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Full Length Research

Effects of three pre-treatment techniques on dormancy and germination of seeds of *Afzelia africana* (Sm. Ex pers)

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The study assessed the effects of three pre-treatment techniques on dormancy and germination of seeds of *Afzelia africana* (Sm. ex Pers), an endangered tree species in Savanna ecozone of Nigeria. The three pre-treatment techniques are: soaking in cold water for 1, 12 and 24 h, soaking in hot water (100°C) for 1, 12 and 24 h and soaking in 10, 50 and 98% concentrated sulphuric acid (H_2SO_4) for 5, 10 and 30 min. Experiments were performed based on a completely randomized design with four replicate. The results show that cold water pre-treatment of *A. africana* seeds only gave a fair germination percentage (33.33 to 53.33%) and reduced dormancy period. On the other hand, the effect of hot water pre-treatment on the seeds gave adverse result (26.67 to 36.67%). Although *A. africana* seeds under control treatment gave an impressive germination, which did not differ significantly from the results of other pre-treatment techniques (F-cal = 2.6777; P-level = 0.1180; P < 0.05), acid pre-treatments yield a more uniform and regular germination. Thus, while the seeds of *A, africana* could be germinated without pre-treatment, to attain higher percentage germination and reduced dormancy period, the seeds should be pre-treated with sulphuric acid.

Key words: Dormancy, pre-treatment techniques, germination percentage, *Afzelia africana*, *ex situ* conservation.

INTRODUCTION

The rapid rates of forest loss and degradation across the tropics have continued to increase both the fragmentation of many populations and the risk of species' extinction. In Nigeria as elsewhere, conservation of forest genetic resources is achieved through the protection of these resources in their natural habitat (*in situ*) or preservation of samples of the genetic diversity of endangered species away from their field habitats (*ex situ*) in facilities such as botanical gardens, seed gene banks, *in-vitro* gene banks, and field gene banks (Owonubi and Otegbeye, 2004). The success of *ex situ* conservation method is dependent on a number of factors including availability of funds, suitable land, appropriate technology and adequate and viable seeds. Seed is of particular importance and a key element in both *in situ* and *ex situ* plant conservation activities. The propagation of most tropical tree species however, is constrained by recalcitrant seed germination as a result of dormancy (Nwoboshi, 1982). The degree of dormancy makes it difficult for seed to germinate evenly and adequately.

In both the forest and savanna ecosystems of Nigeria *Afzelia africana* (Sm. ex Pers) is an endangered species (Gbile et al., 1978). It belongs to the family Fabaceae (sub family- Ceasalpinoidae) in the order Fabales. It is a large tree with a spreading crown having height that varies from 10 to 20 m and a mean average diameter of 36 cm (Keay, 1989a). *A. africana* is a heliophytic species occurring in the humid and dry forests as well as tree savannahs and forest galleries. It is found in well watered sites with a deep sandy soil and can adapt to lateritic soils.

The tree is an invaluable species. The wood is hard, heavy, durable, termite-proof, light brown to red-brown in colour and an excellent timber. Though difficult to work, it is used in carpentry, canoe, house building and furniture making. It is also used in human medicine: febrifugal, analgaesic, anti-hemorrhageic, laxative, emetic, emmenagogic and aphrodisiac. The foliage is good as

cattle forage, particularly before the re-growth of grass in the early rainy season. Wild animals browse the arils, and antelopes eat the young shoots. Pods are rich in ashes used for making soap. The leaves are also rich in nitrogen and are used to enrich the soil. Although locally thought to be inhabited by spirits, it is the hunter's favourite tree (Burkill, 1994). Within the south-eastern zone of Nigeria, seeds of *A. africana* are relished as soup thickener.

Meanwhile, the stock of *A. africana* species has been badly depleted and natural regeneration is constrained by wild animals which rationally feed on it. The trees are not fire resistant and as poacher set fire to the forest to clear the bush for visibility of games, the heat of bush burning destroy the growth of the species. Besides, the nomadic herdsmen that traverse the savanna with their livestock usually lop the species for their livestock to browse. In the light of this, there is an urgent need to consider the *ex situ* conservation option of the species through possible establishment in plantation. There is thus, the need to develop an appropriate pre-treatment technology for breaking dormancy and regularizing germination of *A.africana* seeds, particularly within the savanna ecozone of Nigeria.

MATERIALS AND METHODS

The study area

The study was conducted within the nursery unit of Federal College of Wildlife Management, located on latitude 7°31'- 10°00'N and longitude 4°30'- 4°33'E (Adewetan et al., 1980) in the savanna areas of the Kainji Lake Basin, New Bussa. The climate of the area is tropical with high temperatures, high relative humidity and distinct wet and dry seasons. The average monthly temperature is 34°C with a mean annual relative humidity of 60%. The mean annual rainfall value is 104.45 mm (DRB, 2004). The vegetation of the area has been described as Northern Guinea Savanna (Keay, 1989b).

Experimental procedure and design

Seed collection

A. africana pods were collected under matured trees of the species in field laboratory established by the college within the northern guinea savannah ecozone between December 2007 and February 2008. The collected pods were broken and the seeds harvested decarped and kept under ambient temperature condition for 5 days. The decarped seeds were subjected to viability test through the flotation method; in which case the seeds that floated in water after 24 h of soaking were considered unviable and discarded.

Experiment 1

In this trial, the effects of cold water treatment on A. africana seeds were compared. The treatment involved:

Soaking in cold water (room temperature) for 1 h.
Soaking in cold water (room temperature) for 12 h.
Soaking in cold water (room temperature) for 24 h.

Experiment 2

This trial assessed the effect of hot water treatment on the seeds of *A. africana*. The treatment involved:

Soaking in hot water (100°C) for 1 h.
Soaking in hot water (100°C) for 12 h.
Soaking in hot water (100°C) for 24 h.

Experiment 3

This trial assessed the effect of sulphric acid (H_2SO_4). Three sulphuric acid concentrations (10, 50 and 98%) and three treatment time (5, 10 and 30 min) were compared. The treatment involved:

Soaking in sulphric acid (H_2SO_4) at 10% concentration for 5 min.
Soaking in sulphric acid (H_2SO_4) at 10% concentration for 10 min.
Soaking in sulphric acid (H_2SO_4) at 10% concentration for 30 min.
Soaking in sulphric acid (H_2SO_4) at 50% concentration for 5 min.
Soaking in sulphric acid (H_2SO_4) at 50% concentration for 10 min.
Soaking in sulphric acid (H_2SO_4) at 50% concentration for 30 min.
Soaking in sulphric acid (H_2SO_4) at 98% concentration for 5 min.
Soaking in sulphric acid (H_2SO_4) at 98% concentration for 10 min.
Soaking in sulphric acid (H_2SO_4) at 98% concentration for 30 min.

Control treatment

There was one control treatment, which involved sowing immediately after seed extraction and decarping.

Experimental layout

Since interaction between treatments was not envisaged, the experiment was laid out in a completely randomized design. Thus, each pre-treatment method formed separate experiment with the various levels representing the treatments. A total of 30 seeds was allocated to each treatment and was replicated four times. The seeds were sown in germination pots (of equal dimension) filled with topsoil. Treatments were kept under the weaning shed in order to reduce the rate of evaporation. Watering was done twice daily (morning and evening) throughout the duration of the experiment. Germination count was taken every evening and seedlings were pricked-out after counting to avoid error. Germination was monitored for 42 days, after which the experiment was terminated.

Data analysis

The cumulative germination data were computed and converted to percentage values to determine the germination percentages in each treatment. To ensure conformity with the general assumption of Analysis of Variance (ANOVA) the percentage data were transformed using Arcsine transformation. A one-way ANOVA was carried out to analyze the data and determine the most appropriate pre-treatment method for minimizing dormancy period in *A. africana* seeds within the ecozone.

RESULTS

Effect of cold water treatment

Seed germination under the different cold water treat ment gave a fair germination percentage (33.33 to 53.33%). The longer the cold water treatment, the earlier

Table 1. Germination of *A. africana* seeds in cold water treatment.

Cold water treatment (hour)	Mean number of days taken for first emergence	Mean number of seeds germinated	Germination percentage
1	13	10	33.33
12	12	16	53.33
24	11	12	40.00
Control	14	18	60.00

Table 2. Germination of *A. africana* seeds in hot water treatment.

Hot water treatment (hour)	Mean number of days taken for first emergence	Mean number of seeds germinated	Germination percentage
1	13	11	36.67
12	13	8	26.67
24	13	11	36.67
Control	14	18	60.00

Table 3. Summary of effect of acid concentration and treatment time on days to seedling emergence in *A. africana.*

Treatment time (min)	Acid concentration (%)			Mean number of days taken for first emergence
	10	50	98	
5	12	15	12	**13.00**
10	13	12	10	**11.67**
30	10	10	8	**9.33**
Mean	11.67	12.33	10.00	

the commencement of seed germination, although this was not significant within the treatment. For example, while germination started on the 11th day for seeds soaked for 24 h, it took 12 and 13 days for those soaked in cold water for 12 and 1 h respectively (Table 1). Generally, germination under the control gave higher percentage (60.00%) than those of cold water treatment, though with longer germination commencement period (average of 14 days after sowing).

Effect of hot water treatment

When compared to control and cold water pre-treatment, the effect of hot water on the seeds of *A. africana* could be described as adverse. The highest germination percentage of 36.67% is recorded for both seeds subjected to 1 and 24 h hot water pre-treatment. Seeds subjected to 12 h hot water pre-treatment gave a very low germination percentage of 26.67%. All treatment hours commenced germination on the 13th day (Table 2).

Effect of acid pre-treatment

Under the acid pre-treatment, germination percentage was enhanced by both treatment time and concentration level. Except for the 30 min treatment time, however, commencement of germination when compared to control, cold water and hot water pre-treatments, was not positively impacted by both treatment time and concentration level. The highest germination percentage for the acid pre-treatment was 86.67% at 10% concentration level for 30 min treatment time, while the shortest germination commencement period was 8 days at the 98% concentration level for 30 min treatment time (Tables 3 and 4) (Figures 1 to 4).

From Figure 1, germination of *A. africana* seeds in acid treatment (H_2SO_4) at 10% concentration for 5 min began on the 12th day with total number of 12 seeds germinated (40.00%). Germination at 50% acid concentration began on the 15th day with total number of 7 seeds germinated (23.33%), while at 98% acid concentration germination began on the 12th day with a total of 11 seeds germinated

Table 4. Summary of effect of acid concentration and treatment time on the germination of *A. africana* seeds.

Treatment time (min)	Acid concentration (%)			Mean number of seeds germinated
	10	50	98	
5	40.00	23.33	36.67	33.33
10	66.67	83.33	76.67	75.56
30	86.67	73.33	83.33	81.11
Mean	64.45	60.00	65.56	

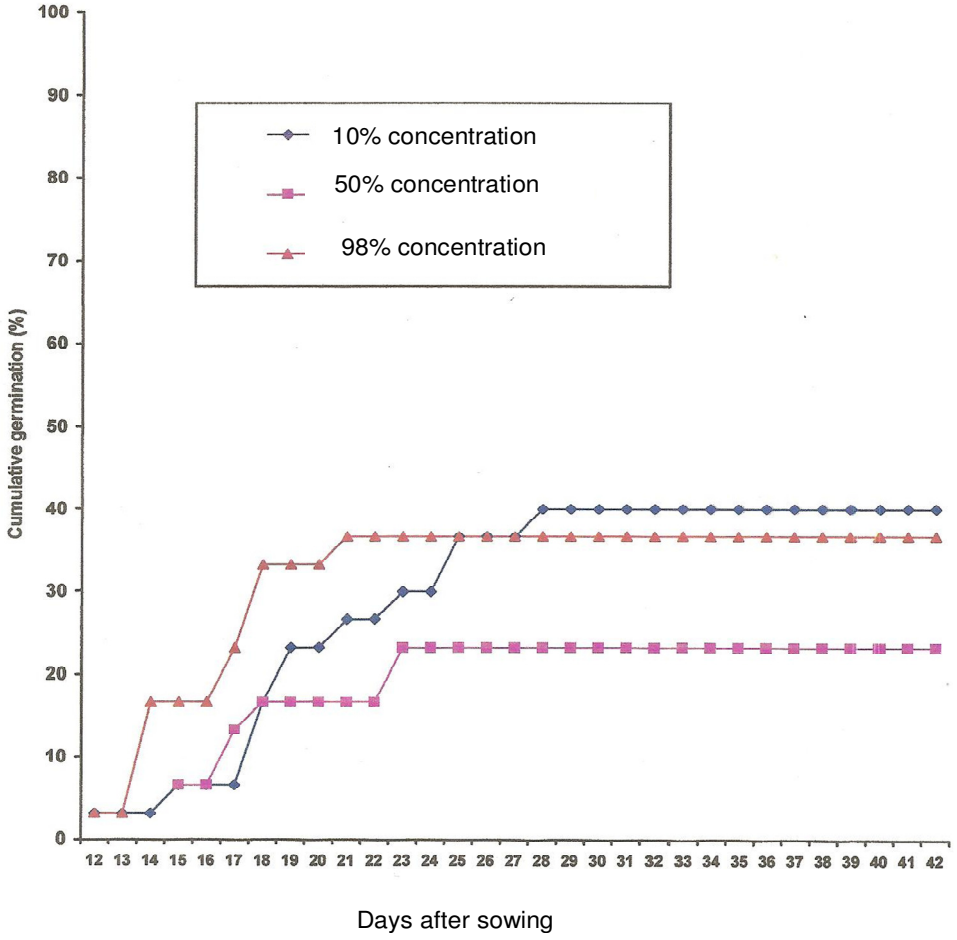

Figure 1. Germination of *A. africana* seeds in acid treatment (H_2SO_4) at 10, 50 and 98% concentration for 5 min.

(36.67%).

From Figure 2, germination of *A. africana* seeds in acid treatment (H_2SO_4) at 10% concentration for 10 min began on the 13th day with total number of 20 seeds germinated (66.67%). Germination at 50% acid concentration began on the 12th day with a total number of 25 seeds germinated (83.33%), while at 98% acid concentration germination began on the 10th day with a total of 23 seeds germinated (76.67%)

From Figure 3, germination of *A. africana* seeds in acid treatment (H_2SO_4) at 10% concentration for 30 min began on the 10th day with total number of 26 seeds germinated (86.67%). Germination at 50% acid concentration began on the 10th day with total number of 22 seeds germinated (73.33%), while at 98% acid concentration germination began on the 8th day with a total of 25 seeds germinated (83.33%).

From Figure 4, germination began on the 14th day with

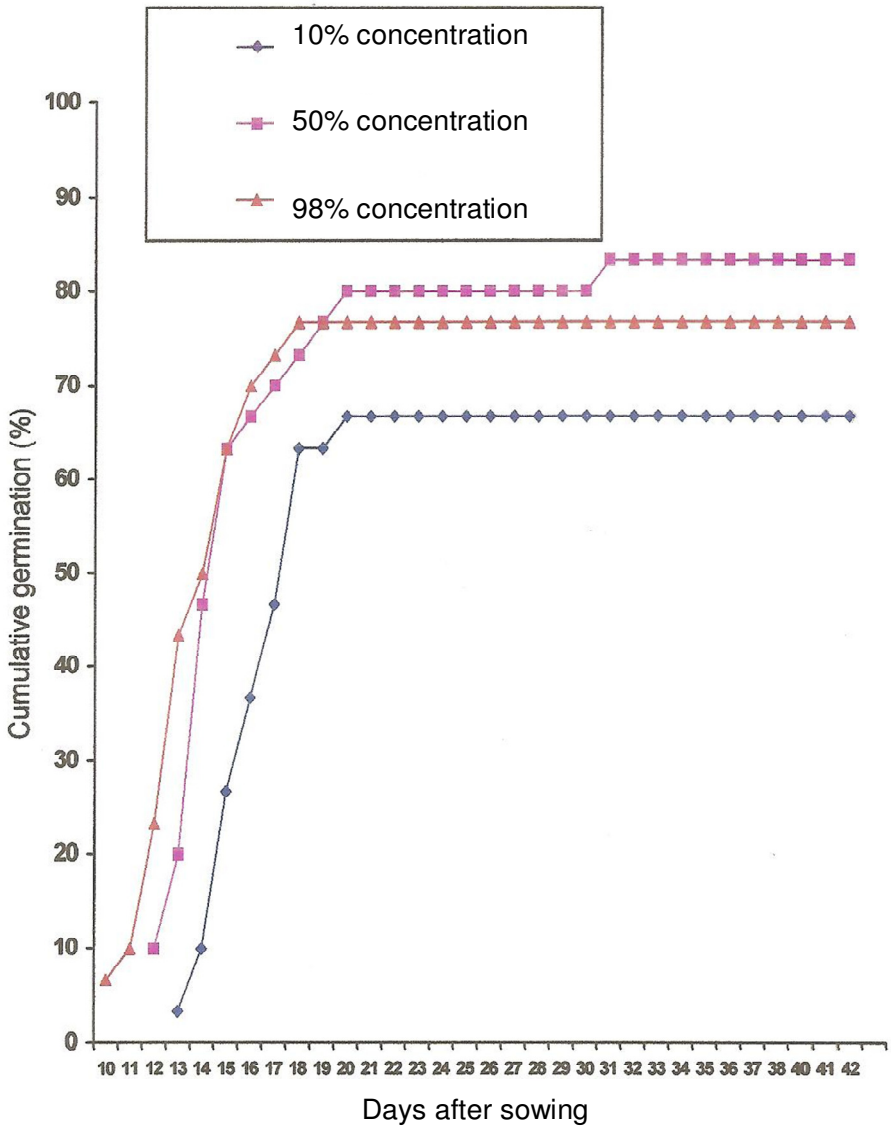

Figure 2. Germination of *Afzelia africana* seeds in acid treatment (H_2SO_4) at 10, 50 and 98% concentration for 10 min.

a total number of 18 seeds germinated (60.00%).

Although acid treatment ranked best among the three pre-treatment techniques assessed in this study, the result of one-way analysis of variance shows that there is no significant different in the overall effect of the three techniques and control treatment (Table 5).

DISCUSSION

Seed dormancy occurs in many tropical tree species to varying degrees (Nwoboshi, 1982). While various pre-treatment methods have been advocated to reduce dormancy and hasten germination, no single pre-treatment technique has been found to be equally effective for all seed species. The preceding findings of this study shows that, although *A. africana* seeds under control treatment gave an impressive germination, which did not differ significantly from the results of other pre-treatment techniques (Table 5), acid pre-treatments yield a more uniform and regular germination. The fact that acid treatment gave highest germination values within shortest time indicates that the more rapidly the seed coat is ruptured, the faster the rate of germination. This is more so, since a very widespread cause of seed dormancy is the presence of hard seed coat which prevents the entrance of water, exchange of gases and /or mechanically constrained the embryo (Mayer and Maber, 1963). Sulphuric acid is thought to disrupt the seed coat and expose the lumens of the macrosclereids

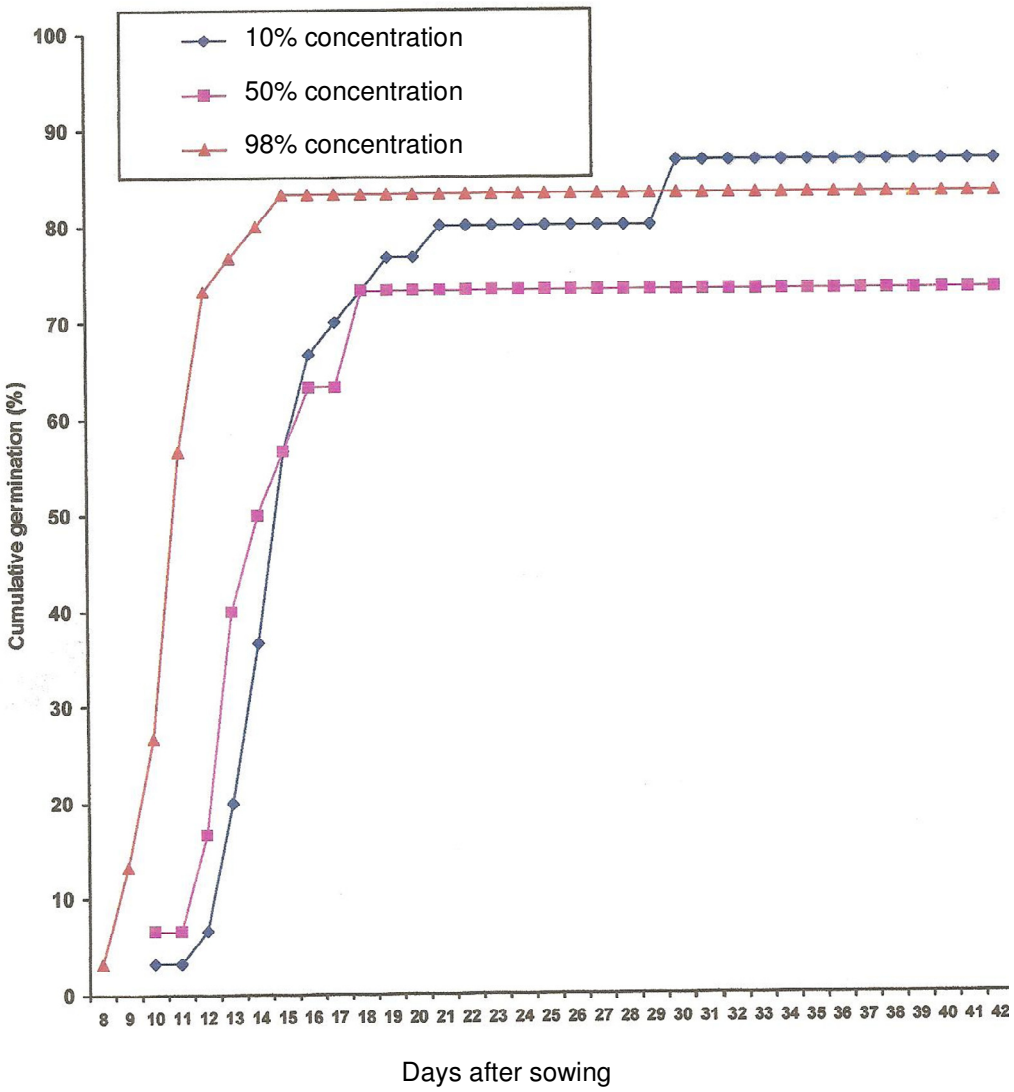

Figure 3. Germination of *Afzelia africana* seeds in acid treatment (H_2SO_4) at 10, 50 and 98% concentration for 30 min.

cells, permitting imbibition of water, which triggers germination (Nikoleave, 1977). Irwin (1982) and Jackson (1994) also found that as soon as the seed coat is softened by scarification, the process of hydrolysis would commence to release simple sugars that could be readily utilized in protein synthesis, thereby encouraging germination.

Cold water pre-treatment of *A. africana* seeds only gave a fair germination percentage and reduced dormancy period (Table 1). The control treatment gave a higher germination percentage. This is in tandem with the observation of Robertson and Small, (1977) that over-soaking seeds in water may reduce germination through oxygen deficiency. However, Owonubi et al. (2005) reported that soaking of *Azadirachta indica* seeds for 1 and 12 h resulted in increasing rate of seed germination corroborating the work of Ibrahim and Otegbeye (2004) on the seeds of *Adansonia digitata.* This implies that different species have varying rates at which their seed coat is permeable to water and gases (Owonubi et al., 2005). Meanwhile, the effect of hot water on the seeds of *A. africana* gave adverse result contrary to what would be obtained in nature (as indicated by the result of the control treatment). This shows that hot water pre-treatment is not an appropriate pre-treatment technology in the seeds of *A. africana*. It perhaps leads to the seed embryo being killed because of prolonged contact with boiled water.

Conclusion

The nature and dimension of hard seed coat dormancy are not fully understood. Pre-germination treatments are

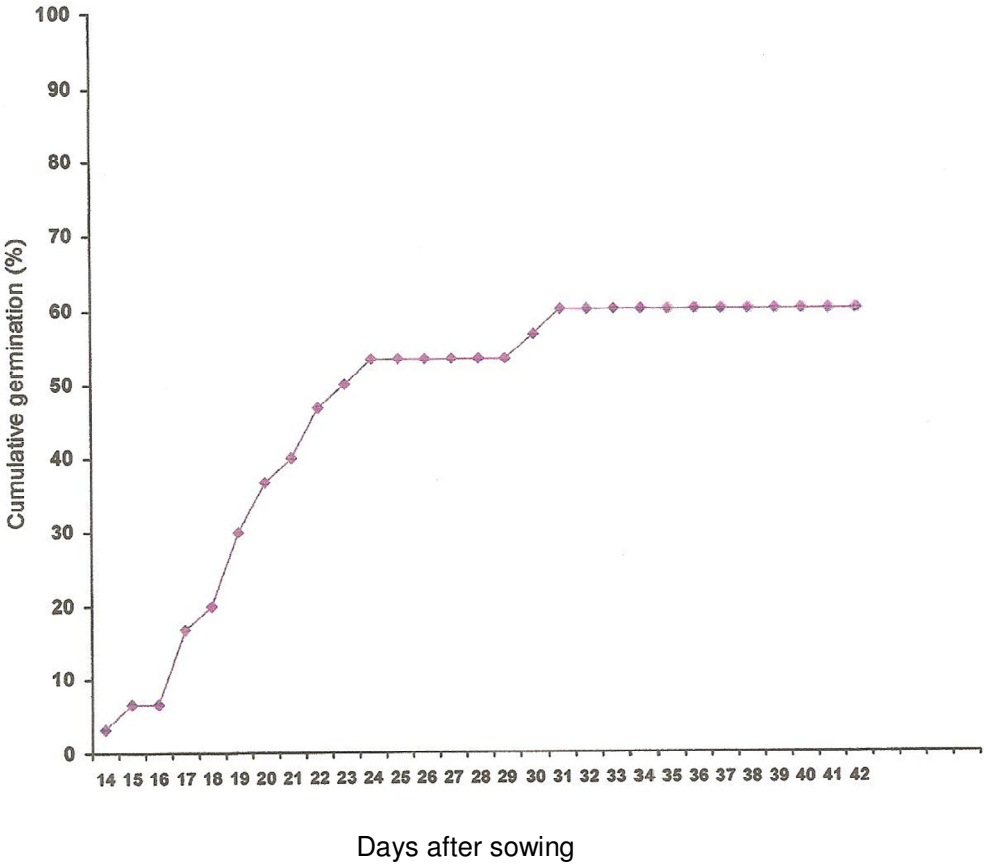

Figure 4. Germination of *A. africana* seeds under control treatment.

Table 5. Analysis of variance for the effect of different pre-treatment techniques on the germination of *Afzelia africana* seeds

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Source of variation	Sum of square	Degree of freedom	Mean Square	F-cal	P-level
Treatment	1847	3	615.7	2.677	0.1180
Residual	1840	8	230.0		
Total	3687	11			

F-cal = 2.6777; P-level = 0.1180. Means are not significantly different ($P < 0.05$).

however, needed to break physical dormancy caused by hard seed coat or pericarps. The result of this study has shown that the seeds of *A. africana* could be germinated without pre-treatment, but to attain higher percentage germination and reduced dormancy period, the seeds should be pre-treated with sulphuric acid. The acid treatment, nonetheless, require further research in order to determine the optimum acid concentration level.

RECOMMENDATION

Based on the findings of this study, the following recommendations are made:

1. Wide range of acid (H_2S0_4) concentration level should be used in order to have a close comparison between the various concentration treatments.
2. Wide range of time should also be used in order to know the effect of time on various levels of concentration.

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