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GERMINATION AND SEEDLING GROWTH RESPONSE OF *Aframomum melegueta* K. SCHUM TO DIFFERENT PRE-TREATMENTS

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ABSTRACT

An experiment was carried out to check the effect of pre-treatments on germination and early seedlings growth of Aframomum melegueta at the Department of Forestry and Wildlife Management of University of Port Harcourt. The research seeks to investigate the effect of pre-germination treatment on seeds of A. melegueta. The treatments used were Hydrogen peroxide (10 minutes), Cold water (24 hours), Methylated spirit (10 minutes), warm sand at 45°C (30 minutes), Nicking and Control. The experimental design was the completely randomized design (CRD) while analysis of variance (ANOVA) was used to test the effect of treatments on seeds and seedlings of A. melegueta. Result from this study indicated that in nicked seeds germination started 11days after sowing while it was 14 days after sowing in hydrogen peroxide treated seeds. Germination duration was lowest in hydrogen peroxide treated seeds (28.67 days) and highest in seeds buried in warm sand (36.33 days) while germination percentage was lowest in nicked seeds (56%) and highest in seeds soaked in cold water (92.67%). Seedlings of A. melegueta subjected to different pre-treatments displayed significant differences ($P \leq 0.05$) in all growth parameters at all stages of growth. Seedlings in warm sand exhibited the greatest seedling height, collar diameter and leaf number while seeds soaked in methylated spirit displayed lowest seedling height and leaf number; and seedlings treated with hydrogen peroxide had the lowest collar diameter. It was observed that seeds of these species did not exhibit dormancy. However, to enable optimum, rapid, uniform germination and good growth, it is recommended that the seeds of A. melegueta should be pre-treated before sowing.

Keywords*: Aframamum melegueta*, pre-treatment, germination, seedling growth.

INTRODUCTION

Aframamum melegueta commonly called alligator pepper is a species in the ginger family, *Zingiberaceae* (Obike *et al.,* 2014). According to Ajayi *et al.*, 2016, the species is native to the tropics and commonly found in swampy habitats of Nigeria, Uganda, Angola, Benin, Gambia, Ghana, Guinea, Cote d'Ivoire (Ivory Coast), Liberia, Sierra Leone, Togo, Cameroon, Congo, Gabon and Guinea-Bissau in West Africa and as far as the Democratic Republic of the Congo and is also widely cultivated in other parts of tropical Africa and South America. It has a tufted leafy stem that grows up to 1.5m high; the leaves are simple, alternate and lanceolate and can grow as long as 40cm and 12cm-15cm wide when mature (Ajayi *et al.,* 2016). It produces purple-coloured flowers which develop into pods as long as 8cm and about 3cm wide. A pod can contain about 300 reddish-brown seeds (Anjah *et al.*, 2015).

The seed looks like cardamom in appearance and pungent and are reddish-brown in colour (Umukoro and Aladeokin, 2011). They are also used for alleviating stomach ache and diarrhoea as well as hypertension, as an aphrodisiac and against measles and leprosy (Kokwaro, 1993). They are taken for excessive lactation and post-partum haemorrhage and are used as purgative, anthelmintic and haemostatic agent (Dzoyem *et al.*, 2017). The seeds contain 1-2% essential oil with a typical odour (Ajaiyeoba and Ekundayo, 1999).

Dormancy is an obstacle to the germination of sown seeds and may be caused by physical or physiological factors. Physical dormancy is as a result of hard impervious seed coat which prevents water and oxygen from entering the embryo, an underdeveloped embryo or some combination of these factors. Dormancy results in irregular germination of seeds and consequently resulting in production of nursery stock of varying ages and sizes. This phenomenon has necessitated the need to device a means of breaking dormancy through seed pre-treatment (Adedire and Oladoye, 2008). Dormancy in seeds can be broken naturally by mechanical abrasion by rocks in the soil, changes in the environmental conditions around the seed, that is, alternate thawing and freezing or in some cases, bacterial action while some artificial methods include the use of hot water, cold water, hydrogen peroxide, tetraoxosulphate IV acid (H_2SO_4), mechanical scarification using knife, plier, sandpaper, pin file, nail cutter, hammer etc. (Schmidt, 2007). Pre-germination treatment has helped silviculturist as well as farmers to hasten germination of their seeds, obtain a more even germination as well as increase productivity. (Ajiboye *et al.*, 2009).

The species is endangered due to deforestation. Forest loss is occurring throughout Africa, knowing that this species is found commonly in the tropical rain forest, this suggests that loss of forest habitat will affect the population of this species. Secondly, seed dormancy is a serious problem of the species in the nursery. Based on the problem mentioned above, there is need as to what can be done to develop an effective propagation technique for its regeneration. The study on the germination and early seedling growth of *A. melegueta* can provide some information on its biology for regeneration and conservation; this justifies the need for the study. The objective of this study was therefore to determine the effect of different pre-treatment methods on germination and early seedlings growth of *A. melegueta.*

MATERIALS AND METHODS

Study Location

The research was carried out at the nursery site of the Department of Forestry and Wildlife Management, Faculty of Agriculture, University of Port Harcourt which lies on Latitudes 4.90794 and 4.90809 N and Longitudes 6.92413 and 6.92432 E in Obio/Akpor Local Government Area of Rivers State (Chima *et al.*, 2017).

Fruit Collection and Seed Processing

The fruit was collected from the swampy forest research station of the Forestry Research Institute of Nigeria (FRIN) Benin city, Edo State, Nigeria which lies between latitudes 6°11 to 6°29 N and longitude 5°33 to 5°47 (Erhabor, 2015). The seeds were extracted manually. The processed seeds were subjected to viability test through floatation method, the seeds that floated after ten minutes of soaking were considered unviable and discarded. The seeds that sank were collected and regarded as viable seeds used for the study.

Experimental Design and Treatment Procedure

The Completely Randomized Design (CRD), involving 6 treatments with 3 replicates was used in this study. The pre-germination treatments involved soaking seeds of *A. melegueta* in hydrogen peroxide (10 minutes (T_1), cold water (24 hours) (T_2), methylated spirit for (10 minutes) (T_3), burying in warm sand at 45^oC (30 minutes) (T_4), Nicking (T_5), and control (untreated) (T_6). A total of one thousand eight hundred (1800) seeds of *A. melegueta* were used for the experiment at 300 seeds per treatment. Pre-treated seeds were sown in germination trays, filled with washed and sterilized river sand to prevent damping off and were placed inside a propagator to conserve moisture, maintain the temperature of about 36^0C and to protect the plants from rodent attack. The trays were monitored and watered daily in the morning to maintain adequate moisture content. Germination was said to have occurred when the plumule emerged from the soil surface.

Early Seedlings Growth

For each treatment, 20 seedlings of uniform height at two (2) leaves stage were transplanted into 20 polypots with each of the polypots taken as a replicate of its own. The polypots were filled with forest topsoil and organic manure in the ratio of 5:1. A total of 120 seedlings were used. Seedlings were watered daily and measured immediately after transplanting and bi-weekly thereafter.

Data Collection

Germination

Germination count was taken daily until no more germination occurred (50 days). Data collected on germination was used to calculate germination percentage (GP), germination emergence (GE) and germination duration (GD) for each treatment using the formulae below.

Germination Percentage (GP) = $\frac{Number\ of\ germinated\ seeds}{Number\ seeds\ sown} * \frac{100}{1}$

Germination emergence (GE) = time to germinate after sowing.

Germination duration (GD) = period of germination emergence to the end of germination.

Early seedling growth

Data was collected on early seedlings growth parameters for twelve (12) weeks. Seedling height was measured from the substrate level to the tip of the youngest leaf using a meter rule; stem collar diameter was measured at the root collar using a digital calliper while leaf and tiller production were determined by directly counting the number of leaves and tillers.

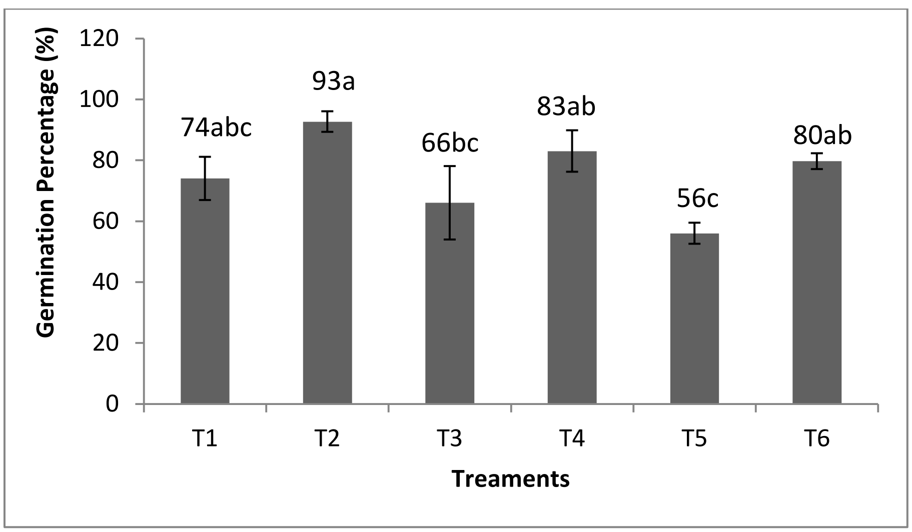
Data analysis

Data collected on germination and early seedling growths were analysed using SPSS statistical software (SPSS version 18, SPSS Inc.). Analysis of variance was carried out to test the effect of treatments on seeds and seedlings of *A. melegueta* and Duncan Multiple Range Test (DMRT) at $p \leq 0.05$ level of significance was used for means separation.

RESULTS

Effect of pre-treatments on germination percentage (GP) of *A. melegueta*

The Analysis of Variance (ANOVA) revealed that germination percentage of *A. melegueta* was affected significantly ($p \leq 0.05$) by the pre-treatments applied in the experiment. Seeds in T_2 exhibited the highest germination percentage (93%), followed by T_4 (83%) while seeds in T_5 exhibited lowest germination percentage (56%) followed by T_3 (66%) as shown in Figure 1.

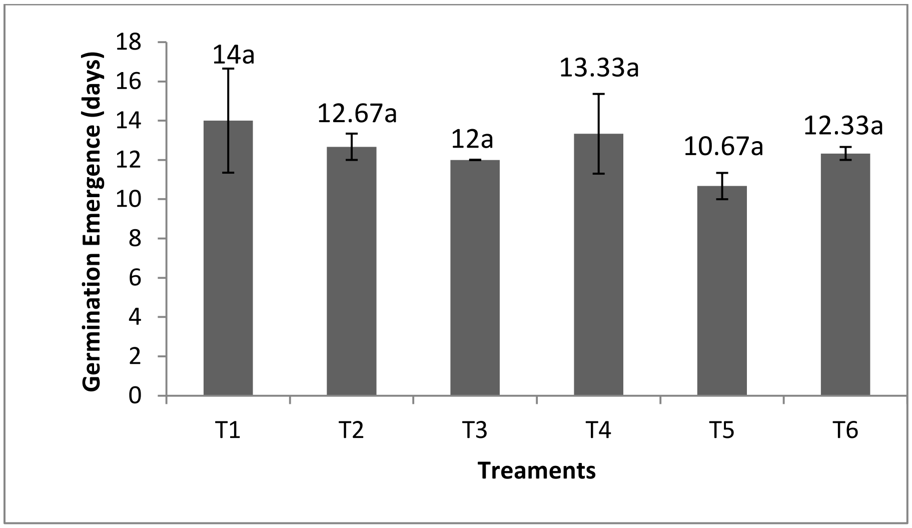


Bars with the same letter (s) are not significantly different at the 0.05 level. Where T_1, T_2, T_3, T_4, T_5 and T_6 correspond to soaking in hydrogen peroxide, soaking in cold water, soaking in methylated spirit, burying in warm sand, nicking, and control respectively

Figure 1. Mean germination percentage of *A. melegueta.*

Effect of pre-treatments on germination emergence (GE) of *A. melegueta*

The results showed that there were no significant differences ($p \geq 0.05$) between the pre-treatments used in the experiment. The number of days in which pre-treated seeds of *A. melegueta* took to emerge after sowing was shortest in T_5 (10.67 days) when compared to T_3 (12 days), T_6 (12.33 days), T_2 (12.67 days) and T_4 (13.33 days) while the longest seedling emergence period was observed in T_1 (14 days) as shown in figure 2.

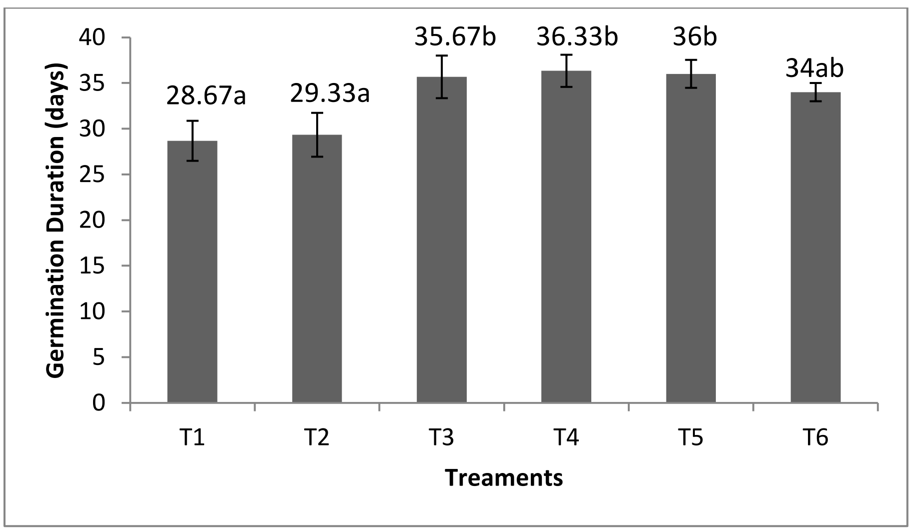


Bars with the same letter (s) are not significantly different at the 0.05 level. Where T_1, T_2, T_3, T_4, T_5 and T_6 correspond to soaking in hydrogen peroxide, soaking in cold water, soaking in methylated spirit, burying in warm sand, nicking, and control respectively.

Figure 2. Mean germination emergence of *A. melegueta.*

Effect of pre-treatments on germination duration (GD) of *A. melegueta*

Seed pre-treatments significantly ($p \leq 0.05$) affected germination duration. Seeds in T_1 exhibited lowest germination duration (28.67 days) followed by seeds in T_2 (29.33 days), T_6 (34 days), T_3 (35.67 days), T_5 (36 days) and T_4 (36.33 days) Summary of this result is presented in Figure 3.



Bars with the same letter (s) are not significantly different at p> 0.05 level. WhereT_1, T_2, T_3, T_4, T_5 and T_6 correspond to soaking in hydrogen peroxide, soaking in cold water, soaking in methylated spirit, burying in warm sand, nicking, and control respectively.

Figure 3. Mean germination duration of *A. melegueta*.

Effect of pre-treatments on seedling height (cm) of *A. melegueta*

Seedlings of *A. melegueta* subjected to different pre-treatments displayed significant difference ($P \leq 0.05$) in height at week 2 - 12 after transplanting. Overall mean seedling height at 2 - 12 weeks varied from 1.43cm at week 2 to 6.03cm at week 12. At week 2, seeds buried in warm sand had highest seedling height (2.22cm), followed by nicked seeds (1.85cm), while hydrogen peroxide treated seeds had lowest seeding height (1.43cm) followed by seeds soaked in cold water (1.54 cm) which was not significantly different from seeds soaked in methylated spirit (1.59cm). At week 4 to 12, highest seeding height was observed in warm sand treated seeds (2.76, 3.05, 5.03, 5.53 and 6.03 cm respectively), followed by nicked seeds (2.22, 2.72, 4.22, 4.72 and 5.22 cm respectively) while lowest height was observed in seeds soaked in cold water (1.90cm) at week 4 and seeds soaked in methylated spirit at month 6 to 12 (2.33, 3.55, 4.05 and 4.550 cm respectively). Summary of this result is presented in Table 1

Table 1. Effect of pre-treatment on mean seedling height (cm) of *A. melegueta* seeds from two to twelve weeks after sowing

Treatment	Seedling Height (cm) (Bi-weekly)					
	2	4	6	8	10	12
Hydrogen peroxide for 10 mins	1.43^{d}	2.17^{b}	2.71^{b}	3.70^{c}	4.20^{c}	4.70^{c}
Cold water (24 hrs)	1.54^{cd}	1.90^{b}	2.52^{bc}	3.60^{c}	4.10^{c}	4.60^{c}
Methylated spirit for 10 mins	1.59^{cd}	1.97^{b}	2.33^{c}	3.55^{c}	4.05^{c}	4.55^{c}
Burying in warm sand at 45^{o}C for 30 mins	2.22^{a}	2.76^{a}	3.05^{a}	5.03^{a}	5.53^{a}	6.03^{a}
Nicking	1.85^{b}	2.22^{b}	2.72^{b}	4.22^{b}	4.72^{b}	5.22^{b}
Control (untreated)	1.72^{bc}	2.14^{b}	2.56^{bc}	4.20^{b}	4.70^{b}	5.20^{b}
Mean	1.72	2.19	2.65	4.05	4.55	5.05
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values in the same column with the same letter (s) are not significantly different at the 0.05 level.

Effect of pre-treatments on seedling leaf number of *A. melegueta*

Seedlings of *A. melegueta* subjected to different pre-treatment displayed significant difference ($P \leq 0.05$) in height at weeks 2 - 12 after transplanting. Overall mean seedlings leaf number varied from 2.50 at week 2 to 7.90 at week 12. At week 2, control had highest seedling leaf number (3.05mm), followed by seeds buried in warm sand (3.00mm) while seeds soaked in methylated spirit had lowest seeding height (2.50mm) followed by seeds soaked in cold water (2.70 mm). At week 4 to 12, highest mean leaf number was observed in seeds buried in warm sand (4.05, 4.90, 5.90, 6.90 and 7.90 respectively) while lowest mean height was observed in seeds soaked in methylated spirit (3.15. 3.60, 5.05, 6.05 and 7.05 respectively) as shown in Table 2.

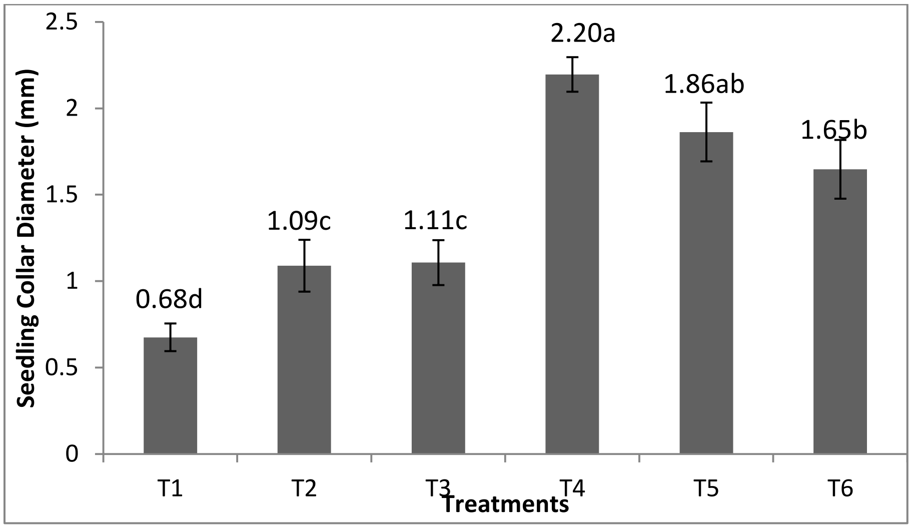
Table 2. Effect of pre-treatment on mean seedling leaf number of *A. melegueta* seeds from two to twelve weeks after sowing

Treatment	Mean Seedling Leaf Number (Bi-weekly)					
	2	4	6	8	10	12
Hydrogen peroxide for 10 mins	2.90^{ab}	3.60^{bc}	4.25^{b}	5.70^{a}	6.70^{a}	7.70^{a}
Cold water (24 hrs)	2.70^{bc}	3.40^{cd}	4.15^{b}	5.45^{ab}	6.45^{ab}	7.45^{ab}
Methylated spirit for 10 mins	2.50^{c}	3.15^{d}	3.60^{c}	5.05^{b}	6.05^{b}	7.05^{b}
Burying in warm sand at 45^{o}C for 30 mins	3.00^{ab}	4.05^{a}	4.90^{a}	5.90^{a}	6.90^{a}	7.90^{a}
Nicking	2.90^{ab}	3.50bc	4.40^{b}	5.75a	6.75^{a}	7.75^{a}
Control (untreated)	3.05^{a}	3.80^{ab}	4.50^{b}	5.80^{a}	6.80^{a}	7.80^{a}
Mean	2.84	3.58	4.30	5.61	6.61	7.61
P-value	0.003	<0.001	<0.001	0.001	0.001	0.001

Values in the same column with the same letter (s) are not significantly different at the 0.05 level.

Effect of pre-treatment on seedling collar diameter (mm) of *A. melegueta* at week 12

The results showed that there was significant difference ($p \leq 0.05$) between the pre-treatments used on *A. melegueta* in the experiment at 12 weeks. Growth in seedling collar diameter was highest in T_4 (2.20mm), followed by T_5 (1.86mm) and lowest in T_1 (0.68mm).



Bars with the same letter (s) are not significantly different at the 0.05 level. Where T_1, T_2, T_3, T_4, T_5 and T_6 correspond to soaking in hydrogen peroxide, soaking in cold water, soaking in methylated spirit, burying in warm sand, nicking, and control respectively.

Figure 4. Mean collar diameter of *A. melegueta* twelve weeks after planting.

DISCUSSION

The six (6) pretreatments including the control were significantly different from each other at 5% level of probability for all parameters except for germination emergence. The range of emergence of *A. melegueta* was between 10.67-14 days. This differed from the results obtained by Omokhua *et al.*, (2015a) who worked on *Maesobytra bateri*; the authors reported a range of 4-22 days. Similarly, Oboho and Igharo (2017) reported a range of 18-31days when *Pycnanthus angolensis* was subjected to pre-germination treatments in the nursery. In addition, Billah *et al.*, (2015) reported a range of 17-28days when *Tectona grandis* seeds were subjected to various pre-germination treatments. The implication of this study is that different forest species respond in various ways to pre-treatments.

Also, duration of germination varied among the 6 pretreatments used on seeds of *A. melegueta* at 5% level of probability. The range of germination duration was 29-36days. Hydrogen peroxide had the best germination performance, with the lowest germination duration of 29days compared to the other pretreatments. This is similar to the work of Omokhua *et al.*, (2015a) and Fredrick *et al.*, (2017) who reported that hydrogen peroxide had the best result on duration of germination in *Maesobytra bateri* (14days) and *Trichilia tessmannii* (7days) respectively compared to other treatments. Also Olatunji *et al.*, (2012) worked on pre-treatments of *Acacia auriculformis* seeds with hydrogen peroxide. The authors reported that hydrogen peroxide treatment was the best with germination duration of 7days. The implication of these findings is that hydrogen peroxide is an effective pre-germination treatment agent for rapid, uniform and maximum propagation of forest species.

The percentage germination of *A. melegueta* was affected by the type of pre-treatments used in this study. Seeds that were treated with cold water had the highest percentage germination, while the least percentage germination was in seeds treated by nicking. This result clearly showed that cold water is an excellent pretreatment for *A. melegueta*. This supports the fact that seed germination is a function of water available to the seeds, the dry dormant seeds absorb moisture by imbibition. This process stimulates the rapid and uniform emergence of the radicle (Hartmann *et al.*, 2002). Billah *et al.*, (2015) observed similar trends with the performance of cold water which had 73.3% germination for *Tectona grandis*. Mabundza *et al.*, (2010) also reported a germination percentage of 71% when cold water was used to treat passion fruit seeds. This result however is contrary to that of Missanjo *et al.*, (2014) and Fredrick *et al.*, 2016 who reported that nicked seeds had highest germination percentage in *Acacia polyacantha* and *F. albida* respectively when compared to other pre-treatment methods used in their study. According to Luna *et al.*, 2009, some necessary conditions which allow seeds to "break" dormancy and germinate can vary greatly among species, within a species, or among seed sources of the same species.

Survival of *A. melegueta* was excellent for all the pretreatments with 100% recorded; this implies that there was no seed mortality after transplanting. This result does not agree with the work of Missanjo *et al.,* (2014). The authors reported a range of seedlings survival percentage of 44.3-97.40% on *Acacia polyacantha* in the nursery.
Seedlings height growth of *A. melegueta* at 12weeks varied from 4.55-6.03cm for the treatments. Seeds treated with warm sand had the highest seedlings growth of 6.03cm. This species is slow-growing compared to a report by Ehiagbanare and Onyibe (2007). The authors reported a range of 18-46cm on seedlings growth of *Tetracarpidum conophorum* at 6weeks. The report is similar to the range of early seedling height growth reported on *Acacia polyacantha* by Missanjo *et al.,* (2014) with 6.46-9.37cm at 12weeks. Also Olatunji *et al.,* (2012) reported early seedling height growth range of 4.61-5.92cm at 4 months after transplanting. However, the implication of this is that the temperature of the warm sand (45^0c) most likely positively affected germination and consequently early growth of *A. melegueta* seedlings.

The study revealed a significant difference at 5% level of probability in leaf production among the treatments which varied from 7.05 in methylated spirit to 7.90 in warm sand. An average of 8 leaves was produced per plant at 12weeks. The mean number of leaves produced monthly was 3 leaves, this is similar to the report by Missanjo *et al.,* (2014), who reported monthly a mean leaf production of 2 leaves in *Acacia polyancatha*. The implication of this study is that the more the leaves, the more the amount of leaf area available for photosynthetic activities, the greater the amount of photosynthate and the higher the growth of the subject forest species. A higher leaf number observed in control seedlings implies that *Aframamum melegueta* seeds do not require much pretreatment to improve in leaf production.

The effect of pretreatments of *A. melegueta on s*eedling collar diameter (mm) revealed significant effect of pretreatment. Warm sand treatment at 45^0C had the highest collar diameter of 2.3mm. The implication of this study is that temperature is important in seed germination and early seedlings growth. Hence, *A. melegueta* seems to have responded well to warm sand treatment in germination and early seedling growth. Several authors have also reported on the different variations recorded on collar diameter in forest species, for instance, Omokhua *et al.,* (2015b) reported a range of 0.48-0.49mm in collar diameter of *Tetrapleura tetraptera*. Similarly Oboho and Igharo (2017) reported collar diameter growth range of 3.51-3.82mm in *Pynacthus angolensis*. Diameter growth is an important characteristic in forest species because it can influence the amount of wood available and can be used to develop growth and yield models. Lowest seedling height and leaf number observed in seedlings treated with methylated spirit is an indication that it does enhance growth in *A. melegueta*. Also, the lowest collar diameter seen in seedlings treated with hydrogen peroxide is an indication that the treatment did not enhance growth in collar diameter of the species.

CONCLUSION

It was observed that seeds of these species did not exhibit dormancy. However, to enable optimum, rapid, uniform germination and good growth, it is important that the seeds of *A. melegueta* are pre-treated before sowing. The pre-treatment of *A. melegueta* seeds using warm sand seems to be the best treatment when compared to other treatments that enhanced germination, seedlings height, seedlings leaf number and collar diameter. This will definitely facilitate availability of seedlings for reforestation and agroforestry projects. Burying seeds in warm sand at 45^0C will be useful to both the local tree planters and silviculturist to break dormancy.

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