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Influence of poultry manure application on the leaf amino acid profile, growth and yield of moringa (*Moringa oleifera* lam) plants plant

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Abstract

The study was carried out in the Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka, to determine the amino acid profile, morphological growth pattern and yield of *Moringa oleifera* plants as influenced by poultry manure application. Three levels of poultry manure (0 tonne/ha, 5 tonnes/ha and 10 tonnes/ha) were used. The moringa seeds used were collected from three locations of Nigeria i.e Nsukka (Enugu State), Dutse (Jigawa State) and Jos (Plateau State). The experiment was a 3 x 3 factorial trial in a randomized complete block design (RCBD) with three replications. Records of first, 50% and 100% seedling emergence were taken between five and 21 days after planting. Morphological growth and yield characteristics were recorded. Succulent and older leaves were sampled monthly for laboratory analysis of amino acid content. There were no significant differences ($p > 0.05$) in the effects of the different poultry manure rates on days to seedling emergence and the total percentage emergence. Moringa plants grown with 10 tonnes/ha of poultry manure had the highest values of plant height, stem girth and leaf number in the first 12 weeks of planting followed by those gown with 5 tonnes/ha and 0 tonne/ha in that order. Moringa plants grown with 5 tonnes/ha of poultry manure flowered most promptly. Plants grown with 10 tonnes/ha of poultry manure had the highest values of all the yield parameters. Levels of poultry manure did not have any significant effect ($p > 0.05$) on essential and non-essential amino acid contents of the leaves. Young succulent leaves had higher values of the amino acids than older leaves except tryptophan, argenine (essential amino acids), aspartic acid, serine and tyrosine (non-essential amino acids).

Key words: ***Amino acids, Moringa oleifera, poultry manure.***

1. Introduction

Moringa oleifera plant is fast growing and can grow up to five meters in the first year of planting. Propagation is either by seeds or vegetative means through cuttings. Viable seeds germinate in five to twelve days. Moringa seeds have no dormancy period, so they can be planted as soon as they are mature and they will retain the ability to germinate for up to one year. An average mature moringa tree can produce between 15 000 and 35 000 seeds per year, with an average seed weight of 0.3 g and kernel to hull ratio of 15:25. The tree is fast-growing and can reach an average height of six to seven meters a year in areas receiving less than 400 mm mean annual rainfall [9]. *Moringa oleifera* Lam is quite prolific in bearing flowers. Its flowers are pleasantly fragrant. They are about two to three centimeters wide and are produced in auxiliary drooping panicles measuring 10 – 25 cm long. A tree can yield between 400 and 600 pods annually in the first three years of planting [5].

Amino acids constitute an important class of organic compounds that contain both the amino ($8NH_2$) and carboxyl (8COOH) groups. They are divided into essential and non-essential amino acids. Essential amino acids are those that cannot be synthesized by the body and, therefore, must be part of the diet. They include lysine, tryptophan, valine, histidine, leucine, isoleucine, phenylalanine, threonine, methionine and arginine. Essential amino acids are found in adequate quantities in animal proteins [7]. Moringa leaves contain high amounts of essential amino acids [8]. Non-essential amino acids are those the body can synthesize in quantities that can allow maximum growth. They include glycine, serine, alanine, asparagine, aspartic acid, glutamic acid, glutamine, proline, lysine and trimethyl-lysine. All the essential and non-essential amino acids had been discovered in the leaves of moringa plants [2; 6; 8 and 10]. Amino acids are required in the body for growth, repair of body tissues, production of enzymes, immunoglobins, hormones and red blood cells [3].

Poultry manure is a rich source of nitrogen (N) and N is precursor of amino acid synthesis. Thus, poultry manure application is expected to have direct effect on amino acid compositions of plants if enough N is released into the soil solution from the manure complex for plant use and the plant systems are functioning properly. Demir et al. [4], however, reported that poultry manure application did not significantly increase the levels of N, magnesium (Mg) and molybdenum (Mo) in leaves and fruits of

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tomato. On the contrary, [1] found that poultry manure application increased the concentrations of N, phosphorus (P), potassium (K), calcium (Ca) and Mg in fluted pumpkin (*Telfairia occidentalis*) leaves. Report from [11] affirmed that there were increases in the levels of N, P, K, zinc (Zn), protein and carbohydrate in maize (*Zea mays)* grain with poultry manure application.

Little information is available on the influence of poultry manure application on amino acid composition of moringa leaves as well as the growth and yield of the plant. The study, therefore, investigated the effects of poultry manure application on the leaf amino acid contents including the growth and yield of the plant.

2. Materials and Methods

The study was carried out in the Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka. The treatments consisted of three levels of poultry manure (0 tonne/ha, 5 tonnes/ha and 10 tonnes/ha) and three accessions of moringa seeds (from Nsukka in Enugu State, Jos in Plateau State and Dutse in Jigawa State). The experiment was 3 x 3 factorial trial laid out in a randomized complete block design (RCBD) with three replications. The moringa seeds were planted directly on the field (two per hole) at a distance of 1m x1m. The seedlings were thinned down to one/hole after emergence. The poultry manure was incorporated into the soil at the different rates stated above before planting.

Determination of morphological and yield characteristics

The morphological characteristics measured were; the plant height (cm), stem girth (cm), leaf number and days to anthesis (flower opening). The plant height was measured with a meter rule, the stem diameter with a pair of venier calipers. The diameter (D) was converted to girth (G) by multiplying with a factor *pi*, where *pi* =22/7.

Thus, G = D*pi* =22/7D.

The number of leaves was counted from the nodal points. The yield parameters included the number of pods/plant, pod length (cm), pod circumference (cm), pod dry weight (g), number of seeds/pod and 100 seed weight (g).

Determination of leaf amino acid content

Succulent leaves (first to the fifth leaf) and older ones (from the base) were sampled monthly for laboratory analysis of amino acid content. Tissue analysis was done at International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The following procedures were adopted in determining the amino acid content of the leaves according to the Association of Analytical Chemists (2005).

1. Hydrolysis/sample extraction
2. Amino acid derivation
3. Separation of obtained amino acids.

Hydrolysis/sample extraction

Thirty milligrams of plant sample was weighed in Eppendorf tubes and kept in ice bath. Chloroform, methanol and water were mixed in the ratio of 20:60:20 mls respectively in the tubes. Internal mixer beads were also added and shaken for 3 minutes after which the mixer beads were removed and the mixture centrifuged for 10 minutes at 14000 rpm. Two hundred micro litres (200*m*l) of the mixture was added to High Profile liquid Chromatography (HPLC) vials and dried in speed-vacuum concentrator.

Amino acid derivation for HPLC analysis

Derivation was performed automatically on the amino acid analyzer by reacting free amino acids, under basic conditions, with phenylisothiocynate (PITC) to produce phenylthiocarbamyl (PTC) - amino acid derivatives. This process took approximately 30 minutes per sample. A standard solution containing a known amount of 17 common amino acids was also loaded on three separate amino acid analyzer sample spots for derivation. These were used to generate a calibration file for determination of amino acid contents of the samples. Methanol solution containing the PTC-amino acids was transferred to a narrow bored HPLC system for separation.

Amino acid separation in the HPLC system

The PTC-amino acids were separated on a reverse phase C18 silica column and the PTC chromophore was detected at 254 nm. Two buffer solutions, A and B were used for separation of the amino acids. Buffer solution A was 120 mls of sodium acetate at pH 5.50 and buffer B was 70% acetonitrile.

The peaks of the different amino acids on the chromatography were read and quantified using a Dionex Chromeleon Data Analysis System attached to the Amino Acid Analyzer System.

3. Results and Discussion

Zero tonne/ha of the poultry manure gave the highest percentage seedling emergence (66.3) followed by 5 tonnes/ha (64.6) and 10 tonnes/ha (58.7) respectively. Zero tonne/ha of poultry manure gave the least number of days to first, 50% and 100% seedling emergence.

Table 1. Main effects of Manure on Days to First, 50% and 100% Emergence and Total Percentage Emergence

Manure Rates (tonnes/ha)	*First*	*50%*	*100%*	*% Emergence at 21 DAP*
0	0.62	8.67	18.81	66.3
5	5.78	9.00	21.44	64.6
10	6.11	9.11	19.78	58.7
Mean	6.04	8.93	20.04	63.0
$LSD_{(0.05)}$	ns	ns	ns	ns

ns = non-significant (p > 0.05)

Table 2. Main Effects of Poultry Manure on Plant Height (PH), Stem Girth (SG) and Number of Leaves (NL) per Plant

Manure Rates (tonnes/ha)	*PH(cm)*	*SG(cm)*	*NL*
0	8.37	3.04	4
5	28.26	8.59	5
10	30.38	8.53	8
MEAN	22.33	6.72	5.67
$FLSD_{(0.05)}$	4.694	ns	ns
8 Weeks after Planting (WAP)			
0	12.60	4.36	9
5	64.60	14.38	11
10	70.80	15.80	13
MEAN	49.30	11.51	11
$FLSD_{(0.05)}$	6.81	1.60	ns
12 Weeks after Planting (WAP)			
0	44.10	7.40	13
5	92.70	15.88	17
10	129.40	22.85	21
MEAN	88.70	15.33	17
$FLSD_{(0.05)}$	29.61	3.66	2.00
16 Weeks after Planting (WAP)			
0	64.30	12.88	17
5	120.20	12.22	22
10	180.20	12.03	25
MEAN	121.70	12.38	21.30
$FLSD_{(0.05)}$	48.89	ns	2.01

ns = Not significant

There were no significant differences (p > 0.05) in the effects of the different poultry manure rates on days to seedling emergence and the total percentage emergence (Table 1).

Moringa plants grown with 10 tonnes/ha of poultry manure had the highest values of plant height, stem girth and leaf number in the first 12 weeks of planting followed by those gown with 5 tonnes/ha and 0 tonne/ha in that order. At the sixteenth week after planting, plants grown with 0 tonne/ha of poultry manure had the highest stem girth value and the least values of the plant height and leaf number. The poultry manure showed no significant differences (p > 0.05) in the number of leaves in the first eight weeks of planting. There were significant differences (p < 0.05) in the stem girth values at the eighth and twelfth week of planting. The plant height differed significantly (p < 0.05) throughout the sixteen weeks of the morphological growth among the different manure rates (Table 2).

Moringa plants grown with 5 tonnes/ha of poultry manure flowered most promptly (within 69.5 days) and took the fewest number of days (114.10 days) to attain 100% anthesis (flower opening) as shown in Table 3. Plants grown with 10 tonnes/ha of poultry manure had the highest values of all the yield parameters. The poultry manure rates showed no significant differences (p > 0.05) in the pod length, number of seeds/pod and 1000 seed weight values (Table 4).

Table 3. Main Effects of Poultry Manure on Days to First, 50% and 100% Flowering, and Days to Ripening of Pods

PM Rate	*Days of flowering*		
	First	**50%**	**100%**
0 tonne/ha	97.6	99.2	118.70
5 tonnes/ha	69.5	103.3	114.10
10tonnes/ha	70.4	100.7	118.10
Mean	79.2	101	117.00
LSD(0.05)	14.36	ns	ns

ns = not significant

Table 4: Main Effects of Poultry Manure on Number of Pods per Plant, Pod Girth, Pod Length, Pod Dry Weight, Number of Seeds per Pod and 1000 Seed-Weight

Manure Rates (tons/ha)	*Number of Pods per Plant*	*Pod Girth (mm)*	*Pod Length (cm)*	*Pod Dry Weight (g)*	*Number of Seeds/ Pod*	*1000 Seed-Weight*
0	0.29	12.80	31.44	6.89	12.11	171.60
5	1.64	15.67	30.98	7.68	13.64	180.40
10	5.25	15.63	33.37	9.43	14.58	213.70
Mean	2.40	14.70	31.93	7.88	13.44	188.57
$FLSD_{(0.05)}$	2.115	1.349	ns	1.956	ns	ns

ns = not significant

Succulent leaves had higher contents of most of the essential and non-essential amino acids except tryptophan, argenine (essential amino acids), aspartic acid, serine and tyrosine (non-essential amino acids) which were greater in the older leaves. There were no significant differences ($t > 0.05$) in the contents of essential and non-essential amino acids of the two leaf types except phenylalanine, tryptophan, histidine (essential amino acids), alanine, glutamine and glycine (non-essential amino acids) which differed significantly ($t < 0.05$) (Table 5).

The different levels of poultry manure did not have any significant effect ($p > 0.05$) on the essential and non-essential amino acid contents of moringa leaves three months after planting (Table 6). Seeds source did not affect the amino acid contents significantly ($p > 0.05$) except histidine which varied significantly ($p < 0.05$) among the various seed sources (Table 7).

The highest values of morphological and yield characteristics obtained in the plants grown in 10 tonnes/ha of poultry manure were clear indications of positive response of moringa plants to poultry manure application. The higher contents of essential and non-essential amino acids in the succulent leaves, compared with older ones, can be associated with active growth and protein synthesis which take place in the younger leaves. Amino acids are the structural units of protein synthesis. The younger leaves also had higher contents of protein in this study compared to the older ones at the base. This result coincided with the report of [2] who also obtained higher protein values in the young leaves of moringa plants.

Table 5: Effects of age on the essential and non-essential amino acids in leaves of *Moringa oleifera*

Essential Amino Acid (%)	***Leaf Age***		$t_{(0.05)}$
	Older	**Succulent**	
Isoleucine	1.395	1.526	ns
Leucine	2.158	2.31	ns
Lysine	1.947	1.997	ns
Phenylalanine	1.52	1.976	0.35
Threonine	1.061	1.515	ns
Tryptophan	1.14	0.817	0.23
Valine	1.639	1.73	ns
Methionine	0.595	0.68	ns
Argenine	1.848	1.719	ns
Histidine	0.446	0.525	0.05
Non-Essential Amino Acid (%)			
Alanine	0.829	1.19	0.19
Asparagine	1.791	1.985	ns
Aspartic Acid	0.489	0.458	ns
Glutamine	0.545	0.627	0.08
Glycine	1.405	1.937	0.29
Glutamic Acid	0.466	0.496	ns
Proline	0.484	0.49	ns
Serine	0.869	0.77	ns
Trymethyl lysine	0.472	0.515	ns
Tyrosine	0.815	0.736	ns

ns = not significant

Table 6: Effects of poultry manure on essential and non-essential amino acid contents of leaves of *Moringa oleifera*

Essential Amino Acids (%)	***Poultry Manure Rate***			*Mean*	$FLSD_{(0.05)}$
	0 ton/ha	5 tons/ha	10 tons/ha		
Isoleucine	1.54	1.58	1.26	1.46	ns
Leucine	2.10	2.29	2.32	2.23	ns
Lysine	2.07	1.97	1.88	1.97	ns
Phenylalanine	1.68	1.86	1.70	1.75	ns
Threonine	1.34	1.24	1.29	1.29	ns
Tryptophan	1.00	0.90	1.04	0.98	ns
Valine	1.59	1.85	1.62	1.68	ns
Methionine	0.66	0.66	0.60	0.64	ns
Histidine	0.50	0.48	0.47	0.49	ns
Argenine	1.80	1.78	1.77	1.78	ns
Non-Essential Amino acid (%)					
Alanine	0.97	1.00	1.05	1.01	ns
Asparagine	1.82	1.92	1.92	1.89	ns
Aspartic Acid	0.47	0.47	0.48	0.47	ns
Glutamine	0.58	0.58	0.60	0.59	ns
Glycine	1.70	1.65	1.67	1.67	ns
Glutamic Acid	0.47	0.47	0.50	0.48	ns
Proline	0.49	0.48	0.50	0.49	ns
Serine	0.84	0.77	0.85	0.82	ns
Trymethyllysine	0.52	0.49	0.47	0.49	ns
Tyrosine	0.86	0.74	0.73	0.78	ns

ns = not significant

Table 7: Effects of seed source on the essential and non-essential amino acids in leaves of *Moringa oleifera* Lam

Essential Amino Acid (%)	***Seed Source***			***Mean***	***FLSD$_{(0.05)}$***
	JOS	**NSK**	**JGW**		
Isoleucine	1.38	1.42	1.58	1.46	ns
Leucine	2.32	2.14	2.24	2.23	ns
Lysine	1.93	2.01	1.98	1.97	ns
Phenylalanine	1.55	1.96	1.74	1.75	ns
Threonine	1.35	1.24	1.27	1.29	ns
Tryptophan	1.01	0.97	0.96	0.98	ns
Valine	1.75	1.66	1.65	1.68	ns
Methionine	0.66	0.61	0.64	0.64	ns
Argenine	1.77	1.77	1.81	0.49	ns
Histidine	0.54	0.49	0.43	1.78	0.65
Non-Essential Amino Acid (%)					
Alanine	1.03	1.00	1.00	1.01	ns
Asparagine	1.93	1.83	1.91	1.89	ns
Aspartic Acid	0.50	0.46	0.46	0.47	ns
Glutamine	0.62	0.58	0.56	0.59	ns
Glycine	1.62	1.69	1.71	1.67	ns
Glutamic Acid	0.50	0.47	0.47	0.48	ns
Proline	0.49	0.51	0.46	0.49	ns
Serine	0.81	0.79	0.86	0.82	ns
Trymethyllysine	0.49	0.50	0.49	0.49	ns
Tyrosine	0.86	0.75	0.72	0.78	ns

ns = not significant

The different levels of poultry manure did not significantly affect the amino acid (both essential and non-essential amino acid) compositions of the leaves.

Demir et al. [4] reported non-significant effect of poultry manure on N-composition of tomato leaves. Nitrogen is the structural component of plant amino acids and proteins and the N content of the plant is a function of N-uptake from the soil solution based on climatic and plant physiological conditions. Probably, a lot of soil N was not taken up by the plants to effect changes in the amino acid contents irrespective of poultry manure level. Poultry manure is also a slow-release fertilizer and might have released little amount of N into the soil solution for plant uptake within the period of the study.

The non-significant effect of the seed source suggested negative phenotypic influences on the genetic constitutions of the seeds. Thus, the amino acids compositions of plants from different seed sources did not vary significantly. However, only histidine content of the leaves varied significantly amongst the three seed sources and this constituted an insignificant ratio (1:20) to the twenty essential and non-essential amino acids considered in the study.

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