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Amerjeet singh
Faculty of Forestry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, J&K India

Mohit Husain
Faculty of Forestry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, J&K India

Asif Ali Gatoo
Faculty of Forestry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, J&K India

Mohd Tariq
Faculty of Forestry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, J&K India

Rafiq A Mir
Faculty of Forestry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, J&K India

Correspondence
Mohit Husain
Faculty of Forestry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, J&K India

Effect of seed storage period under ambient room temperature on seed germination and viability under laboratory conditions in Kashmir Valley, India

Amerjeet singh, Mohit Husain, Asif Ali Gatoo, Mohd Tariq and Rafiq A Mir

Abstract
This study was undertaken in the Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar. This study deals with effect of seven storage periods/ treatments at room temperature (0, 15, 30, 45, 60, 75 and 90 days) on germinability and viability of Allepo pine seeds. The experiment consisted of seven treatments with four replications each and was laid out in completely randomized design in laboratory. The fresh Allepo pine seeds collected possess high viability (79.20 to 80.08%) (Table-1) but low germinability. A maximum of 40.37 per cent germination was observed in mature seeds which declined to 33.25 (Table-1) per cent at the end of 90 days storage period. This indicated that Allepo pine seeds do possess inherent dormancy which further deepened with storage.

Keywords: seed, seed dormancy, viability and seed germination

Introduction
The allepo pine (*Pinus halepensis*) is native to the Mediterranean region. It is generally found at low altitudes mostly from sea level to 200 m but even grow at an altitude of up to 1000 m to 1700 m. Allepo 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. Exotic pine can play the role of restoration of degraded areas and fulfill the local need because of their fast growth rate, long fibered pulp and high volume per hectare. Allepo pine is an exotic conifer/ pine species in India and has been successfully introduced in Kashmir valley. Although the exact date of its introduction has not been ascertained but the tree has been found growing in Shankaracharya reserve forest (Srinagar), situated at an altitude of 1,970 m above MSL. Allepo pine is widely planted for timber in its native areas. 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) [5]. The optimal time to harvest is when a large amount of viable germinable seeds can be collected. 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. There are several factors which effect the viability of seeds in storage. Temperature and moisture are the most critical factors which regulate the seed viability. Seed viability is best retained at low temperature and high concentration of CO_2 (Hadidi, 1996) [7].

Materials and Methods
The valley of Kashmir is situated in western Himalayan range, extends between 32°-20' to 34°-54' N latitude and 73° - 55' to 75° - 35' E longitude. The total geographical area of the valley is about 159480 km^{-2} with the total forest area of 8126 km^{-2} (50.95 %), with an average altitude of 1850 m asl. 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. 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. Seed viability was determined by collecting freshly harvested mature seeds. The seeds were divided into six seed lots and were stored in perforator poly bags at ambient room temperature. The mean room temperature ranged from 2.5 ^{O}C to 22.5 ^{O}C.

Each seed lot were put to germination test at an interval of 15 days. Taking first test on the date of collection (15th March). For the germination test, four replications of 100 seeds for each treatment were used. The seeds were placed on moist filter paper in Petri dishes and kept in BOD incubator maintained at 20±1 ^{O}C. The emergence of the radical was taken as the criterion for germination of seed. The germination per cent was calculated as the number of seeds tested and the number of seeds germinated, expressed in percentage.

Seed viability test was conducted by employing the commonly used tetrazolium test which involves submerging seeds in 1.0 per cent solution of colourless 2, 3, 5 triphenyl tetrazolium chloride. Reduction of this compound to red coloured formazan by the dehydrogenase of viable seeds was an indication of living tissue. This makes it possible to distinguish the red coloured living parts of seed from the colourless dead ones (ISTA, 1993) [8].

The Allepo pine seeds after preconditioning (nipping of seed coat) and preparation were soaked in 1.0 per cent aqueous solution of 2, 3, 5- triphenyl tetrazolium chloride and kept in a hot air oven maintained at 40±2 ^{O}C for 2 hours for staining. The viable and non-viable seeds were grouped as per ISTA (1993) [8] rules.

Germination per cent

Germination per cent was calculated as the number of seeds sown and the number of seeds germinated, expressed in percentage.

Germination capacity

The cumulative number of seeds that germinated during the 28 days incubation period plus the number of viable seeds at the end of the test expressed as percentage of seeds kept for incubation.

Germination energy (GE)

Germination energy 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 a 24 hour period.

$$\text{Germination energy} = \frac{\text{No. of seeds germinated upon the time of peak germination}}{\text{Total no. of seeds sown}} \times 100$$

Germination rate/speed

Germination speed was worked out by the method prescribed by Maguire (1962) [9].

Speed of germination = $\sum (n/t)$

Where,

n = Number of seeds newly germinated at time 'i'

t = Number of days from sowing

Germination value (GV)

Germination value is an index combining speed and completeness of seed germination. Daily germination counts were recorded and germination value was calculated as per Czabator (1962) [3]

GV = PV x MDG

Where, PV = Peak value of germination, MDG = Final mean daily germination

Seed dormancy

High germinability and viability is an imperative physiological criteria of seeds that are subjected to long storage. Keeping this in mind the *Pinus halepensis* fresh seeds (mature) were collected and tested for finding their germinability under laboratory conditions. It was evident from the results that fresh by collected Allepo pine seeds possess high viability (80.08%) but low germinability due to inherent dormancy prevalent in the seeds. It was quite apparent from the data that poor germination success of 40.37 per cent was resulted when fresh seeds were used under laboratory conditions. The problem of low germinability but high viability (79-80%) thus indicates that Allepo pine exhibited internal dormancy in fresh seeds. The results are also in harmony with the finding of Veena (2005) [14] in *Pinus gerardiana* who reported that freshly collected chilgoza seeds failed to germinate even in 28 days period of germination when subjected to test under laboratory conditions.

This has also been demonstrated by Malik (2007) [10] who reported that the freshly collected chilgoza seeds showed poor germination success of 25.33 per cent along with low germinability parameters.

Seed storage

The data presented in Table-1 shows that there was significant decrease in germination percentage as the storage period increased from 15 to 90 days. The germination of freshly collected seeds of *Pinus halepensis* was 40.37 per cent which declines to 39.75 per cent after 15 days in the controlled conditions. The germination at the end of 90 days decline to as low as 33.25 per cent. The loss of germinability during storage at room temperature may be due to much loss of moisture content, wide temperature fluctuation and high activity of mycoflora and fauna present in the seeds which start feeding on endosperm rendering the seeds non-viable. The results thus get support from the work of Tamta *et al.* (2001) [13] who reported that the germination of freshly collected seeds of *Cupressus torulosa* was 49.3 per cent. The germination at the end of 150 days declined to as low as 18.0 per cent. The results are also in line with those of in *Pinus pinaster;* Sahi (1999) in *Acacia catechu;* Paul (2002) in *Phaseolus vulgaris.* Similar results pertaining to poor viability and germination of *Albizia lebbek, Casisia stomea* and *Prosopis specigera* subjected to room temperature was obtained by Arya and Arya (2006) [2]. The other reason for decreased viability with storage period could be associated to depletion of food reserve in seeds during storage and change in respiratory metabolism (Abdul Baki, 1980) [1]. The findings are thus in accordance with the results of Gautam and Bhardwaj (2001) [4] in *Pinus roxburghii* and Gupta and Raturi (1975) [9] who conducted viability tests in number of forest tree species.

Result and Discussion

Seed viability/ dormancy were determined by collecting freshly harvested mature seeds. The freshly mature seeds were divided into six seed lots and were stored in perforated poly bags at ambient room temperature. Each seed lot were put to germination test at an interval of 15 days viz. 0 days, 15 days, 30 days, 45 days, 60 days and 75 days to assess their effect on

viability and germination under laboratory conditions. The experiment was laid out in completely randomized design. It was evident from the results that freshly by collected Allepo pine seeds (S_1) possess high viability (80.08%) but low germinability (40.37%) due to inherent dormancy prevalent in the seeds. The problem of low germinability but high viability thus indicates that Allepo pine exhibited internal dormancy in fresh seeds. Under ambient room temperature, the germination of freshly collected seeds (S_1) was 40.37 per cent which declines to 39.75 per cent after 15 days (S_2) of storage. The germination at the end of 90 days (S_8) declined to low as 33.25 per cent. There was a significant decrease in the per cent germination as the storage period increased from 15 to 90 days (Table-1) (Fig-1).

Conclusion

Freshly collected seeds of *Pinus halepensis* possess high viability (80.08%) (Table-1) but low germinability due to inherent dormancy prevalent in the seeds. Thus indicates that Allepo pine exhibited internal dormancy in fresh seeds. 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%). The seed of Allepo pine do not have any deep dormancy but with the storage dormancy is induced which however, can be overcome by treating the seeds to moist chilling treatment (stratification with media at 2-3+1^{O}C) for a period of 60-75 days, resulting highest seed germinability under laboratory conditions.

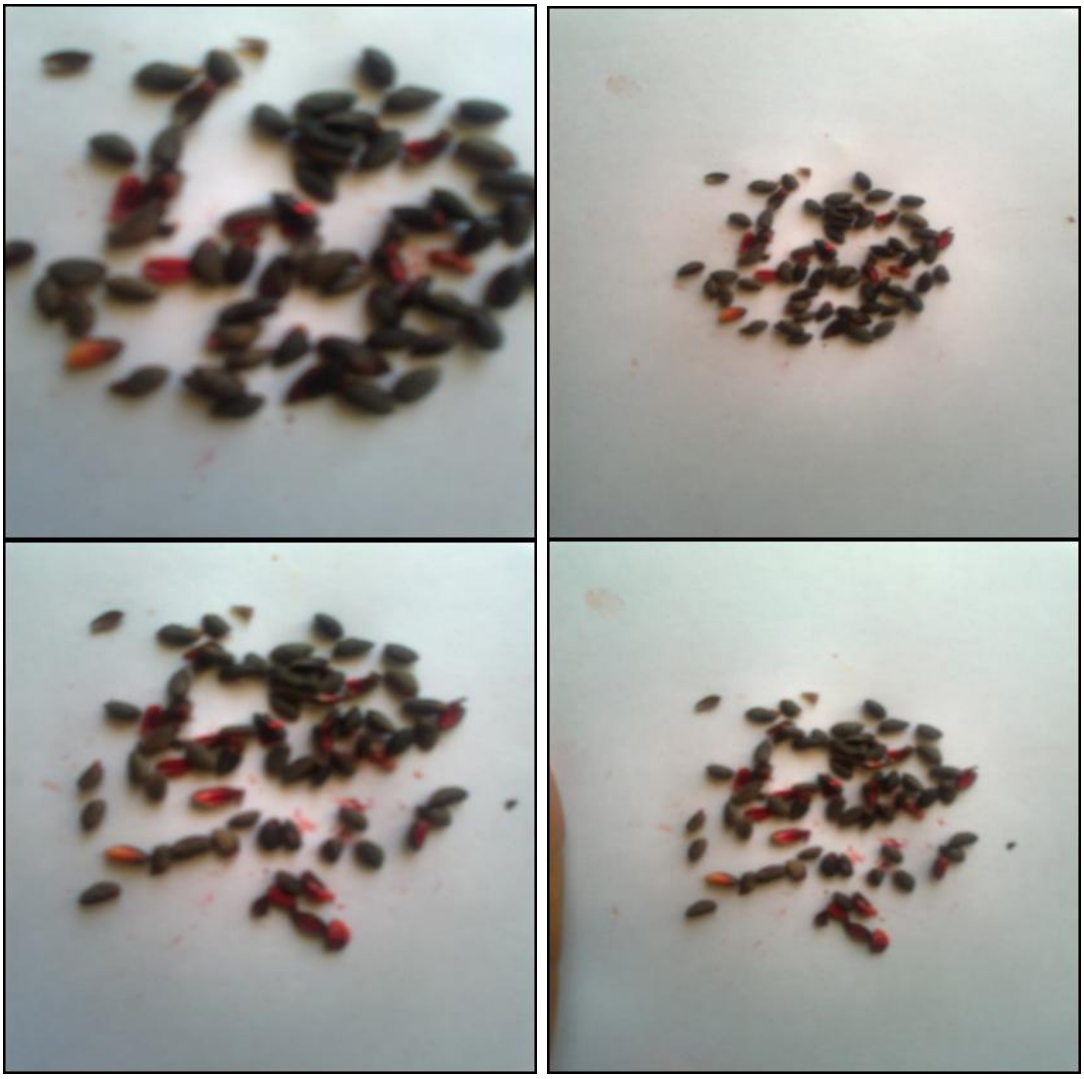

Fig 1: Tetrazolium test showing viable red coloured living seeds of Pinus *halepensis.*

Table 1: Effect of seed storage period under ambient room temperature on germination and viability per cent of *Pinus halepensis* in the laboratory during the year 2009 and 2010 (Average)

Treatment	Germination (%)	Viability (%)
S_1 (0 days)	40.37 (39.45)	80.08 (63.49)
S_2 (15 days)	39.75 (39.08)	79.87 (63.34)
S_3 (30 days)	38.87 (38.57)	79.75 (63.26)
S_4 (45 days)	37.50 (37.76)	79.59 (63.15)
S_5 (60 days)	36.62 (37.24)	79.48 (63.07)
S_6 (75 days)	34.62 (36.04)	79.35 (62.98)
S_7 (90 days)	33.25 (35.21)	79.20 (62.87)
CD ($P \leq 0.05$)	0.406	NS

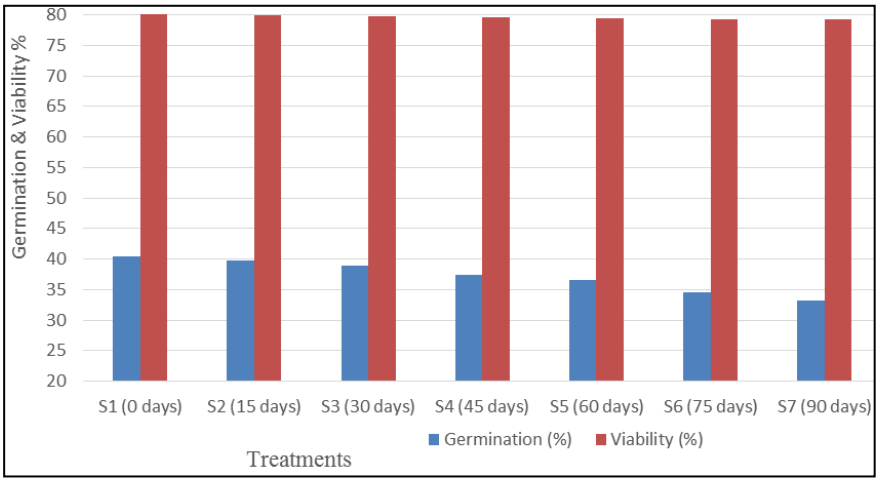

Fig 2: Effect of seed storage period under ambient room temperature on germination per cent of *Pinus halepensis* in laboratory.

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