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EFFECT OF PAWPAW LATEX, PLANTAIN STEM JUICE AND SULPHURIC ACID ON SEED GERMINATION OF *Albizia lebbeck* (L.) BENTH

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ABSTRACT

Albizia lebbeck is an important economic tree known for its medicinal, industrial and environmental benefits but exhibits dormancy problems. The study aimed at determining the effect of pawpaw latex, plantain stem juice and sulphuric acid on seed germination of A. lebbeck to improve seedling regeneration for afforestation programmes. Seeds of A. lebbeck were harvested from mother trees within the University of Ibadan premises and pawpaw latex from the unripe fruits of Carica papaya subjected to various pretreatment methods. The six pretreatment methods used were: T1-50% pawpaw latex (24hours), T2-100% pawpaw latex (24hours), T3-50% plantain stem juice (24hours), T4-100% plantain stem juice (24hours), T5-50% sulphuric acid (10minutes), T6-98% sulphuric acid (10minutes) and control (without pretreatment); with four replicates. Germination variables examined include Germination percentage (GP), Mean Germination Time (MGT), Complete Dormancy Period (CDP), Peak Period of Germination (PPG) and Peak Germination percentage (PGP). Pretreatments with 98% concentration of sulphuric acid recorded the highest GP (76%), early complete dormancy period (CDP) (3days after planting) and PPG (5 days). Seeds pretreated with pawpaw latex at 50% had GP (55%), CDP (7days) and PPG (9 days). Seeds sown without pretreatments (control) recorded 33% GP, CDP (9 days), PPG (13 days) MGT (21.96) and PGP (25%). Breaking of dormancy of A. lebbeck seeds was effective using sulphuric acid at 98% concentration. Results from plant juice were effective and could serve as promising alternatives that can support large scale plantation establishment for plant breeders at all levels.

Keywords: *Albizia lebbeck*, Pretreatment, Seed dormancy, Pawpaw latex, Plantain stem juice, Seed Germination, Sulphuric acid.

INTRODUCTION

Albizia lebbeck (L.) Benth commonly known as lebbeck, siris or woman's tongue tree is inherent to the African tropical regions, Northern Australia and Asia. It is widely spread within sub-humid, semi-arid tropics and subtropical areas with a distinct dry season and a dependable rainy season (Cook *et al*., 2005; Lowry *et al*., 1992). *Albizia lebbeck* belongs to the family of Fabacae and is amongst the most potential and useful fodder trees. Lebbeck has a great ability to protect the soil through its rooting system that holds the soil together and reduces the effect of running water and prevailing winds. It has high nitrogen-fixing ability, provides shade, quality wood and it is used extensively for medicinal purposes. *Albizia lebbeck* plays an important role in recycling soil nutrients (Orwa *et al.,* 2009) in savannah and tropical areas of Nigeria. It is a general-purpose tree species remarkable for conservation to improve its impact on the livelihood and well-being of communities.

Despite these advantages, it still exhibits dormancy problems. Baskin and Baskin (2004) observed that seed coat impermeable to water is a very common type of dormancy in seeds of the Fabaceae family. Seed dormancy is the inability of viable seeds to initiate germination under favourable conditions for seeds to germinate. Germination of lebbeck seed is irregular which makes propagation by seed (sexual) difficult. In artificial regeneration, different forms of seed pretreatment are necessary where dormancy is strong to speed up germination and to achieve uniform germination of seeds.

The use of organic compounds to enhance germination is still a growing aspect of research; its use for pretreatments of seeds of different tree species is been neglected and/or not yet known to so many tree breeders. According to Baskin and Baskin (2001), organic compounds in the soil produced from different sources such as soil organisms, seeds, plant roots and the remains of dead plants have the potentials to stimulate or hinder germination. Studies have shown the use of organic extracts to enhance germination and to break dormancy in different species. The use of bio-extracts containing valuable elements (micro and macro elements) despite the active substances such as volatile compounds, improves the growth of plants through adequate supply of plant nutrients and assist in sustaining soil productivity and environmental health instead of synthetic chemicals (Rady and Seif El-Yazal, 2014).

Carica papaya Linn., commonly known as pawpaw, *ọkwụrụ órù* in Igbo, *ibepe* in Yoruba and *gunda* in Hausa language is a fruit and a vegetable. It is a monoecious, dioecious or hermaphrodite tree and most common species of the family of Caricaceae (Purseglove, 1968; Janick, 1988 and Macalood *et al.*, 2013). Pawpaw latex is a thixotropic fluid that is composed of insoluble particulate fraction whose composition is practically unknown, soluble fraction (carbohydrates (~10%), salts (~10%), lipids (~5%)), and representative biomolecules (cysteine proteinases, glutathione) (~30%), water and many other proteins and enzymes (Macalood *et al.,* 2013). It has a complex mixture of constituents such as proteins, vitamins, carbohydrates, free amino acids, lipids, alkaloids and terpenes (Liggieri *et al.,* 2009). Pawpaw plants are rich in papain and chymopapain which are the major enzymes. Papain is cysteine protease often called papaya proteinase I, composed of 212 amino acid residue chains (Kamphuis, *et al.,* 1984) capable of breaking organic molecules (Amri and Mamboya, 2012; Patel, *et al.,* 2012).
Proteases are enzymes that catalyse the degradation of peptides, proteins; play active role in several physiological processes in the living organisms. They are direct specific and selective modifications of proteins; these include the activation of proenzymes, sanguineous coagulation, germination, senescence, defense against plant pathogens, acquisition of nutrients and apoptosis, secretory protein processing and transport through membranes (Boller, 1986; Baker and Drenth, 1987; Campbell and Szardenings, 2003; Grudkowska and Zagdañska, 2004). Papain is present in all parts of the plant apart from the root (Anonymous, 1992). However, highest papain yield is found in the fruit (Amri and Mamboya, 2012). They are present in both ripe and unripe fruits of pawpaw but are non-active in ripe pawpaw (Nwofia *et al.,* 2012 and Macalood *et al.,* 2013).

Plantain (*Musa paradisiaca* Linn.) is among the staple foods common in Africa. It is a tall plant with a tapering trunk often referred to as pseudostem or false stem, formed by leaf sheaths of the leaves arranged side-by-side (Swennen, and Oritz, 1997; Oladiji *et al*., 2010; Ibisi and Asoluka, 2018). Pseudostem is the conical structure that emerges from the corm and bears the plants' leaves. It is high in fibre and potassium necessary for regulating blood pressure, relaxation of muscles, used in the control of colon cancer and can reduce cholesterol (Ng and Fong, 2000). Studies have shown that bioactive phytochemicals with low-molecular-weight are present in plantain, these include steroids, saponins, flavonoids, alkaloids, tannins, phenolics, terpenoids, glycosides, and phlobatannins (Ramu *et al.,* 2014). The plant has the ability to prevent the activities of enzymes such as α-amylase and α-glucosidase which digests carbohydrate. The phytochemicals have the potential to influence glucose adsorption (Jaber *et al.* 2013) as well as other antioxidant activities (Ayoola *et al.* 2017). However, concentrations of secondary metabolites and bioactivities of acquired extracts depend greatly on environmental factors and species.

Plantain stem juice is the transparent solution contained in the pseudostem of the plant which turns pale brown after prolonged exposure to air. This fluid forms the greater weight of the tree trunk. The chemical composition of pseudostem was determined through the elemental analysis and were found to contain lignin (15 - 16%), cellulose (31-35%), and hemicellulose (14 - 17%) (Bilba *et al.*, 2007).

Previous studies on the influence of different pretreatment techniques in breaking the dormancy of seeds of some forest species proved mechanical scarification as an efficient means of breaking dormancy especially in legumes (Pacheco and Matos, 2009; Azad *et al.*, 2011; Santos *et al.*, 2013; Olatunji *et al.*, 2013). The breaking of seed dormancy of *A. lebbeck* has been found successful with the use of acid scarification (Ajiboye *et al.*, 2009), mechanical scarification (Coelho *et al.*, 2014) and the use of hot water (Missanjo *et al.*, 2013) but all these methods have their limitations.

Thus, there is a need for a more eco-friendly and affordable pretreatment technique that is easily accessible to farmers at all levels for effective germination and regeneration. Though Eyog-Matig *et al.* (2007) successfully used organic compounds in breaking the seed dormancy of *Garcinia kola*, however, this method has not been used in breaking dormancy of *Albizia lebbeck* seeds. Therefore, the study was carried out to evaluate the influence of plant juice in comparison with sulphuric acid on seed germination of *Albizia lebbeck.*

MATERIALS AND METHODS

Study Area

The research was conducted at the Nursery Section of the Department of Forest Production and Products, Faculty of Renewable Natural Resources, University of Ibadan, Nigeria. The University is located within latitude 7˚26ˈ58.0ˈˈ to 7˚26ˈ58.20ˈˈ N and longitude 3˚35ˈ48.56ˈˈ to 3˚53ˈ48.48ˈˈ E; 227m high above sea level (Akinyele, 2010).

Seeds and Material Collection

Matured seeds of *Albizia lebbeck* were harvested from mother trees within the University of Ibadan premises. The seeds were manually extracted from the pod. Pawpaw latex was collected from young unripe fruits of *Carica papaya* plants while plantain stem juice was gotten from the pseudostems of plantain plant. These exercises were carried out within 6:00 a.m. and 7:00 a.m. A sterilized stainless steel knife was used to make incisions on both plant parts used for the experiment. Four to six incisions of 3mm deep longitudinally from the end of the fruit stalk through the tip of the unripe pawpaw fruit. The process was repeated on other parts of the fruits as the latex was channeled to run down through the collecting devices (air-tight containers). The incised plantain stem was severed and the extracts were collected with separate air-tight containers.

Experimental procedure for seed testing and pretreatment

Seed viability was tested using the floating test. The seeds were immersed in water and those that floated were considered to be damaged or bad for germination and as such discarded; while those that settled at the base were considered viable and used for the experiment. A total of 840 viable seeds were obtained and subjected to pretreatments levels as stated below;

- ✓ Soaking in pawpaw latex at 50% concentration for 24 hours (T1)
- ✓ Soaking in pawpaw latex at 100% concentration for 24 hours (T2)
- ✓ Soaking in plantain stem juice at 50% concentration for 24 hours (T3)
- ✓ Soaking in plantain stem juice at 100% concentration for 24 hours (T4)
- ✓ Soaking in H_2SO_4 at 50% concentration for 10 minutes (T5)
- ✓ Soaking in H_2SO_4 at 98% concentration for 10 minutes (T6)
- ✓ Control (no pre-treatment)

The experiment was set in a Completely Randomized Design (CRD) with six treatments (T1, T2, T3, T4, T5, T6, and control) and four replicates of 30 seeds each. The pretreated seeds were sown in germination pots filled with topsoil (Figure 1). This was arranged and kept under the weaning shed to reduce the rate of evapotranspiration. Watering was done daily and germination monitored till no germination was observed.

Figure 1: Experimental layout of the study with emerging *Albizia lebbeck* seedlings.

The variable~~s~~ evaluations were carried out using the formula by ISTA, (1997) and formula by Nadjafi *et al*, (2006). Data were also collected ~~on~~ at peak period of germination (PPG) (Time of highest number of germination), complete dormancy period (CDP) (count of days from seed sowing to the commencement of germination), and peak germination percentage (PGP)

$$GP = \frac{Total\ Seed\ germinated}{Total\ number\ of\ seed\ sown} \times \frac{100}{1} \text{ …..… [1]}$$

$$MTG = \sum \frac{ni \times di}{N} \text{ …………………………… [2]}$$

$$PGP = TSGPP/TNSGPT \text{ .... [3]}$$

Where:
GP = Germination percentage
MTG = Mean germination time
ni = Amount of germinated seeds at days di;
N = sum of germinated seeds in each treatment.
TSGPP =Total Seed Germination at Peak Period
TNSGPT = Total number of Seed germinated per treatment

Data Analysis
The data were subjected to descriptive statistics and Analysis of Variance (ANOVA), and significant means separation was done using Fisher's Least Significant Difference (LSD).

RESULTS
Table 1 illustrates the mean effect of pretreatments on the frequency of germination of *Albizia lebbeck* seeds. The result showed no significant difference at $p<0.05$. The highest mean frequency (3.25±1.18) of germination was observed in T6, followed by 2.68±0.86 for T5 and 2.36±0.70 for T1, while the least was 1.39±0.43 was recorded by T4.

Figure 2 displays the germination patterns and distribution of the various pretreatments. Pretreated seeds germinated earlier than the seeds with no pretreatments (control). The maximum rate of germination occurred in seeds pretreated with H_2SO_4 at 98% concentration, followed by H_2SO_4 at 50% concentration and pawpaw latex at 50% while plantain stem juice at 100% and control had the least.

Table 1: Mean effect of the pretreatments on the rate of germination of *Albizia lebbeck* seeds

Pretreatments	Mean
T1	$2.36^{a} \pm 0.70$
T2	$1.71^{a} \pm 0.76$
T3	$1.82^{a} \pm 0.56$
T4	$1.39^{a} \pm 0.43$
T5	$2.68^{a} \pm 0.86$
T6	$3.25^{a} \pm 1.18$
Control	$1.43^{a} \pm 0.43$

Values were expressed as Mean ± Standard Error. Mean values with dissimilar superscript within the same column are significantly different (P<0.05)

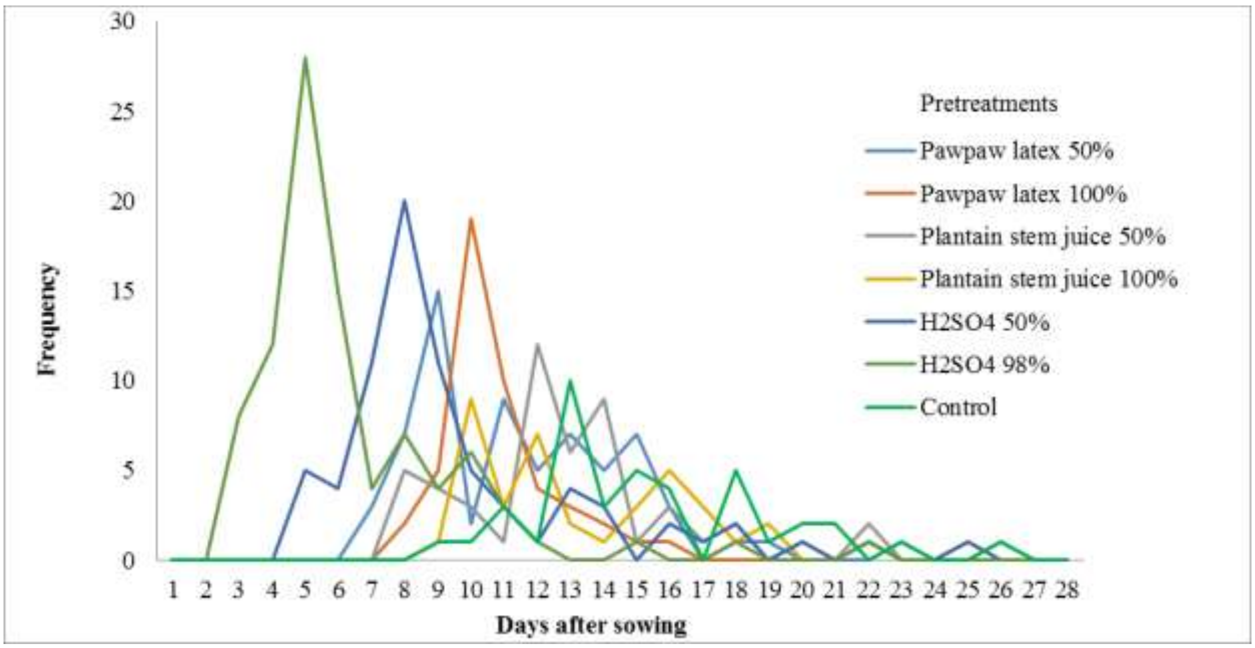

Figure 2: Germination patterns of different pretreatments

Table 2 shows the percentage germination of the six pretreatment methods used in this study. The highest observation (75.5%) was recorded for H_2SO_4 at 98% concentrations, followed by seeds treated with H_2SO_4 at 50% concentrations (62.5%) and soaking in pawpaw latex at 50% concentration (55%). Seeds soaked in plantain stem juice at 100% concentration and the seeds with no treatment (control) recorded 32.5% and 33% germination respectively.

The least complete dormancy periods were observed in seeds pretreated with H_2SO_4 (98%) at 3 days after sowing (DAS), followed by H_2SO_4 (50%) 5 DAS, 7 DAS was recorded at pawpaw latex (50%). Pawpaw latex (100%) and plantain stem juice (50%) sprouted at 8 DAS while the highest 9 DAS were observed for plantain stem juice (100%) and control. Germination capacity reduced after the second week and rate of germination drops (Table 2).

The peak period of germination (PPG) was obtained in 2 weeks for all the pretreatment methods except for soaking in H_2SO_4 at 98% concentration which occurred in the first week. The PPG was recorded at 9, 10, 12, 10, 8, 5 and 13 for pawpaw latex at 50%, pawpaw latex at 100%, plantain stem juice at 50%, plantain stem juice at 100%, H2SO4 at 50%, H2SO4 at 98% and control respectively (Table 2).

Peak germination percentage values were 22.73, 39.58, 23.53, 23.08, 26.67, 30.77 and 25.00% for pawpaw latex at 50%, pawpaw latex at 100%, plantain stem juice at 50%, plantain stem juice at 100%, H2SO4 at 50%, H2SO4 at 98% and control respectively (Table 2). The results showed that H2SO4 at 98% with the most regular and abundant germination range had a lower peak germination percentage (PGP) whereas pawpaw latex at 100% with intermittent spread had the highest value.

Figure 3 shows the mean germination time for the various treatments. Pawpaw latex recorded the highest mean time of germination, followed by H_2SO_4 and plantain stem juice while control has the least. However, T1 showed a mean of 27.32, T3 (23.96), T5 (25.93), while T6 and T4 have means of 20.79 and 19.11 respectively, with the lowest mean 18.89 recorded at T2.

Table 2: Effect of the different pretreatments on the germination parameters of *Albizia lebbeck*

Treatments	GP (%)	CDP (days)	PPG (days)	PGP (%)
Pawpaw latex at 50%	55	7	9	22.73
Pawpaw latex at 100%	40	8	10	39.58
Plantain stem juice at 50%	42.5	8	12	23.53
Plantain stem juice at 100%	32.5	9	10	23.08
H_2SO_4 at 50%	62.5	5	8	26.67
H_2SO_4 at 98%	75.5	3	5	30.77
Control	33	9	13	25.00

Where; GP = Germination Percentage, CDP = Complete Dormancy Period, PPG = Peak Period of Germination, PGP = Peak Germination Percentage

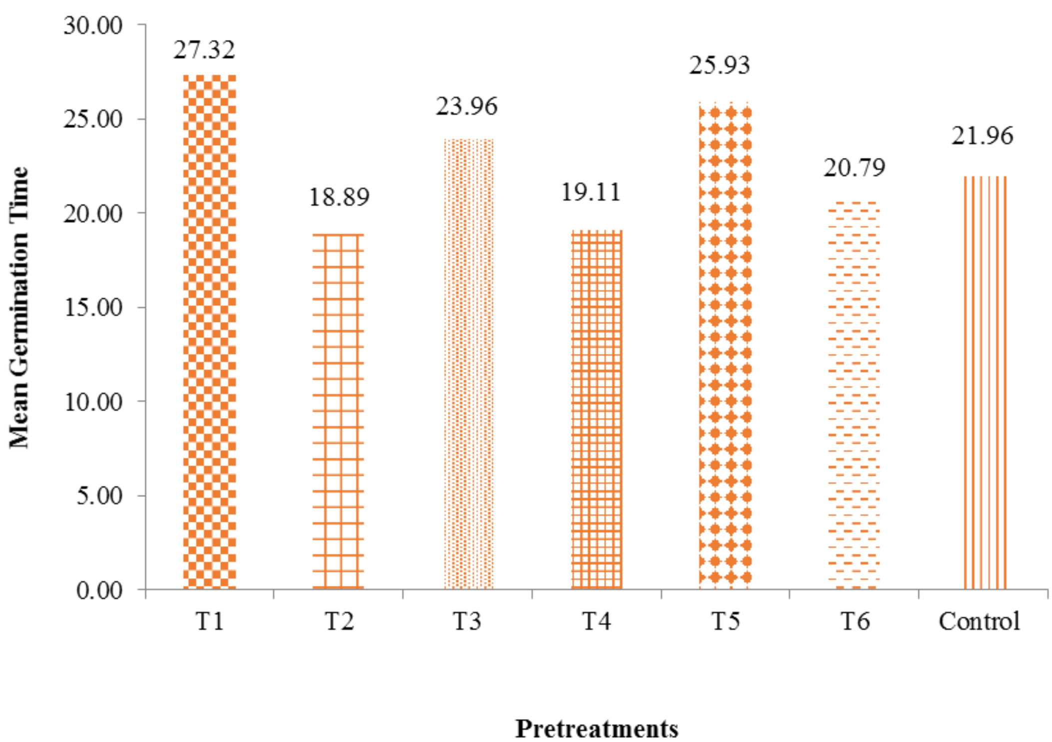

Figure 3: Mean Germination Time for the various treatment

DISCUSSION

The differences observed in the germination percentage of seeds implies there was an impact of the various pretreatments on breaking the seed dormancy, although some appeared negligible (soaking in plantain stem juice at 100% concentration).

The acid scarification of *A. lebbeck* seeds with H_2SO_4 at various concentrations improved seed germination. The maximum germination percentage

(76%) observed in seeds pretreatment with H_2SO_4 at 98% for 10 minutes may be due to the disintegration effects the acid had on seed coat of *Albizia lebbeck* and its ability to stimulate the biochemical and physiological activities necessary for germination. The use of H_2SO_4 has been found to cause a disorder in the seed coat and uncover the lumens of the macrosclereids cells, which allows water to penetrate and initiates seed germination (Nikoleave, 1977). Bhattacharya and Saha (1990) asserted that acid scarification disintegrated the hard seed coat material in *Cassia fistula* seeds.

The best result for pretreatment with organic compounds was observed in soaking in pawpaw latex at 50% for 24 hours. This may be due to the actions of the unknown insoluble particulates in conjunction with the soluble particulates which Macalood *et al.* (2013) stated to contain carbohydrates, lipids, salts, and biomolecules which include glutathione, cysteine proteinases, among other proteins. Proteases according to Macalood *et al.* (2013) played a major part in several physiological processes in the body of living organisms. However, it was confirmed that proteases which are modifications of proteins in direct, selective and specific forms, helps in the activation of proenzymes, sanguineous coagulation, germination, senescence, defense against pathogens in plant, absorption of apoptosis and nutrients, secretion of protein, its processing and transportation through cell membranes (Boller, 1986; Baker and Drenth, 1987; Campbell and Szardenings, 2003; Grudkowska and Zagdañska, 2004). Papain is capable of breaking down organic molecules (Amri and Mamboya, 2012) and it showed greater enzymatic activity when solubilized in water (Maiti *et al.*, 2008). This may be the reason why pretreatment with pawpaw latex at 50% for 24 hours yielded a very good result. Seeds pretreated with plant juice had a more uniform and early germination than the control. This is in line with Eyog-Matig *et al.* (2007) who stated that seeds of *Garcinia kola* pretreated with pineapple juice and those soaked within banana false trunk showed a synchronous and early germination.

The germination rate of *A. lebbeck* was moderate in the control (with no pretreatment). This may be due to the source of seeds (directly from mother tree), time of seed collections and planting. Studies on germination according to Baskin and Baskin (2001) stated that planting should begin within a short period after seeds collection (within 7-10 days) to avoid changes in germination responses during dry storage at room temperature.

CONCLUSION

The result of this study on the effect of various pretreatments on the germination of the seeds of *A. lebbeck* showed that H_2SO_4 had the highest germination. Thus, the use of sulphuric acid in breaking the dormancy of *Albizia lebbeck* is highly effective. However, sulphuric acid is dangerous to users and the environment, difficult to handle and costly. Plant juice, a cheap and easily accessible method used in this study produced a good result, with relatively high percentage germination, early and uniform germination compared to the untreated seeds. Hence, ~~in order~~ to make its plantation establishment more economical, there is need for adopting organic sources for breaking seed dormancy of lebbeck seeds. High-quality seeds are also needed for proper germination of *A. lebbeck.*

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