Breaking Seed Coat Dormancy of Lonchocarpus Cyanescens

O.A. Obembe & B. B. Folorunsho
Adekunle Ajasin University
Akungba Akoko, P.M.B.Oo1
Ondo State, Nigeria
E-mail: olutayobembe@gmail.com olutayobembe@yahoomail.com
Phone: +2348058019336, +2347036152535, +2348100254789

ABSTRACT

Lonchocarpus cyanescens (Schum and Thonn) Benth plant, commonly called the Big Leaf Indigo, Indigo Vine or West African Indigo plant is a shrub of twining nature belonging to the tribe Dalbergiae of the natural Order Fabales, Family Fabaceae and Sub-Family Papilionoideae. Economically important as laundry, textile and hair dyes and for traditional medicine purposes. Seeds of *Lonchocarpus cyanescens* exhibits seed coat dormancy. The seeds were sown in white colored plastic buckets filled with loose and well drained river sand .Bucket diameter was 22cm and bucket depth from the base to the bream was 24cm.4 replications of 100 randomly picked seeds- 25seeds per bucket was used for each treatment. Seed spacing was 2cm by 2cm and at sowing depth of 3 cm.Viable seeds were subjected to pre-treatments using 98% Sulphuric acid, wet heat and physical scarification with rough sand paper for 40seconds. Control treatment was also performed. Results showed that Physical scarification with 53% Germination and lowest Mean Germination Time of 13 days was most efficient. Control experiment gave 19% Germination and Mean Germination Time of 33 days, Sulphuric acid-16% Germination and Mean Germination Time of 22 days. Wet heat or thermal scarification with 9% Germination and Mean Germination Time of 15 days. Thus, Physical scarification pre-treatments because of higher Germination Percentage and reduced spread of days are recommended for the propagation of *Lonchocarpus cyanescens*.

Keywords: *Lonchocarpus cyanescens*, Seed Pre-Treatments, Germination Percentage, Mean Germination Time.

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1. INTRODUCTION

Lonchocarpus cyanescens(Schum and Thonn)Benth, synonym *Philenoptera cyanescens*(Schum and Thonn) Roberty commonly known as Big Leaf Indigo, Indigo Vine or West African Indigo is a shrub of twining nature belonging to the tribe Dalbergieae of the natural Order Fabales, Family Fabaceae and Sub-Family Papilionoideae. The Genus *Lonchocarpus* Kunth comprises of 150 tropical American, West Indies, Australia and Asia (Willis and Airyshaw, 1973). *Lonchocarpus cyanescens* reaches up to 10 to 20metres in height, the plant grows in fringing, transition and Savanna –Forest and it's also found in coastal areas in Southern Nigeria.

Lonchocarpus cyanescens stems are glabrous. Leaves uni- pinnately compound, flowers borne in ample and much branched panicles, lilac-blue in colour, scented and attractive to bees and other insects. Young fruits are bright green in colouration, maturing into grey-brown pods that are indehiscent and with reticulate veined patterns on their surfaces. *Lonchocarpus cyanescens* is economically important for traditional medicine purposes and most prominently as laundry, textile and hair dyes and of great potentials as an industrial staining dye. (Bassey *et. al*., 2012) All the aerial parts of the plant yield an indigo dye. Leaves and young shoots are plucked, they are bruised to a pulp and made into balls of about 10 to 12cm diameter, fermented, then dried or the leaves are kept in a broken state and merely sun-dried, sold in markets as "aro" in Yoruba language (Burkill,1995). Botanically, a seed is a fertilized, ripened and mature ovary containing one or more ovules as in Gymnosperm and Angiosperm plants .A typical seed consist of three basic parts i) an embryo ii) a supply of nutrients for the embryo and (iii) a protective seed coat (testa).Seeds of leguminous plants often develops a split along one side of the pod, really, that is the best time for planting purposes because when seeds are held in long storage, there is gradual decline in their germination ability and vigour. (http.www.)

Seed longevity is the duration of seed viability. Normally, seeds possess maximum germination potential during their physiological maturity and deterioration of seed quality occurs from their point of maturation onwards. The rate of seed deterioration increases due to mechanical injury at the time of harvesting and seed processing, higher moisture content in seeds and exposure of stored seeds to micro-organisms and insects also cause reduction of seed longevity and quality, while seed storage under low moisture content, cool temperature and low oxygen tension promotes seed longevity (Balasubramaniyan and Palaniappan, 2014). Seed viability is defined as the degree to which a seed is metabolically active and capable of germinating under favourable conditions. Seed viability is at highest at the time of physiological maturity. Seeds with high moisture content deteriorates quickly because of energy expenditure and accumulation of breakdown processes by the degradation of mitochondria which permanently loses their ability of swelling and contraction due to the ageing of the mitochondria and as the age of the seed increases, the semi-permeable membrane of the cell organelles loses their selective permeability allowing metabolites to leach out with subsequent loss of seed viability (Balasubramaniyan and Palaniappan, 2014).

Seed vigour is defined as the condition of the seed that permits germination to proceed rapidly and uniformly, allowing production of uniformly seedling stands. The vigour of seeds depends on the genome, history of the individual seed and the environment in which the seed is sown. Vigorous seeds produce seedlings of natural robustness with good health, natural robustness, disease free, physically sound, germinating quickly and producing rapidly developing seedlings (Balasubramaniyan and Palaniappan, 2014). This research focus on breaking seed coat dormancy of *Lonchocarpus cyanescens.*

Many leguminous seeds are notable for their hard seed coat conditions. Their hard seed coat dormancy maintains low moisture content in their seeds and in terms of advantage confers longevity on them, enabling them to survive harsh conditions in dry season; it also allows endozoic dispersal of their seeds and subsequent re-colonization of their habitats after fires (Egley, 1989).

One of the most pertinent questions in the field of germination biology of seeds is what controls the timing of germination of seeds in soils. Many factors such as high level of carbon dioxide in soils, improper aeration, age of seeds, poor seed storage and production of volatile allelo-chemicals have been suggested to prevent germination of seeds in the soil (Holm, 1979).However, in many leguminous seeds, hard seed coat prevents imbibitions of water and exchange of gases, thus preventing initiation of the germination process (Maguire,1975)

Karuiki and Powell (1988) defined Seed Germination as the process by which the dormant embryo grows out of the seed coat and establish itself as a Seedling. That is, Seed Germination involves the growth or transformation of an embryo of a mature seed into a Seedling. Germination is a component of seed quality and it occurs when a viable seed absorbs water, inducing respiration and protein synthesis, leading to the emergence of the radical from the testa (Maguire,1975).Germination incorporates those events that commence with up-take of water by the seed, followed by growth of the embryo, the rupturing of the seed coat and the emergence of a young plant. (Bewley and Black, 1994).
In order to germinate, a seed must fulfill the following requirements i) it must be viable ii) it must be subjected to appropriate environmental conditions and iii) any primary dormancy present in the seed must be overcome.Seed Germination generally measured in percentage is the number of seeds usually out of 100 seeds in a seed lot that are expected to germinate and grow into healthy plants. Viable seeds does germinates when sown, but when they fail to germinate, despite provision of adequate water or moisture, light, temperature, gases, and other suitable materials, such seeds are said to be dormant or are said to exhibit dormancy.

Copeland and McDonald(1985) defined Seed dormancy as a state in which seeds are prevented from germination under environmental conditions normally favourable for germination .Seed dormancy is a block to the completion of germination of an intact viable seed under favourable conditions(Hilhorst,1995). Osonubi and Chukwuka (1999) defined dormancy as the condition whereby seeds fail to germinate because of internal conditions, even though, external conditions such as oxygen, moisture or water, light and temperature are favourable According to Baskin and Baskin, (2004), Seed dormancy is a state in which seeds are unable to germinate in a specific period of time under a combination of environmental factors that are normally favourable for germination. A dormant seed is one that is unable to germinate in a specified period of time under a combination of environmental factors that are normally suitable for the germination of the non-dormant seed (Http.www.).

Dormancy is a period of arrested growth or a period of rest preceding seed germination and a form of survival strategy by seed plants, a mechanism by which seeds can delay germination until the right environmental conditions for seedling growth and development subsist. It is a form of ecological adaptation for staggered germination because if seeds germinate all at once and if there is immediate catastrophe such as severe drought or epidemic diseases, all the new Seedlings will be wiped out at once.

Dormancy may be due to a variety of reasons such as

a) seed coat restrictions based on the seeds morpho-anatomical structure

b) chemical inhibitors within the seed (Physiological Dormancy)

c) light sensitivity

d) embryo dormancy(rudimentary embryo)

Any treatment which reduces or destroys seed impermeability is known as scarification. Scarification treatment is used to soften the seed coat in order to make the seed permeable to water and gases without destroying the embryo (Seedbrock, 2006).Pre-treatments used to break seed dormancy includes usage of nitric acid, sulphuric acid, other acids, and other chemicals, chipping or cutting through with blade, drilling, filing or knife, piercing or puncturing the seed coat with needle or any other sharp objects, grinding seeds with abrasives or brief immersion in hot water (wet heat),soaking in cold water, dilute solutions of caustic soda, sodium hypochlorite, alcohols, hormone applications and solutions of various salts are being used, dry heat, different temperature regimes, drastic temperature shifts or any other thermal scarification methods, physical scarification and stratification. (Gill, 1993, FAO, 1995).

Stratification is a simple and inexpensive technique for over-coming seed dormancy of temperate tree species mostly depending on the type of dormancy
involved, warm stratification is applied for seeds that have immature embryos, cold stratification is used to break physiological dormancy.Combined warm and cold stratification is effective for seeds that have both immature embryos and physiological dormancy. Warm stratification involves the placing of seeds in a moist medium such as sawdust, peat-moss or sand, while cold stratification involves moist chilling of seeds. In the Tropics, seeds are stored in pots or containers with cool soil which causes removal of substances which delays germination as a stratification mechanism.

Since over –exposure of seeds to hot water can kill seeds. It is important not to soak seeds in hot water for too long, also acids can be very dangerous to handle and its usage requires personal protective gears and proper disposal after usage,also prolonged physical abrasion if not well monitored can damage the delicate seed embryo.

The aims and objectives of this study is to determine the influence of scarifications using 98% concentrated sulphuric acid (chemical scarification) wet heat or thermal scarification and physical scarification using rough sand paper in order to enhance germination geared towards prolific propagation of *Lonchocarpus cyanescens*.

2. MATERIALS AND METHODS

2.1 Source of Seeds

Seeds based on availability were collected from Ikare-Akoko, Ondo State (Latitude 7^0 28 E and Longitude 5^0 44 E). Viable seeds determined by floatation method were used for the experiment after Pandey and Sinha, 1972.

2.2 Study Site and Management

The Study was conducted at the Screen House of Plant Science and Biotechnology Department .Seeds were sown in perforated white plastic buckets.Bucket diameter was 22cm and bucket depth from the base to the bream was 24cm.. Buckets were laid out in a randomized block design Four replications of 100 randomly picked seeds-25 seeds per bucket were used for each treatment. Seed spacing was 2 x2 cm and at sowing depth of 2.5cm. Buckets were kept free of weeds and watering was ensured regularly throughout the study period.

3. SEED PRE-TREATMENTS

100 seeds each were subjected to 98% Sulphuric acid (Chemical), Hot water (Wet heat) and Physical abrasion treatments. Another 100 randomly picked seeds were sown as Control treatment based on seed availability.

Chemical (Acid) Scarification Treatment
This was done by immersing the seeds in 98% concentrated Sulphuric acid; the acid was decanted after 40seconds, rinsed several times in distilled water and sown.

Control (Un-Treated) Experiment
100 un-treated seeds chosen at random were sown as control experiment to ascertain the Germination Percentage of intact viable seeds.

Physical Scarification Treatment
Seeds were physically abraded with rough sand paper for 40seconds of 20seconds on either side of each seed and were sown.

Wet Heat (Boiling) Treatment
Boiled water at 100^0 Celsius was introduced to 100 randomly picked seeds, stirred for 40seconds and were sown

Germination Counts
Seeds were recorded as germinated, once the plumule attains a height of 1cm above the soil surface after Missanjo *et.al.,* (2014).Records of seed germination were taken every day for 45 days.

Germination Percentage/Percentage Seedling Emergence
Germination Percentage was recorded as percentage number of germinated seed out of every 100 seeds sown with or without pre-treatments.

Germination Percentage also as Percentage Seedling Emergence recorded as number of seeds which germinated out of a sample of 100 seeds with or without treatment. It was calculated thus,

$$\% \text{ Seedling Emergence} = \frac{\text{Number of Emergent Seedlings}}{\text{Total Number of Seeds Planted}} \times 100$$

Mean Germination Time
Mean Germination time was recorded as the average of summations of the number of days for all the germinated seeds

Statistical Analysis
SPSS Analysis were used to calculate means of distribution and Standard Deviations

4. RESULTS

In terms of germination rate, control experiment gave laggard(slow) time of 33 days, which was the highest mean germination time, sulphuric acid treatment 22 days, wet heat treatment -15 days and physical scarification pre-treatment – 13days, which is the most reduced spread of germination days.

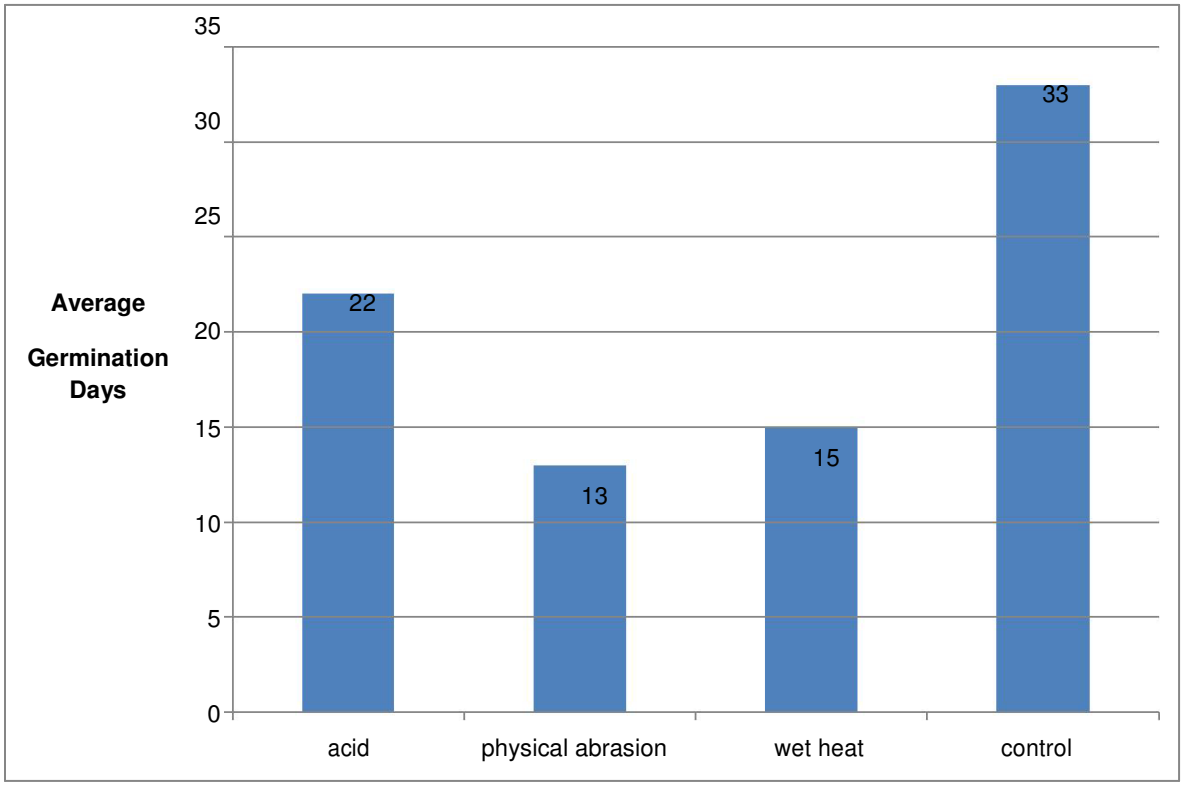

Figure 1: Influence of 40 seconds Seed Pre-Treatments on Germination Rate

40 seconds physical scarification pre-treatment gave the highest yield of 53%,

control (19%), sulphuric acid gave the lowest – 9%

Germination

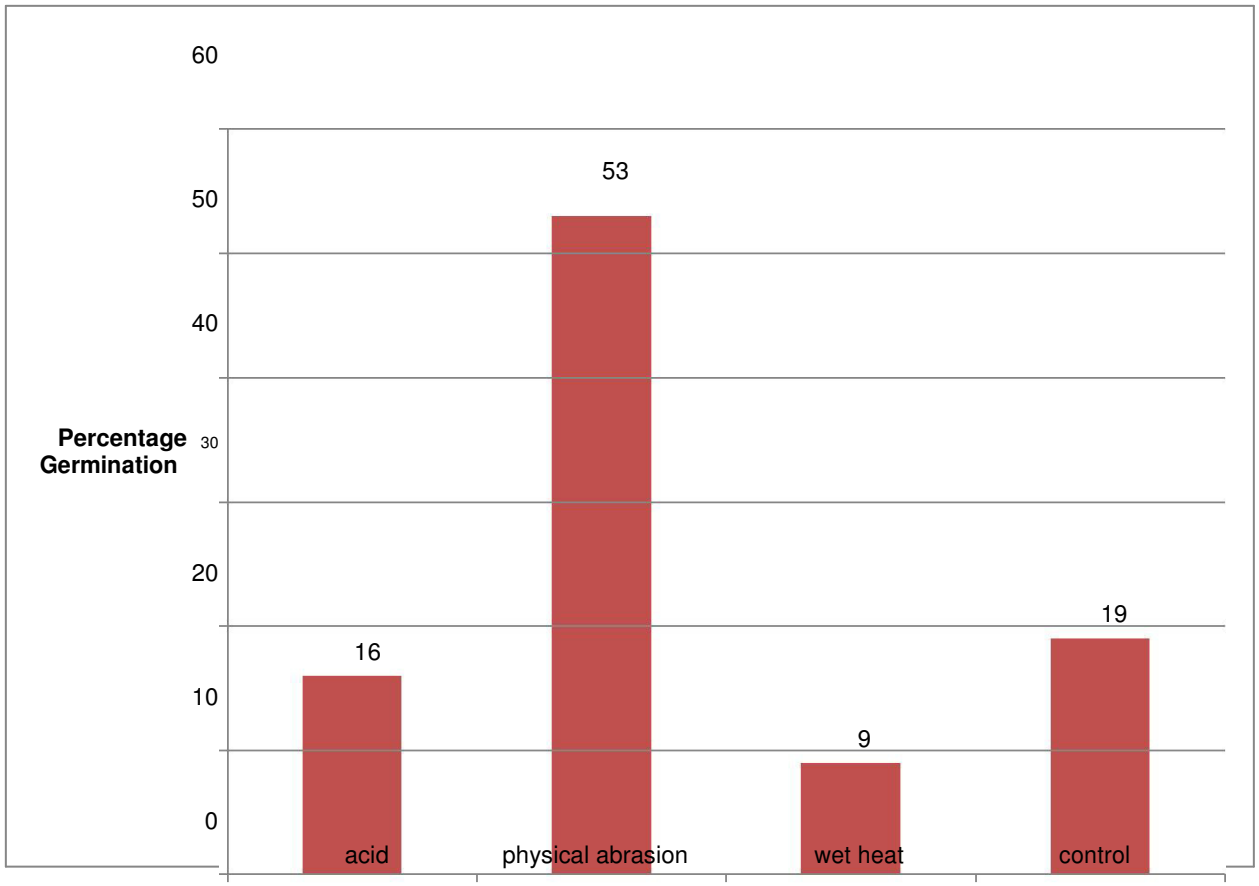

Figure 2: Effects of 40 seconds Seed Pre-Treatments on Percentage

The SPSS Data Analysis, control experiment is represented by To symbol, sulphuric acid treatment as (T1), hot water treatment – (T2) and physical scarification treatment (T3) as represented in Table 1.

Table 1:

Germination	40 seconds	Percentage (%)
T0	7.42 ± 4.95	19 ± 4.69
T1	5.61 ± 2.94	16 ± 4.07
T2	6.28 ± 3.46	9 ± 4.31
T3	14.48 ± 2.72	53 ± 6.55

Based on the mean of the distributions, and standard deviation as presented above, physical scarification gave the highest value of 53%.

5. DISCUSSION

Results showed that impervious seed coat may be the cause of dormancy in

Lonchocarpus cyanescens. The breaking of seed coat dormancy in legumes using Sulphuric acid, hot water(wet heat) and physical abrasion amongst others have been demonstrated by other works (Ajiboye and Agboola, 2010, Ajiboye et. al., 2011, Missanjo et .al., 2014). Copeland (1976) reported that hard seed coat creates barrier to water up-take and entry of gases in most legumes,stating that imperviousness in the seeds of most legumes may be due to deposition of cutin, lignin and suberin in the seed coat membranes or across the micropylar opening of the seed. Presence of continuous layer of tightly packed palisade cells in the seed coat of legumes may also create barriers to water and gases up-take (Egley, 1989). Physical scarification under laboratory conditions here may explain how abrasion of the seed coat by ploughing, harrowing and charring by field burning overcomes hard seed coat dormancy in nature. Conclusively, comparative effectiveness of 40 seconds Physical scarification treatment with 53% Germination and most reduced spread of days with Mean Germination Time of 13 days and its low resources demand makes it more economical and more result oriented treatment for the propapagation of *Lonchocarpus cyanescens*.

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