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EFFECT OF Psorospermum guineense (Hochr.) STEM BARK AND LEAF EXTRACTS ON GERMINATION, SEEDLING VIGOUR AND SEED-BORNE FUNGI ASSOCIATED WITH MAIZE

Titus E.O., Anjorin T.S.* and Asala S.W.
Department of Crop Protection, Faculty of Agriculture,
University of Abuja
oyindamola35@gmail.com; 08062242866

ABSTRACT

This study evaluated the effects of leaf and stem bark extracts of *Psorospermumguineense*Hochr (Christmas berry) on seed-borne fungi, germination and growth characteristics of two maize (*Zea mays* L) varieties. The aqueous extracts of the leaves (LE) and stem bark (SBE) of the plant were prepared at different concentrations of 0%, 25%, 50% and 100%. These seed treatments were repeated four times in the laboratory and three times on the field. Complete Randomized and Randomized Complete Block experimental designs were used in the laboratory and the field respectively. The seed samples were cultured in Petri dishes laden with Whatman filter paper (No 1) and also sown on the field. At 5 Days After Sowing (DAS) the seed treated with 50 and 100 % SBE were observed to have the higher germination percentage (GP) than other treatments and control. The hybrid maize variety applied with *P. guneense*100% aqueous stem bark extracts (SBE) had the highest radicle and plumule length (39 mm) at 9 DAS. At 5 Days After Sowing (DAI), the seed maize treated with 100% stem bark extract showed high reduction in the incidence of *Aspergillus flavus* and comparable with the apron plus treatment.Under 100% SBE treatment, the radial growth of *A. flavus* and *A.niger*were inhibited by 76.29% and 81.5% respectively and not significantly different from that of Apron plus, the synthetic fungicide. From the field experiment, the aqueous SBE (100%) of had the GP of 89.0% compared with 62% in the control plots. This study implies that the SBE of *P. guineense* has fungicidal potential on seed-borne fungi of maize and also enhanced germination probably due to phytotonic organic residues in them.

Keywords: growth characteristics, Psorospermumguineense, extracts, seed-borne fungi, maize

INTRODUCTION

Maize (*Zea mays* L.), family Poaceae is the third most important food crop in the world surpassed only by two other grains, wheat and rice (Tsedaley *et al.*, 2016). It is considered as the most cultivated, consumed cereal in agricultural sector in West Africa (IITA, 2018).It is majorly cultivated in the rainforest and the derived savannah zones of Nigeria (Oladejo and Adetunji, 2012). Maize can be harvested and consumed in the unripe state, when the kernels are fully grown but still soft. It can be processed into a value added product known as corn meal (maize flour) which constitutes a staple food in many regions of the world (Awata *et al.*, 2019).

Despite such benefits of maize, the crop on the field and the seeds are often infected with several pathogenic fungi (Abe *et al*., 2015; Tsedaley and Adugna, 2016) . The negative environmental impact of pesticides used for pest and disease control is intensively increasing every day. For this reason, alternative methods of reducing pesticide are being developed (Firdos *et al*., 2018). Fungicides and other pesticides are expensive and unaffordable to rural farmers in many African countries. Most of

these chemicals have residues that are not biodegradable, posing danger to the environment and to the users. More importantly, increasing instances of pesticide resistance have been reported (Hawkins*et al.*, 2018). Also, pesticides residues and organic priority pollutants of pesticides have been reported on cultivated crops (Gunshit*et al*., 2013).

There is urgent need to find affordable interventions, which can be safely used that is fungicides that does not have residues and are biodegradable. Extracts from plants are viable alternative for such utilization. In Nigeria the use of botanicals as pre-planting seed treatment is very common in rural areas, more especially in populations who cannot afford to buy synthetic chemical (Akpan *et al.,* 2017). *P.guineense* is known to be abundant in the wild and it is locally used by farmers in French Guinea, Senegal and Nigeria as an insecticide and to treat skin infections (Wilcox *et al.,* 2013 and as a source of antioxidant (Elufioye, 2016)**.** The objectives of the study were therefore toassess the effect of *P. guineense* stem bark and leaf extracts on the maize germination and seed-borne fungi in the laboratory, and also on seedlingsvigour on the field.

MATERIALS AND METHOD

Study Area

The research was carried out in the Laboratory of Crop Science Protection Department while the field experiment was on the Teaching and Research Farm of the Faculty of Agriculture, University of Abuja.

Source of Plant Materials

The fresh healthy leaves and stem bark of *P.guineense*(locally called *fukayi* or *kugaye* in Gbagyi) was obtained from Gwako village, Gwagwalada Area Council Abuja, FCT and Zuma village in Bwari Area Council Abuja, FCT.

Source of Planting Material

The seeds used in this trial were Sammaz 14, (open pollinated maize variety) was obtained from Green Pal Global an accredited seed vendor affiliated with the National Agricultural Seed Council Nigeria. Agronomic characteristics of the variety include late maturing, has white coloured grains, large seeds, large cob and good husk cover, matures between 110-120 days, usually attains plant height of 180-200 cm and produce quality protein maize seeds. The local maize variety was obtained from Bwari market,Bwari Area Council, Abuja.

Preparation of leaf Aqueous Extract

Fresh *P. guineense*plant leaves were washed with sterile water, shade-dried at room temperature (25^{o}C) on the disinfected laboratory bench for two weeks to dry properly. The dried leaves were first pulverized in a wooden mortar using a pestle and further milled to fine powder using electric blender (MDL MC – B145). The powder obtained were sieved through a screen with a mesh sizes of 0.4 mm to obtain fine powder (Kuberan*et al*., 2012)

The powdered leaf was stored in an enclosed bottle container at room temperature, pending the time it was used. For the aqueous extraction, one hundred gram of the powdered leaves was added to 100 ml of distilled water in a 500ml glass jar for 24hrs (Rivillas-Acevedo and Soriano-Garcia, 2007). The stock solution (100%) was obtained by evaporating the extract using water bath at 60^{o}C from which 50%, 25% levels of the extracts were prepared by dilution. About 0.2 kg of maize seeds sample were coated with 20 ml of each concentration level of the leaf extract.

Preparation of Stem Bark Extract

Two kilogram (2kg) of stem bark was added to 2litres of distilled water and subjected to heat at 80^{o}C for 90mins to extract the exudate. On heating, the stem bark exudate

float on top of the water and was scooped using a spoon and was left for 20 mins to cool. The viscous exudate was added with Dimethyl sulfoxide (DMSO) as a diluent (10:1 w/w). this formed the 100% stock solution (Moses, 2010).Local farmers used to dissolve the exudate by adding sheabutter (10:1) and heat them from the stock, concentration levels of 50% and 25% were obtained by serial dilution. 0.2 kg of maize seeds sample were added with 20 ml of each concentration level of the stem bark extract, mixed and coated with it.

Determination of the effect of *P. guineense*stem bark and leaf extract on the germination of maize

Sammaz 14 (OPV) hybrid and local maize varieties were planted in a (9cm) Petri dish on a disinfected bench using forceps. The Petri dish was laid with two layers of Whatman filter paper (CAT No 1001110). The prepared100%, 50% and 25% leaf and stem bark extract treatments as earlier described were applied on the seeds. One gram of Apron plus chemical fungicide was applied with the seeds as positive check treatment while the control was applied with distilled water only. Complete Randomized Design (CRD) was adopted involving nine treatments, and with four repetition for each parameter investigated. The Petri dishes were kept in an incubator at room temperature (25^{o}C) with 12hrs alternate light. The seeds in the filter paper were wetted with distilled water daily in the morning and evening during the germination trial.

Germination percentage (GP) were taken 5 days after sowing (DAS). This was calculated using the formula:

$$GP = \frac{\text{Total seeds germinated}}{\text{Number of initial seeds}} \times 100$$

Other parameters measured were radicle and plumule length (mm) taken at 3, 6 and 9 days after sowing (DAS) using a meter rule.

Determination of incidence of fungi associated with the maize seeds

In the laboratory, 39g of Sabroud Dextrose Agar -SDA (Oxoid™ ThermoScientific Product, England, UK) was poured into a conical flask 1000 ml containing l litre of distilled water. It was then sterilized for 15 mins in an autoclave at 121^{o}C. One gram of streptomycin was added to the media to inhibit the growth of bacteria. Then 20 mlmolten agar was poured into the Petri dishes (Ø 9 cm) and allowed to solidify for 20 mins. The Petri dishes were then inoculated with three infected seeds each, repeated four times and labeled accordingly and incubated. At 7 DAS, the colony count were taken.

Sub-culturing to get pure culture of *A. flavus* and *A. niger*

Suspected*A. flavus* and *A. niger*were then sub-cultured in Petri dishes with SDA using an inoculating stick sterilized by flaming in the Bunsen flame. They were incubated at 25°C for 5 days (12 h light/12 h dark photoperiod). At the end of incubation, a speck of mycelium were picked from the actively growing edge and placed on sterile slide, stained with lacto phenol blue and the morphology of the fungi were observed for confirmatory identification under the microscope.

Assessment of *A.flavus* and *A. niger* radial growth inhibition by *P. guineense* stem bark extract

The prepared SBE treatments (100%, 50%, 25%, 0% Apron plus and the control) were added to sterile molten PDA (40^{o}C) using a sterile pipette. The experimental design was CRD involving four repetitions. The

parameters determined at 4, 5 and 6 days after inoculation (DAI) was inhibition of radial growth of the fungi using the formula:

$$\%\text{tage Inhibition} = \frac{C - T}{\mathrm{T}} \text{ x } 100$$

Where

C= Diameter of colony in the control
T= Diameter of colony in the treatment

Field Experimental Procedures.

The experiment was carried out on a total plot area of 480.5m^2 (31m by 15.5m). Each sub-plot was 4m by 4.5m contain six rows with 75cm inter row spacing and 50cm intra row spacing with three crops planted per hole and later thinned to two. The stem bark extract preparation and application was as described earlier for the laboratory experiment. The experimental design was a randomized complete block design involving eight treatments and with three replicates. The treatments were 100%, 50% and 25% leaf extract and stem bark respectively, apron plus (10g) and control.

The parameters measured were:

Germination percentage: The same formula used for laboratory experiment was used.

Plant height at 14 DAS, 21 DAS and 28 DAS: Five plants were sampled from each plot at random, the length of each were measured from the ground level to the flag-leaf, using a meter-rule (Garba and Namo, 2013).

The seedling vigourindex (SVI): was determined using the formula:

SVI = Germination percentage × seedling length/100

Number of leaves at 14 DAS, 21 DAS and 28 DAS: The number of leaves were counted from five noted plants and the means calculated (Johnston, 2018).

Data Analysis.

The standard error of the mean for the incidence of fungi associated with the maize varieties was determined. The data for the germination percentage, the inhibitory effect of the stem bark extract against *A. flavus* and *A. niger*, and also the effect of the stem bark and leaf extracts on the seedling vigour parameters of maize were subjected to univariate analysis of variance (ANOVA)using the generalized linear model (GLM) procedure and significant differences between means were confirmed. Data with zero scores were transformed with the use of $\sqrt{x} + 1/2$ before proceeding with the ANOVA. Differences between the treatments means were determined with Duncan multiple range test (at the 0.05 probability level). The statistical package IBM SPSS statistics 25 (IBM Corp., Armonk, NY, United States) was used for data analysis. Some of the data were graphically represented by the use of bar chart with the aid of excel version, 2016.

RESULTS

The result obtained from the laboratory and field experiments were as follows:

Laboratory experiment:

Germination percentage (GP) of maize treated with *P. guineense*stem bark and leaf extracts at 5 DAS

The GP of improved maize seed variety is slightly higher than those of local variety under all treatment except Apron plus application (Table 1). At 5 DAS the seeds treated with 50 and 100 % SBE were observed to have relatively higher GP than other treatments including those applied with Apron plus.The GP of the seeds under 100% LE treatments were lower than all other treatments including that of the control.

Table 1: Effect of *P. guineense*stem bark (SBE) and leaf extracts (LE) on Germination Percentage of improved and local maize variety at 5DAS

Treatment	Germination %tage	
	Improved variety	Local variety
Control	75.55±0.3* **3	74.44±0.21
25 % LE	77.55±0.11	76.55±0.23
50 % LE	76.77±0.23	75.55±0.34
100 % LE	65.55±0.34	64.44±0.24
25 % SBE	86.77±0.21	85.55±0.22
50 % SBE	88.77±0.33	87.77±0.24
100 % SBE	89.88±0.21	88.77±0.3
Apron plus	79.0±0.3380	80.0±0.34

*Standard Error of the Mean

Effect of *P. guineense* stem bark and leaf extract on radicle length of seeds of local and hybrid maize varieties

There was significant difference ($p \leq 0.05$) in the radicle length of local and hybrid maize seeds due to stem bark extract (SBE) and leaf extract (LE) treatmentsin the laboratory. At 3 and 9 DAS, the radicle length of the seeds applied with 50% SBE was higher than other treatments and the control. But at 6 DAS, the radicle length of the seed applied with 100% SBE was significantly higher than other treatments (Fig. 4.1). The radicle length of 100% LE applied seeds was lower than that of 50% LE.

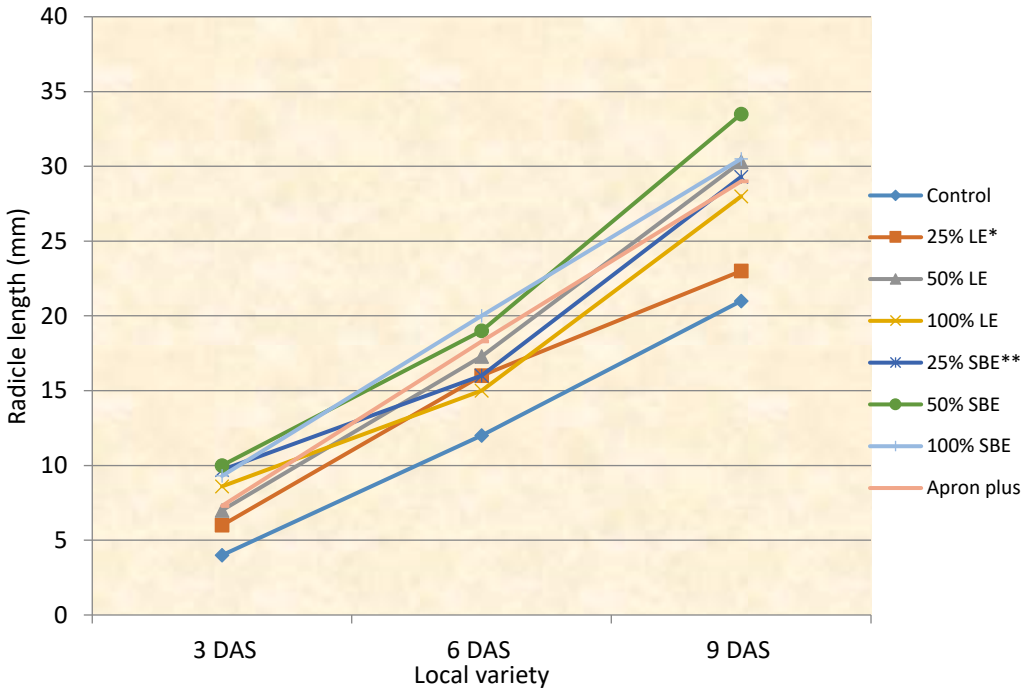

Fig. 1. Effect of *P. guineese*stem bark and leaf extracts on the radicle of local maize varieties

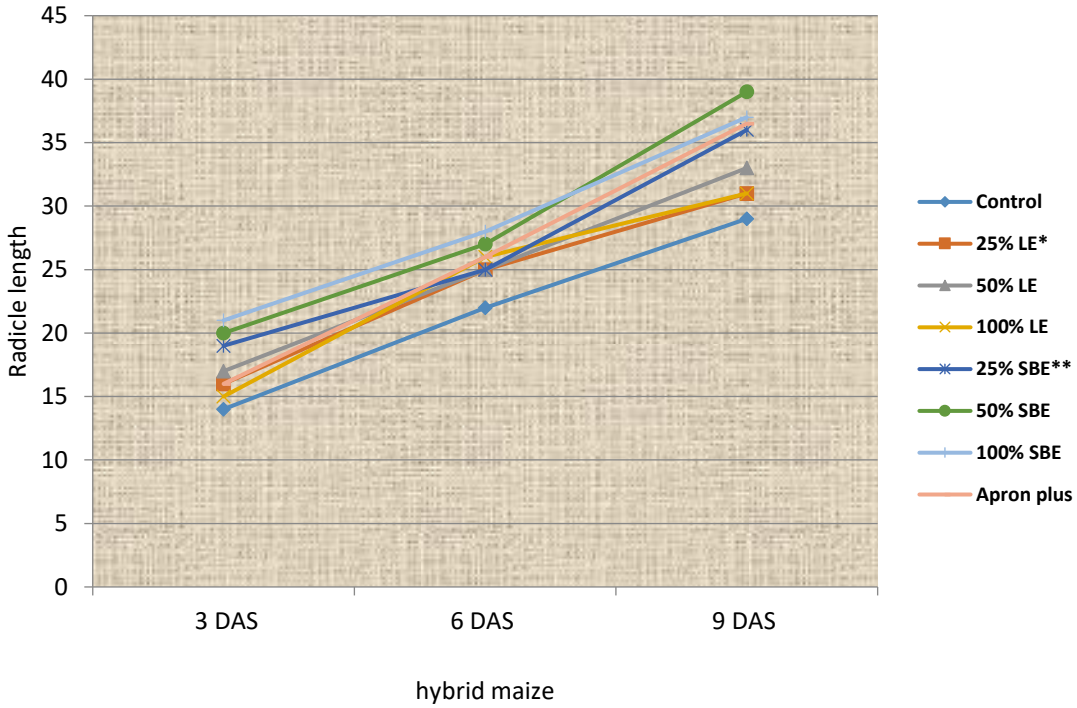

Fig. 2. Effect of *P. guineese*stem bark and leaf extract on the radicle of hybrid maize varieties

Effect of *P. guineense*stem bark and leaf extract on the plumule of local variety of maize.

There was significant difference in the plumule length of local maize varieties applied with the SBE and LE of *P. guineense* (Table 4.3). At 3DAS, the plumule length of the seeds applied with 100% LE and SBE were significantly higher than other treatments. At 9 DAS, the plumule length of the seeds applied with 50% and 100% SBE were significantly higher than other treatments, including those applied with 100% LE.In all, the plumule length of the seeds in the control plots were significantly lower than those from other treatments.

Effect of *P. guineense*stem bark and leaf extract on the plumule of hybrid variety of maize.

There was significant difference ($p \leq 0.05$) in the plumule length of hybrid maize variety applied with *P. guineense*SBE and LE (Table 4.4). At 3 and 6 DAS, the plumule length from the seeds applied with 100% and 50% SBE were significantly higher than other treatments, including the control. At 9 DAS, the plumule length from the seeds applied with 100% and 50% SBE were however not significantly different ($p \geq 0.05$).

In vitro inhibitory effect of *P. guineense*stem bark extract against *A. flavus* and *A. niger*.

There was significant difference ($p \leq 0.05$) on the radial growth inhibition of fungi associated with hybrid maize and local maize variety treated with *P. guineense*SBE (Table 4). Application of 100% SBE and Apron plus significantly inhibited the growth of *A. flavus*by 76.29%compared with 89.20%of the Apron plus.There was similar inhibitory trend of the treatments on *A. niger*

Table 2: Effect of *P. guineense*stem bark (SBE) and leaf extract (LE) on the plumule of local variety of maize

Treatment	Plumule length (mm)		
	3 DAS	6 DAS	9 DAS
Control	4.1^{e}	6.2^{e}	12.0^{g}
25% LE	6.0^{cd}	18.0^{c}	28.7^{d}
50% LE	7.0^{bc}	21.0^{b}	24.0^{e}
100% LE	10.0^{a}	23.0^{a}	38.3^{c}
25% SBE*	6.0^{d}	7.7^{d}	18.0^{f}
50% SBE	7.0^{bc}	7.7^{d}	44.0^{b}
100% SBE	8.0^{b}	20.0^{b}	47.0^{a}
Apron plus	7.0^{bc}	9.3^{d}	22.0^{e}

Means with the same alphabet are not significantly different from one another on the same column using Duncan multiple range test (DMRT) at 5% probability level

Table 3: Effect of *P. guineense*stem bark (SBE) and leaf extract (LE) on the plumule of hybrid variety of maize

Treatment	Plumule length (cm)		
	3 DAS	6 DAS	9 DAS
Control	3.0^{h}	3.0^{g}	21.7^{c}
25% LE	4.0^{gh}	9.0^{de}	12.0^{e}
50% LE	5.3^{fg}	6.0^{f}	29.0^{b}
100% LE	6.0^{ef}	8.0^{e}	20.0^{c}
25% SBE	10.7^{c}	12.0^{c}	21.3^{c}
50% SBE	13.0^{b}	16.0^{b}	46.7^{a}
100% SBE	23.0^{a}	28.0^{a}	47.7^{a}
Apron plus	8.0^{d}	10.0^{d}	17.0^{d}

Means with the same alphabet are not significantly different from one another on the same column using Duncan multiple range test (DMRT) at 5% probability level

Table 4: *In vitro* inhibitory effect of *P. guineense*stem bark extract (SBE) against *A. flavus* and *A. niger*at 5 DAS

Treatment	Inhibition %	
	A. flavus	*A. niger*
Apron plus	89.20^{a}	90.8^{a}
Control	11.72^{c}	12.8^{c}
25% SBE	63.16^{b}	71.5^{b}
50% SBE	69.20^{b}	74.9^{a}
100% SBE	76.29^{ab}	75.4^{a}

Means with the same alphabet are not significantly different from one another on the same column using Duncan multiple range test (DMRT) at 5% probability level.

Field Experiment

Germination percentage of maize treated with *P. guineense*stem bark and leaf extracts at 10 DAS on the field

At 10 DAS, the seeds treated with 50 and 100 % SBE were observed to have relatively higher germination percentage (Fig. 3), while those seeds treated with 25% LEor in the control had relatively low GP.

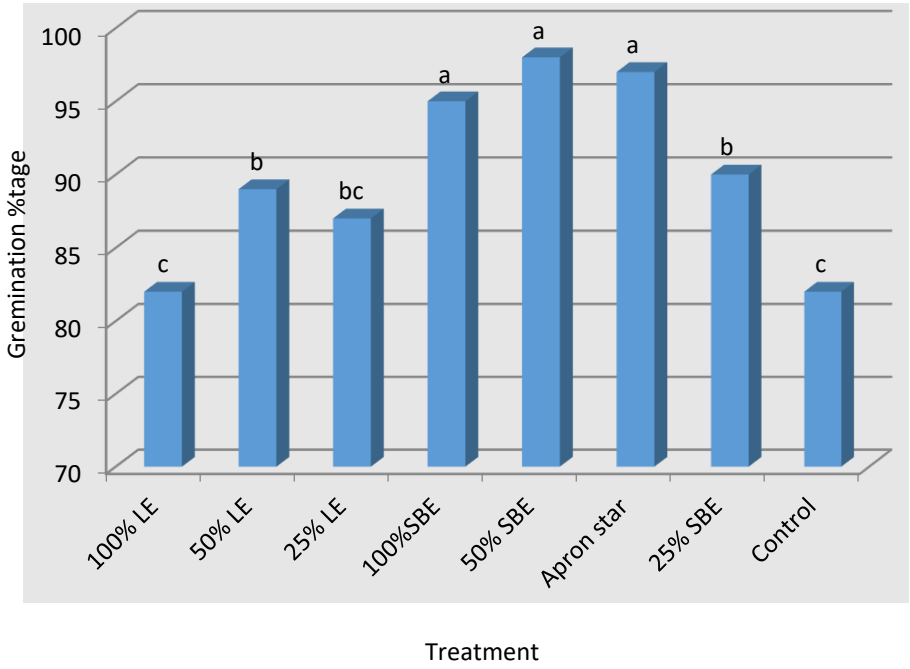

Fig. 3: Effect of *P. guineense*stem bark and leaf extracts on germination percentage of maize at 10 DAS

Effect of *P. guineense* stem bark and leaf extract on plant height of maize on the field.

There was significant difference ($p \leq 0.05$) in the hybrid maize height applied with *P. guineense* SBE and LE. on the field. At 14 DAS, the plant height from the seedling applied with 100% and 50% SBE was significantly higher than other treatments. The plant height from the control seedlings had significantly lower height than other treatments. At 21 DAS, the plant height from the seedling applied with 100%, 50% and 25% SBE were not significantly different ($p>0.05$). At 28 DAS, the maize plant height from the seedling applied with 100% SBE was highly significant compared to other treatments. The plant heights of the seedlings in the control were significantly lower than all other treatments.

Seedling vigour of maize seeds treated with *P.guineense* extracts on the field,

At 14 DAS maize seeds applied with 50 % and 100 % SBE had the relatively higher seedling vigour (Table 4.11). This trend is the same at 21 DAS. Those plots with relatively lower seedling vigour index are from the seeds in the control and 25% LE at 14 DAS

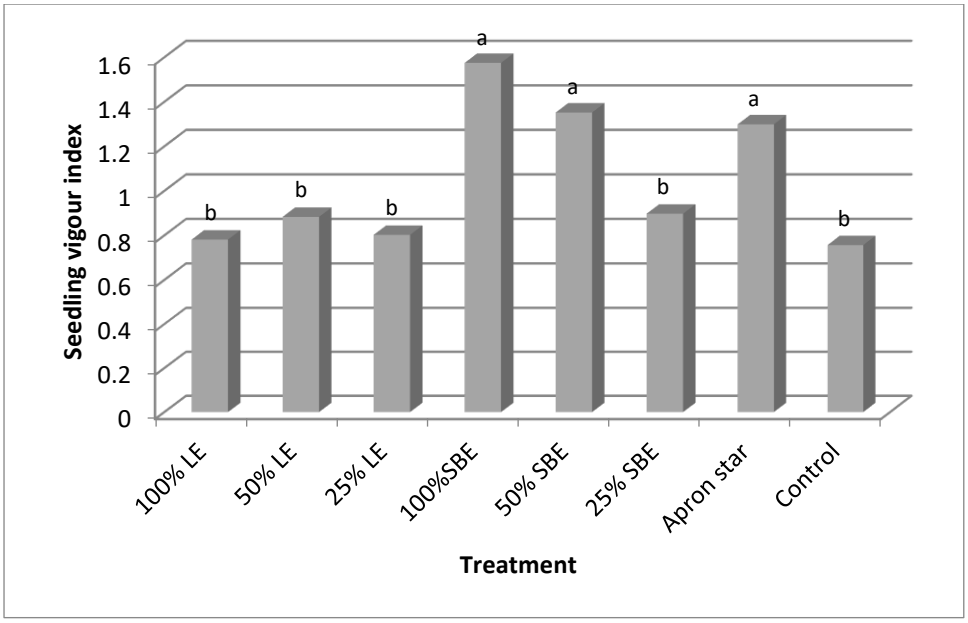

Fig. 4: Seedling vigour index (SVI) of maize seeds treated with *P. guineense*extracts at 14 DAS

Effect of *P. guineense* stem bark and leaf extract on the number of leaves of maize seedling on the field.

There was significant difference ($p<0.05$) in the number of leaves of seedlings of hybrid maize applied with *P. guineense* SBE and LE. At 14 DAS, the number of leaves from maize applied with 100% SBE was highly significant than other treatments. At 28 DAS, the number of leaves of the maize applied with 25% LE and the control were significantly lower than other treatments

Table 5: Effect of *P. guineense* stem bark (SBE) and leaf extract (LE) on the height of maize in the field

Treatment	Plant height (mm)		
	14 DAS	21 DAS	28 DAS
100% LE	12.6^{b}	28.3^{b}	48.1^{b}
50% LE	12.8^{b}	29.0^{b}	49.2^{a}
25% LE	11.8^{b}	28.7^{b}	48.0^{b}
100%SBE	17.5^{a}	33.1^{a}	51.7^{a}
50% SBE	15.2^{a}	32.3^{a}	48.9^{b}
25% SBE	13.2^{ab}	32.3^{a}	44.4^{c}
Apron plus	10.5^{b}	28.3^{b}	47.9^{b}
Control	9.3^{bc}	21.7^{b}	36.7^{d}

Means with the same alphabet are not significantly different from one another on the same column using Duncan multiple range test (DMRT) at 5% probability level

Table 6: Effect of *P. guineense* stem bark (SBE) and leaf extract (LE) on the number of leaves per plant of maize seedling

Treatment	Number of leaves		
	14 DAS	21 DAS	28 DAS
100% LE	2.6[b]	5.0[ab]	7.0[a]
50% LE	2.3[b]	4.7[b]	6.8[a]
25% LE	2.5[b]	5.0[ab]	6.2[b]
100% SBE	3.6[a]	6.0[a]	7.2[a]
50% SBE	2.6[b]	5.0[ab]	7.3[a]
25% SBE	2.7[b]	5.9[a]	7.1[a]
Apron plus	2.3[b]	6.0[a]	7.4[a]
Control	1.9 [c]	5.0[ab]	6.0[b]

Means with the same alphabet are not significantly different from one another on the same column using Duncan multiple range test (DMRT) at 5% probability level.

DISCUSSION

Modern agriculture advocate for seeds treatment for sustainable agriculture (Sharma *et al*., 2015;Yu*et al.,* 2014).In the laboratory the aqueous extract of *P. guineense*100% SBE significantly increased germination of hybrid maize compared to the local maize variety. This finding is similar to that of Talukder*et al*.(2015) who reported that the aqueous extract of Arjuna (*Terminalia arjun*) increased the germination percentage and growth of ladies' finger okra and turnip, while the aqueous extract of Beleric myrobalan (*Terminalia belerica*) increased germination and growth of spinach. In a contrast, Abugre and Quashie-sam, (2010) reported that *Jathrophacurcas*extract at higher concentrations had strong inhibitory effect on germination, radicle and plumule length of *Phaseolus vulgaris, Zea mays, Lycopersicon lycopersicum*and *Hibiscus esculentus*. Also Talukder*et al*.(2015) showed that aqueous extract of *Azadiractaindica* decreased germination and growth of turnip. The increase in the radicle and plumule length due to 50 and 100% SBE might be due to active phytotonic chemicals of the extracts.

In this study, the 50 and 100% of SBE and LE significantly inhibited *A. flavus* and *A niger*and the effect were comparable with the synthetic fungicide (apron plus used). This might be due to presence of active phytotoxic chemicals such as adamaquinone and adamaxanthone present in *Psoropermum*spp with antimicrobial activity (Mongalo*et al.,* 2018). Similar results were obtained by Masum *et al.* (2009) in which neem seed extract reduced incidence of *Fusarium moniliforme* and other seed-borne fungal infection in sorghum and their germination and seedling vigour were boosted. Also Abdurrahman and Hayriye (2016)reported a significant antifungal activity of *P. laurocerasus, T. orientalis, P americana, R. ponticum* and *S. excelsa*leaves, root, fruit and shoots extracts (400 mg/ml respectively against *Aspergillus flavus, A. niger, Curvularialunata, Fusarium moniliforme*and *Penicillium chrysogenum*.Lemon grass was reported to control*Fusarium moniliforme* and other seed borne pathogens of sorghum(Somda*et al.* 2007).

It was discovered from this study that*P. guineense*aqueous SBE had high significant

stimulatory effect on the germination, radicle and plumule length and seedling vigourof local and hybrid maize while the 100% LEand Apron plus had slightly inhibitory effect on the germination up to 14 DAS. Both the SBE and LE did not significantly affect the number of maize leaves. Also the 50 and 100% stem bark and leaf extracts significantly inhibited *A. flavus* and *A.niger* due to their phytotoxicity. More investigation is recommended to find out the pesticidal effect of the fruits and seed oil of *P. guineense*. Also the effects of different methods of extraction and quantity of extract that will be effective should be investigated. The phytochemical constituent of the extract exerting the stimulatory effect on growth parameters and the one responsible for inhibition of pathogenic fungi also requires further study. Purification and standardization of the crude stem or leaf extracts in order to increase their efficacy is imperative.

REFERENCES

Abdurrahman, O.and Hayriye, D.S. 2016. Antifungal activity of some plant extracts against different plant pathogenic fungi. International Journal of Advances in Agricultural and Environmental Engineering. 3(2):284-287.

Abe, C. A. L. , Faria, C. B. and de Castro, F. F. 2015. Fungi Isolated from Maize (*Zea mays* L.) Grains and Production of Associated Enzyme Activities. International Journal of Molecular Sciences.16 (7):15328-15346

Abugre, K.and Quashie-sam, T. 2010.Evaluating the allelopathic effect of *Jatropha curcas*aqueous extract on germination, radicle and plumule length of crops.International Journal of Agriculture and Biology.12(5): 1560- 8530.

Akpan, E.N., Denise, E.M., Ezendiokwelu E.L. andAnyadike, M.C. 2017. Growth response of seedlings of *Zea mays* (L.) to aqueous extract of *Lycopodium clavatum* (L.) Journal of Botany and Ecological Studies, University of Uyo, Nigeria.2 (4):287–289.

Awata, L.A.O, Tongoona P, Danquah E., Ifie B.E, Suresh L.M, Jumbo, M.B, Marchelo D'ragga P.W. and Sitonik C (2019). Understanding tropical maize (*Zea mays* L.): The major monocot in modernization and sustainability of Agriculture in sub-saharan Africa. International Journal of advance Agricultural Research.**7:** 32-77

Baka, Z.A.M. 2014. Plant extract control of the fungi associated with different Egyptian wheat cultivars grains. Journal of Plant Protection Research.54 (3):231–237

El-Abady M.I. 2015. Influence of Maize Seed Size/Shape, Planted at Different Depths and Temperatures on Seed Emergence and Seedling Vigour. Research Journal of Seed Science.8: 1-11

Elufioye T.O. 2016. Evaluation of Antioxidant and Antiacne Activity of *Psorospermum febrifugum*(Spach) and *Psorospermumcorymbiferum* (Hochr.) British Journal of Pharmaceutical Research.12 (5): 1-10.

Garba L.L. and Namo, O.A.T. 2013. Productivity of maize hybrid classes in savannah agro-ecologies of Nigeria. African Crop Science Journal.21 (4): 323-335.

Gunshit (2013). Pesticide residues in food 2013. Joint FAO/WHO Meeting on Pesticide residues. Report 2013. Geneva, Switzerland, from 17 to 26 September, 2013. 1- 625

Hawkins, N.J., Bass, C., Dixon, A. and Neve, P. 2018. The evolutionary origins of

pesticide resistance. Biological reviews of the Cambridge Philosophical Society, Advance online publication.94 **(**1): 135–155.

International Institute for Agriculture (IITA, 2018).Annual report **3:**20-32

Johnston, I.A. 2018.Staging corn growth – Dupont pioneer Pratap University of Agriculture and Technology, Udaipur, Rajasthan-313001, India.pp:7

Kuberan, T., Balamurugan, A., Vidhyapallavi, R., Nepolean, P., Jayanthi, R., Beulah, T.A. and Premkumar, R. 2012. *In vitro* evaluation of certain plant extracts against *Glomerellacingulata*causing brown blight disease of tea. World Journal of Agricultural Sciences8 (5): 464 - 467.

Masum, M.M.I., Islam, S.M.M. and Fakir, M.G.A. 2009. Effect of seed treatment practices in controlling of seed-borne fungi in sorghum. Scientific Research and Essay.4 (1): 22-27

Mongalo, N.I., Dikhoba,P.M. Soyingbe, S.O. Makhafola,T.J.2018. Medicinal plants against mycotoxigenic fungi. University of South Africa, College of Agriculture and Environmental Sciences Laboratories, Private Bag X06, Florida, 0610, South Africa Research, Innovation & Engagements Portfolio, Mangosuthu University of Technology, P O Box 12363, Durban.

Oladejo, J.A. and Adetunji, M.O. 2012. Economic analysis of maize (*Zea mays* L.) production in Oyo State of Nigeria. Agricultural Science Research Journals 2(2): 77-83.

Rivillas-Acevedo L.A. and Soriano-Garcia, M. 2007. Isolation and Biochemical Characterization of an Antifungal Peptide from *Amaranthus hypochondriacus*. Seeds. Journal of Agricultural and Food Chemistry 55(25):10156-10161.

Sharma, K.K., Singh, U.S., Sharma, P.K. Ashish, S and Lalan,L. 2015. Seed treatments for sustainable agriculture-A review VL -7 Journal of Applied and Natural Science.**7** (1): 521- 539.

SomdaL.V. and Sereme, P. 2007 Antifungal Effect of *Cymbopogon citratus* , *Eucalyptus camaldulensis*and *Azadirachtaindica* Oil Extracts on Sorghum Seed-Borne Fungi. Asian Journal of Plant Sciences.6 (8): 1182-1189

Talukder, M.A.I.M. Rahaman, B. Roy and Saha, K.C. 2015. Effects of herbal plant extracts on germination and seedling growth of some vegetables. Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. 6(3):421-425

Tsedaley,B. and Adugna, G. 2016. Detection of fungi infecting maize (*Zea mays*) seeds in different storages around Jimma, Southern Ethiopia. Journal of Plant Pathology Microbial **7**(3):1-6.

Wilcox, F. E. Serena F. and Salvatore, G. 2013.Phytochemistry and pharmacognosy of the genus *Psorospermum*Article in Phytochemistry Reviews.**12:** 676-684

Yu Tian, B. Daowei Z., Junbao, Y., Guangdi, L., and Yujie, L. 2014.Responses of Seed Germination, Seedling Growth, and Seed Yield Traits to Seed Pretreatment in maize (*Zea mays* L.) The Scientific World Journal volume. Yield Traits to Seed Pretreatment in Maize (*Zea mays* L.). 1-8

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SITE OPTIMIZATION FOR SUGARCANE – BASED BIOFUEL PRODUCTION FACILITIES IN NIGERIA USING GIS AND REMOTE SENSING TECHNOLOGIES.

Shehu, B. G. and Williams, M.
Agricultural Research Council of Nigeria Plot 223D Cadastral, Zone B6, Mabushi, Abuja
Centre for Landscape Ecology and GIS. Room G222, Grenville Building, University of Greenwich, Medway Campus. ME4 4TB
mbasuge@gmail.com, +2348034127387.
m.williams@gre.ac.uk, +447510305453.

ABSTRACT:

About 66% of Nigeria's agricultural land is not cultivated. Therefore, land availability for biofuel production in the Country may not be a serious hindrance. However, land areas that may be readily available for the purpose may need to be identified taking other factors into consideration. Nigerian Biofuel Policy (2007) stipulated that one of the responsibilities of the Ministry of Agriculture is to support land acquisition and utilisation strategies by biofuel companies. The aim of this research project was to optimize sites for Sugarcane – based biofuel production facilities in the Country. Datasets were collected from several sources such as NIMet, OFSGoF, NEMA, USGS, SEDAC (NASA), etc. A cell size of 100m was used and thus a nominal scale of 1: 250,000 was selected in the preprocessing data manipulations such as digitization. Three models were developed in GIS environment (ArcMap 10.2.2). The first model excluded constraints by buffering features that may not allow for either crops cultivation or facility establishment. The second model identified suitable areas for cultivation of Sugarcane by weighting the input parameters using Analytical Hierarchy Process (Pairwise comparison), implemented through the weighted overlay function. Third model optimised locations for Sugarcane – based biofuel production facilities by employing service area modelling through network analysis. Demsa Local Government Area of Adamawa State was found to be the most ideal location for a Sugarcane – based biofuel production facility in Nigeria with approximately 15Gg of potential feedstock per harvesting season within a 200km supply area. Based on the results and Nigeria's potentiality in Sugarcane production, it was concluded that the Country can become one of the world's leading Sugarcane – based biofuel producing countries. This will create lots of jobs, substitute importation of refined sugar thereby saving foreign exchange and by implementing E-10 policy, attracts foreign exchange through carbon credit.

Keywords: Bioethanol, Sugarcane, Available land, Multicriteria evaluation, GIS, Remote Sensing, Service area, optimal sites.

INTRODUCTION

There is a growing concern about the environmental impacts of fossil fuels, the footprints of which arise from its exploration to its final use (consumption). Such fuels as petroleum, natural gas, coal, shale oil and bitumen are the main souces of lighting, heating and transport energy. Beside the main constituents such as carbon and hydrogen, these fuels consists of other substances like metals, sulphur and nitrogen compounds [1] which serve as greenhouse gases or pollutants. However, according to [2], there is no alternative source of energy, in sight, that can replace fossil fuels. They argue that fossil fuels account for 86% of the rising global energy demand and there is steady increase in the reserves without immediate depletion in

sight, though they are still finite. Growing world population [3] couple with global awareness about climate change has lead many national energy systems to adopt different carbon dioxide policies and reduction targets [4], planning several forms of renewable energy utilization. The objectives are to address the shortage of and provide sustainable clean energy and to encourage infrastructure development as stipulated in the Kyoto directive towards global decarbonisation [5]. Major arguments against crop-based biofuel development include the impact on food supply and land availability for food crops cultivation. However, according to [6], second generation biofuels such as non-food crops and non-edible vegetable oils does not pose food security concern as they are not used as staple. And according to [7], food-energy analysis in Brazil showed no land conflict between food supply and biofuel crops production. In fact, according to them, there could be direct correlation between biofuel investments and food production in areas with good land availability such as Nigeria. According to [8], Nigeria has a total land area of 91.07 million hectares, of which 77% is cultivable. According to them also, only 44% of the cultivable land is under cultivation, the remaining 66% is under permanent pastures, though it could be argued that some of the pasture lands are used for grazing.

One of the strategies used to ameliorate the environmental concerns of fossil fuels is using biofuels as blend to petroleum fuels. This creates huge potentials for agricultural sector development, especially for a crude oil dependent economy such as Nigeria's with a proven reserve of 36b barrels [9] and prospects for more reserves to be discovered. In 2005, a division was created in the Nigerian National Petroleum Corporation (NNPC) called Renewable Energy Division (RED) to deliver and pioneer development of biofuel industry in the Country. One of the primary aims was to link the oil and gas sector with agricultural sector through commercial production of biofuels from selected energy crops as blend stock for petroleum fuels [10]. Although the biofuel programme in the Country was said to have been evaluated in 2012 as unsatisfactory [7] and the development process was very slow due to regime (government) change [11], huge potential still exists for the industry to be developed given the land availability as seen above. According to NNPC [12], the National Biofuel Programme plans to use Sugarcane, Cassava, Sweet sorghum, Oil palm and Jatropha as feedstock and by 2020, 2% of the arable land will be required for the project. The Nigeria National Biofuel Policy [13] outlined the legality of extraction and use of biofuels as well as the roles of various stakeholders. The Policy stipulated that one of the responsibilities of the Ministry of Agriculture is to support land acquisition and utilisation strategies by biofuel companies. According to the policy also, the ethanol is meant for E-10 (10% ethanol blended with 90% petrol). According to [14], Nigeria has the capacity to produce 47.97 Million Tons of Equivalent (MTOE) from biomass and residues annually. According to [15], no fewer than 20 new bioethanol projects were initiated following the release and implementation of the biofuel policy. Ohimain [15] also concluded, based on some preliminary details of the projects that many of the projects' outputs do not appear to be feasible based on their reported quantity of existing feedstock. Therefore, more sources of feedstock need to be exploited to make those projects a reality. This could be achieved by exploiting all the non-controversial feedstock types and putting the available land areas in to production.

The Brazilian biofuel industry, largely Sugarcane – based, is known to be one of the most successful in the world, though its success could be associated with established favouring policies that support the industry.

Sugarcane requires high levels of nitrogen and potassium and too much water supply (ranging from 1100 to 1500mm of rainfall), therefore, in drier regions irrigation is required [16] [17], leading to higher production costs. Though sugarcane is moderately sensitive to salinity [17], it is the most widely spread biofuel feedstock grown on various soil types and largely concentrated within latitudes 300 north and south [16]. The crop's requirement for water and temperature depend on its growth stage – germination, tillering, stem elongation, grand growth and ripening. The optimal soil pH requirement is 6.5 [17]. Beside ethanol and sugar, other by-products can be used in such industries as chemical, feed, fertilizer and pulp [16]. According to [18], there is growing potential for biofuel sugarcane production which is evident in the number of bilateral deals between Brazil and other countries. The Brazilian biofuel industry is a distinguished example of how suitable Sugarcane is as a biofuel feedstock. The purpose of this research project was to identify land areas suitable for Sugarcane production and optimise sites for Sugarcane – based biofuel production facilities.

DATA, MATERIALS AND METHODS

Agro-meteorological data comprising rainfall, relative humidity, minimum and maximum temperatures were acquired from the Nigerian Meteorological Agency (NiMet). It was 20 years' data (1994 – 2013) collected at 44 weather stations distributed nearly randomly over the country and was supplied in Microsoft Excel format. The soil and soil erosion maps were supplied as jpeg format scan of original paper map by the Office of the Surveyor General of the Federation (OSGoF). According to the Office, the soil map was updated between 2010 and 2011 using data from Geological Survey Agency and Nigerian Bureau of Statistics under Federal Government contract. The data consists of 8 main soil groups - Lithosols, Regosols, Alluvial, Vertisols, Eutrophic, Ferruginous, Ferrasols and Hydromorphic. The groups were classified into 28 sub-classes. The flood map, scale 1: 7,500,000 as jpeg scan of the original paper map, was sourced from the National Emergency Management Agency (NEMA). It was produced based on the 2012 flood extent, considered to be the worst in five (5) decades. It displaced 2.1 million people from an area covering 33 of the 36 states of the Country and many farmland areas, storage facilities and livestock were destroyed. The map of the game reserves, scale 1: 10,000,000 and as jpeg (digital) was downloaded from the website of the National Park Services. The feedstock potential map was extracted in jpeg format from a Powerpoint presentation by Nigerian National Petroleum Corporation (NNPC) at the second Nigerian Alternative Energy Expo, 2012. After considering ASTER Global DEM and GMTED2010, SRTM was found to be more accurate for use as DEM dataset in the study and fortunately the recently (2014) released void-filled, 1-arcsecond (30 metres) global data covered Nigeria. It was downloaded from the USGS Earth Explorer website. The settlement datasets were downloaded from the NASA Socio-Economic Data and Application Centre (SEDAC). The water, transport (roads and rail) and administrative boundary datasets were downloaded from Diva GIS which was the only option for these datasets (Table 1).

ArcGIS 10.2.2 and ERDAS-Imaging 2014 were the two main soft wares used in the analysis, though Microsoft Excel 2013 was used to implement Analytical Hierarchy Process (Pairwise Comparison). The three major pre-processing were digitization (for all the jpeg files), interpolation to create raster surfaces for the agro-meteorological data (rainfall, relative humidity and temperature) and mosaicking 101 tiles covering Nigeria (for the SRTM). All the raster surfaces were resampled to 100

metres. Hence, the nominal map scale of 1: 250,000 was used in the analysis. All the input datasets were aligned to UTM Zone N32 (WGS84).

The analysis was conducted in two stages. Multi-Criteria Evaluation (MCE) through Analytical Hierarchy Process (Pairwise Comparison) was employed and implemented using weighted overlay function in GIS to determine most suitable areas for cultivation of Sugarcane in Nigeria. Based on the principles of the Round-table Sustainable Biofuels (RSB), seven (7) constraints were identified and eliminated by creating buffers around them and applying union function in an ArcMap modeler. These are settlements, reserved areas, flood zones, severe erosion sites, water bodies, roads and railways.

Based on the ecological requirement of the crop and site accessibility, eleven (11) factors were considered in the land suitability analysis. These are soil, rainfall, temperature, relative humidity, elevation, slope, nearness to water bodies, nearness to labour and markets (settlements), nearness to roads and rails. Weights (table 2) were generated using pairwise comparison and a consistency ratio of 0.07376 was achieved. The weights were converted to percentage to make it acceptable to the ArcGIS tool. A model was therefore, developed and Weighted Overlay Function was applied to determine most suitable areas for Sugarcane cultivation in Nigeria.

Table 1: Data and sources

S/N	Data	Format	Description/ Attribute	Attribute Type	Resolution/ Scale	Source
1	Agro-meteorology	Field/Point Data (Excel)	Rainfall, Temperature and Relative Humidity	Numerical	44 weather stations	NiMet
2	DEM	SRTM (BIL)	Elevation and Slope	Numerical	30meters	USGS
3	Soil	Jpeg	Soil Types (Map)	Categorical	1: 6,000,000	OSGoF
4	Erosion	Jpeg	Soil Erosion (Map)	Categorical	1: 6,000,000	OSGoF
5	Flood Zones	Jpeg	Flood Extent (2012)	Categorical	1: 7,500,000	NEMA
6	Game Reserves	Jpeg	Area extent	Categorical	1: 10,000,000	NPS
7	Feedstock	jpeg	Potential Zones	Categorical	1:10,000,000 (estimate)	NNPC
8	Settlement	Raster grid	Urban extent	Categorical	30metres	NASA
9	Settlement	Vector/shp	Settlement points	Categorical	134 points	NASA
10	Water Bodies	Shapefiles	Areas and lines	Categorical	1379 lines 1186 polygons	DIVA GIS
11	Road Network	Shapefile	lines	Categorical	4708 lines	DIVA GIS
12	Rail Line	Shapefile	lines	Categorical	33 lines	DIVA GIS
13	States and LGAs Boundaries	Shapefiles	Polygons	Categorical	37 and 774 polygons respectively	DIVA GIS

Table 2: Criteria weights

Criterion	Weights	Weights (%)	Approximate Weights (%)
Soil	0.204635	20.46353	20
Rainfall	0.229156	22.91563	23
Temp	0.117286	11.72859	12
Humidity	0.085237	8.523708	9
Water	0.1548	15.48005	15
Elevation	0.042025	4.202536	4
Slope	0.026746	2.674579	3
Roads	0.058423	5.842298	6
Settlements	0.047319	4.73192	5
Rail	0.013549	1.35489	1
FeedstockP	0.020823	2.082266	2

In stage two, road transport was used to determine the location with the highest amount of feedstock within 200km service area, based on which the facilities' sites were optimised. Shi et al. [19], in their study used potential biomass as the feedstock, but since the evaluation in this study was based on an agricultural crop, yield was adopted as the potential feedstock amount. According to [20], the average yield of Sugarcane between 2010 and 2012 is 18.89 tons/ha. Service (Supply) area modelling was adopted because of its underlying philosophy that feedstock beyond certain distances are useless to a given location due to transport cost and therefore may not be considered for a facility at that location. The scope of this study covers the whole country and by implication, facilities to be spatially optimised may not viably receive supplies from all the suitable feedstock production areas. Because the analysis is along the roads network, [19] created biomass points along the roads to serve as aggregation centres and therefore potential candidate sites. In this study, Local Government Areas (LGAs) administrative boundaries and their centroids, as in [21], were used as the feedstock aggregation units and centres, respectively. This is based on the fact that road buffers were eliminated as part of the constraints. It was also assumed that the feedstock can viably be transported to any location within each LGA boundary and near analysis showed that all the centroids are within 25 km of at least one road. Because 100 by 100 metres is the cell size, each pixel represents a hectare, and lookup function was used to create a raster surface for the estimated yield. Zonal statistics was used to calculate the amounts of feedstock potentially available in each of the LGAs and the amounts were aggregated to the centroids. Three centroids with the highest feedstock were selected and service areas created around them. All the feedstock within each service area were then aggregated to the leading centroid which serve as the potential facility location.

RESULTS AND DISCUSSION

Figure 1 shows the land suitability index for sugarcane cultivation in Nigeria, while figure 2 shows the potential feedstock amounts in each LGA and it was based on the 'most suitable' areas derived in the land suitability analysis in stage one. The amounts range from 75.56 tons in Fune LGA of Yobe State to 1.6Gg in Wukari LGA of Taraba State. The aggregation centres serve as the potential candidates and service areas were created around the top three (3) candidates. However, overlap was observed between two of the selected candidates. Thus, the candidates' selection was iteratively reapplied until the overlap was removed to avoid competition between

the 'facilities' for supplying sources. The three highest candidates were Demsa (Adamawa State), Wukari (Taraba State) and Gwagwalada (FCT). The amount of feedstock from all other candidates within the service areas were aggregated to the 'facilities' and the three 'facilities' were ranked based on the amount. The top candidate, Demsa is less than 4 km from the road and remained the 'facility' with the highest amount of potential feedstock within its service area, having 15Gg of Sugarcane per harvesting season (Figure 3). It, therefore, serves as the candidate with highest optimisation priority for Sugarcane – based biofuel production facility among 341 candidates. This location was displayed on Google Earth to visually get more understanding of the location (Figure 4) and serves as a means of remote ground validation.

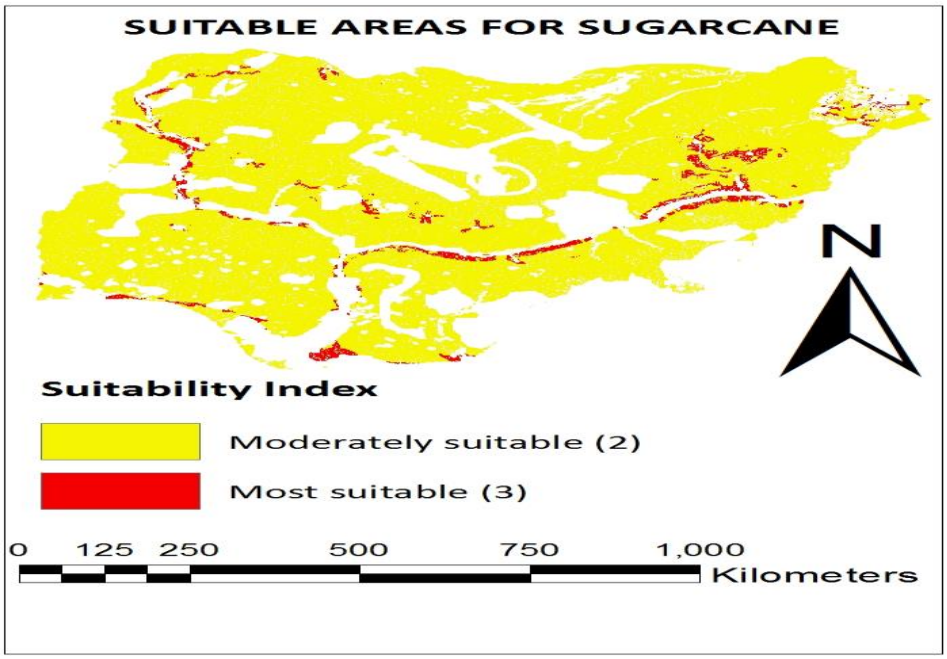

Figure 1: Land suitability for Sugarcane cultivation

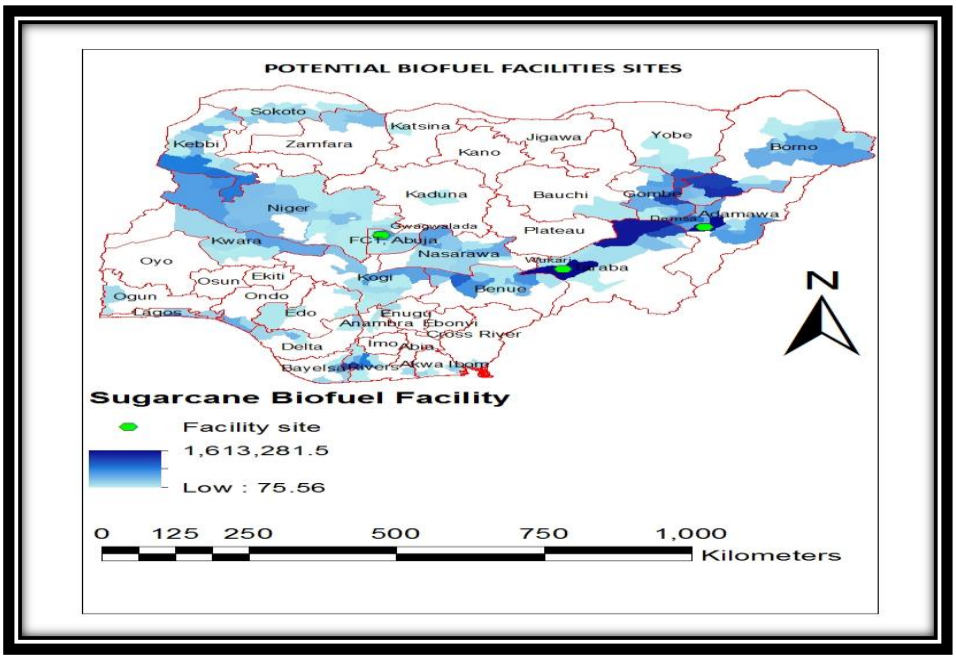

Figure 2: Potential Facility Site

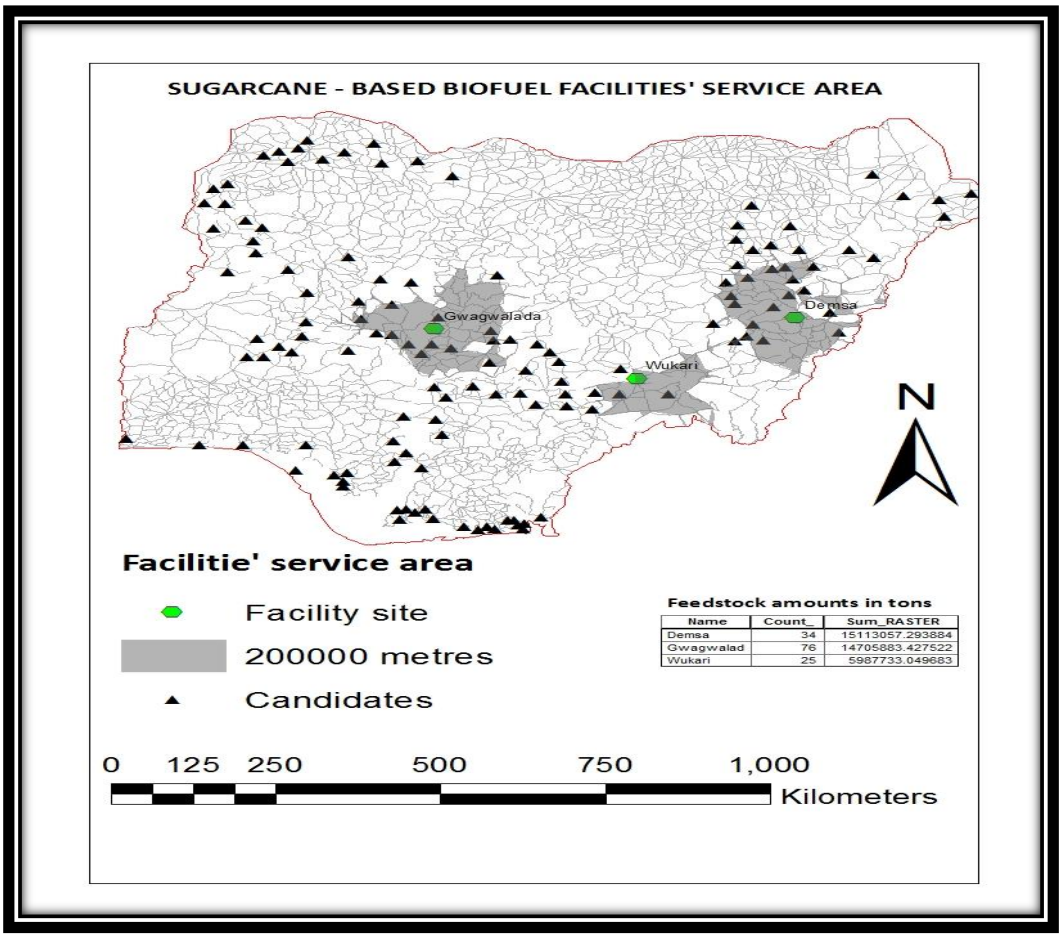

Figure 3: Candidates and Facilities' Service Areas

Figure 4: Google Earth display of the optimized facility site

This location visually (figure 4) appears to be suitable for the purpose; no identified constraint could be seen and the imagery was captured on the 16th of December, 2014 (about 7 months before this analysis was conducted). However, there is need for more localised evaluation of the optimised sites. This is to avoid issues of the limitations on the part of the datasets – availability and the uncertainties in the available data. Round-table Sustainable Biofuel (RSB) principles such as legality and the land rights (regulations), the engineering regulations and the current land values as used in [22], could not be incorporated into the model. Thus, identified suitable areas may need to be confirmed through detailed evaluation considering other important factors such as the engineering requirements. Eight (8) of the selection criteria are directly related to the crop's requirement for growth and development, while the remaining three – settlements, roads and rails – are related to their distance from the suitable areas. Though pairwise comparison, used to generate the criteria weights, is based on the Saaty's scale, some of the issues with it include its being mere decision-makers' judgement of the preference importance between the criteria. However, according to [23], the technique has an added advantage of helping decision makers focus on areas of agreement and disagreement with regards to the weights of the criteria. It also allows for the calculation of consistency ratio which defines the probability that the matrix was randomly generated. The use of centroids of the LGA boundaries in the supply area modelling is a limitation of the GIS functionality. As at the time of this study, the scale, size and technology of the biofuel processing facility was not known. Thus, 200 km was heuristically chosen as the service area extent and due to the nature of Nigeria's transport system, distance variable cost was adopted. Demsa LGA is in guinea savanna vegetation zone and stays under the influence of moisture laden tropical maritime air mass from April to November. For this period, the ITD lies north of the LGA on normal oscillations which can reach up to about 200N. There may be anomalous positions as, for example, was observed in 2015 that the ITD stayed 2 degrees south of its normal positions from March to July [24].

6. Summary and Conclusion

Agriculture is the main employer of the Nigerian populace and crude oil is the country's main revenue source. But there are the environmental concerns of the petroleum industry. Integrating the two sectors is an avenue for agricultural development, improvement of environmental quality and drastic reduction of the unemployment crises in the country thereby stimulating economic development. Blending petroleum fuels with biofuels (ethanol) is a strategy for integrating the two sectors. This forms the background for this study which aims to spatially optimise biofuel production in the country. Different types of feedstocks are used in biofuel production ranging from forest biomass to first, second and third generation biofuels. This article focused on Sugarcane. Based on the aim of the study, principles of the Round-table Sustainable Biofuels (RSB) were used to determine the constraints and a model was developed to eliminate them. Based on the ecological factors, for example soil and rainfall and some spatio-economic factors such as nearness to roads or labour supply (settlements), suitable areas were determined for the cultivation of Sugarcane. One of the major factors of biofuel production – transport – was used to determine optimal sites for locating a Sugarcane – based biofuel production facility. Distance variable cost was used to achieve this objective due to the nature of Nigeria's transportation system and also because the size, scale and technology of the

prospective facilities were not known at the time of the study.

Based on the analysis, it was concluded that Demsa Local Government Area of Adamawa State is the most ideal location for a Sugarcane – based biofuel production facility in Nigeria. Depending on the scale and the production technology to be applied, prospective investors can use this information in collaboration with other stakeholders (such as surveyors and engineers), to identify the most appropriate of the optimised sites for their investment [19]. Because there are many areas in Nigeria much suitable for Sugarcane production, the country can be among the leading Sugarcane – based ethanol producing/exporting countries in the world. This will create lots of jobs, improve environmental quality, generate foreign exchange and attract carbon credit for the country.

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REFERENCES

Chmielewski A. G., 2012. Environmental effects of fossil fuel combustion. Encyclopaedia of Life Support System. Institute of Nuclear Chemistry and Technology and Polland University of Technology, Warsaw, Polland. Available at:<http://www.eolss.net/sample-chapters/c09/e4-23-02.pdf>[Accessed: 16/06/2015].

N. Abas, A. Kalair, N. Khan, 2015. Review of fossil fuels and future energy technologies. Futures 69. 31–49. Available at: <http://ac.els-cdn.com/S0016328715000397/1-s2.0-S0016328715000397 main.pdf?_tid=71c9c07a-143e-11e5 875b00000aab0f01&acdnat=1434469593_f fa0312a9b585b2272d255aae4e53363> [Accessed: 16/06/2015].

V. S. Yaliwal, N. R. Banapurmath, N. M. Gireesh, P. G. Tewari. 2014. Production and utilization of renewable and sustainable gaseousfuel for power generation applications: A review of literature. Renewable and Sustainable Energy Reviews 34. 608–627. Available at:<http://ac.elscdn.com/S1364032114002044/1-s2.0-S1364032114002044-main.pdf?_tid=4e193bb6-1442-11e5-97e600000aacb35e&acdnat=1434471251_430e3697eb0bdd2f07d843f8224377bf> [Accessed: 16/06/2015].

Juan José Vidal-Amaro, Poul Alberg Østergaard, Claudia Sheinbaum-Pardo, 2015. Optimal energy mix for transitioning from fossil fuels to renewable energy sources – The case of the Mexican electricity system. Applied Energy. 150. 80–96. Available at: <http://ac.elscdn.com/S0306261915004353/1-s2.0-S0306261915004353-main.pdf?_tid=74073208-1440-11e5-af8400000aab0f26&acdnat=1434470458_4df67fb081a6e50034af8b76ee5882c9> [Accessed: 16/06/2015].

S. O. Mohammed, E. N. Gajere, E.O. Eguaroje, H. Shaba1, J.O. Ogbole, N.D. Onyeuwaoma and I. S. Kolawole, 2013. A Spatio-Temporal Analysis of the National Parks in Nigeria: A Geo-Information Approach. Proceedings of Global Geospatial Conference, November 4-8, 2013, Addis Ababa, Ethiopia. Available at: <http://www.gsdi.org/gsdiconf/gsdi14/papers/48.pdf> [Accessed: 08/08/2015].

A. K. Azad, M. G. Rasul, M. M. K. Khan, Subhash C. Sharma, M. A. Hazrat, 2015. Prospect of biofuels as an alternative transport fuel in Australia. Renewable and Sustainable Energy Reviews. 43. 331–351. Available at: <http://www.sciencedirect.com/science/article/pii/S1364032114009782>[Accessed: 18/06/2015].

Mofoluwake M. Ishola, Tomas Brandberg, Sikiru A. Sanni, and Mohammad J. Taherzadeh.2013: Biofuels in Nigeria: A critical and strategic evaluation. Renewable Energy (55) 554 – 560. Available at: <http://www.sciencedirect.com/science/article/pii/S0960148112007872>[Accessed: 08/02/2015].

Eric C. Eboh, Kalu O. Oji, Okey G. Oji, Uzochukwu S. Amakom and Oliver C. Ujah, 2004. Towards the ECOWAS Common Agricultural Policy Framework: Nigeria Case Study and Regional Analysis. African Institute for Applied Economics, Enugu. Available at: <http://www.interreseaux.org/IMG/pdf_ECOWAP_Nigeria_Full_Final_Report.pdf> [Accessed: 27/09/2015].

Elijah Ige Ohimain.2013a: A review of the Nigerian biofuel policy and incentive (2007). Renewable and Sustainable Energy Reviews (22) 246–256. Available at:<http://www.sciencedirect.com/science/article/pii/S1364032113000683>[Accessed: 31/01/2015].

NNPC, 2015. Website Available at: <http://www.nnpcgroup.com/NNPCBusiness/MidstreamVentures/RenewableEnergy.aspx> [Accessed: 31/01/2015].

Elijah I. Ohimain.2013b: Can the Nigerian biofuel policy and incentives (2007) transform Nigeria into a biofuel economy? Energy Policy (54) 352–359. Available at: <http://www.sciencedirect.com/science/article/pii/S0301421512010348>[Accessed: 31/01/2015].

NNPC, 2012. Sustainable Biofuel Industry in Nigeria – A Getway to Economic Development. Second Nigerian Alternative Energy Expo. Available at: <http://www.slideshare.net/Mathesisslides/sustainable-biofuels-industry-in-igeria?from_action=save>[Accessed: 14/06/2015].

NNPC.2007: Official Gazette of the Nigerian Bio-fuel Policy and Incentives. Available at: <http://www.sunbirdbioenergy.com/docs/Nigeria_E10_POLICY_GAZETTED.pdf 05/02/2015> [Accessed: 31/01/2015].

K. J. Simonyan and O. Fasina, 2013. Biomass resources and bioenergy potentials in Nigeria. African Journal of Agricultural Research 8(40). 4975 4989. Available at: <http://www.academicjournals.org/article/article1381916826_Simonyan%20and%20Fasina.pdf> [Accessed: 25/06/2015].

E. I. Ohimain, 2015. The Evaluation of Pioneering Bioethanol Projects in Nigeria Following the Announcement and Implementation of the Nigerian Biofuel Policy and Incentives, Energy Sources, Part B: Economics, Planning, and Policy, 10:1, 51-58, DOI: 10.1080/15567249.2010.512904. Available at: <http://www.tandfonline.com/doi/pdf/10.1

080/15567249.2010.512904>[Accessed: 27/06/2015].

Aziz Elbehri, Anna Segerstedt and Pascal Liu, 2013. Biofuel and the sustainability challenge: A global assessment of sustainability issues, trends and policies for biofuels and related feedstocks. FAO, Rome. Available at: <http://www.fao.org/docrep/017/i3126e/i3126e.pdf> [Accessed: 07/07/2015].

NaanDanJain Irrigation, 2013. Sugarcane. Jain Irrigation System Ltd. Available at: <http://www.naandanjain.com/uploads/catalogerfiles/sugar-cane-2/Suger_cane_booklet_100613F.pdf> [Accessed: 06/07/2015].

Anil Hira, 2011. Sugar rush: Prospects for a global ethanol market. Energy Policy (39) 6925–6935. Available at: <http://www.sciencedirect.com/science/article/pii/S0301421510004064>[Accessed: 06/07/2015].

Xun Shi, Andrew Elmore, Xia Li, Nathaniel J. Gorence, Haiming Jin, Xiaohao Zhang, and Fang Wang, 2008. Using Spatial Information Technologies to Select Sites for Biomass Power Plants: A Case Study in Guandong Province, China. Biomass and Bioenergy, Vol. 32 pp 35 – 43. Available at: <http://www.sciencedirect.com/science/article/pii/S0961953407000931>[Accessed: 15/11/2014].

National Bureau of Statistics (NBS), 2013. National Survey on Agricultural Exportable Commodities. Collaborative Survey Conducted by National Bureau of Statistics, Central Bank of Nigeria, Federal Ministry of Agriculture & Rural Development and Federal Ministry of Trade & Investment. Available at: <http://www.nigerianstat.gov.ng/pages/download/144> [Accessed: 19/08/2015].

D. Voivontas, D. Assimacopoulos and E.G. Koukios, 2001. Assessment of biomass potential for power production: a GIS based method. Biomass and Bioenergy Vol. 20 Pp. 101 – 112. Available at: <http://environ.chemeng.ntua.gr/en/Uploads/Doc/Papers/Renewable%20Energy/2001_Assessment%20of%20biomass%20potential%20for%20power%20productio.pdf> [Accessed: 02/09/2015].

Michael A. Borowitzka, Bryan J. Boruff, Navid R. Moheimani, Natasha Pauli, Yunghui Cao, and Helin Smith, 2012. Identification of the Optimum Sites for Industrial Scale Microalgae Biofuel Production in Western Australia Using a GIS Model. Final Report Prepared for the Centre for Research in to Energy for Sustainable Transport (CREST). Murdoch University and University of Western Australia. Available at: <http://www.sciencedirect.com/science/article/pii/S0306261915003918>[Accessed: 30/11/2014].

Samo Drobne and Anka Lisec, 2009. Multi-attribute Decision Analysis in GIS: Weighted Linear Combination and Ordered Weighted Averaging. Informatica (33). 459–474. Available at: <http://www.informatica.si/index.php/informatica/article/download/263/260>[Accessed: 30/06/2015].

2015 Nigeria Climate Review Bulletin. Nigerian Meteorological Agency (NiMet), 2016.

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EFFECTS OF POULTRY DROPPING AND COW DUNG SOIL AMENDMENTS ON GROWTH CHARACTERISTICS AND ROOT-KNOT NEMATODE INFESTATION OF OKRA (Abelmoschus esculentus L. Moench)

Onyekere, U.J. Anjorin T.S.*, Asala, S.W. and Okafor, O.E.
Department of Crop Protection, Faculty of Agriculture,
University of Abuja
oyindamola35@gmail.com; 08062242866

ABSTRACT

This study was conducted to determine the effects of organic soil amendments on the growth characteristics and root-knot nematode (*Meloidogne* spp) infestation of okra (*Abelmoschus esculentus* L. Moench). Cow dung and poultry droppings were applied to the garden soil samples in polypots at the ratio of 80:20, 75:25, 70:30 (w/w) levels. The treatments were laid in a completely randomized design with three replications. The result indicated that soil-poultry dropping application (75:25 w/w) had significantly higher ($p \leq 0.05$) effects on the growth parameters such as plant height, standing leaf and shoot biomass. The amendment significantly reduced the number of withered leaves and the root gall index. It was indicated from the green house study that high growth rate and reduced root-knot nematode infestation of okra could be achieved with the soil-poultry droppings amendment (75:25 w/w). The possibility of extracting the nematotoxic substances in poultry manure and formulating them into organic nematicide deserves further investigation.

Keywords: *Abelmoschus esculentus*, cow dung, growth characteristics, *Meloidogyne* spp, poultry droppings**,** root gall index

INTRODUCTION

Okra, *Abelmoschus esculentus* (L.) Moench, (Family Malvaceae) is a highly rated economic vegetable globally. The most economic produce of okra is the fruit which is usually eaten while green, tender and immature. They are usually used for the preparation of certain soups and sauces (Arapitsas 2008). Okro plays a significant role in human nutrition by providing carbohydrates, protein, fat, minerals and vitamins especially vitamin C that is generally deficient in basic foods (Gopalan *et al*., 2007; Gemede *et al.,* 2015).

Okra are often affected with nematode, viral and fungal diseases and insect pests. Several species of nematodes such as *M. incognita* and *M. javanica* are known to infest okra causing root damage which inhibit the growth and uptake of water and nutrients (Khan *et al.,* 2008). As a result, the growth and yield of okra are greatly hampered. Different management strategies for plant-parasitic nematodes have been developed, some of which involve the use of organic soil amendments, botanicals, crop rotation, solarisation, use of synthetic nematicides and biological control (Hassan *et al*., 2010). These nematicides are now being phased out because of their cost, hazardous nature and high toxicity to non-target organisms as well as environment and ozone layer depletion (Giannakou, 2002; Ufere, 2013).

Organic soil amendments are known to be environment-friendly when used to manage pests. Osman (2011) reported chicken manure to be very effective in controlling nematodes on okra field while Bayuh (2013) and Khalil (2018) found cow dung to be a very good material for maintaining the production capacity of soil and enhancing the microbial population. Adewole and Ilesanmi (2012) reported that the vegetative growth of okra seedling treated with NPK fertilizer grew faster than those applied organic manure in the first three weeks. However, those treated with organic fertilizer grew higher from the sixth week up to the end of the experiment in week 10. They however observed that inorganic fertilizers like NPK worsen soil degradation resulting from higher soil acidity, nutrient imbalance and low crop yield. On the other hand, organic manures promote microbial degradation and the gradual release of nutrients over time.

This study thus assessed the effects of poultry droppings and cow dung on growth characteristics and root-knot infestation in okra. The results obtained can provide new components for integration into effective nematode management systems thus reducing losses in okra yields.

MATERIALS AND METHOD

Experimental Site and Design

The study site was on the 80m by 20m green house of Teaching and Research Farm of Faculty of Agriculture, University of Abuja, Abuja. Complete randomized experimental design was adopted with three levels each of poultry droppings and cow dung in each of the three replicates. Each plot was inoculated with nematode juveniles except the control plots to which ordinary tap water was applied.

Soil Sterilization

The garden soil used for potting medium was sterilized to ensure all nematodes, insect eggs and other pathogens in the soil were killed. The soil samples were packed in a sterilizing can trays at 22.5 cm depth and placed in an autoclave container. The items were autoclaved for 60 mins at 121˚C and 15 psi to achieve sterilization. The sterilized soil was allowed to cool and left to stabilize for 5 days prior to use (Löbmann *et al.*, 2016).

Application of Amendments

Pots filled with sterile soil were set up for sowing seeds of the selected local okra variety. The ratios of soil to poultry manure or cow dung per pot were 80:20 w/w 75: 25 w/w and 70:30 w/w. The soil and the amendments were thoroughly mixed with sterile hand trowel before they were packed in the poly bags. The planting pot medium were wetted slightly and left for 5 days to stabilize before sowing takes place.

Source and Sowing of Okra Seed

The seeds of Gwagwalada local used were sourced from Agricultural Development Project (ADP), Gwagwalada. The variety are known to have spines and about 1.4m tall. Three seeds were sown 2 cm depth per pot and were thinned to 2 seedlings per stand one week after emergence. Treatments were applied accordingly per pot as shown in the layout. The greenhouse was maintained at 22-28°C during the screening.

Preparation of Nematode Inoculum

Nematode inoculum was collected from infected okra plants on a dry season irrigated farm in Chencheyi village, Kwali Area Council. The infected roots with galls and the soil from the rhizosphere of okra showing obvious symptoms of the nematode infection were collected with clean hand

trowel into sterile polystyrene bags. They were then placed in a cooler to protect them from solar rays that can kill nematodes very quickly and taken to Crop Protection laboratory for further analysis.

The roots of okra plants infected with root-knot nematode were gently rinsed under a running tap. The galled root segments were gently placed on Petri dish with some water and under the dissection microscope, the egg masses were removed using scalpel and fine tweezers. The procedure for collecting inoculum was by using diluted sodium hypochlorate (NaOCl) on the picked egg masses from the infected roots before being rinsed well so that hatching will not be affected. The egg masses were carefully placed into distilled water in small vessels and incubate in the dark until the juveniles emerge from 24 - 48 h at 25 ^{o}C.

To measure the quantity of juveniles of the second stage (J_2) required per pot, a clean pipette is used to draw 1mL of homogenized freshly hatched J_2 in a haemocytometer counter and placed under dissecting microscope for counting. For instance, 500 J_2 in a 1mL solution required pipetting 8 ml of the solution to obtain 4000 J_2 used per pot.

Morphological identification of *Meloidogyne* spp

The suspected *Meloidogyne* nematode were taken to the Nematology Laboratory of Department of Crop Protection, Ahmadu Bello University, (ABU) Zaria for confirmatory identification by comparing the features of the specie with reference standard Nematology Manual (Eisenback, 1985). The pear-shaped female at maturity was further characterized by perineal patterning (Eisenbeck 1985) and the use of several morphometric and morphological features of juveniles. The isolated female *Meloidogyne* spp. was placed on the slide glass under a stereoscopic microscope. An incision was made by using a scalpel in the middle of the female body to cut the cuticle into half equatorially. The posterior region consisting of the perennial pattern was carefully cut off and trimmed. The perennial pattern was gently brushed using alcohol to remove any attached debris (Singh, 2009). The perennial pattern was captured by Optilab which was connected to the microscope Olympus BH2 by 400 times magnification. The characteristic features of male such as the form of the labial region, including annulation, and the form of stylet and basal knobs were also used for identification to genera level (Oliveira and Monteiro, 2011; Mutala'liah *et al*., 2019; Aydinli and Mennan 2016; Boubacar, 2019).

Inoculation and establishment of okra seedlings

The nematode inocula were agitated using a magnetic stirring plate at low speed to ensure even distribution. The juvenile (J_2) were then introduced into the root zone of each okra stand each stand of okra root zone through the four holes carefully dug with a sterile fine rod at the moistened base of the seedlings in the pot. They were then covered with soil using a sterile hand trowel. After inoculation, the pots were left to settle for 24h before watering. The plants were maintained for 7 weeks to allow nematode stress reaction such as galling damage on the roots. During the growing period, the pots were watered once in the morning daily and hand weeded occasionally and any leaf eating insects were handpicked and killed. The drainage holes at the bottom of each polypots allowed for draining-off of excess water.

Parameters Measured

The plants were assessed for following parameters:

i. Plant height of the tagged seedlings were measured by metre rule in cm from the stem base to the terminal leaf at 14, 21, 28, 42 DAS and their mean were computed and recorded.

ii. Number of standing leaves of the tagged plants were counted at 14, 28, 42 DAS and their mean were computed and recorded

iii. The percentage of withered/dead plants was determined by using the formula:

$$\%age\ of withered/dead\ leaves = \frac{No.of\ plants\ withered\ leaves}{Total\ plants\ examined\ per\ plot} \text{x}100$$

iv. Galls per plant root/gall index (GI) were determined at 7 weeks after sowing (WAS) i.e. 42 DAS. This was by carefully removing the plants, rub off the soils and examine their roots for galls. then the GI was estimated using a modified Fayzia *et al* ., (2018) scale of 0 to 5:

0 = no galls; 1= traces of infection with some small galls; 2 = 10 - 25% of roots have galls; 3 = 26 to 50% of the roots have galls; 4 = 51 to 75% of the roots have galls and 5 = > 75% of the roots have galls)

v. Extraction of nematode juveniles from the soil was carried out by sieving and sucrose-centrifugation method as described by Lutuf et al., 2016). Each soil samples were thoroughly mixed and passed through coarse sieve to remove stones and debris. A 250 ml subsample of the infested soil was then measured out and placed in a bucket of running water until the soil was covered two times its volume. The solution was then stirred thoroughly until the soil was sufficiently dispersed and then allowed to settle for 3 min. The liquid supernatant was then poured through a 200 mesh sieve nested onto a 400 mesh sieve. The 400 mesh sieve was washed thoroughly with water until as much clay and other fine particles were washed out of the sieve. The remaining sample with the nematodes was then transferred into a 50 ml centrifuge tube. Centrifugation was carried out at 1750 rpm for 7 mins in a MR 23i bench top centrifuge (Jouan-Thermo Scientific, U.S.A.). The supernatant from each sample was decanted before been discarded and centrifuged until a final pellet was obtained from the collective population of nematodes.

The sucrose solution (454g/1L water) was added to the tubes and shaken before the tubes were centrifuged for 3 mins at 1750 rpm. The suspension was poured through the 45 µm sieve and the residue was rinsed from the sieve for examination. The tubes were filled with sucrose solution (450g/1L water) at room temperature and stirred with a spatula to break up the pellet. The sample was centrifuged to 1750 rpm for 3 mins and the supernatant poured through the 45 µm mesh sieve, the residue was rinsed from the sieve and transferred into labelled vials up to 10 ml mark using a fine spray water bottle, ready for examination.

Extraction of nematodes from the roots

Nematodes were extracted from infested okra roots, using modified Baermann funnel method (Whitehead, 1968). The roots were gently washed and chopped into 1 cm pieces using sterile knife and about 10 g was blended for two to five seconds bursts and transferred to a glass funnel lined with a two ply tissue paper placed on a wire mesh. Funnels were left for 48 h and the water (containing nematodes) was poured separately into 250 ml beakers. 2 ml of each suspension were transferred to counting dishes (Hirschmann® 8100210 Bright lined Counting Chamber - haemocytometer, Germany) for counting and recording.

Nematodes selected for identification were mounted in a drop of water on a microscopic

slide and placed on a hot plate at 60 ºC for few seconds, which enabled nematodes to straighten out. Extracted nematodes were examined directly under a compound light microscope (Exacta- OptechBiostar B5P, Germany). Nematodes were identified to the genus level based on their morphological features as described by Siddiqi (1989), Luc *et al,* (1990), and the University of Nebraska Lincoln nematode identification website and recorded.

vii. The shoots or roots collected were weighed, dried at 60°C in an oven for 48 h, and the biomass recorded at 42 DAS.

Data Analysis.

Data collected from the parameters measured in the green house and in the laboratory were subjected to descriptive statistics such as frequency, percentages and bar charts. Also, the data were subjected to analysis of variance (ANOVA). Data from the plots with incomplete record were transformed using $(x+5)^{0.5}$, before performing the statistical analysis. The differences between the means are separated using the Duncan Multiple Range test (DMRT) at 5% probability level using General Statistics (GENSTAT).

RESULTS.

Effects of poultry dropping and cow dung soil amendments on standing/healthy leaves of okra

At 14 and 28 DAS there was no significant difference ($p \geq 0.05$) on the healthy standing leaves due to the effects of poultry dropping and cow dung amendments, but at 42 DAS the treatment of poultry droppings and cow dung at all levels were significantly higher ($p \leq 0.05$) than the one applied with nematode only (Table 1).

Effects of poultry droppings and cow dung soil amendments on stem diameter of okra

At 14 DAS the effects of poultry dropping (25%) had the significant higher stem diameter than the control (Table 2). At 28 and 42 DAS there was no significant difference ($p \geq 0.05$) in the stem diameter due to poultry dropping and cow dung treatments. The nematode-applied plots had the statistically lowest stem diameter (Table 2).

Effects of poultry droppings and cow dung soil amendments on the number of withered/dead leaves of okra.

There was no significant difference ($p \geq 0.05$) on the number of withered leaves at 14 DAS due to the effects of poultry dropping and cow dung amendments at all levels. (Table 3). At 28 and 42 DAS, The withered leaves on plots treated with nematode only were significantly lower than other treatments.

Table 1: Effects of poultry droppings and cow dung soil amendments on healthy standing leaves
* = nematode inoculation;

Treatment	Standing Leaves At 14 DAS	Standing Leaves At 28DAS	Standing Leaves At 42 DAS
N*+Poultrydropping20%	2.667	4.000	5.667[b]
N+Poultrydropping25%	3.667	5.333	7.667[b]
N+Poultrydropping30%	3.333	4.667	6.000[b]
N+Cow dung 20%	2.667	4.000	5.667[b]
N+Cow dung 25%	3.333	4.667	7.000[b]
N+Cow dung30%	3.333	5.000	5.333[b]
Nematode	2.667	4.000	3.000[a]
Control	3.667	4.333	5.667[b]

Means with the same alphabet on a column is not significantly different at p≥0.05by DMRT

Table 2: Effects of poultry dropping and cow dung soil amendments on stem diameter

Treatment	Stem diameter at 14 DAS	Stem diameter at 28DAS	Stem diameter at 42 DAS
N+Poultrydropping20%	1.333[ab]*	2.667	3.500[ab]
N+Poultrydropping25%	2.567[b]	3.333	5.000[b]
N+Poultr dropping30%	1.567[ab]	3.000	4.000[ab]
N+Cow dung 20%	1.167[ab]	2.000	3.000[a]
N+Cow dung 25%	2.067[ab]	3.667	3.500[ab]
N+Cow dung30%	1.667[ab]	2.500	3.333[ab]
Nematode only	1.667[ab]	1.833	2.667[a]
Control	1.000[a]	2.167	3.333[ab]

Means with the same alphabet on a column is not significantly different at p≥0.05by DMRT

Table 3: Effects of poultry dropping and cow dung soil amendments on the number of withered/dead leaves of okra

Treatment	Withered leaves at 14 DAS	Withered leaves leaves at 28DAS	Withered leaves leaves at 42 DAS
N+Poultrydropping20%	0.667	1.333[ab]	1.333[a]
N+Poultrydropping25%	0.667	0.333[a]	1.000[a]
N+Poultrydropping30%	0.667	0.667[ab]	0.667[a]
N+Cow dung 20%	0.667	0.667[ab]	0.667[a]
N+Cow dung 25%	0.333	1.000[ab]	1.000[a]
N+Cow dung 30%	1.333	1.667[ab]	1.333[a]
Nematode only	1.333	2.000[b]	3.333[b]
Control	0.667	1.500[ab]	1.000[a]

Means with the same alphabet on a column is not significantly different at p≥0.05 by DMRT

Effects of poultry droppings and cow dung soil amendments on plant height

At 14, 28 and 28 DAS, the plant height of those applied nematodes only were lower than those that received other treatments (Fig. 4.1.1). Also at these periods, the plant height of okra applied poultry droppings (75:25 w/w) treatment was significantly higher ($p \leq 0.05$) than the nematode applied plots.

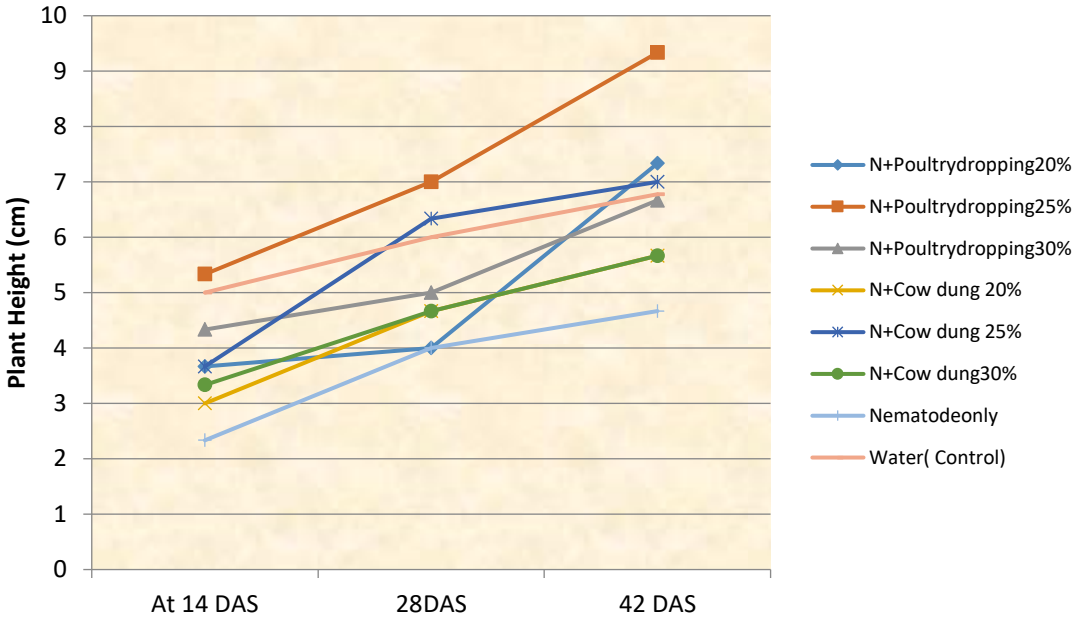

Fig. 1. Effects of poultry dropping and cow dung soil amendments on plant height of okra

Effects of poultry dropping and cow dung soil amendments on gall index of okra at 42 DAS and nematode population per 100cm^3 soil

At 42 DAS, the gall index of the okra roots under the nematode only treated plots were significantly higher ($p \leq 0.05$) than those applied poultry droppings, cow dung and the control (Table 4). The soil nematode population from the plots applied nematodes only was significantly higher ($p \leq 0.05$) than poultry manure and cow dung treatments while the rhizosphere from control plots had no nematode infestation.

Effects of poultry droppings and cow dung soil amendments on shoot biomass at 42 DAS

At 42 DAS, plots applied poultry droppings (75:25; 70:30 w/w) and cow dung70:30 w/w had the highest weight of shoot biomass and closely followed by that of cow dung treated plots, while the nematode only applied plots had the lowest shoot biomass (Table 5). In a contrast, plots applied with nematode only had the highest weight of root biomass, followed by plots applied poultry droppings at 75:25 w/w.

Table 4: Effects of poultry dropping and cow dung soil amendments on gall index at 42 DAS and nematode population per $100cm^3$ soil

Treatment	Gall Index at 42 DAS	Nematode population per $100cm^3$ soil rhizosphere
N+Poultrydropping20%	2.20^{cd}	580^{c}
N+Poultrydropping25%	1.20^{b}	443^{b}
N+Poultrydropping30%	1.80^{b}	548^{b}
N+Cow dung 20%	2.53^{c}	710^{c}
N+Cow dung 25%	2.37^{c}	595^{b}
N+Cow dung30%	2.67^{c}	640^{bc}
Nematode only	4.37^{d}	2003^{d}
Control	0.50a	0.00^{a}

Means with the same alphabet on a column is not significantly different at p≥0.05 by DMRT

Table 5: Effects of poultry dropping and cow dung soil amendments on shoot biomass at 42 DAS

Treatment	Shoot biomass (g) at 42 DAS	Root biomass at 42 DAS
N+Poultrydropping20%	12.000^{ab}	11.333^{ab}
N+Poultrydropping25%	14.667^{b}	12.667^{ab}
N+Poultrydropping30%	13.667^{b}	10.000^{a}
N+Cow dung 20%	12.333^{ab}	10.333^{a}
N+Cow dung 25%	12.000^{ab}	12.000^{ab}
N+Cow dung30%	13.333^{b}	12.000^{ab}
Nematode only	8.3333^{a}	15.667^{b}
Control	11.667^{ab}	11.333^{ab}

*Means with the same alphabet on a column is not significantly different at p≥ 0.05 by DMRT

DISCUSSION

Physico-chemical characteristics of soil can affect the population and incidence of species of nematodes on the field (Asif *et al.*, 2015). It also influences the fertility of a given soil which needed to be sustained, modified or improved upon (Abad *et al*., 2014). In this study, soil amended with poultry dropping (75:25w/w) gave relatively higher mean plant height, number of standing leaves and root diameter while the control had the lowest number of plant height, standing leaves and stem diameter. This result is in agreement with the previous reports that plant height of okra was higher in poultry dropping treated soil among other soil amendments including cow dung (Agboola and Fagbenro, 2016; Heren, 2020). This was attributed to the ready availability of nutrients for the easy absorption by plant root, resulting in an increase in plant growth which also agrees with Kaşkavalcı *et al.* (2009) and Mesfin *et al.* (2016 . In addition, it is believed that high nitrogen content of poultry manure buttresses the vegetative growth of crops.

Morphological identification of *Meloidogyne* spp. to genera level was

conducted in this study using the perennial patterning. Morphological identification is necessary for supporting the molecular identification of nematodes (Kaur et al., 2016; Mutala'liah, 2019). Morphological identification of *Meloidogyne* spp. is however reported to be difficult to distinguish because their perennial pattern looks similar (Oliveira et al. 2011).

The study indicated that incorporation of poultry dropping reduced the root-knot nematode population more than cow dung. This was similar to the report of Chaudhary (2020) that poultry dropping and cow dung applied on *M. incognita*-infested soil sown on field-grown okra reduced the population density of the nematode and increased fruit yield of the plant. This might be due to nemato-toxic substances released by the organic amendments on decomposition and such toxins might have killed the juveniles of *M. incognita* in the soil as reported by Kankam, and Sowley (2016). From this study, reduced nematode population on okra roots and soil was observed from organic amendments. Oka (2010) reported that organic manures were rich in nitrogen and phenolic compounds and on decomposition, nitrogen is converted to toxic ammonia (NH_3) (Khalil, 2018), which can kill several nematode species in the soil (Lazarovits *et al.*, 2012).

Withering, discoloration and death of okra leaves on nematode inoculated plots might be due to above ground symptoms of root-knot nematode infestation. Okra is reported to be highly susceptible to root-knot nematodes with up to 80% yield losses reported in heavily infested soils (Kaskavalci, 2007; Khan *et al*., 2008). Severely affected plants with root-knot nematodes often wilt readily and may also exhibit nutrient deficiency symptoms because the produced galled roots have limited ability to absorb and transport water and nutrients to the rest of the plant according to Coyne *et al*. (2014). The nematodes can make plants more susceptible to damage by plant pathogenic fungi, bacteria, and viruses causing even greater yield losses (Mokrini *et al*., 2018). This may also be contributory reasons to the withering observed.

The findings in this study that okra seedlings on soil applied poultry droppings (75:25 w/w) had the lowest GI and number of nematodes in their rhizosphere validate a similar report by Babatola (2015) that poultry dropping-soil amendment decreased *Meloidogyne* spp population and reduced root galling in okra. Galling of roots is often caused by hyperplasia and hypertrophy of cells of infested roots and this often increase the biomass of the infected roots as observed in this study. The suppression of galling in organic amended soil could be due to the production of substances such as propionic, butyric and acetic acids from poultry dropping which have nematicidal properties as averred to by Orisajo *et al*. (2008) and Herren (2020).

CONCLUSION

The soil-poultry dropping amendment improved the growth performance and reduction of root-knot infestation of *A. esculentus* seedling slightly more than cow dung. The treatment with soil-poultry dropping (75:25w/w) in the study gave the better result. Hence, integration of eco-friendly poultry manure amendment with the use of synthetic fertilizer or bio-control agents in the management of root-knot nematode is recommended. Confirmatory molecular identification of root-knot nematode associated with okra in the FCT Abuja is necessary. The nematotoxic substances in poultry manure should be further investigated in case they could be extracted and formulated into an organic nematicide.

REFERENCES

Abad, J.R.S., Khosravi, H. and Alamdarlou, E.H. 2014. Assessment the effects of land use changes on soil physicochemical properties in Jafarabad of Golestan Province, Iran. Bulletin of Environment, Pharmacology and Life Sciences 3(3): 296 - 300.

Adewole, M.B. and Ilesanmi, A.O. 2012. Effects of different soil amendments on the growth and yield of okra in a tropical rainforest of Southwestern Nigeria. Journal of Agricultural Sciences (Belgrade) 57(3):143-153

Agboola, A.A. and Fagbenro, J.A. 2016. Soil organic matter and its management in the humid tropics with particular reference to Nigeria. *Proceedings ISSS organized by Society of Soil Science of Nigeria*, 21–26 July, Ibadan. 215-283.

Arapitsas, P. 2008. Identification and quantification of polyphenolic compounds from okra seeds and skins. Food Chemistry. 110: 1041-1045.

Asif, M. Rehman, B. Parihar, K., Parihar, K., and Siddiqui, M. A. 2015. Effect of various physico-chemical factors on the incidence of root- knot nematode *Meloidogyne* spp. infesting tomato in District Aligarh (Uttar Pradesh) India. Journal of Plant Sciences 10(6):234 -243

Aydinli G, Mennan S. 2016. Identification of root-knot nematodes (*Meloidogyne* spp.) from greenhouses in the Middle Black Sea Region of Turkey. Turkish J Zool 40: 675-685.

Babatola, J.O. 2015. Effects of some organic manure on nematodes in tomato cultivation. Pakistan Journal Nematology. 7:39-46.

Bayuh, B. Sakhuja, P. K. and Tadele, T. 2013. Integrated management of root-knot nematode (*Meloidogyne incognita*) for tomato production and productivity. Ethiopian Journal of Science and Technology. 6(2): 79-89.

Boubacar, K.T.1. Mohamed, S. M. Doulaye, D. and Hammache M. 2019. Calderón-Urrea A, Vanholme B, Vangestel S, *et al.* (2016). Early development of the root-knot nematode *Meloidogyne incognita*. BioMed Central Dev Biol. 16:10.

Chaudhary, A.D. Narpinderjeet, K.K. Sukhjeet, B. Harwinder, K. 2020. Efficacy of non-chemical methods for management of root-knot nematode (*Meloidogyne incognita*) in tomato in protected cultivation. Journal of Entomology and Zoology Studies. 8(3): 1383-1389

Coyne, D.L. and Ross, J.L. 2014. Protocol for nematode resistance screening: root-knot nematodes, *Meloidogyne* spp. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 27 pp.

Eisenback JD. 1985. Diagnostic characters useful in the identification of four most common species of root-knot nematodes (Meloidogyne spp.). In: Sasser JN, Carter CC. (eds.). An Advanced Treatise on *Meloidogyne*. Volume I. Biology and Control. North Carolina State University Graphics, Raleigh, NC, USA.

Fayzia, M.M, Amira, S.S. Khalil, A.E.M. Shawky, S.M. and Taher, A.A. 2018. Seasonal fluctuation and biological control of root knot nematodes *Meloidogyne incognita* on cucumber. Pakistan Journal of Nematology. 36(1):71-81.

Gemede, H. F. Ratta, N. Haki, G. D., Woldegiorgis, A.Z and Beyene, F. 2015. Nutritional quality and health benefits of okra (*Abelmoschus esculentus*). Journal of Food Processing and Technology, 6:6:1-6

Giannakou, I.O. Sidiropoulos, A. and Prophetou-Athanasiadou, D. 2002. Chemical alternatives to methyl bromide for the control of root-knot nematodes in greenhouses. Pest Management Science, 58(3), 290–296.

Gopalan, C. Sastri, S.B.V. and Balasubramanian, S. 2007. Nutritive value of Indian foods, National Institute of Nutrition (NIN), ICMR, IndiaIBPGR (1991): Report of international workshop on okra genetic resources. International Bureau for Plant genetic Resources (NBPGR), New Delhi, India.

Hassan, M.A. P.S. Chindo, P.S. Marley and Alegbejo, M.D. 2010. Management of root-knot nematodes (*Meloidogyne* spp.) on tomato *(Lycopersicon lycopersicum*) using organic wastes in Zaria, Nigeria. 34-39.

Herren, G.L. Habraken, J. Waeyenberge, L. Haegeman, A. Viaene, N. Cougnon, M. et al. 2020 Effects of synthetic fertilizer and farm compost on soil nematode community in long-term crop rotation plots: A morphological and metabarcoding approach. PLoS ONE 15(3): 0230153.

Kankam, F. and Sowley, E.N.K. 2016. Evaluation of neem (*Azadirachta indica* L.) products for the control of root-knot nematode of chilli pepper (*Capsicum annum* L.), Archives of Phytopathology and Plant Protection, 49:5-6, 111-119.

Kaşkavalcı, G. Tuzel, Y. Dura, O, Oztekin, G. B. 2009. Effects of alternative control methods against *Meloidogyne incognita* in organic tomato production. Ekoloji 18(72):23-31

Kaur S, Kang SS, Dhillon NK, Sharma A. 2016. Detection and characterization of *Meloidogyne* species associated with pepper in Indian Punjab. Nematropica. 46: 209-220.

Khalil, M.S. and Darwesh, D.M. 2018. Some integrated practices to manage root-knot nematodes on tomatoes: A mini review. Innovative Techniques in Agriculture. 3 (2): 618-625

Khan, S.A., Javed, N., Khan, M.A., Kamran, M. and Atif, H.M. 2008. Effects of plant extracts on egg hatch and larval morality of *Meloidogyne incognita*. *Pakistan Journal of Phytopathology* 20: 204-208.

Lazarovits, G. Tenuta, M. and Conn, K. L. 2012. Organic amendments as a disease control strategy for soilborne diseases of high-value agricultural crops. Australia Plant Pathology. 30: 111–117.

Löbmann, M.T., Vetukuri, R.R., Zinger, L., W. Alsanius B.W. Grenville-Briggs, L. J. Walter, A.J. 2016. Applied Soil Ecology. 107: 57- 65.

Luc, M., Sikora, R. A. & Bridge, J. 1990. Reflection on nematology in subtropical and tropical agriculture.In Plant parasitic nematodes in subtropical and tropical agriculture (M., Luc R. A. Sikora & J. Bridge eds.) 2nd ed. pp. 871. *CABI Publishing*, Wallingford, UK.

Lutuf, H., Nyaku, S. T., Cornelius, E. W., Yahaya, S. A. J. & Acheampong, M. A. (2016). Prevalence of plant-parasitic nematodes associated with tomatoes in three agro-ecological zones of Ghana. *Ghana Jnl Agric. Sci.* ***52****, 83-94*.

Mesfin T. G. Hanne, S. David, B. W. and Stefaan, D. (2016). Nematodes enhance plant growth and nutrient uptake under C

and N-rich conditions. Scientific reports. 6: 32862

Mokrini, F. Janati, S, Houari, A and Abdelaziz M. 2018. Management of Plant Parasitic Nematodes by means of organic amendments- A Review. Rev. Mar. Sci. Agron. Vét. 6 (3):337-344.

Mutala'liah, M. Indarti, S. Wibow, A. (2019). The prevalence and species of root-knot nematode which infect on potato seed in Central Java, Indonesia. Biodiversitas Journal of Biological Diversity. 20 (1): 11-16.

Oka, Y. Chet, I. and Spiegel, Y. 2010. Control of root knot nematodes *Meloidogyne javanica* by *Bacillus cereas.* Biocontrol Science and Technology, 3:115-126

Oliveira, C.M.G. Monteiro, A.R and Blok V.C.(2011). Morphological and molecular diagnostics for plant parasitic nematodes: Working together to get the identification done. Tropical Plant Pathology. 36(2):065-073

Orisajo, S.B. Afolami, S.O. Fademi, O.I. and Atungwu, J.J. 2008. Effects of poultry litter and carbofuran soil amendments on *Meloidogyne incognita* attacks on cacao. Journal of Applied Biosciences. 7:214–221.

Osman, H.A. Ameen, H.H. Mohamed, M. *et al*. 2018. Field control of *Meloidogyne incognita* and root rot disease infecting eggplant using nematicide, fertilizers, and microbial agents. Egypt Journal of Biological Pest Control 28**:** 40

Siddiqi, M. R. (1989) *Tylenchida: parasites of plants and insects*. Slough, UK: CAB, International. pp. 385.

Singh SK. 2009. Morphological and molecular characterization of root-knot nematode (*Meloidogyne* spp.) diversity in Fiji. [Thesis]. Faculty of Science, Technology, and Environment, The University of the South Pacific, Fiji.

Ufere, N.U. Kanayo, S.C. and Iwuagwu, M. 2013. Relative effect of organic and inorganic fertilizers on the growth of okra [*Abelmoschus esculentus* (L.) *Moench*]. Journal of Agricultural Sciences (58) 3: 159-166

Whitehead, A.G. 1998. Plant nematode control. CAB International, Wallingford UK, pp: 384.