

GERMINATION AND SEEDLING GROWTH IN *Afzelia africana* SM. EX. PERS.

*Ogbimi, E.R. and Sakpere, A.M.A.

Department of Botany, Obafemi Awolowo University, Ile – Ife, Nigeria.

*Corresponding author's e-mail: ejayisire@yahoo.com

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This study determined the best pre-treatment regime required for germination of the seeds of *Afzelia africana* Sm.Ex.Pers. and also provided information on the early growth parameters of the plant seedlings. Seeds of *A. africana* were collected from Obafemi Awolowo University, Ile – Ife (Lat 7° 32'N, Long 4° 31'E) and authenticated at the IFE-herbarium. One hundred and twenty five (125) seeds were sown per treatment (n=5 with 5 replicates and 5 repeats). Five (5) seeds each were sown in small petri dishes, without pre-treatment (control), or treated by subjecting to mechanical scarification and chemical scarification using Tetraoxosulphate (VI) acid (H₂SO₄) and Trioxonitrate (V) acid (HNO₃) for germination studies. Germination counts were made at an interval of 2 days. For the six different pre-treatments, five seedlings per plastic bowl were transferred into soil in a total of twenty plastic bowls laid out in a randomized design and their growth monitored for 40 weeks. Results showed that pre-treatment of seeds with mechanical scarification gave the highest percentage germination. Significant differences (P < 0.05) occurred in the shoot height and in the number of leaves between 4 and 12 weeks of growth. The study established that pre-treatment with mechanical scarification was the best for uniform germination of seeds of the plant. This study has provided alternative means of pre-treating *A. africana* seeds apart from using H₂SO₄ – the first to subject seeds to mechanical scarification and chemical scarification using HNO₃, in addition to providing information on the germination parameters and the seedling growth rate of *Afzelia africana*.

Key words: Propagation, Growth, Acid, Scarification

INTRODUCTION

Afzelia africana Sm. Ex Pers. known as African mahogany or African oak is a large deciduous tree. It is a leguminous tree which belongs to the family Leguminosae caesalpinoideae or Fabaceae. *Afzelia africana* is found in the humid and dry forests, especially in the forest-savanna borders or semi-deciduous forest (Orwa *et al.*, 2009).

Several studies on *A. africana* showed that its seeds and leaves contain vital nutrients such as proteins, fats, micro and macro minerals. Anti-nutrients such as haemagglutinin, oxalate, phytic acid, saponins, tannins and trypsin inhibitor have been found in the tree foliage (Ikhimioya *et al.*, 2007). Every part of the plant is of great importance (Igbabul *et al.*, 2014). Wood from *Afzelia africana* is used in carpentry, canoe and house building, furniture making, flooring and heavy construction, wood carvings and other traditional uses (Orwa *et al.*, 2009). Seeds are milled into flour which serve as soup thickener (Igwenyi and Akubugwo, 2010) or substitute for wheat flour in biscuits and doughnuts (Orwa *et al.*, 2009). It is a fodder: the leaves, fruits and seeds are browsed by

wildlife, particularly before the regrowth of grass in the early rainy season, also wild animals browse the arils, and antelopes eat the young shoots, while flying foxes and bats eat the flowers (Orwa *et al.*, 2009). The seeds contain about 31% fat and may be a source of seed oil for domestic and industrial uses.

Afzelia africana leaf is one of the non-conventional vegetables obtained from forests, and supplements the conventional ones obtained from farms and home gardens. Seeds of the plant contain extractable oil (Palgrave and Palgrave, 2002). The physicochemical properties of methyl ester derivatives of oils from *Afzelia africana* show its potentials in biodiesel production. The oil can release high amount of heat on combustion and the cetane index shows that the oil can ignite easily in a combustion engine. The iodine and peroxide values show increased stability of the oil during storage and transportation (Igwenyi *et al.*, 2011). As a result of the usefulness of this plant, it has been over exploited, owing to population explosion, urbanization and the resultant degradation of natural forests and the preference

of farmers to produce the conventional vegetables (Umedum *et al.*, 2014).

Afzelia africana is propagated by seeds and vegetative technique via budding. Natural regeneration is poor because seed predation by animals is usually high (Amusa, 2010). *Afzelia africana* seeds are dormant and they become recalcitrant on storage (Orwa *et al.*, 2009). The rate of seed germination in the wild is low and its seedlings rarely develop into saplings. Although treatment with Tetraoxosulphate (VI) acid to overcome dormancy in *Afzelia africana* seeds has been reported by Amusa (2010), no report is documented for treatment with hydrochloric acid and nitric acid. Thus, this study sought to validate the already existing result of overcoming dormancy by treatment with concentrated Tetraoxosulphate (VI) acid as well as compare the effect of treatment with Tetraoxosulphate (VI) acid, and Trioxonitrate (V) acid.

MATERIALS AND METHOD

Seeds of *Afzelia africana* were collected from Obafemi Awolowo University, Ile – Ife (Lat 7° 32' N Long 4° 31' E), in January 2017. The seeds were subjected to six pre-treatments before sowing: soaking of seeds in 10% Trioxonitrate (V) acid (HNO₃) acid for 15 minutes, 10% Trioxonitrate (V) acid for 30 minutes, 10% Tetraoxosulphate (VI) acid (H₂SO₄) acid for 15 minutes, 10% Tetraoxosulphate (VI) acid for 30 minutes, mechanical scarification of seeds with sand paper followed by soaking in water for 30 minutes and finally by sowing of seeds without any treatment (control). Seeds subjected to all pre-treatments were then rinsed thoroughly with water for five minutes. Five seeds each were sown in five small Petri dishes lined with filter paper moistened with distilled water, and the experiment was carried out at room temperature, replicated and repeated five times to give a total of 125 seeds per treatment. All seeds were subjected to dark condition since a preliminary experiment carried out prior to this (unpublished thesis) showed that seeds germinated better in the dark. The experiment was observed every two days for radicle protrusion and after twenty-one days (since germination percentage did not increase after 21 days), the percentage germination was calculated for all treatments.

The data was subjected to arcsine transformation. One – way analysis of variance was used to test for significance in percentage germination among treatments and the means were separated using the Duncan's Multiple Range Test (DMRT) at 5% probability level. Statistical analysis was done with the SAS 2002 package.

Seedlings of *Afzelia africana* were transplanted after 21 days of planting to buckets (23 cm in diameter and 22 cm in depth) filled with top soil. After another 21 days (in February) when the seedlings were established, data on three growth parameters; shoot height, number of leaves and plant leaf area were measured counted and documented every four weeks for a period of 40 weeks. Completely randomized design was used with n=5, with 5 replicates and the experiments were repeated 5 times. The shoot height was estimated as the distance between the base of the shoot at the soil level and the tip of the apical bud of the plant using a meter rule and a thread because some stems were not straight. The leaf area was calculated using the modified Hoyt and Bradfield (1962) formula:

$$\text{Leaf area} = L \times W \times CF$$

where L= length of the leaf, W - width of the leaf, CF - correction factor = 0.66.

The correction factor was determined as follows: the leaf area of 20 leaves was measured by placing each leaf on a graph paper glued to a glass plate. The leaf outline was marked and the boundary was determined by counting the squares to the nearest 1 mm². The length (L) and width (W) of each of the 20 leaves were also measured with a ruler. The ratio of the leaf area determined by using a graph to that obtained by L x W method was calculated for the 20 leaves and the mean (the C.F) obtained. Below is the formula for calculating the germination parameters:

$$\text{Percentage Germination} = \frac{\text{number of seedlings germinated}}{\text{total number of seeds sowed}} \times 100$$

$$\text{Peak value (PV)} = \frac{\text{Highest number of seed germinated}}{\text{number of days taken to germinate}}$$

$$\text{Germination speed (GS)} = \frac{\text{Number of germinated seeds in a day}}{\text{the number of days}}$$

$$\text{Mean daily germination (MDG)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of days}}$$

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

$$\text{Mean germination time (MGT)} =$$

$$\frac{\text{Total number of germin}(n_1 * d_1 + n_2 * d_2 + n_3 * d_3 + \dots \dots \dots)n}{\text{Total number of days}}$$

where n_i is number of germinated seeds in day t_i

$$\text{Germination index (GI)} = \frac{\text{Number of germinated seeds (First count)}}{\text{Days to first count}}$$

RESULTS

Germination and Germination Parameters

The different pre-treatment regimes tested on the seeds of *Azizelia africana* before germination had significant effect on the germination of the seeds after twenty one days (Table 1). The control (seeds not pre-treated) had significantly lower percentage germination (43.45 ± 2.44^d) compared to all the pre-treatments except 10% H_2SO_4 for 30 minutes (51.71 ± 0.87^{cd}) at probability level of $P < 0.05$ (Table 1). Seeds pre-treated with mechanical scarification had significantly higher percentage germination. There was no significant difference in the percentage germination between seeds pre-treated with 10% H_2SO_4 for 15 minutes and 30 minutes. The maximum percentage germination was observed in seeds subjected to mechanical scarification (71.17 ± 0.42).

The mean daily germination of seeds subjected to all pre-treatments were significantly higher than in the control treatment at $P < 0.05$ with the exception of seeds pre-treated with 10% H_2SO_4 for 15 minutes and 30 minutes (Figure 1). The maximum mean daily germination was recorded with mechanical pre-treatment (4.48 ± 0.05) followed by 10% HNO_3 for 30 minutes (3.96 ± 0.34) and 10% HNO_3 for 15 minutes (3.88 ± 0.17)

with the least value in the control treatment (2.40 ± 0.44). The peak value for control seeds (0.13 ± 0.02) was significantly different from all treatments except with seeds treated with 10% H_2SO_4 for 30 minutes (0.19 ± 0.01) (Figure 2). A similar trend was observed with the germination speed and germination value (Table 1). The germination speed and germination value of control seeds were significantly different from all the pre-treatments with the exception of the treatments with 10% H_2SO_4 for 15 minutes and 30 minutes (Table 1). Pre-treatment regime had significant effect on the peak value and germination value. The highest germination speed was obtained in seeds subjected to mechanical pre-treatment (0.40 ± 0.01) followed by 10% HNO_3 for 30 minutes (0.31 ± 0.02) and 10% HNO_3 for 15 minutes (0.30 ± 0.02), although no significant difference was observed among the three pre-treatments (Table 1). The germination value of seeds pre-treated with mechanical scarification was significantly higher compared to all the other pre-treatments. The germination value for control seeds was lowest (0.35 ± 0.09) and highest in seeds subjected to mechanical scarification (1.50 ± 0.10).

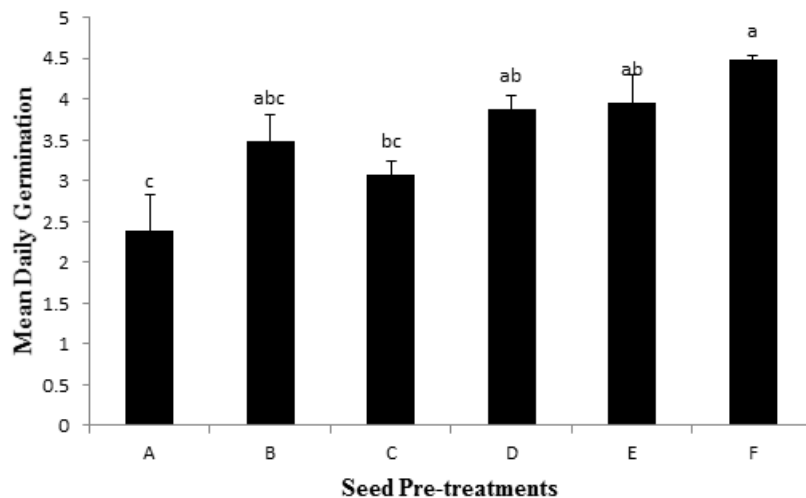
Pre-treatment method did not have any effect on mean germination time and germination index. The mean germination time and the germination index in all the pre-treatments tested were not significantly different among the treatments at $p < 0.05$ significant level (Figures 3 and 4).

The pre-treatment method however had a positive effect on the number of days to seedling emergence (Table 2). Seedlings emerged 14 days after sowing (14 DAS) in control treatment which was the slowest, 10 DAS in 10% H_2SO_4 for 30 minutes, 10% HNO_3 for 15 minutes and mechanical scarification pre-treatment; 9 DAS in 10% HNO_3 for 30 minutes and 8 DAS in 10% H_2SO_4 for 15 minutes, which was the fastest observed.

Table 1: Effect of Different Pre-treatment Regimes on Germination of *Afzelia Africana* Seeds after 21 Days

Treatment	Duration of treatment (Minutes)	Percentage Germination (%)	Germination Speed	Germination Value
Control	-	43.45 ± 2.44 ^d	0.16 ± 0.03 ^c	0.35 ± 0.09 ^c
10% H ₂ SO ₄	15	57.06 ± 1.90 ^{bc}	0.27 ± 0.06 ^{abc}	0.76 ± 0.13 ^{bc}
10% H ₂ SO ₄	30	51.71 ± 0.87 ^{cd}	0.26 ± 0.02 ^{bc}	0.57 ± 0.05 ^{bc}
10% HNO ₃	15	61.97 ± 1.10 ^{abc}	0.30 ± 0.02 ^{ab}	0.88 ± 0.10 ^b
10% HNO ₃	30	63.82 ± 2.08 ^{ab}	0.31 ± 0.02 ^{ab}	0.90 ± 0.14 ^b
Mechanical Scarification	-	71.17 ± 0.42 ^a	0.40 ± 0.01 ^a	1.50 ± 0.10 ^a

*Values with the same superscript in the same column are not significantly different from each other at $P < 0.05$.

**Figure 1:** Mean Daily Germination of Seeds *Afzelia africana*

Keys:

A – Control

B – 10% H₂SO₄ for 15 minutes

C – 10% H₂SO₄ for 30 minutes

D – 10% HNO₃ for 15 minutes

E – 10% HNO₃ for 30 minutes

F – Mechanical scarification

*Values with the same superscript are not significantly different from each other at $P < 0.05$.

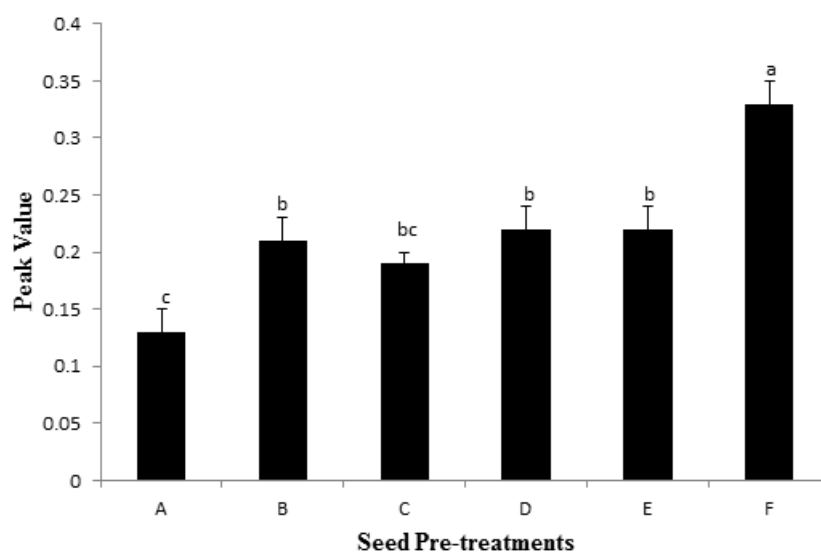


Figure 2: Peak Value of Seeds of *Afzelia africana*

Keys:

A – Control

B – 10% H_2SO_4 for 15 minutes

C – 10% H_2SO_4 for 30 minutes

D – 10% HNO_3 for 15 minutes

E – 10% HNO_3 for 30 minutes

F – Mechanical scarification

*Values with the same superscript are not significantly different from each other at $P < 0.05$.

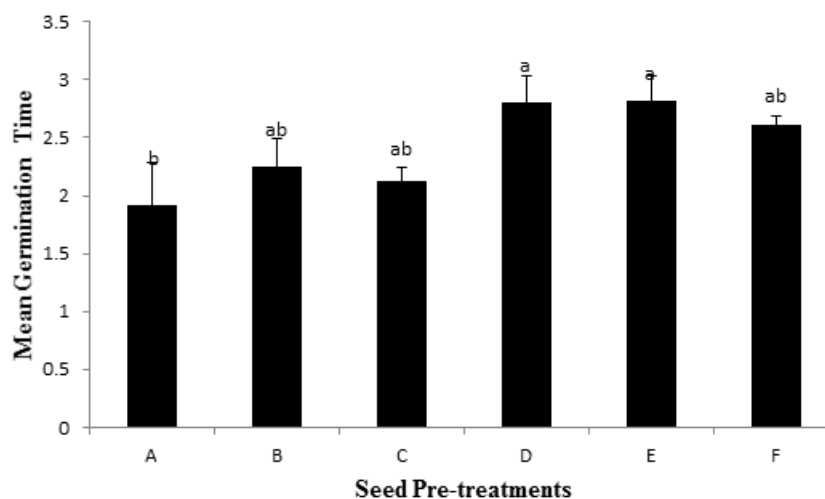


Figure 3: Mean Germination Time of Seeds of *Afzelia africana*

Keys:

A – Control

B – 10% H_2SO_4 for 15 minutes

C – 10% H_2SO_4 for 30 minutes

D – 10% HNO_3 for 15 minutes

E – 10% HNO_3 for 30 minutes

F – Mechanical scarification

*Values with the same superscript are not significantly different from each other at $P < 0.05$.

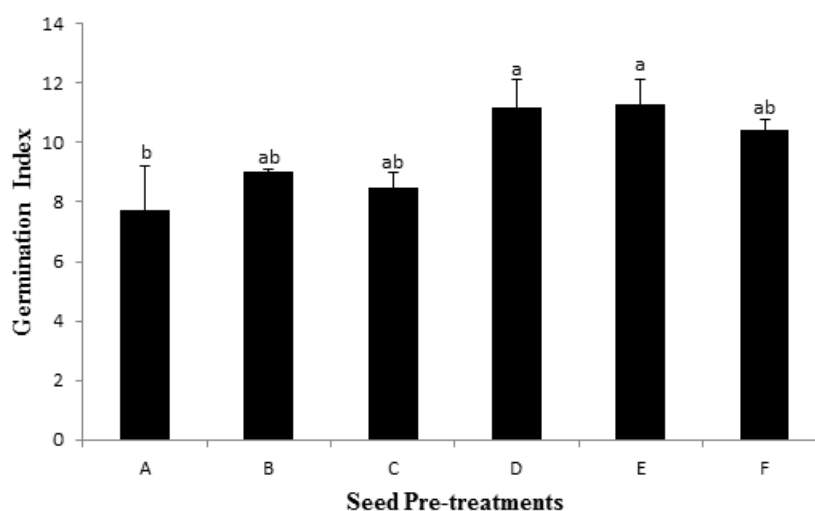


Figure 4: Germination Index of Seeds of *Afzelia africana*

Keys:

A – Control

B – 10% H₂SO₄ for 15 minutes

C – 10% H₂SO₄ for 30 minutes

D – 10% HNO₃ for 15 minutes

E – 10% HNO₃ for 30 minutes

F – Mechanical scarification

*Values with the same superscript are not significantly different from each other at P < 0.05.

Table 2: Effect of Different Seed Pre-treatment Regimes on Mean Number of Days to Seedling Emergence (MNDTSE) of *Afzelia africana*

Treatments	Duration of treatment	MNDTSE
Control	-	14 ± 0.84 ^a
10% H ₂ SO ₄	15 minutes	8 ± 0.61 ^b
10% H ₂ SO ₄	30 minutes	10 ± 0.68 ^b
10% HNO ₃	15 minutes	10 ± 0.54 ^b
10% HNO ₃	30 minutes	9 ± 0.58 ^b
Mechanical scarification	-	10 ± 0.34 ^b

*Values with the same superscript in the same column are not significantly different from each other at P < 0.05.

Abbreviation: MNDTSE – Mean Number of Days to Seedling Emergence

A close observation (Table 1) of the effect of Tetraoxosulphate (VI) acid pre-treatments at the two soaking periods on all the germination parameters tested reveals that the shorter soaking period for *A. africana* seeds (10% H₂SO₄ for 15 minutes) gave higher values but not significantly

different than soaking for a longer period (10% H₂SO₄ for 30 minutes). The shorter soaking period resulted in seedlings emerging faster (though not significantly different from the longer soaking period) (Table 2) with significant difference from that of the control treatment.

Seedling Growth Measurement

The mean shoot height of *Afzelia africana* continued to increase significantly ($P < 0.05$) from the first month (15.69 cm) to the third month (46.11 cm). A decrease was observed at the fourth and fifth month (Figure 5) after which an almost uniform growth was observed through to the tenth month. A significant increase was observed in the shoot height between February to April, after which no significant change was observed till the tenth month (Figure. 5). Also, a significant change was observed between March to April, April to May, May to June and lastly from June to July with respect to the change in shoot height,

with a significant decrease occurring only from April to May.

A similar trend observed in the shoot height was repeated in the number of leaves. A significant increase occurred from the first month through to the third month (February to April), while an increase, though not significant occurred from April (the third month) to May (the fourth month). *Afzelia africana* produced its highest number of leaves (12.80) in the fourth month of growth. A significant change was observed in the change in number of leaves from April to May, and also from September to October.

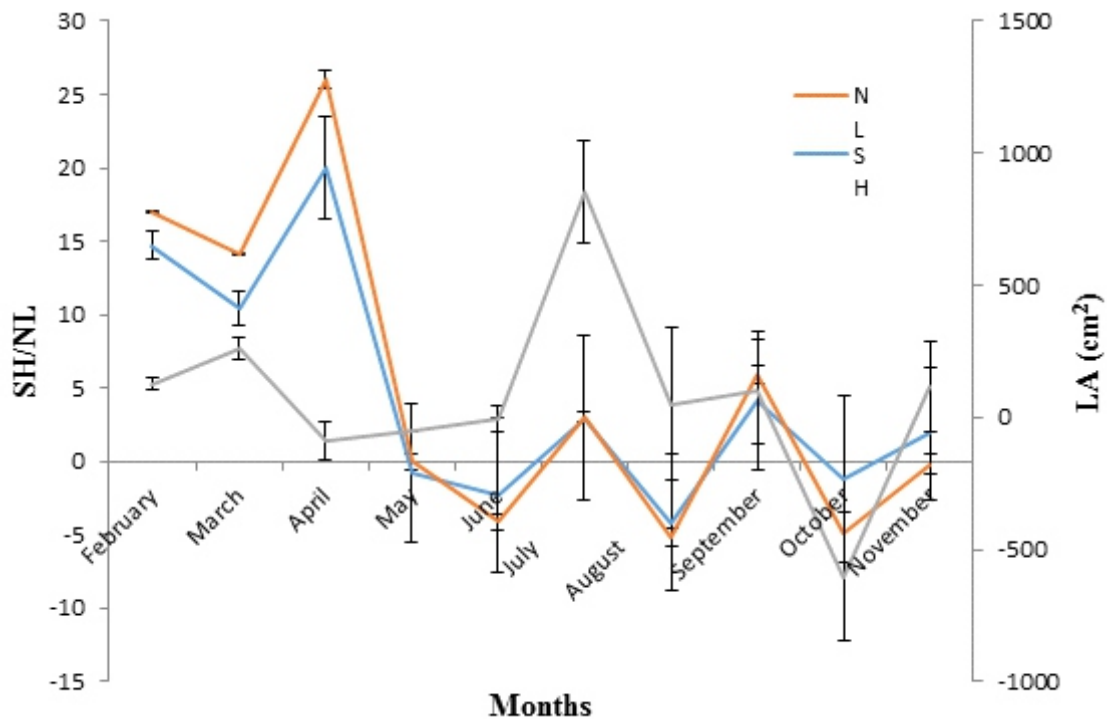


Figure 5: Change in Number of Leaves, Shoot Height and Leaf Area of *Afzelia Africana* Seedlings from the First Month to the Tenth Month.

Abbreviations:

SH – Change in Shoot Height

NL – Change in Number of Leaves

LA – Change in Leaf Area

The leaf area showed an increase (not significant) from the first month (February) to the second month (March), and then it decreased, without significant difference from April to June. A significant increase then occurred from June to July, the increase though not significant continued from July to August and August to September with the leaf area reaching its highest (1239.1 cm²) in August. Thereafter, a significant decrease occurred September to October. With respect to the change in leaf area, significant increases occurred from April to May, May to June, June to July and October to November, while significant decreases occurred from July to August and September to October.

DISCUSSION

The significant difference observed in the percentage germination of *Afzelia africana* seeds not pre-treated before germination (control) compared with seeds that were subjected to mechanical scarification (79.20 ± 0.98) showed that the seeds are dormant; physical dormancy which could result from impermeable hard seed coat which had to be removed prior to germination. The hard seed coat could have imposed a barrier to the entry of water and gases. Delouche (1964) reported the most important cause of seed dormancy in Leguminosae to be impermeability of hard seed coat to water. Seeds of legumes are generally considered to have physical dormancy (Jayasuriya *et al.*, 2013). Thus partial removal of the seed coat through mechanical scarification or softening of the seed coat through chemical scarification allowed more absorption of water resulting in significantly higher percentage germination.

Pre-treatment of the seeds with 10% H₂SO₄ for 15 minutes gave a better response than 10% H₂SO₄ for 30 minutes. The longer exposure of the seeds to H₂SO₄ could have had a damaging effect on the embryo of the seeds thus accounting for the decrease in percentage germination. Azad *et al.*, (2012) and Schmidt (2000) reported that the concentration of the acid and the exposure period of seeds are critical factors to be considered as seeds exposed for a longer period get damaged. Also, Likoswe *et al.*, (2008), who worked with *Terminalia sericea* observed that the duration of exposure of seeds to concentrated H₂SO₄ was

critical because long soaking periods lead to excessive burning of the seed coat and consequently, damage to the embryo of the seeds.

Treatment of seeds with 10% HNO₃ for 30 minutes resulted in significantly higher percentage germination than treatment with 10% H₂SO₄ for 30 minutes. Sulfuric acid is a stronger and more electronegative acid than HNO₃, thus a harsher burning effect on the seed coat of seeds is expected. A significant reduction in the percentage germination was observed in the seeds of *Innula racemosa*, *Bunium persicum*, *Carcum carvi*, *Rheum webbianum* and *Saussurea lappa* when they were pre-treated with Tetraoxosulphate (VI) acid for more than 5 minutes (Ashwani *et al.*, 2016).

Seeds subjected to mechanical scarification gave the best percentage germination in this study because the removal of part of the seed coat exposed the seed to faster entry of water resulting in higher percentage germination. Nicking which is a mechanical pre-treatment method was found to be extremely effective because it made it easier for entry of water and gas exchange thus resulting in enzymatic hydrolysis and enhancing transformation of the embryo to seedling in *Hollarrbena floribunda* (Ayisire *et al.*, 2012). Mechanical scarification has resulted in the highest percentage germination in seeds of *Adesmia* species (Tedesco *et al.*, 2001), *Cassia moschata* (Souza and Silva, 1998), *Glycyrrhiza glabra* (Ghadiri and Torshiz, 2000), *Acacia auriculiformis* and *Acacia tortilis* (Girase *et al.*, 2002).

Pre-treatment method had a positive effect on seedling emergence in this study. Seeds subjected to 10% H₂SO₄ for 15 minutes emerged 8 DAS. This pre-treatment method must have ruptured the seed coat faster than other methods thus allowing for quick entrance of water and gases. This study corroborates that of Amusa (2010), who reported emergence 8 DAS in seeds of *A. africana* subjected to 98% of sulphuric acid pre-treatment.

This study is the first report on pre-treatment of *A. africana* seeds with 10% H₂SO₄ for 15 minutes, 10% HNO₃ for 15 and 30 minutes and with physical scarification - mechanical method. All of these pre-treatments except that with 10% H₂SO₄

for 30 minutes gave significantly higher percentage germination when compared with the control treatment.

The best method for pre-treating *A. africana* seeds is mechanical scarification, this method is cost effective but time consuming and laborious. However, if chemical scarification is opted for, 10% HNO₃ for 30 minutes is recommended, but if this acid is unavailable or when the day of seedling emergence is being considered, 10% H₂SO₄ can be used at a shorter soaking period – 15 minutes.

Considering the seedling growth of *A. africana*, the shoot height of *A. africana* ranged from 15.69 cm in the first month to 46.78 cm in the tenth month and the leaf number ranged from 2.30 in the first month to 12.8 in the fourth month. This information is important in the light of the fact that growth and development studies constitute a basic criterion for evaluating the success of forest reestablishment efforts (Pitto *et al.*, 2004).

The decrease observed in the change in shoot height could be as a result of browsing of the plant by insects, birds and other animals, since seedlings were raised in an open environment. When terminal shoots of plants are browsed upon, apical dominance is released and plants then begin to channel their photosynthates to the growth of axillary branches. Shedding of leaves can also result into decreased shoot height as a result of decrease in number of the leaves photosynthesizing and thus less photosynthate produced.

The significant increase in change in leaf area observed from the month of April to July could be as a result of increased rate of photosynthesis resulting from increased number of leaves (number of leaves were highest between April to July) which will trap more light energy. Poorter *et al.*, (2013) reported that increased light intensity can increase rate of photosynthesis resulting into an increased change in leaf area.

The fluctuations observed in the change in the seedling parameters after the month of June (for shoot height and number of leaves), and July (for leaf area), could be attributed to the onset of the dry season. The reduction in the moisture content

of the soil and the atmosphere will have adverse effect on the shoot height (through shedding of leaves); on the number of leaves and on the leaf area since reduction in the rate of photosynthesis will occur at reduced moisture content.

CONCLUSION

This investigation has confirmed that scarification with acid is effective for the pre-treatment of *A. africana* seeds to overcome dormancy, and HNO₃ is a better alternative than H₂SO₄. However, physical scarification (mechanical) was the most effective method for improving the seed germination of *A. africana* seeds, thus large number of seedlings can be raised in reforestation or restoration programs using this pre-treatment method.

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