RESPONSE OF *Annona muricata* Linn. SEEDS TO DIFFERENT PRE-GERMINATION TREATMENTS

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ABSTRACT
This study assessed the effect of pre-germination treatments on seed germination and early growth of Annona muricata seedlings. Six pre-germination treatments: seeds soaked in water (at room temperature of 30°C) for 24 hours (T1) and 48 hours (T2), scarification (T3), soaked in concentrated H_2SO_4 for 5 minutes (T4) and 10 minutes (T5), and untreated (T6/control), were used in a completely randomized design experiment, replicated five times. The treated seeds were sown and germination rate, seedling height, collar diameter, number of leaves, and biomass (fresh and dry weight) were monitored. Data were analysed using descriptive and inferential statistics. Germination started twenty-eight (28) days after sowing and lasted for 45 days. The T2 seeds had the highest germination rate (97%), followed by T5 seeds (87%) while seeds soaked in T1 had the least (20%). Early growth revealed a significant difference among the different pre-treatments. The highest (13.93 cm) seedling height was obtained for T1, while T6 had the least (9.72 cm). Seeds in T2 had the highest collar diameter (0.76 mm) while T1 had the highest number of leaves (7.39). Seedlings from different treatments significantly differed in fresh and dry weight, root length and lateral roots. Soaking of seeds in water for 48 hours (at room temperature) was most suitable for germination of Annona muricata, while soaking in concentrated H_2SO_4 for 10 minutes was also effective.

Keywords: **Pre-germination, Scarification, Tropical fruit, Sour sop**

INTRODUCTION
Annona muricata (Linn) is the largest-fruit in the family Annonaceae. In Nigeria, the tropical fruit tree is called sour sop, shawa shawa, graviola and sawamsop (Osunwole, 1999). The species is a shrub approximately 5.0 to 9.0 m tall, with low and thin upturned branches. Due to its annonaceous acetogenins content, *A. muricata* prefers a well-drained, loose, deep loamy soil with a pH range of 5 - 6.5. It does not tolerate waterlogged soils and produces a stunted growth with shallow rooting (Orwa *et al*., 2009). The tree blooms and fruits all year round, with more defined seasons based on the altitude (Pinto *et al*., 2005). The fruit consists of 67.5% edible white pulp with a pleasing fragrance and flavour. It contains calcium and phosphorus and is a rich source of vitamins B and C. This 'miracle plant' has medical benefits including anti-cancer (Wang *et al*., 2002), anti-tumour (Kim *et al*., 1998), anti-parasitic (Jaramillo *et al*., 2000), anti-viral (Betancur-Galvis *et al*., 1999), and antioxidant (Gavamukulya *et al*., 2014) qualities.

Seed germination is defined as the process by which dormant embryo becomes activated, grows out of the seed coat and establishes itself as a seedling (Oboho, 2014). The dwindling population of trees has been traced to poor seed germination and seedling survival (Bello *et al*., 2011). The situation is compounded by seed recalcitrance or dormancy. Some tropical seeds would not

easily germinate despite appropriate germination conditions and require to be triggered by particular treatments. Therefore, seed dormancy is broken by natural or artificial techniques through the application of dormancy breaking mechanisms known as pre-germination treatment or pre-treatment. Pre-germination treatment involves the subjection of seeds to external conditions aimed at breaking seed dormancy and enhancing germination within the shortest possible time (Onyekwelu and Akindele, 2002). Pre-treatment methods that have been found appropriate for improving the germination of dormant seeds of tree species include mechanical scarification, chemical scarification, soaking in cold and hot water, alternate soaking and drying as well as mild burning (Aduradola *et al*., 2005; Aduradola and Adejumo, 2005; Agbogidi *et al*., 2007; Oboho, 2014).

Annona muricata seeds require long germination periods in spite of sub-optimal conditions, and could be delayed for 2 - 3 months. This could limit its propagation in home gardens and agroforestry settings (Oboho, 2014). The production of uniform and vigorous seedlings will go a long way in achieving the cultivation of this fruit tree, which has great potential for sustainable production and income generation. This study determined the effect of different seed pre-germination treatments on the seedling emergence and early growth performance of *Annona muricata*.

MATERIALS AND METHODS

Study Site

The experiment was conducted in the nursery of the Department of Forest Resources and Wildlife Management, University of Benin, Benin City, Nigeria. The campus lies between longitude 6.3998°N – 6.2458°N and latitude 5.6099°E – 5.3740°E. The annual rainfall varies from 1000 mm and 2500 mm with mean annual temperature of 27.31°C. Relative humility ranges from 75% at midday to 95% at dawn (UNIBEN Master Plan, 1993). The nursery is located in the tropical lowland rainforest region and experiences a bimodal rainfall pattern (Egharevba *et al.,* 2005).

Collection of Planting Materials and Experimental Design

Fruits of *A. muricata* (Figure 1a) were procured from Ore market in Ogun State, Nigeria. The fruits were allowed to soften before seed extraction and air drying for 48 hours (Figure 1b). Viability test was carried out to identify and select viable seeds. Thirty seeds were each subjected to pre-germination treatments before sowing. Pre-germination treatments included: soaking seeds in water (at room temperature, 30°C) for 24 hours (T1) and 48 hours (T2), scarification of the hilium and distal points on seeds (T3), soaking in pure sulfuric acid (98% H_2SO_4) for 5 minutes (T4) and 10 minutes (T5); as well as untreated seeds (T6). Treated seeds were sown in polypots filled with topsoil. A total of one hundred and eighty (180) poly-pots were arranged in a complete randomized design experiment. Watering was done twice daily and weeding was carried out, when necessary.

Data Collection and Analysis

The number of germinated seeds per day was monitored for seven weeks. The sprouted seedlings were further monitored for seedling height (cm), number of leaves and collar

diameter (mm), for eight weeks. At the end of the experiment, number of lateral roots, length of rootlets, biomass accumulation (fresh and oven dried weights) were determined. Samples were oven dried at 80°C to constant dry weight. Germination data were analysed using descriptive statistics, while a one-way ANOVA was used to analyse the growth data. Duncan multiple range test (DMRT) was used to compare means at 5% level of significance.

RESULTS

Seedling emergence commenced for T2 and T3 seeds at 28 days after sowing (DAS) with 3 and 5 seedlings emerging, respectively, while T1 and T4 seeds germinated after 29 and 30 days, respectively, with 1 seedling each. Emergence of seedlings in T5 occurred 33 DAS with 1 seedling emerging, while T6 emerged after 44 days with 2 seedlings (Figure 2). The highest germination was recorded for T2 (97%), followed by T5 (87%) and T3 (80%). Seeds in T4 had 70%, while T1 was least (20%). There was a significant difference in germination, with T2 and T3 producing the highest emergence (Table 1).

Seeds from T1 produced the highest seedling height (13.93 cm), followed by T2 (12.99 cm) while the least was observed for T6 (9.72 cm). There was a significant difference in seedling height across treatments (Table 2). The seedling collar diameter varied significantly across treatments, with T2 producing the highest (0.76 mm), while T4 was least (0.61 mm). Similarly, there was a significant difference in the number of leaves, with T1 producing the highest (7.39), while T6 (4.83) was least. The fresh and dry biomass, length of the roots and number of lateral roots varied significantly across treatments. Seedlings of T2 had the highest fresh (1.82 g) and dry biomass (0.62 g), while the highest root length (13.94 cm) and number of lateral roots (18.60 cm) were observed in T3 and T6, respectively (Table 3).

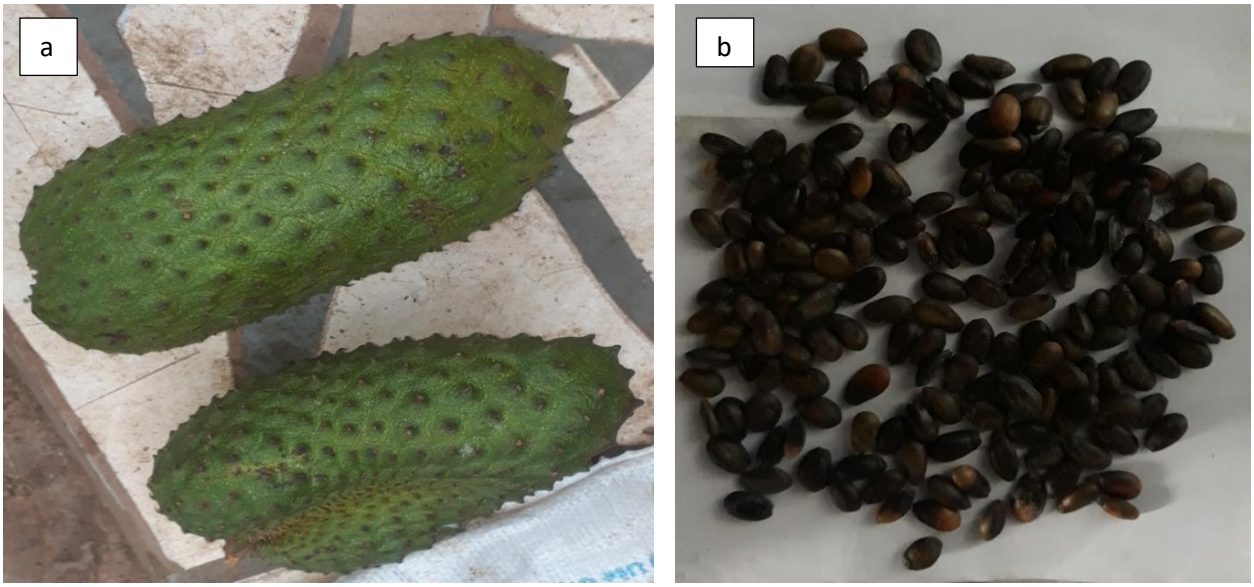

Figure 1. (a) Fruits and (b) seeds of *Annona muricata*

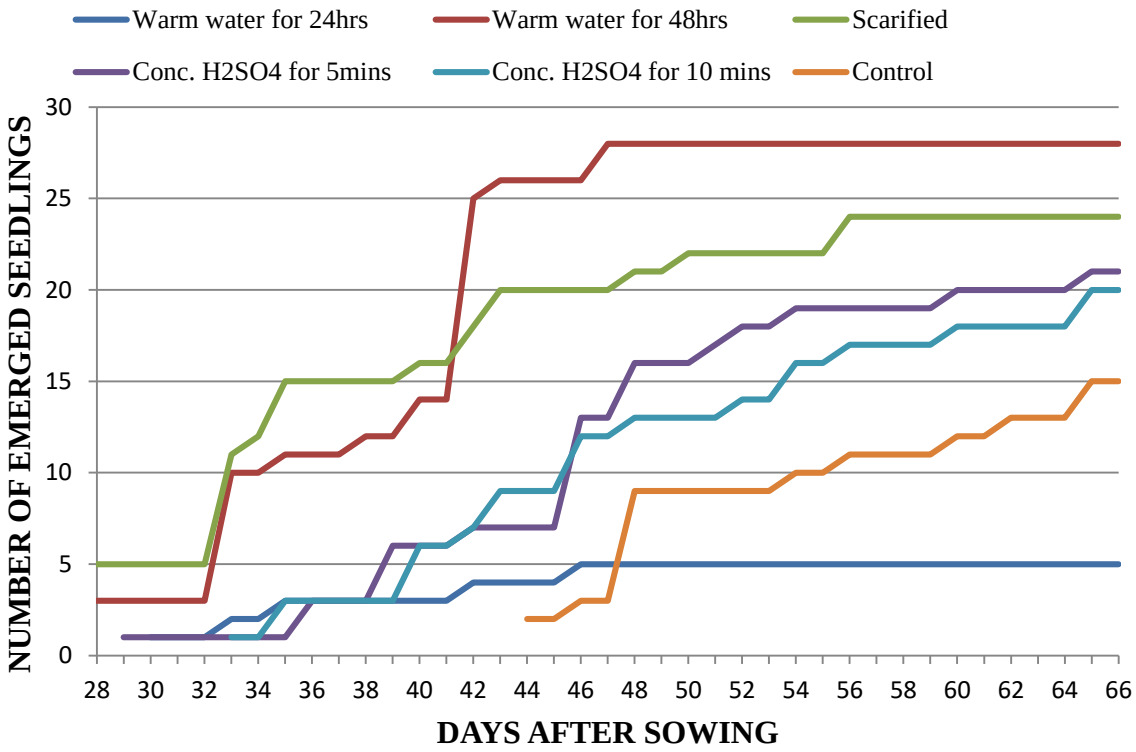

Figure 2. Emergence of seedlings from pre-treated seeds of *Anonna muricata*

Table 1. Effect of pre-treatment on germination of *Anonna muricata* seeds

Pre-treatment	Total	Mean	Germination percentage (%)	Germination rate (%)
Soaking in water for 24 hours (T1)	6	4.23^{c}	20	4.4
Soaking in water for 48 hours (T2)	29	22.00^{a}	97	20.7
Scarification (T3)	24	19.11^{a}	80	23.7
Soaking in H_2SO_4 for 5 mins (T4)	21	13.16^{b}	70	7.7
Soaking in H_2SO_4 for 10 mins (T5)	26	13.68^{b}	87	13.2
Untreated seeds (T6)	17	11.20^{b}	57	13.3

Means along column with the same superscript letter are not significantly different ($P < 0.05$)

Table 2. Growth variables of seedlings from pre-treated seeds of *Anonna muricata*

Growth Parameters	Pre-germination treatments					
	T1	T2	T3	T4	T5	T6
Seedling Height (cm)	13.93^{a}	12.99^{a}	11.52^{b}	10.32^{c}	11.89^{b}	9.72^{c}
Collar Diameter (mm)	0.75^{a}	0.76^{a}	0.67^{ab}	0.61^{b}	0.66^{ab}	0.67^{ab}
Number of Leaves	7.39^{a}	7.36^{a}	6.93^{ab}	5.39^{c}	5.60^{bc}	4.83^{c}

Means with the same superscript letters across rows are not significantly different ($P < 0.05$)

Table 3. Biomass, root length and number of lateral roots of *Annona muricata* seedlings

Variables	T1	T2	T3	T4	T5	T6	SEM
FB (g)	0.90^{c}	1.66^{a}	1.82^{a}	1.58^{a}	1.20^{b}	1.78^{a}	0.10
ODB (g)	0.24^{c}	0.56^{ab}	0.62^{a}	0.54^{ab}	0.43^{b}	0.61^{a}	0.04
RL (cm)	11.40^{c}	12.22^{b}	13.84^{a}	12.04^{b}	12.04^{b}	13.94^{a}	0.45
NLR (cm)	18.60^{a}	21.60^{b}	15.20^{c}	14.40^{c}	23.20^{a}	22.00^{a}	0.90

Means with the same superscript letters across rows are not significantly different ($P < 0.05$)
FB = Fresh biomass, **ODB**= Oven dried biomass, **RL** = Root length, **NLR** = Number of lateral roots

DISCUSSION

The seeds of *A. muricata* showed significant response to the different pre-treatments, where they reduced the period of dormancy, when compared with untreated seeds. Although seeds soaked in water for 48 hours (T2) and scarified seeds (T3) emerged on 28 DAS, the T2 seeds recorded the highest germination (97%). Ibrahim and Otegbeye (2004), affirmed that soaking of *Adansonia digitata* seeds in water aided germination rates. Owonubi *et al*. (2005) also reported that soaking *Azadirachta indica* seeds in water at room temperature for 12 hours increased germination rates. Yisau *et al*. (2020) mentioned that *Synsepalum dulcificum* seeds soaked in water at 20°C for 30 minutes produced the highest germination percentage.

Previous studies have reported the potentials of Sulphuric acid in seed pre-treatment. For instance, Amusa (2011) revealed that even though untreated seeds of *Afzelia Africana* produced impressive germination, sulphuric acid pre-treatments (for 30 minutes) produced a uniform and regular germination, and recorded the highest germination value within the shortest time. Falemara *et al*. (2014) asserted that acid treatment of *Adansonia digitata*, for up to 1 hour produced higher germination percentages. Hence, pre-treatment of *A. muricata* seeds with acid for longer periods than 10 minutes (T5), may result in higher germination rates.

The seed pre-treatment enhanced early growth with higher seedling height, collar diameter, number of leaves, and biomass as well as root length, when compared with untreated seeds. This could be attributed to earlier emergence of pre-treated seeds. The findings corroborate the results of Ibrahim and Otebeye (2004); Agboola and Adebire (1998); Aduradola and Shinkafi (1999). Hence, soaking in water, concentrated acid or scarification enhanced seed coat permeability, aided germination and improved early growth of *Annona muricata*.

CONCLUSION

Seed pre-treatment could enhance the production of healthy and vigorous seedlings for orchards and plantation establishment. The pre-treatment of *A. muricata* seeds influenced the time of emergence, germination rates and percentage. Emergence of seedlings started at 28 days, while peak germination was attained for seeds soaked in

water for 48 hours and scarified seeds. Therefore, soaking in water for 48 hours, acid treatment and mechanical scarification are recommended pre-treatment methods for reducing dormancy, enhancing early growth performance and increasing root development of *A. muricata*.

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