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Original Article

Germination Response of *Juniperus procera* Seed to Temperature and Pre-sowing Treatments

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Seed Pretreatments,
Temperature.

Juniperus procera is an indigenous tree species of Ethiopia that has high economic and ecological importance. Propagation of seedlings had poor germination due to dormancy. Enhancing the germination of Indigenous trees particularly, *Juniperus procera* seeds is the challenge encountered by nurseries. This study was conducted in the laboratory of the Central Ethiopia Forestry Development Center, with the aim to investigate the effect of seed pre-pretreatments (soaking in cold water for 24 hours, scarification, soaking in hot water for 10 minutes, and no seed pre-treatment) and temperature regimes (20°C, 30°C, 40°C, and room temperature) on germination. Twenty-five quality seeds per treatment were sown on a petri dish incubated under temperature regimes using a completely randomized design with four replicates. Germination starting and closing dates, germination period, percentages, mean germination time and index were calculated and analysis of variance was done. The result of ANOVA revealed a significant ($P < 0.05$) effect of pre-treatments and temperature regimes on seed germination closing dates, period, percentage and index over the control. There was a non-significant effect of temperature on germination starting and mean germination time. There was also a non-significant combined effect of pretreatments and temperature on the germination period. Among pre-treatments, scarification achieved the best germination and from temperature regimes, 20°C attained the best result over the other treatments. The highest germination percentage (74%) was obtained under 20°C with scarification. The lowest germination percentage (18 %) was recorded under the control at 30°C. No germination was observed under 40°C. The shortest germination starting, closing dates and period were obtained under 20°C with scarification. The lowest mean germination time and the greatest germination index were recorded under 20°C with scarification. Compared to the control, almost all pre-treatments and temperature regimes increased germination with decreasing temperature regimes. Hence, in raising *Juniperus* seeds, it is recommended to use scarification under 20°C.

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INTRODUCTION

Ethiopia is well-known for its different land types including gorges, river valleys, and various altitude ranges (Shiferaw *et al.*, 2021). These might be the cause of the country to be the origin and diversity of many flora and fauna (Sebsibe *et al.*, 2018). Consequently, around 6500-7000 species of higher plants exist in the country of which about 12% of plant species are indigenous (Kidane *et al.*, 2010). Ethiopian Afromontane forest encompasses tree species such as *Juniperus procera*, *Olea europaea*, *Podocarpus falcatus* etc. (Negash, 2010a).

Juniperus procera is an indigenous tree growing up to a height of 40 meters. It is a dioeciously, evergreen, and wind-pollinated tree of substantial economic and ecological importance (Negash, 2013). It is distributed with an altitude of 1,100–3,500 m and a rainfall of 300–1,200 mm/year. It is found in dry Afromontane (comprising slopes, ridges, and rocky highlands, (Mujwah *et al.*, 2010; Razgour *et al.*, 2021). There is a great interest in reestablishing *Juniper* forests in Ethiopia. Among other things, seed dormancy is the main difficulty in the regeneration of *Juniperus procera* (Kahveci *et al.*, 2018).

The propagation of most tropical trees is inhibited by seed dormancy (Olatunji, 2012; Botsheleng *et al.*, 2014). Germination depends on the physiological state of the seed, which is partially caused by the interaction between the plant genotype and environmental factors, such as temperature, moisture, light, and nutrient availability (Chahtane *et al.*, 2017).

Temperature is one of the main factors affecting the germination percentage and speed, which directly works through seed imbibition and the biochemical reactions that regulate the metabolism in the germination process (Motsa *et al.*, 2015). Most species need a suitable temperature range to achieve maximum germination (Belmehdi *et al.*, 2018). An extensive range of factors may disturb seed coat forced dormancy in seeds (Gama-Arachchige *et al.*, 2012). The seed becomes penetrable to water only when the coat is disturbed in some way, mainly in the strophiole region (Jaganathan *et al.*, 2018). Different pre-treatments were used to break dormancy and enhance the germination of seeds (Fredrick *et al.*, 2017; Mojeremane *et al.*, 2018; Mmolutsi *et al.*, 2020). Effective pre-treatment and suitable seed germinating environmental conditions like temperature should be recognized for those tree species including *Juniperus procera* seeds that have low germination percentages to improve their germination capacity. In this regard, there is no protocol developed on the effect of various seed pre-treatments and temperature regimes on the germination performance of *Juniperus procera* seed.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted in the Central Ethiopia Forest Development seed laboratory, in Addis Ababa, Ethiopia. It is geographically situated at the centre of the nation, 9°2'N latitude and 38°45'E longitude. Its average altitude is 2,400 meters above sea level, with the highest elevations

reaching 3,200 meters. It has a sub-tropical highland climate with a constant moderate temperature of roughly 23°C average high and 11°C average low throughout the year. The main rainy season, Kiremt, is from June to early October, and between early March and mid-April, there is a short period of rainfall called Belg. The average annual rainfall is about 1,200 mm, out of which close to 80% falls during the main rainy season.

Selection of Species for the Study

Juniperus procera was selected based on its economic, commercial, social and ecological importance. And yet it has low germination, slow germination speed, and non-uniform germination compared to other tree species.

Seed Source, Seed Surface Sterilization, and Viability Test

In this study, fresh seeds of *Juniperus procera* were collected from the Menagesha state forest located close to the capital city, Addis Ababa. The seeds collected for the experiment were subjected to a viability test to separate viable seeds using the floating test method (Daneshvar *et al.*, 2014). The test involved immersing the seeds into a container filled with water; seeds that were observed to float were immediately removed as they were considered not viable. The selected viable seeds were well-dried by using a fan at room temperature. Seeds were surface sterilized by placing them in 70% ethanol for 2 min, and then thoroughly washed 2-3 times with distilled water. Sterilized seeds were taken out and dehydrated under a fan at room temperature till they got their initial moisture content. Finally, the seeds were sown on 90 mm Petri dishes.

Experimental Design and Treatment Combination

The experiments consist of two factors; seed pre-treatments (soaking in cold water, soaking in hot water, scarification, and control) and temperature regimes (20°C, 30°C, 40°C, and 25°C (room temperature)). A completely randomized design was employed for this particular study.

Determination of Seed Pre-treatments and Temperature Regimes

Seed pre-treatments (soaking in cold water for 24 hours at room temperature, soaking in hot water for ten minutes, scarification (rubbing with sandpaper), and control with four replications for each were set in the experiment. The Petri dishes containing seeds were arranged in a randomized complete design in a growing chamber at the required temperature. To assess the temperature effect on the germination of seeds, twenty-five seeds were randomly selected and seeds from each treatment were placed in Petri dishes provided with moistened (distilled water) filter paper and kept in various plant growth chambers set at different temperatures (20°C, 30°C, 40°C, and 25°C) for day/night. During the experiment, seeds were exposed to lamp light while conducting observations. A careful follow-up was done for consecutive days and seeds were considered germinated when a healthy white radicle was observed emerging through the integument. Germinated seeds were counted every five days. The response variables are the proportion of germinated seeds at the end of the experiment.

Procedures and Description of Seed Pre-treatments

Cold and Hot Water Pre-treatments

Hundred seeds of *Juniperus procera* were placed in different beakers containing cold water and the seeds were immersed for 24 hours at room temperature. Water was then removed and the seeds were sown on the same date. For the hot water treatment, water was boiled to 100 °C and then poured into beakers holding a hundred seeds that were left to stay for 10 minutes.

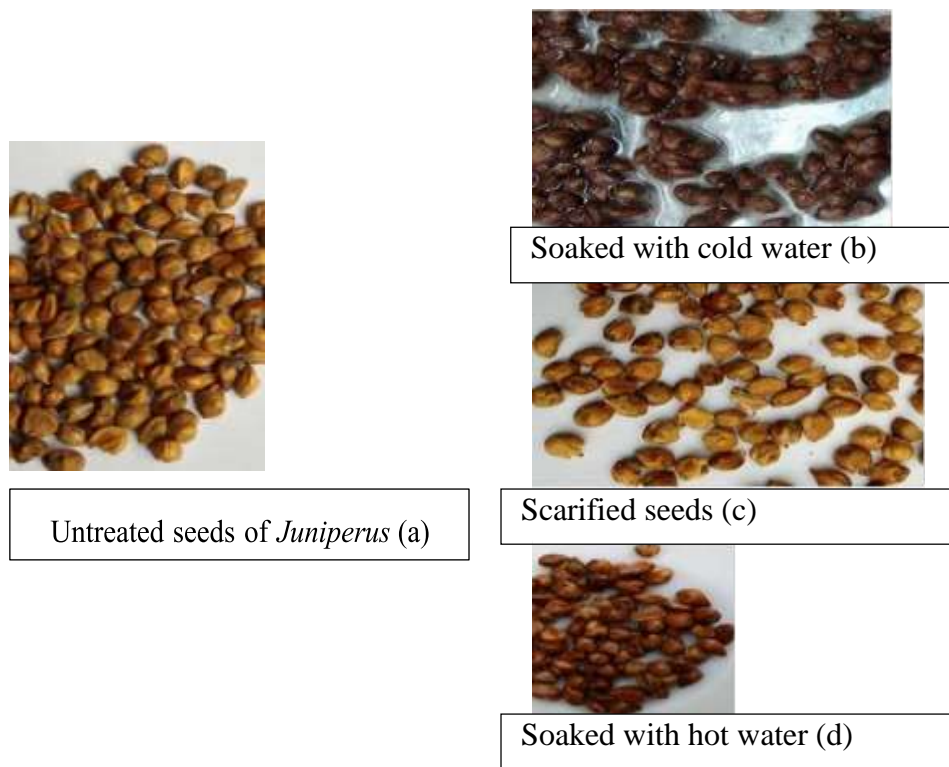
Mechanical Scarification

Juniperus procera seeds were scarified (rubbed with sandpaper) before sowing in Petri dishes. The scarified seeds were sown immediately.

Control

Seeds were sown without applying any seed pre-treatment

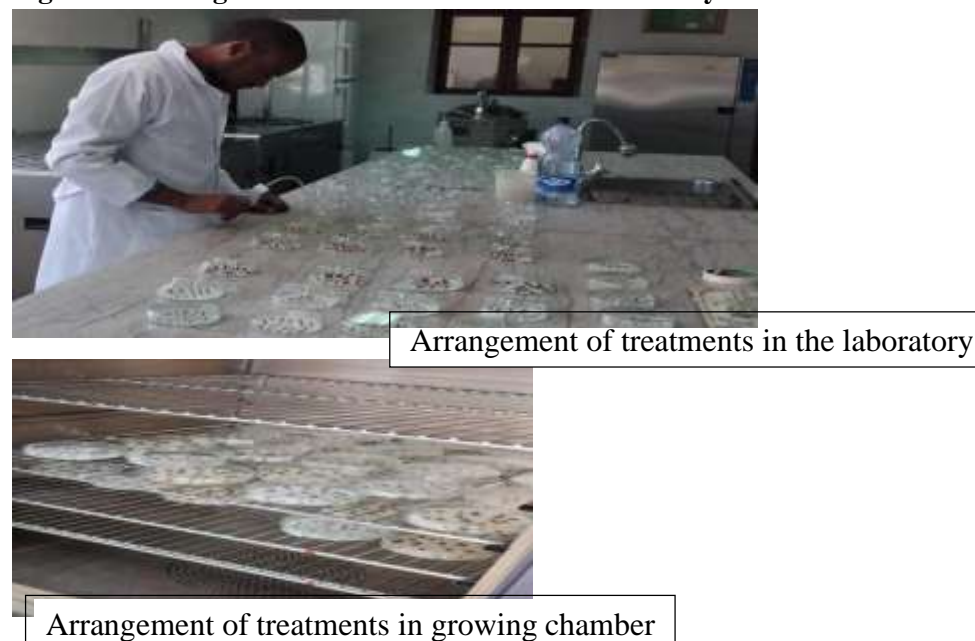
Figure 1: Seeds of *Juniperus procera* (a) Untreated Seeds (b) Seeds Soaked in Cold Water (c) Scarified Seeds and (d) Seeds Soaked in Hot Water



Arrangement of Treatments in the Laboratory

The treatments with four replicates were arranged and placed in a growing chamber.

Figure 2: Arrangement of Treatments in the Laboratory



DATA COLLECTION AND GERMINATION PARAMETERS

Data collection from laboratory experiments focused on the germination potential of various treatments so that beginning from the date of sowing up to the end of the experiment germination counts were done. For the next count, germinated seeds were excluded and the same data recording technique was followed. Data on the newly regenerated count was added to the previous data and the cumulative germination was noted down so that the last record showed the total count. Germination responses were described in germination starting dates, closing dates, germination period, germination percentage, mean germination time and index of germination. Germination percentage, mean germination time and germination index were stated following the formulae used by (Tanaka-Oda *et al.*, 2009; Botsheleng *et al.*, 2014; Rupinta *et al.*, 2014) respectively. Germination percentage (Gp): the number of germinated seeds as a percentage of the total number of tested seeds given as:

$$Gp = n/N * 100 \text{ equation 1 Where,}$$

n=total number of germinated seeds in the trial;

N=total number of seeds in the sample (total No. of seeds sown) Mean germination time (MGT) implies the time taken for seeds to germinate.

$$MG = \sum (n*d)/N \text{ equation 2 Where,}$$

n= number of seeds germinated on each day from the beginning of the test; d= number of days since the germination experiment started;

N= total number of seeds at the end experiment.

Germination index (GI) - was calculated for each treatment using the following equation; $GI = (G1/1) + (G2/2) + \dots + (Gx/x)$ equation 3

Where G is the germination day 1, 2..., and x represents the corresponding day of germination.

Data Analysis

IBM SPSS Statistics 26 software was used for data analysis. And also, the mean \pm SD (standard deviation) was computed for treatments. A two-

way ANOVA was conducted to analyze the effects of seed pre-treatments, temperature regimes, and combination effects on germination starting dates, closing dates, germination period, germination percentages, mean germination time, and index of germination. Tukey's test was conducted to compare mean germination starting dates, closing dates, period, germination percentages, mean germination time, and index of germination of seed pre-treatments effects, temperature as well as those interactions between temperature and pre-treatments. Differences among pre-treatments and temperature regimes were considered significant when $p < 0.05$.

RESULTS

Effect of Seed Pre-treatments on Germination Performance

Germination started earlier for Scarified seeds (P2) (21.9 days) over the other three pre-treatments. The control took a longer time to start germination (Table 1). Two-way ANOVAs revealed significant variation in germination starting date ($p < 0.05$). Tukey's test indicated non-significant differences in seed germination starting date between soaking in hot water (P3) and the control (P0). A statistically significant difference was observed between scarification (P2) and the other three treatments (P1, P3, and P0). A significant difference was also observed between soaking in cold water (P1) and the other three treatments. Germination was closed earlier for the scarified seeds (P2). Germination ceased later for non-treated seeds (P0). Germination ceasing dates significantly differed among pretreatments ($P < 0.05$). Tukey's test showed that there was a statistically significant difference among scarification, cold water, hot water and the control ($P < 0.05$) (Table 1).

The shortest germination period was recorded in scarification whereas the longest was in the control (P0). Seed pre-treatments affected the germination period significantly. Scarification has a significant difference over the other pretreatments. However, soaking in cold, and hot water and the control showed non-significant

differences in their germination durations (Table 1).

Results indicated that scarification (P2) distinctively increased germination percentage over all other treatments. The highest germination percentage (50%) was obtained in scarification and the least was recorded in control 20 %. There was a significant influence of pre-treatments on germination percentage ($P < 0.05$). All treatment means namely, scarification (P2), cold water (P1), hot water (P3) and (P0) control were significantly different (Table 1).

The least mean germination time was obtained from the seeds treated with scarification (P2) and

the highest was obtained from seeds soaked in hot water (P3). Seed pre-treatments affect the mean germination time ($p < 0.05$). A significant difference was observed between scarification (P2) and hot water (P3) as well as between cold water (P1) and hot water (P3) treatments (Table 1). However, a statistically non-significant difference was observed between P3 and P0. Seed pre-retreatments affect the germination index. The highest germination index recorded in seeds scarified (P2) was statistically different compared to the other pretreatments. There was a non-significant difference between (P3) and (P0) in the germination index.

Table 1: Summary of Effect of Pre-treatments on Germination Performance Parameters

Treatments	Starting date	Ceasing date	Germination period	MGT	Germination index	G %
P1	26 ^b	50 ^b	23 ^{ab}	7 ^{ab}	1.02 ^a	42 ^b
P2	21 ^a	41 ^a	20 ^a	6.2 ^a	1.19 ^a	50 ^a
P3	32 ^c	54 ^c	23 ^{ab}	8.6 ^c	0.58 ^b	31.6 ^c
P0	33 ^c	58 ^d	25 ^b	7.8 ^{bc}	0.45 ^b	20 ^d

Where MGT is the mean germination time and G% is the germination percent

Note: within a column, identical letters show a non-significant difference based on Tukey's test at a 5% level of significance.

Effect of Temperature Regimes on Germination Performance

Seeds started germinating earlier at 20°C regime. There was a statistically significant difference among germination starting dates ($p < 0.05$). Germination at 20°C (T1) was significantly different from other regimes. However, a non-significant difference was observed between the effect of 30°C (T2) and room temperature (RT) (Table 2). Germination ended up in advance in seeds treated with 20°C (T1) (Table 2). Temperature regimes affect the germination closing date ($P < 0.05$). There was a non-significant difference between the effect of 30°C (T2) and room temperature regimes (RT) in the germination ceasing dates.

The shortest germination period was observed at 20°C (21 days). Temperature regimes affect the germination period ($P < 0.05$). There was a non-

significant difference between 20°C (T1) and 30°C (T2) effect in the germination period. There was also a non-significant difference between 30°C (T2) and room temperature (RT) effect. Temperature regimes significantly affect germination percentage ($P < 0.05$). There was a significant difference among all temperature regime effects. The highest germination (50%) was observed at 20°C and after days of imbibition decreased with increasing temperature (Table 2). The lowest mean germination time was recorded under 20°C (T1). There was a non-significant difference in the effect of temperature regimes on mean germination time ($P > 0.05$). Non-significant difference was observed among temperature regime effects (Table 2). The highest germination index (1.15) was detected in seeds germinated at (T1) 20 °C. The germination index was also influenced by variations in temperature regimes ($P < 0.05$). There was a significant difference among temperature regime effects (Table 2).

Table 2: Effect of Temperature Regimes on Germination Performance

Treatments	Starting date	Ceasing date	Germination period	MGT	Germination index	G %
T1	25 ^a	46 ^a	21 ^a	7.11 ^a	1.15 ^a	50 ^a
T2	31 ^b	53 ^b	23 ^{ab}	7.5 ^a	0.53 ^b	23.5 ^c
RT	29 ^b	53 ^b	24 ^b	7.7 ^a	0.75 ^c	32.5 ^b

Where MGT is the mean germination time and G% is the germination percent

Note: within a column, identical letters show a non-significant difference based on Tukey's test at a 5% level of significance.

N.B. under T3 (temperature 40°C) germination failed and was not included in the analysis

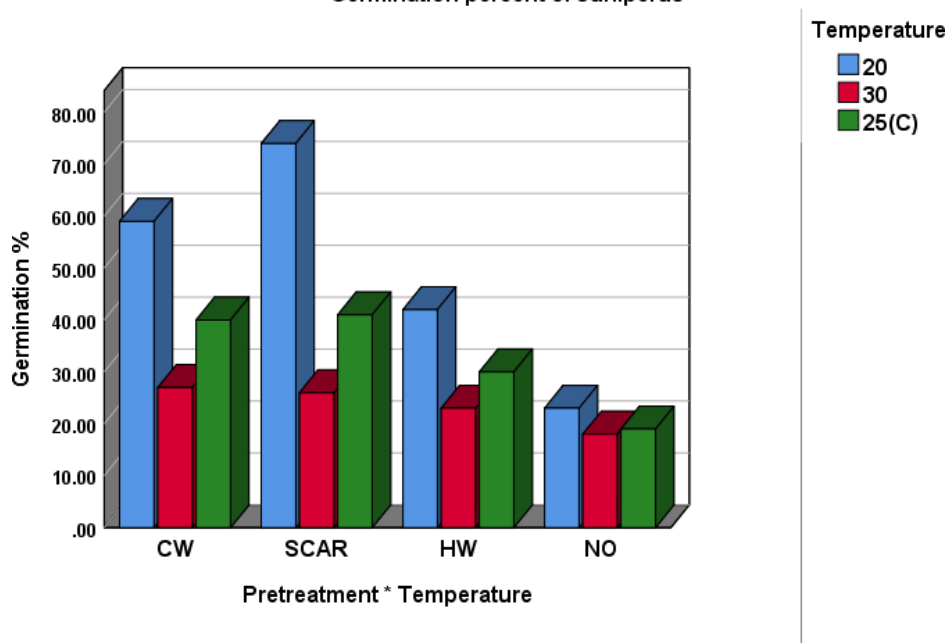
Combined Effect of Seed Pre-treatments and Temperature Regimes on Germination Performance

Germination began sooner, proceeded at a quicker rate, and the final level was higher at the 20°C regime with scarification. Germination started after fifteen days. The germination closing date was affected by the combination of pre-treatments and temperature. Germination closed earlier (30 days) after sowing in the combined effect of scarification with 20°C temperature regime. The longest germination closing date was observed in

control with 30°C. The combined effect of pre-treatments and temperature regimes had a non-significant difference in the germination period ($p > 0.05$). The germination period of scarified seeds grown under 20°C temperature was more rapid during imbibition than other treatment combinations.

The combination of pre-treatments and temperature regimes effect revealed a significant difference ($p < 0.05$) in germination percentage. Scarified seeds germinated better in 20°C regimes than in other treatment combinations (Figure 3). The highest germination percentage (74%) and the lowest (18%) were obtained in scarified seeds with 20°C (P2T1) and control with 30°C respectively.

Figure 3: Combined Effect of Seed Pre-treatments and Temperature on Germination Percentage
Germination percent of Juniperus



Note: CW=cold water SCAR =scarification HW = hot water NO = no treatment

N.B. under T3 (temperature 40°C) germination failed and was not included in the analysis

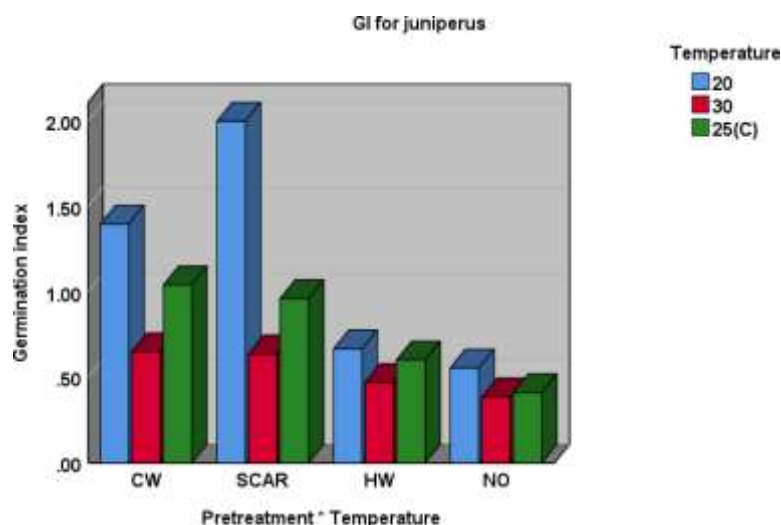
Figure 4: Germinated Seeds of *Juniperus procera* Seeds



The results of the analysis of variance showed that the combined effect of pre-treatments and temperature regimes affected the mean germination time ($P < 0.05$). The shortest (4.9) mean germination time was observed in scarification with 20°C. The longest mean germination time was observed in hot water

treatment with 20°C. There was a significantly different germination index among the effect of seed pre-treatments and incubation temperatures. The highest germination index (1.99) was recorded in the scarification with 20°C while the lowest germination index (0.31) was observed in the control with 30 °C regime (Figure 5).

Figure 5: Combined Effect of Seed Pre-treatments and Temperature on Germination Index



Note: CW=cold water SCAR =scarification HW = hot water NO = no treatment

N.B. under T3 (temperature 40°C) germination failed and was not included in the analysis

DISCUSSION

Germination involves numerous phases that initiate with water uptake by the seed and end up with the emergence of the embryonic axis (Scheler *et al.*, 2015) However, even under suitable situations, water uptake is prohibited in seeds of numerous species (Willis *et al.*, 2014). To

increase the germination capability of this tree species, such kind of problem must be removed. Hence, it is very essential to determine which technique and situation are appropriate for this species. Therefore, several seed pre-treatment techniques were applied. The results of this study revealed that germination of *Juniperus procera* seeds was significantly affected by various seed

pre-treatments ($p < 0.05$). This was in agreement with Nasr *et al.* (2013) who stated that final germination percentages were significantly affected by seed pre-treatments. Among the four pre-treatments, scarification showed the highest germination percentage. It is the most effective seed pretreatment method in enhancing seed germination. The absorption of water after disrupting the seed coat is known to be active in germination, but water may not be presented to the embryo in the untreated seeds. The present finding was in agreement with (Missanjo *et al.*, 2014; Fredrick *et al.*, 2017). Scarification helps enzymatic hydrolysis and thus transforms the embryo into a seedling. Physical dormancy (seed coat dormancy) is broken by scarification in several tree species (Aref *et al.*, 2011; Odirile *et al.*, 2019; Teketay *et al.*, 2020). In contrast, Thangjam, & Sahoo (2017) found little effect of scarification on germination performance in *Parkia timoriana* seeds.

Soaking softens the hard seed coat and makes it permeable to water. Similar views have been expressed by Thakur *et al.* (2019) who reported that soaking in water for 24 hours at room temperature enhanced germination performance of *Tectona grandis*. The research report of Hasnat *et al.* (2016) also showed that seeds of *Canarium resiniferum* germination started at first soaked in cold water for 24 hours and germination was accomplished within a shorter time. On the contrary, the germination of *Pouteria campechiana* seeds soaked for 24 h was less compared to the control (Amoakoh *et al.*, 2017). The hot water seed pretreatment method in this study produced a higher germination percentage than the control. This was in line with Missanjo *et al.* (2013) who found improved germination in *Albizia lebbeck* seeds soaked in hot water seed pretreatment. The positive effect of hot water pretreatment on the germination of impermeable seeds has been observed by (Gilani *et al.*, 2019; Singh *et al.*, 2019). Nevertheless, the germination percentages of the selected tree seeds obtained in the hot water treatment were lower compared to the numbers stated in some of the above-cited studies, perhaps due to the short soaking periods

adopted in the current study. In contrast, Rasebeka *et al.* (2019) found that hot water did not significantly increase the germination of *Acacia* species.

The study revealed the presence of a significant effect of temperature variation on seed germination percentage ($p < 0.05$). The result was in line with Catara *et al.* (2016) and Cristaudo *et al.* (2019) who reported temperature as the main factor in controlling germination percentage. The germination under the 20°C regime was effective since it had a direct effect on germination. However, germination failed as the growing temperature increased and no germinated seeds were found under the highest temperature (40°C). The Present study was in agreement with Guo *et al.* (2020) who showed that seeds germinate to a high percentage at temperatures up to 20°C, whereas higher temperatures hindered radicle protrusion and resulted in the germination percentage decreasing sharply in *Pinus bungeana* tree seeds. Ribeiro, & Costa (2015) Also reported that higher final germination was obtained at a temperature of 20°C, whereas significantly lower germination occurred at higher temperatures then decreased with increasing temperature in tropical tree *Myrsine parvifolia*. In contrast, Gairola *et al.* (2011) indicated that there was a sequential decrease in germination with the decrease in temperature, and germination failed to take place at lower temperature regimes in *Jatropha curcas*. Daibes *et al.* (2019) also stated that high germination capacity was obtained under temperatures of 40°C in *Astronium lecointei* and *Parkia nitida* timber trees.

The result of this study indicated that the combined effect of pre-treatments and temperature regimes significantly affects seed germination starting and closing dates, germination percentage and index ($p < 0.05$). The combination of seed pretreatments and temperature regimes had non- non-significant effect on the germination period. The present investigation was in agreement with Mewded *et al.* (2019) who found that breaking dormancy of *Terminalia laxiflora* attain good germination results when it is pretreated with the combined

effect of temperature and pre-treatments. This study was also in agreement with Bian *et al.* (2013) who revealed that the combination of pretreatment and temperature effect significantly differed in germination percentages in *Sorbus pohuashanensis*. The highest germination in this study may arise from the pretreatments and temperature regimes effects since there was a significant influence of both factors' variation on different features of seed germination. However, all seeds under a combination of pretreatments and T3 (40°C) failed to germinate under seemingly favourable seed pre-treatment conditions due to high temperature. This was in agreement with De Oliveira *et al.* (2013) who showed that temperature influences germination by affecting water uptake, thereby influencing the physiological processes and biochemical reactions that determine the germination of tree seeds. This also might be the reason for inhibiting seeds from germinating at high temperatures.

CONCLUSION

The present study revealed that there were significant differences in the effect of seed pre-treatments on seed germination starting and closing dates, germination period, germination percentage, mean germination time and germination index ($p < 0.05$). Among the seed pre-treatments, scarification performed best over the other pretreatments. Commonly, the untreated seeds of *Juniperus procera* germinate late and unevenly. There was a significant difference in seed germination among the effects of scarification, soaking in cold water for 24 hours, and soaking in hot water for 10 minutes.

In this study temperature was the critical factor for germination of *Juniperus procera* seeds. There was a significant effect of temperature regimes on seed germination starting and closing dates, germination period, germination percentage, and germination index ($p < 0.05$). However, there was a non-significant effect of temperature variation on mean germination time. Within the specified temperature regimes in this study, the optimum temperature for germination was found to be 20°C. Nevertheless, a higher incubation

temperature (40°C) led to a radical decline in germination and caused thermo-inhibition since seeds were not metabolically active in this high-temperature regime. There was a significant difference in germination performance among temperature regimes effect (20, 30, and 25 °C) based on Tukey's test at 5% significance level.

The result of this study also indicated that there was statistically significant combined effect of pre-treatments and temperature regimes on seed germination starting and closing dates, germination percentage and germination index ($p < 0.05$). From all combination effects of seed pre-treatments and temperature regimes, the shortest germination starting and closing dates, germination period and mean germination time were obtained under 20°C with scarification. The highest germination percentages (74%) and germination index were obtained under 20°C with scarification. Therefore, it is recommended that these techniques be followed by small-scale forest nursery owners, farmers, Government and NGO nurseries to enhance the germination of *Juniperus procera* and other tree seeds with identical dormancy problems.

Declaration of Interest statement

The authors declare no conflict of interest.

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