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INFLUENCE OF THE (PGPRS) TREATED-CUTTINGS ON WOOD ANATOMICAL STRUCTURES OF *TRECULIA AFRICANA (DECNE)*

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Abstract:

The purpose of this study to evaluates the influence of (PGPRS) on the anatomical structure of wood elements of Treculiar africana. (PGPRS) micro-organism-treated cuttings of T. africana were investigated using three rhizobacteria species; Pseudomonas fluorescens, P. cissicola and P. corrugate for the inoculation. Parameters measured were plant height and collar diameter using a meter rule and a digital caliper respectively. The T. africana stem samples were sectioned into three planes transverse, tangential and radial sections using Riechert sledge microtome, 20 microns thick fibres were measured after 32 weeks of planting. The results showed that collar diameter values of seedlings produced from inoculation of P. fluorescens (M1), P. cissicola (M2), P. corrugate (M3), M1/M2, M1/M3, M2/M3, M1/M2/M3, M4 (Control) were 9.42cm, 8.78cm, 8.13cm, 7.90 cm, 8.48cm, 7.58cm, 7.93cm and 6.14cm respectively, while the values for seedling height in P. fluorescens (M1), P. cissicola (M2), P. corrugate (M3), M1/M2, M1/M3, M2/M3, M1/M2/M3, M4 (Control) were 39.65cm, 43.23cm, 41.90cm, 45.40cm, 39.95cm, 40.75cm, 38.28cm and 28.10cm respectively. Anatomical structures revealed that the oculations (Pseudomonas cissicola, Pseudomonas corrugate, Pseudomonas fluorescens) used on the cuttings influenced deposit materials which were more in parenchyma of seedlings inoculated with P. cissicola as seen in the micrographs. This might have informed why the largest collar diameter and the highest value of seedling length were recorded for seedlings inoculated with P. cissicola, masking the materials inside the parenchyma cells could be food materials induced as a result of inoculation while the least values of collar diameter and seedling height were recorded for seedlings that were not inoculated at all. Besides, uninoculated seedlings had smaller indistinct vessels and rays, more uniseriate rays than any other inoculated seedlings. Hence, the observed outcome will aid the tree growth and improvement of this species for forest establishments and forest product utilization.

Key words: *collars; height; parenchyma; pseudomonas; seedlings; vessels.*

INTRODUCTION

Plant growth-promoting rhizomes (PGPRs) are a group of rhizosphere colonizing bacteria that produce phytohormones, siderophores, antibiotics, solubilize phosphate, inhibit plant ethylene synthesis, fixes Nitrogen, and induce plant systemic resistances to pathogens (Saharan and Nehra 2011, Bhattacharyy 2012). Jang and Woo (2018) had once reported that plant growth-promoting rhizobacteria *Bacillus subtilis* on the growth and physiological changes of some species of Poplar seedlings promoted the growth of the species. Also, bacterial inoculation has been found to increase shoot diameter from 7.0 to 16.3% when compared to control. All the inoculated PGPR strains contributed to the increase in fruit yield of apples when compared to control (Aslantas *et al*. 2007).

The wood of *Treculia africana* Decne (African breadfruit) is a large tree in the family Moraceae and grows up to 30m high in the rain forest zone, particularly the swamp zone. It is also widely grown in Southern Nigeria and North of the Democratic Republic of the Congo for its seeds. The tree species are known by various tribal names in Nigeria (Irvine 1981; Onweluzo and Odume 2008) such as Afon (Yoruba), Barafuta (Hausa), Ize (Bini), Eyo (Igala), Ediang (Efik), and Ukwa (Igbo) and popularly known as Boimbo (Mongo) in the Democratic Republic of the Congo, fruit collection is possible throughout the year with a period of heavy fruiting between February and August alternating with that of light fruiting between September and January

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(Okafor 1985, cited in Nzekwe, Ojeifor, and Nworie 2010). *Treculia africana* is increasingly becoming commercially important in Africa due to the potential use of its seeds, leaves, timber, roots, and bark. It constitutes a cheap source of vitamins, minerals, proteins, carbohydrates, fats and is non-poisonous (Osabor *et al*. 2009). It is made into flour to be used: as a soup thickener, Imitation milk, in bakery and Pharmaceutical industry. It provides fodder for animals and the wood is put into various uses. It also has various medicinal uses. Yet, it was enlisted as a high valued endangered indigenous fruit tree that needs to be domesticated (Nuga and Ofodili 2010, Meregini 2005). No study has been carried out on the account of the features of these micro-organisms inside the plants. Of course, anatomical features of *Tectona grandis* that was infected with some fungi has been investigated (Adeniyi *et al*. 2015), investigating the cells of inoculated wood of tree seedlings of *T. africana* may reveal what happens when these organisms relate with wood cells.

OBJECTIVE

The objective of this study is to examine the wood cells of *T. africana* that are enhanced with PGPR with those other wood cells that are not treated with the organisms as the outcome of this study will provide additional information on what influence the organisms has on plant cells.

MATERIALS AND METHODS

The PGPRs used for this study were isolated from a soil sample collected from fruit trees rhizosphere in the physiology fruit tree nursery of the Forestry Research Institute of Nigeria (FRIN) Ibadan Oyo state, located on latitude 7 ^{o}23'15'' to 7 ^{o}24'00''N and longitude 3 ^{o}51'00'' to 3^{o}52'15''E of the equator. The plant growth-promoting rhizobacteria were isolated using nutrient agar and Kings B medium and molecularly identified at IITA Ibadan Oyo state. They were confirmed to be nitrogen-fixing bacteria by sub-culturing on Burk's N-free medium while their plant growth-promoting trait were ascertained by confirming their ability to solubilize phosphate, produce Indoleacetic Acid (IAA), hydrogen cyanide (HCN), and Ammonia. The rhizobacteria species were molecularly identified to be *Pseudomonas cissicola, Pseudomonas fluorescens,* and *Pseudomonas corrugate.* In the present study, 10^{8} cfu/ ml of PGPR were then used for the inoculation of African breadfruit seeds using sterile distilled water as a control (distilled water). Sterile topsoil was used as the sowing media. Inoculation was done by soaking the seeds in PGPR treatments using the Quick Dip Method according to Gholami *et al*. 2009. Watering was done once daily.

The following variables were assessed at two weeks interval for 32 weeks:

i. Plant height using a meter rule;
ii. Collar diameter using a digital caliper.

While the following abbreviations tag were used for easy identification:

A. M_1: P. fluorescens.
B. M_2: P. cissicola.
C. M_3: P. corrugate.
D. M_1/M_2: Co-inoculation of the mixture of *P. fluorescens* and P. cissicola.
E. M_1/M_3: Co-inoculation of the mixture of *P. fluorescens* and P. corrugate.
F. M_2/M_3: Co-inoculation of the mixture of *P. cissicola* and P. corrugate.
G. M_1/M_2M_3: Co-inoculation of mixture of P. cissicola, *P. Corrugate* and P. fluorescens.
H. M_4: CONTROL.

SECTIONING

Wood samples from some seedling stems of *T. africana* were sectioned into 20 microns thick at the end of the 8th month using a Riechert sledge microtome. Samples were prepared into three planes namely transverse, tangential and radial sections. The sections were later covered with safarin stain for two minutes after which series of concentrations of ethanol were used for dehydration. The cleaning and proper clarity was done using vegetable oil (Adeniyi *et al*. 2016). The specimens were embedded with Canadan balsam on a microscopic slide and examined under a light microscope.

RESULTS AND DISCUSSIONS

At the end of the 32 weeks, the results showed that values of collar diameter of seedlings produced from inoculation of *P. fluorescens* (M_1), *P. cissicola* (M_2), *P. corrugate* (M_3), M_1/M_2, M_1/M_3, M_2/M_3, $M_1/M_2/M_3$, M_4 (Control) were 9.42cm, 8.78cm, 8.13cm, 7.90cm, 8.48cm, 7.58cm, 7.93cm, and 6.14cm respectively, while the values for seedling height in *P. fluorescens* (M_1), *P. cissicola* (M_2), P. corrugate(M_3), M_1/M_2, M_1/M_3, M_2/M_3, $M_1/M_2/M_3$, M_4 (Control) were (39.65) cm, (43.23) cm, (41.90) cm, (45.40) cm, (39.95) cm, (40.75) cm, (38.28) cm and (28.10) cm respectively.

According to Table 1, seedling collar diameter was highest in *Pseudomonas* fluorescens (9.42) cm and least at M_1/M_3 (8.48cm) which is the mixture of *Pseudomonas* fluorescens and *P. corrugate*. Control

produced the least collar diameter of (6.14) cm, while M_1, M_3, and M_4 were within the same range (7.58 to 7.93) cm. Seedling height was highest in M_1/M_2 (45.40) cm which is the mixture of *P. fluorescens* and *P. cissicola*. This was followed by (M_2) *P. cissicola* (43.23) cm, while the least value of seedling height (28.10cm) was recorded in Control.

Table 1

Mean values of seedling parameters of *T. africana* PGPR-treated seeds

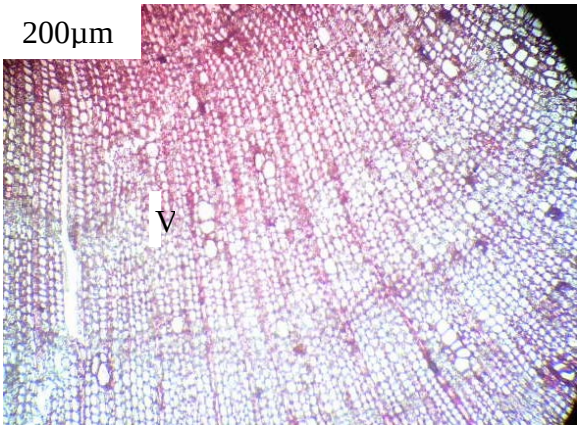
No	Treatment	Collar Diameter (cm)	Seedling Height (cm)
M_1	*P. fluorescens*	9.42	39.65
M_2	*P. cissicola*	8.78	43.23
M_3	*P. corrugate*	8.13	41.90
M_1/M_2	*P. fluorescens* / P. Cissicola	7.90	45.40
M_1/M_3	P.fluorescens / P. Corrugate	8.48	39.95
M_2/M_3	*P. cissicola* / P. Corrugate	7.58	40.75
$M_1/M_2/M_3$	*P.fluorescens*/*P.cissicola*/P. *Corrugate*	7.93	38.28
M_4	Control	6.14	28.10

Generally, anatomical features of the seedlings investigated in this study showed that vessels were diffuse, rays were mostly uniseriate. Mucilage cells were present though not well developed; deposit materials were inside some parenchyma cells. Comparatively, rays from a mature *T. africana* wood species are normally 2-3 cell wide, with mucilage cells; axial parenchyma cells in mature trees are both paratracheal and apotracheal, while some gum deposits are found inside some rays. Uniseriate rays in mature wood are rear. Also in the seedlings, vessels are not as big as found in a mature tree, and in addition to these, axial parenchyma cells in seedlings easily blend with other ground tissues like fibres such that the pattern of parenchyma cells is not paratracheal unlike a mature tree.

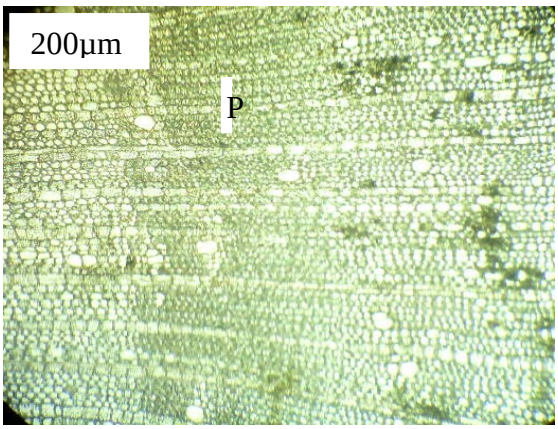
Furthermore, the innoculations (*Pseudomonas cissicola, Pseudomonas corrugate, Pseudomonas fluorescens*) used on the seeds suggest that deposit materials observed were more in parenchyma of seedlings inoculated with *P. cissicola* (Fig. 1, (c), Fig. 2, (a), and Fig. 3,(b and e) than the rest of the treatments. An abundance of such deposit materials was also noticed in seedlings inoculated with the mixture ($M_1/M_2/M_3$) p*. cissicola, P. corrugate,* and *P. fluorescens* (Fig. 1, (a), Fig. 3, (c and d). This might inform why the largest collar diameter (8.78) cm was recorded for seedlings inoculated with *P. cissicola* followed by seedlings inoculated with the mixture which produced a collar diameter average of about (8.22) cm.

Perhaps the materials inside the parenchyma cells were food materials induced as a result of inoculation which affect the vessel sizes and pore as seen in transverse, tangential and radial sectioning ((Fig. 1, (a-e), Fig. 2, (a-e), and Fig. 3,(a-e), respectively.

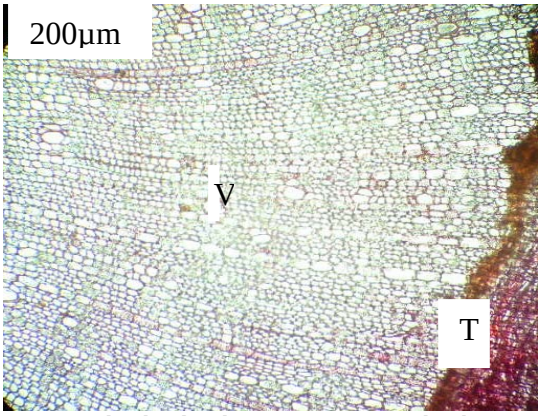
From Table 1, the highest height of seedlings was recorded using *P. cissicola* and the least values of collar diameter and seedling height were recorded for seedlings that were not inoculated at all. Moreover, seedlings that were not inoculated seemed to be characterized by smaller vessels and rays, and with more uniseriate rays than any other inoculated seedlings. This observation is similar to Areo (2019) in *Artocarpus altilis* wood.



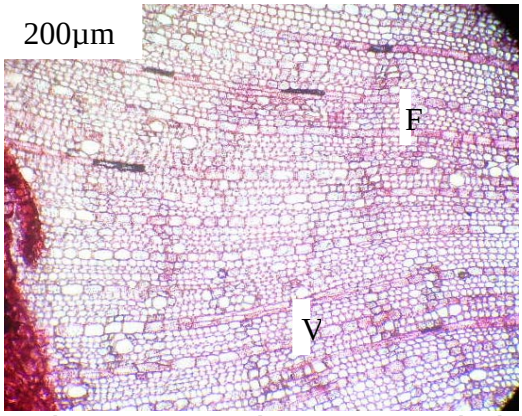
a - Mixture (transverse section)



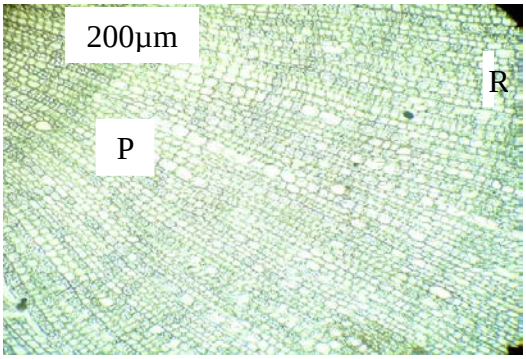
b - P. fluorescens (transverse section)



c - P. cissicola (transverse section)



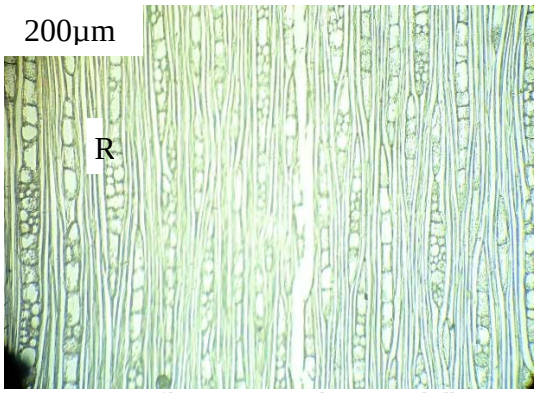
d - P. corrugate (transverse section)



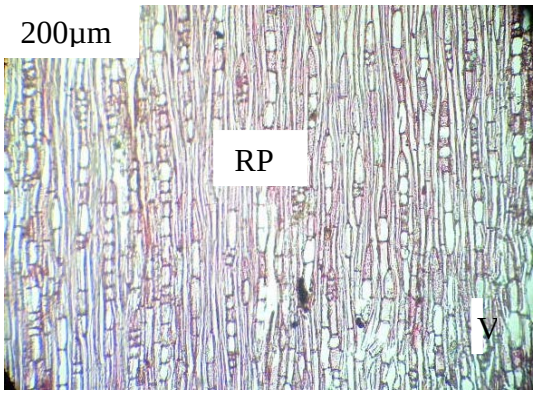
e – CONTROL (transverse section)

V: Vessel, R: Rays, P: Parenchyma, T: Tyloses, F: Fibre

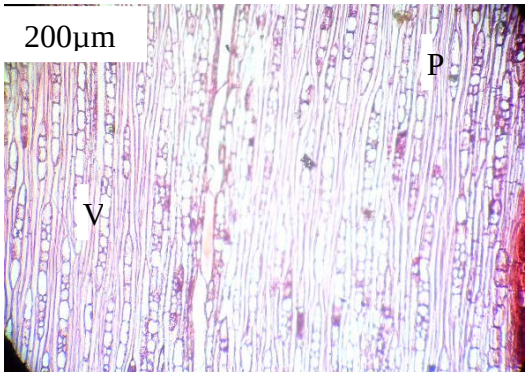
Fig. 1.
(a-e): Transverse section showing micrographic features observed from the tree cuttings of T. africana treated with PGPR.



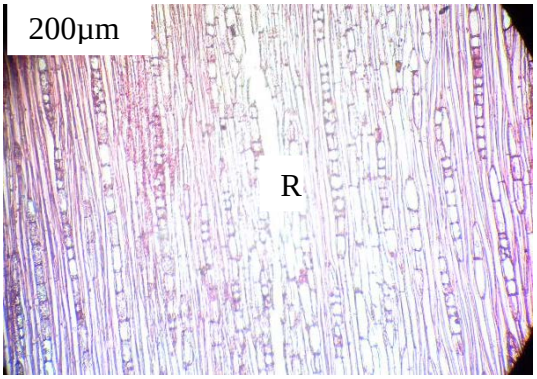
a - P. fluorescens (tangential)



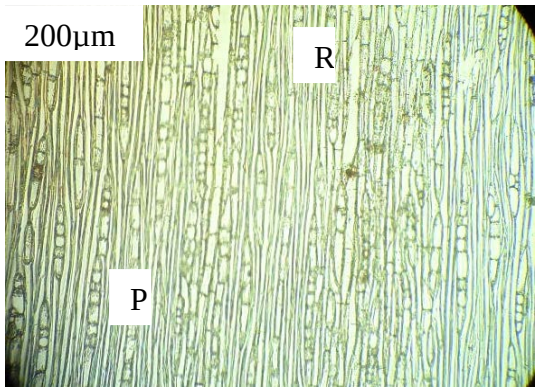
b - P. cissicola (tangential)



c - P. corrugate (tangential)



d - Mixture (tangential)

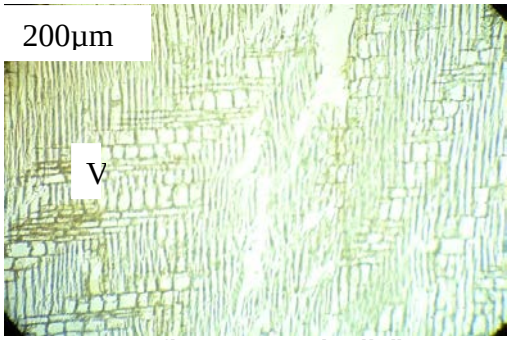


V: Vessel, R: Rays, RP: Ring Porous, P: Parenchyma

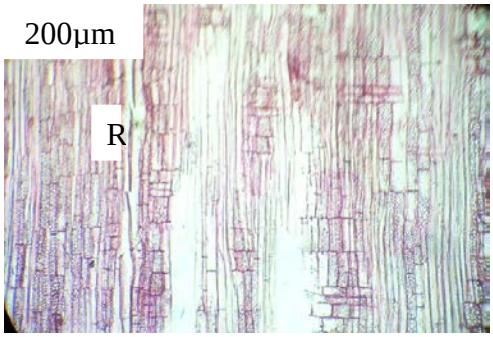
e – CONTROL (tangential)

Fig. 2.

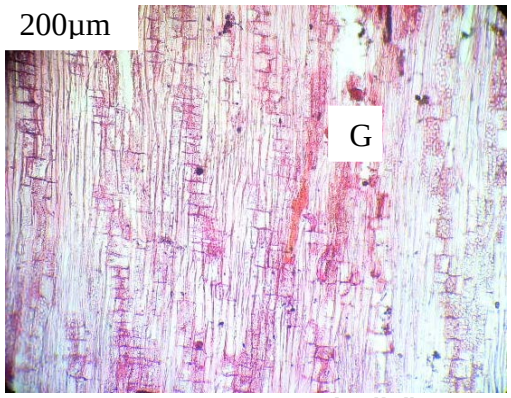
(a-e): Tangential section showing micrographic features observed from the tree cuttings of T. africana treated with PGPR.



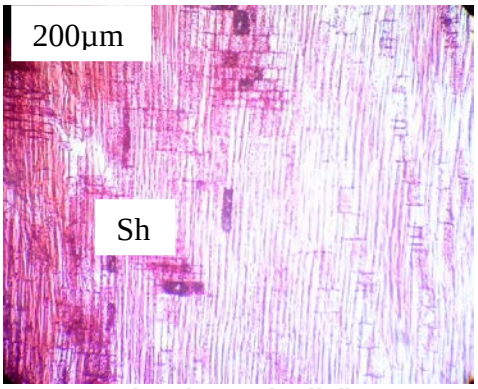
a - P. fluorescens (radial)



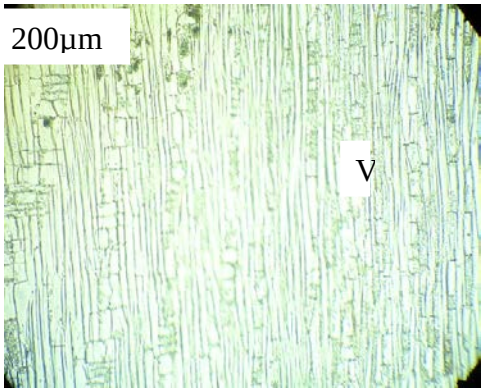
b - P. cissicola (radial)



c - P. corrugate (radial)



d - Mixture (radial)



V: Vessel, R: Rays, G: Gum deposit, Sh: Shealth cell

e - CONTROL (radial)

Fig. 3.

(a-e): Radial section showing micrographic features observed from the tree cuttings of T. africana treated with PGPR.

Plant growth-promoting rhizobacteria (PGPR) have been used in conjunction with the cultivation of many important agricultural crops. They are commonly introduced through seed and soil inoculation, Sanginga *et al*. (2009). In the present study, the effects of inoculations of three different *Pseudomonas* species having plant growth-promoting traits on seedlings of African breadfruit (*T. africana*) have also influenced wood cells especially the parenchyma cells as shown in (Fig. 1, (b) and Fig. 3,(a and d). This was evident by some patches of materials that were confined to the parenchyma cells alone as shown in (Fig. 2, (a-e) as also discovered by Adeniyi *et al*. (2015) and Areo (2019).

CONCLUSION

This present study found that inoculation of co-inoculants of PGPR *Pseudomonas cissicola, Pseudomonas corrugate, and P. fluorescens* which promoted seedling growth influenced the wood cells. They majorly reflect in the contents of the parenchyma cells which serve as food storage for plants. Hence, the amount of food contents within the cells has a direct relationship with collar diameter, seedling height, and sizes of some wood cells such as the pores and rays. The PGPR that we have identified therefore assists in our understanding of the role of tree breeding and improvement on the timber growth properties which will further enhance the wood quality and technical performance for timber users and the wood industry.

RECOMMENDATION

The scope of wood anatomy in this study was limited to qualitative investigation, but there is a need to go further in the area of quantitative anatomy for more information in the area of data analysis.

REFERENCES

Adeniyi IM, Areo OS, Oluwadare AO, Okorie NA, Alao OJ (2015) Wood Anatomy of Mistletoe-Infected Wood of Tectona grandis Linn. F. Elixir Environ. & Forestry 88:36527-36530.

Adeniyi IM, Adejoba OR, Akinlabi FM, Alao OJ (2016) Vegetable oils as clearing agents. Achievements in Life Sciences. www.elsevier.com/locate/als 10:1-6.

Aslant R, Cakmakci R, Sahin F (2007) Effect of plant growth promoting rhizobacteria on young tree growth and fruit yeild under orchard conditions. Scientia Horticulturae 111:371-377.

Agbogidi OM, Onomeregbor VA (2008) Morphological Changes in the Seedlings of *T. africana* Grown in Crude Oil Impacted. In: Popoola, L. (ed), *Climate Change and Sustainable Renewable Natural Resources Management.* Paper presented at the proceeding of the 32nd Annual Conference of the Forestry Association of Nigeria, UmuahiaAbia-state State, Nigeria. 20 – 24 October 2008, pp. 170-182.

Ahemad M, Kibret M (2013) Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective *Journal of King Saud University of Science* 26:1-20.

Appiah F, Oduro I, Ellis WO (2011) Pasting properties of Treculia africana seedflour in Ghana and the production of a breakfast meal.Agriculture and biology *Journal of North America*. Retrieved 10 July, 2014 from http: // www.scihub.scihub.org/abjna. 54 *Journal of Forestry Research and Management. Vol. 14(2):46-54; 2017, ISSN 0189-8418 www.frin.gov.ng/frin1/journals.html*

Areo OS (2019) Wood Properties and Natural Durability of *Artocarpus altilis* (Parkinson Ex F.A. Zorn) Fosberg, Unpublished Thesis. University of Ibadan, pp. 111-114.

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology Biotechnology* 28:1327-1350.

Giami SY, Adindu MN, Akusu MO, Emelike JN (2000) Compositional, functional and storage properties of flours from raw and heat processed African breadfruit (*Treculia Africana* Decne) seeds. *Journal of Plant foods for human nutrition* 55(4):357-368.

Gholami A, Shahsavani S, Nezarat S (2009) The effect of plant growth promoting Rhizobacteria PGPR) on germination, seedling growth and yield of Maize*. World Academy of Science Engineering and Technology* 49:19-24.

Irvine JI (1981) Comparative Study of the Chemical Composition and Mineral Element Cotnent of **Treculia africana** Seeds and Seed Oils. Journal of Food Engineering, 40:241-244.

Jang JH, Woo SY (2018) Physiological changes and growth promotion induced in poplar seedlings by the plant growth-promoting rhizobacteria *Bacillus subtilis* JS. Photosynthetica 56:1188-1203.

Jean DB (2014) *Treculia africana* - The Potentials of a Multipurpose Indigenous Trees Species in Africa. Nutrecul Agroforestry publication. Retrieved 22 May, 2015 from www.slideshare.net//nutrecu/*treculia-africana*.

Meregini AOA (2005) Some Endangered Plants Producing Edible Fruits and Seeds in Southern Nigeria. *Fruits* 60:211-220.

Nuga OO, Ofodile EAU (2010) Potentials of *Treculia Africana* Decne - An endangered species of Southern Nigeria. Journal of Agricultural and Social Research. 10(2):91-99 https://www.ajol.info/index.php/jasr/index

Nzekwe U, Ojeifor IM, Nworie HE (2010) Assessment of the gestation period and economic yield of African breadfruit, Reculia Africana, var. Africana Decne, moraceae. Journal of Agricultural and Social Research. Vol. 9 No. 1 https://www.ajol.info/index.php/jasr/index DOI: 10.4314/as.v9i1.57450

Odume L, Onweluzo LJC (2008) Method of Extraction and Demucilagination of Treculia africana: Effect on Composition. www.bioline.org.br/request?nf07008.

Okafor JC (1985) Selection and Improvement of Indigenous Tropical Fruit Trees: Problems and Prospects. Journ. For. Res, 1(2):87-95.

Osabor VN, Ogar DA, Peter CO, Eneji E (2009) Profile of the African Bread Fruit (*Treculia africana*). Pakistan Journal of Nutrition 8(7):1005-1008 DOI:10.3923/pjn.2009.1005.1008

Onweluzo LJC, Odume L (2008) Method of Extraction and Demucilagination of *Treculia africana*: Effect on Composition. Retrieved 22 October, www.bioline.org.br/request?nf07008.

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. https://agris.fao.org/agris-search/search.do?recordID=DJ2012070623

Sanginga N, Woomer PL (2009) Integrated Soil Fertility Management in Africa: Principles, Practices, and Development Process. Tropical Soil Biology Fertility Institute of the International Center for Tropical Agriculture, Narobi, pp. 273.

Sumbele SA (2012) Effects of Auxins and Leaf Size on Rooting of *Treculia Africana* Dcene Stem Cuttings. *Science Journal of Environmental Engineering Research*, pp. 210.