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# Positive effect of warm water and acid scarification on seed germination of *Canarium schweinfurthii* Engl is diminished by sand substrate

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## Abstract

*Canarium schweinfurthii* is a multipurpose tree of the Burseraceae family whose population has declined tremendously in the Western Cameroon Highlands. The situation is exacerbated by a poor germination behavior, making propagation a valuable alternative for supply of regeneration material. This study examined the combined effect of pre-sowing treatments and substrates on germination and root development of the species. Treatments were comprised of three substrates (soil, sawdust, sand) and five pre-sowing treatments (control, water warm, acid, dry heat at 100 °C, dry heat at 60 °C). Sand significantly reduced Final Germination Percentage (*FGP*) while it increased Mean Germination Time (*MGT*). Germination Rate Index (*GRI*) declined from soil to sand and sawdust which did not differ from each other for this trait. Root length and number of roots were highest in sawdust while the latter was lowest in soil. As for the pre-sowing treatments, values of *FGP* and *MGT* were highest in warm water and lowest in the dry heat treatments in which germination was almost completely absent. No other parameter was significantly affected by pre-sowing treatment. There were significant 2-factor interactions for *FGP* and *MGT*. Although sand attenuated the effects of all three non-dry heat treatments on *FGP*, the margin was highest in warm water. On the other hand, the increase of *MGT* by sand was limited to the control and acid pre-treated seeds. The results suggest that sand may not be a suitable germination medium for pre-treated seeds of *C. schweinfurthii* while early post-germination root development of the species may be favoured by sawdust.

**Keywords:** dormancy, growth medium, pre-sowing treatment, seedling emergence

## Introduction

*Canarium schweinfurthii* is a fruit bearing tree species of the Burseraceae family. This family of angiosperms is comprised of about 18 genera of resin ducts trees and shrubs. The deciduous and perennial tree is found throughout tropical Africa [1, 2, 3], in rainforest, gallery forest and transitional forest from Senegal to Cameroon and extending to Ethiopia, Tanzania and Angola [4]. They contain seeds and fruits that are rich in protein, fats and oil, minerals and vitamins. Some of the species are used traditionally in the treatment of various illnesses. Extracts of the trees have been found to possess antimicrobial and antioxidant properties [5]. *Canarium schweinfurthii* is among the riverine fruit trees that are commonly found in the diet of most inhabitants of the Western Highlands of Cameroon. In addition to the direct consumption of its fruits, substantial amounts of money are generated from sale by many households. All parts of the tree are of medicinal importance: a decoction of the bark is used for treatment of hypertension, dysentery, gonorrhea, coughs, chest pains, pulmonary affections, stomach complaints, and food poisoning while the pounded bark is used against leprosy and ulcers; the root is used against adenitis; leaves are boiled with other herbs and the decoction used to treat coughs; powder from its roasted and pounded seeds is mixed with skin oil or jelly to treat wounds [6]. The mahogany-like wood is used as for furniture, mortars, drums, planks, canoes, fuel, and general utility purposes while the resin is used as a fumigant to ward off mosquitoes [7]. Seeds are also strung into necklaces, blinds or attached to traditional instruments. Due to the wide range of benefits, the exploitation of *C. schweinfurthii* has reduced its population and, in many cases, degraded the hosting ecosystem, making restoration a priority.

*Canarium schweinfurthii* can be propagated by sexual or vegetative methods, sexual propagation by seeds being important as it maintains genetic diversity and is also cheaper than asexual techniques [8]. Propagation through seeds is, however, both very slow and difficult because of the hard outer shell which inhibits water and oxygen uptake that translates to a low germination ability [2]. The impermeable layers of seed coat were developed during maturation and seed drying. Seed coat dormancy may be overcome with several pre-sowing treatments including dry heat, hot water, acid, and mechanical scarification methods which may help in removing or softening the seed coat [9, 10, 11]. In a study on the effect of pre-sowing treatment on germination of *C. schweinfurthii*, warm water and acid scarification significantly increased germination percentage, with germination time decreased and germination rate increased by the acid treatment [12].

Seed germination is a complex process controlled both by the internal properties of seeds and by an array of environmental factors including light quality, soil moisture, temperature, soil chemistry, and substrate [13, 14]. In particular, a good germination substrate needs to have adequate aeration and moisture [15]. Root growth after germination and survival of seedlings are also strongly substrate dependent. This study was therefore aimed at investigating the interactive effect of pre-sowing treatment and substrate on the germination of *Canarium schwienfurthii* seeds. However, abiotic factors often interact in affecting physiological processes and growth of plants in ways that do not directly reflect the sum of main effects. This study was aimed at exploring the combined effects of pre-sowing treatment and substrate on germination and root growth of *C. schweinfurthii*.

## Material and Method

**Description of study site.** The experiment was conducted at the Reforestation Task Force (RETAFO) nursery located at Mile 6 Nkwen in Bamenda III Sub Division. Bamenda III is one of the three Sub Divisions that make up the Bamenda council area in Mezam Division, North West Region, Cameroon. Bamenda III Sub Division is located between latitude 6°15' and 6°25'N of the equator and longitude 10° 02' and 10°15'E of the Greenwhich Meridian. It is bounded by the following Sub Divisions: Tubah to the West, Bamenda I to the north, Bamenda II to the east, and Bafut to the south. The Bamenda III municipality shows great ecological variations with a Guinea-Savannah type climate that is characterized by two distinct seasons, the dry season extending from mid-November to mid-March and the wet season from mid-March to mid-November resulting in a yearly rainfall of *ca* 2567 mm. and average annual temperature is 19.3 °C. A detailed account of the weather and climatic conditions for Bamenda are presented in Table 1.

**Table 1. Bamenda monthly weather and climate data for 1991 – 2021** [16]

| Month/ Condition | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Avg. Temperature (°C) | 21 | 21.6 | 20.9 | 19.6 | 19.1 | 18.3 | 17.7 | 17.6 | 17.9 | 18.2 | 19.2 | 20.3 |
| Min. Temperature (°C) | 14.6 | 15.6 | 15.8 | 15.8 | 15.7 | 15.4 | 15 | 15.1 | 15.1 | 14.8 | 14.3 | 14.6 |
| Max. Temperature (°C) | 28.5 | 29.1 | 27.7 | 25.6 | 24.6 | 23.2 | 22.1 | 21.9 | 22.7 | 23.5 | 25 | 27.3 |
| Precipitation / Rainfall (mm) | 9 | 29 | 129 | 293 | 298 | 332 | 358 | 346 | 380 | 298 | 86 | 9 |
| Humidity (%) | 39 | 42 | 61 | 82 | 87 | 89 | 90 | 90 | 90 | 87 | 71 | 47 |

**Experimental design.** The experiment was comprised of four pre-sowing treatments (warm water, acid, dry heat at 100 °C, dry heat at 60 °C), a control and three substrates (soil, sawdust, sand). The experiment that was carried out in a non-mist propagator followed a split-plot design with substrate as the main plot and pre-sowing treatments as the sub-plots. The propagator was made up of three chambers representing the substrates. Each chamber was subdivided into five subunits in which the pre-sowing and control treatments were assigned. The experiment was comprised of three replications. The experimental material was comprised of seeds collected from *C. schweinfurthii* trees in Bambili village (5º 59′ N, 10º 15′) in Tubah Sub-Division. The warm water treatment was administered by immersing the seeds in a plastic bucket containing water at 60 ˚C for 2 hours. The temperature was kept constant by circulating the temperature-regulated water through the bucket with the use of an electric pump whose heating function was adjusted to the target. As for the acid treatment, the seeds were soaked in dilute HCl in a bowl for 2 hours. The bowl containing the seeds-acid combo was agitated occasionally to prevent the seeds from sticking together and then sieved after the required duration. The dry heat at 100 °C was achieved by heating seeds in an oven for 2 hours. To ensure that the sowing was done at the same time for all the pre-treatments, the dry heat at 60 °C treatment had been started the previous day since it required oven-heating for 24 hours. Seeds in the control were untreated. There were 50 seeds in each treatment combination. Irrigation was done as per an indicator gauge built in the propagator. Any weeds emerging from the substrates were hand-picked. The experimental set-up was situated in a shade house roofed with alternating rows of aluminum and transparent plastic sheets.

**Data collection.** The number of seedlings that emerged from the substrate was recorded daily. When there was no further emergence for a period of three weeks in any treatment combination, the seeds from which no parts had protruded the substrate were carefully taken out and examined for germination. Since none of dug-up seeds showed a sign of germination, the process was considered complete and the experiment was terminated. Root growth measurements were performed in the control, warm water and acid pre-treatments but not the dry heat treatments in which germination was generally absent. Five seedlings were randomly chosen from the control, warm water and acid pre-treatments and carefully uplifted from the substrate. Each seedling was rinsed free of substrate after which root system length (Rl) and number of roots (Nr) were determined. The dry heat pre-sowing treatments were excluded from root growth measurement because of a general absence of germination.

The following parameters were calculated from the germination data:

$$Final\ Germination\ Percentage\ (FGP) = \frac{SG}{TP} \times 100$$

where SG = seeds germinated, and TS = total seeds sown.

$$Mean\ Germination\ Time\ (MGT) = \Sigma(n\ \times d)/N$$

where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment [17].

$$Germination\ Rate\ Index\ (GRI) = [(G_1/1) +\ (G_2/2) +\ (G_3/3) + \ldots +\ (G_x/X)]$$

where G = germination on each day after sowing; 1, 2, 3, *X* = corresponding day of germination [18].

**Data analysis.** The data were examined for homoscedasticity and normality using histograms and normal probability plots before being subjected to split-plot ANOVA. When the results of the ANOVA was significant for a particular parameter, means separation was conducted with Tukey HSD. All the tests were run in Data desk 6.0 at $p \leq 0.05$.

## Results

**Final Germination Percentage.** Pre-sowing treatment and substrate significantly affected *FGP* both in isolation and in combination (Table 2). Unlike in the 60 °C and 100 °C dry heat pre-treatments where germination was almost completely absent, *FGP* of untreated seeds and those treated with warm water and acid was significantly reduced by sand (Figure 1).

**Table 2. ANOVA *F*-values for the effect of substrate, pre-sowing treatment, and their interaction on germination and root growth**

| Source | *FGP* | *MGT* | *GRI* | Rl | Nr |
|---|---|---|---|---|---|
| Substrate | 45.458*** | 8.1971*** | 5.5419** | 6.9237** | 17.623*** |
| Pre-treatment | 121.65*** | 551.49*** | 1.5625$^{ns}$ | 1.4689$^{ns}$ | 0.90629$^{ns}$ |
| Substrate × Pre-treatment | 9.3137*** | 6.8382*** | 2.0231$^{ns}$ | 1.3787$^{ns}$ | 0.35954$^{ns}$ |

* $p \leq 0.05$; ** $p \leq 0.01$; $p \leq 0.001$; $p > 0.05$.

*FGP* = Final Germination Percentage; *MGT* = Mean Germination Time; *GRI* = Germination Rate Index; Rl = Root length; Nr = Number of roots.

The ranking of pre-sowing treatments for this parameter was dependent on substrate. In soil, it was highest in warm water and lowest in the dry heat pre-treatments. However, the difference between warm water and acid was statistically significant in this substrate treatment. A similar trend was observed in sawdust with the difference that the pre-treatments that displayed a comparable response of this trait were acid and the control.  As for sand, the acid pre-treatment resulted in the highest *FGP*, the dry heat treatments resulted in the least while the warm water and the control treatments were not different from each other (Figure 1).

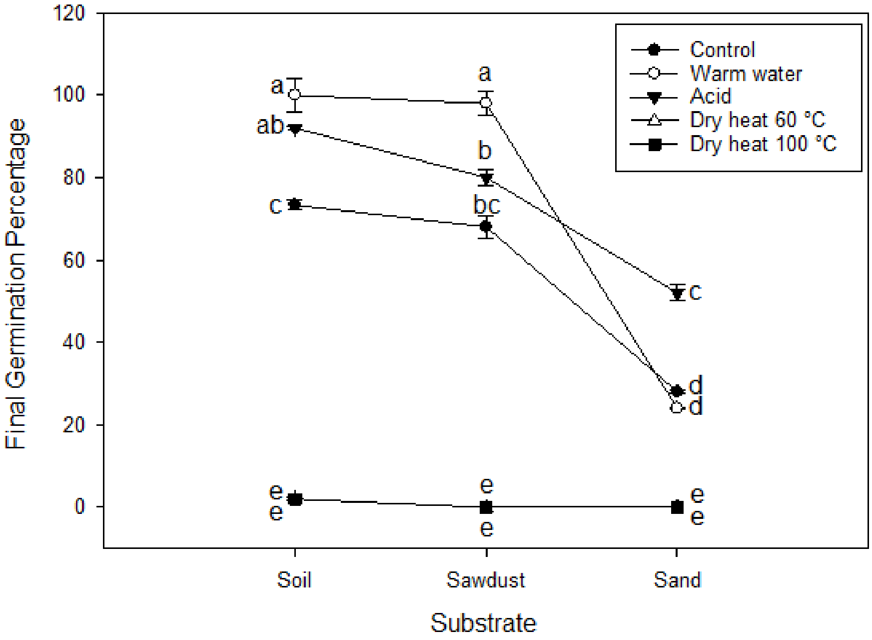

**Fig. 1. Effect of substrate and pre-sowing treatment on FGP.**
**Values are mean ± SE. Different letters indicate significant differences between combinations of substrate and pre-sowing treatment.**

**Mean Germination Time.** There was a significant effect of substrate, pre-sowing treatment, and their interaction on *MGT* (Table 2). Like FGP, the dry heat treatments suppressed *MGT* in all substrates. For the other three pre-treatments, values of *MGT* were highest for warm water in sand and lowest for acid in sawdust (Figure 2). However, the differences in *MGT* between the acid pre-treatment in sawdust and either acid or control in soil was statistically insignificant. Similarly, the warm water pre-treatment was not significantly different from warm water in soil which displayed comparable responses of *MGT* with the other remaining treatments (Figure 2).

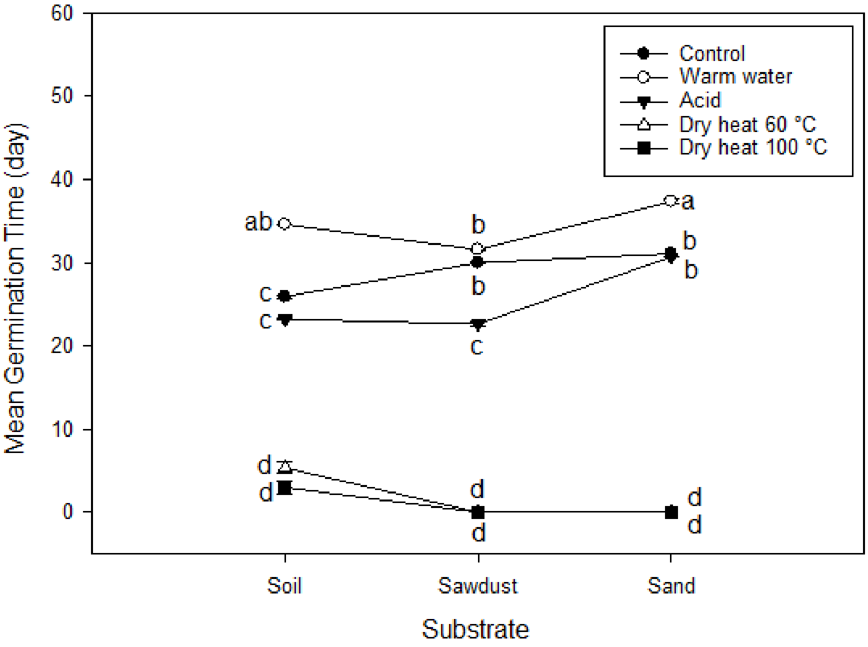

**Fig. 2. Effect of substrate and pre-sowing treatment on MGT. Values are mean ± SE. Different letters indicate significant differences between combinations of substrate and pre-sowing treatment.**

**Germination Rate Index:** GRI was significantly affected by substrate. It declined from soil to sawdust and sand which did not differ for the trait (Figure 3). There was no significant main or interactive effect of pre-sowing treatment on GRI (Table 2).

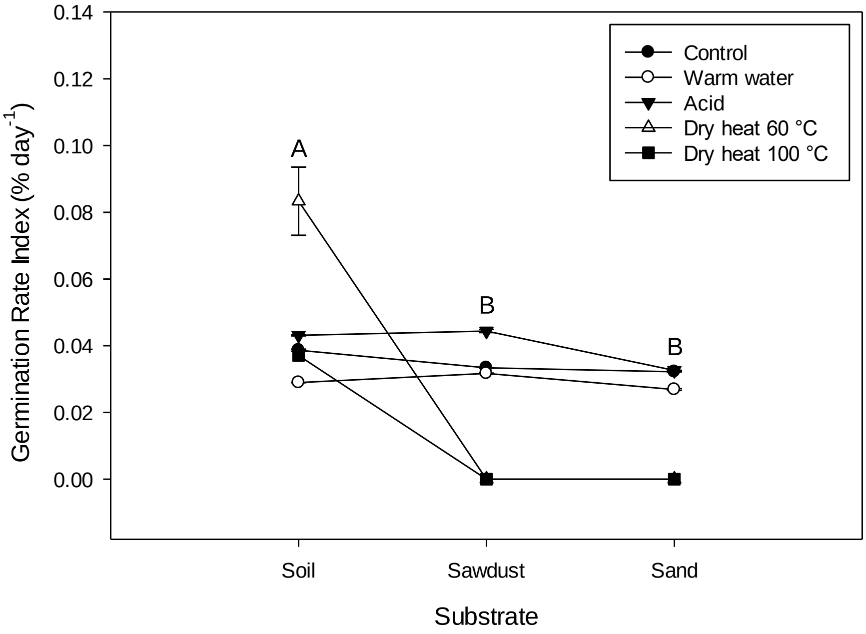

**Fig. 3. Effect of substrate and pre-sowing treatment on GRI. Values are mean ± SE. Different letters indicate significant differences between substrates.**

**Root growth.** The length and count of roots responded to substrate but not to pre-sowing treatment or its interaction with substrate (Table 2). Rl was highest in sawdust and lowest in sand. However, differences between soil and either sand or sawdust were not significant (Figure 4 *i*). As for Nr, all the three substrates differed from one another with values highest in sawdust and lowest in soil (Figure 4 *ii*).

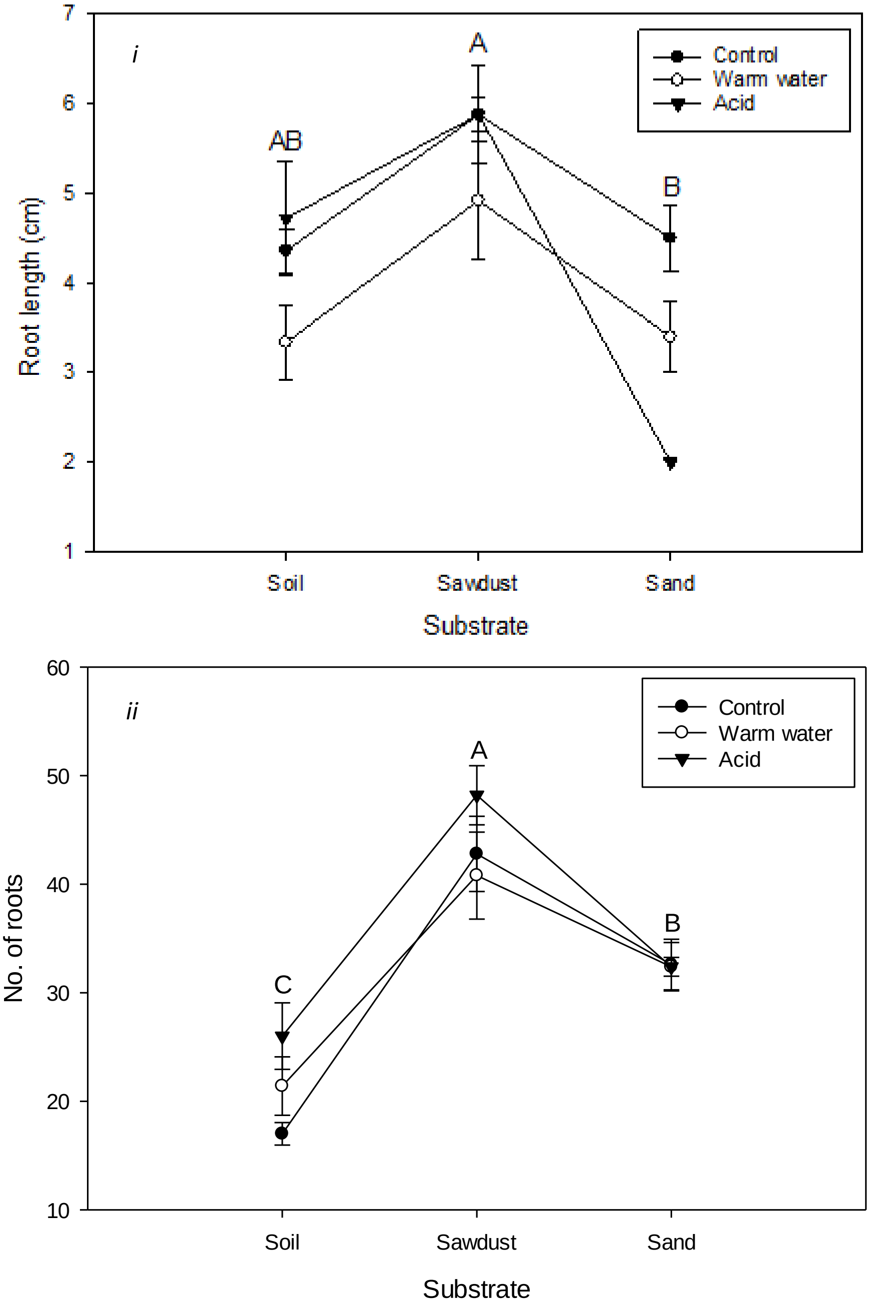

**Fig. 4. Effect of substrate and pre-sowing treatment on Rl (i) and Nr (ii).**
**Values are mean ± SE. Different letters indicate significant differences between substrates.**

## Discussion

**Effect of pre-sowing treatment.** The nut of *Canarium schweinfurthii* has a hard shell which is highly lignified and contains holocellulose [19], making it impermeable to water and oxygen. For germination to take place, the hard shell needs to be broken. To ensure proper germination and healthy seedling establishment of species with hard seed dormancy, the seeds are subjected seeds to various kinds of pre-sowing treatment by mechanical means or with the use water and different chemicals [20, 21, 22, 23, 24]. Pre-sowing soaking or scarification treatments improves rate of germination by encouraging the metabolic process in the seed [25].

The beneficial role of warm water in improving germination, as observed in *C. schweinfurthii*, is associated with its ability to inflict cracks in seed coat while leaving the anatomy of the micropyle unaltered [26]. The breaking of the physical barrier to the entry of water and oxygen into the seed activates metabolic processes for germination [27]. As also detected in this investigation, acid scarification can be a useful alternative to the warm water pre-treatment for breaking seed coat dormancy in *C. schweinfurthii* as both treatments resulted in statistically comparable germination percentages. Additionally, the incubation of the seeds with hydrochloric acid speeded up the germination process so that the time for its completion was substantially shortened, a phenomenon that has been reported by other investigators [28, 29, 30]. Acid reduces the thickness of the water-repelling testa, permitting the uptake of water and respiratory gases [30, 31, 32, 33].

In contrast to the other pre-sowing treatments, the dry heat scarification treatments inhibited germination of *C. schweinfurthii* seeds. Instead of causing the seed coat to develop cracks as is the case with the other form of thermal scarification, the oven-drying dehydrated the seed to an extent that enzyme activation was adversely affected [34]. Furthermore, dry heating has the potential to further harden the seed coat with the outcome that the impermeability is enhanced.

**Effect of substrate.** Sand trailed behind soil and sawdust in final germination percentage of *Canarium schweinfurthii* seeds. Similar results have been reported for seeds of many others species [15, 35], although sand has suggested to be a suitable medium for seed germination in some species [36]. The finding of the present study may be attributed to differences in physical properties of the substrates [37]. A suitable substrate for seed germination should be characterized by adequate aeration and moisture [15]. The non-mist propagator was constructed such that irrigation water was taken up by substrates through capillary action from an underlying water table. With a high water holding capacity, the soil and sand remained moist throughout the experiment. On the other hand, an abundance of air-filled macrospores in sand left it with a low capacity for water absorption. However, a greater bulk density of soil than sawdust resulted in lower root growth in the former.

**Effect of pre-treatment and substrate.** Variable pre-sowing treatment related interactions on germination and seedling growth have been reported for different plant species, including *Acacia cyanophylla* (pre-sowing treatment × temperature; [38]), *Jatropha cucas* (pre-sowing treatment × genotype; [39]), pre-sowing treatment × seed size × potting mixture [40], *Khaya senegalensis* (pre-sowing treatment × seed orientation; [41]), *Albizia Lebbek* (pre-sowing treatment × potting medium; [42]), and *Garcinia cola* (pre-sowing treatment × potting medium; [43]). For *Canarium schweinfurthii*, it has been determined from this study that the beneficial effect of warm water and acid pre-treatment on germination of may be suppressed by sand.

## Conclusions

This study has explored the effects of pre-sowing treatments and substrates on seed germination and root development of *Canarium schweinfurthii.* While warm water and acid scarification were found to be the most effective for enhancing germination, oven-heating appeared to be damaging to the process as it resulted in approximately which was far below values for untreated seeds. However, the positive effects of the pre-treatments may diminish if sand rather than soil or sawdust are used as substrate.

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