

Soil Application of *Moringa* Leaf Extract on Root Development and Root Exudates of Soybean (*Glycine max* L.)

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Abstract – The Study of Foliar Application of *Moringa* Leaf (*Moringa oleifera* Lam) extract as plant growth hormone has attracted attention of agronomist more especially in the developing countries where crops yields are very low, than soil application. Field experiment in 2014 cropping season was carried out at the Teaching and Research Farm, Faculty of Agriculture, Imo State University to study soil application of *moringa* leaf extract on root development and yield of soybean (*Glycine max* L.). The experiment consisted of a Randomized Complete Block Design with four replications. Treatment levels were 0% (control), 10% MLE, 20% and 30% MLE. Subsequent to planting of soybean after 14 days of interval, the growth analysis of the soybean crop was done at 2 weeks of interval. The underground parameters measured are number of roots, root length and root dry weight, also root exudates (crude protein, carbohydrates and phenolics) were also measured. It was observed that *moringa* leaf extract (MLE) improved the root development parameters which were not significantly difference from control. Production of root exudates was found to be significantly improved from root of soybean treated with *moringa* leaf extract. Soil physico-chemical properties were also found to be improved by application of *moringa* leaf extract (MLE). The results suggest that soil application of *moringa* leaf extract as soil exogenous nutrient could enhance growth process of soybean root parameters leading to increase mineralization of nutrients.

Keywords – *Moringa* Leaf Extract, Yield, Soil Application, Root Development, Root Exudates.

I. INTRODUCTION

Moringa (*Moringa oleifera* Lam) is gaining significance in the world of agriculture. The extract from the plant is use as a plant growth hormone which enhances seed germination, growth and yield of crops (Foidl et al., 2001; Edward and Jenny, 2009; Muhamman et al., 2009; Phiri and Mbewe, 2010).

Soybean (*Glycine max*) is one of the important vegetable protein crops in Nigeria and has several momentary research attentions by such agricultural research organisation and the international institute for tropical agriculture (IITA) Ibadan. It belongs to the legume family, and legume crops are cultivated on 12-15% of the arable land throughout the world. Legume species including soybean and peanut (*Arachis hypogaeae*), provide more than one third of the processed vegetable oil throughout the world's market (Graham and Vivanco 2003).

Moringa leaves gathered from various parts of the world were found to have high Zeatin concentration of between 5 mcg and 200 mcg/g of leaves (Fuglie, 2000). Martin (2000) and Foidl et al., (2001) reported that juice from fresh

moringa leaves produce an effective plant growth hormone, increasing yields by 25-30% for nearly all crops. In a similar study Muhamman et al. (2009 a and b) reported a significant increase in some growth characters of cowpea and tomato with *Moringa* extract.

Plant hormones can be used to increase yield per unit because they influence every phase of plant growth development. Traditionally, there are five groups of growth regulators which are listed; auxins, gibberellins, absciscic acid, ethylene and cytokinins (Prosecus, 2006). Cytokinins enhance food production. *Zeatin* is one form of the most common forms of naturally occurring cytokinin in plants, fresh *Moringa oleifera* leave have been shown to have high *Zeatin* content.

Chemicals secreted into the soil by roots are broadly referred to as root exudates. Through the exudation of a wide variety of compounds in roots may regulate the soil microbial community in their immediate vicinity, cope with herbivores, encourage beneficial symbiosis, change the chemical and physical properties of the soil, and inhibit the growth of competing plant species (Nardi et al., 2000). Compounds secreted by plant roots serve important roles as chemical attractants and repellents in the rhizosphere, and the narrow zone of soil immediately surrounding the root system (Bais et al., 2001).

Since release of root exudates, which are mainly derived from photosynthesis, is a significant carbon (C) cost for plants, root exudates are believed to have important functions in regulation of plant growth (both directly or indirectly), although most of these functions are beginning to be investigated (Bertin et al., 2003; Walker et al., 2003). Uren (2007) hypothesized that root exudation was involved in the regulation of internal plant metabolic processes, such as respiration and nutrient acquisition. Additionally, root exudates (e.g. phytoalexins) can also be a mechanism of plant defence against soil-borne pathogens and can stimulate or inhibit interactions with other soil organisms (Bais et al., 2004, Bertin et al., 2003; Rengel 2002). There is a dearth of information on the direct application of MLE on soil and its effect on growth of soybean and root production of exudates. Hence this research is aimed at breaching this gap.

II. MATERIALS AND METHODS

Location

The experiment was conducted during early may, planting season of 2014 at the Teaching and Research Farm of the Faculty of Agriculture and Veterinary medicine, Imo

State University Owerri. Owerri lies between latitude 5010'N and 60 0'N and longitude 6033'E and 700'E within the South-East rain forest agricultural zone of Nigeria. The average temperature, annual rainfall and relative humidity of Owerri are 270 c, 2500 mm and 75% respectively (Meteorological unity Ministry of Land and Survey, 2006).

Experimental Design and Procedure

The experiment consists of four treatment levels of MLE with four replications and fitted into a Randomized Complete Block Design (RCBD). Which consist of four treatment levels replicated four times.

The experimental site was cleared conventionally using cutlass and hoes. Prior to planting, soil samples from the experimental plot was collected randomly and analysed for some physico-chemical properties.

The Soybean seeds were sown at a spacing of 30 cm by 30 cm along rows and 2 seeds will be sown per hole and later thinned down to 1 per stand 7 days after germination. The area of the land will be 7m x 7m (49m²) and each bed will be separated from each other by 1m.

Treatment Materials

The treatment materials was leaf extract of *Moringa Oleifera* which was randomly distributed at the root of the plant with the following concentrations: 0% (T₁) which served as the control, 10% (T₂), 20% (T₃) and 30% (T₄) at the rate of 25 ml per plant. Treatments was randomized or distributed randomly in each block or replicate, one week after planting and continue until 8 weeks after planting.

Data Collection

The following parameters were measured and data collected.

Root Length: This was measured using ruler (graduated in centimeter).

Number of Root: This was obtained by visual counting of the roots using the handle.

Root Dry Weight: This was obtained by drying the root in the Oven at a temperature of 80oc for 24 hours and then measured with a weighing balance

Total Phenolic: This was analyzed from the soil using spectrophotometer method described by Satory (1982)

Crude Protein: This was analyzed from the soil using Bradford's method (1976) described by Bacilio Jimenez et al., (2003)

Carbohydrate: This was performed using the Anthrone Colorimetric assay as described by Aulakh *et al.* (2001).

Data Analysis: The data collected was subjected to analysis of variance to test the significance of treatment effects. Data will be analysed as completely Randomized design according to Onuh and Igwemma, (2001).

III. RESULTS

Physical and Chemical Properties of the Soil before Experiment

As presented in table 1, the experimental soil was slightly acidic with pH value of 5.19 at the beginning of the experiment having a sand percentage of 82.80 with 4.00% silt and 13.20% clay. The organic carbon (OC) and organic matter (OM) were at 1.28 and 2.20% respectively. Total exchangeable acid (TEA) was at 1.30% and aluminum ion (Al³⁺) was 0.90% level while the hydrogen ion (H⁺) stood at 0.40% with a total Nitrogen (TN) level of 0.11%. The cation levels were 2.60, 1.20, 0.23 and 0.14 meg/100 g soil for calcium levels were 2.60, 1.20, 0.23 and 0.14 meg/100 g soil for calcium ion (Ca²⁺), magnesium ion (Mg²⁺), potassium ion (K⁺) and sodium ion (Na⁺) respectively. The base saturation (BS) was at 76.2% with cation exchange capacity (CEC) of 5.47 meg/100g soil and available phosphorus of 4.63 ppm (Table 1).

After Experiment

In the 10% MLE treated plots, the soil pH increased slightly to 5.71 with sand increasing to 86.80% and silt 2.00% while clay lowered to 11.20%. Having OC level of 1.35% and OM level of 2.34%. The TEA stood at 0.90% while Al³⁺ and H⁺ were at 0.50 and 0.40% respectively. The TN was 0.11% while the Ca²⁺, Mg²⁺, K⁺ and Na⁺ levels were 2.00, 1.20, 0.27 and 0.21 meg/100 soil respectively. Available phosphorus was 4.63 ppm with BS level of 80.70% (Table 1).

In the 20% MLE treated plots, pH value was 5.16 with 82.00% sand, 4.00% silt and 13.20% clay. OC, OM, TEA, Al³⁺ and H⁺ levels were 1.41, 2.44, 0.70, 0.40 and 0.30% respectively with 0.12% TN.

Ca²⁺, Mg²⁺, K⁺ and Na⁺ levels remained at 2.60, 1.80, 0.18 and 0.11 meg/100 g soil respectively (Table 1). BS and CEC levels were at 87.10% and 5.15 meg/100 g soil.

In the 30% MLE treated plots, pH level was 4.84 with sand, silt and clay levels at 84.80%, 2.00% and 13.20% respectively (Table 1). OC, OM, TEA, Al³⁺ and H⁺ were recorded at 1.63, 2.82, 1.40, 0.90 and 0.50% respectively while the TN was at 0.14%. Ca²⁺ Mg²⁺, K⁺, Na⁺ and CEC were recorded at 4.20, 2.60, 0.28, 0.19 and 8.65 meg /100 g soil respectively (Table 1). Available phosphorus was 3.76 ppm with BS of 84.00% (Table 1).

In the control plots, pH value was 5.36 while sand, silt and clay levels were 84.80%, 4% and 11.20% respectively (Table 1).

OC and OM were recorded as 1.64 and 2.87% respectively. TEA, Al³⁺ and H⁺ were observed as 1.20, 0.70 and 0.50% respectively with TN of 0.12%. Ca²⁺, Mg²⁺, K⁺, Na⁺ and CEC were recorded as 3.60, 1.60, 0.21, 0.14 and 6.75 meg/100 g soil respectively while BS and phosphorus were recorded at 82.20% and 4.54 ppm respectively (Table 1).

Table 1. Soil Physico-chemical properties before and after the experiment

Soil parameters	Before experiment	After Experiment			Control
		10% MLE	20% MLE	30% MLE	
Ph (H ₂ O)	5.19	5.71	5.16	4.84	5.36
Sand (%)	82.80	86.80	82.80	84.80	84.80
Silt (%)	4.00	2.00	4.00	2.00	4.00
Clay (%)	13.20	11.20	13.20	13.20	11.20
O.C (%)	1.28	1.35	1.41	1.63	1.64
O.M (%)	2.20	2.34	2.44	2.82	2.87
TEA (%)	1.30	0.90	0.70	1.40	1.20
Al ³⁺ (%)	0.90	0.50	0.40	0.90	0.70
H ⁺ (%)	0.40	0.40	0.30	0.50	0.50
TN (%)	0.11	0.11	0.12	0.14	0.12
Ca ²⁺ meg/100gsoil	2.60	2.00	2.60	4.20	3.60
Mg ²⁺ meg/100gsoil	1.20	1.20	1.60	2.60	1.60
K ⁺ meg/100gsoil	0.23	0.27	0.18	0.28	0.21
Na ⁺ meg/100gsoil	0.14	0.21	0.11	0.19	0.14
CEC meg/100gsoil	5.47	4.56	5.15	8.65	6.75
BS (%)	76.20	80.70	87.10	84.10	82.20
P (ppm)	4.63	2.96	3.44	3.76	4.54

Effects of MLE Application on the Number of Roots of Soybean

The highest (45.50) mean number of roots per plant at 2 WAP was recorded from the 20% MLE treated plots while the lowest (35.00) mean number of roots was recorded from the 30% MLE treated plots and those showed no significant difference ($P < 0.05$) at 4 WAP, the 20 and 30% MLE treated plots recorded the highest (66.00) mean number of roots per plant which was not significantly different from the lowest (59.25) mean number of roots recorded from the control (Table 7). At 6 WAP, 30% MLE treated plots recorded the

highest (80.75) mean number of roots which was not significantly different from the lowest (55.75) mean number of roots from the 10% MLE treated plots (Table 7). At 8 WAP, the 10% MLE treated plots recorded the highest (110.25) mean number of roots per plant which did not show significant difference ($P < 0.05$) from the lowest (57.75) mean number of roots from the 20% MLE treated plots. At the 10 WAP, the 10% MLE treated plots recorded the highest (114.25) mean number of root per plant which did not show significant difference from the lowest (51.00) mean number of roots per plant recorded from the control plots (Table 2).

Table 2. Number of Roots of Soybean as Influenced by MLE Application

Treatments	MEAN NUMBER OF ROOTS /PLANT				
	2WAP	4WAP	6WAP	8WAP	10WAP
Control (T ₁)	36.25 ^a	59.25 ^a	62.50 ^a	89.00 ^a	51.00 ^a
10% MLE (T ₂)	39.25 ^a	62.00 ^a	55.75 ^a	110.25 ^a	114.25 ^a
20% MLE (T ₃)	45.50 ^a	66.00 ^a	70.25 ^a	57.75 ^a	63.50 ^a
30% MLE (T ₄)	35.00 ^a	66.00 ^a	80.75 ^a	81.00 ^a	74.00 ^a

Means in the column are with the same letter are not significantly different ($P < 0.05$)

Effects of MLE Application on Root Length of Soybean

At 4 WAP, 20% MLE treated plots recorded the highest (11.050 cm) mean root length which was not significantly difference from the lowest (8.475 cm) from control plots. At 4 WAP, the 30% MLE recorded the highest mean root length (17.30 cm) which was not significantly different ($P < 0.05$) from the lowest (14.58 cm) recorded from control plots.

At 6 WAP, 30% MLE treated plots also recorded the highest (22.56 cm) mean root length which was not significantly different ($P < 0.05$) from the lowest 18.400 recorded from control.

At 8 WAP, the highest (30.20 cm) mean root length was recorded from the 30% MLE treated plots which showed significance difference ($P < 0.05$) from the lowest (18.20 cm) mean root length recorded from the control treated plots (Table 8).

At 10 WAP, the highest (27.25 cm) mean root length was recorded from the 30% MLE treated plots which did not show significant difference ($P < 0.05$) from the lowest (24.00 cm) mean root length recorded from the 20% MLE treated plots (Table 3).

Table 3. Root Length of Soybean Plant as Influence by MLE Application

Treatments	MEAN ROOT LENGTH (cm)				
	2WAP	4WAP	6WAP	8WAP	10WAP
Control (T ₁)	8.475 ^a	14.58 ^a	18.40 ^a	25.450 ^a	24.15 ^a
10% MLE (T ₂)	8.850 ^a	17.15 ^a	18.475 ^a	22.15 ^{ab}	24.18 ^a
20% MLE (T ₃)	11.050 ^a	19.900 ^a	25.450 ^a	25.450 ^a	24.60 ^a
30% MLE (T ₄)	9.200 ^a	22.50 ^a	30.20 ^a	30.20 ^a	27.25 ^a

Means in the column with same letter(s) superscript are not significantly different (P<0.05)

Effect of MLE Application on Root Dry Weight of Soybean

At 2 WAP, 10% MLE gave the highest root dry weight (0.13 g) followed by 20% MLE (0.12 g) which was significantly different from the lowest (0.10 g) recorded from control.

The highest (0.22 g) mean root dry weight was recorded from the 10% MLE treated plots which did not show significant difference (P<0.05) from the lowest (0.16 g)

mean root dry weight (Table 3). At 6 WAP, the 20% MLE treated plots recorded the highest (0.41 g) mean root dry weight which did not show significant difference (P<0.05) from the lowest (0.29 g) mean root dry weight recorded from control.

At 8 WAP, the 10% MLE treated plots recorded the highest (0.58 g) mean root dry weight which did not show significant difference (P<0.05) from the lowest (0.287 g) recorded from the 30% MLE treated plots (Table 4).

Table 4. Root Dry Weight of Soybean is influenced by MLE

Treatments	MEAN ROOT DRY WEIGHT (g)				
	2WAP	4WAP	6WAP	8WAP	10WAP
Control (T ₁)	0.10 ^a	0.16 ^a	0.29 ^a	0.54 ^a	0.37 ^a
10% MLE (T ₂)	0.13 ^a	0.22 ^a	0.39 ^a	0.58 ^a	0.39 ^a
20% MLE (T ₃)	0.12 ^a	0.20 ^a	0.41 ^a	0.39 ^a	0.32 ^a
30% MLE (T ₄)	0.11 ^a	0.19 ^a	0.39 ^a	0.287 ^a	0.56 ^a
LSD	0.09	0.07	0.17	0.46	0.28

Means in the column with same letter(s) superscript are not significantly different (P<0.05)

Effects of MLE Application on the Root Protein Exudates

Root protein exudates at 2 WAP was lowest (0.2828) from the control plots which showed significant difference (P<0.05) from the highest (0.4143) mean root protein recorded from 30% MLE. At 4 WAP, the highest (0.3850) mean root protein was recorded from the control and 20% MLE treated plots however, these did not show significant difference (P<0.05) from the lowest (0.3175) mean root protein. At 6 WAP, the 10% MLE treated plots recorded the highest (0.3925) which showed significant difference

(P<0.05) from the lowest (0.2900) mean root protein obtained from the 20% MLE treated plots (table 5). At 8 WAP, the control and 30% MLE treated plots recorded the highest (0.5450) mean root protein which showed significant difference (P<0.05) from the lowest (0.3450) mean root protein recorded from 20% MLE treated plots (Table 5). At 10 WAP, 30% MLE treated plots recorded the highest (0.4425) mean root protein and this did not show significant difference (P<0.05) from the lowest (0.2050) mean root protein recorded from the 20% MLE treated plots (Table 5).

Table 5. Root Protein Exudates of Soybean as Influence by MLE Application

Treatments	MEAN ROOT PROTEIN EXUDATES (%)				
	2WAP	4WAP	6WAP	8WAP	10WAP
Control (T ₁)	0.2828 ^b	0.3850 ^a	0.3000 ^{ab}	0.5450 ^a	0.3488 ^a
10% MLE (T ₂)	0.3118 ^a	0.3400 ^a	0.3925 ^a	0.3650 ^b	0.3178 ^a
20% MLE (T ₃)	0.3795 ^a	0.3850 ^a	0.2900 ^b	0.3450 ^b	0.2050 ^a
30% MLE (T ₄)	0.4143 ^a	0.3175 ^a	0.3900 ^a	0.5450 ^a	0.4425 ^a
LSD	0.01	0.0687	0.0413	0.1225	0.4554

Means in the column with same letter(s) superscript are not significantly different (P<0.05)

Effects of MLE Application on Root Carbohydrate Exudates of Soybean

At 2 WAP, the highest (24.767%) mean root carbohydrate was recorded from the 30% MLE treated plots which showed significant difference (P<0.05) from the other plots while the lowest (8.150%) mean root carbohydrate was recorded from the control plots. At 4 WAP, the control plots recorded the highest (15.500%)

mean root carbohydrate which showed significant difference (P<0.05) from the lowest (10.680%) mean root carbohydrate (Table 6). At 6 WAP, the highest (13.608%) mean root carbohydrate which showed significant difference from the lowest (10.738%) mean root carbohydrate recorded from the 10% MLE treated plots. At 8 WAP, the 20% MLE treated plots also recorded the highest (13.323%) mean root carbohydrate which showed

significant difference ($P < 0.05$) from the lowest (9.730) mean root carbohydrate recorded from the control plot (Table 6). At 10 WAP, the 10% MLE treated plots recorded the highest (0.087) mean root carbohydrate which was

significantly different ($P < 0.05$) from the lowest (0.014) mean root carbohydrate recorded from the control plots (Table 6).

Table 6. Root Carbohydrate Exudates of Soybean as Influenced by MLE Applicant

MEAN ROOT CARBOHYDRATE EXUDATES (%)					
Treatments	2WAP	4WAP	6WAP	8WAP	10WAP
Control (T ₁)	8.50 ^d	15.800 ^a	11.950 ^b	9.730	0.014 ^b
10% MLE (T ₂)	15.510 ^c	11.725 ^b	10.738 ^b	12.963 ^a	0.087 ^a
20% MLE (T ₃)	20.430 ^b	12.510 ^b	13.608 ^a	13.323 ^a	0.023 ^{ab}
30% MLE (T ₄)	24.767 ^a	10.680 ^b	11.298 ^b	10.300 ^b	0.018 ^{ab}

Means in the column with same letter(s) superscript are not significantly different ($P < 0.05$)

Effects of MLE on the Root Phenolic Exudates of Soybean

The mean root phenolic exudates of soybean recorded at 2 WAP was highest (14.825%) with the control plots which showed significant difference ($P < 0.05$) from the lowest (2.778%) mean root phenolic exudates recorded from the 30% MLE treated plots.

At 4 WAP, the control plots also recorded the highest (1.113) mean root phenolic exudates which was significantly higher ($P < 0.05$) than the lowest (0.858%)

mean root phenolic exudates recorded from the 30% MLE treated plots (Table 7).

At 6 WAP, the control plots recorded the lowest (0.8580) mean root phenolic exudates which showed significant difference from the highest (0.963) mean root phenolic exudates which was recorded from the 10% MLE treated plots. At 10 WAP, the 10% MLE treated plots recorded the highest (0.576) mean root phenolic exudates which did not show significant difference ($P < 0.05$) from the lowest (0.186) mean root phenolic exudates which was recorded from the 30% MLE treated plots (Table 7).

Table 7. Root Phenolic Exudates as Influenced by MLE Application

MEAN ROOT PHENOLIC EXUDATES (%)					
Treatments	2WAP	4WAP	6WAP	8WAP	10WAP
Control (T ₁)	14.825 ^a	1.113 ^a	0.858 ^b	0.845 ^b	0.415 ^a
10% MLE (T ₂)	6.800 ^b	0.865 ^b	0.963 ^a	0.870 ^b	0.576 ^a
20% MLE (T ₃)	3.855 ^c	0.955 ^{ab}	0.868 ^b	1.010 ^a	0.309 ^a
30% MLE (T ₄)	2.778 ^d	0.858 ^b	0.895 ^{ab}	0.895 ^b	0.186 ^a

Means in the column with same letter(s) superscript are not significantly different ($P < 0.05$)

IV. DISCUSSION

The major limiting factor of crop production in the tropics is the deficiency of soil nutrient resulting from land degradation which affects the growth, nutrient content, and uptake of the plant. Low levels of Nitrogen, Phosphorus and organic carbon were observed in the soil used for the experiment which substantiates the claims of Aduayi *et al.* (2002) who reported that most of Nigerian soil is deficient in Nitrogen potassium, and even organic matter. Application of MLE at 30% concentration significantly increased the organic carbon and organic matter content of the soil. The cation exchange capacity was also significantly increased with 30% MLE application. The application of MLE generally improved physical and chemical properties of soil especially at 30% level of concentration. This finding confirms the findings of Anyaegbu (2014) who reported that application of Moringa extract increased the availability of micro and macro nutrients in the soil for plant uptake.

Although, MLE application did not significantly influence the number of root, root length and root dry weight of soybean. Higher values of these parameters were observed to be recorded from the MLE treated plots which also showed that MLE was able to boost the root

development of soybean. This slight increased in growth is in agreement with work of Muhamman *et al.* (2009 a and b) who reported a significant increase in some growth characters of cowpea and tomato with *Moringa* extract

However, significant influence of the MLE application was observed with the production of root exudates. The MLE treated plots in comparison with the control plots recorded higher values for root protein, carbohydrate and phenol. This is in line with the claims of Dakora and Philips (2002), that application of organic materials can improve the production of root exudates.

The number of roots, root length and dry weight in treated plants were found to be higher than in control, this could be due to release of crude protein and carbohydrate which influence root system architecture (RSA) in search of more nutrients needed to enhance their growth in heterogeneous environment like soil.

The high level of concentration of root carbohydrate exudates recorded from treated plots could cause improvement in the growth recorded in plant roots which help in absorption of mineral elements irrespective of acidic nature of soil. This agreed with Pierret *et al.*, 2000 that release of organic compound from root is a key factor in mineralization and in mediating plant – microbe interactions.

Since the soil of study area is acidic, going by high level of Al^{3+} , it suffices to say that the growth in plant height recorded (data not shown) could be due to production of root exudates which enhanced availability of nutrients needed for soybean growth, was stimulated by soil application of Moringa leaf extract organic exudates can influence nutrient availability (eg. iron, phosphorus in rhizosphere and assist in nutrient uptake by plants (Jones and Darrah, 1995; Dinkelake et al., 1997).

V. CONCLUSION

The study indicates that MLE application greatly improved soil condition which in turn improved the root growth parameters. However, MLE application was not able to increase soil pH in this study which in turns created room for aluminum toxicity. This aluminum toxicity could be responsible for the limited phenolics exudates production compared to crude protein and carbohydrate production.

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