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Effect of seed sources and different presowing treatments on the early growth performance of *Plukenetia conophora (P.conophora)*

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Abstract

This study evaluated the effect of seed sources and different pre-sowing treatments on the early growth performance of Plukenetia conophora from three different locations in Ondo State, Nigeria. The seeds used for this experiment were collected from three (3) different sources namely; Aba-Oyo (Akure South Local government Area), Owena (Ijesa-Osun LGA) and Ilara-Mokin (Ifedore LGA). The pre-sowing treatments used for this research include control (T_1), soaking in cold water for 30 min (T_2), 6 hrs (T_3) and 24 hrs (T_4), boiling in hot water for 20 secs (T_5), 40 secs (T_6), and 80 secs (T_7), soaking in concentrated acid for 5 mins (T_8), 10 mins (T_9) and 20 mins (T_{10}) were applied across all the seed sources under this experiment. Therefore, this experiment was performed in a Randomized Completely Blocked Designed (RCBD). Across all the different pre-sowing treatments used for this experiment, analysis of variance revealed the highest mean germination percentage value in the seeds pretreated for 20 minutes (T_{10}) concentrated H_2S0_4 (77.78%) followed by the seeds pretreated for 5 minutes (T_8) concentrated H_2S0_4 (68.89%) while the seeds soaked in cold water at thirty (30) minutes (T_2) had a closer mean germination percentage value (62.22%) to T_8. Finally, the lowest mean germination percentage value was seen in the seeds boiled in hot water at 80 seconds (T_7) with no germination percentage value (0%) in Table-2. For increase in productivity and germination percentage. This study, therefore, recommends that P.conophora seeds be soaked in concentrated sulphuric acid for 20 minutes.

Keywords: Seed sources, early growth performance, pretreatments, *Plukenetia conophora*.

Introduction

Across the tropics, forest destruction and degradation have increased faster, including the fragmentation of many populations and the risk of plant extinction. As such, in Nigeria and other countries, the conservation of forest genetic resources is acquired if the seeds are protected in natural habitat (in-situ) or the samples of the genetic diversity of endangered species are preserved from their habitats (ex-situ) in facilities like botanical gardens, seed gene banks, in vitro gene banks, and field gene banks[1].

Plukenetia conophora is a wild woody perennial climber (liana) belonging to the family of *Euphobiaceae* with length ranges between 12m-30m. Depending on cultural differences, the popular name differs. However, it is called African walnut, "conophora nut," which varies according to tribes. Therefore, it is called "Awusa" (Sierra Leone), "Asala" (Yoruba), and "Ukpa" (Ibo). It is a non-timber forest species that serves as a good source of income to the natural populace. As such, it is protected on farmland and sparingly cultivated for its oil-rich fruits[2].

Okafor and Okorie[3] investigated that the macerated leaves and roots of *P.conophora* are traditionally used for medicinal preparations of asthma and hypertension. However, the significant challenge for this valuable under-exploited forest fruit and plant is large-scale deforestation. Therefore, *P.conophora* being a wild indigenous species, its maximum production and availability will be inevitable when production practices for future exploitation of its potentials are acquired through domestication. In *P.conophora*, there is some degree of dormancy, which hinders its adequate seed germination, necessitating the use of seed treatment(s)[4]. Thus, for nursery establishment and production of the maximum number of quality seedlings with minimum cost, time, and labour, the use of adequate seed pre-treatments is important[5].

As presented by the plus trees or parental material, seed sources should represent the best available genetic material for planting. Therefore, (seed sources) are regarded as the supply of seed for planting and replications of forest plants and genetic materials that are of utmost important[6]. Occasionally, it is hard for the seeds to germinate, though they have a favourable condition known as dormancy[7]. According to Hossain et. al[5], this delay is irregular germination of seeds in the nursery, and it is a significant constraint of efficient nursery management and plantation establishment. Therefore, to get the expected quantity and good germination of seedlings in the nursery, dormant seeds must be pretreated before sowing[8]. When inhibitions disrupt the apparent metabolic dormancy of desiccated seeds, seed germination occurs, and it is essential of most stages in the life cycle of plants[9,10]. After sowing, dormancy breaking is

expedient when rapid germination is required[11]. Therefore, the general objective of this research is to study the effect of different pre-sowing treatments on the early growth performance of *Plukenetia conophora*.

The specific objectives are to: i. investigate the effect of different pre-sowing treatments on the seed germination of *Plukenetia conophora* with the view to determining the best treatment(s) for the germination improvement of the seed species; ii. examine the provenance (location) differences in the germination of *Plukenetia conophora* from three different seed sources of Aba-Oyo, Owena, and Ilara.

Methodology

Study area: The experiment for this study was performed at the permanent nursery site (Obanla) of the Department of Forestry and Wood Technology, Federal University of Technology, Akure, Ondo State, Nigeria. The University is located between latitude 70 17'N and longitude 050 18'E. The forest is about 9.34ha in size. Generally, the vegetation zone is the tropical humid lowland forest ecosystem. The forest is very rich in tree and animal species diversity[12].

Method of data collection: Seed Collection: One hundred and fifty viable seeds of *P.conophora* were collected from each location, namely; Aba-Oyo (Akure South LGA), Ilara-Mokin (Ifedore LGA), and Owena (Ijesa-Osun LGA), Ondo State, Nigeria. The seeds were therefore subjected to visibility test through the floatation method[13]. Seeds that floated over the water were discarded and regarded as unviable and unfit for the experiment[13]. Fifteen seeds per treatment per location were designated for this experiment. Thus, a total of four hundred and fifty seeds were subjected to ten (10) pre-sowing treatments of the seed species. Thus, one seed was sown per polypot for this experiment. Four hundred and fifty poly pots were filled in total for the experiment with sieved topsoil as the sowing media. As described below, the seeds collected from each location were distributed equally to the pre-sowing treatments used in this experiment.

Seed Treatment: Experiment 1 (Control Treatment): This experiment was done to have a comparable effect of no pre-treatments known as control (T_1) from the three locations. Fifteen seeds of *Plukenetia conophora* from each location were planted without allotted time intervals in the following manner, Aba-Oyo (15 seeds), Owena (15 seeds), and Ilara (15 seeds).

Experiment 2 (Cold Water Treatment): Fifteen seeds of *Plukenetia conophora* from three different locations, namely; (Aba-Oyo, Owena, and Ilara) were soaked in clean tap water at ambient temperature (28^0C) for three other times; 30 mins (T_2), 6 hrs (T_3), and 24 hrs (T_4).

Experiment 3 (Hot Water Treatment): Fifteen seeds of *P.conophora* each from the three different locations were boiled in water at (100^0C), and allowed to soak for three different times, 20 secs (T_5), 40 secs (T_6), and 80 secs (T_7).

Experiment 4 (Acid Treatment): Fifteen seeds of *P.conophora* collected from the three different locations were soaked in 5% sulphuric acid concentration at three different times in the following manner, 5 mins (T_8), 10 mins (T_9), and 20 mins (T_{10}) and sown per treatment. After soaking, the seeds were removed, washed, and rinsed in water to remove any (traces) remaining acid.

Data collection and assessment of the early growth performance on *P.conophora* seedlings: Germination count(s) for this experiment were taken and recorded daily from the 1st day of germination (19^{th} day after sowing) to the 42^{nd} day when the seeds stopped germinating across the three seed sources under this study. Cumulative germination counts were obtained by summing up daily germination for each treatment in each location. Once counted, seedlings were labelled to avoid double counting. The sown seeds were watered twice daily (morning and evening), and weeding was regularly done on the nursery site throughout the seedlings germination period.

Parameters that were assessed: The assessed parameters in the course of the experiment include: i. Days of Emergence (Numbers of days taken for first emergence), ii. Total Number of seeds germinated, iii. Germination percentage/Emergence percentage (E%), iv. Emergence index, v. Emergence rate index.

Fakorede and Ayoola[14] Formulae was adopted in calculating the emergence parameters as follows:

$$E\% = \frac{No\ of\ seedlings\ emerged}{Total\ number\ of\ seeds\ planted} \times 100$$

$$(EI) = \frac{\Sigma\,(Number\ emerged)(DAP)}{Total\ seedlings\ emerged})$$

$$ERI = \frac{EI}{E\%(in\ decimal)}$$

Where, E%= Emergence percentage, EI = Emergence index, ERI = Emergence rate index, and DAP = Days after planting.

Experimental Design and Statistical Analysis: Model for Experiments in Randomized Complete Blocked Design (RCBD)

Where; Yij= μ + Bi + Ti + £ij

Yij = Individual Observation; μ = Group Mean; Bi = Block Effect; Ti = Treatment.

Hypothesis of the treatment(s) in the mean (Ho & Ha): Ho= There is significant different among the treatments, Ha = There is no significance difference among the treatments.

Statistical analysis: The number of days it took the first seed to emerge and the total emergence of the seeds per treatment based

on sowing time and location was represented in tables (MS Word) and graphically (cumulative frequency curve (line graph) using Microsoft Excel. Data on cumulative germination percentages collected were computed and subjected to one-way Analysis of Variance (ANOVA) using the Randomized Complete Block Design (RCBD). Under this research, Mean Separation was used to test the significant difference among the seeds collected from each location. Duncan's Multiple Range Test (DMRT) was used as a follow-up to determine more suitable pre-treatment techniques for the early growth performance of *P.conophora* seeds. The resulting data of the experimental design were analysed using Microsoft Excel and SPSS (Statistical package for scientific search) window version 21.

Results and discussion

Effect of seed sources and presowing treatments on the early growth performance on *P.conophora:* Seeds germination varied differently across each seed source for different pre-sowing treatments used for this experiment. On the 19th day after planting (DAP.), germination was recorded in Aba-oyo progeny under T_8 and T_9, while from the Ilara source, only T_9 germinated on the same day. Seed germination commenced on the 20th DAP, under T_8 only, from Owena source, while no germination was recorded observed under T_6 and T_7 from the same seed source throughout the germination assessment period (Table-1 and Figure-1,2,3). Across different treatments, seed germination (%) of the seedlings varied significantly ($p<0.05$). From the Owena source, the highest cumulative germination percentage (80%) was recorded under T_8 followed by T_1, T_4 and T_{10} with equal cumulative germination percentage (66.67%); meanwhile, T_2, T_3 and T_9 also had the same germination percentage (53.33%), but lesser to T_1, T_4 and T_{10}. While none of the seed under T_6 and T_7 germinated from the same source (Table-1 and Figure-2).

From Owena seed source, T_2 had the highest emergence index rate (ERI) of (4.29) followed by T_9 with (3.06), while none was seen in T_6 and T_7. As such, the highest emergence index (EI) was recorded in T_8 (2.35), while for both treatments (T_6 and T_7), none was recorded. On the other hand, in the Aba-Oyo seed source, T_2 and T_{10} had the highest cumulative germination percentage (80%) followed by T_9 (73.33%); T_3 and T_8 had cumulative germination percentage (60%) while the least was seen in T_5 and T_7 with no germination % throughout the germination assessment period. Also, in the Aba-Oyo location, T_6 had the highest emergence rate index (3.98) followed by T_4 (3.83), while T_5 and T_7 had none throughout the experimentation and the emergence index varies across each treatment with respect to the seed sources (Table-1). Likewise, from the Ilara source, T_{10} had the highest cumulative germination percentage (86.67%) followed by T_4 and T_8 with the same cumulative germination (66.67%), T_2 had (53.33%), T_3 and T_9 had (46.67%), T_1 had (26.67%) while in T_5, T_6 and T_7, none was recorded (Figure-3 and Table-1). Finally, under the Ilara progeny, T_1 had the highest emergence rate index (4.16) followed by T_3 with (3.34) while none was recorded for T_5, T_6 and T_7. From the Ilara progeny, T_{10} had the highest emergence index (1.94) followed by T_8 (1.70), T_3 had (1.56), while in T_5, T_6 and T_7 the least were recorded (Table-1).

From (Table-2), the we result we got from analysis of variance (ANOVA) showed highest mean germination percentage value in T_{10} (77.78) among for all the pre-sowing treatments across the seed sources studied, T_8 followed this with a mean value (68.89), mean while, 62.22% (mean germination percentage value) was recorded for T_2; T_4 had 57.78, T_9 had 57.77, T_3 had 53.33, T_1 had 44.45, T_5 and T_6both had the same mean germination value (4.444), while mean germination percentage value was not recorded for T_7 when subjected to analysis of variance (Mean Separation). Across the seed sources, the mean germination percentage value for the ten pretreatments showed no significant difference among T_7, T_6, and T_5. T_1 was not significantly different from T_4, T_3, T_9, and T_2. T_3 was not significantly different from T_4, T_2, T_8, and T_9. Also, T_9 is not significantly different from T_2, T_8, T_{10}, and T_4.

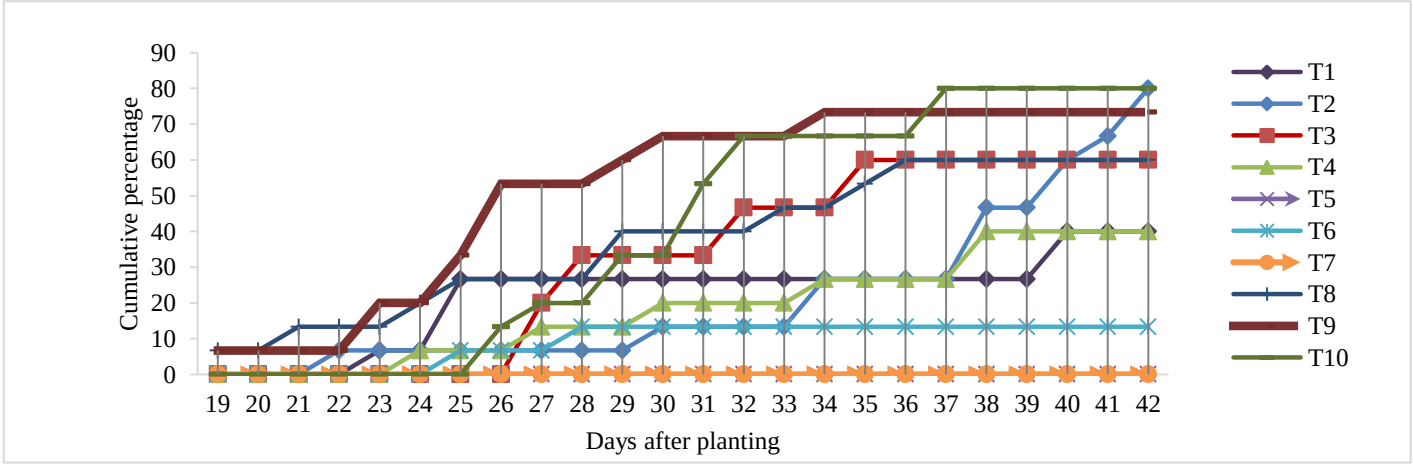

Figure-1: Cumulative germination percentage of *P.conophora* seeds from Aba-Oyo under different pre-sowing treatments.

Table-1: Differences in seed emergence across each treatments and seed source.

Source	Treatments	No. of days taken for the 1st germination	Total seeds germinated	Germination Percentage (E %)	Emergence Index (EI)	Emergence Rate Index (ERI)
Aba-Oyo	T1	23	6	40	0.88	2.2
	T2	22	12	80	2.47	3.09
	T3	27	9	60	1.22	2.03
	T4	24	6	40	1.53	3.83
	T5	0	0	0	0	0
	T6	25	2	13.33	0.53	3.98
	T7	0	0	0	0	0
	T8	19	9	60	2.22	3.7
	T9	19	11	73.33	1.86	2.54
	T10	26	12	80	1.83	2.29
Owena	T1	23	10	66.67	1.04	1.56
	T2	26	8	53.33	2.29	4.29
	T3	24	8	53.33	0.86	1.61
	T4	24	10	66.67	1.82	2.73
	T5	39	2	13.33	0.39	2.93
	T6	0	0	0	0	0
	T7	0	0	0	0	0
	T8	20	12	80	2.35	2.94
	T9	21	8	53.33	1.63	3.06
	T10	23	10	66.67	1.68	2.52
Ilara	T1	35	4	26.67	1.11	4.16
	T2	22	8	53.33	1.39	2.61
	T3	22	7	46.67	1.56	3.34
	T4	24	10	66.67	1.51	2.26
	T5	0	0	0	0	0
	T6	0	0	0	0	0
	T7	0	0	0	0	0
	T8	21	10	66.67	1.70	2.55
	T9	19	7	46.67	1.40	3.00
	T10	24	13	86.67	1.94	2.24

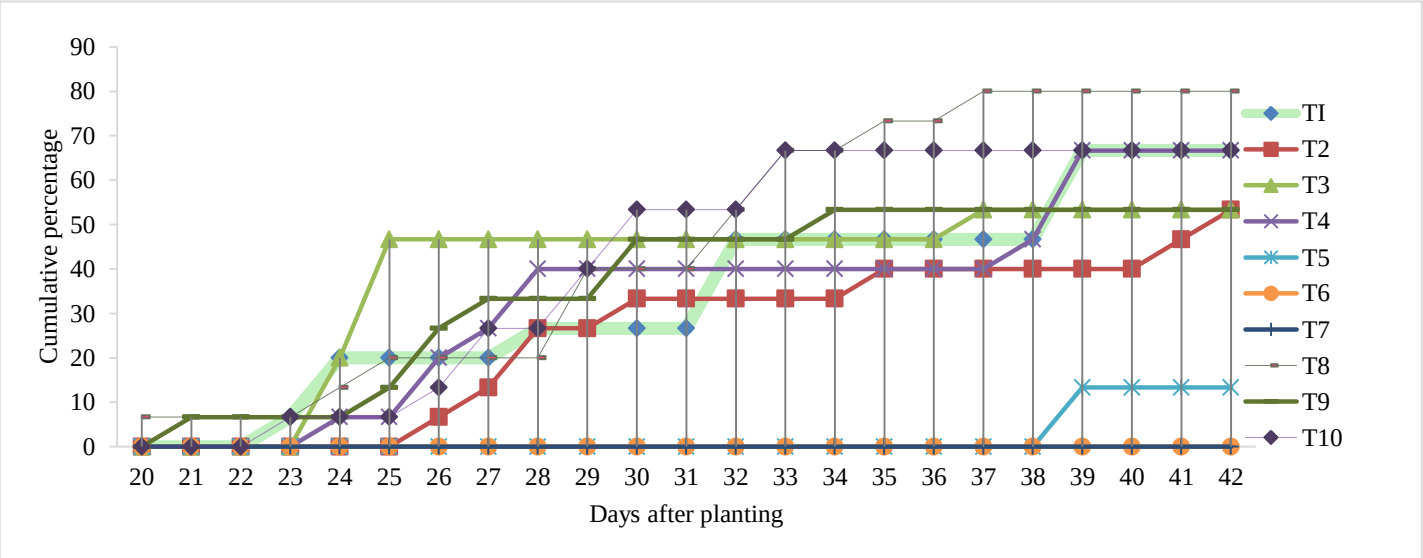

Figure-2: Cumulative germination percentage of *P.conophora* seeds from Owena under different pre-sowing treatments.

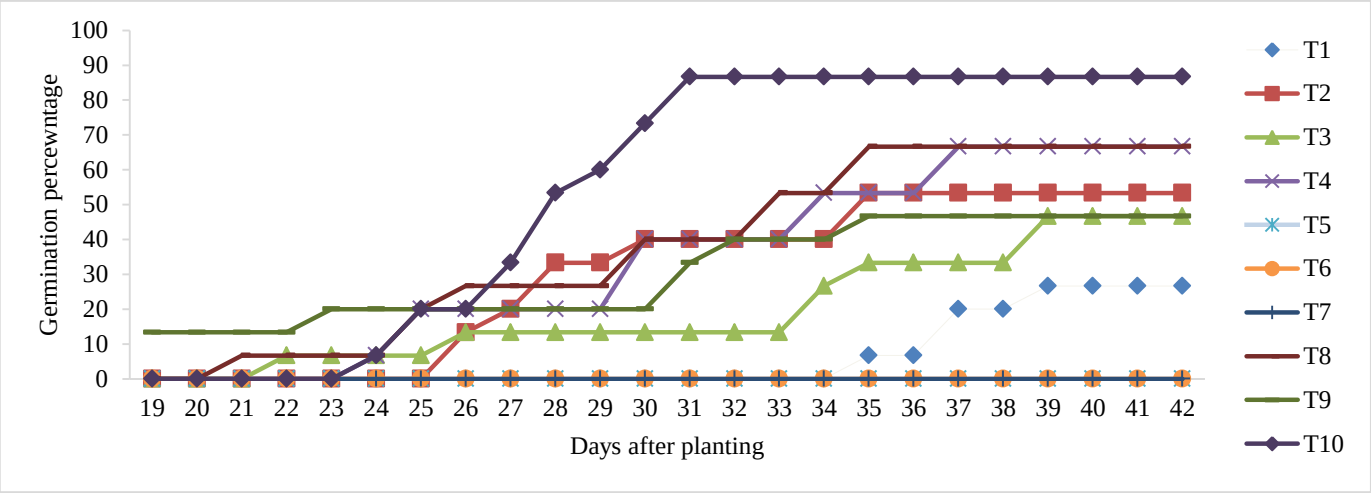

Figure 3: Cumulative germination percentage of *P.conophora* seeds from Ilara under different pre-sowing treatments.

Table-2: Differences in mean germination percentage across the ten (10) pretreatments.

Treatment	Mean germination percent (percent)
(T1) (Control)	44.45^{c}
(T2) 30 minutes CW	62.22^{abc}
(T3) 6 hours CW	53.33^{bc}
(T4) 24 hours CW	57.78^{abc}
(T5) 20 seconds HW	4.444^{d}
(T6) 40 seconds HW	4.444^{d}
(T7) 80 seconds HW	0.00^{d}
(T8) 5 minutes H_2S0_4	68.89^{ab}
(T9) 10 minutes H_2S0_4	57.77^{abc}
(T10) 20 minutes H_2S0_4	77.78^{a}

Note: Values in the same column carrying the same alphabet are not significantly different at (P>0.05).

Table-3: Follow- up analysis result of effect of seed source on germination percentage of *P.conophora*

Source	Mean Germination Percentage
Aba-Oyo	44.67^{a}
Owena	45.33^{a}
Ilara	39.34^{a}

Note: Values in the same column carrying the same alphabet are not significantly different at (P>0.05).

Discussion: Effect of seed sources on growth performance of *Plukenetia conophora:* Table-1 and Figures-1, 2, and 3 revealed that seeds collected from different sources showed differences in mean germination percentage and pattern (i.e., germinated at a varying time). The findings agree with the work of Weinert *et. al*[15], who reported in their work that differences in seed germination patterns and seedling growth rate might be due to climatic and geographic influences or, more importantly, even genetic differences. The variation in growth pattern (germination percentage) could be attributed to the provenance of the seeds. This finding corroborates with the report of Loha *et. al*[16], who observed that the germination of a seed is due to the effect of the seed source. Finally, a similar report was given by Benowicz *et. al*[17] that seeds vary in degree of permeability in most plant species between and within populations and between and within individuals.

Additionally, analysis of variance from mean germination percentage in (Table-3) showed that no significant difference (>0.05) occurred among the seeds obtained from the three different sources of Aba-Oyo, Owena, and Ilara. Although, seedlings from Owena had the highest mean germination percentage (45.33), followed by seeds procured from Aba-Oyo with a mean germination percentage (44.67), while the least was observed from the Ilara source (39.44). This finding support the work of Oyun[18], who reported the same differential pattern of growth in seedlings of *Parkia biglobosa* collected from different sources in Nigeria. Wright[19] reported that general geographical trends in growth are evident when trees from regions up to one hundred kilometers apart, especially regions with differences in climate, are compared.

Effect of Hot water pre-sowing treatments on growth performance on *Plukenetia conophora:* Throughout the seeds germination assessment period, seeds pretreated with hot water from Aba-Oyo source under T_5 and T_7 failed to germinate. Also, T_6 and T_7 from Owena did not sprout, while from the Ilara

source, germination was not recorded under T_5, T_6, and T_7 (Table-1). Additionally, across the ten (10) pre-sowing treatments, analysis of variance revealed that T_5 and T_6bothhad the same mean germination value (4.444), while none (mean value) was recorded for T_7 (Table-2). The failure in germination observed across the hot water pre-sowing treatment was in conformance with the work of Gill *et. al*[20], who reported failure in germination when seeds of *Callindra prototricensis* were soaked in hot water. Moreso, across the ten pretreatments under this study, analysis of variance revealed the lowest mean germination value in hot water pretreatments generally when compared to other treatments (Table-2). This report conforms to the findings of Agboola and Adedire[21], where the least germination percentage was recorded for *Parkia biglobosa* when treated in hot water. However, it can be deduced that hot water is not a good pre-sowing treatment for the growth performance of *Plukenetia conophora* seeds as it produced the lowest mean germination percentage when compared to other pre-sowing treatments under this research. Therefore, hot water pre-sowing treatments at T_5, T_6, and T_7 should not be used for raising *P.conophora.*

Effect of cold water pre-sowing treatments on growth performance on *Plukenetia conophora*: An experiment period on the cold water soaking duration of seeds of *Plukenetia conophora* showed that T_2 gave the highest mean germination value (62.22) followed by T_4 that gave (57.78), then, T_3 with the least mean germination value (53.33). Still, there was no significant difference between T_2, T_3, and T_4 (Table-2). From the result in Table-2 above, seeds soaked in cold water for 24hrs (T_4) had a higher germination percentage than those soaked in cold water for 6hrs based on observation. Though not significantly different from one another. The result of these findings is, however, contrary to the report given by Robertson and small[22] that over-soaking of seeds in cold water reduces germination. It conforms to the findings of Owonubi et al[23] who observed variability in the rate at which the seed coat of different seed species absorb water and gases.

Effect of concentrated sulphuric acid pre-sowing treatments on growth performance of *Plukenetia conophora:* Across all the different pre-sowing treatments used for this experiment, ANOVA revealed that soaking the seeds in concentrated sulphuric acid at 20 minutes (T_{10}) gave the best mean germination value. The highest mean germination value (77.78) was recorded for the seeds soaked in concentrated sulphuric acid for 20 minutes (T_{10}) followed by T_8 (68.89), and the least was found in the seed soaked in concentrated sulphuric acid (T_9) with a mean germination value (57.77) as shown in (Table-2). Although, T_{10} was not significantly different ($P>0.05$) from T_8 and T_9. The findings of this work disagree with the work of Iroko et. al[24], where they recorded the highest germination percentage when the seeds of *V.paradoxa* were soaked in concentrated acid for 10 minutes compared to when the seeds were pretreated in concentrated sulphuric acid for 15 minutes and 20 minutes. But, it aligns with the finding of Rusdy[25], *were soaking the seeds of Leucaena leucocephala* in sulfuric acid for 20 mins and 24 mins gave the best and highest results.

Effect of untreated seed (control) on growth performance on*plukenetia conophora*: In this study, across the ten pre-sowing treatments used, the ANOVA table (Table-2) revealed that the control had a better mean germination value (44.45) than the seeds soaked in hot water. From this study, the results agree with the findings of El-Nour, *et. al*[26], where he recorded that the control (untreated seeds) of *Balamites aegyptiaca* have higher germination than the seeds boiled in hot water.

Conclusion

Plukenetia conophora is known to contain a hard seed coat, which prolongs its development. Hence, this study revealed the expediency of pre-sowing treatments on the seeds of *P.conophora* before planting/sowing so that the best pre-sowing treatments are known in other to prevent materials/resources wastage. Across all the different pretreatments used for this experiment, concentrated acid (T_{10}) produced the highest mean germination value (77.78). Therefore, this research recommends that the seeds be soaked in concentrated H_2S0_4 for 20 minutes before sowing.

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