



Effect of Three Pregermination Treatments on the Growth of Emerging Seedlings of *Acacia auriculiformis* (Cunn. Ex. Benth)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors YOA and AMA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JAY performed the statistical analysis, managed the analyses of the study. Authors RAH and SOA assist with field work, drafting of manuscript as well as managed the literature searched. All authors read and approved the final manuscript.

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ABSTRACT

This research was carried out to determine the effect of some pregermination treatments on the seeds of *Acacia auriculiformis* as it affects growth. The treatments involve the effect of sulphuric acid (concentration and time), mechanical scarification (at different sides), and hot water treatment (volume and time). Split plot design was used for acid treatment and hot water treatment, while Randomized Complete Block Design (RCBD) was used for mechanical scarification. The experiment was carried out at Federal University of Agriculture, Abeokuta, Nigeria. Parameters assessed include; plant height, stem height, leaf number. Acid treatment was carried out using different concentration of sulphuric acid (98%, 50% and 20%) and different treatment time (2 minutes, 5 minutes and 10 minutes). Concentration of acid was significant ($p < 0.05$) on plant height, stem height and leaf number, higher concentration of acid (98%) enhanced morphological parameters of *Acacia auriculiformis*. Result showed that mechanical scarification significantly affect

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stem height ($p < 0.05$), however, while mechanical scarification does not significantly affect plant height, the highest mean plant height (8.98 cm) was observed in seeds that were mechanically scarified at the micropyle; early germination of seeds was also noticed. Hot water treatment was carried out using different volume of water (50 cl and 1 litre) and varying treatment time (2 minutes, 4 minutes, 6 minutes and 10 minutes), the treatment showed no significant difference, and this method is not favourable for breaking seed dormancy of *Acacia auriculiformis*. Result showed that 100% germination could be achieved within seven (7) days when seeds are mechanically scarified at the micropyle, circumference, distal-end and micropyle+distalend, provided the seeds are viable. This research conclude that, acid scarification using H_2SO_4 at 98% concentration is the best method yielding better morphological parameters in *Acacia auriculiformis*, followed by mechanical scarification (especially when scarified at the micropyle).

Keywords: *Acacia auriculiformis*; pregermination treatment; growth rate; seed dormancy; morphological parameter.

1. INTRODUCTION

Acacia auriculiformis A. Cunn ex. Benth (Family - Fabaceae) is a leguminous and multipurpose tree which is found in many tropical zones of Southeast Asia, Africa and Latin America [1,2]. It is a fast growing species, with the ability to fix nitrogen, surviving on infertile, acid, alkaline, saline and waterlogged soils [3]. A growth rate of 2–3m per year has been reported on low fertility soil in plantations [4].

Natural hybridization has been reported between *Acacia mangium* and *Acacia auriculiformis* in the late 1970s; first reported in Sabah, Malaysia [5]. *Acacia mangium* was identified as the female parent and *Acacia auriculiformis* as the male parent of the natural *Acacia* hybrid [6]. Vietnam has over 400 000 ha of *Acacia* plantations, including over 220 000 hectares of clonal *Acacia* hybrid. *Acacia* plantations have the potential to provide several environmental benefits; its plantations are expected to reduce the pressure on native forests as a source of industrial raw materials. The capacity of *Acacia* to improve infertile soils could be an advantage on low fertility soil [7].

Seeds are very important in forest regeneration, seed germination and early seedling growth phases are considered critical for raising a successful forest plantation as they directly determine the stand density and consequently the yield [3]. *Acacia* species generally have a water-impermeable seed coat, which means that physical and/or chemical pre-germination treatments are necessary in order to overcome seed dormancy and obtain rapid and synchronous germination. Soaking seeds in boiling water, sulphuric acid, or mechanical scarification has been demonstrated to promote

germination [8,9,10,11]. Physical dormancy is caused mainly by impermeable seed coats that prevent water uptake. Thick and lignified cell walls are responsible for this physical barrier; water repellent compounds (waxes, cutin and suberin) that cover the seed can also limit water uptake, which in turn slows down the process of germination. Until the external layers become permeable to water by temperature oscillation, by seed going through the digestive system of animals, or by freezing and thawing cycles, the germination process will not start [12]. There is need to research into the most effective method of breaking the dormancy of *Acacia auriculiformis* as well as the best method that promote its growth parameters.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out at the Forest nursery site of the Federal University of Agriculture, Abeokuta, Ogun state, Nigeria. The area is located within latitude $7^{\circ}N$ and $7^{\circ}58'N$ and longitude $3^{\circ}20' E$ and $3^{\circ}27'E$. It has annual rainfall of 1200 mm with peaks in June and July; there is a dry season of two or three months. The relative humidity of the area is 82.54% and an average monthly temperature of $35.8^{\circ}C$. It has a gentle undulating landscape and mild slope. The site is punctuated in parts by ridges, isolated, residual hills, valleys and lowlands. The soils are sand and clay with crystalline basement complex. The Forestry Nursery is located about 50 m from the main gate of the campus.

2.2 Experimental Methods and Layout

This experiment was carried out at the peak of rainy season (July through September) under

tree shade at nursery site. Seeds were collected from College of Environmental Resources Management Students' Association (COLERMSA) garden, at the Federal University of Agriculture, Abeokuta, Nigeria. Float test was carried out by pouring the seeds in a beaker containing water to remove unviable floating seeds. A total of 86 pretreated seeds were sown in polythene bags, parameters assessed include; plant height, stem height, and leaf number. Germination was recorded weekly. Germination percentage was calculated using Maguire [13] equation:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Number of seeds sown}} \times 100$$

Mean germination time was calculated using $MGT = \sum T_i N_i / \sum N_i$, where N_i is the number of newly germinated seeds at time T_i [14,15]. Energy of germination was calculated as the percentage of germinating seeds a week (7 days) after sowing relative to the number of seeds tested. Mean germination rate (MGR) was calculated as thus; $CV/100=1/T$ where T is mean germination time and CV is coefficient of germination velocity. Germination index was calculated using the following formula:

$$\text{Germination index (GI)} = \sum (Gt/Tt)$$

Gt is the number of seeds germinated on day t and Tt is the number of days. Split plot design was used for acid treatment and hot water treatment, while Randomized Complete Block Design (RCBD) was used for mechanical scarification.

Experiment 1: Acid pre-treatment, using H_2SO_4 . A split plot design was used with varying concentration of acid as follows; 20%, 50% and 98% (main plot treatment). The seeds was submerged in H_2SO_4 for a period of 2 minutes, 5 minutes and 10 minutes (sub-plot treatment) for each concentration of acid, each with three replicates. The aim of this experiment is to observe the effect of concentration of H_2SO_4 and treatment time as it affects the growth of *Acacia auriculiformis*.

Experiment 2: Mechanical scarification using sand-paper (seeds were rubbed against sand-paper at different sides). There were seven (7) treatments; scarification at the micropyle, circumference, distal-end, micropyle and distal-end, micropyle and circumference, distal-end and circumference and combination of

micropyle +distal-end and circumference. The treatments were replicated five (5) times. Randomized Complete Block Design was used. The aim is to determine the best scarification position/point which brings about the best growth rate in *Acacia auriculiformis*.

Experiment 3: hot water at temperature of $100^\circ C$. Split plot design was used with varying volume of water as follows; 50 cl, 1 litre (main plot treatment), soaked in hot water for a period of 2 minutes, 4 minutes, 6 minutes and 10 minutes (sub-plot treatment), each replicated three (3) times. The aim is to determine the effect of hot water volume and treatment time on the growth rate of *Acacia auriculiformis*.

2.3 Data Collection

The experiment lasted for a period of 12 weeks. Morphological parameters were taken on a weekly basis. Morphological parameters taken include; Plant height, stem height, leaf number. Plant height and stem height was assessed using calibrated ruler.

2.4 Data Analysis

Data collected were subjected to statistical Analysis of Variances on the general linear model of SAS Software. Means that were significantly different were separated using Least Significant Difference (LSD).

3. RESULTS AND DISCUSSION

3.1 Effect of Treatments on Growth Parameters

The effect of acid concentration on plant height, stem height, and leaf number was significant ($p < 0.05$). Seeds treated in 98% concentration of sulphuric acid resulted in the highest mean value for plant height (6.72 cm), stem height (3.50 cm), and leaf number (4.56) Table 1.

Effect of acid timing does not significantly ($p < 0.05$) affect the morphological parameters taken, however, the highest mean value for plant height (5.48 cm), stem height (2.80 cm), leaf number (3.58) was observed in seeds treated for 10 minutes, while the least mean values were recorded in seeds treated for 2 minutes (Table 2).

The interaction shows no significant ($p < 0.05$) difference, however, the highest mean value for plant height was recorded in seeds treated with 98% of sulphuric acid for 10 minutes (8.57cm). Seeds treated for 5 minutes in 98% concentration resulted in the highest mean value for stem height (4.47 cm) and leaf number (6.47) among the acid treated seeds (Table 3).

Mechanical scarification does not significantly ($p < 0.05$) affect plant height and leaf number, however, result shows that it significantly ($p < 0.05$) affect stem height. The highest mean values recorded for plant height (8.98 cm), stem

height (5.14 cm), leaf number (5.79) in the mechanically scarified seeds were observed in seeds scarified at the micropyle, while the least mean values were recorded in those collectively scarified at the micropyle circumference and distal-end (Table 4).

Water volume had no significant effect on the parameters taken, however, the highest mean value for plant height (3.37 cm) and stem height (1.70 cm) was recorded in seeds treated in 50 cl volume of hot water, while 1 litre volume of hot water resulted in the highest mean value for leaf number (2.43) Table 5.

Table 1. Effect of acid concentration on the growth parameters

Concentration	Concentration	Stem height (cm)	Leaf number
98%	6.72 ^a	3.50 ^a	4.56 ^a
50%	1.80 ^b	1.02 ^b	1.15 ^b
20%	2.64 ^b	1.53 ^b	1.62 ^b

Mean values with the same superscript in each column are not significantly different ($p > 0.05$). LSD

Table 2. Effect of acid timing on the growth parameters

Treatment time	Plant height (cm)	Stem height (cm)	Leaf number
10 minutes	5.48 ^a	2.79 ^a	3.58 ^a
5 minutes	3.37 ^a	1.79 ^a	2.65 ^a
2 minutes	2.31 ^a	1.46 ^a	1.10 ^a

Mean values with the same superscript in each column are not significantly different ($p > 0.05$). LSD

Table 3. Interaction between acid concentration and treatment time

Interaction	Plant height (cm)	Stem height (cm)	Leaf number
20% 2 minutes	1.31 ^a	1.11 ^a	0.00 ^a
20% 5 minutes	1.80 ^a	0.98 ^a	1.50 ^a
20% 10 minutes	4.71 ^a	2.52 ^a	3.36 ^a
50% 2 minutes	2.28 ^a	1.61 ^a	1.33 ^a
50% 5 minutes	0.00 ^a	0.00 ^a	0.00 ^a
50% 10 minutes	3.08 ^a	1.55 ^a	2.13 ^a
98% 2 minutes	3.21 ^a	1.72 ^a	1.97 ^a
98% 5 minutes	8.16 ^a	4.47 ^a	6.47 ^a
98% 10 minutes	8.57 ^a	4.36 ^a	5.25 ^a

Mean values with the same superscript in each column are not significantly different ($p > 0.05$). LSD

Table 4. Effect of mechanical scarification on growth parameters

Scarification point	Plant height (cm)	Stem height (cm)	Leaf number
M	8.98 ^a	5.13 ^a	5.79 ^a
C	5.17 ^a	2.89 ^{abc}	3.20 ^a
D	6.15 ^a	3.78 ^{ab}	3.50 ^a
MD	6.21 ^a	3.80 ^{ab}	3.58 ^a
MC	5.16 ^a	2.87 ^{abc}	2.81 ^a
DC	3.21 ^a	1.83 ^{bc}	1.85 ^a
MDC	1.66 ^a	3.72 ^c	1.21 ^a

Mean values with the same superscript in each column are not significantly different ($p > 0.05$). LSD

M: Micropyle, C: Circumference, D: Distal-end, MD: Micropyle and Distal-end, MC: Micropyle and Circumference, DC: Distal-end and Circumference, MDC: Micropyle + Distal-end and Circumference

Table 5. Effect of hot water volume on growth parameters

Volume	Plant height (cm)	Stem height (cm)	Leaf number
50 cl	3.36 ^a	1.70 ^a	2.29 ^a
1 litre	2.93 ^a	1.66 ^a	2.43 ^a

Mean values with the same superscript in each column are not significantly different ($p>0.05$). LSD

Table 6. Effect of hot water timing on growth parameters

Treatment time	Plant height (cm)	Stem height (cm)	Leaf number
2 minutes	2.01 ^a	1.15 ^a	1.27 ^a
4 minutes	4.93 ^a	2.58 ^a	3.54 ^a
6 minutes	2.33 ^a	1.08 ^a	1.63 ^a
10 minutes	3.32 ^a	1.91 ^a	2.98 ^a

Mean values with the same superscript in each column are not significantly different ($p>0.05$). LSD

Table 7. Interaction between volume of hot water and treatment time

Interaction	Plant height (cm)	Stem height (cm)	Leaf number
50 cl 2 minutes	1.74 ^a	0.91 ^a	0.97 ^a
50 cl 4 minutes	6.64 ^a	3.19 ^a	4.80 ^a
50 cl 6 minutes	2.56 ^a	1.17 ^a	1.63 ^a
50 cl 10 minutes	2.40 ^a	1.40 ^a	1.75 ^a
1 Litre 2 minutes	2.14 ^a	1.30 ^a	1.58 ^a
1 Litre 4 minutes	3.13 ^a	1.92 ^a	2.27 ^a
1 Litre 6 minutes	2.08 ^a	0.99 ^a	1.63 ^a
1 Litre 10 minutes	4.26 ^a	2.43 ^a	4.22 ^a

Mean values with the same superscript in each column are not significantly different ($p>0.05$). LSD

Treatment time does not significantly ($p<0.05$) affect the growth parameters taken, however, the highest mean value recorded for plant height (4.94 cm), stem height (2.59 cm), and leaf number (3.54) was observed in seeds treated for 4 minutes (Table 6).

The interaction between hot water volume and treatment time was not significant ($p<0.05$), however, seeds treated in 50 cl volume of hot water for 4 minutes yielded the highest mean value for plant height (6.64 cm), stem height (3.19 cm), and leaf number (4.80) among the hot water treated seeds (Table 7).

Effect of treatments on seed germination (Table 8) shows that maximum germination was recorded in seeds treated with; 98% concentrated sulphuric acid, mechanical scarification at the micropyle, circumference, distal-end, micropyle +distal-end, as well as 4 minutes timing with 50 cl of hot water, however, their energy of germination and mean germination time differs. Result showed that 100% germination could be achieved within seven (7) days when seeds are mechanically scarified at the micropyle, circumference, distal end and micropyle+distal-end, provided the seeds are viable.

Concentration of acid had significant ($p<0.05$) effect on plant height, stem height and leaf number of *Acacia auriculiformis*. The seeds that were treated with concentrated sulphuric acid (98%) performed better compared to 20% and 50% concentration. Mechanical scarification significantly ($p<0.05$) affect stem height. The seeds that were collectively scarified at the micropyle+distal-end+circumference resulted in the least mean total height, stem height and leaf number. This could be that the scarification resulted in greater imbibition of moisture than its requirement, while the scarified sides serve as entry points for harmful micro-organisms such as fungi (e.g *Rhizoctonia spp*, *Fusarium spp*) which deteriorate the seed vigour before it could germinate.

It was observed that treating *Acacia auriculiformis* seeds in 1litre and 50cl volume of hot water for period of 2minutes, 4minutes, 6minutes and 10minutes does not significantly affect the treated seeds, this agrees with the report of Osman et al. [16] that treating *Lupinus varius* seeds in hot water does not completely overcome seed coat impermeability in any durations of boiling time (4, 6, 8 and 10 minutes). While [17] Baskin et al reported that boiling seeds of *Senna marilandica* and *Senna*

Table 8. Effect of treatments on seed germination of *Acacia auriculiformis*

Treatments	Germination (%)	Energy of germination (%)	Mean germination time (days)	Mean germination rate	Germination index (days)
Acid (H₂SO₄)					
20%(2 minutes)	67	0	21.00	0.048	0.11
20%(5 minutes)	33	0	28.00	0.036	0.04
20%(10 minutes)	67	0	14.00	0.071	0.14
50%(2 minutes)	33	33	7.00	0.143	0.14
50%(5 minutes)	0	0	0.00	0.000	0.00
50%(10 minutes)	33	33	7.00	0.143	0.14
98%(2 minutes)	100	67	9.33	0.107	0.36
98%(5 minutes)	100	67	9.33	0.107	0.36
98%(10 minutes)	100	67	9.33	0.107	0.36
Mechanical scarification					
M	100	100	7.00	0.143	0.71
C	100	100	7.00	0.143	0.71
D	100	100	7.00	0.143	0.71
MD	100	100	7.00	0.143	0.71
MC	80	60	8.75	0.114	0.50
DC	60	20	11.67	0.086	0.29
MDC	20	20	7.00	0.143	0.14
Hot water treatment					
50 cl(2 minutes)	33	0	28.00	0.036	0.04
50 cl(4 minutes)	100	0	28.00	0.036	0.13
50 cl(6 minutes)	33	0	21.00	0.048	0.05
50 cl(10 minutes)	33	0	21.00	0.048	0.05
1 litre(2 minutes)	67	0	28.00	0.036	0.08
1 litre(4 minutes)	67	0	21.00	0.048	0.11
1 litre(6 minutes)	33	0	28.00	0.036	0.04
1 litre(10 minutes)	67	0	21.00	0.048	0.10

obtusifolia for duration between 20seconds- 240 seconds was detrimental, it can be deduced that treating seeds of *Acacia auriculiformis* in hot water for duration between 2-10 minutes is not favourable and higher treatment time might be required. Seed coat permeability differs from species to species [18], it can be deduced that the seed coat of *Acacia auriculiformis* differs from that of *Senna marilandica* and *Senna obtusifolia*.

Among acid treated seeds, 50% 2 minutes and 50% 10 minutes resulted in shortest Mean Germination Time, however, Percentage Germination and Energy of Germination is lower compared to 98% concentration with higher Percentage Germination and Energy of Germination, with a slight longer Mean germination time. Among the mechanically scarified seeds, result showed that Energy of Germination, Mean Germination Time and Germination Index for seeds scarified at the micropyle, circumference, distal-end, and micropyle+distal-end were more favourable than most other treatments, which implies that higher germination within shorter period of time can be achieved when scarification is done in the above mentioned points. Yisau et al. [19] reported that the highest germination for mechanically scarified seeds of *Albizia zygia* was recorded on seeds scarified at the circumference, seeds of *Acacia auriculiformis* that were scarified at the micropyle, circumference, distal-end, and micropyle+distal-end resulted in the highest germination within shortest possible days. This agree with the report of Okunlola et al. [20], Amoakoh et al., [21], and Osman et al. (2004) that, faster germination of mechanically scarified seeds was noticed in *Parkia biglobosa*, *Pouteria campachiana*, and *Lupinus varius* as compared with other treatments employed. This suggests that dormancy resulting from impermeable seed coat (physical dormancy) may be overcome by peeling-off the seed coat [22]. Result showed that while hot water treatment brings about germination, the Energy of Germination appears to be the lowest among all methods employed, and Mean Germination Time was longer. This suggests that hot water treatment is not favourable for breaking dormancy of *Acacia auriculiformis* seeds within the shortest possible time. This agree with the report of Osman et al. (2004), that values obtained for Energy of germination and Germination Index indicate that boiling treatment was not adequate for rapid and uniform germination of *Lupinus varius*.

4. CONCLUSION

This study conclude that the method which result in the best morphological parameters of *Acacia auriculiformis* is acid scarification using sulphuric acid at concentration of 98%, followed by mechanical scarification (especially when scarified at the micropyle), and that 100% germination can be achieved within 7 days when seeds are treated mechanically at the micropyle, or circumference, or distal-end, or combination of micropyle+distal-end, provided the seeds are viable. Hot water treatment is not effective for breaking dormancy of *Acacia auriculiformis*.

This research recommends the use of sulphuric acid at 98% concentration to promote morphological parameters of emerging *Acacia auriculiformis* seedlings. However, where concern is raised about the effect of sulphuric acid on the environment, this research suggests that:

- Mechanical scarification at the micropyle serves as an alternative means for sulphuric acid treatment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Appendix 1. Analysis of variance (ANOVA) table for acid treatment

Independent variable	Source	df	Type III SS	MS	F	P
Plant height	Main Effects					
	Concentration	2	124.6208755	62.310438	5.788567	.0115 *
	Time	2	46.80804128	23.404021	2.1742062	.1426 ns
	Interaction					
	Concentration * Time	4	44.07996353	11.019991	1.0237443	.4217 ns
	Error	18	193.7591609	10.764398<-		
	Total	26	409.2680412			
Stem height	Main Effects					
	Concentration	2	30.75630635	15.378153	4.8579993	.0206 *
	Time	2	8.698305684	4.3491528	1.3739089	.2784 ns
	Interaction					
	Concentration * Time	4	14.71750948	3.6793774	1.162325	.3601 ns
	Error	18	56.97957993	3.1655322<-		
	Total	26	111.1517014			
Leaf number	Main Effects					
	Concentration	2	61.48353737	30.741769	5.8295857	.0112 *
	Time	2	28.30452868	14.152264	2.683705	.0955 ns
	Interaction					
	Concentration * Time	4	28.19547554	7.0488689	1.3366825	.2946 ns
	Error	18	94.92129709	5.2734054<-		
	Total	26	212.9048387			

Appendix 2. Analysis of variance (ANOVA) table for mechanical scarification

Independent variable	Source	df	Type III SS	MS	F	P
Plant height	Main Effects					
	Treatments	6	149.1634573	24.860576	1.937254	.1095 ns
	Error	28	359.3210477	12.832895<-		
	Total	34	508.484505			
Stem height	Main Effects					
	Treatments	6	57.29560686	9.5492678	2.6925789	.0343 *
	Error	28	99.30238157	3.5465136<-		
	Total	34	156.5979884			
Leaf number	Main Effects					
	Treatments	6	56.93846056	9.4897434	1.8020339	.1350 ns
	Error	28	147.4516168	5.2661292<-		
	Total	34	204.3900773			

Appendix 3. Analysis of variance (ANOVA) table for hot water treatment

Independent variable	Source	df	Type III SS	MS	F	P
Plant height	Main Effects					
	Treatments	1	1.087293993	1.087294	0.0899168	.7681 ns
	Time	3	31.12190705	10.373969	0.8579043	.4829 ns
	Interaction					
	Treatments * Time	3	22.8603093	7.6201031	0.6301657	.6061 ns
	Error	16	193.4755477	12.092222<-		
	Total	23	248.545058			
Stem height	Main Effects					
	Treatments	1	0.007824026	0.007824	0.0022468	.9628 ns
	Time	3	9.049132253	3.0163774	0.8661895	.4789 ns
	Interaction					
	Treatments * Time	3	4.312060059	1.4373534	0.4127535	.7461 ns
	Error	16	55.71764282	3.4823527<-		
	Total	23	69.08665916			
Leaf number	Main Effects					
	Treatments	1	0.115740648	0.1157406	0.0157586	.9017 ns
	Time	3	20.87731466	6.9591049	0.9475107	.4411 ns
	Interaction					
	Treatments * Time	3	19.19675666	6.3989189	0.8712391	.4764 ns
	Error	16	117.5138946	7.3446184<-		
	Total	23	157.70			

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