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Effects of Different Pre- sowing Treatments on Seed Germination of African Grape (*Lannea microcarpa* L.)

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Abstract

Over-exploitation coupled with adverse effect of increased human activities and lack of standard plantation endangers the germination of Lannea microcarpa seeds. An experiment was carried out to check the effect of different pre-sowing treatments on seed germination of African Grape (Lannea microcarpa L.). Pot trials was conducted during dry season at Botanic garden, Department of Plant Science and Biotechnology, Faculty of Life Science, Federal University Dutsin-Ma, Katsina State using completely randomized design (CRD). Seeds were subjected to three different concentrations of sulphuric acid, hot water, cold water, physical treatment and untreated seed as control and were analyzed independently to obtain the best treatment periods. To remove the fungal spore and exudate, a total of nine hundred and sixty (960) viable seeds were selected and surface sterilized in 1.0% Sodium-hypochlorite (NaOCl) for 1 minute and rinsed three (3) times in sterile distilled water. 20 viable seeds were sown for each polypot. In chemical pre-sowing treatments, maximum germination percentage (85.00%) and early days to seedling emergence (8.00 days) were significant ($P \leq 0.05$) in seed treated with 50% dilute H_2SO_4 for 1 hour when compared to control and other time intervals. Similarly, in physical pre-sowing treatment, days to early emergence of the seedling were recorded in seed with full-sided scarification (6.00 days). Highest germination percentage (90.00 %) and early days to seedling emergence (6.00 days) were recorded in seed immersed in hot water for 10 minutes when compared to untreated (15.00 days). In Seeds immersed in cold water, the result did not produce significance differences ($P > 0.05$) among the treatments except for seeds immersed in cold water for 8.00 hours (80.00%) and early days to seedling emergence (6.00 days) when compared to other treatments and the untreated seed (15.00 days). These results suggested that seed with full-sided scarification (6.00 days), seed immersed in hot water for 10 minutes (6.00 days) and cold water for 8.00 hours (6.00 days) used

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in this study were optimum for the best treatments inducing emergence breakage of seed coat and the technology could be applied to generate seedling for future breeding programme.

Keywords: Pre-sowing treatment, Emergence, African grape (*Lannea microcarpa* L.), Scarification.

INTRODUCTION

African grape (*Lannea microcarpa* L.) is a shrub or small deciduous tree that belongs to the anacardiaceae family and genus *Lannea*. It is an economic multipurpose tree of West Africa and an important tree that has the capacity to withstand drought because of its deep tap root system and ability to restrict transpiration (Arbonnier and Michel, 2004). More attention has been given to economic important species of tree plants for a sustainable use and integrated management due to an increasing recognition of its contribution to fulfill basic needs of people, household economics, food security and conservation of natural resources (Arbonnier, 2014). It is a common species in agro-forestry which plays vital role to food security, supply of timber, firewood, fodder, drugs, and dyes as well as restoration of fertility (Maroyi and Alfred, 2018). Approximately, the genus *Lannea* consists of about 40 different species which are usually trees or shrubs, occupying different habitats in Sub-Saharan Africa, with only one species recorded in Tropical Asia and several species introduced throughout the world (Pale, *et. al*., 1998). The tree is multipurpose indigenous to Nigeria, Niger, Gambia, Ghana, Guinea, Mali, Benin, Burkina Faso, Cameroon and Central African Republic (Bhardwaj, 2014). Many species of lannea have been recorded in bushy grassland, woody savannah, forest edges and rocky environment (Joshi and Joshi, 2009). In West African local communities, it is widely used as a source of dye, food, medicines, and other commodities (Kunz, 2009). The fruit is resinous, slightly acidic with pleasant taste in which both fresh and dried fruits are eaten raw or made into juicy, jamy, or puddy substances while the young leaves are cooked as leafy vegetables (Maroyi and Alfred, 2018).

A frican Grape (*Lannea microcarpa* L.) seed possess an exogenous dormancy, a hard seed coat structure that prevents the seed from germination. As a result of this problem, there is a loosing of genetic hereditary information and rapid depletion of natural population of the tree (Okunlola *et. al*., 2011). The hardness of the seed coat prevents the seed from fast and uniform germination at vegetative stage. Seed dormancy is produced from low permeability of seed coat to water and gases, immaturity and mechanical resistance to the growth of embryo of the seed. The problems of seed dormancy can be tackled by establishing plantation of indigenous tree there by successful production of vigorous and healthy emergence seedling by ensuring viability at nursery stage (Amonun *et. al*., 2016). This research is aimed at providing solution by breaking the dormancy using the four different pre-sowing treatment methods.

MATERIALS AND METHODS

Study Area

The experiment was carried out during 2021 dry season at Botanic garden, Department of Plant Science and Biotechnology, Faculty of Life Science Federal University Dutsin-Ma, Katsina State. The area is typically Sudan savanna ecological zone located in the north-western region of Nigeria which lies between latitude 12 27′ 14.11″ N and longitude of 07 29′50.03″ with an average elevation of 1,744 feet above sea level. The climate of the area is tropical sub-humid with high temperatures and high humidity. Wet season is humid and mostly cloudy, the dry season is partly cloudy and it is hot year round. The mean annual rainfall value is between May and September with a peak in August. The average annual rainfall is about 700 mm (Nnaji, 2001). The pattern of rainfall in the area is highly variable. This can result in severe and widespread droughts that can impose serious socio-economic constraints (Abaje *et. al.*, 2014). The mean annual temperature ranges from 29 °C – 31 °C. The highest air temperature normally occurs in April/May and the lowest in December through February. Evapo-transpiration is generally high throughout the year. The highest amount of evaporation occurs during the dry season. The vegetation of the area is the Sudan Savanna type which combines the characteristics and species of both the Guinea and Sahel Savanna (Abaje *et. al.*, 2014).

Seed Collection and Polypot Preparation

African Grape (*Lannea microcarpa* L.) fruits for the experiment were collected from Dutsin-Ma community of Katsina State. Riped fruits were selected and cracked to remove the seeds for pre-treatment prior to the germination experiment. Polythene bags were filled with top soil. Seeds were sown in polypots (30 x 30 x 40cm) containing 7kg of the mixture of top soil and compost manure (ratio 3:1). All standard cultural practices were maintained based on the procedure described by Chude *et. al.*, (2012).

Viability Test

The seeds collected for the experiment were subjected to the viability test and those not viable were liberated. This involved immersion of seeds into water in a container, the seeds that were observed to float were immediately removed and considered not viable (Usman *et. al.*, 2010). N The seeds that were observed to be viable were dried for further used in the experiment.

Seeds Preparation, Seed Sowing and Weed Control

Pot trials and sowing of seeds were carried out .To remove the fungal spores and exudate, total of nine hundred and sixty (960) viable seeds were selected and surface sterilized in 1.0% Sodium-hypochlorite (NaOCl) for 1 minute and rinsed three (3) times in sterile distilled water. 20 viable seeds were sown for each polypot. The depth of the sowing was 1 cm and few weed germinated with seeds were removed by hand picking.

Irrigation

For better emergence and performance of the lannea seed germination, moisture supply was very essential. Constant watering of the polypots was crucial throughout the period of the experiment because the experiment was carried out during dry season. Polypots were watered twice, morning and evening every day.

Experimental Design

The experimental layout was Completely Randomized Design (CRD) with equal replications. The seeds were subjected to three (3) pre-treatment methods at three (3) pre-treatment durations with untreated seeds as control and were analyzed independently to get the best treatment time for the four pretreatment periods.

Data Analysis

Data on days to early emergence of seeds were collected at vegetative stage and subjected to Analysis of Variance (ANOVA) using SPSS soft were version 23 (www.ibm.com) and significant means were separated using Turkey's Honestly significant diffrence (HSD).

Chemical Pre-Treatment

50% dilute Sulphuric acid was poured in a beaker containing viable lannea seeds. The seeds were fully immersed and left in an acid for 0.0, 1.0, 2.0 and 3.0 hours after which they were removed and rinsed thoroughly in a cold water for 3 to 5 to remove the excess chemicals.

Physical Pre-Treatrrment
For physical treatment, one side, two sides and full sides of the seeds were scrapped so that some parts of the seed coats were removed and healthy radicals were left and used for the evaluation.

Hot Water Pre-Treatment
Water was boiled at 100 ˚C and the seeds were soaked in boiled water (100 ˚C). Subsequently, the source of heat was removed to allow the immersion of the seeds. The seeds were then immersed in the hot water for a period of 0.0, 10.0, 15.0 and 20 minutes in a 100ml beaker. The seeds were then dried upon removal from hot water.

Cold Water Pre- Treatment
For the cold water pre-treatment, the seeds were soaked in cold water at room temperature for 8, 16, 24 hours. As in hot water, the seeds were then dried upon removal from cold water.

RESULT AND DISCUSSION
The result on the effects of chemical treatments (50% dilute H_2SO_4) on seed germination of African grape (*Lannea microcarpa* L.) is presented in Table 1. The result showed significance differences ($P\leq 0.05$) in *lannea* seeds treated with 50% dilute H_2SO_4 at different time interval. On the percentage of seed germinated, the result showed statistical significant effect when compared to untreated seeds. Maximum germination percentage (85.00%) and early days to seedling emergence (8.00 days) were recorded in seed treated with 50% Sulphuric acid in dilution for 1 hour when compared to control and other time intervals. Maximum days to late emergence were recorded in untreated seeds (21.00 days). This result observed that, *Lannea microcarpa* seed dormancy might have been associated with hardness of the seed coat. Seed pre-sowing treatment in 50% dilute H_2SO_4 induced germination with effective breakage of seed coat. This was in line with work of Amonum *et. al*., (2016) in *Parkia biglobosa* seeds, who reported that germination must have occurred as a result partial exposure of the seed that permit hydrolysis in the seed thereby increasing the activities of hormone such as Auxin and Ethylene gas which contribute to increase in synthesis of proteins and releasing nucleic acid metabolisms in the cells.

TABLE 1. Effect of Chemical Treatments on Germination Percentage and Days to Early Emergence in Seeds of African Grape (*Lannea microcarpa* L.)

Treatments 50%Sulphuric acid in (Hours)	No. of Seed Germinated	% of Seed Germinated	Days to Early Emergence of Seeds	Days to late emergence seeds
T_0	$9.00^d\pm1.20$	$45.00^d\pm1.70$	$15.00^a\pm1.90$	$21.00^a\pm1.33$
T_1	$17.00^a\pm1.23$	$85.00^a\pm1.71$	$8.00^d\pm1.91$	$14.00^d\pm1.30$
T_2	$11.00^c\pm1.21$	$55.00^c\pm1.72$	$10.00^c\pm1.71$	$17.00^c\pm1.23$
T_3	$14.00^b\pm1.22$	$70.00^b\pm1.71$	$13.00^b\pm1.73$	$19.00^b\pm1.22$

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant difference (HSD) $P\leq 0.05$. T_0 = untreated seeds (control), T_1 = treatment with 50% dilute H_2SO4 for 1 hour, T2 = treatment with 50% dilute H2SO4 for 2 hours, T_3 = treatment with 50% dilute H_2SO4 for 3 hours, % = percentage, SE± = standard error.

On the effect of physical treatments on seed germination of African grape (*Lannea microcarpa* L.), the result is presented in Table 2. The result obtained shows significant increase ($P\leq 0.05$) in seeds germination with full-sided scarification (15.00), two-sided scarification (16.00) and one-sided scarification (11.00) when compared to non-sided (7.00) scarification with few number of seeds germinated. Highest germination percentage was also recorded in seeds with full-sided scarification (75.00%), two-sided scarification (80.00%) and one-sided scarification (55.00%) when compared to non-sided (35.00%). This might be attributed to the hardness of the seed coat that prevents the rupture of the seed and uptake of water into the thick palisade layer which reduces the germination performances. The research was in agreement with work of Usman *et. al.*, (2010) in *Acacia senegal* seeds who reported that, the germination performance must have occurred due to partial exposure of seed coat to different scarification methods that increase the process of hydrolysis and hormonal activity of auxin. On the days to early emergence of the seedling, the result shows significant variation (P≤ 0.05) among the different scarification methods. Days to early emergence of the seed were recorded low in seed with full scarification (6.00 days) and the seed treated with two-sided scarifications (8.00 days) when compared to non-sided scarification (15.00 days), while the maximum days to late emergence recorded in non-sided scarification. This might have observed that, the palisade layers in the seed coat were lignified with malpighian cells tightly packed together and impregnated with water repellant in the seed. The full-side scarification might have functioned as a water-gap associated with the impermeable layers of the seed that prevent the uptake of water. This work was in conformity with work of Ubaidillah *et. al.*, 2020 on physical scarification method of *Moringa oleifera* seed. Bhardwaj, (2014) reported that, breaking physical dormancy involves disruption of a specialized structures of the seed.

TABLE 2. Effect of Physical Treatments on Germination Percentage and Days to Early Emergence in Seeds of African Grape (*Lannea microcarpa* L.)

Physical Scarification	No. of Seed Germinated	% of Seed Germinated	Days to Emergence of the Seeds	Days to late emergence of the seeds
Non-sided	7.00^{ab}±1.80	35.00^{ab}±1.80	15.00^{a}±1.22	22.00^{a}±1.21
One side	11.00^{a}±1.90	55.00^{a}±1.63	12.00^{b}±1.23	21.00^{a}±1.21
Two sides	16.00^{a}±1.80	80.00^{a}±1.60	8.00^{c}±1.22	14.00^{b}±1.20
Full sides	15.00^{a}±1.70	75.00^{a}±1.8	6.00^{c}±1.20	14.00^{b}±1.19

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant differenc e (HSD) P≤ 0.05, % = percentage, SE± ═ standard error.

The result on the effect of hot water as pre-sowing treatment is presented (Table 3). Hot water significantly (P≤ 0.05) affects the seed germination, germination percentages and early seedling emergence. Highest germination percentage (90.00 %) and early days to seedling emergence were recorded in seed immersed in hot water for 10 minutes (6.00 days) when compared to untreated (15.00 days) while maximum days to late emergence also recorded in untreated seed (21.00 days). This might have been attributed to physiological potential from the seed micropyle which speed the germination. This result agrees with the work of Amonun *et. al.*, (2016), who stated that germination performance with highest days to germination, is as a result of seed treated with hot water for 20 minutes to above, resulting in the rupturing and damaging the embryo.

TABLE 3. Effect of Hot Water Treatments on Germination Percentage and Days to Early Emergence in Seeds of African Grape (*Lannea microcarpa* L.)

Hot Water Treatment (100°C) in min	No. of Seed Germinated	% of Seed Germinated	Days to early Emergence of the Seeds	Days to late emergence of the seeds
0.00	8.00^{c}±2.73	40.00^{c}±2.56	11.00^{a}±1.73	21.00^{a}±1.72
10.00	18.00^{a}±2.50	90.00^{a}±2.55	6.00^{c}±1.73	14.00^{b}±1.72
15.00	13.00^{b}±2.52	65.00^{b}±2..40	8.00^{b}±1.72	14.00^{b}±1.73
20.00	13.00^{b}±2.55	65.00^{b}±2.56	9.00^{b}±1.73	15.00^{b}±1.72

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant difference (HSD) P≤ 0.05, % = percentage, SE± ═ standard error.

On the effect of cold water on germination percentage and days to early seedling emergence is presented in table 4. The result did not produce significance differences (P> 0.05) among the treatments except for seeds immersed in cold water for 8.00 hours (80.00%) and (6.00 days) which produced early days to seedling emergence when compared to seed treated for 24 hours (13.00 days) and the untreated control (15.00 days), whereas the days to late emergence were recorded to be (21.00 days). This might be as a result of impermeability of the seeds, palisade cell with hard pectinaceous outer layer and higher lignin content in the seed coat. This work was in line with work of Kimura and Islam, (2012) in Long bean (*Vigna sinensis*), who reported that germination of seeds treated with hot water for long time could be decreased by developing macro-cracks on the seed coat. Novita *et. al*., (2021) also reported that, treatment with cold water might have removed the thick palisade layer of seed coat. Effectiveness of the seed dormancy may vary within the cultivars of same species, depending on their seed coat structures. Each type of seed from various plants has a different level of seed coat hardness, and then this affects the sensitivity of the seed coat to start an imbibition in the germination process.

TABLE 4. Effect of Cold Water Treatments on Germination Percentage and Days to Early Emergence in Seeds of African Grape (*Lannea microcarpa* L.)

Treatments Cold Water in (Hours)	No. of Seed Germinated	% of Seed Germinated	Days to Emergence of the Seeds	Days to late emergence of the seeds
0.00	7.00^{b}±2.70	35.00^{c}±2.70	15.00^{a}±0.22	23.00^{a}±0.22
8.00	16.00^{a}±2.71	80.00^{a}±2.71	6.00^{b}±0.21	14.00^{b}±0.21
16.00	9.00^{b}±2.40	45.00^{b}±2.40	10.00^{a}±0.21	15.00^{b}±0.21
24.00	11.00^{b}±2.60	55.00^{b}±2.60	13.00^{a}±0.23	23.00^{a}±0.22

Means followed by the same letter (superscripts) are not significantly different using Turkey's honestly significant difference (HSD) P≤ 0.05, % = percentage, SE± ═ standard error.

CONCLUSION

This study demonstrated that, in chemical pre-sowing treatments maximum germination percentage (85.00%) and early days to seedling emergence (8.00 days) were significant (P≤ 0.05) in seed treated with 50% dilute H_2SO_4 for 1 hour when compared to control and other time intervals. Similarly, in physical pre-sowing treatment, days to early emergence of the seedling were recorded higher in seed with full-sided scarification (6.00 days). Highest germination percentage (90.00 %) and early days to seedling emergence (6.00 days) were recorded in seed immersed in hot water for 10 minutes when compared to untreated (15.00 days). For Seeds immersed in cold water, the result did not produce significance differences (P> 0.05) among the treatments except for seeds immersed in cold water for 8.00 hours (80.00%) and early days to seedling emergence (6.00 days) when compared to other treatments and the untreated seeds (15.00 days). These results suggested that seed with full-sided scarification, seed immersed in hot water for 10 minutes and cold water for 8.00 hours used in this study are the best pre-sowing treatments for breaking the dormancy of African grape (*Lannea microcarpa* L.) seed.

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