

Effects of Neem Extracts on Soil Properties, Microbial Populations and Leaf Area of Fluted Pumpkin (*Telfairia occidentalis*)

¹M.G. Solomon, ¹Paul B. Okon and ²S.B.A. Umoetok

¹Department of Soil Science, University of Calabar, P.M.B. 1115, Calabar, Cross River State, 540-001, Nigeria

²Department of Crop Science, University of Calabar, P.M.B. 1115, Calabar, Cross River State, 540-001, Nigeria

Abstract: Potted followed by field experiments were set up to investigate the effects of various neem (*Azadirachta indica*) extracts on an acid soil physicochemical properties and microbial populations. *Telfairia occidentalis* was planted to test the impacts of the treatments. The results indicated highly significant positive improvements in soil physicochemical properties. Exchangeable Calcium (Ca), Magnesium (Mg) and other cations were added to the soil, which elicited an improvement of the soil pH and boosted soil fertility. Calcium significantly increased from initial 1.70 to 3.20 cmol kg⁻¹, causing improvement in Effective Cation Exchange Capacity (ECEC) and Base Saturation (BS) statuses of the soil, at more than 60 and 50% ,respectively. Leaf area of *Telfairia* was significantly improved as it shifted from initial mean of 4.00 to 7.50 cm². There was generally increase in the population of the carbon degrading organisms with time while free living organisms like *Streptococcus* sp. and *Klebsiella* sp. had initial depression in population. *Micrococuss* sp. was eliminated from the soil. *Bacillus* sp. increased from 7×10⁶ in the control to 18 × 10⁶ cfu g⁻¹ at 6 weeks after treatments. It was concluded that crops by-products such as neem extracts are environmentally safe soil amendments and fertility booster.

Key words: Neem extracts, *Azadirachta indica*, soil physicochemical properties, soil microbial population, *Telfairia* sp., typic paleudults

INTRODUCTION

The use of natural resources and products to minimize external inputs into the farming cycle and to enhance sustainable agricultural system became apparent in the 1990s. Indian farmers have traditionally used de-oiled Neem (*Azadirachta indica*) cake as a fertilizer in their fields and some commercial neem formulation, such as Neemix®, are already tested to be effective (Kreutzweiser *et al.*, 2005). The dual activity of neem cake as fertilizer and pest control has made it a favoured input in agricultural production. Neem leaves have also been used to enrich the soil; it also protects plants roots from nematodes and termites and brings about mold-free dry products (Soon and Bottrell, 1994).

According to NRC (1992), Radwanski and Wickens (1981) neem cake contains more N, P, K, Ca and Mg than farmyard manure or sewage sludge, hence its unique promises as a fertilizer. In some areas of India's Karnataka

State, people grow neem trees mainly for their green leaves and twigs, which they puddle into flooded rice, field before the rice seedlings are transplanted. In spite of all the apparent promises, Umeh and Ivbijaro (1998) reported that the effectiveness of botanicals used in farming still require more scientific research in order to improve on their potentials. It is envisaged that increased use of neem products will reduce environmental pollution and serve as alternative agricultural input.

This research was therefore, conducted with the following objectives:

- To investigate the effects of neem extracts (neem seed and neem bark extracts) on the physicochemical and biological properties of the Acid Sands soil of Calabar.
- To investigate the wholesome effects of applying the neem extracts on soil inhabiting microbial populations.

MATERIALS AND METHODS

Study area: The potted experiment was carried out in the Faculty of Agriculture Laboratory under a controlled environment while the field experiments were conducted in the University of Calabar Teaching and Research Farm (Long. 08°21' 04 E; Lat 04°56' 50 N) in 2004 and 2005. The soils were the Typic Paleudult type of the Acid Sands (Udo and Sobulo, 1981) of Southern Nigeria. Clay in the soil increases with increase in depth and has been variously reported as Ferralitic (in the FAO classification), deep porous brown soils derived from sand deposits (Enwezor *et al.*, 1989). The already acid-sandy soil has additional problems of leaching, having developed from Coastal Plain Sand with elaborate depth. The mean annual atmospheric temperature range is 25°C to 33°C. Mean range of rainfall is 2750 to 3050 mm per annum (FAAN, 2006). Land type was arable.

Potted experiment: Twenty-one labelled pots were filled with 2 kg each of bulked soil and divided into three replications. The treatments were applied using randomized complete block design. The general treatment structure as developed by GenStat (2006) Procedure Library was followed in laying out the treatments.

Procedures: The soil was first wetted to field capacity and kept for 24 h before applying the crude neem extracts treatments. One hundred and fifty milliliter of each concentration levels of the crude extracts was sprayed on the soil. The untreated soil served as the control. Soil samples were taken out and analysed bi-weekly for 6 weeks.

Treatment materials: Three different concentrations of the neem crude extracts each of the Neem Leaves Extract (NLE) and Neem Bark Extracts (NBE) were prepared. The concentrations of the extracts were 1.25, 2.5 and 5%. Fifty g each of neem leaves and bark were ground into paste, soaked in 1000 mL of distilled water for 24 h and then filtered with a muslin cloth to obtain the 5% concentrations. Twenty-five gram and 12.5 g of leaves and bark were used to prepare the 2.5 and 1.25% of the NLE and NBE.

Microbial studies: The samples were processed for plating using sterile distilled water as diluents; then serially diluted and plated out. Standard Plate Count Agar (SPCA), nutrient agar and malt extract agar were used for the aerobic incubation at 37°C for 24 h.

For quantitative analysis, isolation was done for general characterization and identification of bacteria types. Bacteria and fungi were assessed numerically and

grouped according to degree of occurrence and frequency in samples. Qualitative analysis of microbial flora using the gram stain reaction and biochemical tests (methyl red test, Voges Proskauer test, motility test, sugar fermentation and oxidase tests) were done according to standard procedures (Weaver *et al.*, 1994).

Laboratory physicochemical analysis of the soil samples:

The soil samples were air-dried and passed through 2 mm sieve. For organic carbon determination, a 0.5 mm sieve was used. The pH_w was determined by using the electrometric measurement method in 1:1, soil: water (v/v) suspension (Thomas, 1996). Particle size analysis was by the modified hydrometer method (Gee and Bauder, 1986). Organic carbon was estimated by the Walkley-Black wet oxidation method (ITTA, 1979). The regular Kjeldahl analytical method was used in determining total nitrogen (Bremner, 1996). Available phosphorus (P) was extracted using Bray No.1 (0.03 N NH_4F + 0.025 N HCl) extractant and the available P determined by the vanadomolybdophosphoric Acid method of Kuo (1996). Exchangeable acidity was determined by the KCl extraction method and titrated with sodium hydroxide (NaOH) as laid out by IITA (1979) Exchangeable cations were extracted with the 1 N NH_4OAc at pH 7.0 leachate; Calcium (Ca) and Magnesium (Mg) were determined by EDTA-titration, while sodium (Na) and potassium (K) were determined with a Flame Photometer. The Effective Cation Exchange Capacity (ECEC) and Base Saturation (BS) were obtained by summation method (ITTA, 1979).

Field experiment: Twenty-seven plots, each plot measured 4 m × 3 m (12.0 m²) were used. The treatments were laid out in the field in Randomized Complete Block (RCB) design. The two-way experimental design was generated by GenStat Procedure Library Release PL 12.2 (GenStat, 2005) to enable appropriate analysis. There were two factors; drenched soil and foliar spray. Each factor had three levels giving nine treatments and replicated three times.

The test crop cultivation: One seed of *Telfairia occidentalis* Hook.f. commonly called Ubong Annang was sown per stand at a depth of 4 to 5 cm using a spacing of 1.0 m × 1.0 m; this gave a plant population of 10,000 per ha. The test crop is a main staple vegetable in the region, grown mainly by resource poor farmers. Detailed botanic and agronomic information have been documented in the literature (Tindall, 1968; Grubben *et al.*, 2004). Leaf harvesting begins at one month and continued at 3-4 weeks intervals; best done by pruning. Fresh shoot yield of 500-1000 kg ha⁻¹ is documented for 18 or more harvests.

Preparation and application of treatment materials: The dried fruits were pounded lightly in mortar to release the seeds, which were then grounded into a paste. The Neem Seed Paste (NSP) treatment were made up of 0g, 172.8 g and 345.6g NSP for the 0% (D0), 50% (D1) and 100% (D2) drenching, respectively as recommended by NRC (1992). The Neem Seed Extract (NSE) treatment was obtained by steeping overnight 250 and 500 g of seed into 10 litres of de-ionized water each for the 50 and 100% extract, respectively (NRC, 1992).

Drenching: The NSP was soil drenched forth nightly into a ring, 10 cm deep and 15 cm around and at the base of the crop. The ring was covered with soil after drenching.

Spraying: Thirty, fifteen and zero gram NSE were applied per the 12 m² plots. NSE was foliarly sprayed on the crop using hand knapsack sprayer. The control had no extract applied.

Telfairia leaf and yield data collection methods: Six middle stands of each plot constituted the sampling units. Data on the leaf area were collected from the plots for 10 weeks. Both the leaves and the damaged portions were traced on graph sheets. The total leaf area and damaged portions were known by counting the total square boxes within which each leaf was covered. The leaf area damaged was calculated as the percentage of the leaf area thus:

$$\text{Leaf area damaged (\%)} = \frac{\text{Leaf area damaged (cm}^2\text{)}}{\text{Total leaf area (cm}^2\text{)}}$$

Statistical data analysis: For both experiments, GenStat release 4.2 DE (9) was used for all data analysis. All results were subjected to ANOVA procedure. The effects of each of the treatments, their interactions and standard errors were estimated.

RESULTS

Effects of neem treatment on soil properties: Table 1 to 3 show the importance of treating soils with neem extracts. From Table 2, the concentrations of neem products (NBE and NLE) in the soil significantly ($p < 0.01$) increased the BS by 5.88 units above the grand mean of all the treatments. Similar positive effects were observed for soil pH (Table 1) and exchangeable Ca (Table 3) and some other soil physicochemical properties studied such as soil exchangeable Mg, ECEC and soil total nitrogen as a result of application of either the NBE or NLE into the soil. Agbenin *et al.* (1999) a greenhouse study observed large

Table 1: Effect of Neem Bark Extract (NBE) and Leaf Extract (NLE) on soil pH; [n = 21]

Concn (%)	Control	NBE	NLE	Mean effects
0.00	5.027			5.027
1.25		4.750	4.683	4.717
2.50		4.597	4.247	4.422
5.00		4.527	4.137	4.332

CV (%) = 8.854; LSD_{0.05} = 0.50; SE_{mean} = 0.088; Grand mean = 4.567; $p < 0.01$

Table 2: Effect of Neem Bark Extract (NBE) and Leaf Extract (NLE) on soil percentage base saturation (%); [n = 21]

Concn (%)	Control	NBE	NLE	Mean effects
0.00	45.22			45.22
1.25		45.41	47.80	46.60
2.50		48.15	47.56	47.85
5.00		55.62	54.50	55.06

CV (%) = 9.587; LSD_{0.05} = 4.982; SE_{mean} = 1.029; Grand mean = 49.18; $p < 0.001$

Table 3: Effect of Neem Bark Extract (NBE) and Leaf Extract (NLE) on soil Calcium content (Ca) (cmol kg⁻¹); [n = 21]

Concn (%)	Control (cmol kg ⁻¹)	NBE (cmol kg ⁻¹)	NLE (cmol kg ⁻¹)	Mean effects (cmol kg ⁻¹)
0.00	1.700			1.700
1.25		2.133	2.067	2.100
2.50		2.333	2.133	2.233
5.00		2.600	3.200	2.900

CV (%) = 22.90; LSD_{0.05} = 0.5307; SE_{mean} = 0.115; Grand mean = 2.310; $p < 0.001$

Table 4: Soil physicochemical properties of the field before the field experiment

Soil property	Unit	Top soil (0-15 cm)	Sub-soil (15-30 cm)
Sand	%	84.6	82.6
Silt	%	7.7	5.7
Clay	%	7.7	11.7
Texture		Loamy sand	Loamy sand
pH _w	5.12	5.06	
Total nitrogen	%	0.08	0.07
Organic carbon	%	1.00	0.86
Available phosphorus	mg kg ⁻¹	59.57	66.75
Ca	cmol kg ⁻¹	1.20	1.20
Mg	cmol kg ⁻¹	0.40	0.40
Na	cmol kg ⁻¹	0.12	0.11
K	cmol kg ⁻¹	0.13	0.12
Exchange acidity	cmol kg ⁻¹	2.32	2.84
ECEC	cmol kg ⁻¹	4.17	4.67
Base Saturation (BS)	%	44	39

maize biomass yield and N uptake, suggesting rapid N mineralization from neem residue to meet plant nutrition.

In the field, there was no net significant effect of either the drenching or spraying of the NSP and NSE on the soil physicochemical properties. The texture of the soil as indicated by sand, silt and clay contents remained loamy sand throughout, irrespective of the time and treatment plots sampled (Table 5). The soil chemical cum fertility indicators were also not affected significantly by any of the treatments. Soil pH remained strongly acidic with a range of 4.80-5.42 and grand mean of 5.196. The Total Nitrogen (TN), Organic Carbon (OC) and ECEC and BS were generally critically low and remained so before

Table 5: Effects of neem extract on selected soil physicochemical properties after the field experiment (neem extracts were both drenched and sprayed on the soil as indicated)

Treatment		Sand (%)	Clay (%)	OC(%)	Avail P. Ca pH _w	ECEC TN(%)	(mg kg ⁻¹)	(mg kg ⁻¹)	(cmol kg ⁻¹)
Drenched	Sprayed	N=27	N=27	N=27	N=27	N=27	N = 27	N = 27	N = 27
0	0	86.30	6.33	0.867	5.167	0.073	66.8	1.133	4.673
	1	84.63	7.33	0.920	5.147	0.080	60.4	0.933	5.087
	2	85.30	6.67	0.800	5.250	0.070	61.0	1.400	4.523
1	0	85.30	6.33	0.893	4.970	0.076	59.8	0.933	4.617
	1	84.97	7.67	0.807	5.287	0.067	61.0	1.400	5.293
	2	85.30	6.33	1.007	5.320	0.087	60.1	1.200	4.610
2	0	84.63	6.67	0.953	5.217	0.083	63.7	1.133	4.610
	1	85.63	7.67	0.967	5.150	0.083	78.5	1.067	4.563
	2	85.97	5.67	0.893	5.253	0.077	52.5	1.800	4.720
Grand mean		85.34	6.74	0.901	5.196	0.077	62.6	1.22	4.74
CV %		1.4	19.8	15.6	3.0	15.7	17.9	26.9	8.1
s. e. d.		0.941	1.091	0.114	0.126	0.009	9.17	0.268	0.314
Plevel *(p<0.05)		NS	(0.10)	NS	(0.13)	NS	NS	0.04*	(0.10)

Table 6: Bacterial species isolated per gram of soil (cfu g⁻¹) treated with different concentrations of neem extracts at six weeks. [cfu/(x 10⁶g)]

Bacterial species (× 10 ⁶)	Concentrations of neem extracts (%)							
	Treatment	Control	NBE			NLE		
	Mean	0	1.25	2.50	5.00	1.25	2.50	5.00
<i>Bacillus subtilis</i>	15.00 ^{ab}	7.0	18.0	17.0	18.0	16.0	19.0	18.0
<i>Micrococcus</i> sp.	1.75 ^d	7.0	-	-	-	-	-	-
<i>Arthrobacter</i> sp.	18.25 ^a	14.0	20.0	20.0	20.0	20.0	19.0	19.0
<i>Pseudomonas</i> sp.	16.25 ^a	10.0	18.0	18.0	18.0	20.0	18.0	18.0
<i>Klebsiella aerogenes</i>	5.81 ^c	7.0	5.0	5.0	5.5	6	5.5	5.5
<i>Streptococcus aureus</i>	6.12 ^c	7.0	5.0	6.0	6.0	6.0	6.0	6.0
Grand mean	10.53							

LDS_{0.05} for comparing bacteria population = 3.19×10^6 cfu g⁻¹; CV% = 29.9; SE = 3.15; p<0.001

Enwezor *et al.* (1989) in their fertility classification rate this soil as having low fertility indices that are below the critical level needed to elicit optimum growth and yields of crops. The grand means for TN, OC, ECEC and BS were 0.077, 0.901, 4.744 cmol kg⁻¹ and 48.6%, respectively. Tables 4 to 5 show the detailed soil physical and chemical properties.

Effects of neem treatment on microbial populations:

Bacterial species isolated per gram of soil treated with neem extracts are shown in Table 6. *Bacillus subtilis*, *Arthrobacter* sp. and *Pseudomonas* sp. are the carbon degraders which were increased with increase in neem application. On the other hand, *Micrococcus* sp. was completely eliminated by neem extracts treatments. Treatments with NBE and NLE at 2.5 and 5.0 % concentrations increased the population of *Arthrobacter* sp. relative to the control. The population of *Klebsiella aerogenes* was reduced at all concentrations of the treatments. Neem extracts favoured the metabolism of isolated organisms at this time. The microbial population showed an initial depression followed by an increase then a gentle decrease. Generally, there was high significant difference (p<0.001) among the populations of bacteria found in the soil. When compared with other bacteria species, *Bacillus* sp. recorded a significant (p<0.01)

increase from 7.0×10^6 cfu g⁻¹ of soil to 19.0×10^6 cfu g⁻¹ also *Pseudomonas* sp. recorded similar increases from 10×10^6 cfu g⁻¹ to 20×10^6 cfu g⁻¹. This indicated that these organisms were capable of mineralizing and utilising the neem products added to the soil within a period of just 6 weeks. On the other hand, *Streptococcus aureus* with a possible anthropogenic origin was degraded. This contaminants' reduction from 7.0×10^6 cfu g⁻¹ to 6.0×10^6 cfu g⁻¹ was favourable to the soil. This shows that neem can serve and a possible sterilising agent to enable a balance of the soil living system.

For soil fertility indicators, the ANOVA revealed that interactions effect were not significantly different. In effect, spraying at 100% had greatest positive impact on the crop, soil and microbial populations. Therefore, the treatment combination of first drenching the soil at 100% and then spraying the crops at 100% elicited the highest Ca addition to the soil. Drenching the soil and spraying the crops with the optimum treatment materials elicited significant (p<0.001) increases in contents of BS and Ca; from the initial BS of 45.22 to 55.06 % (Table 2) and from initial Ca content of 1.7 cmol kg⁻¹ to 3.2 cmol kg⁻¹ (Table 3). The addition of Ca in the soil as a result of the Neem treatments is a very good development in the region, which is dominated by strong acid soils (Udo and Sobulo, 1981). The pH of the soils was far below the

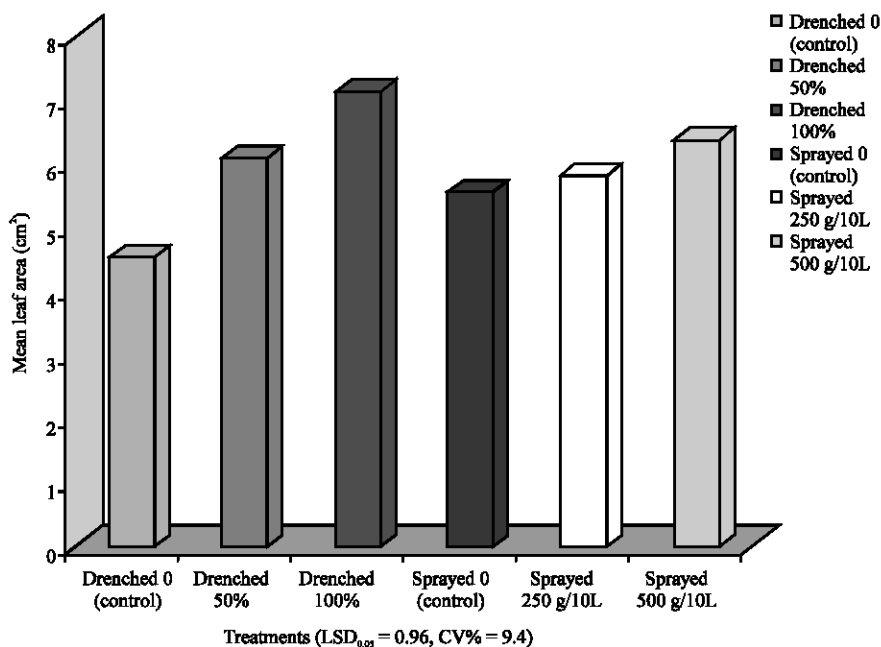


Fig.1: Effects of different neem treatment on *Telfairia* leaf area (cm²)

optimum (5.5-6.5) needed for optimum crops growth and yield (Table 1 and 4). But free Ca can react with hydroxides of Fe and Al to knock-off active soil acidity (Okon *et al.*, 2005). This free Ca could be obtained with increased neem extracts application (Table 5).

Effects of neem treatment on leaf area of *Telfairia occidentalis*: Leaf area increased from a mean of 4.00 cm² in the control to 7.50 cm² when treated with the combination of spraying and drenching and 100% (Fig. 1). ANOVA indicated that drenching was superior to spraying as drenching was significant at $p < 0.001$ and elicited an effect that was 1.21 cm² higher than the mean increase in leaf area, while spraying was significant at $p < 0.01$ and elicited an increment of 0.46 cm² higher than the mean improvement in leaf area of the crop. An improvement in leaf area translates into higher yields as there is wider surface area for photosynthetic reactions to take place. Moreover, in the study area, the larger the *Telfairia* biomass, the higher the economic value of this highly valued vegetable.

DISCUSSION

Neem has demonstrated considerable potential as a fertilizer and neem-seed cake and neem leaves are especially promising (NRC, 1992). Many insecticides have been used in agriculture but seldom do they have additional benefit to the soil apart from their target

organisms. For instance, Atrazine (herbicide) was observed (20), on the same Acid Sand soil, to have no significant effect on soil pH, available potassium and available phosphorus levels, but OC increased significantly, perhaps due to additions by dead and decomposing vegetation and not the chemical per se.

Mechanisms for the strong fertilizing effects of neem products: Addition of organic matter from the bark and leaves of neem leads to increase in soil organic matter. Mineralization took place as evident in the increase in carbon degrading micro organisms. Nutrient in the added organic matter was decomposed and added to the soil. There was generally an increase in the population of the carbon degrading micro organisms with increase in time, while the free-living organisms like *streptococcus* and *Klebsiella* had initial depression in population and even by the 6th week had not reached the controlled population. This was attributed to a slow adaptation of the organism to the added amendment. That was how strong the fertilizing effect of neem products was. They lacked the enzyme that could stand degrading the amendment. However, *Micrococcus* was eliminated from the soil microbial population. This was clearly an organism incapable of utilising the added amendments of neem products. Similar observation was made by Solomon (1999) in which microbial populations showed initial depression followed by an increase and a gentle decrease.

CONCLUSION

In this study, the effects of neem extracts were tested both in the soil and crop. The results showed improvements in soil physical, chemical and biological properties. Microbes were added but fungi were eliminated. The leaf area of *Telfairia* sp. was better off than in the control with additional benefits of expelling the insects, which escaped from the soil physically during the drenching. This confirms earlier report that neem products possess strong insecticidal properties (Durmusoglu *et al.*, 2003) and have shown impressive inputs into the soil. Addition of calcium helped in offsetting the acidic conditions of the study area irrespective of the method of application. It amended soil condition such as acidity and are environmentally safe, constitute no health hazard to the user or the benefiting media. It is therefore strongly recommended that farmers should look inwards and start using these crop by-products to boost food production.

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