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Effects of Type and Size of Growing Media on Seed Germination and Seedling Growth of *Pinus gerardiana*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chilgoza pine (*Pinus gerardiana* Wall.) is an important but endangered tree species in Afghanistan. Due to poor natural regeneration, seed-related dormancy and anthropogenic factors, the IUCN put this species on the red list. Therefore, this study was undertaken to investigate the effect of growing medium and polybag sizes on seed germination and seedling growth. In this study, four growing media of soil (So), compost (Co), forest soil (Fs) and sand (Sa), *viz*., M_1 (So + Co + Fs); M_2 (Fs + Co + Sa); M_3 (So + Fs + Sa); and M_4 (Sa + Co + Fs) and three polybag sizes including P_1 (16 cm×16 cm), P_2 (18 cm×18 cm), and P_3 (21 cm×26 cm) were used and arranged in the frame of 4×3 factorial design to assess their effects on germination and seedling growth. The results revealed that among different growing media treatments, higher germination and seedling growth parameters were observed on treatment M_4 (Sand + Compost + Forest Soil). Seedling growth parameters were significantly affected by polybag size, and the highest seedling growth parameters were obtained for Large Polybag size (P3).

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Keywords: Chilghoza; media; polybag; seed germination; seedling growth; forest soil.

1. INTRODUCTION

Pinus gerardiana is the most economically and ecologically important tree species in Afghanistan, commonly known as Chilgoza, chalghoza, pine nuts, and Himalayan pine nuts [1]. Chilgoza is one of the 110 pine species of the Pinus genus, found at 1800 to 3350 m elevation in the temperate forests of the Himalayan region [2]. It produces edible kernels that are rich in many essential nutrients such as proteins, carbohydrates, fats, minerals, and fibers [3]. Fresh kernels contain sugars (4.07%), proteins (13.03%), oils (52.15%) and moisture (25.36%) [4].

Furthermore, Chilgoza plays an important role in the socio-economic development of the local communities and the national economy of Afghanistan; the livelihood of approximately 120,600 households directly depends on the Chilgoza forest. Each family obtains 444-555 USD income per year from this species [5]. Poor natural regeneration, seed-related dormancy, and other anthropogenic factors cause the degradation of the species ([6,7,8,9,10]. On the other hand, seed germination and seedling survivability in ex-situ are too low even uneconomical. Different factors affect seed germination and seedling growth [11]. Therefore, it is important to improve the seed germination and seedling growth of this species. The type and size of the growth medium are important factors for seed germination and seedling growth. Seed germination and seedling growth are greatly affected by the sowing medium and GA_3 [10]. Chilgoza pine seedlings require adequate drainage in nurseries [8]. Based on the latest research, soil media, and growth-promoting chemicals can improve seed germination; however, the size of the media is also important to investigate and define the proper type and size of the growing media.

2. MATERIALS AND METHOD

2.1 Study Area

The study was conducted during 2021–2022, in the research farm of the agriculture faculty of Paktia University, Gardez City, Paktia, Afghanistan, which is located at 33^{o} 38 48 N, 69 13 56 E, and 2390m altitude. In this study, we used a completely randomized design (CRD) with three replications.

2.1.1 Treatment properties

Two factors were included in this experiment. The first factor was sowing medium, with four combinations: ground soil: compost: forest soil (M_1), ground soil: compost: sand (M_2), ground soil: forest soil: sand (M_3), and sand: compost: forest soil (M_4). Second factor was polybag size: P_1(16×16), P_2(18×18), and P_3(21×21).

Seeds: Seeds were collected from the forest area of the province. Seeds were sorted and graded based on their healthiness, size and physical properties, respectively. Healthy seeds were sown in polybags filled with the prepared sowing media. Irrigation was performed using a hand sprinkler.

2.1.1.1 Studied parameters and data collection

In this study seed germination and seedling growth parameters were measured, presented in Table 1.

The first germination of seeds was observed 21 days after seed sowing and continued up to the 48^{th} day and 8 months after seed sowing, seedlings were removed from polybags for measurement of some growth parameters. Root and shoot dry weights were assessed after oven drying at 68 °C for 48 h.

3. RESULTS AND DISCUSSION

Analysis of variance tables showed that seed germination was affected only by growing media, and seedling growth was significantly affected by both growing media and ploy bag size (Tables 2 and 3). High germination percentage was observed in (sand: compost: forest soil) and M_3 (soil: forest soil: sand) media, in all polybag sizes. However, germination decreased in M1 (soil: compost: forest soil) medium (Table 4). Seedling growth parameters were significantly affected by media and polybag size and were higher in M4 (sand: compost: forest soil) and P3 (26 cm × 21 cm) polybag sizes (Table 5).

3.1 Effect of Growing Medium

In M_4 (sand: compost: forest soil) and M_3 (sand: forest soil: sand) growing media, more seeds germinated than in the other growing media, and less germination occurred in M_1 (sand: compost: forest soil). A 17% increase in

germination was observed in M_4 (sand: compost: forest soil) compared to soil: compost: forest soil (Table 4).

Collar diameter, root dry weight, and seedling quality index in M4 (sand: compost: forest soil) growing medium were significantly higher than those in other growing media. Shoot length, root fresh weight, and shoot dry weight were statistically similar in M4 (sand: compost: forest soil) and M3 (soil: forest soil: sand). Shoot fresh weight, Root: Shoot ratio and sturdiness quotient were not significantly different in the different growing media (Table 5).

3.1.1 Effect of polybag size

Polybag size did not affect germination parameters (Table 4). The growth parameters were significantly affected by the polybag size. Collar diameter, root fresh weight, shoot dry weight, and seedling quality index were significantly affected by P3 (26 cm × 21 cm). Seedling height and root dry weight were measured and parred in P2 and P3 polybags. Shoot fresh weight, Root: Shoot ratio and sturdiness quotient were not significant for any polybag size (Table 5).

3.1.1.1 Interaction effect

The effects of the growing media and polybag size on germination and seedling growth are shown in Tables 4 and 5, respectively. Germination parameters (MGT and GI) were observed to be greater in M2 (soil: compost: sand) for P2 (18 cm × 18 cm) and M3 (soil: forest soil: sand) for P1 (16 cm × 16 cm) polybag sizes (Table 6).

The collar diameter and seedling height were greater in M4 (sand: compost: forest soil) for P3 (26 cm × 21 cm) polybag size. Shoot fresh weight interaction was better in M3 (soil: forest soil: sand) for P1 (16 × 16 cm) polybag size. The sturdiness quotient was greater in M3 (soil: forest soil: sand) for P3 (26 × 21 cm) polybag size (Table 7).

Table 1. Seed germination and seedling growth parameters, formulas, and units

Studied parameters	Calculation formula	Unit of Measurement	Used References
Germination Percent (GP)	$GP = \frac{Ni}{N} \times 100$	%	[12]
Mean Germination Time (MGT)	$MGT = \frac{\sum ni \times di}{N} \times 100$	Day	[13]
Germination index	GI = ∑ (Gt/Dt)	Day	[14]
Collar diameter	Digital clipper	mm	[10]
Shoot Length	Ruler	cm	[10]
Shoot fresh Weight	Precision scale	gr	[10]
Root fresh weight	Precision scale	gr	[10]
Shoot dry weight	Precision scale	gr	[10]
Root dry weight	Precision scale	gr	[10]

N: Total number of seeds and Ni: germinated seeds at the end of counting days, ni: germinated seeds per day and di: counting day, Cpsgt: final germination percent and T: Total number of days, Gt is corresponding number of seeds germinated in the t day; Dt is time corresponding to Gt in days

Table 2. Analysis of variance (ANOVA) for growing media (M), polybag size (P), and their interaction (M×P) for seed germination parameters

SOV	DF	Mean Squares (MS)		
		Germination (%)	MGT	GI
M	3	620.2*	0.19 ^{ns}	82.54
P	2	19.8 ^{ns}	0.02 ^{ns}	9.44
M×P	6	248.6 ^{ns}	0.49**	96.27**
Error	24	204.9	0.1130	23.84

**, **, and ^{ns} show significant differences at 5% and 1% levels of probability and non-significance, respectively*

Table 3. Analysis of variance (ANOVA) for growing media (M), polybag size (P), and their interaction (M×P) for seed germination parameters

SOV	DF	MS								
		Diameter	Seedling Height	Seedling Fresh Weight	Root Fresh Weight	Seedling Dry Weight	Root Dry Weight	Root: Shoot ratio	Sturdiness Quotient	Seedling Quality Index
M	3	0.3188**	0.4016*	0.0098 ^{ns}	0.0087*	0.0119*	0.0022*	0.0043 ^{ns}	0.1159 ^{ns}	0.0011**
P	2	0.5**	0.61*	0.02 ^{ns}	0.02**	0.02**	0.0025*	0.01 ^{ns}	0.44 ^{ns}	0.002**
M×P	6	0.2916**	0.3414*	0.056**	0.002 ^{ns}	0.003 ^{ns}	0.0005 ^{ns}	0.0054 ^{ns}	1.2524*	0.0005
Error	24	0.0611	0.1224	0.0134	0.0024	0.0030	0.0007	0.0067	0.4518	0.0002

**, **, and ^{ns} show significant differences at 5% and 1% levels of probability and non-significance, respectively*

Table 4. Effect of growing medium and polybag Size on germination of *Pinus gerardiana* seeds

Sowing media	Germination (%)	Mean germination time	Germination index
Soil: compost: forest soil	64.44 b	2.25 ab	26.18 ab
Soil: compost: sand	77.78 ab	2.30 a	28.93 ab
Soil: forest soil: sand	80.74 a	2.17 ab	33.35 a
Sand: compost: forest soil	82.96 a	1.97 ab	28.16 ab
P1(16cm*16cm)	75.00 a	2.15 a	28.75 a
P2(18cm*18cm)	77.22 a	2.15 a	30.17 a
P3(21cm*26cm)	77.22 a	2.22 a	28.54 a

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

Growing media and polybag size	Diameter	Seedling height	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Root: shoot ratio	Sturdiness quotient	Seedling quality index
Soil: compost: forest soil	1.62 b	5.11 b	0.69 a	0.44 b	0.48 b	0.30 b	0.62 a	3.29 a	0.10 b
Soil: compost: sand	1.62 b	5.22 b	0.66 a	0.47 ab	0.51 b	0.31 b	0.61 a	3.27 a	0.10 b
Soil: forest soil: sand	1.67 b	5.33 ab	0.71 a	0.47 ab	0.53 ab	0.30 b	0.58 a	3.22 a	0.11 b
Sand: compost: forest soil	2.01 a	5.60 a	0.64 a	0.51 a	0.57 a	0.33 a	0.58 a	3.04 a	0.13 a
P1(16cm*16cm)	1.53 b	5.08 b	0.65 a	0.44 b	0.49 b	0.30 b	0.62 a	3.41 a	0.10 b
P2(18cm*18cm)	1.72 b	5.33 ab	0.65 a	0.45 b	0.51 b	0.31 ab	0.61 a	3.16 a	0.11 b
P3(21cm*26cm)	1.94 a	5.53 a	0.73 a	0.52 a	0.57 a	0.32 a	0.56 a	3.03 a	0.12 a

Table 6. Interaction effect of growing medium and seed size on germination of *Pinus gerardiana* seeds

Soil media	Poly bag size	Germination (%)	Mean germination time	Germination index
Soil: compost: forest soil	P1(16cm*16cm)	51.11 b	1.77 c	17.44 c
	P2(18cm*18cm)	77.78 a	2.42 ab	32.44 ab
	P3(21cm*26cm)	64.44 ab	2.54 ab	28.64 b
Soil: compost: sand	P1(16cm*16cm)	80.00 a	2.17 abc	32.18 ab
	P2(18cm*18cm)	68.89 ab	2.67 a	28.18 b
	P3(21cm*26cm)	84.44 a	2.08 bc	26.42 b
Soil: forest soil: sand	P1(16cm*16cm)	82.22 a	2.58 ab	38.77 a
	P2(18cm*18cm)	80.00 a	1.85 c	29.16 b
	P3(21cm*26cm)	80.00 a	2.06 bc	32.13 ab
Sand: compost: forest soil	P1(16cm*16cm)	86.67 a	2.07 bc	26.60 b
	P2(18cm*18cm)	82.22 a	1.65 c	30.91 ab
	P3(21cm*26cm)	80.00 a	2.18 abc	26.98 b

Table 7. Interaction effect of growing medium and seed size on seedling growth of *Pinus gerardiana*

Soil media	Poly bag size	Diameter	Seedling height	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Root: shoot ratio	Sturdiness quotient	See ling quality index
Soil: compost: forest soil	P1(16cm*16cm)	1.50 cd	4.33 c	0.80 ab	0.40 d	0.40 d	0.27 c	0.69 a	2.94 bc	0.09 d
	P2(18cm*18cm)	1.57 bcd	5.33 ab	0.54 ef	0.45 bcd	0.49 cd	0.30 bc	0.63 ab	3.41 ab	0.10 cd
	P3(21cm*26cm)	1.53 bcd	5.25 b	0.60 cdef	0.4 cd	0.49 bc	0.30 bc	0.61 ab	3.43 ab	0.10 cd
Soil: compost: sand	P1(16cm*16cm)	1.53 bcd	5.40 ab	0.66 bcdef	0.49 bc	0.58 abc	0.31 bc	0.54 b	3.86 ab	0.11 bcd
	P2(18cm*18cm)	1.93 b	5.40 ab	0.56 def	0.44 cd	0.50 bc	0.31 bc	0.62 ab	2.80 bc	0.11 bcd
	P3(21cm*26cm)	1.47 cd	5.17 b	0.65 bcdef	0.45 bcd	0.49 bc	0.31 bc	0.64 ab	3.56 ab	0.10 cd
Soil: forest soil: sand	P1(16cm*16cm)	1.60 bcd	5.23 b	0.87 a	0.45 bcd	0.51 bc	0.28 bc	0.56 ab	3.28 abc	0.10 bcd
	P2(18cm*18cm)	1.87 bc	5.53 ab	0.52 f	0.46 bcd	0.53 abc	0.32 ab	0.61 ab	3.02 abc	0.12 bc
	P3(21cm*26cm)	1.43 d	5.58 ab	0.71 abcde	0.47 bcd	0.55 abc	0.31 bc	0.56 ab	4.12 a	0.10 cd
Sand: compost: forest soil	P1(16cm*16cm)	1.83 bcd	5.17 b	0.78 abc	0.49 bc	0.56 abc	0.31 bc	0.55 ab	2.83 bc	0.12 b
	P2(18cm*18cm)	1.87 bc	5.50 ab	0.67 bcdef	0.53 ab	0.58 ab	0.32 ab	0.55 ab	2.95 bc	0.12 b
	P3(21cm*26cm)	2.63 a	5.87 a	0.75 abcd	0.59 a	0.60 a	0.36 a	0.59 ab	2.23 c	0.15 a

4. DISCUSSION

4.1 Effect of Growing Medium

Our findings showed that the germination percentage was higher in those media treatments which had sand as a combinational portion like M_3 (soil + forest soil + sand) and M_4(sand + compost + forest soil). In media treatments with no sand portion, germination percentage was better in forest soil-containing treatments. This betterment in germination might be attributed to forest soil and sand that in turn prepare *in situ* conditions and good aeration for seed germination. These results are in harmony with those of [15] and [16].

Our results also showed that sand-containing treatments (M_4) resulted in higher growth parameters and seedling quality index as compared with treatments not contained sand portions. Media with sand (M_4) causes aeration leading to longer primary root and good respirational activity. Similar results have been reported by Abebe [17] in *Azadirachta indica* Nemm.

4.1.1 Effect of polybag size

Our findings regarding seed germination showed that polybag size had no significant effects on germination parameters whilst polybag size significantly influenced seedling growth. The highest growth parameters were obtained by treatment P_3 (26 cm × 21 cm). The larger the polybag size, the higher the seedling growth. This assumed to be due to adequate space for the growth of both (above and below ground) portions. These findings are parallel with the results of Abera et al. [18].

4.1.1.1 Effect of interaction of container size and potting media on growth and development of seedlings

Results of this study showed that the interaction of growing media and polybag size was significantly effective on diameter and height. P_3×M_4 interaction resulted in the highest diameter and height. Large polybag size with good growing media, paves the way for good water-holding capacity, porosity, and root growth as reported by Ilyas et al. [19]. Although various interactions had different effects so the results made inconclusive. For precise estimation of interaction effects, additional studies are needed to be run in the future.

5. CONCLUSION

The results of the study conclude that germination and seedling growth of *P. gerardiana* seeds depend on growing medium and polybag size. The application of different growing mediums and the use of different polybag sizes significantly affected the germination and seedling growth of the chilgoza. In general, in the present study, M4 (sand: compost: forest soil) is recommended for seed germination and seedling growth, but P3 (26 cm x 21cm) polybag size is recommended only for enhancing seedling growth parameters.So polybag size should be selected according to the nursery space and economic prospects of the grower. For precise estimation of interaction effects, further research is needed to be done in a reasonable design and well-equipped laboratory conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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