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Soil Augmentation with *Moringa oleifera* Lam. Leaf Materials for Assessment of Pod Yield, Nitrate Reductase Activity and Proximate Fractions of Bambara Groundnut (*Vigna subterranea* (L.) Verdc.)

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ABSTRACT: Application of dry ground leaves of *Moringa oleifera* (Lam.) as soil nutrient augment for the productive assessment of seven accessions of bambara groundnut (*Vigna subterranea* (L.) Verdc.) was studied. The experimental pots (containing 4 kg of soil) were treated with 0 g (control), 10 g, 20 g and 35 g of dry powdered leaves in three replicates, arranged as completely randomised block design. Data collected include percent germination, number of days to flower bud formation, number of flower buds and flowers formed, number of pods produced per plant and 100-pod fresh and dry weights. Other parameters analyzed were acid phosphatase activity, nitrate reductase activity and proximate contents of harvested pods. One hundred percent germination was recorded for bambara seeds sown in soils augmented with *M. oleifera* in all accessions assessed. Early flower bud formation was observed in five accessions. Fresh and dry weights of 100-pod had no significant difference among the treatments in all the accessions studied. Higher numbers of pods were recorded for plants grown in the augmented soils with *M. oleifera*. Significantly lower values of acid phosphatase activity (µmol/min/g), and higher values of nitrate reductase activity (µmol/min/g) were observed in accessions grown in augmented soils. Values for proximate content were observed to increase with the proportions of *M. oleifera* employed. Furthermore, an increase in pod size was observed in accession (TVSU-466) grown in soil amended with 35 g dry ground powdered leaves of *M. oleifera*. The result obtained in this study demonstrated that using dry ground leaves of *M. oleifera* as soil augment is a good soil enhancer and has the capacity to boost plant yield.

Keywords: *Moringa oleifera; Vigna subterranea*; Soil augmentation; Nitrate reductase; Acid phosphatase

Introduction

Declining soil fertility has become a subject of global research focus and an environmental condition that is dear to African scientists (Sanchez *et al.*, 1997). The world`s population is growing fast, and more food is required to maintain a balance between economic development and poverty eradication for a healthy and sustainable ecosystem (Ogwu *et al*., 2018). In a view to achieving a proper balance, there has been vigorous campaign against deforestation and the need to practice eco-friendly agriculture through reduction in the use of inorganic fertilizers, (Abdalla, 2015; Aluko *et al*., 2017; Muthalagu *et al.*, 2018).
Agricultural production continuously faces challenges such as climate change, decreasing number of farms due to herdsmen and farmers crises (like in Nigeria), increasing production costs (including energy), fluctuations of price in global markets, increasing demand for biofuels, environmental conservation issues and biodiversity protection (Trostle, 2008; FAO, 2009a; Tester and Langridge, 2010). In most regions of the world, availability of arable farmlands (including engagement of marginal lands) has limited crop cultivation, and this has

necessitated continuous cropping of the land resources. These have resulted in soil fertility decline and it is perceived to be widespread, especially in sub-Saharan Africa, (Pieri, 1989; Henao and Baanante, 1999; Smaling, 1993). Declining soil fertility is the major cause of yield reductions observed in crops grown on most soils, (Lal, 1989; Sanchez, 2002). Lal (1989) and Sanchez (2002) emphasized the dearth of research on addressing decline in soil fertility unlike attention accorded soil erosion. The reason for this is connected to the fact that soil fertility decline is more complicated to assess since chemical properties of most soils change very slowly or have large seasonal fluctuations.

To address the loss or unavailability of nutrients in soils, farmers use inorganic fertilizers. Farmers have recorded appreciable yields in crops treated with inorganic fertilizers and for this cause; it remains promoted by many as an indispensable soil revitalizer. When applied, they become immediately available in the soils for uptake by choice crops (Aluko *et al*., 2017). Inorganic fertilizer is not without its demerits as several scientific investigations into the impact of their continual or inappropriate use revealed that they can lead to soil acidification, nutrient imbalance, underground water pollution and trace elements deficiencies (Shehu and Okafor, 2017). Soil nutrient availability and solubility of elements is a function of soil pH (Ayuba *et al*., 2014). The sole dependence on the application of inorganic fertilizer as a source of plant nutrients by farmers is affected by the high cost of fertilizers and is not easily available to farmers. Thus, there is continuous need to search for alternative safe natural sources of plant nutrients (Abdalla, 2015; Aluko *et al*., 2017; Muthalagu *et al.*, 2018).

The present direction of scientific research is to identify organic materials that can serve as alternative nutrient sources for crop production (Aluko *et al*., 2017). Materials from agricultural and domestic wastes have been investigated for soil fertility improvement (Ayuba *et al*., 2014). Organic materials from agricultural and domestic wastes have been found to improve the availability of nutrients in the soil by increasing crop yields and improving activities of soil micro-organisms through amelioration of the problem of soil pH associated with inorganic fertilizers. These natural extracts have been proven to be useful substitutes of chemical fertilizers for healthy crop production (Sakr *et al*., 2018). Organic manures are relatively resistant to microbial degradation but are essential for enhancing soil nutrient availability and maintaining optimum soil physical conditions. Poultry manure, a type of animal manure, is a very cheap and effective source of nutrients, especially nitrogen but its availability remains an important issue since large amounts must be applied to give optimum yield (Aluko *et al*., 2017). Organic manure contains phosphorus and other plant nutrients, and crop production can benefit from their application. Phosphorus is one of their critical nutrients' elements needed in Nigerian soil as tropical soils are highly weathered, low in cation exchange capacity, low base saturation, pH and phosphorus availability. The low pH accounts partly for the low P availability because of its fixation. Organic manures sources are known to play critical roles in the moderation of growth and yield of various crops through their indirect effect on the physical, chemical and biological properties of soil. Manures are also high in exchangeable cations such as Ca, K, Mg, Na and other macro and micronutrients for vegetable production (Ali *et al*., 2014).

Livestock manure, irrespective of its well-researched advantages, is not without its ills. Livestock manures are either not available or too expensive for subsistence farmers in the tropics (Ayuba *et al*., 2014). Livestock manure can also be harmful due to climate change as the emergence of endemic zoonotic diseases pose a potential threat to the use of their wastes for agricultural purposes (Ali *et al*., 2014).

The demerits of animal sources of organic manure justify the continuous search for alternatives which are safe and natural sources of plant nutrient growth enhancers with the ability to ward off pests and diseases (Muthalagu *et al*., 2018). Extracts of plants like *Aloe vera*, have been shown to improve the germination, vegetative growth and flowering of plants (Sakr *et al*., 2018). Plant residues such banana peels, *Senna siamea*, *Leucaena leucocephala*, *Gliricidia sepium* and *Caulerpa taxifolia* have been shown to be good sources of nutrients, indispensable in crop improvement (Aluko *et al*., 2017).

Moringa oleifera, one of the world's most useful trees (Biswas *et al*., 2016), has attracted many research interests in recent times. The basic science, nutritional and medicinal benefits of this wonder crop, including its germplasm has been studied (Baiyeri *et al*., 2015). It is a multipurpose plant widely known for its ethno-medicinal and culinary properties. *M. oleifera* can survive in less fertile soils and exhibit drought tolerance. It serves alimentary, medical or industrial purposes (Moyo *et al*., 2011). *M. oleifera* has the capacity to boost food security, foster rural development and support sustainable land care. It has also proven to be economically beneficial to man, livestock feed and help overall plant performance (Baiyeri *et al*., 2015). On the subject of crop nutritional benefits, *M. oleifera* has been reported to possess wide adaptations and high nutrient composition in its biomass and unlike poultry droppings, is renewable and more environmentally friendly (Ayuba *et al*., 2014). *M. oleifera* has been shown to possess the potential to help crops in dire need of improvement where conventional propagation has met with limitations (Muthalagu *et al*., 2018). *Moringa oleifera* is a good source of green manure as it compares very well with other green manure crops such as lablab beans (*Lablab purpureus* L). *M. oleifera* leaves are rich in zeatin, a naturally occurring cytokinin, auxins, abscisic acid (ABA) and other compounds such as ascorbates, vitamin E and phenolics which confer on the leaf

extract the status of a natural plant growth enhancer (Aluko *et al*., 2017). *Moringa* leaf extract application has proven to be a cheap and environmentally friendly organic technology which increases growth of most vegetable crops like grape (*Vinis vinifera*), cabbage (*Brassica oleracea* var. *capitata*) and tomato (*Solanum lycopersicum*) and field crops like *Zea mays* and common beans (*Phaseolus vulgaris*) (Biswas *et al*., 2016), which has prompted this research. The objective is to investigate yield and nutritional quality of bambara groundnut grown in soils supplemented with leaf materials of *Moringa oleifera.*

Materials and methods

Experimental site: The field experiments were conducted at the Botanic Garden, Department of Plant Biology and Biotechnology, (N: 00265107, E: 00354280) Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.
Plant material: Plant materials used include seeds of seven accessions of bambara groundnut (*Vigna subterranea* (L.) Verdc.), collected from IITA, Ibadan; and identified as follows:
1: Accession **A**. Passport No. TVSU- 466 (big seed size, deep cream with black eye)
2: Accession **B**. Passport No. TVSU-562 (Big seed size, dark brown with white eye)
3: Accession **C.** Passport No. TVSU-1416 (medium seed size, deep cream with black eye)
4: Accession **D.** Passport No. TVSU-1155 (medium seed size, brownish with white eye).
5: Accession**E.** Passport No. TVSU -75 (medium seed size, reddish brown with white eye.6:
6: Accession **F**. Passport No. TVSU-1496 (big seed size, deep cream with ash eye)
7: Accession **G**. Passport No. TVSU-303 (big seed size, deep cream with white eye)
Collection of leaves of Moringa oleifera (Lam.): Fresh leaves of *Moringa oleifera* (Lam.) were collected from home gardens in EDPA Ugbowo Housing Estate, (N:00265125 E: 00353124) Benin City, Edo State.
Processing of Moringa oleifera (Lam.) leaves into powder as soil amendment: Fresh leaves of *Moringa oleifera* (Lam.) were air dried under shade for one week followed by sun drying for two days. The dried leaves were reduced to powdered form by dry milling.
Collection of soil: Top soil (0-15 cm depth) samples were collected from a fallow farm, Faculty of Agriculture, University of Benin, Benin City (N:00265168 E:00355952). The soil was clay silt in texture with an average pH 7.34, organic matter content of 1. 91%, total N = 0.39%, available P = 5.75 mg/ Kg, and available K = 1.58 Cmol/Kg.
Packaging and preparation of experimental pots: A total of 84 plastic bowls were used in the experiment. Soil samples were air-dried and sieved to remove stones and other particles. Four kilogrammes (4 Kg) of the sieved soil was then weighed with a top loading scale and packaged into each of the perforated bowls.
*Application of biofertilizer to the soil***:** Known weights (0, 10, 20, and 35 g) of powdered leaves of *Moringa oleifera* (Lam.) were mixed with soils as soil nutrient amendments. Twenty-one experimental pots were amended with 10 g each, another 21 pots with 20 g each, and 21 pots with 35 g each, while the remaining 21 pots received no soil amendments. These were taken as control. The prepared experimental pots were taken to the field, watered as necessary and continually mixed at weekly intervals and allowed to stand in the field for a period of 30 days. This activity was done to create uniform soil matrix degradation before planting.
Planting of bambara groundnut seeds into experimental pots: Seeds of bambara groundnut were tested for viability by the flotation method. Five (5) viable seeds were hand sown into each experimental pot at a depth of about 3-4 cm. Sowing was carried out between 6.30 - 7.30 a.m.
Experimental design and treatment: A completely randomized design (CRD) using seven varieties of Bambara groundnut were used with three (3) replications. Plot size was 4 m × 4 m. Individual plots, within a block were separated by 0.5 m, Seeds were sown in experimental pots with inter-row spacing of 50 cm and intra-row spacing of 20 cm.
Thinning of experimental pots: Four weeks after planting (28 Days after planting, DAP) the germinated seedlings were thinned to two seedlings per pot.
Hand weeding and watering of experimental pots: The experimental site was cleared of weeds as often as necessary. Pots were watered as often required and necessary till maturity. The weeds found in experimental pots were removed by hand. The developing pods that were above the soil level were covered with soil.
Collection of plant data
Percent germination: The appearance of plumule above the soil surface was recorded as germination. The number of seeds that germinated per pot were counted and the percentage of germination calculated. The germination record was taken every day for 14 days.
Number of days to flower bud formation: The number of days to flower bud formation was recorded by counting.

Number of flower buds formed: The number of flower buds formed per plant was counted and this was recorded.
Number of days to flower formation: The number of days to flowering was taken as the number of days that elapsed, after sowing when 50% of the plants began to produce flowers.
Number of flowers formed: The number of flowers formed per plant in each experimental pot was counted and recorded.

Measurement of yield parameters

Number of pods harvested per plant: One hundred and twenty (120) days after planting (DAP), the number of pods harvested per experimental pot was counted and recorded when plant was matured and ready for harvest.
Fresh weight of 100 – pod harvested per plant: Harvested pods sampled at 120 days after planting were cleaned and fresh weight of 100-pod was determined.
Dry weight of 100 – pod harvested per plant: Harvested pods collected 120 days after planting were cleaned, dried at 80 ºC for 48 h to a constant weight in an oven to obtain their dry weights and 100-seed weight was determined.

Biochemical determinations

Acid phosphatase

Enzyme extraction for non-specific Acid phosphatase (EC 3.1.3.2): A weight of 0. 5 g of the leaves of *Vigna subterranean* were ground in a chilled mortar, with acid washed sand and 2 ml of chilled 50 mM Tris - HCl buffer (pH 7.6) containing 1 mM EDTA . The homogenate was filtered through double layers of cheesecloth and centrifuged at 20,000 g for 20 min. The supernatant was used as the crude extract for the enzyme assay (Murray, 1980).
Enzyme assay for non-specific Acid phosphatase (EC 3.1.3.2): Acid phosphatase activity was assayed by adding 100 µl (0.1 ml) of the enzyme extract to 1 ml of 3 mM α-naphthylphosphate in 60 mM sodium citrate (pH 5.3). The reaction mixture was incubated at 37 °C for 5 min, after which absorbance was read at 450 nm every minute for another 5 min to determine the change in absorbance per minute (ΔA/minute). The assay was performed in duplicate and acid phosphatase activity expressed as µMol α-naphthol released $min^{-1}g^{-1}$ fresh weight.

Nitrate reductase

Harvesting of plants was done at 12.00 noon. The peak of photosynthetic activity in plants as suggested by Hennessy and Field (1991). Leaves and roots of each were separated and labelled.
Preparation of nitrite standard curve: Standard curve of nitrite concentration was obtained following the method of He (2005). A volume of 14.2 ml of potassium nitrite standard solutions (containing 0 to 30 µmoles NO_2^-) were reacted with 7.9 ml of 1 % sulfanilamide dissolved in 3 N HCl and 7.9 ml 0.02 % N- (1-Naphtyl) ethylene diamine dihydrochloride monomethanoate in distilled water. Absorbance was read at 540 nm after 15 minutes incubation period. Blank was 14.2 ml distilled water 7.9 ml 1% sulphanilamide in 3 N HCl plus 7.9 ml of 0.02 % N-(1-Naphtyl) ethylenediamine dihydrochloride monomethanoate. Each point on the graph refers to mean values of three (3) replications.
Assay for nitrate reductase activity: *In vivo* nitrate reductase (NR EC.1.6.6.1.) activity was determined following the method of Srivastava (1974). A weight of 0.3 g fresh leaves was washed with distilled water, blotted with tissue paper and homogenized with a mortar and pestle and put in 5 ml tubes filled with incubation medium (0.1 M Phosphate buffer at pH 7.5 and 200 mM KNO_3 and 0.5% n-propanol) and left in dark for two hours at 30 ºC. After incubation, to 1 ml aliquot, 1 ml sulphanilamide (1% in 3 N HCl) and 1 ml 0.02% naphthylethylenediamine hydrochloride was added and shaken thoroughly. After keeping for twenty-five minutes for colour development, absorbance was read at 540 nm. NR activity was calculated employing standard curve of nitrate and expressed in micromole/minute/grammes (µmol/min/g) on a fresh weight basis. For each experiment an average of 3 replications was estimated and standard error computed.

Proximate analyses of harvested pods

Determination of moisture content: The moisture content was measured at a temperature of 105 ºC, according to method described by Udo and Ogunwele (1986).
Determination of ash content: The ash content was measured after combustion of the dried pods sample in a furnace at a temperature of 600 °C for 3 h, according to the method described by James (1995).
Determination of crude lipids/fat: The crude lipid content in the sample was extracted using soxhlet extraction procedure described by Udo and Ogunwele (1986).
Determination of crude fibre content: Percentage of crude fibre was determined by the method of Udo and Ogunwele (1986).
Determination of crude protein content: The crude protein of the sample was determined using the micro – Kjeldahl method described by AOAC (1990).
Determination of carbohydrates (as nitrogen free extract): The method of James (1995) was adopted where the total proportion of carbohydrate in the leaves sample was obtained by calculation using the percentage weight method.

Statistical analysis: Mean and standard error were calculated for the data collected. Analysis of variance (ANOVA) was done using GENSTAT (version 12). Differences between treatment means were determined using Duncan multiple range (DMR) test (Alika, 2006).

Results

The percentage germination of seven different accessions of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) in soil amended with *Moringa oleifera* (Lam.) are presented in Table 1. The seeds germinated within the first seven days after planting (7 DAP). Many of the accessions gave almost 100% germination twelve days after planting (12 DAP).

Table 1: Percentage germination of seeds of seven accessions of Bambara groundnut grown on soil augmented with *Moringa oleifera*

Treatment	Accession	10 DAP	11 DAP	12 DAP	14 DAP	28 DAP
Control	TVSU-1155	80.00±34.64^{a}	80.00±34.64^{a}	80.00±34.64^{a}	80.00±34.64^{a}	80.00±34.64^{a}
Control	TVSU-1416	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
Control	TVSU-1496	86.67±11.55^{ab}	86.67±11.55^{ab}	86.67±11.55^{ab}	86.67±11.55^{ab}	86.67±11.55^{ab}
Control	TVSU-303	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
Control	TVSU-466	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
Control	TVSU-562	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
Control	TVSU-75	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
10 g	TVSU-1155	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
10 g	TVSU-1416	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
10 g	TVSU-1496	86.67±11.55^{ab}	86.67±11.55^{ab}	86.67±11.55^{ab}	86.67±11.55^{ab}	86.67±11.55^{ab}
10 g	TVSU-303	86.67±11.55^{ab}	86.67±11.55^{ab}	86.67±11.55^{ab}	86.67±11.55^{ab}	86.67±11.55^{ab}
10 g	TVSU-466	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
10 g	TVSU-562	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
10 g	TVSU-75	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
20 g	TVSU-1155	86.67±23.09^{ab}	86.67±23.09^{ab}	86.67±23.09^{ab}	86.67±23.09^{ab}	86.67±23.09^{ab}
20 g	TVSU-1416	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
20 g	TVSU-1496	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
20 g	TVSU-303	93.33±11.55^{ab}	93.33±11.55^{ab}	93.33±11.55^{ab}	93.33±11.55^{ab}	93.33±11.55^{ab}
20 g	TVSU-466	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
20 g	TVSU-562	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
20 g	TVSU-75	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
35 g	TVSU-1155	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
35 g	TVSU-1416	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
35 g	TVSU-1496	93.33±11.55^{ab}	93.33±11.55^{ab}	93.33±11.55^{ab}	93.33±11.55^{ab}	93.33±11.55^{ab}
35 g	TVSU-303	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
35 g	TVSU-466	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
35 g	TVSU-562	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
35 g	TVSU-75	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}

Values are mean ± S.E. (n=3). Values with same alphabets as superscripts in one column are not significantly different using DMR test (α=0.05)

The results of *Moringa oleifera* soil amendments on the number of days to flower bud formation are shown in Table 2. The results showed no significant differences between the treatments and the control. However, early flower buds formation was observed in accessions; TVSU-466, TVSU-1416, TVSU-1155, TVSU-75 and TVSU-1496 when compared to the control.

Table 2: Number of days to flower buds formation of Bambara groundnut plants grown on soil augmented with *Moringa oleifera*.

	Accessions						
Soil amendment (g)	**TVSU-466**	**TVSU-562**	**TVSU-1416**	**TVSU-1155**	**TVSU-75**	**TVSU-1496**	**TVSU-303**
0	50.33±8.08[a]	41.00±1.00[a]	45.67±8.08[a]	45.67±8.08[a]	50.33±8.08[a]	60.67±34.06[a]	41.00±0.00[a]
10	50.33±8.08[a]	41.33±0.58[a]	50.33±8.08[a]	45.67±8.08[a]	55.00±0.00[b]	60.67±34.06[a]	41.00±0.00[a]
20	41.00±0.00[b]	45.67±8.08[a]	50.33±8.08[a]	41.00±0.00[a]	45.67±8.08[a]	60.67±34.06[a]	60.67±34.06[b]
35	45.67±8.08[a]	41.00±0.00[a]	41.00±0.00[b]	41-00±0.00[a]	45.67±8.08[a]	41.00±0.00[b]	41.00±0.00[a]

Values are mean ± S.E. (n=3). Values with same alphabets as superscripts in one column are not significantly different using DMR test (α=0.05)

The effect of *Moringa oleifera* soil amendments on the number of flower bud formed by plants of seven different accessions of Bambara groundnut at 55 and 100 DAP is presented in Table 3. Significantly higher numbers of flower buds were recorded in the treatment with 35g *Moringa oleifera* compared to the control treatment in accession TVSU-303.

Table 3: Number of flower buds formed by plants of seven accessions of bambara groundnut grown on soil augmented *Moringa oleifera* at 55 DAP and 100 DAP

Accession	Soil Amendment (g)	55 DAP	100 DAP	Accession	Soil Amendment (g)	55 DAP	100 DAP
TVSU-466	0	7	14	TVSU-1155	20	1	2
TVSU-466	10	10	15	TVSU-1155	35	7	14
TVSU-466	20	12	13	TVSU-75	0	0	2
TVSU-466	35	7	15	TVSU-75	10	0	3
TVSU-562	0	6	9	TVSU-75	20	0	0
TVSU-562	10	2	9	TVSU-75	35	2	5
TVSU-562	20	4	14	TVSU-1496	0	1	3
TVSU-562	35	2	5	TVSU-1496	10	7	9
TVSU-1416	0	0	1	TVSU-1496	20	4	9
TVSU-1416	10	0	1	TVSU-1496	35	13	17
TVSU-1416	20	0	2	TVSU-303	0	0	2
TVSU-1416	35	0	0	TVSU-303	10	0	0
TVSU-1155	0	1	3	TVSU-303	20	0	3
TVSU-1155	10	0	2	TVSU-303	35	2	4

Values are cumulative amounts of flower buds formed by 3 plants.

The result of the number of flowers formed at 55 and 100 DAP in soils amended with *Moringa oleifera* are presented in Table 4. No significant difference was observed among plants grown in the treatments. However, the highest value for the numbers of flowers formed were recorded in plants grown in soils augmented with *Moringa oleifera* for all the accessions of Bambara groundnut studied.

Table 4: Number of flowers formed by plants of seven accessions of bambara groundnut grown on soil augmented with *Moringa oleifera* at 55 DAP and 100 DAP.

Accession	Soil augment applied (g)	55 DAP	100 DAP	Accession	Soil augment applied (g)	55 DAP	100 DAP
TVSU-466	0	2	7	TVSU-1155	20	10	20
TVSU-466	10	6	17	TVSU-1155	35	8	16
TVSU-466	20	0	7	TVSU-75	0	0	6
TVSU-466	35	5	12	TVSU-75	10	0	6
TVSU-562	0	1	3	TVSU-75	20	1	2
TVSU-562	10	5	14	TVSU-75	35	5	8
TVSU-562	20	8	15	TVSU-1496	0	0	7
TVSU-562	35	1	11	TVSU-1496	10	3	11
TVSU-1416	0	0	7	TVSU-1496	20	6	15
TVSU-1416	10	0	5	TVSU-1496	35	3	17
TVSU-1416	20	1	13	TVSU-303	0	3	7
TVSU-1416	35	0	3	TVSU-303	10	2	4
TVSU-1155	0	0	3	TVSU-303	20	5	5
TVSU-1155	10	0	4	TVSU-303	35	8	16

Values are cumulative amounts of flowers formed by 3 plants.

.

The numbers of harvested pods per plant of seven accessions studied are presented in Table 5. Significantly higher number of pods was recorded in plants grown in all soils augmented with *Moringa oleifera* for accession TVSU1416 compared with the control. For plants of all other accessions, soil augmentation with *Moringa oleifera* had no significant effect on the number of pods harvested.

Table 5: Number of harvested pods per plant of seven accessions of bambara groundnut grown in soil augmented with *Moringa oleifera*

	Accessions						
Soil amendment (g)	**TVSU-466**	**TVSU-562**	**TVSU-1416**	**TVSU-1155**	**TVSU-75**	**TVSU-1496**	**TVSU-303**
0	2.67±3.79^{a}	10.33±6.81^{a}	2.33±3.22^{a}	8.67±3.06^{a}	15.33±2.89^{a}	13.33±10.26^{a}	20.33±10.97^{a}
10	5.33±1.53^{a}	8.00±6.00^{a}	7.00±3.00^{ab}	3.67±2.31^{a}	14.67±3.51^{a}	24.67±23.46^{b}	16.33±11.02^{b}
20	9.33±4.93^{b}	6.00±7.21^{a}	15.00±6.08^{b}	7.67±8.62^{a}	16.33±8.51^{a}	5.67±2.89^{a}	22.67±8.74^{a}
35	3.67±6.35^{a}	11.00±9.54^{b}	5.67±7.37^{ab}	15.67±14.01^{b}	21.00±2.65^{b}	28.00±16.64^{b}	27.67±10.97^{a}

Values are mean ± S.E. (n=3). Values with same alphabets as superscripts in one column are not significantly different using DMR test (α=0.05).

The fresh weights of pods harvested from plants of seven accessions of Bambara groundnut grown in soil amended with *Moringa oleifera* are shown in Figure 1. The fresh weight of pods showed no significant difference among the treatments statistically. However, the pods obtained from plants grown in *Moringa oleifera* augmented soils had the highest fresh weights of pods in the seven accessions studied.

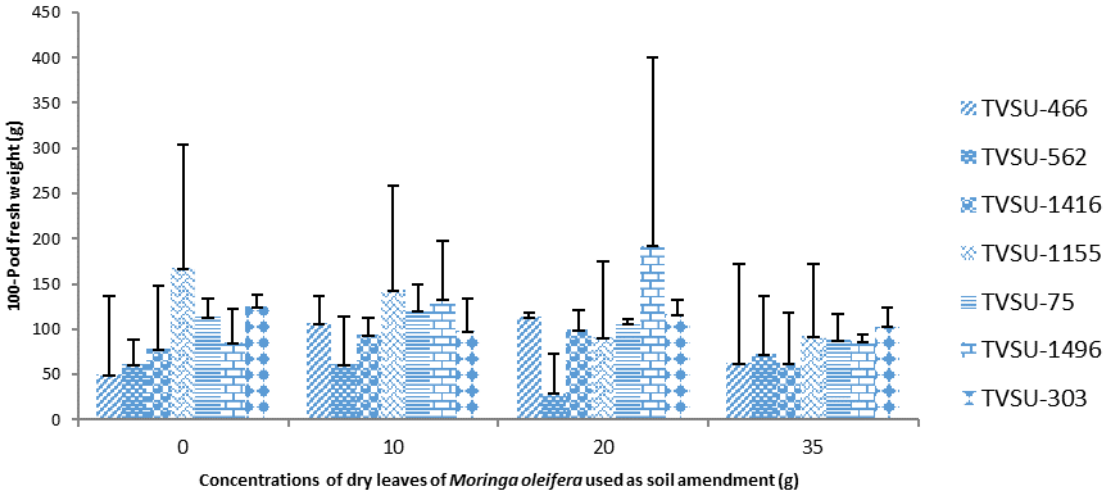

Figure 1: Yield measurement recorded as 100-pod fresh weights of *Vigna subterranea* (bambara groundnut) plants of seven accessions grown in soil augmented with dry leaves of *Moringa oleifera*

The dry weights of harvested pods of seven accessions of bambara groundnut are shown in Figure 2. The results obtained for plants of the seven accessions of Bambara groundnut showed significantly higher dry weight of pods at 35 g soil augmentation with *Moringa oleifera* and accession TVSU-303 had the highest pod weights. The least values of pod weights were recorded for accessions TVSU-466, TSVU-562 and TVSU-1416 plants grown under control soil conditions.

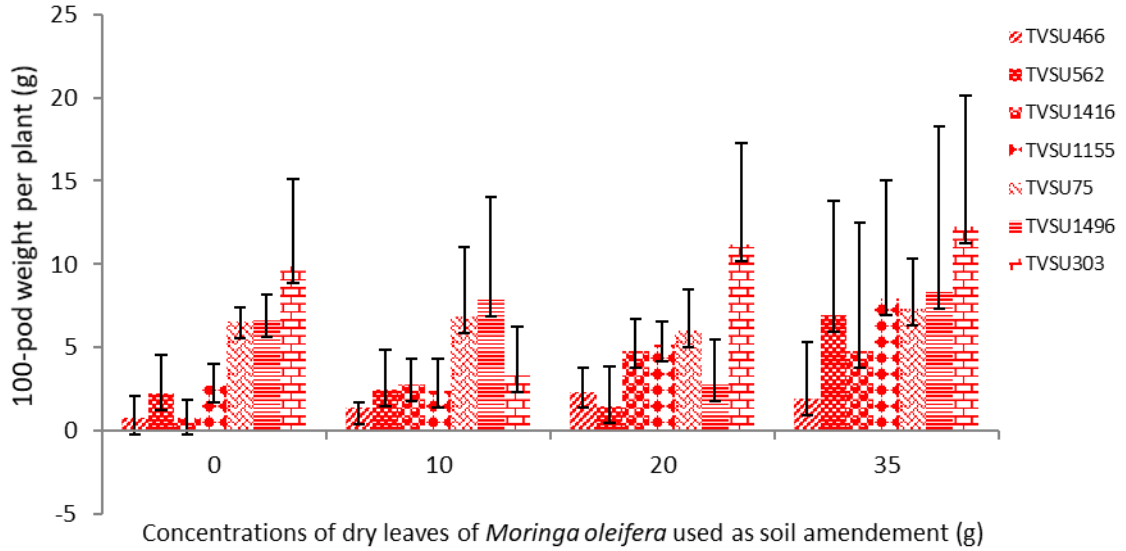

Figure 2: Yield measurement recorded as 100-pod dry weights of *Vigna subterranea* (bambara groundnut) plants grown in soil augmented with dry leaves of *Moringa oleifera* .

The acid phosphatase activity (µmol/min/g) in the leaves of plants of the seven accessions of Bambara groundnut grown on soil amended with *Moringa oleifera* is shown in Figure 3. Among plants of the seven accessions, acid phosphatase activities in leaves were clearly high in TSVU-466 and TSVU-562 plants in 10 g, 20 g augmented and control soils. Plants of four of the accessions consistently recorded low activities of acid phosphatase in leaves in all soil conditions.

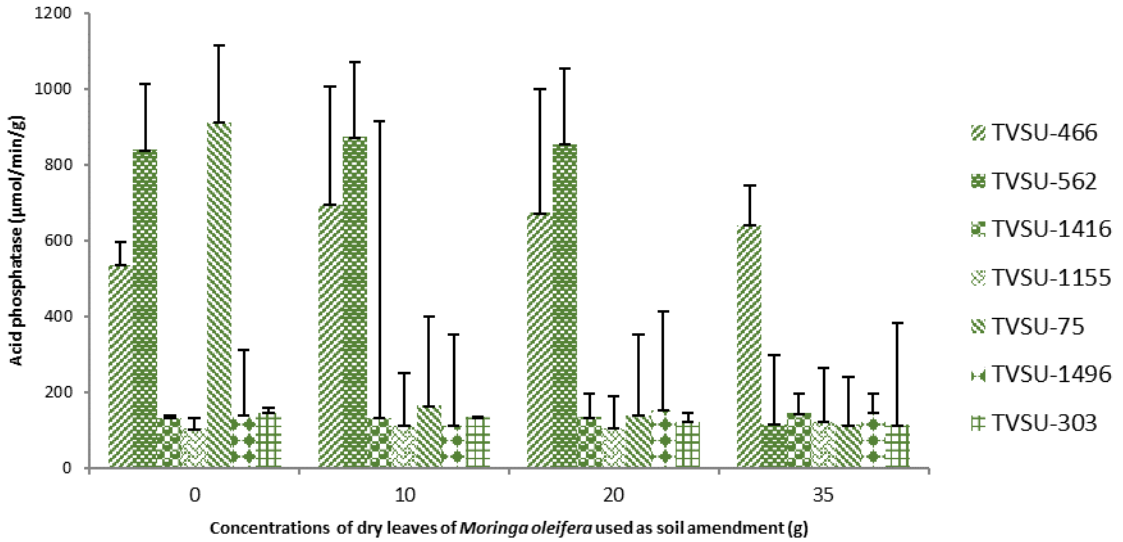

Figure 3: Acid phosphatase (µmol/min/g) activity in the leaves of seven different accessions of Bambara groundnut grown in soil augmented with *Moringa oleifera*.

The activity of nitrate reductase (µmol/min/g) on the leaves of the seven accessions of Bambara groundnut is shown in Figure 4. The highest values were recorded for plants of TVSU-1496 and TVSU-303 in 35 g augmented soils.

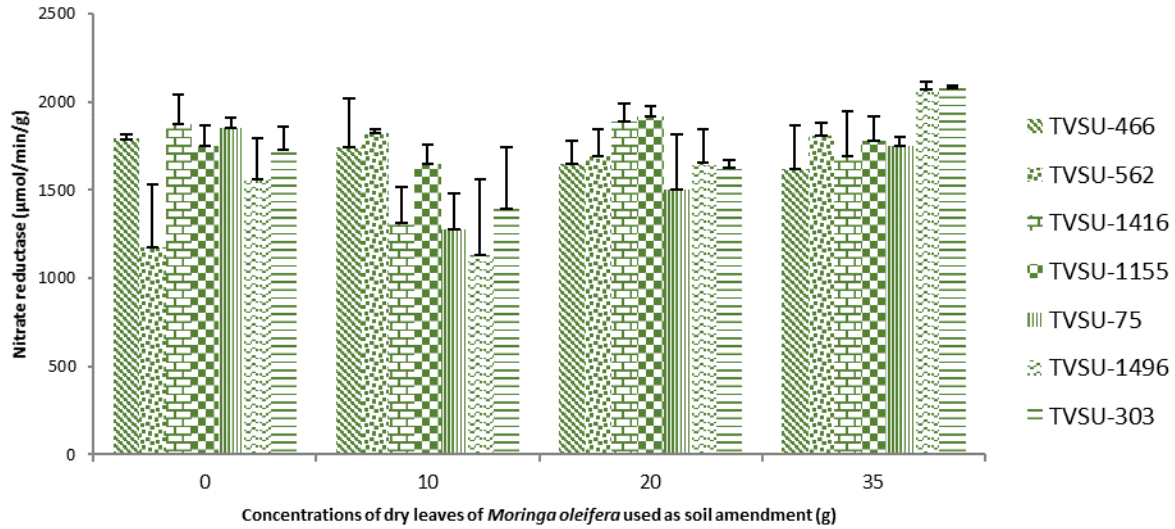

Figure 4: Nitrate reductase (µmol/min/g) activity in the leaves of seven accessions of *Vigna subterranea* grown in soils augmented with *Moringa oleifera*.

The values obtained for proximate analyses of the harvested pods of plants of seven different accessions of Bambara groundnut grown on soil amended with *Moringa oleifera* are presented in Table 6. Plant growth in augmented soils failed to show an increase in the proximate contents generally. Moisture contents were higher in pods harvested from plants grown in control soils except for TVSU-75, TVSU-1496 and TVSU-1155 plants. Higher protein contents were observed for pods harvested from plants grown in control soils. Carbohydrate contents (estimated as NFE) increased as soil augmentation increased.

Table 6: Proximate analyses of harvested pods of Bambara groundnut plants of seven different accessions of grown on soils augmented with *Moringa oleifera*.

Accession	Treatment (g)	% Moisture content	%Ash	% Fat	% Protein	% Fibre	% N.F.E
TVSU-466	0	7.92	4.37	4.98	18.66	5..55	58.5
	10	7.27	3.18	3.21	15.10	2.49	68.8
	20	5.87	4.96	5.98	17.33	5.55	60.3
	35	5.88	4.12	4.89	14.43	1.48	69.2
TVSU-562	0	8.06	4..31	5.79	18.21	3.57	60.1
	10	6.13	4.14	4.12	15.75	5.15	64.7
	20	6.78	4.38	5.09	15.58	4.58	63.6
	35	6.89	4.88	3.98	14.89	3.57	65.8
TVSU-1416	0	6.01	4.30	5.30	17.98	4.85	61.6
	10	6.17	4.32	3.03	15.01	5.53	65.9
	20	6.22	4.98	4.29	15.25	4.63	64.6
	35	5.54	4.37	6.02	18.62	3.55	61.9
TVSU-1155	0	6.54	4.14	4.98	16.42	5.67	62.3
	10	7.88	5.32	3.54	14.89	4.86	63.5
	20	8.55	4.52	4.71	14.22	4.44	62.7
	35	7.61	4.41	4.11	15.51	5.89	62.5
TVSU-75	0	5.35	5.44	4.39	19.11	4.61	61.1
	10	8.10	5.22	4.96	16.65	2.67	62.4
	20	7.10	3.11	5.44	14.55	5.31	64.5
	35	5.88	3.25	3.55	18.87	3.47	65.0
TVSU-1496	0	6.17	5.71	3.89	18.75	3.45	62.0
	10	5.15	4.33	6.25	14.97	4.53	64.8
	20	6.15	4.89	3.56	15.53	3.59	66.3
	35	8.01	4.71	3.88	15.33	1.54	65.9
TVSU-303	0	8.37	4.31	3.44	16.23	4.98	62.7
	10	8.12	5.03	6.12	21.54	5.65	53.5
	20	8.17	4.01	3.28	14.91	4.57	65.1
	35	6.22	4.51	6.51	15.96	3.41	60.1

Values are means of triplicate analysis of each level of soil augment.

Plate 1 shows the increase in size observed in harvested pods of Bambara groundnut plants of accession (TVSU-466) grown in soils amended with 35 g *Moringa oleifera* ground leaf materials when compared with the sizes of pods obtained for other accessions shown in Plate 2.

Plate 1: Harvested pods of Bambara groundnut plants of accession (TVSU-466) grown in soil amended with 35 g *Moringa oleifera* leaf materials

Plate 2: Harvested pods (mixed) of Bambara groundnut plants of other six accessions grown in soils augmented with 35 g *Moringa oleifera* leaf materials.

Discussion

Soil amendments are added to improve the physical characteristics of soil such as water retention, water infiltration, drainage, structure, permeability and aeration. These help to enhance soil fertility and provide a good environment for roots optimal function (Bulluck *et al*., 2002b; Mandal *et al*., 2007). The leaves of *Moringa* plant are useful soil amendment for the remediation of soil acidity and for sustainable production of plants. The findings observed in this study on the effect of *Moringa oleifera* soil amendment on the percentage of germinated seeds of Bambara groundnut grown on it, showed no significant difference among treatment. This is contrary to the work of Sarmin (2014) who reported that the germination percentage and plumule length were reduced when compared to the control when *M. oleifera* plant extract was sprayed on the seeds of *Triticum aestivum* (wheat). The highest sprouted seeds were observed in the control, thus no significant difference among treatments. The effects of soil augmentation on germination were not apparently defined.

The effects of *M. oleifera* amended soils on the number of days to flower bud formation, number flower buds and flower formation by plants assess transition from vegetative to reproductive stage and there is no visible literature on the flower bud and flowering response of plants to *M. oleifera* soil amendment. Generally, soil stress conditions stimulate plants to commit to early flowering and in this work, the soil treatments applied were ameliorative conditions. The augmentation supported some accessions to commence early flowering indications. For example, 20 g and 30 g augmentations in soils supported plants of TVSU-466 and TVSU-1496 respectively to form flower buds earlier significantly as compared to plants under control soils.

The results of yield, in terms of number of pods per plant as observed in this study, agrees with the findings of Biswas *et al*. (2016) who reported that the spray of *M. oleifera* aqueous leaf extracts on *Zea mays* plant at 2 weeks after emergence and at every 2 weeks thereafter, increased the yield components like number of grains per cob. Similar results obtained by Dunsin and Odeghe (2015) recorded high yield of sweet bell pepper (*Capsicum annum)* by the application of *Moringa* aqueous leaf extracts and Organo-Bio Degradable fertilizer (OBD+). Also, Ali *et al*. (2014) reported that poultry droppings (15 t ha^{-1}) and *Moringa* leaf (20 t ha^{-1}) are alternative and safe supply of nitrogen in soil for the growth of *Solanum aethiopicum* (garden egg) in Nigeria. Earlier reports from Ozobia (2014) observed significant plant improvement in *Solanum melongena* due to foliar and soil spray of *Moringa* aqueous extracts. The soil characteristics were also improved, with pH influenced from 5.4 to 6.7.

However, the results obtained in terms of dry weight of harvested pod agrees with the report of Ogbuehi and Agbim (2018), who reported that the average dry seed weight of soybean (*Glycine max* L.) treated with 30% *M. oleifera* extracts recorded the highest seed weight. Similar reports by Yasmeen *et al*. (2011) and Anyaegbu *et al*. (2013) also recorded an increase in 100-grain weight in wheat *(Triticum aestivum)* and *Telfaria occidentalis* respectively, due to application of aqueous *M. oleifera* extract. The results obtained in the case of fresh weight of bambara groundnuts observed in this study contradicts the earlier report of Mishra *et al*. (2013) who observed increased fresh weight of pea (*Pisum sativum* L.) pods due to foliar application of Moringa leaves aqueous extract. The reason for the difference in effect may be the method of application and the concentration of the *M. oleifera* leaf supplement used; as shown in the experiment of Kanchani *et al*. (2019) in okra (*Abelmoschus esculentus*) plant.

The size of seeds can affect germination, emergence and further development of plants for improved plant performance. Seed size can vary among species and even within the same lot of a variety. Variations could be genetically or environmentally influenced (Kolawole *et al*., 2011). Fertilisation improves yield and increase the size of seeds and pods (Abayomi *et al*., 2008) and this gives credit to the result obtained in this study in which increased pod size was obtained for accession (TVSU-466) in the presence of 35 g soil amendments. Furthermore, this observation is confirmation to the earlier claim of Zaku *et al*. (2015), that aqueous leaf extract of *Moringa* can influences fruit size and increase yield when used as plant growth enhancers.

Moringa oleifera leaf extract has been reported to increased soluble sugar, potassium, phosphorus and sodium in legumes. This was reported by Abd-El-Hack *et al*. (2018) who reported an increase in soluble sugars, nitrogen content, phosphorus, potassium and sodium in pea (*Pisum sativum* L.). Ogbuehi and Agbim (2018) also revealed that *Moringa* leaf extract application boosted the protein, carbohydrate and chlorophyll content of soybean (*Glycine max*) leaves. This report supports the results obtained for carbohydrates contents of harvested pods of bambara groundnut plants in this study.

Moringa oleifera leaf extracts have been reported to influence stress enzymes in plants. Abdalla (2014) reported that *M. oleifera* leaf extracts brought about an increase in catalase, POD and SOD of *Eruca vesicaria* spp. *sativa*. This supports the increased nitrate reductase activities in leaves of TVSU-1496 and TVSU-303 plants following growth in 35 g soil augmentation with *M. oleifera*. Acid phosphatase is an enzyme that catalyses the hydrolysis of phosphate esters to produce inorganic phosphate and its major role in plants is to supply the inorganic phosphate needed for the maintenance of cellular activity (Tabaldi *et al*., 2007; Mishra *et al*., 2008). Agoreyo (2010) reported that acid phosphatase performs important physiological and biochemical functions

during fruits ripening of *Musa paradisiaca* L. The reduced acid phosphatase activities in leaves of bambara groundnut plants gown in soils augmented with *Moringa* observed in this study is not popular expectation. The work of Agoreyo and Nweke (2014) suggested that acid phosphatase is the major non-specific phosphatase that stimulates the production and supply of inorganic phosphate in the unripe stage of *Carica papaya* fruits, while alkaline phosphatase is responsible for the production and supply of inorganic phosphate in the over ripe stage. This study suggests that the soil augmentation with *Moringa* creates metabolic environments in leaves that lower acid phosphatase activities, whether this effect is concentration dependent is not profound.

The application of *Moringa* leaf as soil amendment generally ameliorates the physical and chemical characteristics of soil especially at higher concentration. Undie *et al*. (2013) recommended application of fresh *Moringa* leaves at different rates as a good soil amendment for the remediation of soil acidity and to produce garden egg. Anyaegbu (2014) emphasized that incorporating *Moringa* extracts into soil by spraying aqueous solution influences soil properties like pH and improve yields of *Solanum melongena*. Studies by Mridha (2015) and Ndaginna and Ozobia (2017) reported that *Moringa* influences soil properties when used as green manure or when the seed cake is used as fertilizer. *Moringa* leaf materials can also be used as an alley with other crops to help check erosion and consequently amend the soil.

Conclusion

The result obtained in this study reveals that this plant has some vital nutritional components that can improve soil and thereby boost plant growth. The higher the amount of *Moringa oleifera* soil amendment, the higher the yield in terms of number of harvested pods. Increase pod size was observed in one of the accessions. More research is needed to bring to light the mechanism behind the physiological and biochemical effects of *M. oleifera* on legumes and other crops especially in our present day where we truly need an alternative to inorganic fertilizer.

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