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Influence of Growing Medium and Seed Size on Germination and Seedling Growth of *Pinus gerardiana* Wall.

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ABSTRACT

Pinus gerardiana Wall. is an important nut-producing pine having restricted distribution in the world. It has been observed that natural regeneration in the species is extremely poor or entirely lacking. The species has erratic and infrequent seed years, dormancy-related problems, and slow growth of seedling, which reduces its regeneration process in natural habitats. Therefore, we investigated the effect of growing medium and seed size on germination and seedling growth of the *Pinus gerardiana*. The seeds were categorized into two sizes, viz, small (<2.35 cm) and large (>2.35 cm) and five growing medium treatments were used, viz, Soil:Sand:FYM; Soil:Sand:Moss:FYM; Soil:Moss:Vermicompost; Soil:Sand:Vermicompost; and Soil:Sand:Moss:Vermicompost, for assessing their impact on germination and seedling growth. It was observed that, among different growing medium treatments, higher germination and seedling growth parameters were recorded, when Soil:Sand:Moss:Vermicompost was used for the study. Between different seed sizes, higher germination and seedling growth were recorded when seed size was large.

Introduction

Chilgoza pine (*Pinus gerardiana* Wall.) is an important ecological and economic tree species found in the dry temperate North-Western Himalayas of India, Pakistan, and Afghanistan. It is a small- to medium-sized evergreen tree of 17 to 27 m in height and 2–4 m in girth (Bhattacharrya et al. 1988). It has been observed that natural regeneration in the species is very poor or entirely lacking. The most important factor responsible for this is the collection of cones by the local people (Kumar et al. 2013). Severe biotic interference and lack of regeneration have resulted in the disappearing/extinction of this valuable species in Kinnaur (Sehgal and Chauhan 1989; Kumar et al. 2014). Therefore, it is important to improve regeneration by devising afforestation strategies to prevent it from extinction. For successful of afforestation, a species should have greater germination and seedling growth.

Growing medium is a major factor influencing seed germination and seedling growth, and quality of seedlings in the nursery (Aklibasinda et al. 2011; Bulut and Damir 2007; Robbins and Micheal 2009). For successful establishment of plantation in an area, quality planting material is of immense importance as tolerance to adverse conditions in the field is very important for establishment of plants (Heiskanen and Rikala 1998). The quality of planting material is dependent on composition of the growing medium used, which may vary depending on several other factors. Farm yard manure (FYM) is the composition of growing media presently used in forest nurseries in India. Chilgoza pine is a slow growing species and requires 3–4 years to achieve a plantable size (Luna 2008). Therefore, immediate research is required for reducing the time period to attain a plantable size and production of quality planting material in nursery conditions. Aeration and water retention under low matric potentials of media are the most important factors for plant growth (Kuslu et al. 2005). Chilgoza pine seedlings require adequate drainage in the nursery. In this regard, Moss has a great potential to drain excess water from media. For enhancing growth of the seedling, vermicompost has been recommended by

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many researchers (Arancon et al. 2008; Atiyeh et al. 2002; Cannellas et al. 2002; Donald and Visser 1989; Hashemimajd et al. 2004; Hidaglo et al. 2006; Zeller 2007). Lazcano et al. (2010) concluded that vermicompost enhanced the germination of *Pinus pinaster*. Donald and Visser (1989) observed that vermicompost had contrasting effects in the development of *Acacia mearnsii*, *Pinus patula*, and *Eucalyptus grandis* seedling, and found that the effect of vermicompost varies with the species. The effects of vermicompost on seedling growth have been studied mostly in agriculture and horticultural plant species, while negligible information is available regarding its effect on the germination and nursery growth of forest trees, and specifically the effects of vermicompost on Chilgoza pine have not been studied until now.

Growth responses of seedlings emerging from seeds of different sizes may differ markedly when seedlings are exposed and acclimated to varying climatic conditions (Khurana and Singh 2000; Morrison and Xue 2007). The effect of seed size on germination and seedling growth has been investigated by many researchers in various species (Guberac et al. 1998; Kaydan and Yamur 2008; Larsen and Andreasen 2004; Roy et al. 1996; Willenborg et al. 2005). A positive effect of large seeds on germination and plant growth has been reported (Negi and Todaria 1997; Seiwa 2000; Parker et al. 2004).

Seed size and growing medium may interact in their effects on germination and early seedling growth of *P. gerardiana*. However, no such work has been attempted so far in Chilgoza pine. Thus, the present investigation was conducted to determine the effect of seed size and growing medium on germination and seedling growth of *P. gerardiana*.

Material and Methods

The experiment was conducted at a farm nursery of RHRS, Sharbo (Reckong peo) (31° 54′N; 78° 27′ E) situated at 2450 m AMSL during the years 2009–2011. The area is characterized by long winters from October to April and short summers from June to August. Though rains are scanty, precipitation is received mostly in the form of snow during winter.

Treatment Details

P. gerardiana seeds were obtained from identified plus trees; those were on average better in height and diameter than the other trees in the natural stand. The seeds from plus trees provides improved quality planting material for afforestation. Seeds were brought to the laboratory and subjected to cold, moist treatment for 12–24 h for breaking the dormancy. After breaking dormancy, seeds were categorized into two classes, i.e., small (<2.35 cm) and large (>2.35 cm). The treatments along with their physicochemical properties of growing medium are given in table 1.

Evaluation of Seed Germination and Seedling Properties

All treatments were examined daily for recording germination. Seeds were considered germinated when the radicle was 5 mm long (Sosa et al. 2005). Germination percentage (GP) (equation (1)) and mean daily germination (MDG) (equation (2)) was calculated using the following formula (Hossain et al. 2005; Li et al. 2006):

$$GP = \frac{n}{N} \times 100, \tag{1}$$

$$MDG = \frac{n}{D} \times 100, \tag{2}$$

where n is the number of germinated seeds, N is the total number of seeds, GP is the germination percentage, and D is the number of days to final germination percentage.

Peak value (PV) is the maximum mean daily germination reached at any time during the period of the test. Germination speed (GS) (equation (3)) was

Table 1. Physicochemical analysis of different growing medium.

Growing medium	Ratio	Organic carbon (%)	Nitrogen (Kg ha^{-1})	Phosphorus (Kg ha^{-1})	Potassium (Kg ha^{-1})
Soil:Sand:FYM	1:1:1	2.99	251.80	39.12	273.10
Soil:Sand:Moss:FYM	1:1:1:1	2.65	298.10	46.90	278.70
Soil:Moss:Vermicompost	1:1:1	3.42	347.10	54.80	296.60
Soil:Sand:Vermicompost	1:1:1	3.89	360.47	51.48	298.42
Soil:Sand:Moss:Vermicompost	1:1:1:1	3.92	350.46	52.41	295.64

determined by the method prescribed by Maguire (1962):

$$GS = (n / t), \quad (3)$$

where n is the number of seeds newly germinating at time (i) and t is number of days from sowing. The germination value (GV) (equation (4)) was calculated, as per the formula given by Czabator (1962):

$$\text{Germination value (GV)} = PV \times MDG, \quad (4)$$

where PV is the peak value of germination and MDG is mean daily germination. Germination index (GI) was calculated by dividing the total number of seeds germinated at the end of the experiment with the time taken for 50% germination.

The observation of seedling parameters was recorded after one growing season. Seedlings were harvested and height, collar diameter, and root length recorded; dry shoot and root weight were recorded after oven-drying at 60°C for 48 h to determine root-shoot ratio and total biomass.

Experimental Design

The experiment was laid out in a factorial randomized block design (RBD) of ten treatments and three replications. Each combination of the experimental factors (growing medium and seed size) was applied to one pot with three replicates resulting in a total of thirty pots (five growing medium × two seed size × three replicates). Five seeds were sown in each pot at a depth of 1.5 cm from the surface, with a distance of 3 cm among them. Data were statistically analyzed using the GLM procedure in SAS 9.3 software. Tukey's HSD test was used to compare means within and among treatments at 5% level of significance.

Results

Analysis of variance showed that germination and seedling growth of *P. gerardiana* were significantly affected by growing medium and seed size (table 2). Most seeds were germinated in Soil:Sand:Moss:Vermicompost medium when large-sized seeds were used, but when seed size decreased to small and growing medium changed to control, a substantial reduction of seed germination occurred. In other growing medium, germination was higher than the control, but less than the Soil:Sand:Moss:Vermicompost medium (tables 3 and 4). Seedling growth was also significantly affected by growing medium and these were higher in Soil:Sand:Moss:Vermicompost medium (tables 5 and 6).

Effect of Growing Medium

In Soil:Sand:Moss:Vermicompost growing medium, more seeds germinated than other growing medium and less germination occurred in Soil:Sand:FYM (control). A 20% increase of germination was observed in Soil:Sand:Moss:Vermicompost over Soil:Sand:FYM (control). Germination speed and peak value of germination of seeds in Soil:Sand:Moss:Vermicompost medium was significantly greater than Soil:Sand:Vermicompost, Soil:Moss:Vermicompost, Soil:Sand:Moss:FYM, and Soil:Sand:FYM (control). Mean daily germination and germination value in Soil:Sand:Moss:Vermicompost medium differed significantly from Soil:Sand:FYM (control) (table 3). Germination index in Soil:Sand:Moss:Vermicompost medium was significantly greater than in other growing mediums.

Table 2. ANOVA result of effect for growing medium and seed size on germination and seedling growth of *Pinus gerardiana*.

	Growing medium					Seed size				
Source of variation	DF	Anova SS	Mean square	*F* value	*Pr* > *F*	DF	Anova SS	Mean square	*F* value	*Pr* > *F*
Germination (%)	4	2170.47	542.62	97.35	<.001	1	97.20	97.20	17.44	0.001
Germination speed	4	135.94	33.99	6.80	0.002	1	27.94	27.94	5.59	0.030
Peak value	4	1.53	0.38	28.09	<.001	1	0.04	0.04	2.72	0.116
Mean daily germination	4	1.59	0.40	22.55	<.001	1	0.07	0.07	4.16	0.056
Germination value	4	37.54	9.39	24.51	<.001	1	1.47	1.47	3.85	0.066
Germination index	4	3.84	0.96	22.55	<.001	1	0.18	0.18	4.16	0.056
Plant height (cm)	4	89.35	22.34	24.89	<.001	1	8.71	8.71	9.71	0.006
Collar diameter (mm)	4	2.18	0.55	3.45	0.029	1	0.23	0.23	1.44	0.246
Root length (cm)	4	11.58	2.89	4.81	0.008	1	0.52	0.52	0.86	0.366
Shoot:root ratio	4	11.58	2.89	4.81	0.008	1	0.52	0.52	0.86	0.366
Total biomass (g)	4	1.28	0.32	10.50	<.001	1	0.05	0.05	1.61	0.220

Table 3. Effect of growing medium and seed size on germination of *Pinus gerardiana* seeds.

	Germination (%)	Germination speed	Peak value	Mean daily germination	Germination value	Germination index
Growing medium						
Soil:Sand:FYM	43.00c ± 1.41	5.78c ± 3.65	1.59d ± 0.05	2.46c ± 0.04	3.85c ± 0.13	2.44c ± 0.06
Soil:Sand:Moss:FYM	44.83c ± 2.32	6.20bc ± 0.98	1.65cd ± 0.07	2.57bc ± 0.06	4.21c ± 0.25	2.61bc ± 0.09
Soil:Moss:Vermicompost	43.67c ± 3.61	8.15bc ± 1.23	1.74bc ± 0.15	2.73b ± 0.19	4.45bc ± 0.41	2.86b ± 0.30
Soil:Sand:Vermicompost	54.00b ± 5.51	8.89b ± 2.96	1.80b ± 0.21	2.59bc ± 0.15	5.02b ± 1.06	2.63bc ± 0.23
Soil:Sand:Moss:Vermicompost	65.17a ± 3.19	11.71a ± 1.80	2.23a ± 0.14	3.12a ± 0.14	7.01a ± 0.73	3.46a ± 0.22
Seed size						
Small	48.33b ± 2.19	7.18b ± 3.05	1.83a ± 0.32	2.64a ± 0.25	4.69a ± 1.18	2.72a ± 0.39
Large	51.93a ± 2.14	9.11a ± 2.75	1.76a ± 0.20	2.74a ± 0.28	5.13a ± 1.36	2.88a ± 0.43

Note: Values are means ± standard error. Different letters indicate significant differences at $p < 0.05$.

Seedlings height in Soil:Sand:Moss:Vermicompost and Soil:Sand:Vermicompost growing medium was significantly more than that of other growing mediums. A 4.75-cm increment in plant height was recorded in Soil:Sand:Moss:Vermicompost in comparison to Soil:Sand:FYM (control). Collar diameter recorded was significantly greater in Soil:Sand:Moss: Vermicompost, Soil:Sand:Vermicompost, and Soil: Moss:Vermicompost than Soil:Sand:FYM (control). Root length differed significantly in Soil:Sand:Moss: Vermicompost from that in Soil:Sand:FYM (control). Root:shoot ratio in Soil:Sand:Moss:Vermicompost growing medium was more than that of other growing medium treatments. Moreover, seedlings' biomass was significantly decreased as growing medium containing Soil:Sand:FYM (control) was used to grow the seedlings (table 5).

Effect of Seed Size

Seed size significantly affected germination percent and higher germination was recorded in large-sized seed than small-sized seed. Germination speed was significantly varied with different seed size. Peak value, mean daily germination, and germination index revealed non-significant effect of seed size (table 3). Seedlings' height and collar diameter were strongly affected by different seed size. Root length and root: shoot ratio and total biomass were non-significantly different with increasing seed size (table 5).

Interaction Effect

Effect of growing medium and seed size on germination and seedling growth has been analyzed in tables 4 and 6. Germination parameters were observed to be greater in Soil:Sand:Moss:Vermicompost for large-sized seeds. Similarly, greater seedling growth was recorded in Soil:Sand:Moss:Vermicompost for large-sized seeds.

Discussion

Effect of Growing Medium

Germination percentage was found to be generally higher for *P. gerardiana* seeds in the Soil:Sand:Moss: Vermicompost treatment than the other treatments (table 4). Similar results were obtained in *Pinus pinaster* by Cristina et al. (2010). They found that the addition of vermocompost in growing medium increased the germination and also promotes the emergence of seeds. Scheuerell and Mahafee (2004)

Table 4. Interaction effect of growing medium and seed size on germination of *Pinus gerardiana* seeds.

Growing medium	Seed size	Germination (%)	Germination speed	Peak value	Mean daily germination	Germination value	Germination index
Soil:Sand:FYM	Small	42.00f ± 1.00	4.02d ± 0.82	1.55d ± 0.03	2.43c ± 0.02	3.74c ± 0.08	2.39c ± 0.03
	Large	44.00ef ± 1.00	7.55bcd ± 4.83	1.63d ± 0.03	2.49c ± 0.02	3.95c ± 0.08	2.48c ± 0.03
Soil:Sand: Moss:FYM	Small	43.00ef ± 1.00	5.51cd ± 0.32	1.60d ± 0.06	2.54c ± 0.07	4.17c ± 0.27	2.56c ± 0.11
	Large	46.67de ± 1.53	6.89bcd ± 0.91	1.70d ± 0.04	2.59c ± 0.04	4.25c ± 0.27	2.65c ± 0.06
Soil:Moss: Vermicompost	Small	44.33ef ± 5.51	7.08bcd ± 0.47	1.74cd ± 0.11	2.63bc ± 0.11	4.22c ± 0.05	2.70bc ± 0.17
	Large	43.00ef ± 1.00	9.22abc ± 0.28	1.73d ± 0.22	2.83b ± 0.23	4.67bc ± 0.53	3.01b ± 0.35
Soil:Sand: Vermicompost	Small	50.00d ± 3.46	7.71bcd ± 3.77	1.93bc ± 0.23	2.54c ± 0.10	4.48c ± 0.70	2.56c ± 0.16
	Large	58.00c ± 4.00	10.08ab ± 1.84	1.67d ± 0.08	2.63bc ± 0.20	5.56b ± 1.20	2.70bc ± 0.31
Soil:Sand:Moss: Vermicompost	Small	62.33b ± 0.58	11.61a ± 0.92	2.35a ± 0.04	3.07a ± 0.18	6.81a ± 0.59	3.39a ± 0.29
	Large	68.00a ± 1.00	11.82a ± 1.43	2.10b ± 0.05	3.17a ± 0.10	7.21a ± 0.93	3.54a ± 0.15

Note: Values are means ± standard error. Different letters indicate significant differences at $p < 0.05$.

Table 5. Effect of growing medium and seed size on seedling growth of *Pinus gerardiana*.

	Plant height (cm)	Collar diameter (mm)	Root length (cm)	Shoot:root ratio	Total biomass (g)
Growing medium					
Soil:Sand:FYM	8.35c ± 1.66	3.02c ± 0.29	11.48c ± 0.77	2.20a ± 0.53	1.28c ± 0.09
Soil:Sand:Moss:FYM	11.26b ± 0.81	3.19bc ± 0.59	11.89bc ± 0.91	2.59ab ± 0.32	1.46bc ± 0.11
Soil:Moss:Vermicompost	11.84b ± 1.72	3.66ab ± 0.25	12.00bc ± 0.76	2.77a ± 0.24	1.65b ± 0.20
Soil:Sand:Vermicompost	13.00a ± 0.39	3.69a ± 0.38	12.75ab ± 0.81	2.65ab ± 0.31	1.66b ± 0.22
Soil:Sand:Moss:Vermicompost	13.10a ± 1.05	3.57ab ± 0.88	13.20a ± 0.87	2.79a ± 0.34	1.90a ± 0.22
Seed size					
Small	10.97b ± 2.19	3.34a ± 0.61	12.13a ± 1.01	2.54a ± 0.42	1.55a ± 0.26
Large	12.05a ± 1.92	3.51a ± 0.52	12.40a ± 0.99	2.66a ± 0.39	1.63a ± 0.28

Note: Values are means ± standard error. Different letters indicate significant differences at $p < 0.05$.

also confirmed that the addition of vermicompost affects germination and root elongation. The influence of growing medium can be attributed to the presence of nutrients, which might have created a better environment for *P. gerardiana* seeds. Our research shows that the presence of vermicompost in growing medium promoted the germination and growth of seeds. Seedlings in growing medium containing vermicompost had greater height and diameter than those without vermicompost treatments (table 5), as previously reported by Malik (2007), who studied the response of *P. gerardiana* seedlings to different growing medium treatments. In that study, it was detected that seedling development is reduced when pure forest soil was used.

As shown in table 4, the seeds of *P. gerardiana* responded to different growing medium treatments and less germination occurred in Soil:Sand:FYM (control) growing medium. The most seeds germinated in Soil:Sand:Moss:Vermicompost because their germination index and germination value were more in Soil:Sand:FYM (control) growing medium. Moreover, when Soil:Sand:Moss:Vermicompost growing medium was used, a substantial increase in the germination speed occurred. These results are in agreement with the finding of Mohammad et al. (2013), who reported that the rate and percentage of seed germination were significantly increased if vermicompost was used. Greater germination in growing medium containing vermicompost was attributed to better germination condition of seeds (Alves and Passoni 1997; Lazcano et al. 2010).

In our study, the seedlings' height and collar diameter in Soil:Sand:Moss:Vermicompost medium was more than that of other growing medium treatments (table 6). Moreover, root length was significantly increased with an increase in nutrients of the growing medium. This suggests that, if the growing medium changed from Soil:Sand:FYM to Soil:Sand:Moss:Vermicompost, the seeds of *P. gerardiana* responded better. The present results support Aklibasinda et al.'s (2011) observation that there was a decrease in final seedling height absence of proper growing medium. Root:shoot ratio and biomass were significantly affected by different growing medium treatments. Root:shoot ratio and biomass were significantly increased after incorporating vermicompost in the growing medium (table 6). Shamet et al. (1994) proved that, in *P. gerardiana*, growing medium effect at the seedling stage appears to be greater than at the germination stage.

Table 6. Interaction effect of growing medium and seed size on seedling growth of *Pinus gerardiana*.

Growing medium	Seed size	Plant height (cm)	Collar diameter (mm)	Root length (cm)	Shoot:root ratio	Total biomass (g)
Soil:Sand:FYM	Small	7.72d ± 1.74	2.84c ± 0.20	11.17d ± 0.48	2.16b ± 0.64	1.24f ± 0.07
	Large	8.98cd ± 1.64	3.19bc ± 0.27	11.79bcd ± 0.97	2.23ab ± 0.54	1.32ef ± 0.11
Soil:Sand:Moss:FYM	Small	10.66b ± 0.50	3.21bc ± 0.68	12.08bcd ± 1.22	2.62ab ± 0.50	1.45def ± 0.12
	Large	11.87ab ± 0.54	3.18bc ± 0.64	11.71bcd ± 0.69	2.56ab ± 0.04	1.47cdef ± 0.13
Soil:Moss:Vermicompost	Small	10.59bc ± 0.88	3.59ab ± 0.35	11.60cd ± 0.70	2.62ab ± 0.18	1.64bcd ± 0.27
	Large	13.10a ± 1.38	3.73ab ± 0.13	12.40abcd ± 0.69	2.92a ± 0.21	1.66abcd ± 0.17
Soil:Sand:Vermicompost	Small	13.05a ± 0.44	3.81ab ± 0.27	12.94ab ± 0.64	2.58ab ± 0.29	1.57bcde ± 0.17
	Large	12.95a ± 0.43	3.57ab ± 0.49	12.56abc ± 1.07	2.71ab ± 0.38	1.75abc ± 0.27
Soil:Sand:Moss:Vermicompost	Small	12.85a ± 1.17	3.25abc ± 1.05	12.88abc ± 0.97	2.72ab ± 0.42	1.85ab ± 0.24
	Large	13.36a ± 1.09	3.89a ± 0.73	13.52a ± 0.82	2.87a ± 0.31	1.95a ± 0.23

Note: Values are means ± standard error. Different letters indicate significant differences at $p < 0.05$.

Effect of Seed Size

More seeds germinated when seed size was large, because seed size is related to the total nutrient pool and energy, which affects germination. Moreover, germination of *P. gerardiana* is strongly influenced by characteristics of seed and their nature (Singh and Chaudhary 1993). Mcrae (2005) found that the largest seed germinated faster and produce the highest final germination.

Our results showed that the height of seedlings raised from large-sized seeds was significantly greater than the small-sized seeds. Seedlings raised from large-sized seeds showed significantly higher collar diameter compared to small-sized seeds, confirming similar results reported by Malik (2007). He found significant differences among seedlings raised from small- and large-sized seeds. Seedlings' root length was greater when raised from large-sized seeds. Greater seedling root length from large seeds was the result of high influence of seed size on root growth observed by Leishman and Westoby (1994). An increase in root biomass may be due to a greater potential of large seed to supply nutrients (Khurana and Singh 2000). Root:shoot ratio and total biomass of seedlings were greater in large-sized seeds, because large-sized seeds having a greater nutrients reserve enable the seedling to grow faster and to use available resources earlier as reported by Moles et al. (2005).

Conclusion

The results of the study conclude that germination and seedling growth of *P. gerardiana* seeds depends on growing medium and seed size. The application of different growing medium and use of different sized seeds significantly affected germination and seedling growth of the species. In general, the present study recommends Soil:Sand:Moss:Vermicompost as the growing medium and use of large-sized seeds for enhancing germination and seedling growth of the species. Additional research on effect of growing medium and seed size could further elucidate protocol for faster germination and seedling growth of *P. gerardiana*.

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