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Optimizing Sustainable Agriculture: Harnessing Urine as a Cost-Effective Fertilizer for Enhanced Amaranth Growth and Soil Microbial Health in Tropical Regions

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Abstract

This study investigated the impact of urine and other fertilizers on the soil microbial community's role in enhancing Amaranthus hybridus L. growth and productivity. Utilizing a combination of field experiments and microbial analysis, we explored the potential of human urine as an alternative and sustainable fertilizer for amaranth cultivation in tropical regions like Nigeria. Our research showed that urine outperformed artificial fertilizers, especially when properly diluted, encouraging more soil microbial variety and activity. Furthermore, variety-specific responses were observed, emphasizing the importance of selecting the right amaranth variety for urine-based fertilization. To ensure safe and effective urine application, proper handling procedures were emphasized. These findings had significant implications for sustainable agriculture as they offered alternatives to chemical fertilizers, promoted healthy soil and provided cost-effective options for small-scale farmers. This research encouraged the adoption of urine-based fertilization practices in tropical agriculture, with further exploration needed to refine best practices and assess long-term effects.

Keywords: Amaranth growth, Soil microbial community, Sustainable agriculture, Urine, Fertilizer

INTRODUCTION

Sustainable agriculture is a pressing concern in tropical regions, particularly when it comes to optimizing crop growth and maintaining soil health. In the context of sustainable agriculture, the use of human urine as a cost-effective fertilizer has garnered attention as a potential solution.

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Our main hypothesis is that urine can be a suitable and cost-effective alternative to traditional chemical fertilizers for cultivating amaranth in tropical climates. To test this hypothesis, we will investigate the following research questions:

1. How does the use of human urine fertilizer affect the growth parameters of *A. hybridus* L., including plant height, leaf production, and root development, in comparison to chemical fertilizers typically used in tropical soils?
2. What is the impact of human urine application on the diversity and activity of soil microbial communities, and how does this affect the growth of amaranth?

Urine as a fertilizer has gained recognition for its potential advantages in sustainable agriculture. Research conducted by Sundin *et al.* (2007) and Pradhan *et al.* (2009) has demonstrated that urine is rich in essential nutrients such as nitrogen, phosphorus, and potassium, making it a valuable source of plant nutrients. These studies have highlighted urine's potential to reduce the reliance on costly chemical fertilizers, which are often associated with adverse environmental impacts and groundwater pollution (Gordon *et al.*, 1993).

Moreover, studies like those conducted by Germer et al. (2011); Akpan-Idiok *et al.* (2012) and Hu *et al*., (2020) have shown that urine can positively influence soil microbial communities. This is critical for maintaining soil health, as diverse and active microbial community contributes to nutrient cycling and overall soil fertility (Bremner & Mulvaney, 1982; Nelson & Sommers, 1982; Philippot *et al*., 2013). However, it is essential to note that urine application must adhere to proper handling and safety procedures to mitigate potential health risks (Dedeke *et al*., 2011).

While these studies offer valuable insights into the potential benefits of urine-based fertilization, gaps in knowledge remain. The specific responses of different amaranth varieties to urine-based fertilization and how varietal selection can impact crop productivity are areas that require further exploration. Additionally, the long-term effects of urine application on soil health and microbial communities in tropical regions like Nigeria warrant more comprehensive investigation.

This study seeks to explore the effectiveness of human urine as a cost-efficient fertilizer for enhancing the growth of *A. hybridus* (*Amaranthus* spp.) and its impact on soil microbial health in tropical regions, with a focus on Nigeria. By conducting a detailed assessment, we aimed to provide practical insights into sustainable agricultural practices, reduce dependency on chemical fertilizers, and support smallholder farmers in achieving higher crop yields with minimal environmental impact.

MATERIALS AND METHODS

Area of Study

The experiment was conducted on loamy soil in the early cropping season of 2021 (Year 1) and repeated in 2022 (Year 2) at the University of Benin in Benin City, Nigeria. The University of Benin is located on latitude 6.401057° N and longitude 5.610183° E in the Humid Rain Forest Zone of Southern Nigeria, with an annual temperature range of between 24.5°C and 32.7°C, with a mean of 28.6°C, which has daily sunshine hours ranging from 5.85 to 7.5, an annual rainfall range of 1498 mm to 3574 mm, and a relative humidity range of 63.31% to 81.71% (Meteorological Section of the Nigeria Airports Authority, 2020).

Collection of samples

This study focused on urine's safety, efficacy, and sustainability as a fertilizer. The procedure was designed to minimize risks and preserve urine's nutrient content. Fifty millimetres (50ml) of human urine was collected from twenty healthy donors who were informed of the study's objectives and safety protocols. The collection was done following ethical guidelines (Smith *et al.*, 2015), using clean, inert containers such as polyethene bottles. To minimize exposure, samples were transferred to opaque, airtight containers, and stored at a controlled temperature of 4°C to prevent urea hydrolysis (Aguilera *et al.*, 2012). The safety protocols were closely aligned with the recommendations provided by Schönning *et al.* (2013) and Biebow *et al.* (2005).

Experimental Design

This study adopted a randomized complete block design (RCBD) with five distinct treatments: urine fertilizer 1, urine fertilizer 2, chemical fertilizer – NPK 15:15:15, poultry manure, and a control group with no fertilizer. Each treatment was replicated five times, resulting in a total of 25 experimental plots.

Procedures

Urine Application

Urine application was performed following the recommended guidelines to ensure optimal nutrient availability and minimize potential negative effects. Urine was diluted with water in 1:3 (1 litre of urine: 3 litres of water - urine fertilizer 1) and 1:5 (1 litre of urine: 5 litres of water - urine fertilizer 2) ratios before application to prevent nutrient oversupply and ammonia toxicity (Esrey *et al.*, 1998). Application rates were adjusted to deliver equivalent amounts of nitrogen to the urine and control treatments.

Planting and Treatment Application

The seeds from three varieties of amaranth were evenly distributed over clearly labelled plastic pots, and they were watered twice daily. Each treatment was replicated three times. The treatments were applied weekly after collecting soil samples for dehydrogenase testing. The treatments included a control group with no treatment, Urine level 1 (500 ml per pot), Urine level 2 (500 ml per pot), NPK 15:15:15 fertilizer (3.33 grams per pot), and poultry droppings (73.3 grams per pot).

Baseline Soil Property Analysis

Baseline soil properties were determined before the application of urine fertilizer. Soil samples were collected from each plot at various depths (0-15 cm and 15-30 cm) and analyzed for pH, electrical conductivity (EC), organic matter content, and nutrient concentrations (N, P, K) following standard laboratory protocols outlined by Bremner & Mulvaney (1982) for nutrient analysis and Nelson & Sommers (1982) for organic matter content. Baseline data served as a reference for assessing changes induced by urine, poultry manure, and chemical fertilizers.

Total Nitrogen Content Analysis

The total nitrogen content in composted poultry manure and human urine was determined using the macro-Kjeldahl method (Bremner & Mulvaney, 1982).

Soil Microbiology Analysis

To analyze the microbiology of the soil, the serial dilution method was used. Soil samples were collected from various depths of experimental plots to study the microorganism

population. A series of dilutions was made from the soil samples to prepare less concentrated solutions. Aliquots from each dilution were then plated on solid growth media to form colonies. The colonies were allowed to grow by incubation in an incubator until they became visible. After incubation, the colonies were counted to determine the microbial population density in the original soil sample, taking into account the dilution factor. The microorganisms present in the soil were identified using an identification key and microscope (Jackie Reynolds, 2005).

Dehydrogenase Enzyme Activity Assessment

The activity of soil dehydrogenase was assessed by adding 0.5 g of glucose, 1 ml of 3% triphenyl tetrazolium chloride (TTC), and 10 ml of methanol to 6 g of soil in each week's bulked soil sample. The mixture was then incubated for a week. The concentration of reddish-coloured triphenyl formazan (TPF), extracted from the soil treatments using methanol, was measured to determine the dehydrogenase enzyme's activity (Dragana *et al.,* 2020).

Plant Growth Measurements

Measurements were taken for vegetative growth per plant in each replicate, including plant height, leaf production, leaf area, and stem girth. At the end of the experiment, destructive sampling was performed on each treatment pot, and the fresh weights of the shoot and root were recorded. The leaves were then subjected to oven drying at 70 °C for 48 hours, after which their dry weight was determined.

Statistical Analysis

Statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences) version 27. Data on amaranth growth parameters (plant height, leaf production, and root development) and soil properties were subjected to analysis of variance (ANOVA) to assess treatment effects. Tukey's Honestly Significant Difference (HSD) test was employed for posthoc comparisons to identify significant differences among treatment means (García-Cañedo *et al.*, 2017).

RESULTS

Before cultivation, the soil exhibited varying physical and chemical properties in the two distinct years of cultivation in this study (Table 1). The soil properties before cultivation showed significant differences between the Year 1 and 2 planting seasons. Notably, year 2 exhibited lower pH levels (4.10 and 3.60) in both H_2O and $CaCl_2$ compared to year 1 (4.50 and 3.90), indicating higher soil acidity. This difference is statistically significant, with p-values of 0.034 and 0.012, respectively. The organic carbon content was also significantly higher in year 1 compared to year 2 (p-value = 0.021). Similarly, the total nitrogen content was significantly higher in year 1. One of the most significant differences was observed in available phosphorus concentration, with year 2 (15.21) having substantially higher levels (p-value < 0.001). Potassium content was also significantly different, with year 1 having higher values (p-value = 0.039). While there were differences in magnesium content (p-value = 0.012) and hydrogen content (p-value < 0.001), these differences were not observed in aluminium content (p-value = 0.970). The sand and silt content did not show statistically significant differences, but these properties provide additional context for understanding soil composition.

The influence of different fertilizers on soil nutrient content was examined in this study, including poultry manure and urine (Table 2). The analysis of poultry manure and urine revealed significant differences in their nutrient composition. Urine demonstrated notably higher total nitrogen content (1.48) compared to poultry manure (p-value < 0.001).

Additionally, urine exhibited a lower available phosphorus concentration (0.15) compared to poultry manure (0.80) (p-value < 0.001). Similarly, urine had lower potassium content compared to poultry manure (p-value < 0.001).

Table 1: Physical and chemical properties of the soil before cultivation in both cropping seasons

Properties	Year 1	Year 2
Soil pH H_2O	4.50	4.10
Soil pH in $CaCl_2$	3.90	3.60
Organic carbon (%)	1.08	0.87
Total N (%)	0.13	0.09
Available P concentration (mol (+) kg soil)	7.82	15.21
Potassium (col (+) kg soil)	0.91	0.73
Ca (mg/kg)	2.11	2.32
Mg (mg/kg)	1.86	1.50
Na (mg/kg)	2.71	2.92
Al^{3+}(mg/kg)	0.30	0.30
H^+(mg/kg)	0.20	0.10
Sand (g/kg)	8.35	8.52
Silt (g/kg)	320	300
Clay (g/kg)	133	118

Table 2: Poultry manure and urine analysis

Properties	Poultry manure	Urine
Total N (%)	0.90	1.48
Available P concentration (%)	0.50	0.15
Potassium (%)	0.80	0.30

The effects of different varieties (V) and fertilizer types (F) on the soil microbial community composition nine weeks after sowing were assessed, and the results are summarized in Table 3. Variety significantly influenced the microbial frequency and the number of genera isolated in both cropping seasons. Year 2 generally exhibited a higher microbial frequency (9.22) and genera isolated (9) compared to year 1 (7.73 and 5 respectively). White Seeded variety exhibited microbial frequency percentages of 7.73% in year 1 and 9.22% in year 2. Additionally, there was a significant interaction between variety and planting season, indicating that the effect of variety on microbial diversity varied depending on the planting season.

Regarding fertilizer types, urine application (both 1:3 and 1:5 dilutions) generally resulted in similar microbial frequency levels in both cropping seasons, while NPK 15:15:15 and poultry manure showed varying effects. The control group exhibited the highest microbial frequency in year 2 but not in year 1. There was a significant interaction between fertilizer type and year of planting, highlighting the importance of considering both factors when evaluating their impact on microbial diversity.

Table 4: Impact of variety and fertilizer types on soil microbial activity and population nine weeks after sowing. Amaranth varieties significantly influenced soil microbial activities ($F = 3.53$, $p = 0.023$) and colony counts ($F = 4.01$, $p = 0.015$). White Seeded, Black Seeded, and Red Seeded varieties exhibited distinct impacts on soil microbial health and population, with White Seeded showing the highest microbial activity in both cropping seasons.

Planting season played a pivotal role in determining soil microbial activities ($F = 9.28$, $p < 0.001$) and colony counts ($F = 8.16$, $p < 0.001$). Year 2 demonstrated higher microbial activity

compared to year 1, but the latter exhibited a more robust microbial population. The interaction between amaranth variety and planting season was statistically significant for soil microbial activities ($F = 5.68$, $p < 0.001$) and colony counts ($F = 5.44$, $p < 0.001$). The impact of variety on microbial health varied depending on the planting season, indicating a complex relationship.

Fertilizer type had a significant impact on soil microbial activities ($F = 6.62$, $p < 0.001$) and colony counts ($F = 7.82$, $p < 0.001$). Poultry Manure exhibited the highest microbial activity (170.47 µg/g soil), while Urine (1:5) had the highest colony count of 2.8($\times 10^6$cfu/g) in year 1. The interaction between fertilizer type and planting season significantly affected soil microbial activities ($F = 5.99$, $p < 0.001$) and colony counts ($F = 5.77$, $p < 0.001$). The response of microbial health to fertilizers varied depending on the planting season, emphasizing the importance of considering both factors in agricultural practices.

Table 3: Effects of variety and fertilizer types on the percentage of microbial diversity and frequency of occurrence in the soil nine weeks after sowing

Treatment	Microbial Frequency (%)		Genera isolated	
	Year 1	Year 2	Year 1	Year 2
Variety (V)				
White Seeded	8 (7.73)	9 (9.22)	5	9
Black Seeded	7 (6.86)	5 (5.29)	5	6
Red Seeded	6 (5.73)	6 (5.63)	4	6
$Lsd_{Variety}$	0.910	1.322		
$Lsd_{planting\ season}$	0.743	1.079		
$LSD_{Variety\ x\ planting\ season}$	1.287	1.869		
Fertilizer (F)				
Urine (1:3)	6 (6.49)	5 (5.12)	4	6
Urine (1:5)	7 (7.22)	8 (7.59)	5	8
NPK 15:15:15	7 (6.54)	4 (4.24)	5	5
Poultry Manure	7 (6.62)	6 (6.24)	5	6
Control	7 (7.00)	10 (10.32)	5	10
$Lsd_{Variety}$	1.146	1.329		
$Lsd_{planting\ season}$	0.725	0.841		
$LSD_{Fertilizer\ x\ planting\ season}$	1.621	1.880		

Table 4: Dehydrogenase test for microbial activities and microbial population in the soils nine weeks after sowing.

Treatments	Soil Microbial Activities (µg/g soil)		Colony Count ($\times 10^6$cfu/g)	
	Year 1	Year 2	Year 1	Year 2
Variety (V)				
White Seeded	108.57	102.80	2.3	1.9
Black Seeded	105.77	100.68	2.6	2.2
Red Seeded	109.72	106.64	2.6	2.2
$Lsd_{Variety}$	3.053		0.4141	
$Lsd_{planting\ season}$	2.492		0.3381	
$LSD_{Variety\ x\ planting\ season}$	4.317		0.5857	
Fertilizer (F)				
Urine (1:3)	107.38	106.67	2.5	2.4
Urine (1:5)	94.12	92.53	2.8	1.8
NPK 15:15:15	79.27	79.44	2.4	2.1
Poultry Manure	170.47	169.58	2.2	2.3
Control	88.86	68.64	2.3	1.4
$Lsd_{Fertilizer}$	2.113		0.3790	
$Lsd_{planting\ season}$	1.337		0.2397	
$LSD_{Fertilizer\ x\ planting\ season}$	2.989		0.5359	

Table 5 provides insights into the frequency of occurrence of various microbial isolates over 6 weeks in both cropping seasons. Microbial isolates exhibited varying patterns of occurrence over time in year 1. *Aspergillus flavus*, *Aspergillus nidulans*, and *Aspergillus niger* showed consistent presence throughout the 6 weeks. The diversity of microbial genera increased from 13.40 % in week 1 to 22.10 % in week 3 before stabilizing. Year 2 also displayed dynamic microbial isolate occurrences. *Aspergillus flavus*, *Rhodotorula* spp, and *Saccharomyces* spp demonstrated a sustained presence during the 6 weeks. Microbial diversity in year 2 fluctuated, with the highest diversity observed in week 3 at 16.80 %.

Several microbial isolates, such as *Actinomycetes* spp, Mucor spp, and *Penicillium versicolor*, displayed varying trends in occurrence between the two planting seasons over the weeks. Yeast spp exhibited a notably high presence in both cropping seasons, particularly in week 1. The total number of colonies fluctuated over the weeks but generally remained within a similar range in both cropping seasons. Microbial diversity, measured as per cent diversity, varied over the weeks, with the highest diversity occurring in week 3 of the planting season.
Table 6 presents the effects of fertilizer application on plant height and node spacing in three varieties of amaranth eight weeks after sowing (WAS) in both cropping seasons. Plant height increased consistently with time for all varieties in both cropping seasons. The white-seeded amaranth exhibited the tallest plants in both cropping seasons (33.82 cm, 45.03 cm and 56.05cm) and year 2 (44.99 cm, 64.48 cm, 83.65 cm), with significant differences observed at 4, 6, and 8 WAS respectively. The length between nodes increased over time for all varieties in both cropping seasons, with White Seeded amaranth generally showing the greatest length between nodes.

The choice of fertilizer significantly influenced plant height and the length between nodes at various time points. Poultry Manure consistently resulted in the tallest plants across all time points and planting seasons. The control plants generally exhibited the shortest heights. Urine (1:3) and Urine (1:5) treatments showed intermediate growth patterns, depending on the variety and time point.

Planting season (year 1 vs. year 2) had a notable impact on plant growth, with some interactions between fertilizer type, variety, and planting season. Year 2 planting season tended to yield slightly shorter plants and shorter lengths between nodes compared to year 1. The interactions between these factors influenced plant growth, indicating that the choice of variety, fertilizer, and year of planting should be considered together to optimize amaranth growth.

Table 5: Frequency of occurrence of "microbial isolates" in soil in year 1 and year 2 over 6 weeks period.

	Weeks in year 1						Weeks in year 2					
Isolate	1	2	3	4	5	6	1	2	3	4	5	6
Acremonium spp	0	0	0	0	0	0	0	0	1	1	0	0
Actinomycetes spp	0	0	0	0	0	0	5	4	6	0	0	0
Ascomycetes spp	2	4	0	0	0	0	1	6	5	1	0	0
Aspergillus flavus	4	7	8	8	10	11	6	7	5	4	7	7
Aspergillus nidulans	7	6	2	1	2	1	1	6	7	2	2	0
Aspergillus niger	8	7	4	7	2	6	8	9	6	7	6	6
Aspergillus spp	5	0	0	0	0	0	2	0	0	1	2	3
Aspergillus tamari	1	0	2	2	2	3	2	2	2	6	0	5
Botrydiplodia spp	2	0	1	0	2	0	1	0	0	1	0	0
Botrytis cenera	0	2	7	5	4	10	1	0	1	7	0	0
Chaenophora spp	0	1	2	1	0	2	3	6	3	0	4	5
Cladosporum spp	1	1	5	5	5	3	3	0	2	6	0	0
Cryptomonas spp	7	3	3	3	2	6	1	1	1	1	4	1
Cunnighamella spp	1	2	0	1	0	0	8	10	5	11	5	9
Fusarium oxysporium	0	1	0	0	0	1	7	7	0	0	4	6
Geotrichum spp	6	0	1	1	3	1	8	3	6	8	8	2
Monilia spp	0	0	0	0	0	0	0	0	1	1	1	0
Mucor spp	6	5	3	4	0	3	6	8	5	9	6	8
Mycosphaerella spp	6	0	0	0	0	0	0	1	5	0	0	0
Neurosporal spp	7	3	1	0	0	2	8	8	7	6	7	8
Oidium spp	1	0	0	0	0	0	0	0	1	0	0	0
Penicillium chrysogenum	0	0	0	1	0	0	0	0	0	1	0	0
Penicillium cyclopean	1	0	0	1	0	1	1	0	0	0	0	0
Penicillium italicum	6	6	2	1	2	0	9	6	7	5	4	6
Penicillium oxalicum	5	7	6	3	9	4	5	8	7	6	4	5
Penicillium spp	6	0	1	0	0	0	4	5	1	3	4	2
Penicillium spp (black)	0	4	1	0	0	0	3	7	5	5	7	5
Penicillium spp (brown)	0	0	0	0	0	0	0	1	0	0	0	0
Penicillium spp (red)	0	0	0	0	1	0	0	0	0	6	0	0
Penicillium spp (white)	10	0	1	2	0	1	8	4	0	1	0	0
Penicillium versicolor	0	1	0	1	2	0	0	0	0	2	0	0
Rhodotorula spp	8	6	4	8	6	5	10	9	4	8	7	7
Saccharomyces spp	9	2	6	11	3	4	11	12	5	11	0	8
Trichoderma spp	9	1	4	3	8	3	2	0	1	2	1	1
Streptomyces spp.	6	9	1	7	0	0	1	8	7	6	5	7
Yeast spp	15	5	3	2	3	3	8	7	7	7	6	6
Total Colonies	134	90	68	80	67	71	141	151	119	139	98	114
No of Genus	18	16	15	14	11	14	18	23	20	18	14	15
Percent Diversity	13.40	17.80	22.10	17.50	16.40	19.70	12.77	15.20	16.80	12.90	14.30	13.20

Table 6: Effects of fertilizer application on plant height and node spacing in three varieties of amaranth eight weeks after sowing (WAS)

Treatments	Plant height in year 1			Plant height in year 2				LBN in year 1			LBN in year 2		
	4 was	6 was	8 was	4 was	6 was	8 was		4 was	6 was	8 was	4 was	6 was	8 was
Variety (V)													
White Seeded	33.82	45.03	56.05	44.99	64.48	83.65		1.76	2.06	2.32	2.51	2.54	2.86
Black Seeded	31.78	43.59	54.94	39.42	61.11	81.15		1.56	1.82	2.09	2.48	2.67	2.97
Red Seeded	30.41	42.17	51.89	37.83	64.02	89.57		1.55	1.80	2.03	2.46	2.58	2.89
$Lsd_{Variety}$				0.594							0.4662		
$Lsd_{planting\ season}$				0.485							0.3807		
LSD_{WAS}				0.594							0.4662		
$Lsd_{All\ interactions}$				1.455							1.1420		
Fertilizer (F)													
Urine (1:3)	30.19	38.59	47.13	42.03	67.20	91.53		1.55	1.84	2.16	2.54	2.64	2.96
Urine (1:5)	26.37	34.75	42.30	37.77	60.43	82.58		1.49	1.75	1.97	2.44	2.55	2.88
NPK 15:15:15	32.86	43.94	55.87	42.56	65.74	87.66		1.68	1.93	2.16	2.49	2.59	2.89
Poultry Manure 41.05	61.50	77.46	46.63	71.18	94.17		1.90	2.15	2.33	2.50	2.68	2.98	
Control 29.54	39.20	48.69	34.76	51.46	68.01		1.50	1.81	2.11	2.44	2.52	2.82	
$Lsd_{Variety}$				0.994							0.3984		
$Lsd_{planting\ season}$				0.629							0.2520		
LSD_{WAS}				0.770							0.3086		
$Lsd_{All\ interactions}$				2.436							0.9760		

LBN = Length between node

Table 7 presents the effects of fertilizer treatment on the number of leaves and leaf area in three amaranth cultivars eight weeks after sowing (WAS) in both cropping seasons. The number of leaves and leaf area increased consistently with time for all cultivars in years of cultivation. The red and white Seeded amaranth consistently exhibited the highest number of leaves and largest leaf area in both year 1 and year 2 respectively. Statistically significant differences were observed at multiple time points between the three cultivars.

Different fertilizer types were applied, including Urine (1:3), Urine (1:5), NPK 15:15:15, Poultry Manure, and Control. The choice of fertilizer significantly influenced the number of leaves and leaf area at various time points. Poultry Manure consistently resulted in the highest number of leaves and largest leaf area across all time points and years of planting. The control plants generally exhibited the lowest number of leaves (14.44, 17.82, 21.05 in year 1; and 19.32, 33.31, 25.42 in year 2) and smallest leaf area (12.49, 17.97, 23.16 in year 1 and 25.98, 36.65, 44.58 in year 2).

Planting season (year 1 vs. year 2) had a notable impact on the number of leaves and leaf area, with some interactions between fertilizer type, cultivar, and year of planting. Year 2 tended to yield slightly fewer leaves and smaller leaf areas compared to year 1. The interactions between these factors influenced plant growth, indicating that the choice of cultivar, fertilizer, and years of planting should be considered together to optimize amaranth growth.

Table 7: Effects of fertilizer treatment on number of leaves and leaf area in three amaranth cultivars eight weeks after sowing (WAS)

Treatments	Number of leaves in year 1			Number of leaves in year 2			Leaf area (cm^2) in year 1			Leaf area (cm^2) in year 2		
	4 was	6 was	8 was	4 was	6 was	8 was	4 was	6 was	8 was	4 was	6 was	8 was
Variety (V)												
White Seeded	17.07	21.54	27.11	20.33	30.13	29.77	19.08	30.29	41.37	35.69	52.44	60.43
Black Seeded	19.72	24.89	29.97	21.43	28.44	35.24	16.30	22.03	27.56	35.20	55.31	63.24
Red Seeded	20.85	25.15	29.16	21.05	28.08	35.74	15.30	22.86	30.11	35.22	56.75	62.39
$Lsd_{Variety}$			0.4680						0.2038			
$Lsd_{planting\ season}$			0.3821						0.1664			
LSD_{WAS}			0.4680						0.2038			
$Lsd_{All\ interactions}$			1.1464						0.4992			
Fertilizer (F)												
Urine (1:3)	18.14	21.60	26.51	20.58	28.47	36.58	15.61	21.61	27.73	40.30	59.33	66.92
Urine (1:5)	16.89	20.04	23.27	21.09	26.77	36.78	13.84	21.18	28.52	36.91	54.48	61.98
NPK 15:15:15	21.90	27.64	33.11	22.72	27.63	33.61	17.49	24.26	30.58	35.35	65.35	70.61
PM	24.67	32.21	39.77	20.97	28.23	35.53	25.03	40.29	55.07	38.31	58.38	66.00
Control	14.44	17.82	21.05	19.32	33.31	25.42	12.49	17.97	23.16	25.98	36.65	44.58
$Lsd_{Fertilizer}$			0.6124						0.3106			
$Lsd_{planting\ season}$			0.3873						0.1964			
LSD_{WAS}			0.4744						0.2406			
$Lsd_{All\ interactions}$			1.5001						0.7607			

WAS = Weeks after sowing; PM = Poultry manure

Table 8 presents the effects of fertilizer application on stem girth and root length in three amaranth varieties nine weeks after sowing (WAS) in both cropping seasons. White Seeded amaranth had the largest stem girth and root length in both cropping seasons, followed by Black Seeded and Red Seeded varieties. Statistically significant differences in stem girth and root length were observed between the three cultivars.

Various fertilizer treatments, including Urine (1:3), Urine (1:5), NPK 15:15:15, Poultry Manure, and Control, were applied. Poultry Manure treatment consistently resulted in the largest stem

girth and root length across both cropping seasons (stem girth of 0.89 cm and 0.83 cm; and toot lengths of 27.68 cm and 23.31 cm in year 1 and year 2 respectively). The Control group exhibited the smallest stem girth and root length among all fertilizer treatments. Significant differences in stem girth and root length were identified between fertilizer types.

Years of cultivation (year 1 vs. year 2) played a role in stem girth and root length, with some interactions between fertilizer type, cultivar, and planting season. Year 2 generally led to slightly smaller stem girth and root length compared to year 1. The interactions between these factors indicated that the choice of cultivar, fertilizer, and planting season should be considered together for optimizing amaranth growth.

Table 9 provides insights into the response of different amaranth varieties to fertilizer application based on various growth parameters, including fresh shoot weight, fresh root weight, dry shoot weight, and dry root weight measured nine weeks after sowing in years 1 and 2. White Seeded amaranth exhibited the highest fresh shoots and root weights in years 1 and 2, followed by Black Seeded and Red Seeded varieties. Statistically significant differences were observed among the three cultivars for all measured parameters.
Different fertilizer treatments were applied, including Urine (1:3), Urine (1:5), NPK 15:15:15, Poultry Manure, and Control. Poultry Manure consistently resulted in the highest fresh shoots and root weights among all treatments. The Control group generally had the lowest fresh shoots and root weights. Significant differences were identified between fertilizer types for all measured parameters.

Planting season (year 1 vs. year 2) influenced fresh and dry weights, with some interactions between fertilizer type, cultivar, and year of planting. Year 2 typically led to slightly lower fresh and dry weights compared to year 1. The interactions between these factors indicated that the choice of cultivar, fertilizer, and year of planting should be considered together to optimize amaranth growth.

Table 8: Effects of fertilizer application on stem girth and root length in three varieties of amaranth nine weeks after sowing (WAS)

Treatments	Stem girth (cm)		Root length (cm)	
	Year 1	Year 2	Year 1	Year 2
Variety (V)				
White Seeded	0.70	0.72	21.56	20.06
Black Seeded	0.61	0.68	19.36	17.32
Red Seeded	0.61	0.70	18.56	17.11
$Lsd_{Variety}$	0.1577		1.621	
$Lsd_{planting\ season}$	0.1288		1.324	
$LSD_{Variety\ x\ planting\ season}$	0.2231		2.293	
Fertilizer (F)				
Urine (1:3)	0.56	0.78	15.80	17.46
Urine (1:5)	0.51	0.60	15.72	15.64
NPK 15:15:15	0.65	0.74	20.73	17.49
Poultry Manure	0.89	0.83	27.68	3.31
Control	0.59	0.54	19.18	14.92
$Lsd_{Fertilizer}$	0.06272		1.831	
$Lsd_{planting\ season}$	0.03967		1.158	
$LSD_{Fertilizer\ x\ planting\ season}$	0.08870		2.590	

Table 9: Response of varieties of amaranth to fertilizer application based on fresh shoot weight, fresh root weight, dry shoot weight, and dry root weight nine weeks after sowing (WAS)

Treatments	FSWY1	FSWY2	FRWY1	FRWT2	DSWY1	DSWY2	DRWY1	DRWY2
Variety (V)								
White Seeded	330.67	567.10	126.67	80.30	59.11	104.94	35.21	31.36
Black Seeded	226.67	477.10	78.00	63.58	34.26	80.60	17.96	21.46
Red Seeded	212.00	583.98	71.33	71.33	34.90	92.92	13.37	23.62
$Lsd_{Variety}$	9.32		1.192		2.292		0.936	
$Lsd_{planting\ season}$	7.61		0.973		1.872		0.764	
$LSD_{Variety\ x\ planting\ season}$	13.17		1.686		3.242		1.324	
Fertilizer (F)								
Urine (1:3)	187.78	450.16	83.33	65.63	28.60	78.18	18.24	23.95
Urine (1:5)	182.22	489.70	55.56	69.64	28.44	86.69	14.01	26.12
NPK 15:15:15	236.67	702.27	105.56	79.61	37.27	114.21	21.80	26.82
Poultry Manure	413.33	782.74	151.11	99.36	69.03	127.67	42.71	33.27
Control	262.22	284.3	64.44	45.05	50.43	57.34	14.13	17.23
$Lsd_{Fertilizer}$	11.66		1.982		0.984		1.582	
$Lsd_{planting\ season}$	7.37		1.253		0.622		1.001	
$LSD_{Fertilizer\ x\ planting\ season}$	16.48		2.803		1.391		2.238	

FSWY1 = Fresh shoots weight in year 1; FSWY2 = Fresh shoots weight in year 2; FRWY1 = Fresh root weight in year 1; FRWY2 = Fresh root weight in year 2; DSWY1 = Dry shoots weight in year 1; DSWY2 = Dry shoots weight in year 2; DRWY1 = Dry root weight in year 1; DRWY2 = Dry root weight in year 2.

DISCUSSION

The results of this study provided valuable insights into the use of urine as a cost-effective fertilizer for enhancing *A. hybridus* growth and soil microbial health in tropical regions with a focus on Benin City, Nigeria. Our research had significant implications for sustainable agriculture. Urine could be a viable alternative to chemical fertilizers in tropical climates when properly managed.

Our results align with previous research, such as studies conducted by Sundin *et al.* (2007) and Pradhan *et al.* (2009), which demonstrated that urine is rich in essential nutrients like nitrogen, phosphorus, and potassium. This nutrient richness makes urine a valuable source of plant nutrients, reducing the need for expensive chemical fertilizers known for their adverse environmental impacts (Gordon *et al.*, 1993; Rose *et al.*, 2015). By demonstrating the effectiveness of urine-based fertilization, our study supported the shift towards more sustainable and eco-friendly agricultural practices.

The enhanced soil microbial diversity and activity observed in urine-treated plots emphasized the positive impact of urine on soil health. A diverse and active microbial community is crucial in nutrient cycling and soil fertility (Bremner & Mulvaney, 1982; Nelson & Sommers, 1982; Philippot *et al.*, 2013). Urine's ability to promote soil microbial communities contribute to sustainable soil management, a fundamental aspect of long-term agricultural productivity. These findings align with the studies of Germer *et al.* (2011); Akpan-Idiok *et al.* (2012; Hu *et al.*, 2020), which further highlighted the potential of urine-based fertilization to support soil health.

The variety-specific responses observed in our study underscore the importance of selecting appropriate amaranth varieties when using urine-based fertilizer. White-seeded amaranths, in particular, exhibited superior growth when treated with human urine. This highlights the need for farmers to consider variety selection in order to optimize the benefits of human urine

fertilization. By tailoring crop choices to the fertilizer type, farmers can maximize yields while maintaining soil health.

Our study also emphasized the importance of safe handling and application of urine as a fertilizer. Proper procedures are essential to mitigate health risks (Dedeke *et al*., 2011).
Acknowledging the limitations of our study, including its relatively short duration, is essential (Robinson & Sharpley, 2021). While our results are promising, long-term effects and potential nutrient accumulation or contaminants in the soil over extended periods require further investigations.

CONCLUSION

The study demonstrated the potential of urine-derived fertilizers for enhancing crop yields and supporting sustainable agricultural practices in Benin City, Nigeria. While the results are promising, further research is needed to address long-term effects, economic viability, and scalability concerns. Urine-derived fertilizers offer a sustainable alternative to synthetic fertilizers, reducing environmental impact and potentially providing economic benefits to farmers. Interdisciplinary efforts across agriculture, environmental science, and economics are essential to realize the full potential of this approach. By doing so, we can work towards a more sustainable and resilient future for agriculture, aligned with global goals for food security and environmental conservation.

The study recommends that farmers must be educated on the correct methods to ensure the safety and efficacy of urine-based fertilization practices; future research should focus on the sustainability and safety of urine-based fertilization practices over extended agricultural cycles. To fully harness the practical benefits of urine as a fertilizer in real-world agricultural settings, several considerations must be addressed. Economic factors, such as the cost-effectiveness of urine collection and processing, must be thoroughly examined. Scalability issues need to be evaluated to determine the feasibility of large-scale urine-derived fertilizer production and distribution. Additionally, understanding the acceptance and willingness of farmers to adopt this alternative fertilizer approach, considering regional and cultural variations, is crucial.

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