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Cone and seed maturity indices in *Pinus wallichiana* under temperate conditions of Kashmir Himalayas, India

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Abstract: *Pinus wallichiana* (Blue pine) does not have a good seed every year and hence it becomes necessary to collect abundant quantity of seed during good seed year. It becomes necessary to know the exact time of seed maturity. To overcome this problem, the present investigation was conducted in Kashmir valley at four different altitudes and locations i.e. (1,600-2,000 masl–KFD), (2,000-2,400 masl -LFD), (2,400-2,800-PFD) and (2,800-3200 masl–SFD). The results revealed that seed collection clearly showed wide variation in the maturity of cones. Cone colour served as an indicator of maturity and it changed from light green to green and green with brown patches at maturity. Seed colour changed from whitish to light brown and dark brown at maturity. The mean cone weight (118.67-88.17gm) and specific gravity (1.13-0.90) decreased as the cones proceeded towards maturity. The mean seed weight of 21.79 to 57.13gm increased at all altitudes as the cones advanced towards maturity. Cone length, cone diameter and germination percent differed (P≤0.05) significantly between altitudes and increased when the cones advanced towards maturity. The germination per cent was recorded more at altitudinal range of 1,600-2,400 masl (67.25-70.26%) at maturity, while as it was recorded lower at higher altitudes (42.12-47.25%). It is concluded that the altitudinal range of 1,600-2,400 masl is best sites for collection of phenotypically superior seeds in terms of maximum cone length (18.18cm), diameter (5.23mm) and weight (108.94gm), number of seeds per cone (117.72), seed weight (79.99) and germinability (68.75).

Keywords: Altitude, Cone characters, Germination, Maturity, *Pinus wallichiana*, Seeds

INTRODUCTION

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. It also occurs in Balipora tract of Assam. Its bark is smooth and resinous in young stem, turning grey and corky with shallow fissures with maturity, leaves are needle like in clusters of five, abnormal fascicles containing 4, 6 or 7 needles occasionally. In the western Himalayas, the altitudinal range between 1,800 and 2,500 m the tree is gregarious, often forming extensive pure crops, owing to its capacity to come up in dense even aged masses. Conifers do not have a regular seed year and they produce good seed crop at intervals and hence, it becomes important to collect mature/ripe seeds during good seed years. The knowledge of exact time and stage of seed maturity is essential for collection of abundant quantity of healthy and vigorous seeds. The early collection may lead to the collection of immature fruit/seeds, while delayed collection always lead to loss of crop. The immature seeds have low viability and often produce low vigour and deformed seedlings (Singh and Kachari, 2006; Mirgall *et al*., 2016). The mature seeds collected retain viability longer than immature seeds (Sajad *et al*., 2016). Maturity of cones is ascertained by seed dispersal, which is the common indicator of seed maturity in conifers (Edwards, 1980). The mature seeds have higher vigour and potential for establishment of seedlings as compared to immature seeds (Sofi *et al*., 2016; Mumtaz *et al*., 2009). Fruit collection should only be started when seeds are sufficiently mature. Therefore, indicators of maturity for individual tree species are must so that collection is made at right time. Problem in conifers is that once the cone matures, they open up and the seed is dispersed, therefore mature seed collection difficult. Therefore, the quality seed harvesting requires an understanding of seed ripening and dispersal characteristics. Seed quality at the physiological level includes seed viability, germinability and vigour. The viability of seed is simply its capacity for growth and development (Malik *et al*., 2013). Seed maturity indices which include physical as well as chemical characteristics of seed/fruit are key attributes for successful collection. Therefore, indicators of maturity for individual tree species are must so that collection is made at right time. An attempt was made in the present study to find the best time of collection of mature cones of Blue pine from different

locations of Kashmir valley situated at different altitudes.

MATERIALS AND METHODS

Description of study area: The research work was conducted in Kashmir valley of India situated between 32˚17′ and 37˚6′ North latitude and 73˚26′ and 80˚36′ east longitude during the years 2011 and 2012. The climate in general is temperate type. Winter is severe extending from December to March. The region faces a wide temperature range from a minimum of -8^{0}C in winter to a maximum of 33^{0}C in the summer. Winter frost is common and medium to heavy snowfall is witnessed. The area receives an annual precipitation of 675 mm to 1193 mm.

Methodology adopted: Three different aspects were selected viz., North Kashmir, Central Kashmir and South Kashmir. Among three aspects four altitudinal sites were selected i.e. A_1;1,600-2,000 masl (Kehmil Forest Division-KFD), A_2; 2,000-2,400 masl (Lidder Forest Division-LDF), A_3; 2,400-2,800 (Pirpanchal Forest Division-PFD) and A_4; 2,800-3200 masl (Sindh Forest Division-SFD) to record the observations. The cones were collected from all the altitudes fortnightly from the month of August and continued till maturation. On each collection date 100 cones were collected from 20 phenotypically superior trees at least 100 m apart from one another and subjected to observations in the Forest laboratory of Faculty of Forestry, Shalimar campus of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir.

Observation recorded: The Ocular estimates for colour changes in cone, seed, radical and plumule were observed on each collection date. The cones were weighed (g) by using top pan balance. However, Float test or water displacement method (Barnet, 1979) was used to determine the cone specific gravity at each collection date and value was determined by the ratio of weight of cone to the weight of volume displaced by cones. Cone length of 20 collected cones in a replicated manner from each site was measured with the help of measuring tape for their maximum length in cent meter (cm) up to one decimal place, while cone diameter was recorded up to two decimal places in mile meter (mm) with the help of electronic digital Vernier calliper. The seed number per cone was determined after manual extraction of seeds from 20 cones from each site replicated four times and their seed weight (g) of 100 seeds was recorded using eight replicates of 100 seeds each with the help of sensitive top pan balance and was finally transformed into 1,000 seed weight by multiplying the weight of 800 seed by a factor of 1.25 (ISTA, 1993). Finally, the germination test was conducted at all the dates of collection to confirm its maturity. Only healthy seeds were used for germination test. All seeds were surface sterilized with 0.04% $HgCl_2$ (1 min) followed by washing thoroughly with double distilled water. The test was conducted in glass Petri plates on top of a germination paper. Four replicates of 100 seeds each were used for the test. The seeds were counted as germinated when radical emerged. The experiment was undertaken in Complete Randomized Design.

Data analysis: The data were analyzed statistically using MS Excel 2000. The 2 years results (2011 and 2012) were subjected to the analysis of variance (ANOVA) technique. To determine significant difference among mean values of the various treatments, the Duncan test ($P \leq 0.05$) was used. Data as percentages were transformed to arcsine $(9/100)^{0.5}$.

RESULTS AND DISCUSSION

The results recorded for cone collection revealed that at altitude A_1 the Blue pine cones were Light green in colour which changed to various shades of green and brown with advancement of maturity. Cone colour was observed light green to green for A_1 and A2 altitudes (1st fortnight of August to 2nd fortnight August) and green with brown patches from (1st fortnight of September to 1st fortnight of October) and finally to chocolate brown (2nd fortnight of October) (Table-1). The results pertaining in the present study indicated that cone colour changed towards maturity. At maturity when cone colour changed to chocolate brown, the cones were found open on the trees. At other two altitudes viz., A_3 and A_4 the cone colour changed from light green to green (1st fortnight of August to 2nd fortnight September) to green with brown patches (1st fort

Plate 1. (a). *Immature cones,* ***(b).*** *Mature cones,* ***(c).*** *Cone collection,* ***(d).*** *Empty cones and* ***(e).*** *Seed extracted from*

Table 1. Altitudinal variation in physical characteristics of cone and seeds of *Pinus wallichiana* at different stages of maturity.

Altitude	1st fortnight August	2nd fortnight August	1st fortnight September	2nd fortnight September	1st fortnight October	2nd fortnight October
Cone colour						
A1	Light green	Green	Green with brown patches	Green with brown patches	Green with brown patches	Chocolate brown (Cones opened on trees)
A2	Light green	Green	Green with brown patches	Green with brown patches	Green with brown patches	Chocolate brown (Cones opened on trees)
A3	Light green	Light green	Green	Green with brown patches	Green with brown patches	Green with brown patches
A4	Light green	Light green	Green	Green	Green with brown patches	Green with brown patches
Seed colour						
A1	Whitish	Light brown	Light brown	Chocolate brown	Chocolate brown	Chocolate brown
A2	Whitish	Light brown	Light brown	Chocolate brown	Chocolate brown	Chocolate brown
A3	Whitish	Light brown	Light brown	Chocolate brown	Chocolate brown	Chocolate brown
A4	Whitish	Light brown	Light brown	Chocolate brown	Chocolate brown	Chocolate brown
Radical colour						
A1	Yellowish	Yellowish	Yellow	Yellow	Dark yellow	Dark yellow
A2	Yellowish	Yellowish	Yellow	Yellow	Dark yellow	Dark yellow
A3	Whitish	Yellowish	Yellow	Yellow	Yellow	Dark yellow
A4	Whitish	Yellowish	Yellow	Yellow	Yellow	Dark yellow
Plumule colour						
A1	Yellow	Yellow	Hazy green	Hazy green	Green	Green
A2	Yellow	Yellow	Hazy green	Hazy green	Green	Green
A3	Yellow	Yellow	Hazy green	Hazy green	Green	Green
A4	Yellow	Yellow	Hazy green	Hazy green	Green	Green

Altitude A_1: Kehmil Forest Division Kupwara (1600-2000 masl); Altitude A_2: Lidder Forest Division Phalgam (2000-2400 masl); Altitude A_3: Pir panchal Forest Division Dooghganga range (2400-2800 masl) and Altitude A_4: Sindh Forest Division Sonamarg (2800-3200 and above masl

night of October to 2nd fortnight of October. The data further revealed that as soon as cone colour changed from light green to green with brown patches germination percentage increased reasonably in relation to colour change from green to green with brown patches. Seed colour on first collection (1st fortnight of August) was whitish and it changed to light brown and finally to chocolate brown at final collection in the month September at lower altitudes and in October at higher altitudes (A_3 and A_4). The radical colour changed from yellowish in the month of August to dark yellow at maturity in the month of October at all sites (Plate 1). In *Pinus banksiana* Lamb. cones ripe when half or more of the cone surface is brown (Stockler and Jones, 1957), In case of *Bauhinia restua* (semla), change in fruit colour from green to dark red and the seed colour from green to whitish brown is useful indicator of seed maturity (Upadhayay *et al.*, 2006). Similarly, Mirgall *et al.,* (2007) reported that the cones of Cryptomeria should be collected in the month of October under temperate conditions of Kashmir valley, when cone colour changed from dark green to green and finally light green in colour. While as, (Singh, 2011) has reported that glossy red brown coloured cones of *Pinus halepensis* collected between 15th March to 1st April are mature.

The data recorded for cone and seeds weights are presented in (Fig.1) revealed that cone weight was significantly (P≤0.05) differ at different altitudes and it showed a significant decrease towards maturity at all altitudes. The fortnightly decrease in cone weight from 1st fortnight of August to 2nd fortnight of October with maximum cone weight decreased significantly (P≤0.05) from 151.17 gm to 133.25 gm at maturity at A_2 altitude followed by 123.76- gm to 103.39 gm at A_1 and 100.75 gm to 68.56 gm at A_3 altitude at maturity. While as the significantly (P≤0.05) minimum decrease in cone weight of 98.99 gm to 66.26 gm was recorded at A_4 altitude. Decrease in cone weight is result of moisture loss due to desiccation. Mughal and Thapliyal (2006) have also reported a decrease in cone weight of *Cedrus deodara* cones as they advanced towards maturity at different altitudes of Kashmir valley. Joshi (2000) reported that moisture loss coincided with maturity in *Dalbergia sisso* seed in norther India. However, the Seed mass affect different juvenile and adult characters and is one of the earliest indicators of offspring quality. The perusal of the data presented in (Fig. 1) indicated that the fresh weight of 1000 seeds (g) increased significantly (P≤0.05) towards the maturity in all the four altitudes. The increase in seed weight was significant at P≤0.05 level of significance. The seed weight increased significantly (P≤0.05) from 21.06 to 85.35 and 26.59 to 74.64 g/1000 seeds at A_2 and A_1 altitudes respectively. While as it increased from 18.92 to 34.98 and 20.60 to 33.58 g/1000 seeds at A_4 and A_3 altitudes respectively. Similarly, Mirgall *et al.,* 2016 reported that larger seeds produce maximum germination as compared to smaller seeds in *Saraca asoca*. Increase in seed weight and size is a function of resource allocation. During developmental stage most of the nutrients are translocated from the cones into the

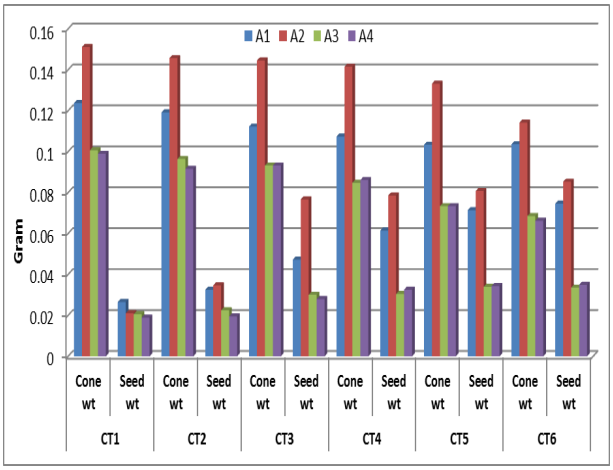

Fig. 1. *Altitudinal variation in cone weight (gm) and seed weight (gm/1000 seeds) during different stages of cones maturity in Pinus wallichiana.*

Altitude A_1: Kehmil Forest Division Kupwara (1600-2000 masl); Altitude A_2: Lidder Forest Division Phalgam (2000-2400 masl); Altitude A_3: Pir panchal Forest Division Dooghganga range (2400-2800 masl) and Altitude A_4: Sindh Forest Division Sonamarg (2800-3200 and above masl); CT1:1st fortnight August; CT2: 2nd fortnight August; CT3:1st fortnight September; CT4:2nd fortnight September; CT5:1st fortnight October; CT6:2nd fortnight October

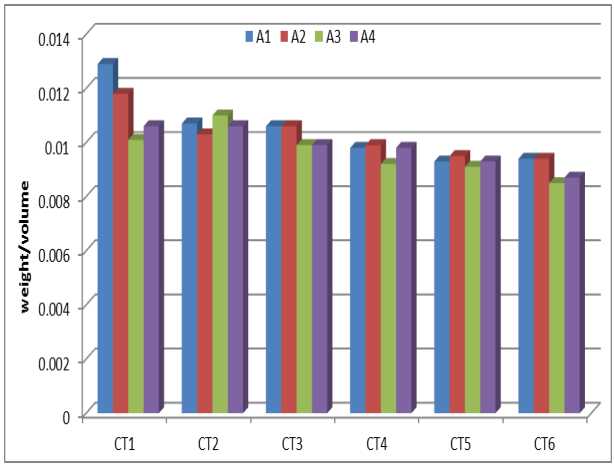

Fig. 2. *Altitudinal variation in specific gravity at different stages of cones maturity in Pinus wallichiana.*

Altitude A_1: Kehmil Forest Division Kupwara (1600-2000 masl); Altitude A_2: Lidder Forest Division Phalgam (2000-2400 masl); Altitude A_3: Pir panchal Forest Division Dooghganga range (2400-2800 masl) and Altitude A_4: Sindh Forest Division Sonamarg (2800-3200 and above masl); CT1:1st fortnight August; CT2: 2nd fortnight August; CT3:1st fortnight September; CT4:2nd fortnight September; CT5:1st fortnight October; CT6:2nd fortnight October

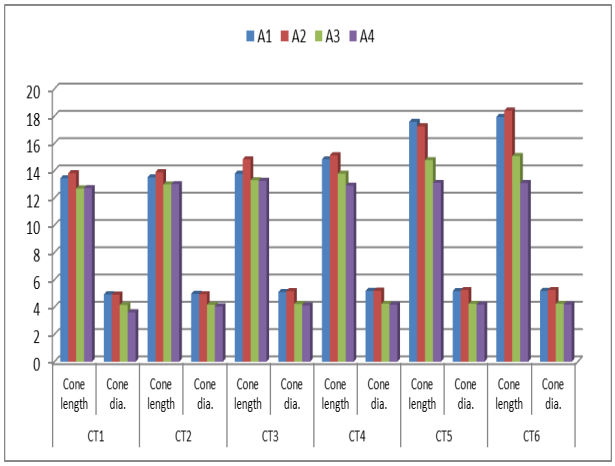

Fig. 3. *Altitudinal Variation in cone length (cm) and cone diameter (mm) at different stages of cone maturity of Pinus wallichiana.*

Altitude A_1: Kehmil Forest Division Kupwara (1600-2000 masl); Altitude A_2: Lidder Forest Division Phalgam (2000-2400 masl); Altitude A_3: Pir panchal Forest Division Dooghganga range (2400-2800 masl) and Altitude A_4: Sindh Forest Division Sonamarg (2800-3200 and above masl); CT1:1st fortnight August; CT2: 2nd fortnight August; CT3:1st fortnight September; CT4:2nd fortnight September; CT5:1st fortnight October; CT6:2nd fortnight October

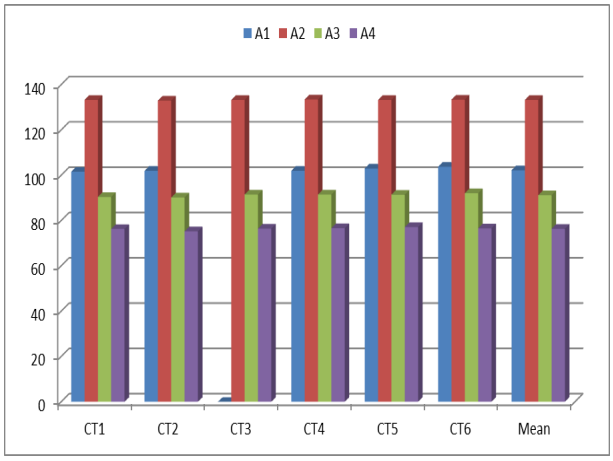

Fig. 4. *Altitudinal variation in number of seeds per cone at different stages of cone maturity in Pinus wallichiana.*

Altitude A_1: Kehmil Forest Division Kupwara (1600-2000 masl); Altitude A_2: Lidder Forest Division Phalgam (2000-2400 masl); Altitude A_3: Pir panchal Forest Division Dooghganga range (2400-2800 masl) and Altitude A_4: Sindh Forest Division Sonamarg (2800-3200 and above masl); CT1:1st fortnight August; CT2: 2nd fortnight August; CT3:1st fortnight September; CT4:2nd fortnight September; CT5:1st fortnight October; CT6:2nd fortnight October.

seeds thereby increasing their weight. The cone specific gravity also followed the cone weight pattern. It is evident from data in (Fig. 2) that different collection dates exert significant effect on the specific gravity of cones. Cone specific gravity also followed the cone weight pattern as it also decreased significantly ($P \leq 0.05$) as the cone proceeded towards the maturity. Specific gravity decreased significantly ($P \leq 0.05$) from 1.29 (immature) to 0.94 with the maturity of cones/ seeds at altitudes A_1 and 1.18 to 0.94 at altitude A_2. While as, at A_3 and A_4 it decreased from 1.01 to 0.85 and 1.06 to 0.87 respectively. Altitudinal effect on specific gravity is also significant and pronounced with lower specific gravity at higher altitudes and higher specific gravity at lower altitudes. Specific gravity in Abies concolor is 0.85 at maturity while it is 0.90 for Abies grandis and 0.75 *for Abies magnifica* (Oliver, 1974; Rediska and Nicholson, 1964). Singh (1998) reported decrease in cone specific gravity *of Abies pindrow* from 1.04 in August to 0.97 at maturity in October. The cone length of the blue pine cones collected from different altitudes on different collection dates differ significantly ($P \leq 0.05$) (Figure-3). At A_1 and A_2 altitudes cone length increased from 13.45 to 17.95 and 13.82 to 18.41 cm respectively at maturity. While as at other two altitudes viz., A_4 and A_3 it increased from 12.73 and 12.70 (Ist fortnight of August) to 13.10 and 15.08 cm in (2ndfortnight of October) respectively. Altitude seems to have its effect on size of cones as small sized cones were recorded at higher altitudes. Cone diameter also showed an increasing trend towards maturity (Fig.3) and it increased from 4.94 and 4.92 mm to 5.20 and 5.26 mm at maturity in case of A_1 and A_2 altitudes. While as cone diameter increased from 3.63 and 4.16 mm at (Ist collection date) to 4.21 and 4.25 mm at maturity at altitudes A_4 and A_3 respectively. Results further indicate that with the increase in altitude there is significant ($P \leq 0.05$) decrease in diameter of cones. The results further revealed that with the increase in both cone length and cone diameter the germination percentage were also increased. The number of seeds per cone of Blue pine collected from four different altitudes differed significantly ($P \leq 0.05$) (Fig. 4). However the number of seeds does not show any significant ($P \leq 0.05$) increase with maturity. The number of seeds per cone was dependent on the length and collar diameter of cones. More the length, more be the number of seeds per cones (Fig. 3). The number of seeds ranged from (76.38) at A_4 altitude to a maximum of 133.26 in A_2 altitude followed by 102.26 and 91.17 in A_1 and A_3 altitudes respectively.

The moisture percentage of seeds collected on different dates (Fig. 5) from different altitudes varied significantly ($P \leq 0.05$). Moisture percentage was maximum in immature seed with 33.34, 25.91, 37.92 and 40.22 percent of moisture A_1, A_2, A_3 and A_4 altitude respectively, during 1st fortnight of August. This moisture content decreased to 14.8, 13.56, 16.65 and 17.42 percent at the time of maturity for A_1, A_2, A_3, and A_4 altitude respectively. The moisture content of the seeds reduced significantly ($P \leq 0.05$) as the seed proceeded

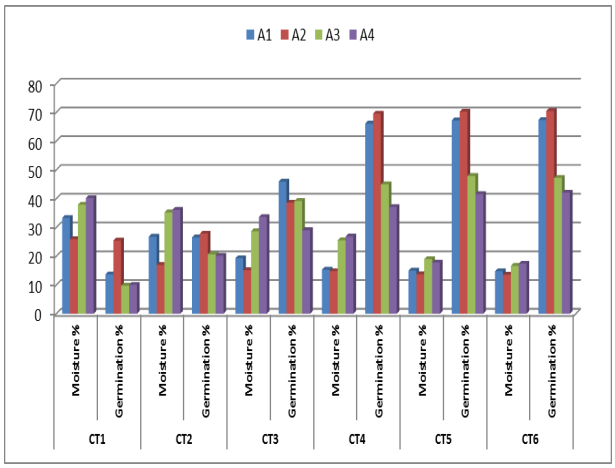

Fig. 5. *Altitudinal variation in moisture content and germination percent at various stages of cone maturity in Pinus wallichiana seeds.*

Altitude A_1: Kehmil Forest Division Kupwara (1600-2000 masl); Altitude A_2: Lidder Forest Division Phalgam (2000-2400 masl); Altitude A_3: Pir panchal Forest Division Dooghganga range (2400-2800 masl) and Altitude A_4: Sindh Forest Division Sonamarg (2800-3200 and above masl); CT1: 1st fortnight August; CT2: 2nd fortnight August; CT3: 1st fortnight September; CT4: 2nd fortnight September; CT5: 1st fortnight October; CT6: 2nd fortnight October

towards maturity. This study further revealed that moisture percent and germination percent are negatively correlated. The germination percentage increased with the decrease in moisture percentage. However lower germination was observed when moisture percentage was recorded higher. The results are in line with the findings of Singh and Kachari (2006) who reported that the moisture content of khasi pine seeds collected form Uttrahkund on different dates varied from 36.42 per cent of immature seeds to 9.58 of mature seeds. Similarly Mirgal *et al.*, 2016 reported in *Antiaris toxicaria* that seed characteristic showed significant variation in germination in the species. Lavania *et al.,* (2010) also recorded a loss of moisture content up to 12.30 in seeds of Blue pine at Teri Garwal Uttrakhand of Indian Himalaya. The seed germination per cent increased significantly ($P \leq 0.05$) as the cones and seeds proceeded towards maturity (Fig. 5). A number of researchers (Sofi *et al*., 2016 in *Acer caesium*; Ahirwar, 2012 in *Alangium lamarckii* and Mumtaz *et al*., 2009 in *Aesculus indica*) have correlated seed germination percent with maturity of seeds. At altitude A_1 germination per cent of 13.71 per cent was recorded at first collection in the first fortnight of August and subsequently increased to 67.37 percent at maturity. At altitude A_2 germination percent increased from 25.47 to 70.50 per cent at maturity in the month of October. Similarly at altitudes A_4 and A_3 the germination percentage increased from 10.06 and 9.79 per cent to 42.12 and 47.25 per cent at maturity respectively. The last three collections dates i.e. 2nd fortnight of September and 1st and 2nd fortnight of October didn't exhibit any significant increase. This pattern of germination can also be correlated with specific gravity i.e. with decrease in the specific gravity there is an increase in seed germination per cent. Studies conducted on seed germination and seedling establishment in natural forests of spruce and silver fir in Kotgarh Forest Division H.P showed that in both the species seed dispersed in October had lower germination percentage than seeds dispersed in November (Singh and Singh, 1984). Singh (1998) obtained similar results in Abies pindrow cones, with germination per cent increasing from 0.50 in August to 32.14 in October at maturity at 2600 m elevation in Kotgarh Forest Division, Himachal Pradesh.

Conclusion

The study concluded that the cones of Blue pine should be collected in the month of September when the colour of cones is green with brown patches at an altitudinal range of 1,600-2,400 masl. While as, collection of cones at higher altitudinal range (2400-3200 masl) can be done upto October. However, the cone length, cone diameter, cone weight, number of seeds per cone and number of scales per cone were recorded maximum at lower altitudinal range of 1,600-2,400 masl.

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