under controlled conditions in Kashmir Valley, India

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**Abstract**
The mature cones of Aleppo pine were collected during the first fortnight of March from Shankar Acharyas hill forest of Kashmir valley. The cones were packed in gunny bags and were brought to the laboratory of Faculty of Forestry, SKUAST-K, Shalimar. The 15 cones per experimental unit in three replications were used. Oven with rotator fan was used for the cones subjected to various seed extraction methods ($T_1$-$T_6$) at constant temperatures. Analysis of variance was carried out using completely randomized design. The seeds extracted by the different methods ($T_1$-$T_7$) were subjected to germination test in the laboratory. The experiment was laid out in completely randomized design. Among different seed extraction methods, the maximum seed extraction (per cent basis) in minimum number of days was recorded as 74.23 per cent and 63.03 per cent under treatment $T_6$ (alternate dipping for 10 minute followed by drying at 50 $^{O}$C) and $T_3$ (dry heating at 50 $^{O}$C for 24 hours), respectively. The significantly minimum rate of seed extraction of 1.12 per cent was observed when the cones were dried in the sun ($T_7$). The almost similar trend was observed in both the years of investigation i.e. 2009 and 2010 (Table-1).

**Keywords:** Aleppo pine, seed extraction. Seed treatments, temperature and percent extraction

## Introduction
Aleppo pine is a small to medium sized tree reaching 15-25 m tall and with a trunk diameter of up to 60 cm exceptionally up to 1 m. The bark is an organ red, thick and deeply fissured at the base of the trunk and flanky in the upper crown, the leaves (needles) are very slender 6-12 cm long distinctly yellowish green. Aleppo pine is widely planted for timber in its native areas. It is also a popular ornamental tree extensively planted in parks and gardens in hot dry areas. It is also extensively grown as wind break and for soil conservation. A resin from the trunk is used for chewing and flavoring wine. The turpentine obtained from the resin is antiseptic, diuretic, rubefacient and vermifuge. It is also used for complaints of respiratory systems, kidney and bladder complaints (Grieve, 1984) [6]. Drying using either natural or artificial heat sources, must be used in the extraction of seeds of many important tree species and is almost always used for the cones of pines and other conifers (Turnbull, 1975) [12]. Drying of cones in heated kilns may be necessary for a variety of species where the climate is not suited to air drying i.e. in cool moist climates. It may also be necessary for a few refractory species which will not respond to sun-drying even in a dry climate. Some conifers have serotinous cones which require the high temperature of a kiln before they open (Turnbull and Martensz, 1983) [13]. In general, serotinous cones and fruits are morphologically dehiscent, but their dehiscence requires an exceptionally high temperature, in nature encountered during forest fires. Serotinous cones occur in many pine species, *Pinus halepensis* is one such species. In these 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 temperatures of at least 70 $^{O}$C to 80 $^{O}$C for several hours (Schmidt, 2000) [8]. An increase in the humidity of the air may cause a re-closing of the cones. Artificial heating in contrast permits control of air, moisture and temperature. 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 safe temperature for all cones is about 30 $^{O}$C, rising to 60 $^{O}$C when the moisture content of cones is below 10 per cent (Aldhous, 1972) [1]. The present investigation on Aleppo pine indicated that seed extraction methods exert significant influence

on number of seed extraction on cumulative basis and on germination parameters. It was evident from the data in Table-4 that for achieving complete seed extraction, the drying cones at 30 $^{O}$C, 40 $^{O}$C and 50 $^{O}$C required merely more number of days than the alternate wetting and drying procedures at the same temperatures.

## Materials and Methods

The experimental site is located between 34.08$^{O}$N latitude and 74.83$^{O}$E longitude at an altitude of about 1587 m above mean sea level whereas the average altitude of Kashmir valley (valley zone) ranges between 1500 to 2300 m above mean sea level. The minimum and maximum temperature ranged between–4.41 $^{O}$C in January to 29.34 $^{O}$C in July respectively. The average annual precipitation ranges between 949 –1,100 mm mostly in the form of snow, which covers the mountainous belts for 160-195 days/year (Baba and Mohit, 2017). Mature cones were collected during the first fortnight of March from well-grown trees of *Pinus halepensis* in Shankaracharya hill forest (Srinagar). The cones were closed at that time. The cones were packed in gunny bags and were brought to the laboratory of Faculty of Forestry, SKUAST-K, Shalimar. Forty-five cones, consisting of 15 cones per experimental unit in three replications were used per treatment. Oven with rotator fan was used for heating the cones at constant temperature in treatments $T_1$ to $T_6$. Inside the oven, cones were not enclosed in bags; artificial barriers were created between cones in every tray to prevent movement of seeds from one cone to another. Shaking of cone was done by hand for about 15 seconds every day on each treatment to release the 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 the cones. These results were in harmony with the works done by Seward (1980) [9] who reported that the cones of *Pinus patula, Pinus elliotii* and *Pinus taeda* require sun drying on large scale in open sides shed covered with clear plastic roofs supporting on wire netting. The results also get support from Turnbull and Martensz (1983) [13] who reported that the cones of *Casuarina* require the higher temperature of kiln. In general the present findings are in consonance with those of Kumar *et al.* (2006) [7] who reported that the wetting of cones for 10 minutes by submerging them in water followed by drying at 60 $^{O}$C in 24 hours cycles was a safe procedure for quick extraction of seeds from the cones of *Pinus roxburghii.* The findings are thus in accordance with the results of Goor and Basney, 1976 in Mediterranean pines, Gera *et al.* (2003) [4] in *Pinus roxburghii* and Stein *et al.* (1974) [11] in number of forest tree species. Artificial heating requires expensive equipments and installations which are not used throughout the year. This makes their unit cost exceptionally high. A very careful appraisal must therefore, be made of the capital cost before establishing large permanent kiln (FAO, 1985) [12]. Since hot air ovens or kilns are not available in most of the nurseries in India, drying of cones at controlled temperature is not feasible. There is however, a scope for adapting the best procedure found in this study to many nursery conditions. There was significant increase in number of seeds extraction on cumulative per cent basis as the temperature was increased from 30 $^{O}$C to 50 $^{O}$C. For complete seed extraction, drying cones at 50 $^{O}$C ($T_3$) required merely two days more than the alternate wetting and drying procedure at the same temperature. Same was the case with cones subjected to 30 $^{O}$C and 40 $^{O}$C for complete seed extraction. However, the alternate wetting and drying procedure of cones at 30 $^{O}$C ($T_4$) and 40 $^{O}$C ($T_5$) required less number of days for achieving complete seed extraction, the procedures also significantly produced the more number of seeds on cumulative basis. For achieving complete seed extraction, drying cones at 50 $^{O}$C ($T_3$) required merely two days more than the alternate wetting and drying procedure at same temperature ($T_6$). However, the former is less suitable due to the possibility of greater damage to seeds at higher temperature. Treatment combination $T_4D_8$, $T_5D_7$ and $T_6D_5$ are significantly superior to the treatment combination of $T_1D_{12}$, $T_2D_{10}$ and $T_3D_6$ as the former treatment combinations required slightly lesser number of days for seed extraction.

**Table 1:** Effect of seed extraction methods at various number of days on cumulative number (per cent basis) of seed extracted during the year 2009 and 2010 (Average)

| Days/Treatment | Per cent extraction (cumulative) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ | $D_7$ | $D_8$ | $D_9$ | $D_{10}$ | $D_{11}$ | $D_{12}$ | Mean |
| Heating at 30$^{O}$C for 24 hrs | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 4.21 (9.68) | 16.75 (24.12) | 39.47 (38.90) | 70.62 (57.18) | 89.53 (71.39) | 98.42 (85.81) | 100.00 (89.96) | **31.42** |
| Heating at 40$^{O}$C for 24 hrs | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.99 (2.35) | 15.16 (22.64) | 35.50 (36.42) | 71.13 (57.87) | 88.54 (70.87) | 96.63 (82.53) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | **45.21** |
| Heating at 50$^{O}$C for 24 hrs | 0.00 (0.00) | 0.00 (0.00) | 14.33 (22.16) | 53.46 (46.99) | 83.40 (66.27) | 95.42 (81.18) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | **63.03** |
| Dipping for 10 min followed by dry at 30$^{O}$C | 0.00 (0.00) | 0.00 (0.00) | 2.56 (6.41) | 19.49 (26.12) | 40.67 (39.59) | 88.40 (70.14) | 99.32 (87.24) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | **56.61** |
| Dipping for 10 min followed by dry at 40$^{O}$C | 0.00 (0.00) | 0.00 (0.00) | 6.99 (15.30) | 65.08 (53.79) | 91.28 (73.33) | 97.55 (83.59) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | **63.81** |
| Dipping for 10 min followed by dry at 50$^{O}$C | 0.00 (0.00) | 16.86 (23.96) | 80.35 (64.01) | 96.87 (83.10) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) | **74.23** |
| Sun Drying | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.51 (1.69) | 1.18 (2.57) | 7.02 (13.87) | 10.02 (16.59) | **2.89** |
| Mean | 0.00 | 3.42 | 15.41 | 30.33 | 41.68 | 52.99 | 62.73 | 67.09 | 71.61 | 74.82 | 78.50 | 79.48 | |
| CD ($P \leq 0.05$) | Treatment | (T) | = | 0.845 | Days | (D) | = | 1.107 | Days x Treatment | (D x T) | = | 2.928 | |

Figures in parenthesis are arc sine transformed values.

** Value calculated on the basis of total number of seeds extracted at the end of each treatment

**Note**: Drying in the sun required 36 sunny days for complete seed extraction.

**Table 2:** Effect of seed extraction methods on the rate of number of seed extraction in *Pinus halepensis* during the year 2009 and 2010 (Average)

| Treatment | Rate of seed extraction |
|---|---|
| $T_1$ (Heating at 30$^{O}$C for 24 hrs.) | 8.83 |
| $T_2$ (Heating at 40$^{O}$C for 24 hrs.) | 10.55 |
| $T_3$ (Heating at 50$^{O}$C for 24 hrs.) | 15.47 |
| $T_4$ (Dipping for 10 min followed by dry at 30$^{O}$C) | 13.69 |
| $T_5$ (Dipping for 10 min followed by dry at 40$^{O}$C) | 15.47 |
| $T_6$ (Dipping for 10 min followed by dry at 50$^{O}$C) | 22.50 |
| $T_7$ (Sun drying) | 1.12 |
| CD ($P \leq 0.05$) | 0.576 |

**Table 3:** Variation in rate of seed extraction (number of seeds) during different methods of seed extraction

| Treatment | 2009 | 2010 |
|---|---|---|
| $T_1$ | 9.09 | 8.58 |
| $T_2$ | 11.10 | 10.00 |
| $T_3$ | 16.66 | 14.28 |
| $T_4$ | 14.28 | 13.09 |
| $T_5$ | 16.66 | 14.28 |
| $T_6$ | 25.00 | 20.00 |
| $T_7$ | 1.12 | 1.12 |
| C. D. 0.05 | 0.251 | 0.984 |

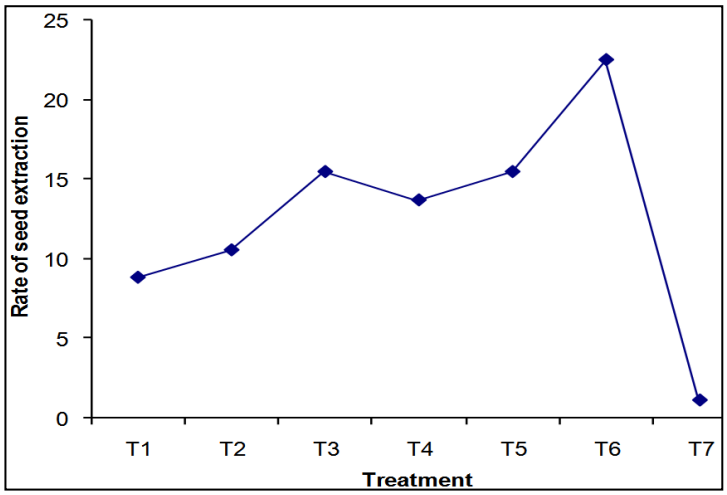


**Fig I:** Effect of seed extraction methods on the rate of seed extraction in *Pinus halepensis*.

## Results and Discussion

The conventional practice of extracting seeds from cones of *Pinus halepensis* consists in drying of cones in the sun. The process ($T_7$) takes about five weeks for complete seed extraction. Among different seed extraction methods, the maximum seed extraction (per cent basis) in minimum number of days was recorded as 74.23 per cent and 63.03 per cent under treatment $T_6$ (alternate dipping for 10 minute followed by drying at 50 $^{O}$C) and $T_3$ (dry heating at 50 $^{O}$C for 24 hours), respectively (Table-1). The rate of seed extraction was in the order of $T_7 < T_1 < T_2 < T_4 < T_3 < T_5 < T_6$. However, for achieving the complete seed extraction, the drying of cones at 30 $^{O}$C ($T_1$), 40 $^{O}$C ($T_2$) and 50 $^{O}$C ($T_3$) required merely more number of days as compared to the procedure ($T_4$, $T_5$, $T_6$) of alternate wetting and trying at the same temperature. As far as the procedure of extracting maximum number of seeds in minimum number of days and having maximum germination per cent was concerned, a new technique has been developed which achieves seed extraction in eight days. The new technique ($T_4$) involves dipping of cones in water for 10 minutes followed by drying at 30 $^{O}$C for 23:50 hours and shaking daily. However, the process takes about five weeks for complete seed extraction. Hence, the $T_4$ method of dipping of cones in water for 10 minutes followed by drying at 30 $^{O}$C for 23:50 hours and shacking daily was a safe procedure for quick extraction of seeds from the cones of *Pinus halepensis.* Although drying of cones at high temperature of 50 $^{O}$C and 40 $^{O}$C procedure produced the more number of seeds in minimum number of days. However, there was a significant decrease in the germination per cent as the temperature was increased from 30 to 50 $^{O}$C (Table-2). Hence $T_4$ method which require wetting of cones for 10 minutes by submerging them in water followed by drying at 30 $^{O}$C in 24 hours cycles was a safe procedure for quick extraction of seeds from the cones of *Pinus halepensis.* The results also revealed that the rate of seed extraction (Table-2) on the basis of number of seeds was in the order of $T_7 < T_1 < T_2 < T_4 < T_3 < T_5 <$ T6. Seed extraction by conventional method of drying cones in the sun ($T_7$) required 36 sunny days for almost complete seed extraction. The significantly maximum rate of seed extraction (22.50%) was recorded when the cones were dried at 50 $^{O}$C with alternate wetting and drying procedure ($T_6$). While as the minimum rate of seed extraction of 1.12 per cent was observed during sun drying ($T_7$) (Table-2). However, the later procedure (sun drying) was suitable due to the possibility of minimum damage to seeds because of low temperature. Similarly the results of the experiment regarding the rate of seed extraction presented in Table-2, Fig. I shows that the rate of seeds extraction was in the order of $T_7 < T_1 < T_2 < T_4 < T_3 < T_5 < T_6$. The significantly maximum rate of seed extraction 22.50 per cent was recorded when the cones were dipped in water for 10 minutes followed by drying at 50

$^{O}$C for 23:50 hours ($T_6$) which is closely followed by $T_5$, $T_3$ having 15.47 per cent rate of seed extraction each and $T_1$ (8.83%) giving values in descending order. The treatment $T_3$ (continuous heating at 50 $^{O}$C for 24 hours) was however statistically at par with $T_5$ (dipping in water for 10 minutes followed by drying at 40 $^{O}$C for 23:50 hours). The almost similar trend was observed in both the years of investigation i.e. 2009 and 2010 (Table-3).

**Conclusion**

The germination percentage of freshly collected seeds stored under ambient room temperature decreased as the storage period was extended from 15 to 90 days. However, the un-germinated seeds showed high viability of seeds (79.20-80.08%). Submerging of *Pinus halepensis* cones in water for 10 minutes followed by drying at 30 $^{O}$C and shaking for few seconds, in 24 hours cycles, is recommended for quick extraction of seeds. The procedure requires eight days for complete extraction of seeds from the cones. This is in contrast to five weeks period required by the conventional method of exposing cones to the sun. The new procedure has not shown any adverse effect on seed germination.

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