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EFFECTS OF FOUR PRE-SOWING TREATMENT METHODS (DOUBLE PHASE) ON THE GERMINATION OF THE SEEDS OF *Afzelia africana* Sm. ex Pers

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ABSTRACT

The study investigated the effects of four pre-treatment methods on the germination response of the seeds of Afzelia africana (Sm. ex Pers) in the savannah ecozones of Nigeria. The experiment was carried out in two phases. Phase I involved; 50% concentrated sulphuric acid for 10 minutes, hot water at 100ºC for 3 minutes, cold water at room temperature for 24 hours, Mechanical scarification (rubbing on abrasive concrete surface). Phase II of the experiment was carried out based on the result of phase I. Mechanical scarification, which performed best among the treatments used in the first phase, was closely examined. The various mechanical scarifications used were rubbing the seeds on abrasive concrete surface to the length of 1mm into the seed coat, 2mm, 3mm and 4mm. The experimental layout used was Completely Randomized Design. For phase I, mechanical scarification had 25% germination rate and had the highest germination compared to other treatments. Due to the performance of mechanical scarification in phase I, varying mechanical scarification treatments were further investigated in phase II of the experiment. The result of the analysis of variance indicated a significant difference between the varying mechanical scarification treatments (p≤0.05). Separation of the means showed that 1mm mechanical scarification treatment differed significantly from all other treatments. 1mm mechanical scarification treatment gave an average germination of 50% and early emergence of 9 days. Therefore, 1mm mechanical scarification treatment was regarded as the best mechanical scarification treatment for the germination of Afzelia africana seeds.

Keywords: pre-treatment, dormancy, germination, *Afzelia africana*

INTRODUCTION

Seed germination is one of the most important stages in life cycles of plants. Germination is affected by all of the environmental factors affecting vegetative growth. Germination requires favourable temperature, oxygen, water and lack of inhibitory substances in the environment. Seeds of many plant species cannot germinate despite favourable environmental conditions required for germination. Main reasons for this problem, which is termed as seed dormancy, are hard and impermeable seed coat and presence of immature or dormant embryo (Olmez *et al*, 2008).

Afzelia africana sm ex pers (Family Caesalpinaceae) is an endangered species. It is a medium to large spreading deciduous tree attaining 24–35m height and 1−1.6m diameter breast height (Gerard and Louppe, 2011). *Afzelia africana* is one of the most threatened multipurpose tree species in Africa used by local people for animal feeding (Sinsin, 1993; Onana, 1998), traditional medicine (Sinsin et al., 2002) and wood (Ahouangonou and Bris, 1997; Bayer and Waters-Bayer, 1999). Seeds are used as thickening agent (African Regional Workshop, 1996). The species is found in several types of natural forests ranging from the dense forest of the guineo-congolian zone to the woodland

forest of the sudanian zone (White, 1983). The multiple uses of *Afzelia africana* in Nigeria and West Africa as a whole led to a permanent pressure on its natural populations. It is frequent to observe adult trees of *Afzelia africana* in savannah as well as in woodland and dense forests but its natural regeneration is rarely observed within the same habitats. This situation was noticed within the species range of distribution in Africa (Sinsin, 1993; Bationo *et al.*, 2000).

Natural regeneration and independent germination of *Afzelia africana* is poor (Bationo *et al.,* 2000). The seeds are known to stay dormant in the soil for long time before germination. Cultivation of *Afzelia africana* necessitates that the seeds be pretreated to enhance the accessibility of water and oxygen before planting. The species is facing a high risk of extinction because of lack of its natural regeneration and hence practical ex situ conservation measures are urgently needed to preserve genetic diversity and maintain multiple specimens. Thus, the need to investigate the effects of pretreatment techniques on breaking dormancy, germination response and growth rate of *Afzelia africana*.

MATERIALS AND METHODS

Study Area

The study was carried out at the nursery unit of the department of Forestry Technology, Federal College of Wildlife Management, located between latitude 7°31'- 10°00'N and longitude 4°30'- 4°33'E. It is situated in the savanna area of the Kainji Lake Basin, New Bussa, Niger State. The study area has a tropical continental climate characterized by a shorter wet season (May to September) and a longer dry season (October to April) with a temperature of 15ºC to nearly 40ºC. Annual rainfall is about 1000mm but there is considerable variation in amount and distribution of rainfall among years. Rainfall is concentrated in the months of June, July and August. The town is bordered by the Kainji Lake created by the damming of River Niger at Kainji. The vegetation in New-Bussa is guinea savannah which is characterized by undistributed woodland with trees 15m – 18m tall (Nnaji and Omotugba, 2014).

Experimental Procedure and Design

Seed Collection

Afzelia africana pods were collected under matured mother trees within the premises of Federal College of Wildlife Management, New-Bussa, Niger State. The collected pods were broken and the seeds extracted and kept under ambient temperature condition for five days. The extracted seeds were subjected to viability test through the flotation method; in which case the seeds that floated in water after 24 hours of soaking were considered unviable and discarded.

Experimental Layout

The experiment was carried out in two phases. Experiments under the two phases were arranged in completely randomized design. Phase I involved four pre-treatments: 50% concentrated sulphuric acid for 10 minutes, hot water at 100 °C for 3 minutes, cold water at room temperature for 24 hours, Mechanical scarification (rubbing on abrasive concrete surface). Another set of seeds with no pre-treatment was sown, which served as the control. Each treatment was replicated four times. A total of 120 seeds were allocated to each treatment with 30 seeds per replicate. A total of 600 seeds were sown in the first phase. The first phase lasted for 30 days. Phase II of the experiment was carried out based on the result of phase I. Mechanical scarification (rubbing on abrasive concrete surface), which performed best among the treatments used in the first phase, was closely examined. The various mechanical scarifications used were rubbing the seeds on abrasive surface (concrete) to the length of 1mm into the seed coat (cuticle), 2mm, 3mm and 4mm. Also another set of seeds, which served as the control was sown without treatment. 80 seeds were subjected to each treatment with 20 seeds per replicate. Each treatment was also replicated four times. A total of 400 seeds were sown in phase II. This phase also lasted for 30 days.

Watering in both phases was done twice daily (morning and evening) throughout the duration of the experiment. Germination count was taken every evening. Seedlings were pricked-out after counting. This was done to avoid error. Cumulative data was recorded for 30 days for each phase.

Data Analysis

Cumulative data obtained from both phases of the experiment was converted to percentage values to

determine the germination percentage of the seeds. For the purpose of analysis of variance, Statistical Packages for Social Scientists (SPSS version 20) and Microsoft Office Excel were used. The data were entered into Excel Spreadsheet and appropriately arranged to conform to the layout of Completely Randomized Design. The data were copied into the data view of the SPSS, the percentage values were transformed into Arcsine values in SPSS and then analyzed. The data was analysed using one-way analysis of variance (Completely Randomized Design). Fisher's Least Significant Difference and Duncan's means were used to separate means of data found to differ significantly.

RESULTS

Phase 1

Effects of the pre-treatment techniques on the germination response of the seeds of *Afzelia africana.*

Seed dormancy occurs in many tropical tree species to varying degrees (Nwoboshi, 1982). While various pretreatment methods have been advocated to reduce dormancy and hasten germination, no single pretreatment technique has been found to be equally effective for all seed species.

In phase I of the experiment, high germination percentage were not attained. However, mechanical scarification treatment resulted in earlier and more uniform germination than other treatments, including control. Germination started on the 10th day for mechanical scarification treatment and had 25% germination rate (table 1). As for cold water treatment, 10% germination rate was attained and germination started on the 11th day. Germination started in the hot water and control treatment on the 15th day, hot water treatment had very low germination of 4.17% while control gave 12.50%, acid treatment (50% concentration) also gave a low germination rate of 6.67 %. The experiment was in agreement with Tigabu and Oden (2001) findings who concluded that scarification treatments improved the germination capacity and vigour, the highest being mechanical scarification, followed by sulphuric acid and 50°C hot water treatments.

The preceding findings of this study carried out by Amusa (2011) revealed that acid treatment (50% H2SO4) for 10 minutes had 83.33% germination rate and emergence of 12 days. In contrast, the same treatment gave a low germination percentage of 6.67% in this experiment, but with a close emergence of 13 days. Amusa (2011) also found out in his research that soaking of *Afzelia africana* in cold water for 24 hours gave 40% germination rate and 11 days emergence, which was also in contrast with this experiment that gave 10% germination but recorded the same 11 days of emergence. Hot water treatment gave a very low germination percentage of 4.17% with a longer days of emergence (15 days), this is quite in agreement with the findings of Amusa (2011) who described the effect of hot water treatment as adverse due to its low germination rate of 26.67% with 13 days of emergence.

Table 1: Germination of *Afzelia africana* seeds in phase I

Method of pretreatment	Days germination started	Germination %
Acid (50% H2SO4, 10 minutes)	13	6.67
Hot water (3 minutes)	15	4.17
Cold water (24 hours)	11	10.00
Mechanical scarification	10	25.00
Control	15	12.50

Method of pre-treatment with the earliest days of germination

As revealed in table 1, mechanical scarification (rubbing on abrasive concrete surface) had the earliest days of germination (10 days). Germination was also higher in seeds subjected to mechanical scarification (25%) and differ significantly from other treatments (tables 1 and 3).

Percentage of germinated seeds can be considered low as regards this experiment, however, mechanical scarification is a safer and more

practical alternative for small scale plantation establishment (Hermansen *et al.*, 2000) in that it is a simple, low-cost, and effective method to promote rapid and uniform germination.

Table 3: Effects of pre-treatments on the germination of seeds of *Afzelia africana* 30 days after sowing

Treatment	Mean
Hot water	11.63 ± 2.23^{a}
Acid	14.72 ± 3.25^{ab}
Cold water	18.31 ± 2.64^{ab}
Control	20.60 ± 2.72^{b}
Mechanical scarification	29.41 ± 8.56^{c}

Each value is a mean of four replicates ± standard error
Values with the same letter are not significantly different based on Fisher's LSD

Best pre-treatment method for the germination of *Afzelia africana* seeds

As revealed in table 1 and figure 1, mechanical scarification (rubbing on abrasive concrete surface) had the best germination percentage and hence the best pre-treatment in the experiment carried out in phase I (25%). Although 25% germination is considered low but when compared with other treatments (cold water, 10%, control, 12.5%, hot water, 4.17%, and acid, 6.67%) mechanical scarification gave the highest germination percentage.

The result of the analysis of variance (table 2) indicated a significant difference between the treatments ($p \leq 0.05$). Separation of the means showed that mechanical scarification differed significantly from all other treatments, which indicates it is regarded as the best pre-treatment method (table 3). Due to the performance of mechanical scarification in phase I, varying mechanical scarification techniques were further investigated in phase II of the experiment

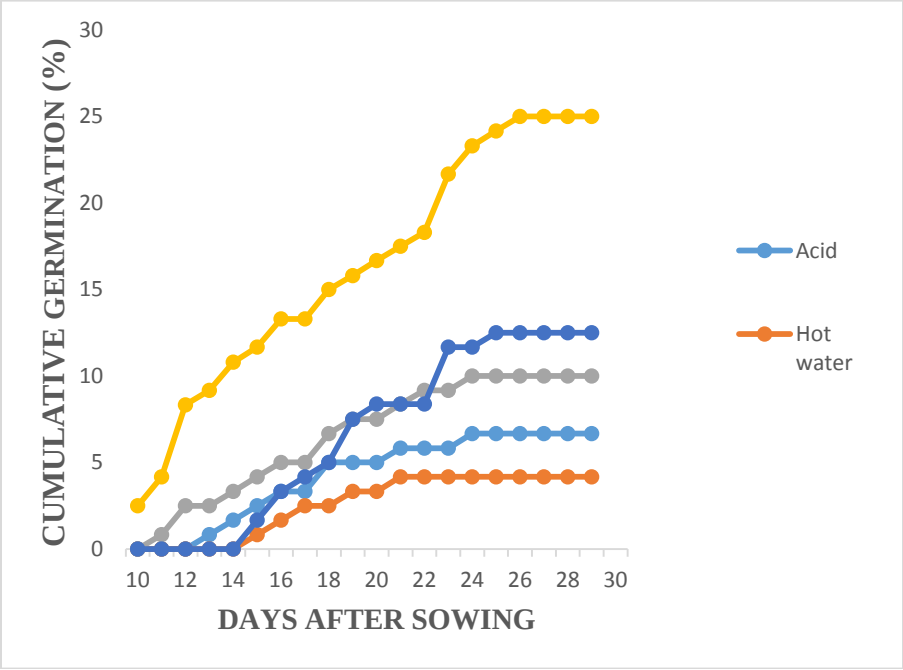

Figure 1: Effects of various pre-treatments on the germination of *Afzelia africana* seeds.

Table 2: Analysis of variance (ANOVA) Table for the Effect of Pre-Treatments on the Seeds of *Afzelia africana* 30 days after planting

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F-Value	Sig.
Treatment	4	736.146	184.036	8.916	0.001*
Error	15	309.614	20.641		
Total	19	1045.760			

***Significant (p<0.05)**

Phase II

Effects of varying mechanical scarification treatments (rubbing on abrasive concrete) on the seeds of *Afzelia africana*.

Due to the performance of mechanical scarification in phase I, varying mechanical scarification treatments were further investigated in phase II of the experiment. Germination started on the 9th day for 1mm and 2mm mechanical scarification treatments, all other treatments started germinating between 10th and 13th day (table 4). 1mm mechanical scarification treatment gave an average germination of 50%, 2mm mechanical scarification treatment had 37.5%, 3mm mechanical scarification treatment gave 27.5%, and 4mm mechanical scarification treatment gave 13.75%. The control gave 15% (table 4). 1mm mechanical scarification treatment resulted in earlier and more uniform germination than other treatments, including control (figure 2). Germination started in the 3mm mechanical scarification treatment on the 10th day, on the 13th day for 4mm and 12th day for the control.

Albizia gummifera which possess seed coat imposed dormancy was able to have complete, rapid and uniform germination as a result of application of mechanical scarification treatment. Tigabu and Oden (2001) in their study, therefore concluded that scarification treatments improved the germination capacity and vigour, the highest being mechanical scarification, followed by sulphuric acid and 50°C hot water treatments.

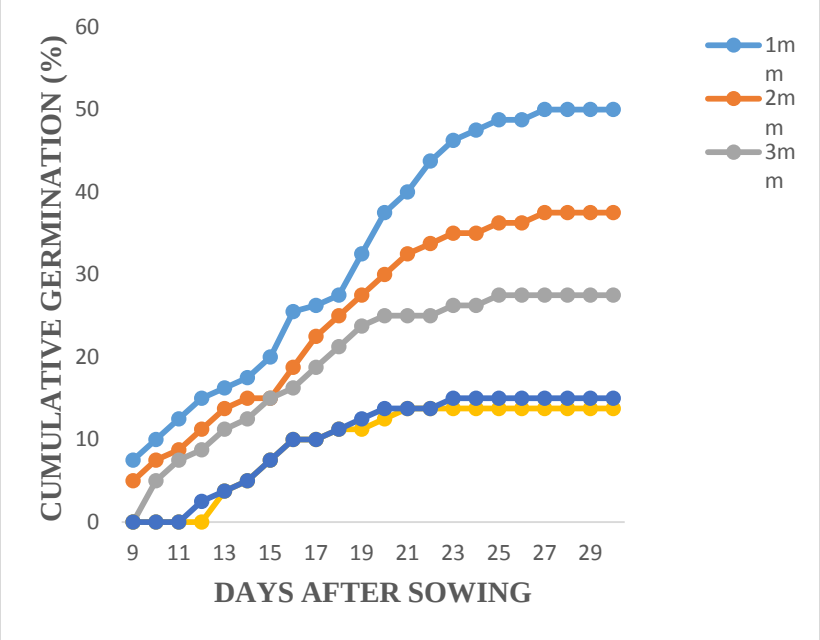

Figure 2: Effects of Varying Mechanical Scarification Treatments (rubbing on abrasive concrete) on the Germination of *Afzelia africana* Seeds

Method of pre-treatment with the earliest days of germination

As indicated in table 4, 1mm and 2mm mechanical scarification treatments had the earliest germination days, germination started on the 9th day for both treatments. Physical dormancy is caused by a hard and impermeable seed-coat or fruit enclosure which prevents imbibition and sometimes also gaseous exchange. Mechanical scarification treatment applied on the seed cause it to pierce the seed-coat to an extent that will render it permeable to water so that imbibition can take place. This results in early germination (Cremer 1990).

Table 4: Germination of *Afzelia africana* seeds in phase II

Method of pretreatment	Days germination started	Germination %
1mm mechanical scarification	9	50.00
2mm mechanical scarification	9	37.50
3mm mechanical scarification	10	27.50
4mm mechanical scarification	13	13.75
Control	12	15.00

Best mechanical scarification treatment for the germination of *Afzelia africana* seeds

As revealed in table 4, 1mm mechanical scarification treatment gave an average germination of 50% and early emergence of 9 days. The result from the ANOVA in table 5 also indicated that 1mm mechanical scarification treatment differed significantly from other treatments having the highest mean of 45 (table 6). Therefore, 1mm mechanical scarification treatment was regarded as the best mechanical scarification treatment for the germination of *Afzelia africana* seeds. This is in agreement with Sandiford (1988) who carried out a comparative study of hot wire scarification and conventional pretreatment methods of 10 hard seeded species and found out that hot wire scarification was shown to be the most effective for all species.

In *Trichilia emetica*, a non-legume with physical dormancy, it has been found that the aril has a strong influence on dormancy. Removal of the aril was sufficient to break dormancy in the majority of seeds, while the remaining seeds needed an additional scarification (Masanga and Maghembe 1993). The importance of the aril in imposing physical dormancy is also known in e.g. *Afzelia xylocarpa* and *Sindora siamensis* (Pukittayacamee 1990).

Table 5: ANOVA table for the effect of varying mechanical scarification treatments (rubbing on abrasive concrete) on the seeds of *Afzelia africana* 30 days after planting.

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F-Value	Sig.
Treatment	4	1588.972	397.243	21.155	0.000*
Error	15	281.662	18.77		
Total	19	1870.634			

***Significant (p<0.05)**

Table 6: Effects of varying Mechanical Scarification Treatments (rubbing on abrasive concrete) on the Germination of seeds of *Afzelia africana* 30 Days after Sowing

Treatment	Mean
1mm mechanical scarification	45.00 ± 4.71^{c}
2mm mechanical scarification	37.65 ± 5.28^{b}
3mm mechanical scarification	31.51 ± 4.18^{b}
4mm mechanical scarification	21.56 ± 3.92^{a}
Control	22.64 ± 3.32^{a}

Each value is a mean of four replicates ± standard error Values with the same letter are not significantly different based on Fisher's LSD

CONCLUSION

Mechanical scarification treatment (rubbing on abrasive concrete) is the best treatment for the seeds of *Afzelia africana*, 1mm mechanical scarification treatment gave an average germination of 50% and early emergence of 9 days. The result from the ANOVA in table 6 also indicated that 1mm mechanical scarification treatment differed significantly from other treatments having the highest mean of 45. Therefore, 1mm mechanical scarification treatment was regarded as the best mechanical scarification treatment for the germination of *Afzelia africana* seeds. 2mm and 3mm mechanical scarification treatments did not differ significantly from each other and hence can as well be recommended for the germination of *Afzelia africana* seeds. All other treatments gave low germination percentage and hence not suitable for the germination of *Afzelia africana* seeds.

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