Effect of Acid Pretreatment of Seeds on the Germination and Early Growth of *Albizia lebbek (L) Benth* in Makurdi, Benue State-Nigeria

G. Dachung [a], J. I. Amonum [a*], I. B. Chenge [a] and P. Adeka [a]

[a] *Department of Forest Production and Products, College of Forestry and Fisheries, Federal University of Agriculture Makurdi, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The seeds of *A. lebbeck* exhibit physical dormancy, which leads to poor seed germination due to a tough seed coat, which has prevented the species from being domesticated in the study area. Pre-sowing treatments are therefore necessary to increase seed germination and reduce germination period. This study aims at the evaluation of the impact of acid pretreatment on seed dormancy and growing seedlings of *A. lebbeck* for the establishment of plantations in Benue state, Nigeria, where it is lacking. The experiment was laid out in a completely randomized design with six pretreatments (soaked) with the used of acids. The pretreatments include: sulphuric acid (70 % H_2SO_4) for 2 minutes, 5 minutes, and 10 minutes; nitric acid (70 % HNO_3) for 2 minutes, 5 minutes, and 10 minutes; and untreated seeds (control); and replicated thrice. Two seeds were sown in each plastic pot and later thinned to one seed per pot after germination. A total of 144 plastic pots were used. The immersion in 70 % nitric acid for 5 minutes as pretreatment of A. *lebbeck*yielded the highest

Corresponding author: E-mail: jamonng@yahoo.com;

germination percentage (50%) and immersion in nitric acid for 2 minutes had the highest emergence index (38). According to the study's findings, pre-treating A. *lebbeck* with 70 % nitric acid breaks dormancy better and promotes faster growth. Thus, it is recommended that pretreatment of *A. lebbeck* seeds with 70% nitric acid for five minutes before sowing should be adopted to grow the species faster in the study area.

Keywords: Germination; growth; forest; plantation; sustainable.

1. INTRODUCTION

Widely distributed *Albizia lebbeck* is a deciduous woody tree with wide leaves, the flowers are fragrant cluster of greenish-yellow flowers, white, many stamens, and lengthy seed pods. It is native to tropical Asia and is commonly grown and naturalized in other tropical and subtropical climates, including Malawi [1] and Nigeria. The tree species is a member of the Leguminoseae family [2]. At maturity, *A. lebbeck* has an estimated bole diameter between 50 cm to 1 m and could reach a height of 20 to 35 m. The leaves have one to four pairs of pinnae and are 7 to 15 cm long. Each pinna has six to eight leaflets [3].

The fruit could content about five to twelve seeds, which are used for various purposes. The seeds are contained in fruit pods that are 15 to 30 cm long and 2.5 to 5.0 cm wide [3]. The species has traditionally been used to treat allergy conditions like asthma, colds, and coughs. The medicinal properties of *A. lebbeck* extend to its flowers, bark, fruits, roots, and stems [4]. As widely practice, skin issues are treated with a leaf paste. *A. Lebbeck* is renowned for treating allergies and respiratory issues [3]. Other plant parts are also utilized to treat eye issues, cleanse the blood, and improve tooth health. The most significant benefit is that the ethanol extract from the pods can effectively treat various cancer types [5]. Because the leaves include proteins, calcium, phosphorus, and amino acids, they are nourishing [2].

Due to the existence of a hard seed coat, it has been observed that the seeds of *A. lebbeck* exhibit physical dormancy, which leads to poor seed germination [6]. Due to the delayed and subpar germination, raising seedlings from such seeds becomes problematic. Despite their significance, the seeds are very dormant because of their tough seed coat, which has prevented the species from becoming domesticated. When seeds of *Albizia* species were sown without any pre-treatment, several studies found low seed germination [7-12].

Pre-sowing treatments are therefore necessary to increase seed germination and reduce germination times. Acid scarification, mechanical scarification, and submersion in water are a few techniques for overcoming the physical dormancy of seeds [13]. Therefore, the purpose of this study is to evaluate the impact of acid pretreatment on releasing seed dormancy and growing seedlings for the establishment of plantations in Benue state which is lacking. Find out if sulphuric and nitric acid pretreatment influence the germination and early growth of the seeds in the study area.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Forestry Nursery, Joseph SarwuanTarka University, Makurdi. Makurdi covers an area of 804 square kilometers and it lies between Latitude 07° 21' - 08° N, and Longitude 08 °21' - 09 °E. It is situate on 98m above sea level. Details of the study area was described in Dau and Chenge [14]; Agera et al. [15] and Kuje et al. [16].

2.2 Seed Collection, Pretreatment of *A. lebbeck* and Experimental Design

The seeds of *A. lebbeck* were collected directly from mature mother tree of superior phenotypic traits within the study area; between the month of March and May. The seed were sun dried for 48 hours, and stored under normal room temperature for 3 months, until the planting period. The seeds were given pre-sowing treatments (soaked) in sulphuric acid (70 % H_2SO_4) for 2 minutes (T_1);sulphuric acid (70 % H_2SO_4) for 5 minutes (T_2); sulphuric acid (70 % H_2SO_4) for 10 minutes (T_3);nitric acid (70 % HNO_3) for 2 minutes (T_4); nitric acid (70 % HNO_3) for 5 minutes (T_5); nitric acid (70 % HNO_3) for 10 minutes (T_6), and untreated seeds which served as control (T_7). After immersion, the solution was drained off, and seeds repeatedly rinsed in running tap water until considered safe

to handle and subsequent dried for a period of 2hours before the seeds were sown in plastic pots containing 1kg of soil. The experiment was conducted in plastic pots containing 1kg of soil each.

The experiment was laid out in Completely Randomized Design (CRD) with six (6) treatments and replicated three (3) times. Two seeds were sown in plastic pot and later thinned to one stand per pot after germination [15]. A total of one hundred and forty four (144) plastic pots were used and two hundred and eighty eight (288) seeds were sown.

2.3 Data Collection and Analyses

The experiment was monitored for eight (8) weeks and the germination data was observed in an interval of two days from the 7th day when germination commenced till 10th week. Germination data were collected based on treatments. The number of germinated seeds, used to determine germination percentage of seedlings, emergence index and emergence rate index of *A. lebbec* seeds in the study area.

$$\text{Germination percentage of seedling}=$$
$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds planted}} \times \frac{100}{1} \quad [17]$$

$$\text{Emergence index} =$$
$$\sum \frac{\text{Number of seedling germinated per day}}{\text{Total number of seedling planted}} \times \frac{\text{day after planting}}{1}$$

$$\text{Emergence rate index}= \frac{\text{Emergence index}}{\text{Germination percentage}}$$

Three months old seedling growth variables of interest were measured as follows: seedling heights were measured using a meter rule, number of leaves per seedling were counted, leaves length were also measured with a meter rule, collar diameters of the seedlings were measuring using veneer caliper and graph sheets were used to determined leaf area for sampled seedlings in the study area [16]. Data collected were subjected to Shapiro wilk and Levene's test to check if the data met the requirement of the ANOVA (Analysis of variance). The data was then subjected to analysis of variance (ANOVA). Mean separation was carried out to determine the best suitable acid pretreatment for germination and early growth of *A. lebbeck* using post-hoc test at a significance level of 0.05.

3. RESULTS

3.1 Germination Indices of *A. lebbeck*

The result of this finding on germination is shown on Table 1. The first germination commenced after six days of planting. The highest number of germination occurred in T_5 (immersion in Nitric acid for 5 minutes) with a germination percentage of 50% and the least was recorded in T_7 (Control) and with a germination potential of 5%. The highest emergence rate was recorded in T_3 (Immersion in sulphuric acid for 10 minutes) 31.9 and the least were recorded in the case of T_7 (control) 0.8. For emergence rate index, the highest was recorded in T_4 (immersion in Nitric acid for 2 minutes) with a value of 0.84 and the lowest was in control with a value of 0.16.

The result on summary statistic (Table 2) of seedling heights indicates the highest mean seedling height of 17.2 cm as recorded under T_3, this was followed by 14 cm and 13.1 cm recorded under T_1 and T_2, respectively. The minimum and maximum seedling heights of 5 cm (T_4) and 30.8 cm T_3, respectively, were recorded from this study. The highest mean seedling diameter of 2.5 cm (T_3) and lowest mean diameter of 1.8 cm (Control) were recorded; with the minimum and maximum seedling diameters of 1.1 cm (T_3) and 3.9 cm (T_5). The mean number of leaves was at its highest under $T_1 T_2$ T_3 and T_5 while T_6 and control (T_7) yielded the lowest mean number of leaves. The minimum and maximum number of leaves recorded from this study indicates 5 (T_6 and control) and 11 (T_4), respectively. The highest mean leaf area of 3.7 cm^2 (T_3) was observed and control was yielded the lowest mean leaf area.

3.2 Seedling Height of *A. lebbeck*

The Shapiro-wilk normality test revealed a P-value of 0.1884 which is greater than the significance level of 0.05. This implies that the data are not significantly different from the normal distribution. The box plot (Fig. 1) provides a graphical summary of the seedling heights distribution of each treatment. The box plot indicates that, the observation in each treatment was not significantly far from each other. The Levene's test was used to check the homogeneity of variance revealed a P-value of 0.08038 is not less than the level of significance level of 0.05. This implies that the variance across the groups is statistically not significantly different. The ANOVA result in shown in Table 3;

which showed the difference between the mean which were not statistically significantly (*P*=0.172). The effect of the treatment on the height growth of the *A. lebbeck* seedlings across the treatment groups was not significantly different.

3.3 Seedling Collar Diameter of *A. lebbeck*

The Shapiro-wilk normality test revealed a *P-value* of 0.005 which is greater than the significance level of 0.05. This implies that the data are not significantly different from the normal distribution of the seedlings collar diameter. The box plot (Fig. 2) shows a graphical summary of the data distribution of each treatment. The box plot suggests that, the observation in each treatment is not far from each other. The Levene's test was used to check the homogeneity of variance revealed a P-value of 0.064 is not less than the level of significance level of 0.05. This implies that the variance across the groups is statistically not significantly different. The ANOVA result in Table 4 shows the difference between the mean are not statistically significantly different (P-value=0.128). The effect of the treatments on the collar diameter of the *A. lebbeck* seedlings across the treatment groups is not significantly different.

Table 1. Germination indices of *Albizia lebbeck* seedlings sown

| Treatments	GP (%)	EI	ERI
T$_1$	45	28.8	0.64
T$_2$	35	27.2	0.78
T$_3$	45	31.9	0.71
T$_4$	45	38	0.84
T$_5$	50	28.2	0.56
T$_6$	30	13.4	0.38
Control	5	0.8	0.16

Where: GP (germination percentage), EI(emergence index), ERI(emergence rate index), T$_1$- Seeds pretreated with sulphuric acid for 2 minutes, T$_2$- Seeds pretreated with sulphuric acid for 5 minutes, T$_3$- Seeds pretreated with sulphuric acid for 10 minutes,T$_4$- Seeds pretreated with nitric acid for 2 minutes, T$_5$-Seeds pre-treated with nitric acid for 5 minutes, T$_6$- Seeds pretreated with nitric acid for 10 minutes,T$_7$- Seeds that did not undergo any pretreatment

**Table 2. Summary of the statistical result for the various treatment groups**

Variables	Treatments						
	T$_1$	T$_2$	T$_3$	T$_4$	T$_5$	T$_6$	T$_7$
Height (cm)							
Mean	14.0	13.1	17.1	10.7	12.5	12.1	12.0
Standard Deviation	3.3	3.9	7.9	4.7	5.2	3.2	0.0
Minimum	9.6	9.5	10.6	5.0	5.6	9.0	12.0
Maximum	19.0	21.3	30.8	19.5	19.3	16.2	12.0
Diameter (cm)							
Mean	2.3	2.2	2.5	1.9	2.5	1.9	1.8
Standard Deviation	0.5	0.8	0.6	0.4	0.9	0.5	0.0
Minimum	1.7	1.4	1.7	1.5	1.3	1.1	1.8
Maximum	3.0	3.2	3.4	2.8	3.9	2.6	1.8
Number of Leaves							
Mean	8	8	8	7	8	5	5
Standard Deviation	1	0	0	2	0	0	0
Minimum	7	7	8	6	7	5	5
Maximum	8	8	8	11	8	5	5
Leaf Area							
Mean	2.1	2.7	3.7	3.0	2.8	1.4	1.2
Standard Deviation	0.6	1.2	0.7	0.6	0.6	0.4	0.0
Minimum	1.3	1.1	2.6	2.1	2.0	1.0	1.2
Maximum	3.3	3.8	5.0	3.8	3.8	2.0	1.2

Table 3. ANOVA test results for seedling height of *Albizia lebbeck*

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	223.86	6	37.309	1.585	0.17	2.290
Within Groups	1153.46	49	23.54			
Total	**1377.32**	**55**				
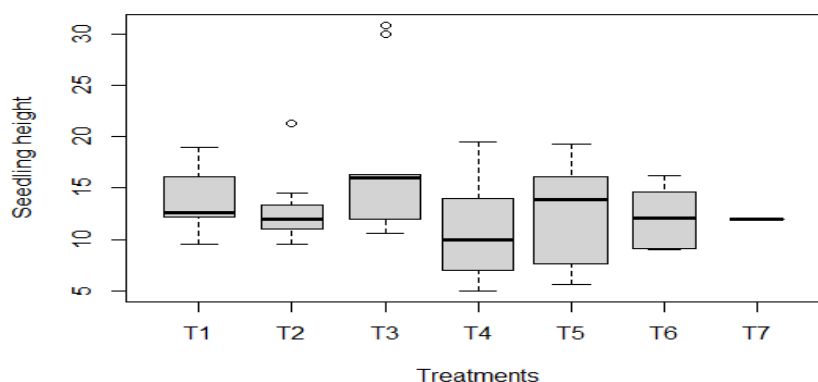
Fig. 1. Box plot for seedling height by treatment groups
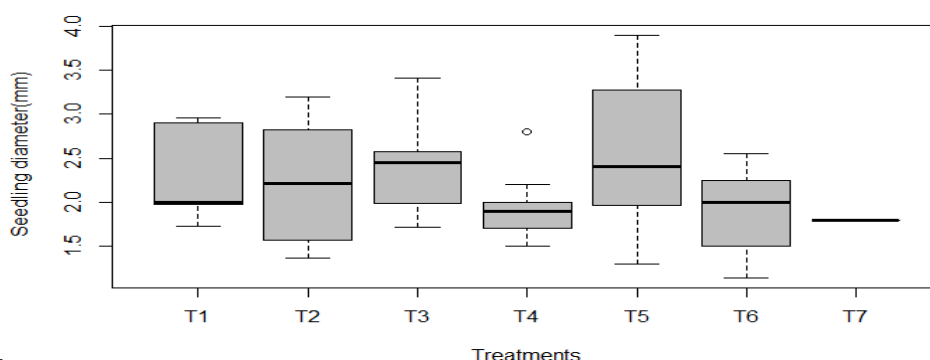
Fig. 2. Box plot of seedling collar diameter by treatment groups

3.4 Seedling Number of Leaves of *A. lebbeck*

The Shapiro-wilk normality test revealed a P-value of 0.0834 which is greater than the significance level of 0.05. This implies that the data are not significantly different from the normal distribution. The Levene's test was used to check the homogeneity of variance revealed a P-value of 0.0600 is not less than the level of significance level of 0.05. This implies that the variance across the groups is statistically not significantly different.

The post hoc test conducted in Table 6 shows that T_1, T_2, T_3, T_4, T_5 are not significantly different from each other, T_6 and T_7 are not significantly different from each other but are significantly different from the first five treatment groups. The ANOVA result in Table 5 shows the difference between the mean are statistically significantly different (P-value=0.14E-12). The effect of the treatment on the number of leaves of the *A. lebbeck* seedlings across the treatment groups is significantly different.

3.5 Seedling Leaf Area of *A. lebbeck*

The Shapiro-wilk normality test revealed a P-value of 0.1421 which is greater than the significance level of 0.05. This implies that the data are not significantly different from the normal distribution. The box plot (Fig. 3) provides a graphical summary of the data distribution of each treatment. The box plot indicates that, the observation under each treatment differed from each other. The Levene's test was used to check the homogeneity of variance revealed a P-value of 1.421 is not less than the significance level of 0.05. This implies that the variance across the groups is statistically not different.

A post hoc test conducted in Table 8 reveals that T_2, T_3, T_4, T_5 are not significantly different from each other, T_1, T_6 and T_7 are not significantly different from each other. The ANOVA result in Table 7 shows the difference between the mean are not statistically significantly different (P-value=0.2.61E.08). The effect of the treatment on the leaf area of the *A. labbeck* seedlings across the treatment groups is significantly different.

4. DISCUSSION

Acid pre-treatments influence the early growth and germination of *A. lebbeck* seedlings by breaking dormancy and influencing the physical characteristics of the seedlings that are visually determinable of nursery tree seeding. The major morphological criteria often used to describe tree seeding potentials for timber production are height, number of leaves, and seeding crown width. Height is an important phenotypic plant character (although generally controlled genetically) which differs within varieties. Under this present study, the mean height, collar diameter were tested using ANOVA and the results were not significantly different, number of leaves and leaf area were tested using ANOVA and the result was significantly different this was influenced by the type of acid pretreatment and duration of soaking significance level of 0.05.

This is an indication that both Nitric acid and sulphuric acid at different duration of seed soaking acid improve the early growth and germination of the *A. lebbeck* seeding. This present study corresponds with the findings of Ajiboye et al. [8] who reported that, most pre-treatments significantly reduced hard seed content and improved germination percentage compared to untreated seeds. This is also in agreement with the findings of Edward et al. [18] which revealed that the effect of sulphuric acid on promotion of seed germination, seedling growth, and survival at nursery stage might be due to the highly desiccant effect of the acid on the seed coat thereby allowing easier water uptake and oxygen diffusion in species of *Acacia polycantha*. This is in contrast to the findings of Olatunji et al. [19], who reported higher seed germination seedling growth, and survival in immersion in concentrated sulphuric acid than other treatments for the seed species.

Table 4. ANOVA test results for seedling collar diameter of *Albizia lebbeck* seedlings sown

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	3.927	6	0.655	1.756	0.128	2.290
Within Groups	18.261	49	0.33			
Total	22.189	55				

Table 5. ANOVA test results for number of leaves of *A. lebbeck* seedlings sown

Source of Variation	SS	Df	MS	F	P-value	F. critical
Between Groups	72.112	6	12.019	22.639	1.4E-12***	2.290
Within Groups	26.013	49	0.531			
Total	98.125	55				
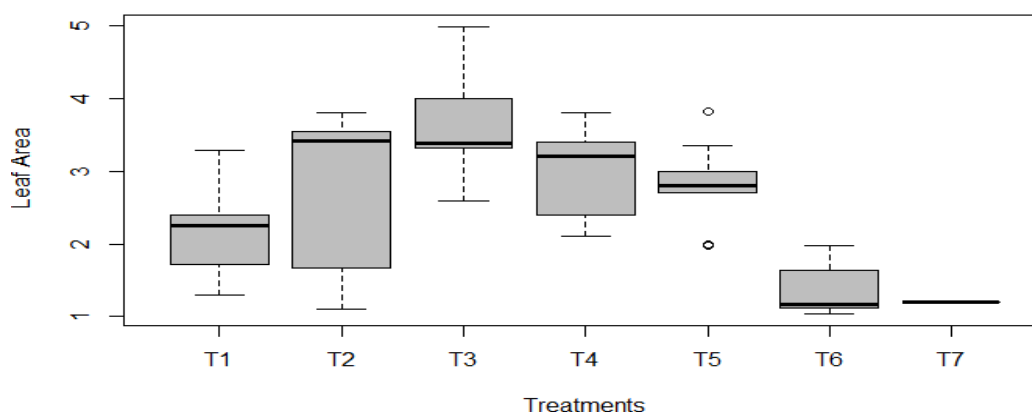
Fig. 3. Box plot of leaf area by treatment groups

Table 6. Post-hoc test results for number of leaves: Tukey (Tukey's honest significance difference) multiple comparisons of means

Treatments	Means
T_1	7.7 a
T_2	7.9 a
T_3	8.0 a
T_4	7.2 a
T_5	7.8 a
T_6	5.0 b
Control (T_7)	5.0 b

Pairs of treatments that are not significantly different from each other share the same letter

Table 7. ANOVA test results for leaf area of seedlings sown

Source of Variation	SS	D.f	MS	F	P-value	F. critical
Between Groups	33.879	6	5.647	12.131	2.61E.08	2.290
Within Groups	22.807	49	0.466			
Total	56.687	55				

Table 8. Post-hoc test results for leaf area of *A. lebbeck*: Tukey (Tukey's honest significance difference) multiple comparisons of means

Treatments	Mean
T_1	2.1 bc
T_2	2.7 ab
T_3	3.7 a
T_4	3.0 ab
T_5	2.8 ab
T_6	1.4 c
Control (T_7)	1.2 c

Pairs of treatments that is not significantly different from one another share the same letter

Acid pre-treatments are aimed at making the seed coat permeable either naturally or artificially and hence enhances germination [20]. Agbogidi et al. [21] also agrees with the present study, the results also indicated that acid treatment of *D. edulis* seeds significantly (P≤ 0.05) improved the performance of the seedlings as regards plant height, number of leaves, leaf area and collar diameter for *D. Edulis* seedlings. Amira et al. [22] reported similar results on *Cassia fistula* and *P.biglobosa* respectively. This is dissimilar to the finding of Pierre et al. [23] which revealed that the use of sulphuric acid was not as effective alternative to manual scarification. The result gotten in this study is similar to the findings of Usman and Asan [24] revealed that H_2SO_4 was to enhance germination percentage (93.33%) and growth performance in terms of plant height (15cm), number of leaves (6) and leaf length (6cm) respectively in *A. digitata.*

5. CONCLUSION

This study revealed that the pretreatment of *A. lebbeck* T_5 (Immersion in nitric acid for 5 minutes) had the highest germination percentage and T_4 (Immersion in nitric acid for 2 minutes) had the highest emergence index. Based on the results obtained in the study, pre-treating of *A. lebbeck* with 70% concentration of Nitric acid breaks dormancy better and enhance faster growth. Thus, it is recommended that, pretreatment of *A. lebbeck* seeds with 70% Nitric acid for five minutes before sowing should be adopted to grow species faster in the study area.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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