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THE EFFECT OF PRE-SOWING TREATMENT AND SEED SIZES ON THE GERMINATION OF Canarium schweinfurthii (Engl.) SEEDS IN JOS PLATEAU STATE

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Abstract:

Canarium schweinfurthii (Engl.), a forest tree from the Burseraceae family, is valued across Africa for its medicinal uses, including treating dysentery, coughs, chest pain, and skin conditions. This study examined the impact of various pre-sowing treatments on breaking seed dormancy and enhancing the development of C. schweinfurthii in Jos, Plateau State, Nigeria, from January to July 2023. Fieldwork at the Federal College of Forestry (latitude 9.5650380 N, longitude 8.533409 E) utilized a Completely Randomized Block Design (CRBD) with 18 treatment combinations replicated three times, resulting in 54 plots. Six pre-sowing treatments were evaluated: control (Untreated), hot water soaking, cold-water soaking for 48 hours, sulfuric acid (H_2SO_4) soaking, coconut water soaking for 12 hours, and mechanical abrasion. Parameters assessed included germination count, seedling height, leaf count, stem girth, leaf length, and width. Mechanical abrasion and cold-water treatment significantly improved germination and seedling growth ($P \leq 0.05$), with germination counts of 3.556 and 2.778, respectively, at 25 weeks after planting. Conversely, hot water treatment was least effective, with a germination count of 0.333 and negligible influence on seedling parameters. The study concludes that cold-water soaking for 48 hours and mechanical abrasion are the most effective techniques for breaking C. schweinfurthii seed dormancy. These findings contribute to optimizing the propagation of this valuable species, supporting agroforestry and conservation initiatives.

Keywords: Canarium schweinfurthii; seed dormancy; germination enhancement; pre-sowing treatment methods; seed size effects

1. Introduction

Canarium schweinfurthii (Engl.) is an important tropical forest tree species belonging to the family Burseraceae and is widely distributed across Africa. In Nigeria, the species is known by several local names, including Mupun (Paat), Berom (Pwat), Hausa (Atile or Atilis), Igbo (Ube agba), and Yoruba

(Origbo, Elemi, or Agbababubu) (Gbile, 1984). It is also commonly referred to as torchwood, frankincense, black olive, bush candle tree, or forest pear. The species predominantly occurs in riverine forests, moist evergreen and semi deciduous forests, secondary forests, gallery forests, and dry forest margins. In Nigeria, its distribution spans Bauchi, Southern Kaduna, Niger, Oyo, and Plateau States, with notable populations in Pankshin Local Government Area of Plateau State and parts of southeastern Nigeria, including Enugu, Ebonyi, and Anambra States (Nyam, *et al.,* 2014; Dawang, 2016).

Despite its wide ecological amplitude and adaptability to both rocky and flatland terrains, *C. schweinfurthii* increasingly occurs as isolated individuals or fragmented populations due to deforestation, habitat degradation, and land-use changes. Although the species possesses considerable ecological, nutritional, medicinal, and economic potential, it remains underutilized and poorly domesticated. Its oleoresin, edible seeds, and medicinal attributes are often overlooked in favour of more commonly exploited wild fruits and oilseed species (Nyam, *et al*., 2014; Kuete, *et al*., 2015). The large evergreen tree can attain heights exceeding 45–50 m, producing ellipsoid drupes that change from green to bluish-purple at maturity. The seeds are edible and widely traded in local markets, either consumed raw or softened by soaking to improve flavour.

A major constraint to the sustainable utilization and domestication of *C. schweinfurthii* is poor natural regeneration, largely attributed to seed dormancy and low germination rates. The seeds exhibit primary dormancy, enabling them to survive unfavourable environmental conditions between maturation and seedling establishment (Angel, 2020). Understanding the factors influencing seed germination is therefore critical for improving propagation, enhancing population sustainability, and supporting conservation efforts. Consequently, this study aims to assess the effects of seed size and different pre-sowing treatments on germination and early seedling development of *C. schweinfurthii*. The findings are expected to provide insights into seed viability, dormancy mechanisms, and effective propagation strategies essential for domestication and ecosystem restoration.

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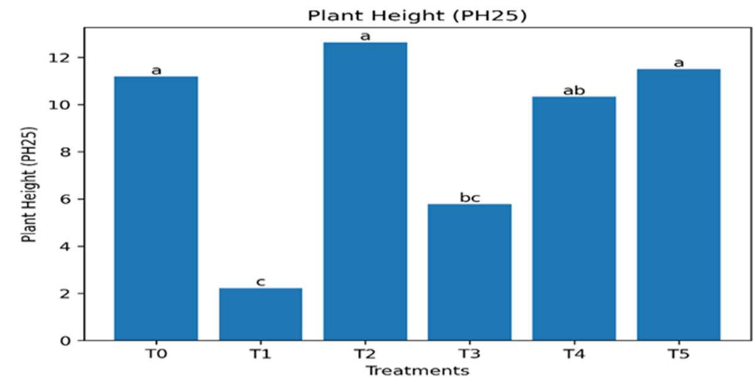

Figure 1. Effect of treatments on final plant height (PH25). Bars represent mean ± SE. Bars sharing the same letters are not significantly different at $P \leq 0.05$ (LSD).

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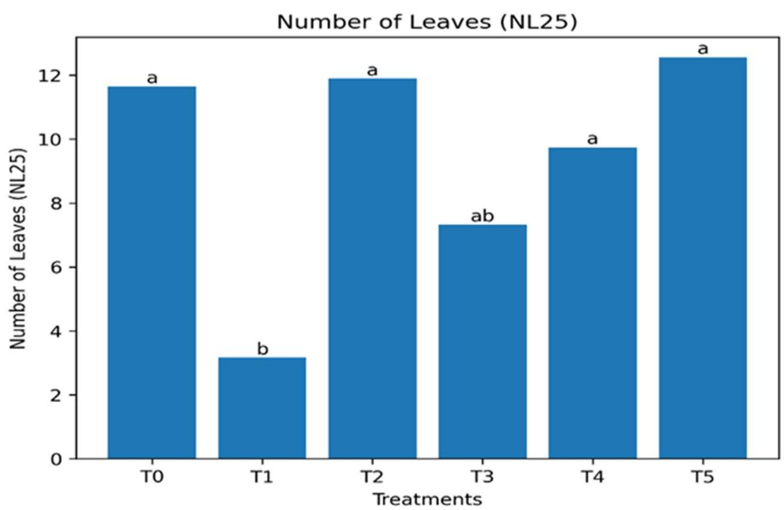

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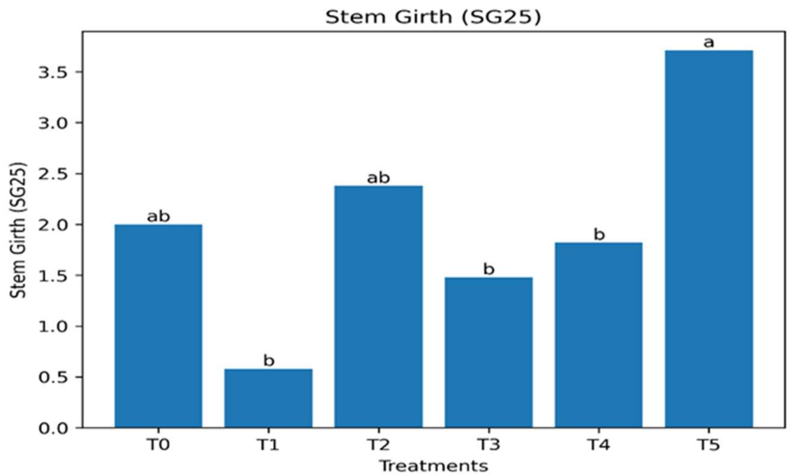

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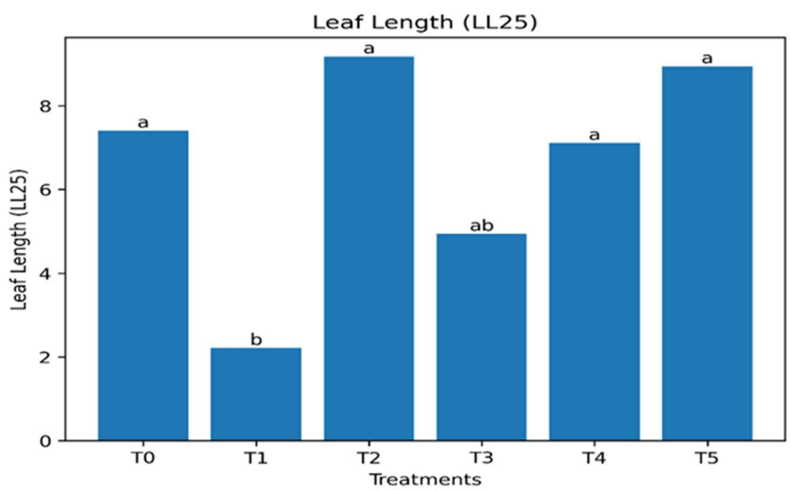

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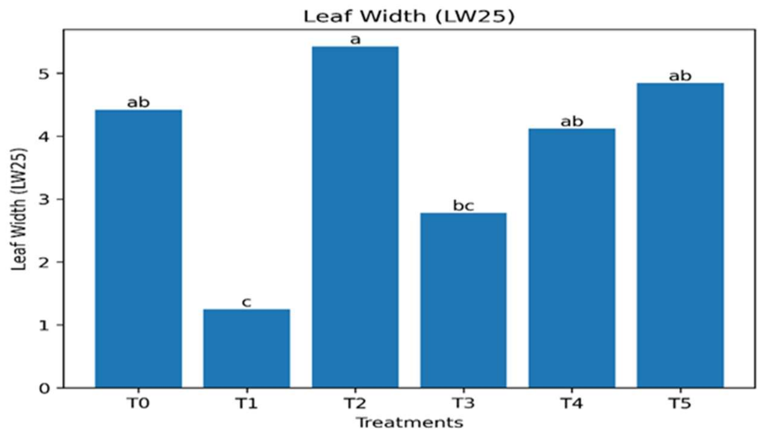

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2. Literature Review

Botanically, *Canarium schweinfurthii* is a large dioecious tree characterized by a straight cylindrical bole, dense crown, and thick grey bark that exudes an aromatic oleoresin when injured (Burkill, 1985; Orwa, *et al*., 2009). The fruits are ellipsoid drupes containing a thick, hard, rigorous endocarp that encloses one to three oily seeds (Nyam and Wonang, 2004). This hard seed coat plays a crucial role in limiting water uptake and gaseous exchange, thereby influencing germination behavior. As observed in many tropical woody species, such structural barriers often result in physical or combined dormancy, leading to delayed, uneven, or low germination under natural conditions.

Canarium schweinfurthii is a highly valued multipurpose species with extensive ethnomedicinal, nutritional, and economic importance across tropical Africa. Various plant parts including leaves, bark, roots, fruits, seeds, and resin are widely used in traditional medicine for treating ailments such as malaria, anaemia, gastrointestinal disorders, diabetes mellitus, hypertension, rheumatism, skin diseases, and post-partum complications (Ngbebe, *et al*., 2008; Koudou, *et al*., 2005; Okullo, *et al*., 2014). The resin is commonly used as an emollient, fumigant, antiparasitic agent, and wound dressing (Ken Fern, 2014), while the wood serves as raw material for furniture, plywood, fuelwood, and canoe construction (Tchouamo, *et al*., 2000). Continuous exploitation of these resources, coupled with minimal deliberate cultivation, has intensified pressure on wild populations.

Phytochemical investigations have revealed that *C. schweinfurthii* contains numerous bioactive compounds, including flavonoids, tannins, saponins, alkaloids, phenolics, cardiac glycosides, steroids, and triterpenes (Ngbebe, *et al*., 2008; Atawodi, 2010; Kouambou, *et al*., 2007; Okoli, *et al*., 2015). Specific compounds such as triterpenoic acids and canarene have been isolated from the resin and seeds, respectively (Tamboue, *et al*., 2000; Yousuf, 2011). These phytochemicals are responsible for a wide range of pharmacological activities, including antimalarial, antioxidant, antimicrobial, antidiabetic, analgesic, and nephroprotective effects (Obame, *et al*., 2007; Kamtchouing, *et al*., 2007; Ramadhan, *et al*., 2015; Dzotam, *et al*., 2015; Okwuosa, *et al*., 2009). The high medicinal and economic value of the species further underscores the importance of its conservation and domestication.

Seed dormancy is a common adaptive strategy that enables seeds to survive adverse environmental conditions and synchronize germination with favourable periods (Bentsink, 2008; Baskin and Baskin, 2014). In tropical tree species such as *C. schweinfurthii*, dormancy is often associated with hard seed coats and physiological constraints. Dormancy may be primary or secondary and can be classified as exogenous (physical or chemical) or endogenous (physiological, morphological, or morpho physiological) (Abubakar, 2022). Physical dormancy, caused by seed coat impermeability to water and gases, is particularly relevant in *C. schweinfurthii* due to its thick endocarp. Consequently, pre-sowing treatments such as soaking, mechanical or chemical scarification, stratification, and the use of growth regulators have been recommended to enhance imbibition and stimulate germination.

Traditional processing of *C. schweinfurthii* fruits commonly involves soaking to soften the hardened mesocarp and facilitate seed extraction. During soaking, water is absorbed through diffusion across cell walls and intercellular spaces until saturation is achieved, a process influenced by fruit size, maturity, structural characteristics, temperature, and chemical composition (Ehiem, *et al*., 2019). From a germination perspective, restricted water uptake due to seed coat hardness may limit imbibition, the initial and most critical stage of germination. Therefore, evaluating seed size and pre-sowing treatments is essential for identifying effective methods to overcome dormancy and improve germination performance.

Although considerable research has focused on the ethnomedicinal and pharmacological properties of *C. schweinfurthii*, information on its seed germination ecology and dormancy management remains limited. Given the species' poor natural regeneration, increasing exploitation pressure, and the influence of seed size and dormancy on germination success, systematic studies on pre-sowing treatments are necessary. This study contributes to existing knowledge by examining the effects of seed size and pre-sowing treatments on germination, thereby supporting improved nursery propagation, conservation, and sustainable domestication of *Canarium schweinfurthii*.

3. Materials and Methods

Study Site: The study was carried out at the nursery of the Federal College of Forestry, Jos Plateau State.

Sources of Planting Materials: Matured ripe fruits of *Canarium schweinfurthii* (Eng.) were collected randomly from a phenotypically superior mother tree in Jos Plateau State.

Seed Preparation: The seeds were sorted out into 3 sizes: small, medium and large based on physical structure. The seeds were then counted into the different quantity per replication. Each of the sizes had 90 seeds weight per replication. The small sized seed ranged between the weight **2g-4g** each, with the total weight of 90 seeds **176g.** The medium sized seed ranged between the weight of **7g-8.5g** each, with the total weight of 90 seeds **224g** and the large sized seeds ranged between the weight **9.5g-12g** with the total weight of 90 seeds, **264g.**

Soil Preparation: Top soil was mix with manure (cow dung) at the ratio of 2:1 and used for this experiment. The mixed soil was filled using a hand trowel into pots measuring 30cm in length and 25cm width, the pots were perforated to ensure proper drainage of water, and avoid water logging during watering. The mixed sand was filled into the pots and watered before the planting and after planting, the seeds were watered regularly in order to aid dormancy break.

Experimental Design and Treatment: Seeds were sorted according sizes (Small, Medium and Large). A Factorial Randomised Block design (FRBD) was used for this research. The experiment was made up of 6 pre-sowing treatment and 3 sizes giving 18 treatments combinations replicated 3 times giving a total of 54 field lay out. A total number of **270 seeds,** was sown. **Total number of pots used = 54 (**18 pots per replication, 5seeds per bag, 3 replications, 90 seeds per replication). **Total number of seeds planted: 270** (18 x 3 = 54bags, 18 x 5 =90seeds and 90 seeds x 3 replication = 270 / 54 x 5 = 270 total number of seeds planted)

The pre-sowing treatments used include: i. Control Treatment (T0): No treatment was applied to the seeds. **ii. Cold Water Treatment (T1):** Seeds were soaked in cold water for 48 hours (2 days). **iii. Hot Water Treatment (T2):** Seeds were soaked in boiled water until the water cooled. **iv. Sulfuric Acid Treatment (T3):** Seeds were soaked in sulfuric acid for 7 to 8 hours and then rubbed with sandpaper to reduce the outer layer. **v. Hormonal Treatment with Coconut Water (T4):** Seeds were soaked in coconut water for 12 hours, serving as a rooting hormone. **vi. Mechanical Abrasion Treatment with Sandpaper (T5):** Seeds were rubbed with sandpaper to reduce the seed coat.

Data Collection and Recording: Data was collected based on germination assessment. Germinated seeds were counted and recorded from the date of first germination until there was no more germination. A seed was considered to have germinated when the tip of the radicle emerges free from the seed coat. The germination parameters investigated are: Germination count, Number of leaves, Plant height, Stem girth, Leaf length and Leaf width.

Statistical Analysis: Data collected were subjected to Analysis of variance (ANOVA). Means were separated using Least Significant Difference (LSD) at 0.05 level of probability using R programming software.

4. Results and Analysis

Table 1 presents the summary of analysis of variance (ANOVA) for germination and early growth traits of *Canarium schweinfurthii* evaluated under a factorial randomized block design(FRBD), showing the effects of seed size (SIZE), pre-sowing treatment (TREAT), and their interaction (SIZE × TREAT).

For germination traits (GM5 - GM25), seed size had no significant effect, indicating that differences in seed mass did not influence germination progression. In contrast, pre-sowing treatments exerted a highly significant effect (**P ≤ 0.01), demonstrating that treatment-induced physiological modification of the seed was the primary factor controlling germination. The significant SIZE × TREAT interaction (*P ≤ 0.05) suggests that treatment responses varied slightly among seed size classes during germination, although seed size alone was not a determining factor.

For vegetative growth traits, including plant height (PH5 - PH25), number of leaves (NL5-NL25), stem girth (SG5 - SG25), leaf length (LL5 - LL25), and leaf width (LW5–LW25), seed size showed no significant effect across all measurement stages. Pre-sowing treatments, however, had significant to highly significant effects on all growth traits (P ≤ 0.05 to P ≤ 0.01), indicating that early physiological advantages gained during germination were sustained during seedling development.

The absence of significant SIZE × TREAT interactions for most growth traits suggests that treatment effects were consistent across seed sizes, implying that seedling growth responses were largely independent of seed size variation once germination was established.

Overall, the ANOVA results indicate that pre-sowing treatment was the dominant factor influencing both germination and early seedling growth in *C. schweinfurthii*, while seed size played a negligible role. These findings underscore the importance of treatment optimization rather than seed size selection for improving germination efficiency and early seedling performance in this species.

Table 1. Summary of ANOVA significance for germination and growth traits under FRBD

Trait group	**Traits**	**SIZE**	**TREAT**	**SIZE × TREAT**
Germination (GM)	GM5–GM25	NS	**	*
Plant height (PH)	PH5–PH25	NS	–*	NS
Number of leaves (NL)	NL5–NL25	NS	–**	NS
Stem girth (SG)	SG5–SG25	NS	–**	NS
Leaf length (LL)	LL5–LL25	NS	–**	NS
Leaf width (LW)	LW5–LW25	NS	–**	NS

Keynotes:

NS = not significant (P > 0.05);

*, **, *** = significant at P ≤ 0.05, 0.01 and 0.001, respectively.

Effect of treatments on germination percentage (%) at different days after sowing

Table 2 shows the effect of pre-sowing treatments on germination percentage of *Canarium schweinfurthii* at different days after sowing (GM5 - GM25). Significant differences among treatments were observed at all assessment periods ($P \leq 0.05$), indicating that germination response varied depending on the applied treatment.

Cold-water treatment consistently recorded the highest germination percentages throughout the observation period, with values increasing progressively from GM5 (1.11%) to GM25 (2.56%). At each germination stage, Cold-water treatment was statistically superior to most other treatments, indicating a faster and more uniform germination response.

Mechanical abrasion also resulted in relatively high germination percentages, particularly at early stages (GM5 and GM10), where it did not differ significantly from Cold-water treatment. However, from GM15 onward, germination under Mechanical abrasion increased more slowly and remained lower than that of Cold-water treatment, although still significantly higher than several other treatments.

Untreated seeds exhibited moderate germination across all sampling dates, with gradual increases from GM5 to GM25. Germination under Untreated seeds was generally lower than that of cold-water treatment and mechanical abrasion but higher than that observed under hot water treatment and Sulfuric acid scarification (H2SO4) treatment at most stages, indicating the inherent ability of *C. schweinfurthii* seeds to germinate without treatment, albeit at a reduced rate.

Acid scarification and hot water treatment resulted in low to moderate germination percentages across all days after sowing. These treatments showed limited improvement over time and were often not significantly different from each other, particularly at later germination stages.

The poorest germination performance was consistently observed in hot water treatment, which recorded minimal germination throughout the study period and remained significantly lower than all other treatments at all sampling dates.

Overall, the results indicate that cold-water treatment was the most effective treatment for enhancing germination of *C. schweinfurthii*, followed by mechanical abrasion, while acid and hot-water treatments were comparatively less effective.

Table 2. Effect of treatments on germination percentage (%) at different days after sowing

Treatment	GM5	GM10	GM15	GM20	GM25
T0	0.89 ab	1.67 ab	1.67 a	1.78 ab	1.78 ab
T1	0.00 c	0.11 c	0.33 c	0.33 c	0.33 c
T2	1.11 a	1.78 a	2.33 a	2.33 a	2.56 a
T3	0.44 bc	0.56 c	0.56 bc	0.56 bc	0.56 bc
T4	0.33 c	0.89 bc	1.33 abc	1.56 abc	1.56 abc
T5	1.00 a	1.44 ab	1.56 ab	1.56 abc	1.56 abc

Values are means; means followed by the same letter(s) are not significantly different at **$P \leq 0.05$ (LSD)**.

Effect of treatments on Final germination and vegetative growth responses to treatments

Table 3 summarizes the effects of pre-sowing treatments on final germination (GM25) and vegetative growth traits of *Canarium schweinfurthii* at 25 days after sowing. Significant differences among treatments were observed for all measured traits ($P \leq 0.05$).

Final germination percentage (GM25) varied significantly among treatments. Cold-water treatment recorded the highest germination value (2.56), which was significantly superior to most other treatments. Mechanical abrasion and hot-water treatment produced intermediate germination values and did not differ significantly from the control. The lowest germination was observed under Hot water treatment, followed by H2SO4 acid scarification.

Plant height at 25 days (PH25) was significantly influenced by treatment. Treatments Cold-water, Mechanical abrasion, Untreated Treatment, and Coconut water recorded higher plant height values and were statistically comparable, whereas Hot water treatment produced significantly shorter seedlings. Acid scarification resulted in intermediate plant height.

The number of leaves (NL25) also differed significantly among treatments. The highest leaf numbers were recorded under Mechanical abrasion, Cold water, Untreated, and Coconut water, which were statistically similar. Hot water produced the lowest number of leaves, while Sulfuric acid scarification showed intermediate performance.

Stem girth (SG25) exhibited significant treatment effects, with mechanical abrasion producing the highest value and being significantly superior to most other treatments. Treatments Cold water and Untreated treatment showed moderate stem girth, whereas Hot water, Sulfuric acid scarification, and Coconut water recorded comparatively lower values.

Leaf morphological traits were similarly affected by treatments. Leaf length (LL25) was highest under Cold water, Mechanical abrasion, untreated, and Coconut water, with no significant differences among these treatments. Hot water treatment recorded the shortest leaves. Leaf width (LW25) followed a comparable pattern, with Cold water treatment producing the highest value, Mechanical abrasion and Untreated treatment showing intermediate values, and Hot water remaining significantly lower than all other treatments.

Overall, the results indicate that pre-sowing treatments significantly influenced final germination and early vegetative growth traits of *C. schweinfurthii*, with cold water soaking and mechanical abrasion consistently associated with superior performance across multiple traits.

Table 3. Effect of treatments on final germination and vegetative growth traits under FRBD

Treatment	GM25	PH25	NL25	SG25	LL25	LW25
T0	1.78 ab	11.18 a	11.65 a	2.00 ab	7.40 a	4.42 ab
T1	0.33 c	2.22 c	3.17 b	0.58 b	2.22 b	1.25 c
T2	2.56 a	12.63 a	11.89 a	2.38 ab	9.17 a	5.42 a
T3	0.56 bc	5.78 bc	7.33 ab	1.48 b	4.94 ab	2.78 bc
T4	1.56 abc	10.33 ab	9.74 a	1.82 b	7.11 a	4.12 ab
T5	1.56 abc	11.50 a	12.56 a	3.71 a	8.94 a	4.84 ab

Values are means.
Means followed by the same letter(s) within a column are **not significantly different at $P \leq 0.05$ (LSD)**.

5. DISCUSSION

The present study investigated the effects of seed size and pre-sowing treatments on germination and early seedling growth of *Canarium schweinfurthii* (Engl.), a tropical tree species of ecological and genetic importance whose regeneration is constrained by poor germination. The results clearly demonstrated that germination and early growth responses were significantly influenced by pre-sowing treatments, whereas seed size had no significant effect, indicating that physiological dormancy imposed by the seed coat plays a more dominant role than maternal seed size variation.

Seed germination in *C. schweinfurthii* was generally low, as reflected by the low germination value recorded across treatments and seed sizes. Low germination value is indicative of delayed and uneven germination, a characteristic commonly associated with hard-coated seeds of forest tree species (Asiedu, *et al*. 2012). Such dormancy is an adaptive trait that enhances survival under natural conditions but poses a challenge for domestication, conservation, and genetic improvement programmes. Differences in seed coat permeability to water and gases among species have been reported as key factors regulating germination behaviour (Owonubi, *et al*. 2005), and this appears to be a major constraint in *C. schweinfurthii*.

Among the pre-sowing treatments evaluated, cold water soaking was consistently superior, resulting in the highest germination percentages and enhanced early seedling growth. Cold water soaking likely promoted gradual imbibition, leading to seed coat softening and activation of embryo metabolism. Similar moisture driven physiological responses have been reported in other hard seeded tropical species, where controlled imbibition enhances enzymatic activity and facilitates radicle emergence (Ehiem, *et al.* 2019). The improved performance of seedlings arising from cold-water soaking for 48 hours treated seeds suggests that enhanced germination translated into sustained vegetative growth, an important consideration for nursery establishment and ex situ conservation.

Mechanical abrasion also significantly improved germination and early growth, supporting the hypothesis that dormancy in *C. schweinfurthii* is primarily physical. Scarification likely reduced mechanical resistance of the seed coat, allowing direct water uptake and oxygen diffusion to the embryo. Mechanical disruption of hard seed coats has been widely reported as an effective dormancy-breaking method in woody species (Ghadiri and Torshiz 2000), and its effectiveness in the present study confirms its applicability for *C. schweinfurthii* propagation.

In contrast, acid scarification using 80% H_2SO_4 resulted in poor germination, suggesting embryo damage due to the corrosive nature of the treatment and prolonged exposure duration. Acid penetration through the micropyle may have adversely affected embryo viability, as previously reported for sensitive tree seeds (Muhammad and Amusa 2003). These findings indicate that high-concentration acid treatments are unsuitable for *C. schweinfurthii* and may compromise genetic material intended for conservation.

Untreated seeds exhibited moderate but delayed germination, confirming that *C. schweinfurthii* is capable of natural germination without intervention, albeit at a slow rate. Hot water treatment was ineffective, likely due to insufficient disruption of the hard seed coat, further emphasizing the importance of treatment specificity for this species.

The absence of significant seed size and SIZE × TREAT interaction effects across most traits suggests that treatment-induced physiological responses were stable across seed size classes. This finding has important implications for germplasm utilization, as it indicates that seed size selection may not be necessary for improving germination efficiency in *C. schweinfurthii*. Instead, optimizing pre-sowing treatments offers a more reliable approach for enhancing regeneration.

Overall, the study highlights the critical role of appropriate dormancy-breaking treatments in improving germination and early growth of *C. schweinfurthii*. The consistent superiority of cold-water soaking provides a simple, low-cost, and nondestructive method suitable for nursery propagation, conservation, and potential domestication of this underutilized and threatened tree species. These findings contribute valuable knowledge toward the sustainable management and conservation of *Canarium* genetic resources.

6. Conclusion

This study examined the growth response of *Canarium schweinfurthii* (Engl) seedlings to various pre-sowing treatments, revealing a moderate growth rate overall. Cold-water treatment, where seeds were soaked for 48 hours, proved to be the most effective method for enhancing growth. In contrast, treatments that drastically altered the seed coat, particularly the acid treatment, negatively impacted seedling growth. This suggests that the seed coat plays a protective role essential to the early growth stages, particularly for species like *C. schweinfurthii* with epigeal germination and foliaceous seedlings.

The findings align with Oboho (2015), indicating that while seedlings displayed moderate growth potential, there was no significant difference in growth response across different seed sizes. However, pre-sowing treatments did yield significant differences in growth outcomes. Therefore, any seed size of *C. schweinfurthii* can be utilized for planting, as growth response remained consistent across sizes in this study.

The early growth characteristics of *Canarium schweinfurthii* (Engl.) indicate a moderate growth rate in nursery conditions. Seedlings from small, medium, and large seed sources generally performed well, with growth parameters showing a similar trend across these sizes. While no significant differences were observed between the sizes and their interactions, there were notable differences among the treatments applied to the growth parameters studied.

To achieve rapid and uniform germination, various techniques and treatments are often applied to overcome seed dormancy, which can otherwise delay germination. These methods help reduce dormancy, though they may also lead to early sprouting if seeds encounter wet conditions before harvest. Given the importance of seed germination and dormancy traits, these practices are essential for optimizing seedling establishment and growth.

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Authors Contribution

The authors declare the following contributions to this manuscript: **Satdom, S.M. (Author 1)** conceptualized and designed the study, supervised the research, and contributed to manuscript drafting and revision; Izang, C. **(Author 2)** contributed to data collection, analysis, and initial drafting of the manuscript; Deshi, K.E. **(Author 3)** performed statistical analyses and contributed to the interpretation of findings; Nanbol, K.K. **(Author 4)** provided critical revisions and helped in refining the manuscript; **Odesina, S. I. (Author 5)** participated in study design, reviewed the manuscript critically and ensured alignment with the study's objectives; **Edward, N. B.** (**Author 6)** coordinated the overall execution of the study, contributed to writing, and approved the final manuscript. All authors have read and approved the submitted manuscript and agree to be accountable for all aspects of the work

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript**.**

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