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Effect of pre-sowing chemical treatments and environmental parameters on seed germination and survival of *Alnus nepalensis* D.Don and *Ligustrum lucidum* W.T. Aiton: A landslide control and an ethnomedicinally important tree species of the Himalayan highlands

M Wanlambok Sanglyne, Carefulness M Dirborne, Hriiziini Monica*
Department of Botany, North Eastern Hill University, Shillong, Meghalaya, India

Abstract
The present study was carried out in order to ascertain the effect of different pre-sowing chemical treatments, growth regulators and environmental parameters on seed germination, response and survival of seeds of *Alnus nepalensis* and *Ligustrum lucidum*, two priced tree species of the Eastern Himalayas. Treatments with different chemicals like Pottasium nitrate (KNO_3), Hydrochloric acid (HCl), Sulphuric acid (H_2SO_4), Nitric acid (HNO_3), Plant growth regulators (PGRs) like Gibberellic acid (GA_3), α-Napthaleneacetic acid (NAA), and environmental conditions like storage temperature, seed moisture content were performed on the seeds. Results of this investigation revealed that some treatments have a negative effect while others have a positive effect on seed germination and survival of seeds of these two tree species. Lower storage temperature and seed moisture content along with treatment involving dipping in Gibberellic acids (GA_3) prior to sowing are strongly encourage for the successful germination, survival, growth and establishment of these tree species.

Keywords: *Alnus nepalensis* D. Don, *Ligustrum lucidum* W.T. Aiton, seed germination, survival, environmental parameters, chemical treatments, PGRs

Introduction

The Indian Himalayan region is characterized by its rich diversity and heterogeneous area in terms of geology, lithology, rainfall distribution, land cover, soil properties, road and stream networks, which makes it highly vulnerable to landslides [1]. Further, most of the area is seismically active and subject to extreme precipitations and the situation has been further worsened by ruthless anthropogenic activities and rapid climate change. According to the Ministry of Environment, Forest and Climate Change (MoEF&CC), Government of India, the region covers 16.2% (~500,000 km^2) of India's landmass as well as 10 of its states. The region extends 26°200'-35°400' N and 74°500'-95°400' E and was divided into two distinct regions i.e. the Western Himalayas and the Eastern Himalayas. The Western Himalayan ranges extend over Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Shiwaliks of Punjab as well as Haryana up to the western border of Nepal. The Eastern Himalayan ranges cover north-eastern hill states of Arunachal Pradesh, Manipur, Arunachal Pradesh, Mizoram, Nagaland, Sikkim, Tripura, and parts of Assam and West Bengal [2]. The study sites include the north-eastern states *viz*., Arunachal Pradesh, Assam, Meghalaya, Nagaland and Sikkim situated in the Eastern Himalayan region (Fig 1).

Landslides are hazardous geologic process that caused significant damage to any affected landscape. Landslide perils are face by the Himalayan region every year which affect at least 15% of our country-an area which exceeds 0.49 million km^2 subsequently leading to the loss of human life, extensive damage to properties and biodiversity loss [3, 4, 5]. Landslides of different types are frequent in geo-dynamically active domains in the Himalayan and Arakan-Yoma belt of the North-Eastern parts of the country as well as in the relatively stable domains of the Meghalaya Plateau, Western Ghats and Nilgiri Hills [6].

In addition to rich biodiversity reservoir, Indian Himalayas ethnic inhabitants are deeply rooted with traditional and indigenous knowledge. Traditional ecological knowledge is a rich cultural heritage about a place accumulated over many generations. The indigenous technical knowledge acquired is local, or confined to specific site and plays an undeniable role in the management of natural resources [7, 8, 9, 10]. With pertinent systematic studies and implementation of remedial or mitigation measures, landslides can be predicted which can minimized the damage and can even be averted [11]. Thus, integrating traditional and scientific knowledge is imperative for better understanding and improving adaptation strategies for land and natural resource management which is the key to sustainable development as well as conservation of biodiversity. To decrease the hazards of landslides and to improve the fertility of the soil many plant species are planted in the Indian Himalayan regions eg. *Alnus nepalensis*, *Zanthoxylum* spp, *Clerodendrum cordatum*, *Populus ciliata*, *Desmodium sp*, *Emblica officinalis*, *Dendro calanus*, *Calotropis procera* etc.

Alnus nepalensis D. Don. belongs to family Betulaceae, a rapid growing early successional species mostly found in landslide affected areas with nutrient impoverished soil conditions in most Himalayan forests below 2500m [12, 13, 14, 15] (Fig 2). *Alnus* roots have association with *Frankia* which fix nitrogen and have stronger impact on soil physicochemical characteristics [13, 16, 17]. Therefore, it is widely used as an effective agent in agroforestry, forest management and restoration of the ecosystem, *Alnus* spp. has also been traditionally used as an inter-cropping tree species [12, 18, 19, 20]. Further, it also mobilizes available phosphorus, changes soil properties by increasing nitrogen

levels and organic matter in the soil [21, 22]. In addition to landslide control and soil management factor *A. nepalensis* is also an important ethno-medicinal plant. The leaf, roots and bark are used in dysentery, stomach ache and diarrhoea in ayurveda [23]. Leaf paste is applied in cuts and wounds [24]. Barks are boiled, and the gelatinous liquid is applied to burns [25]. Diarylheptanoids are characteristic components of *Alnus* spp.

Important biologically active diarylheptanoids *viz*., Platyphyllenone, alusenone, hirustenone, and hirsutanonol are present in *A. nepalensis* which are anti-influenzal, antioxidant, cytotoxic and has hepatoprotective effects [26, 27, 28, 29].

It is known for its dyeing and tanning purpose [30].

Ligustrum lucidum W.T.Aiton also known as glossy privet is an important ethnomedicinal wild tree species which belongs to the family Oleaceae. In traditional Chinese medicine the fruits are used as a tonic for the treatment of chronic bronchitis, hepatitis, diarrhoea, kidney and liver diseases and has an antiproliferative potential against human carcinoma [31] (Fig 3). Ailments such as dizziness, tinnitus, vertigo, hair fall, premature greying hair, joints pain, weakness of the lower back, knees, bones injuries/bone fracture etc. were also treated using the seeds of this species [32, 33]. Owing to the presence of important bioactive compounds *viz*., botulin, flavonoids, iridoid, ligustrin, lupeol, oleanolic, secoiridoid glycosides, syringin, triterpenoid, ursolic acid etc. It has been reported that the species exhibit anti-inflammatory, antidiabetic, antibacterial, antiviral and hepatoprotective properties [34, 35, 36].

Seed germination, a comprehensive developmental process in the lifecycle of plants providing a fundamental link in population dynamics leading to successful establishment of new individuals thus, the founding of populations which are significantly important from both an economic and ecological point of views [37, 38, 39]. Seed germination is species specific and the rate of germination depends on number of internal factors like dormancy, genotype, maturity of the seeds and external factors, includes temperature, salinity, light, moisture conditions and seed maintenance [40,41]. Enhancing the seeds germination and development of the vigorous seedlings is crucial which decides the increase or decrease of the plant population. Suitable pre-sowing treatment can ensure fast and uniform germination [42]. So, it is vital to find a suitable pre-sowing treatment technique. To fasten the process of germination and to bring about uniformity chemical, physical and environmental pre-sowing treatment of seeds have been carried out in many plant species [43, 44, 45, 46].

It has been reported that nursery techniques with appropriate pre-sowing treatments is the ultimate way to improve germination percentage and to save economically valuable plant species from becoming extinct [47]. Therefore, studies on pre-sowing treatments of economically and ethnobotanically important plants to find the optimum conditions that maximize the germination rate becomes a necessity for increasing the economy and conservation purposes. Thus, the objective of this study was to find out the appropriate pre-sowing treatments of two important tree species of the Himalayan highlands -*Alnus nepalensis* D.Don and *Ligustrum lucidum* W.T.Aiton: A landslide controlling and an ethnomedicinally important tree species that will enhanced total germination and effect on seedlings growth.

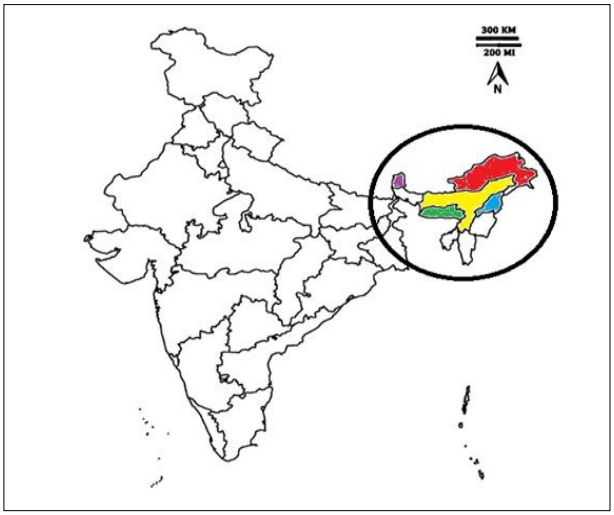

Fig 1: Map showing the study sites (Red: Arunachal Pradesh; Yellow: Assam; Blue: Nagaland; Green: Meghalaya and Purple: Sikkim).

Fig 2: *Alnus nepalensis* D.Don A: Mature trees in the wild; B: Immature fruits resembling Pine cones; C: Mature fruits; D: Mature fruits with inflorescence (catkin) E: Seeds of *Alnus nepalensis*.

Fig 3: *Ligustrum lucidum* W.T. Aiton A: Tree with immature fruits; B: Tree with mature fruits; C: Mature fruits.

Materials and methods

Collection of samples

Seeds of *Alnus nepalensis* D.Don and *Ligustrum lucidum* W.T. Aiton were collected from fruits and fruiting catkins of mature trees located in the state of Assam, Meghalaya, Sikkim, Nagaland and Arunachal Pradesh from May 2016 to Jan 2019. The seeds were sun dried to remove the pulp (for

L. lucidum) and were then brought to the laboratory in an air tight container in order to prevent the gain or loss of moisture during transit or storage. In the laboratory, the following observations were then carried out.

Pre-sowing chemical treatments

The collected seeds of *Alnus nepalensis* and *Ligustrum lucidum* were subjected to various pre-sowing chemical treatments *viz*., soaking in water for 24, 48, 72, 96 and 120 hours, Pottasium nitrate (KNO_3) (24,48,72,96 and 120 hours), acid scarification to remove hard seed coats with 0.1N nitric acid (HNO_3) (1,2,3,4 and minutes), 0.1N hydrochloric acid (HCl) (1,2,3,4 and 5 minutes), concentrated sulphuric acid (H_2SO_4)(30,60,90,120 and 150 seconds), dipping in growth regulators like Gibberellic acid (GA_3) (100,200,300,400 and 500 ppm) and α-Napthaleneacetic acid (NAA) (100,200,300,400 and 500 ppm) respectively. All seed germination experiments that followed were performed at room temperature. The chemically treated seeds were not sterilized before use and were sown directly into natural soil immediately after above treatments. Experiments were conducted in triplicates and the results shown are the mean values of these replicates. Results from three replicates were taken each after 40 days of sowing [48].

Effect of environmental parameters on Seed germination and survival

The effect of two environmental parameters on the survival and seed germination of the two studied species was determined by performing a number of seeds testing experiments discuss below.

Seed moisture determination

The seed moisture content of the fresh and conditioned seeds was determined by the standard method prescribed by the International Seed Testing Association [49].

4 - 5 g seeds were weighted in the moisture bottle. The bottles containing seeds were transferred to the oven maintained at 103± 2°C for 17 hours. The weight of the dried samples and bottles were again taken and the moisture content was calculated according to the formula below:

$$Seed\ Moisture\ content\ (\%) = \frac{W1 - W2}{W1 - W3} X100$$

Where,

'W1' is the weight of seeds and moisture bottle

'W2' is the weight of dried seeds and moisture bottle and

'W3' is the weight of empty moisture bottle.

The results were expressed in percentage (fresh weight basis) and this was regarded as the original seed moisture content.

Germination test

Seeds were sterilized in 0.1% $HgCl_2$ solution followed by repeated washing (about three times) in distilled water. Routinely, 50 seeds in three replicates were rolled into a moistened towel paper (germination paper) and incubated at 25°C. On the 14th day, germination was evaluated by classifying seedlings into normal, abnormal, dead seeds (if any) and hard seeds (if any). Each category was expressed in percentage. The percentage of normal seedlings represents the germination percentage of the lot. This is to be considered as the original germination percentage.

Seedlings length

The seedlings length was determined by measuring the length of each seedling and the mean of means of each of the three replicates of 10 was reported and expressed in gram per seedling.

Seedlings dry weight

Three replicated of ten seedlings each are dried for 24 hours in an oven maintained at 90°C. The mean of three replicates of 10 seedlings was reported and expressed in gram per seedling.

The seedling length and dry weight were taken as indices of seed vigour as it cannot be taken directly.

Conditioning of seeds and storage

Conditioning of seeds to higher desirable seed moisture content was achieved by adding water using the following formula by Varier *et al*. [50].

$$W = S(mf - mi)/100$$

Where,

'W' is the amount of water to be added

'S' is the weight of seeds

'MI' is the initial seeds moisture content

'MF' is the final seed moisture/desirable seed moisture content.

The seeds so conditioned to desired moisture content by the above formula were kept at 4°C for moisture calibration for 1 or 2 days. The seeds so calibrated were divided into two lots, one lot was subjected to 10°C and another to 40°C for three days respectively, following which germination, seedlings length and seedling dry weight were evaluated on the 14st day after incubation for germination as discussed above.

Statistical analysis

All the results obtained were subjected to two-way ANOVA.

Results and Discussion

From our observation, seeds of *Alnus nepalensis* and *Ligustrum lucidum* showed an original seed moisture content of 10.54% and 16.95% which is based on a fresh weight basis and an original germination percentage of 44.88% and 83.33% based on normal seedlings germination percentage at room temperature. Since the seeds of both the species survived their respective seed moisture content discussed above, it may be argue that both their seeds belong to the orthodox category. Seed moisture content and storage temperature are the two determining factors that affect the longevity and viability of seeds. Therefore, the present investigation aims to investigate the seed storage behaviour under different combination of seed moisture content and storage temperature. In this study, it was observed that for any seed moisture content of 10.54% and 20.40% (*Alnus nepalensis*) 16.95% and 20.40 % (*Ligustrum lucidum*), there was a decreasing trend in seed viability during storage with increasing storage temperature ranging from 10 to 40°C in term of normal seedlings evaluated after 14 days of incubation (Table 1,2,3 and 4). Abnormal seedlings increased with increasing seed moisture content and storage temperature. A similar trend was observed in

the percentage of dead seeds (Table 1, 2, 3 and 4; Fig 4, 5). This observation that involves the loss of seed vigour with higher storage temperature and moisture content has also been reported in many plant species [51, 52]. So far as seedling length was concerned it was observed that the decrease in seedling length was most affected by a combination of 20.40% of seed moisture content and 40°C of storage temperature. The seedling length was minimum (2.45 cm and 0.25 cm) at storage temperature of 40°C and seed moisture content of 20.40% for both *Ligustrum lucidum* and *Alnus nepalensis* while it was maximum (9.00cm) at storage temperature of 10°C and seed moisture content of 20.40% for *L. lucidum* and a maximum length of 1.54 cm at storage temperature of 20°C and seed moisture content of 10.54% for *A. nepalensis* respectively (Table 5, 6; Fig 6). The seedlings dry weight has been found to be affected by storage temperature of 40°C. Higher storage temperature yield lower seedlings dry weight. This is true for both the species under investigation. A combination of a higher seed moisture content of 20.40% and an optimum storage temperature of 20°C was found to be the best combination that yielded heavier seedlings dry weight. For *L. lucidum*, maximum (0.96g) seedlings dry weight was recorded at a storage temperature of 20°C and seed moisture content of 20.40%. This same trend was also observed in *A. nepalensis* in which maximum (0.069g) seedling dry weight was recorded at the same storage temperature and seed moisture content (Table 7,8; Fig 7). Cooper *et al.*, argued that vigorous seeds i.e. those seeds that could maintain their seed vigour status as a results of favourable/optimum conditions requirements would produce healthy long seedlings and high dry weight as compared to those less vigorous ones [53]. Different pre-sowing treatments have different effects on seed germination and survival of seeds of both the tree species under investigation. Before we interpret the effects of these chemical and different pre-sowing treatments on seeds germination and survival in both the species, it is to be noted that the seeds of *L. lucidum* is far more superior in size compare to those of *A. nepalensis*. Because of this acid scarification techniques may damage seeds of *A. nepalensis* far more significantly than it would have done for the seeds of *L. lucidum* which may explain the low germination percentage during acids scarification treatment in *A. nepalensis* seeds. It has been found through this investigation that the best germination percentages after pre-sowing treatments in both *Alnus nepalensis* and *Ligustrum* lucidum comes from those plots that had been treated with 500 ppm Gibberellic acid (GA_3) with 63.52% and 86.64% respectively. On the other hand, scarification using conc. Sulphuric acid (H_2SO_4) for 150 seconds seem to have a detrimental effect on seed germination and survival in both the species with minimum germination percentage of 3.74% (for *A.nepalensis*) and 24.32% respectively. It has been observed that different pre-sowing treatments have varying effects on the seeds germination and their survival rates (Table 9; Fig 8). Success while using Gibberellic acid (GA_3) and other related plant growth regulators (PGRs) in seed germination has been extolled by many other in numerous plant species [46,47]. Since seeds are the basic units of reproduction/propagation, basic understanding of their physiological behaviours under different conditions could help improved their germination success rate and aid in their successful proliferation, survival and growth. Seedlings sprouts of many economically important, medicinal or even endangered plant species failed to establish themselves in poly houses conditions even with added chemicals, fertilizers and soil enhancers. This may be due to a number of reasons, one such reasons is the loss of seed viability (i.e. Seed ageing) which is a result of unfavourable storage conditions of seeds and the mechanisms that are involved during such storage of seeds in unfavourable environments. Seeds has been reported to undergo different physiological and biochemical changes during transit or storage in unfavourable or favourable environmental conditions, changes that occurs during unfavourable conditions during storage or transit led to seed deterioration and ultimately to their death [54]. This mechanism of seed deterioration during storage or transit has been attributed to numerous enzyme activities. In addition to the combine effects of biochemical and environmental changes/processes/mechanisms/ conditions, seeds of many plant species had also been reported to have lost their membrane stability and permeability during storage and transits. This physiological changes in seeds characteristics led to the leaching out of important biomolecules from the tissues which if otherwise present would have greatly benefitted in their survival, growth and establishment in nature [55, 56]. The present study hope to shed some light on the importance of optimum conditions requirements and appropriate seed care in the successful germination, survival, growth and establishments of seedling of important tree species. Studies such as this could greatly benefit farmers, agriculturist, foresters and environmentalist in ensuring that planted seeds would thrive survive, grow and establish themselves in any given environment provided that appropriate care is taken at every steps of the developmental process.

Table 1: Showing the germination percentage of normal seedlings of *Ligustrum lucidum* from seeds of 16.95% (original) seed moisture content stored at different temperatures of 10°C, 20°C and 40°C for a period of 3 days (mean of three replicates: readings taken on the 14th day).

Storage Temperature °C	**Normal Seedlings %**	**Abnormal Seedlings %**	**Dead Seeds %**	**Hard Seeds %**
10	83.33(±1.44)	6.00(±2.50)	9.30(±0.54)	1.37(±0.054)
20	83.33(±1.44)	5.60(±1.88)	9.74(±0.68)	1.33(±0.054)
40	22.00(±0.89)	19.48(±0.98)	57.07(±1.62)	1.45(±0.021)

(Values in parenthesis indicate Standard error)

Table 2: Showing the germination percentage of normal seedlings of *Alnus nepalensis* from seeds of 10.54% (original) seed moisture content stored at different temperatures of 10°C, 20°C and 40°C for a period of 3 days (mean of three replicates: readings taken on the 14th day).

Storage Temperature °C	**Normal Seedlings %**	**Abnormal Seedlings %**	**Dead Seeds %**	**Hard Seeds %**
10	44.27 (±0.08)	25.28 (±0.02)	22.48 (±1.24)	7.97 (±0.11)
20	44.88 (±0.07)	26.88 (±0.62)	19.56 (±0.89)	8.68 (±0.29)
40	17.56 (±1.02)	35.88 (±0.89)	40.64 (±0.04)	5.92 (±0.98)

(Values in parenthesis indicate Standard error)

Table 3: Showing the germination percentage of normal seedlings of *Ligustrum lucidum* from seeds of 20.40% (After conditioning) seed moisture content stored at different temperatures of 10°C, 20°C and 40°C for a period of 3 days (mean of three replicates: readings taken on the 14th day).

Storage Temperature °C	Normal Seedlings %	Abnormal Seedlings %	Dead Seeds %	Hard Seeds %
10	77.33(±1.44)	4.67(±1.44)	10.00(±3.41)	1.20(±0.63)
20	66.90(±1.27)	15.09(±1.67)	16.71(±2.67)	1.30(±0.63)
40	11.29(±1.78)	31.22(±1.27)	56.19(±2.12)	1.30(±0.49)

(Values in parenthesis indicate Standard error)

Table 4: Showing the germination percentage of normal seedlings of *Alnus nepalensis* from seeds of 20.40% (After conditioning) seed moisture content stored at different temperatures of 10°C, 20°C and 40°C for a period of 3 days (mean of three replicates: readings taken on the 14th day).

Storage Temperature °C	Normal Seedlings %	Abnormal Seedlings %	Dead Seeds %	Hard Seeds %
10	35.28 (±1.74)	11.58 (±1.01)	45.98 (±0.88)	7.16 (±1.12)
20	30.66 (±0.55)	22.85 (±0.81)	39.98 (±0.79)	6.51 (±1.63)
40	9.86 (±0.63)	41.56 (±074)	46.07 (±0.23)	2.51 (±1.23)

(Values in parenthesis indicate Standard error)

Table 5: Showing seedling length of *Ligustrum lucidum* (cm) from seeds of different moisture content stored at different temperatures for 3 days (mean of three replicates: readings taken on the 14th day).

Seed Moisture Content (%)	Storage Temperature		
	10°C	20°C	40°C
16.95	8.85 (±0.095)	8.90 (±0.15)	6.65 (±0.56)
20.40	9.00 (±0.34)	6.87 (±0.09)	2.45 (±0.34)

(Values in parenthesis indicate Standard error)

Table 6: Showing seedling length of *Alnus nepalensis* (cm) from seeds of different moisture content stored at different temperatures for 3 days (mean of three replicates: readings taken on the 14th day).

Seed Moisture Content (%)	Storage Temperature		
	10°C	20°C	40°C
10.54	1.28 (±0.023)	1.54 (±0.52)	0.97 (±0.44)
20.40	1.15 (±0.25)	1.01 (±0.08)	0.25 (±0.25)

(Values in parenthesis indicate Standard error)

Table 7: Showing seedlings dry weight of *Ligustrum lucidum* (g) from seeds of different moisture content stored at different temperatures for 3 days (mean of three replicates: readings taken on the 14th day).

Seed Moisture Content (%)	Storage Temperature		
	10°C	20°C	40°C
16.95	0.129 (±0.003)	0.130 (±0.003)	0.076 (±0.002)
20.40	0.133 (±0.006)	0.96 (±0.001)	0.035 (±0)

(Values in parenthesis indicate Standard error)

Table 8: Showing seedlings dry weight of *Alnus nepalensis* (g) from seeds of different moisture content stored at different temperatures for 3 days (mean of three replicates: readings taken on the 14th day).

Seed Moisture Content (%)	Storage Temperature		
	10°C	20°C	40°C
10.54	0.022 (±0.002)	0.029 (±0.007)	0.011 (±0.002)
20.40	0.054 (±0.008)	0.069 (±0.001)	0.017 (±0.001)

(Values in parenthesis indicate Standard error)

Table 9: Showing the effect of various pre-sowing treatments on seed germination of *Alnus nepalensis* and *Ligustrum lucidum* (mean of three replicates) after 40 days of sowing.

Pre-sowing treatments	Seeds	
	Alnus nepalensis	*Ligustrum lucidum*
Control	43.27 (± 0.202)	84.82 (±0.048)
Water Soaking		
24 hours	45.23 (±0.033)	85.61 (±0.302)
48 hours	46.21 (±0.036)	85.99 (±0.012)
72 hours	46.07 (±0.061)	85.85 (±0.078)
96 hours	31.11 (±0.429)	72.69 (±0.096)
120 hours	18.98 (±0.140)	61.40 (±0.157)
0.1 N HNO_3 Scarification		
1 minute	17.20 (±0.038)	43.34 (±0.051)
2 minutes	16.15 (±0.038)	42.09 (±0.009)
3 minutes	18.24 (±0.073)	41.41 (±0.185)
4 minutes	19.06 (±0.029)	40.80 (±0.163)
5 minutes	19.65 (±0.061)	41.66 (±0.211)
0.1 N HCl Scarification		
1 minute	20.33 (±0.078)	50.65 (±0.075)
2 minutes	20.55 (±0.191)	50.20 (±0.355)
3 minutes	18.87 (±0.205)	48.35 (±0.182)
4 minutes	16.23 (±1.111)	44.29 (±0.046)
5 minutes	11.48 (±0.994)	40.47 (±0.031)
Conc. H_2SO_4 Scarification		
30 seconds	10.43 (±0.177)	30.60 (±0.240)
60 seconds	7.32 (±0.212)	28.70 (±0.104)
90 seconds	5.94 (±0.089)	27.64 (±0.132)
120 seconds	4.37 (±0.319)	25.65 (±0.324)
150 seconds	3.74 (±0.148)	24.32 (±0.327)
24 hours GA_3 dipping		
100 ppm	51.83 (±0.157)	84.85 (±0.061)
200ppm	54.05 (±0.205)	85.09 (±0.028)
300 ppm	55.12 (±0.047)	85.33 (±0.043)
400 ppm	60.31 (±0.382)	85.89 (±0.069)
500 ppm	63.52 (±0.225)	86.64 (±0.321)
24 hours NAA dipping		
100 ppm	48.49 (±0.813)	77.49 (±0.164)
200 ppm	51.81 (±1.750)	78.34 (±0.038)
300 ppm	51.61 (±1.829)	76.99 (±0.729)
400 ppm	52.64 (±1.835)	79.11 (±0.058)
500 ppm	51.22 (±2.097)	78.48 (±0.507)
KNO_3 soaking		
24 hours	30.74 (±0.415)	43.65 (±2.415)
48 hours	31.88 (±0.207)	46.90 (±0.041)
72 hours	30.52 (±0.249)	44.24 (±0.035)
96 hours	31.74 (±0.153)	43.90 (±0.059)
120 hours	28.83 (±0.243)	40.13 (±0.120)

(Values in parenthesis indicate Standard error)

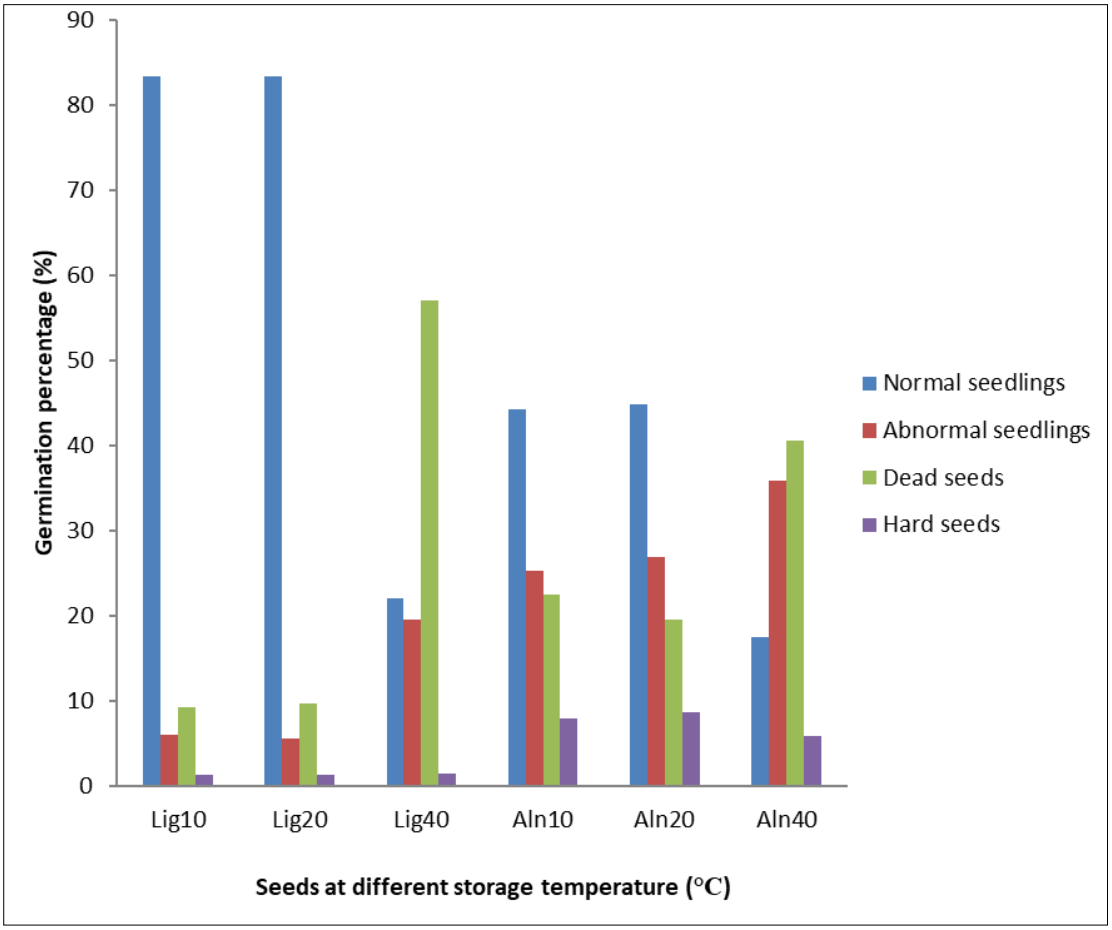

Fig 4: A comparative graphical representation of the germination percentages exhibited by the seeds of *Alnus nepalensis* (with original seed moisture content 10.54%) and *Ligustrum lucidum* (with original seed moisture content of 16.95%) stored at varying storage temperature (10°C, 20°C and 40°C): Lig10: seeds of *L. lucidum* stored at 10°C; Lig20: Seeds of *L. lucidum* stored at 20°C; Lig40: Seeds of *L. lucidum* stored at 40°C; Aln10: Seeds of *A. nepalensis* stored at 10°C; Aln20: Seeds of *A. nepalensis* stored at 20°C; Aln40: Seeds of *A.nepalensis* stored at 40°C.

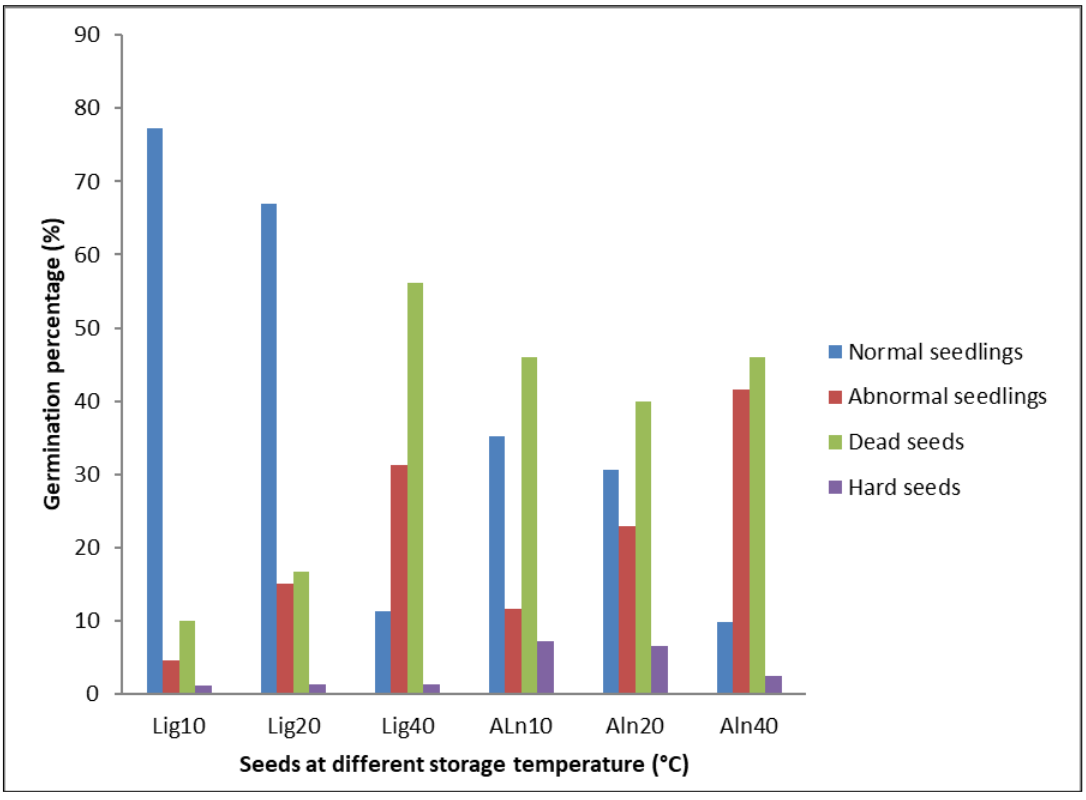

Fig 5: A comparative graphical representation of the germination percentages exhibited by the seeds of *Alnus nepalensis* (with after conditioning seed moisture content of 20.40 %) and *Ligustrum lucidum* (with after conditioning seed moisture content of 20.40%) stored at varying storage temperature (10°C, 20°C and 40°C): Lig10: seeds of *L. lucidum* stored at 10°C; Lig20: Seeds of *L. lucidum* stored at 20°C; Lig40: Seeds of *L. lucidum* stored at 40°C; Aln10: Seeds of *A. nepalensis* stored at 10°C; Aln20: Seeds of *A. nepalensis* stored at 20°C; Aln40: Seeds of *A.nepalensis* stored at 40°C.

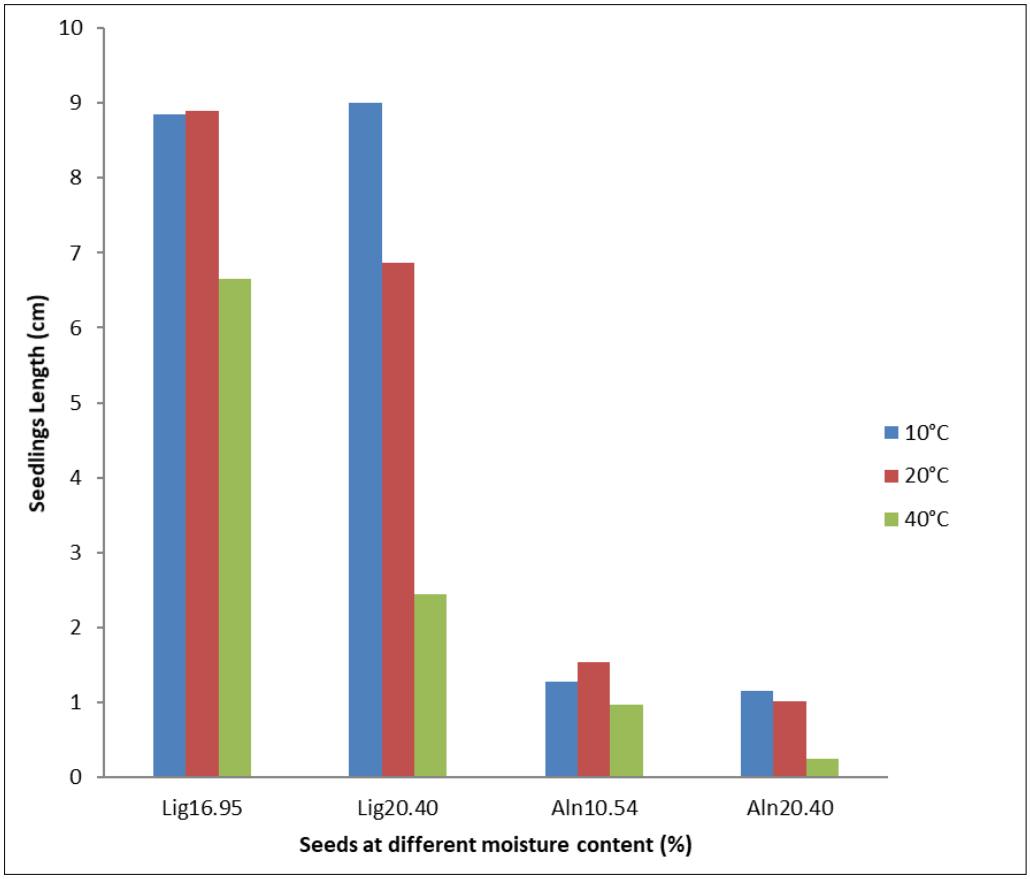

Fig 6: A comparative graphical representation of seedlings length of *Alnus nepalensis* and *Ligustrum lucidum* of seeds with original (10.54% and 16.95%) and conditioned (20.40%) moisture contents stored at varying storage temperature (10°C, 20°C and 40°C): Lig16.95: Seeds of *L. lucidum* with 16.95% moisture content; Lig20.40:Seeds of *L. lucidum* with 20.40% moisture content; Aln10.54: Seeds of *A. nepalensis* with 10.54% moisture content; Aln20.40: Seeds of *A. nepalensis* with 20.40% moisture content.

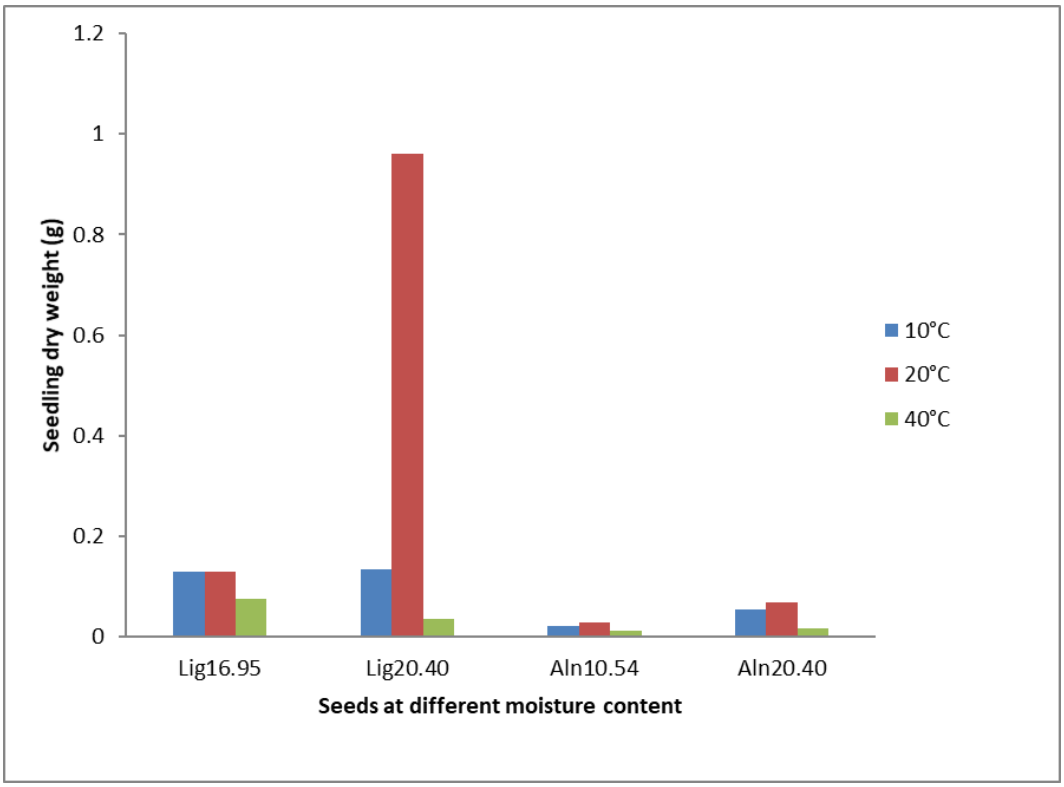

Fig 7: A comparative graphical representation of seedlings dry weight (g) of *Alnus nepalensis* and *Ligustrum lucidum* arising from seeds with original (10.54% and 16.95%) and conditioned (20.40%) moisture contents stored at varying storage temperature (10°C, 20°C and 40°C): Lig16.95:Seeds of *L. lucidum* with 16.95% moisture content; Lig20.40: Seeds of *L. lucidum* with 20.40% moisture content; Aln10.54: Seeds of *A. nepalensis* with 10.54% moisture content; Aln20.40: Seeds of *A. nepalensis* with 20.40% moisture content.

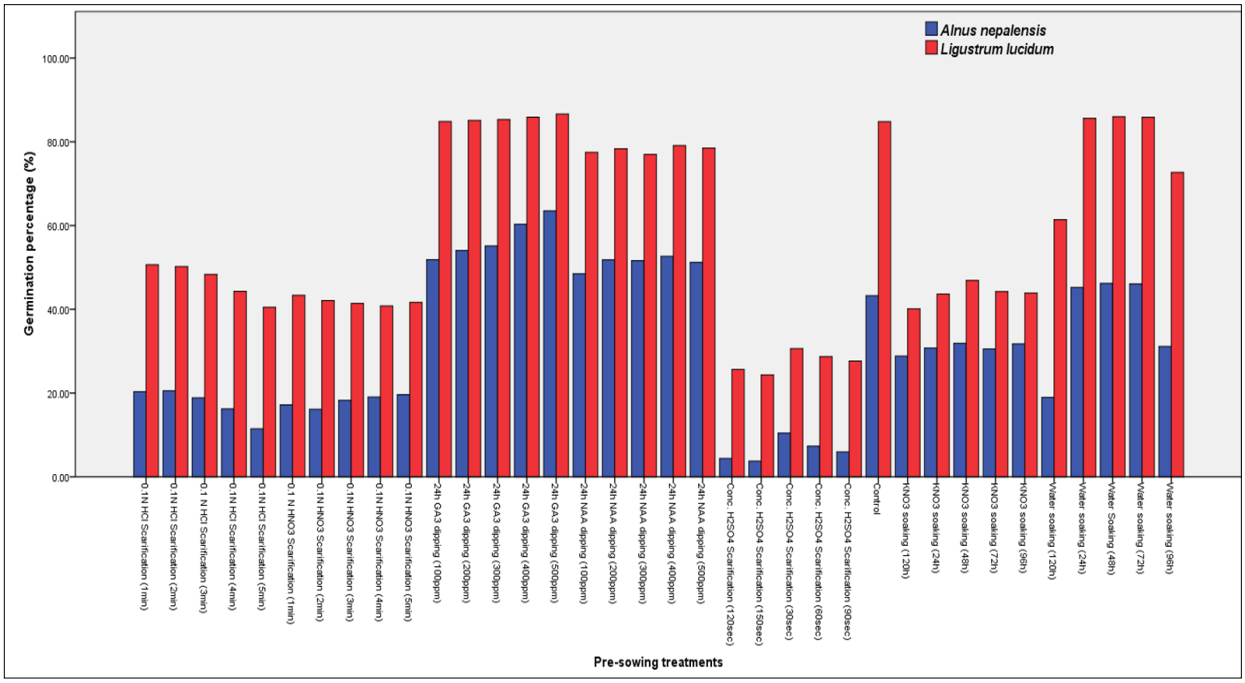

Fig 8: Graphical representation of the effects of various pre-sowing chemical treatments on seed germination of *Alnus nepalensis* and *Lugustrum lucidum*.

Conclusion

From this investigative study, it may be concluded that the seeds of both *Alnus nepalensis* D.Don and *Ligustrum lucidum* W.T. Aiton belongs to the orthodox seed category. Further, analysis of germination tests with regards to storage temperature and seed moisture content indicated that lower moisture content and lower storage temperature showed more successful germination rates in term of percentages than conditions of higher temperature and higher seeds moisture content. Therefore storing of seeds of both the species under conditions of low storage temperature and moisture content should be encourage. Lastly, when it comes to pre-sowing seeds chemical treatments, this study strongly suggest dipping the seeds in Gibberellic acids (GA_3) prepared at higher concentration of parts per million (ppm) eg. at 500ppm which yielded maximum responses and survival in both the tree species.

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