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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(4): 3060-3068. **OPEN ACCESS**

Effects of seed origin on the germination, seedling development and morphology of African Breadfruit (*Treculia africana* decne, moraceae) seeds

Amujiri Angela N., Eze, Perpetua C., Amadi, Chibugo C and Njoku E.U.

Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, **Nigeria**

***Correspondence:** angela.amujiri@unn.edu.ng **Received 14-08-2020, Revised: 05-12-2020, Accepted: 10-12-2020 e-Published: 20-12-2020**

Treculiaafricana is a multipurpose plant with food, medicinal and industrial attributes which has not been introduced into many ecological zones despite its potentials. This study investigated the effects of seed origin on germination, seedling emergence and morphology, when sown in petri dishes and poly pots. Soil samples and species fruit heads were collected from five Southeastern States of Nigeria. Seed extraction was by splitting and exposing the fruits to ferment, which eased the extraction without damages. EZ seeds germinated 4 ± 0.58 days after sowing; AG seeds had highest percentage germination (76.66 ± 0.38), shoot and root length in petri dishes. Seeds emerged 6 ± 1.00 days after sowing, AG produced highest leaf parameters, height and percentage emergence (96.80 ±1.04) in poly pots. Generally, EZ seeds had the lowest growth parameters, AG seeds took the longest period to accomplish germination and emergence, while seeds had similar sprouting rate and morphological feature. Soil recorded significantly ($P < 0.05$) highest percentage of clay (26%) and silt (39%) in NE, fine sand (62%) in EZ, and coarse sand (60%) in AG. Soil chemical properties showed; no significant difference in N, Na, K, Al and H values, high significant Ca, Mg and P in EZ, base saturation in IS, carbon content (1.82%) and organic matter (3.13) in OK. Soil pH was acidic except in EZ (pH 7.10). This study observed that species seeds readily germinate, adapt and yield large resources that can be conserved and biotechnologically exploited when sown in other ecological zones.

Keywords: *Treculia africana*, seed origin, germination, emergence, morphology, conservation, ecological zone.

INTRODUCTION

African breadfruit (*Treculia africana,* var. *africana,* Decne), belongs to the family Moraceae. The species is a wild neglected and under exploited tropical evergreen tree with immense nutritional potential for man (Osabor et al. 2009; Olapade and Umeonuorah, 2014) and distributed in the humid forest zones of West Africa. It grows to about 30 m tall, dichotomously branched with fluted trunks adorned with patches of white colouration that have no discernable pattern. The species produces big infructescences that are borne on the main trunks and big lateral branches, whose ripe fruits are traditionally not harvested but allowed to drop from the trees which produces heavy bang (Ogbonnia et al. 2008; Nuga and Ofodile, 2010). The fruit is light green in colour at juvenile stage and yellowish- green when ripe, (Efemena, 2013; Amujiri et al*.* 2018).

The most highly sought after parts of the species are the seeds, leaves, wood and latex in the order of priority (Hassain, 2002; Anon, 2005; Enibe et al. 2013). The seeds can be prepared as pottage, as sole food, or as food compliment, when cooked together with maize grains or beans, and can be roasted and eaten together with coconut bread or fresh palm kernels as snack (Baiyeri and Mbah, 2006; Obi and Akubuo, 2018). *Treculia africana* plants can significantly mitigates seasonal food scarcity (Amujiri et al. 2018). It ranks first among popularly consumed indigenous foods (Nzekwe and Amujiri, 2011; NAC, 2013) and has been recognized as the food of choice for royalty especially at celebration or during social obligations (Nwosu, 1990). In most hotels, eating houses, feasts, celebration etc within Nigeria, *Treculia* based food is highly cherished. Virtually every part of the species plays important role in traditional health care delivery (Anon, 2005). Latex and wood are indispensible in bone setting; aqueous extract of the boiled seeds and root decoction respectively, are effective in treating ulcer, inflammation and bacterial infection; leaf decoction is effective in the treatment of hypertension and gastro-intestinal infection; and the wood has potential for pulp and paper making (Hassain, 2002; Anon, 2005; Nzekwe and Amujiri, 2011).

Apart from food, the species plays very important role in man's socio-economy, as substantial revenue is generated from trade on the species seeds (Ogbonnia et al. 2008). The seeds are of great strategic importance in the alleviation of poverty as rural dwellers rely on them for their sustenance in the wet season when most of the conventional staple food sources are in the farms (Marenah, 1989; Amujiri et al. 2018). *Treculia africana* is currently included in the list of endangered and under-exploited indigenous economic species in Southern Nigeria with scanty presence in other ecological zones (Nzekwe and Amujiri, 2011). Therefore, there is need for urgent identification and conservation of *Treculia* species in the view of the current population increase, mass unemployment and food insecurity.

This study investigated the effects of seed origin on germination, seedling emergence and morphology of the species seedlings. This result can no doubt easy identification of the *Treculia* seedlings, motivates people to introduce the species to other ecozones and It will also help in conserving the species to makes available, larger quantity of trees whose resources can sustain biotechnological.

MATERIALS AND METHODS

Experimental site

This study was carried out in the Botanic Garden of the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka.

Fruits Collection, Processing and Seed Viability Test

Eighteen fruit heads of *T. africana* species composing of three (3) fruit heads each and soil samples were collected from one town in each of the five States of Southeastern States of Nigeria namely, Abia (Isulabor), Anambra (Agulu), Ebonyi (Ezzamgbo), Enugu (Nenwe) and Imo (Okigwe) and from the tree in Botanic Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka (UNN), the control. Seeds were extracted manually by scooping out seeds with mucilaginous mesocarp, and washing under running tap water. Seeds from each town were mixed thoroughly to form a seed lot. Floatation method was used to test for viability of seeds before sowing by soaking inside water for 10 minutes. Seeds that floated were regarded and discarded as not viable, while the ones that sank were used for the study.

Experimental Design

A total of 1800 randomly selected seeds were used for the experiment, 300 seeds per treatment (location) at 50 seeds for petri dish and 250 seeds for poly pots. Each location had five (5) replicates and each replication had ten (10) seeds for petri dish and fifty (50) seeds for poly pots. In poly pots, common medium, 2:1:1 mixture of top forest soil (TS), sawdust (SD) and composted poultry manure (PM) was formulated and used by mixing 200 kg of TS and 100 kg each of SD and PM. Seeds were sown in poly pots measuring 25 x 12.5cm at the rate of ten (10) seeds per poly pot to a depth of about 2.5–3cm. The experimental design was a Completely Randomized Design (CRD). The set up was displayed under partial shade, monitored and watered in the morning at an interval of two days, except when it rained to maintain adequate moisture content. Weeds were handpicked during watering.

Observations were made on periods of initial, final and rate of seed germination, percentage germination and seedling emergence, leaf production, leaf area, lengths and girth of plumule (shoot), radicle (root) circumference and morphology. Seeds were considered germinated on appearance of radicle and considered emerged when the plumule emerges to about 2-3 cm above the medium. Percentage seed germination and emergence was calculated by using the formula of Loha et al*.* (2006) as follow

% germination (PG) or emergence (PE) = $\frac{\text{Total seeds germinated or emerged}}{\text{Total seeds sown}} \times \frac{100}{1}$

Soil samples Collection

Soil samples randomly collected from five points in each location were lumped to form a lot. The soil samples were analyzed in the Soil Analysis Laboratory of the Department of Soil Science, University of Nigeria, Nsukka. They were analysed for their physicochemical contents: mechanical particles, pH, and Exchangeable cations (Ca^{++}, Mg, Na, K) following the normal procedure (Nwajei et al. 2012). The cations, Na and K were determined by flame test method, (photometric method), while other cations were determined by titrimetic method (Udo et al. 2009).

Data analysis

Data obtained were analysed using SPSS statistical software (SPSS version 18). One way analysis of variance (ANOVA) was used to determine variation and if mean value was significant at P<0.05. Duncan multiple range test (DMRT) was used to indicate levels of differences.

RESULTS

In the petri dish experiments (Table 1), EZ seeds had the earliest germination (4 ± 0.58), followed by IS, NE and OK seeds (5 ± 0.00) while PSB seeds, the control took the longest period (7 ±1.15) to germinate. Periods of final seed germination showed that seeds collected from IS, NE and OK were first to terminate germination on 16 ± 0.58 day after sowing, while AG and PSB seeds took the longest period (18 ± 0.33) to terminate germination.

Percentage germination (76.66 ± 0.38), shoot length (2.50 ± 0.04) and root length (1.60 ± 0.11) of the seedlings from AG was the highest while EZ seedlings had the least. Results showed that leaf area of seedlings in different locations was the same. Ok seedlings were significantly ($p \leq 0.05$) highest in shoot circumference (0.59 ±0.41) and root circumference while EZ seedlings had the least.

In the Poly pots experiment (Table 2), the earliest seedling emergence was obtained from IS and OK seeds (6 ± 1.03), followed by AG and NE (7± 0.48) seeds while EZ seeds took the longest period (9 ± 0.00) to emerge. Seedling emergence terminated within 28 ± 0.00 and 31 ± 1.94 days. Seeds from NE took the shortest period (21±0.00), while AG seeds took the longest period 24± 1.73 to terminate emergence. Mean percentage emergence showed that seeds collected from AG (96.80 ± 1.04) had the highest percentage emergence, followed by seeds from OK and PSB (95.40 ± 0.35), while EZ (88.80 ± 0.69) seeds had the least. Results of the morphological features of the emerging seedlings of *T. africana* showed thatOK seedlings recorded significantly($p < 0.05$) the highest leaf area, seedling height and number of leaves, followed by AG seedlings while EZ produced significantly($p < 0.05$) the least of the above vegetative parameters. Generally, AG seedlings had highest stem girth and root circumference that are not significantly ($p < 0.05$) different from other locations. NE seedlings produced least stem girth while EZ and PSB, the control were significantly ($p < 0.05$) the least in the root parameters.

Rate of seed germination and seedling emergence

Results showed that *Treculia africana* had similar pattern in their germination and emergence rate (Figures 2 and 3). They began gradually within a week, increased rapidly and attained peak at different periods (weeks), before declining sharply. EZ seeds (28.00 ± 0.577) had highest germination peak in the second week, followed by IS (27.00 ± 1.155) seeds, while seeds from AG (22.00 ± 0.000) had the least peak. In the third week, AG, OK, NE, IS and EZ in this order produced the highest peak, when most of the seed germinations terminated.

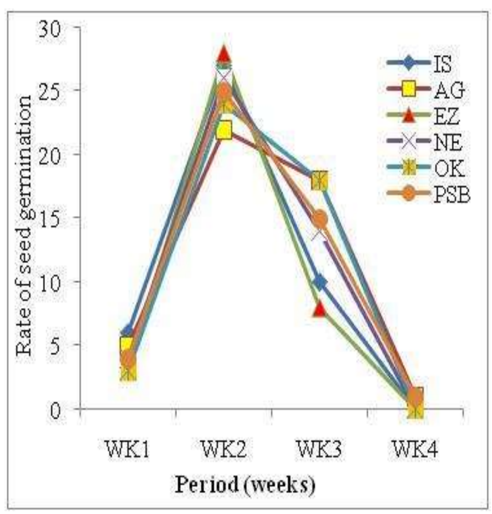

Figure 1:Germination rate of *Treculia*seedsof *Treculia* seeds from six different locationsin

petri dishes

Result showed that seeds that emerged earlier in the first week (NE (75 ± 2.887), OK (56 ± 1.732) and IS (54 ± 1.732) attained their emergence peak in the second week, whereas seeds that emerged within the second week (EZ, AG and PSB) got their peak in the third week. Highest emergence peak in the second was from NE, followed by OK and IS seeds. In the third week, EZ, AG, OK, IS and NE in this order produced the highest peak. Seedling emergence in all the locations was accomplished in the fifth week.

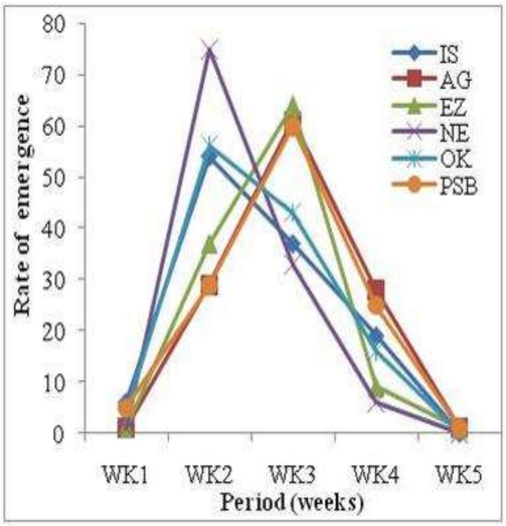

Figure 2: Emergence rate of *Treculia*seedscollected from six different locations in polypots

Result of Soil Analysis

Results showed that the soil samples were heterogeneous i.e. they contained physical and chemical properties but in varied percentages. Results of the physical properties classified soil particle size into: Sandy (S) topsoil, Loamy (L) and Clay (C) subsoil. AG, IS and EZ had the same soil texture class (SL), NE had (L) while OK had (SCL). Results of particle size showed that NE soil significantly ($P < 0.05$) had the highest percentage clay content (26%), silt (39%) with least percentages of fine sand (20%) and coarse sand (15%) content. EZ had highest percentage fine sand (62%) with least percentages of clay content (6%) and silt (9%), while AG produced highest percentage coarse sand (60%).

Results of the chemical properties showed that all other soil samples were acidic (range, pH 5.10 to pH.6.70) except EZ soil which was slightly alkaline (pH 7.10). N, Na, K, Al and H values had no significant difference in all the locations. EZ, produced significantly highest Ca, Mg and P while AG had least Ca and P. IS soils produced significantly highest percentage base saturation, followed by EZ while NE soil had the least. OK had the highest percentage of the carbon content (1.82%) and Organic matter (3.13) with zero Phosphorus contents, while EZ had the least percentage carbon (0.89%) and Organic matter (1.54%) with highest Phosphorus content (30.78). For exchangeable bases, NE had highest Nitrogen (0.210%) content, followed by OK (0.112%) while AG had the least.

Morphological Studies on Germinating seeds and emerging seedlings

Results of *Treculia africana* showed protrusion of the seed micropylar region and increase in the seed size in 2 days (Plate 1); protrusion of radicle, the outgrowth of root primordium from the concave end of the seed as the first external visible sign of germination within 4 to 6 days (Plate 2). Results observed that as the cells of the root apex of the embryo elongate, the seed coat ruptured and growth extension continued, resulting to a curved radicle from the 7^{th} to 9^{th} day (Plate 3). Ruptured seed showed feeding root on curved radicle and a prominent bulge of initial green-coloured hypocotyls as the radicle grows longer in 10 to 12 days (Plate 4). The bulged hypocotyls appeared to enlarge by forming a hook-shape (Plate 5a) or non hook-shaped hypocotyls (Plate 5b) as the radicle elongates in 13 to 14 days.

The elongating hypocotyls carried cotyledons with ruptured testa upward and a developed lateral root system downward (Plate 6) in 15 to 26. Results showed that the ruptured testa fell off as the internode elongated to show the prominent plumule (Plate 7). Results observed that emerged seedling of *Treculia* showed green coloured hook-shaped hypocotyls (HSH) as external sign of seedling emergence for seeds planted in poly pots (Plate 8), followed hypocotyls carrying the cotyledons above the soil level i.e. Epigeal germination (Plate 9). Finally, results observed that two seedlings came out from one seed coat; one of the seedlings was larger than the other as a result of seed polyembryo (Plate 10a and b).

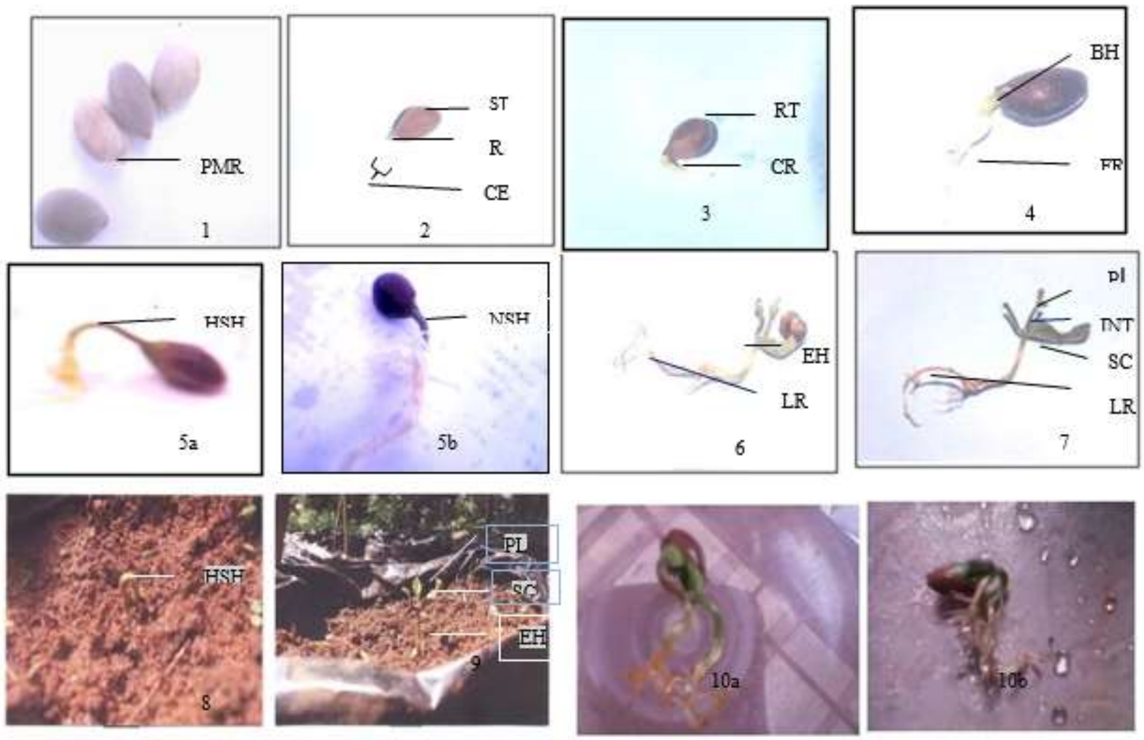

Figure 3: Germinating and emerging view of *T.africana* seedlings

Key: PMR = protruded micropylar region; ST = seed testa; R = radicle; CE = concave end; RT = Ruptured testa; CR = curved radicle; BH = bulged hypocotyls; FR = feeding roots; HSH = Hook-shaped hypocotyls, NSH = Non hook-shaped hypocotyls; RT = ruptured testa; SC = seed cotyledon; PL = plumule; INT = internode; EH = elongating hypocotyls, LR = Lateral root

Table 1: Effects of location on the germination and seedling development of *Treculiaafricana* sown in Petri dishes.

Location	Germination periods			Percentage Germination (%)	Leaf area (cm^2)	Shoot length (cm)	Root Length (cm)	Shoot circumference (cm)	Root circumference (cm)
	Initial	Final	Duration						
IS	5 ±0.00^{bc}	21±0.00^{b}	16± 0.58^{b}	71.60 ± 92^{ab}	0.01 ± 0.00^{a}	2.33± 0.09^{a}	1.55 ± 0.30^{a}	0.20±0.01^{b}	0.19 ±0.01^{a}
AG	6 ± 0.45^{b}	24±0.00^{a}	18± 0.00^{a}	76.66 ± 0.38^{a}	0.01 ± 0.00^{a}	2.50± 0.04^{a}	1.60 ± 0.11^{a}	0.19±0.00^{b}	0.19 ±0.00^{b}
EZ	4 ± 0.58^{c}	21±0.36^{b}	17±0.50^{ab}	66.66 ± 0.20^{b}	0.01 ± 0.00^{a}	2.01±0.05^{b}	1.14± 0.04^{cs}	0.19±0.00^{b}	0.17 ±0.00^{b}
NE	5± 0.00^{bc}	21±0.00^{b}	16± 0.00^{b}	73.33 ± 3.08^{a}	0.01 ± 0.00^{a}	2.46 ±0.07^{a}	1.40± 0.11^{b}	0.20±0.01^{b}	0.18 ±0.01^{a}
OK	5± 0.00^{bc}	21±0.00^{b}	16± 0.00^{b}	76.00 ± 2.89^{a}	0.01 ± 0.00^{a}	2.29± 0.06^{a}	1.42± 0.30^{b}	0.59±0.41^{a}	0.21 ±0.01^{b}
PSB	7 ± 1.15^{a}	25±0.58^{a}		75.50 ± 0.87^{a}	0.01 ± 0.00^{a}	2.44± 0.08^{a}	1.60 ± 0.11^{a}	0.40±0.41^{ab}	0.18 ±0.00^{b}

Values represent means ± standard error. Means with different alphabets in the same column differed significantly using the DMRT

Table 2: Effects of location on seedling emergence and morphological development of *Treculiaafricana* grown in poly pots for 5 weeks

Locations	Initial	Final	Duration	Percentage emergence	No. of leaves	Leaf area (cm^2)	Seedling height (cm)	Stem girth (cm)	Root length (cm)	Root Circircumference (cm)
IS	6 ± 1.03^{b}	28 ± 0.55^{b}	22 ± 0.00^{a}	92.8 ± 0.46^{b}	5.6 ± 1.32^{b}	43.9 ± 3.36^{b}	17.0 ± 4.06^{b}	1.3 ± 0.11^{a}	18.9 ± 2.74^{a}	1.0 ± 0.21^{a}
AG	7 ± 0.48^{ab}	31 ± 0.58^{a}	24 ± 1.73^{a}	96.8 ± 1.04^{a}	6.8 ± 3.58^{a}	49.6 ± 2.91^{a}	18.5 ± 3.76^{a}	1.4 ± 0.18^{a}	19.3 ± 0.89^{a}	1.8 ± 0.13^{a}
EZ	9 ± 0.00^{a}	31 ± 1.16^{b}	22 ± 0.58^{a}	88.8 ± 0.69^{c}	5.0 ± 1.73^{c}	39.8 ± 1.21^{c}	14.6 ± 2.17^{c}	1.2 ± 0.21^{a}	16.5 ± 0.93^{b}	$1.08\pm0.\ 1.1\pm0.19^{b}$
NE	7 ± 1.55^{ab}	28 ± 0.00^{b}	21 ± 0.00^{a}	92.0 ± 0.58^{b}	6.4 ± 2.35^{b}	44.3 ± 1.88^{b}	16.7 ± 7.57^{b}	1.0 ± 0.07^{b}	20.0 ± 1.49^{a}	1.2 ± 0.20^{c}
OK	6 ± 0.00^{b}	28 ± 0.00^{b}	22 ± 1.16^{a}	95.2 ± 0.12^{a}	7.0 ± 2.95^{a}	50.9 ± 2.01^{a}	18.9 ± 1.96^{a}	1.2 ± 0.09^{a}	18.9 ± 1.71^{a}	1.4 ± 0.10^{a}
PSB	8 ± 0.73^{ab}	31 ± 1.94^{a}	23 ± 0.33^{a}	95.4 ± 0.35^{a}	6.2 ± 2.29^{b}	42.7 ± 3.27^{b}	16.6 ± 5.71^{b}	1.2 ± 0.18^{a}	15.8 ± 0.55^{b}	1.0 ± 0.13^{b}

Values represent means ± standard error. Means with different alphabets in the same column differed significantly using DMRT ($p \leq 0.05$).

Table 3: Result of soil analyses collected from the study locations

Sample description	Texture Class	Particle size (%)				pH value		Organic matter (%)		N % %	Exchangeable Base (mg/100g)				B CEC 100g	Exchangeable Acidity (me/100g)		P	Base saturation
		Cl Clay	Silt	Fine sand	Coarse sand	H_2O	KCl	C	OM	%	Na^+	K^+	Ca^{2+}	Mg^{3+}	100g	Al^{3+}	H^+	Pp m	%
IS	LS	8.00	5.00	58.0	29.0	6.70	5.90	0.96	1.66	0.10	0.10	0.11	8.00	4.00	1 3.20	_	1.20	25.2	92.5
AG	LS	8.00	5.00	27.0	60.0	6.70	5.70	0.96	1.66	0.07	0.06	0.12	5.20	1.20	1 0.00	_	1.40	12.1	65.8
EZ	LS	6.00	9.00	62.0	23.0	7.10	6.20	0.89	1.54	0.10	0.06	0.06	8.40	4.00	1 4.60	_	1.40	30.8	85.8
NE	L	26.0	39.0	20.0	15.0	5.60	4.50	1.34	2.30	0.21	0.06	0.15	7.20	1.20	3 0.40	1.20	1.60	26.1	28.3
OK	SCL	12.0	13.0	46.0	29.0	5.10	4.20	1.82	3.13	0.11	0.08	0.04	6.40	1.20	1 8.40	0.80	1.80	_	42.0

KEY: N = Nitrogen, CEC= Cation exchangeable capacity, P = Phosphorous, C = Carbon, H_2O = water, H^+=Hydrogen, KCL= Potassium Chloride, OM= Organic matter, Na= Sodium, K= Potassium, Al^{3+} = AluminiumCa^2= Cacium, Mg$_3$= Magnesium, LS = loamy soil, L = loamy, CL = clay, - = none, mg = milligram

DISCUSSION

In all the locations, initial and final germination and emergence, percentage germination and emergence, and other vegetative parameters varied. The high percentage germination and seedling emergence obtained agreed with (Ezumah, 1986) who investigated the effects of seed origin on the germination of Neem (*Azadirachta indica*) seeds collected from 12 different towns in Nigeria and reported that seed origin had no effect on seed germination. Moreover, in the mid-70s, agents of Malaysian Government introduced oil palm nuts from the Nigerian Institute for Oil Palm Research (NIFOR), Benin City to Malaysia. The seeds geminated, established so well that Malaysia displaced Nigeria as the world's highest palm oil producer. Again, the high percentage germination and emergence obtained can be attributed to the common medium, 2:1:1 mixture of top forest soil, sawdust and composted poultry manure used. Reports have shown advantages of amended media over single medium in improving moisture retention, nutrients, healthy and better growth and restoring of soil structure (Baiyeri and Mbah, 2006; Agbogidi et al. 2007; Osaigbovo et al. 2010; Nzekwe et al. 2016; Fredrick et al*.* 2017; Fredrick et al. 2018). This implies that with soil amendment, seedlings of uniform height can be established.

The results of this study showed that germination of *Treculia* was epigeal. Earlier reports on the morphology of germinating seeds are scanty. Most of the earlier studies focused mainly on the external features leading to seedling establishment (Ameh, 2007) without correlating these with the physiological processes that ensure germination. Okafor (1981) had similar report that the species had an elongated opposite dimorphic cotyledons, purplish brown hypocotyls and light green epicotyls during his description of *Treculia* varieties*.* Though, the author's report was on the seed emergence features and was silent on the proper germination as was obtained in this petri dish study. The development of the hook-shaped and non hook-shaped hypocotyls of *T. africana* seedlings was attributed to the position of seeds at sowing. Results showed that seed that rested upward on the edge of the petri dish did not develop hook-shaped hypocotyls (Plate 5b) while seeds that were sown flat did (Plate 5a). Jeremi (2017) made similar observations that position of a seed at sowing can affect morphology and germination. Result observed polyembryonic seedlings which were attributed to the genetic inherit of the germinating seeds rather than to the position of seeds at sowing. This implies that seeds from one parent may have differences embryonic composition due to inherited genes as can obtained from other living things.

Therefore, findings of this study showed that *Treculia*seeds readily germinate and can emerge early. Thus the species propagules when introduced into new ecological zones can germinate, establish and reproduce well since it has been found that "readily seed germination" is an inherited character of the species in all the locations. This implies that *Treculia* seeds support food security as it can yield large quantity of uniform seedlings for its conservation in plantations, which will yield sustainable resources for future biotechnological exploitation in the production of acceptable, assessable and affordable products.

CONCLUSION

In conclusion, this finding observed that seed origin, method of seed extraction and soil compositions had no effects on seed germination of *T. africana* species. The results suggest that further work be carried out on the seed shelf-life of the species so as to determine the seeds storage period in order to introduce them to other ecological zones. Also, based on the findings established from this study more studies of this nature should be carried out in all the indigenous plants of the Tropical rainforest to ensure availability of their resources and proper conservation of their resources.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

We wish to humbly thank all visited towns and owners of trees from whom we sourced fruits and information which formed the backbone of our paper. Equally, we appreciate ourselves who contributed to make funding of this paper possible.

AUTHOR CONTRIBUTIONS

Amujiri, A. N. conceived the design of the topic and surveyed the study area. Amujiri, A. N and Eze, P.C. took part in sample collection and identification, wrote the manuscript introduction,

discussion and abstract. Amadi, C. C and Njoku E.U helped in compilation of data, results and references. Finally, all authors read and approved the manuscript without conflict.

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