

Evaluation of the Growth and Foliage Yield of *Artemisia Annua* (Asteraceae) Grown with Varying Levels of Two Types of Organic Manure in Jos, Plateau State, Nigeria

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ABSTRACT

The evaluation of the growth and foliage yield of Artemisia annua was carried out in Jos Plateau State, Nigeria using varying levels of two types of organic manure from December 2015 to June 2016. Poultry droppings and Sheep droppings were mixed separately with soil in the potting ratio of 3:2:1 i.e loam, river sand and organic manures respectively and put in bags. The various manure levels added were 0t/ha (control), 10t/ha, 15t/ha, and 20t/ha respectively in each case. These combinations were replicated three times and laid out in the format of a Randomized Complete Block Design (RCBD) before the seedlings of Artemisia annua plant were transplanted into the bags. The differences within the means for the various parameters examined were largely not significant statistically, though the performance of plants treated with Sheep droppings were slightly better than those of Poultry droppings in almost all the parameters studied. It is recommended that either poultry droppings or sheep droppings could be used in the cultivation of A.annua and the best level for either of them is 10t/ha.

Keywords: *Artemisia annua, Organic manure levels, Foliage yield, Sweet annie*

INTRODUCTION

Malaria has posed a major health problem in many developing countries especially in Africa and South-east Asia Prabhakar et al. [1]. According to World Health organization, approximately 3.2 billion people are at risk of malaria. 214 million cases of malaria were reported in 2015 in 95 countries and more than 400,000 people died of it [2]. The first potent drug for malaria treatment was quinine, but Plasmodium falciparum has developed resistance against chloroquine and phadoxine/pyrimethamine [1]. Artemisinin which is isolated from the foliage of *Artemisia annua* is used in the production of Artemisinin Combination Therapy that treats uncomplicated *Plasmodium falciparum* malaria [2,3] reported that the genus *Artemisia* is among the largest and most widely distributed genera of the Asteraceae family. It comprise of over 450 diverse species of medicinal and aromatic plants. *Artemisia annua* is an annual, vigorous, aromatic herb reaching a height of about 1-3m and has antibacterial properties, natural pesticides and is used for the production of anti-malaria drugs. The plant has its origin from China and is distributed worldwide. It is naturalized in many countries like; Argentina, France, Austria etc. Its other common names are; Sweet wormwood, Annual wormwood, Sweet annie, Qing Hao [4]. Sweet annie has been used over 2000 years as a Traditional Chinese Medicine (TCM) for treating many ailments including malaria [5]. The active ingredients that are extracted from the plant are mainly artemisinin and essential oil. The essential oil however, is used as food additive; also in perfumery and as flavouring in spirits [4,5] reported that TuYouyou, a Chinese scientist was the first person to extract artemisinin from *A. annua* in the 1970's and distinguished its anti-malaria effect. Report from Nobelprize.org [6] stated that TuYouyou in her research discovered artemisinin mainly in the leaves of *A.annua*. Also, Mannam et al. [7] reported the highest concentration of artemisinin in the leaves of *A.annua* when compared with other parts of the plant. The artemisinin extracted from *A.annua* by Pharmaceutical companies is used in combination therapy with

some other drugs (Artemisinin Combination Therapies, (ACTs)) which are used in the treatment of malaria and some other ailments [8,3] stated that *A. annua* is the only plant that artemisinin can be extracted from. The problems posing the commercialization of the drugs are the expensive production methods and low yield of artemisinin by the plant [1,9] reported that the ACTs are safe and have quick cure for fever and parasitaemia.

Organic manure such as poultry droppings and sheep droppings are known to contain high percentage of Nitrogen 1.5% [10] and 1.4% [11] respectively. [12] discovered that for *A. annua* to attain its best growth and yield, it will require about 165kg/ha of NPK fertilizer or more. In view of this high quantity and cost of inorganic fertilizer required for its cultivation, there is need to explore other sources of nourishment which will provide equivalent high amount of nitrogen to the plant and also give quantitative and qualitative foliage yield. Poultry droppings and Sheep droppings may eventually provide a lot of nitrogen to the plant and make a possible replacement for the costly inorganic fertilizer. They are easily available and cheap to farmers due to the ever increasing number of both poultry and sheep farms unlike inorganic fertilizers. Organic manures help improve soil structure. They also encourage microbial activities that add nutrients to the soil which aids in plant nutrition. Since the artemisinin content of the plant is highest in the leaves there is need to find out the quantity of organic manure that can be used to produce *A. annua* with high foliage yield [7,1].

MATERIALS AND METHODS

The experiment was carried out from December, 2015 to June, 2016; at the nursery of School of Forestry adjacent to University of Jos, Plateau State; which is located at an altitude of 1,159 meters above sea level, 09°57'N and 08°53'E. The seeds of *Artemisia annua* obtained from a research farm belonging to Centre for Biotechnology and Genetic Engineering (CBGE), University of Jos, at Gangnim; Langtang-South Local Government Area of Plateau State, Nigeria; were sown in 1.5m wide, 2.0m long and 20.0cm high nursery bed that was located under shade by broadcast (scattering) method at the rate of 1g of seed per meter square. The soil was enriched with a mixture of poultry droppings and sheep droppings in order to ensure that the miniature seeds have adequate nourishment prior to broadcast. The nursery bed was mulched using grasses in order to conserve soil moisture and also to prevent insect attack. The nursery bed was subsequently watered twice a day morning and evening using a vapourizer EABL [13].

A portion of the soil used for the pots was sent to Department of Agronomy in Ahmadu Bello University, Zaria, Kaduna State-Nigeria; where it was subjected to physical and chemical analysis. The result of the analysis showed soil pH, H₂O, % Nitrogen, Sulphur, % Organic carbon, Potassium, Phosphorus, and exchangeable Na, K, and Ca²⁺ contents; also Mg²⁺, CaCl²⁻, CEC (Cation exchange capacity) and particle size distribution [14].

The soil materials collected were mixed with the organic manures which were collected from Poultry and Sheep farms in Jos separately in the potting ratio of 3:2:1, i.e. loam; river-sand; and organic manures respectively as suggested by Mathew and Karikari [15]. The manure used in this potting mixture was 50% of the overall manure for the separate treatments as basal dose. This was done by Prabhakar et al. [1].

The overall manure applications for the three different levels were as follows: 0 tons/ha: i.e 0kg/bag designated L₀; 10tons/ha: i.e 1.1kg/bag designated L₁; 15tons/ha: i.e 1.65kg/bag designated L₂ and 20tons/ha: i.e 2.2kg/bag as L₃ respectively. The two manures used were designated as follows: Poultry droppings as F₁; Sheep droppings as F₂, The treatment combinations for the manures and the manure levels were: F₀L₀ (control), F₁L₁, F₁L₂, F₁L₃ and F₂L₁, F₂L₂, F₂L₃, for Poultry and Sheep droppings respectively.

The above treatment combinations were replicated three times and the various bags were laid out in the format of a Randomized Complete Block Design (RCBD). Before transplanting was done, the nursery beds were moistened with water so that the lifting of the seedlings will be easy and also to ensure that the seedlings were lifted together with soil at the immediate root region; which will help ensure that the transplants easily adapt to the new environment. The bags containing the soil manure mixture in their various combinations were irrigated before transplanting. A hole of 9-12cm was made in each bag with a hoe and the seedlings approximately 10-13cm tall were transplanted to the bags. They were watered immediately after transplanting and subsequently on daily basis. Weeds were removed from time to time with hands in order to reduce the incidence of diseases and pests and also to reduce the rate of competition for nutrients between *A. annua* plants and the weeds. Different growth parameters were measured on each plant starting from 5WAT (weeks after transplanting) and subsequently at an interval of 2 weeks till 21WAT. Data were collected on each parameter and recorded as follows:

Plant Height (cm)

The plant height was obtained by measuring each plant stand from the base of the plant at ground level to the apex of the plant with a flexible measuring tape.

Number of Branches Per Plant

The number of branches per plant were counted and recorded for each plant stand.

Stem Girth Per Plant (cm)

The stem girth of each plant was obtained by measuring the circumference of each plant close to the base of the plant, using a white thread (which was later spread on a metre rule) and the reading of the length was recorded Rahman et al.[16].

Plant Foliage Diameter (cm)

The diameter of each of the plant foliage was measured by placing a flexible tape horizontally at the widest part of the plant and the readings recorded.

Volume of Foliage Per Plant (cm³)

The volume of foliage was calculated using the formula; $V = \frac{1}{3} \pi r^2 h$. Where, ' π ' (pie)=22/7, ' r '=radius (half of the plant foliage diameter) and ' h ' is the height of the plant. The above was calculated for each of the plant in the 3 replicates for the various treatments and recorded.

Fresh and Dry Weight of Leaves at Harvest (g)

Fresh weight of leaves for each of the plant at harvest was recorded. The leaves were later dried under shade and average dry weight was also calculated and recorded.

Correlations

Correlation coefficient (r) was calculated which was used to compare the parameters.

Phytochemical Analysis

The leaves of the plant treated with Poultry and sheep droppings respectively and also control (no treatment) were harvested, weighed and labeled. They were also dried under shade, until a constant weight was obtained; and their various weights were recorded. The dried leaves of *A. annua* were blended into powder form and subjected to phytochemical screening at the toxicology laboratory of the Department of Biochemistry, National Veterinary Research Institute, Vom, Plateau State in order to identify the compounds contained in the leaves. The chemical tests were carried out with aqueous extracts and Organic solvents. Standard qualitative procedures used for the test were that described by Senguttuvan et al. [17].

The leaves of *Artemisia annua* were screened for the following constituents: Alkaloids, Tannins, Saponins, Cardiac glycosides, Steroids, Terpenes, Flavonoids, and Anthraquinone.

Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) to test the treatment effects for significance at 95%/99% confidence limit using the 'F' Test as described by Snedecor and Cochran [18]. Significant mean difference were tested using Least Significant Difference (LSD) [19].

RESULTS

Table 1 Shows the effect of varying levels of Poultry and Sheep droppings on the mean plant height of *Artemisia annua* at 5, 7, 9, 11, 13, 15, 17, 19 and 21WAT.

Table1: Plant Height of *Artemisia annua* as affected by varying levels of Poultry and Sheep droppings (organic manures) at 5,7,9,11,13,15,17,19,and 21WAT in Jos , Plateau State.

Mean Plant height (cm)									
Manure(M)	5WAT ¹	7WAT	9WAT	11WAT	13WAT	15WAT	17WAT	19WAT	21WAT
PD	46.1	59.0	65.2	79.4	93.6	109.1	117.5	124.5	125.2
SD	47.2	63.5	69.3	80.5	96.0	112.1	120.1	128.1	128.9
LSD	-	-	-	-	-	-	-	-	-
Levels (L)									
0t/ha	43.4b ²	54.6	61.2	70.1	79.8	92.5	102.5	113.2	113.2
10t/ha	43.8b	59.1	67.2	80.0	90.9	107.3	116.6	124.1	125.1
15t/ha	43.5b	60.4	64.9	78.7	94.5	112.6	124.4	127.2	127.5
20t/ha	52.6a	64.3	69.6	81.1	99.1	112.0	120.8	127.7	128.6
LSD	7.28	-	-	-	-	-	-	-	-
Interaction									
MxL	NS	NS	NS	NS	NS	NS	NS	NS	NS

1-WAT: Weeks after Transplanting, PD: Poultry Droppings, SD: Sheep Droppings, LSD: Least Significant Difference, NS: Not Significant, 2: Means followed by the same letter(s) within the same column and treatments are not significantly different at 5% level of probability using LSD, a and b are ranking of means using the Least Significant Difference, MxL: Interaction between manure types and manure levels.

The differences in the mean plant height, due to manure type were not statistically different in all the stages of growth. The differences in the mean plant height due to varying levels of organic manure were statistically significant ($p=0.05$) only at 5WAT stage of growth, where, 20t/ha recorded significantly taller plants than 15t/ha, 10t/ha and 0t/ha levels which were statistically the same.

The mean number of branches per plant of *Artemisia annua* as affected by manure types and varying levels of manure (poultry and sheep droppings) at 5, 7, 9, 11, 13, 15, 17, 19 and 21WAT is given in Table 2.

Table 2: Number of branches per plant of *Artemisia annua* as affected by varying levels of Poultry and Sheep droppings (organic manures) at 5,7, 9,11, 13,15,17,19,and 21 WAT1 in Jos, Plateau State.

Mean Number of Branches Per Plant									
Manure(M)	5WAT ¹	7WAT	9WAT	11WAT	13WAT	15WAT	17WAT	19WAT	21WAT
PD	24.0b ²	30.8	34.6	36.6	39.7	44.0	46.4	49.3	50.3a
SD	27.0a	32.4	35.1	37.3	39.7	42.2	44.0	46.2	48.1b
LSD	2.84	-	-	-	-	-	-	-	1.91
Levels (L)									
0t/ha	22.0b	30.0b	32.3	34.2	35.1	39.3	41.8	44.4	47.5
10t/ha	21.2b	29.3b	32.5	34.8	37.8	41.0	43.5	46.3	47.8
15t/ha	26.7a	32.3ab	35.8	37.7	40.5	44.3	46.2	48.5	50.0
20t/ha	28.7a	33.2a	36.2	38.3	40.7	44.0	46.0	48.5	49.8
LSD	3.48	3.14	-	-	-	-	-	-	-
Interaction									
MxL	NS	NS	NS	NS	NS	NS	NS	NS	NS

1: WAT-Weeks after Transplanting, PD: Poultry Droppings, SD: Sheep Droppings, LSD: Least Significant Difference, NS: Not Significant, 2: Means followed by the same letter(s) within the same column and treatments are not significantly different at 5% level of probability using LSD, a and b are ranking of means using the Least Significant Difference, MxL: Interaction between manure types and manure levels.

The differences due to the different manure types were significant ($p=0.05$) only at 5 and 21 WAT growth stages. At the 5WAT stage, the sheep droppings produced significantly higher number of branches than the poultry droppings while the reverse was the case at 21WAT stage.

The differences in the mean number of branches due to the varying levels of manure were significant ($p=0.05$) only at the early growth stages (5 and 7 WAT). At these growth stages, the 20t/ha produced significantly higher number of branches and was statistically the same as the 15t/ha level. 20t/ha manure level also recorded the highest number of branches at 7 WAT stage and it was statistically the same with 15t/ha level. Table 3 shows the effect of manure type and varying levels of organic manure on stem girth per plant.

Table 3: Stem girth per plant of *Artemisia annua* as affected by varying levels of Poultry and Sheep droppings (organic manures),at 5,7,9,11,13,15,17,19,and 21WAT¹ in Jos, Plateau State.

Mean Stem Girth Per Plant (cm)									
Manure(M)	5WAT ¹	7WAT	9WAT	11WAT	13WAT	15WAT	17WAT	19WAT	21WAT
PD	3.22b ²	4.13	4.49	4.87	5.41b	6.22b	6.94b	7.47b	7.60b
SD	3.88a	4.44	5.11	5.53	6.31a	7.62a	8.12a	8.52a	8.47a
LSD	0.57	-	-	-	0.74	0.64	0.55	0.52	0.42
Levels (L)									
0t/ha	3.40b	4.61a	4.93	5.20	5.52	6.23	6.75	7.14	7.58
10t/ha	3.27b	4.20ab	4.47	5.10	5.59	6.69	7.42	7.87	7.85
15t/ha	3.18b	4.03b	4.68	5.08	5.70	6.89	7.50	7.92	8.02
20t/ha	4.20a	4.63a	5.30	5.42	6.30	7.20	7.69	8.20	8.23
LSD	0.70	0.45	-	-	-	-	-	-	-
Interaction									
MxL	NS	NS	NS	NS	NS	NS	NS	NS	NS

1: WAT- Weeks after Transplanting, PD: Poultry Droppings, SD: Sheep Droppings, LSD: Least Significant Difference,NS: Not Significant, 2: Means followed by the same letter(s) within the same column and treatments are not significantly different at 5% level of probability using LSD, a and b are ranking of means using the Least Significant Difference, MxL:Interaction between manure types and manure levels.

The differences in stem girth per plant due to manure types were significant ($p=0.05$) at 5 and 13WAT stages of growth. There were also significant differences ($p=0.01$) at 15, 17, 19 and 21 WAT stages of growth. At all these growth stages, the sheep droppings produced significantly thicker stems than the poultry dropping. For the manure levels, there were significant differences ($p=0.05$) only at 5 and 7WAT stages of growth. At these stages of growth, 20t/ha recorded the thickest stem girths per plant. At 7WAT growth stage, 0t/ha and 20t/ha produced the thickest stem girths and were statistically at par with 10t/ha level.

The effect of different manure types and varying levels of organic manure (poultry and sheep droppings) on the diameter of plant foliage is given in Table 4.

Table 4: Plant Foliage Diameter per plant of *Artemisia annua* as affected by varying levels of Poultry and Sheep droppings (organic manures) ,at 5,7,9,11,13,15,17,19,and 21WAT¹ in Jos, Plateau State.

Mean Plant Foliage Diameter(cm)									
Manure(M)	5WAT ¹	7WAT	9WAT	11WAT	13WAT	15WAT	17WAT	19WAT	21WAT
PD	34.9	46.9	53.2	58.6	64.5	70.8b ²	77.8b	89.7b	89.5b
SD	41.4	51.1	56.2	63.1	68.8	79.5a	87.4a	99.4a	99.2a
LSD	-	-	-	-	-	7.70	8.55	9.17	9.02
Levels (L)									
0t/ha	35.8	46.5b	46.8b	50.3c	58.6	68.2	74.8	83.1	85.7
10t/ha	32.9	45.2b	52.6b	58.0b	64.3	73.9	82.0	95.4	95.1
15t/ha	37.1	45.2b	50.0b	58.4b	66.5	77.3	84.5	95.4	95.2
20t/ha	44.5	54.6a	61.6a	66.2a	69.1	74.2	81.4	93.0	92.7
LSD	-	7.46	7.29	6.04	-	-	-	-	-
Interaction									
MxL	*	NS	NS	NS	NS	NS	NS	NS	NS

1: WAT-Weeks After Transplanting, PD: Poultry Droppings, SD: Sheep Droppings, LSD: Least Significant Difference,NS: Not Significant, *: Significant at 5% level of probability, 2:Means followed by the same letter(s) within the same column and treatments are not significantly different at 5% level of probability using LSD,a and b are ranking of means using the Least Significant Difference, MxL:Interaction between manure types and manure levels.

Significant differences in mean foliage diameter, at 5% level of probability were recorded at 15, 17, 19, and 21WAT due to manure types. At these stipulated stages of growth, sheep droppings produced significantly higher foliage diameter than poultry droppings. The increase in plant foliage was higher with sheep droppings throughout the growth stages of the experiment.The differences in the diameter of plant foliage due to the varying manure levels were significant ($p=0.05$) at 7, 9, and 11WAT stages of growth where 20t/ha recorded statistically higher foliage diameter; 0t/ha, 10t/ha and 15t/ha produced lesser foliage diameter and were statistically at par. While at 11WAT growth stage, 20t/ha produced highest foliage diameter followed by 15t/ha and 10t/ha which were statistically the same. 0t/ha

however recorded the least foliage diameter. Here also, there were steady increases in the diameter of plant foliage all through the period of experiment.

Significant interactions between manure types and varying manure levels at 5% level of probability were recorded only at 5WAT stage of growth in only plant foliage diameter.

Table 5: Interaction between manure Types and manure Levels on Foliage diameter of *Artemisia annua* plant at 5 WAT1.

Interaction Effects of Manure and Manure Levels			
	10t/ha	15t/ha	20t/ha
PD	24.4e ²	31.5bcde	48.9a
SD	41.3abc	42.7ab	40.1abcd
LSD	14.1	-	-

1-WAT: Weeks after Transplanting, 2:Means followed by the same letter(s) within the same column and treatments are not significantly different at 5% level of probability using LSD,PD : Poultry Droppings,SD : Sheep Droppings,LSD: Least Significant Difference. a , b,c,d and e are ranking of means statistically, using the Least Significant Difference(LSD).

Table 5 shows the details of the interaction between manure type and the varying levels of manure on the mean plant foliage diameter at 5WAT.The foliage diameter for the poultry droppings continued to increase as the manure concentration increased. For the sheep droppings however, the foliage diameter increased from 10t/ha to 15t/ha level but dropped at 20t/ha manure level. When the different manure types were considered against the different manure levels, the sheep droppings recorded significantly higher foliage diameter with 10t/ha and 15t/ha levels of manure but it was lower at the 20t/ha level. The effect of varying levels of organic manure on the volume of plant foliage is shown in Table 6.

Table 6: Volume of Foliage of *Artemisia annua* as affected by varying levels of Poultry and Sheep droppings (organicmanures) ,at 5,7,9,11,13,15,17,19,and 21WAT¹ in Jos, Plateau State.

Mean Volume of plant foliage(m ³)									
Manure(M)	5WAT ¹	7WAT	9WAT	11WAT	13WAT	15WAT	17WAT	19WAT	21WAT
PD	0.02	0.04	0.05	0.07	0.10	0.15	0.19	0.26	0.26
SD	0.02	0.04	0.06	0.07	0.12	0.19	0.24	0.34	0.34
LSD	-	-	-	-	-	-	-	-	-
Levels (L)									
0t/ha	0.01b ²	0.03b	0.03c	0.05b	0.07	0.11	0.15	0.21	0.22
10t/ha	0.01b	0.03b	0.05b	0.06b	0.10	0.16	0.21	0.31	0.62
15t/ha	0.02b	0.04b	0.04b	0.07ab	0.11	0.18	0.23	0.31	0.62
20t/ha	0.03a	0.05a	0.07a	0.09a	0.13	0.17	0.21	0.29	0.59
LSD	0.01	0.02	0.02	0.03	-	-	-	-	-
Interaction									
MxL	NS	NS	NS	NS	NS	NS	NS	NS	NS

1: WAT-Weeks after Transplanting, PD: Poultry Droppings, SD: Sheep Droppings, LSD: Least Significant Difference,NS: Not Significant, 2: Means followed by the same letter(s) within the same column and treatments are not significantly different at 5% level of probability using LSD,a and b are ranking of means using the Least Significant Difference, MxL:Interaction between manure types and manure levels.

There were no statistically significant differences on the mean volume of foliage due to manure types all through the stages of growth. The differences in the mean volume of plant foliage due to the varying levels of manure were significant (p=0.05) only at 5, 7, 9 and 11WAT stages of growth. At these growth stages, 20t/ha produced plants with the highest foliage volume.

Table 7 shows the effect of manure types and varying levels of poultry and sheep droppings on the mean number of days between transplanting and date of first flowering. There was no significant difference due to manure types on the mean number of days to first flowering. Also, no significant differences were recorded due to the varying levels of organic manure.

Table 7: Number of days to first flowering of *Artemisia annua* as affected by varying levels of Poultry and Sheep droppings (organic manures) in Jos, Plateau State:

Mean Number of Days Between Transplanting and Date of First Flowering	
Manure(M)	NODFF ¹
PD	97.2
SD	102.7
LSD	-
Levels (L)	
0t/ha	78.3
10t/ha	105.0
15t/ha	100.4
20t/ha	94.5
LSD	-
Interaction	
MxL	NS

1-Number of days to first flowering, PD: Poultry Droppings, SD: Sheep Droppings, LSD: Least Significant Difference, NS: Not Significant. MxL:Interaction between manure types and manure levels.

The effect of manure types and varying levels of organic manure on the yield (fresh and dry weight of leaves) is given in Table 8.

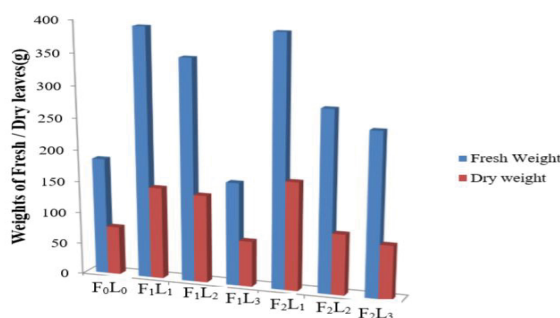
Table 8: Fresh and dry weight of leaves(g) per plant of *Artemisia annua* as affected by varying levels of Poultry and Sheep droppings (organic manures)in Jos, Plateau State:

Mean Fresh and Dry Weight of Leaves at Harvest (g)		
Manure(M)	Fresh Weight	Dry Weight
PD	301	119
SD	311	123
LSD	-	-
Levels (L)		
0t/ha	185b ¹	76.9
10t/ha	392a	158
15t/ha	317ab	118
20t/ha	210b	79.0
LSD	143	-
Interaction		
MxL	NS	NS

1- Means followed by the same letter(s) within the same column and treatments are not significantly different at 5% level of probability using LSD, PD: Poultry Droppings, SD: Sheep Droppings, LSD: Least Significant Difference,NS:Not Significant, MxL :Interaction between manure types and manure levels.

There were no significant differences on the mean fresh and dry leaf weight due to manure types. There were significant differences ($p=0.05$) on the fresh weight of leaves due to the varying level of manure. 10t/ha manure level recorded significantly higher fresh weight and was statistically at par with 15t/ha than 0t/ha and 20t/ha which produced statistically the same fresh weight at harvest. No significant differences were recorded in the dry weights of leaves due to varying levels of organic manure.

Figure 1 shows the histogram presentation of the combined effects of manure types and varying levels of organic manure on the mean fresh weight and dry weight of leaves per plant at harvest. 10t/ha poultry droppings recorded the highest fresh weight of leaves, this was followed by 10t/ha sheep droppings, then 15t/ha poultry droppings, 15t/ha sheep droppings, 20t/ha sheep droppings, 0t/ha (control) and lastly 20t/ha poultry droppings recorded the least weight of fresh leaves. The dry weight of leaves showed a similar pattern with fresh leaf weights.



F₁: Poultry droppings, F₂: Sheep droppings, L₁: 10t/ha; 1.1kg/bag, L₂: 15t/ha; 1.65kg/bag, L₃: 20t/ha; 2.2kg/bag.

Figure 1: Combined effects of manure types and manure levels on Fresh and Dry weight (g) of leaves per plant.

Correlations

The correlation coefficients between leaf yield and some parameters and among parameters are presented in Table 9. Slightly positive and insignificant correlation occurred between fresh weight of leaves, number of days to first flowering and volume of plant foliage; while plant height, number of branches per plant, stem girth per plant and diameter of plant foliage had negative correlations with leaf yield. Plant height had slightly positive and insignificant correlation with number of branches per plant, stem girth per plant, plant foliage diameter, volume of plant foliage and number of days to first flowering. The correlation between number of branches per plant, stem girth per plant, plant foliage diameter, volume of plant foliage and number of days to first flowering is negative. Positive correlations exist between stem girth per plant, plant foliage diameter and number of days to first flowering while there is a negative correlation between stem girth per plant and volume of plant foliage. There is a positive correlation between diameter of plant foliage and number of days to first flowering while that between diameter of plant foliage and volume of foliage is negative. Positive correlations exist between volume of plant foliage and number of days to first flowering.

Phytochemical Screening of *A. annua* leaves.

The chemical components of crushed dry leaves from *Artemisia annua* plant is shown in Table 10. Results from the screening showed the presence of Alkaloids, Tannins, cardiac glycosides, Steroids, Terpenes, flavonoids in the leaves of plants treated with poultry droppings, sheep droppings and control. Anthraquinone was found negative in the leaves of plant treated with poultry manure, sheep manure and control.

Table 9: Correlation analysis of quantitative characters of *A. annua* evaluated with different treatments.

Sr. No.	Quantitative Characters	1	2	3	4	5	6	7	8
1	fresh weight of leaves	1							
2	Dry weight of leaves	0.070 ^{NS}	1						
3	Plant height	-0.001 ^{NS}	0.0003 ^{NS}	1					
4	Number of branches per plant	-0.003 ^{NS}	-0.001 ^{NS}	0.0003 ^{NS}	1				
5	Stem girth per plant	-0.006 ^{NS}	-0.00003 ^{NS}	0.0014 ^{NS}	-0.043 ^{NS}	1			
6	Diameter of foliage per plant	-0.0001 ^{NS}	-0.003 ^{NS}	0.001 ^{NS}	-0.001 ^{NS}	0.004 ^{NS}	1		
7	Volume of plant foliage	0.002 ^{NS}	0.013 ^{NS}	0.005 ^{NS}	-0.003 ^{NS}	-0.045 ^{NS}	-0.218 ^{NS}	1	
8	Number of days to first flowering	0.006 ^{NS}	0.013 ^{NS}	0.001 ^{NS}	-0.001 ^{NS}	0.003 ^{NS}	0.004 ^{NS}	0.015 ^{NS}	1

NS: Not Significant.

Table 10: Phytochemical components of *Artemisia annua* leaves from plants transplanted in bags.

Phytochemical Components	Poultry Droppings	Sheep Droppings	Control
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Cardiac glycosides	+	+	+
Steroids	+	+	+
Terpenes	+	+	+
Flavonoids	+	+	+
Anthraquinone	-	-	-

+: indicates presence of compound, -: indicates absence of compounds.

DISCUSSION AND CONCLUSION

Effect of Manure Types

The differences between the means for the two manure types were largely not significant for plant height, number of branches, foliage diameter and foliage volume probably because there were no remarkable differences in the nitrogen content of the two as reported by Foreman and Long [10]. They gave the nitrogen content of poultry droppings as 1.5% while Schoenian [11]; gave the nitrogen content of sheep droppings as 1.4%. The values recorded for various parameters in this work are comparable with those recorded for results of works done by Prabhakar et al. [1,8] who reported that organic manures release their nutrients to the plants slowly but steadily. There were steady increases in the growth rates over the period of experiment because, it has been shown in the earlier studies that the addition of organic matter improves soil properties such as aggregation, water holding capacity, bulk density, the degree of compaction, fertility and resistance to water and soil erosion. These soil factors positively affect the growth and development of plant roots and shoots [20-22].

Moreover, organic manure may be helpful in conserving soil moisture and lowering the soil temperature, resulting in absorption of more water and nutrients by the plants from soil [23-25].

There were significant differences in the mean stem girth between the two manure types mainly at the later stages of growth. This may be because, the plants were obtaining better nourishment which made the differences in the stem girth to be more pronounced.

The position of the slight superiority of the sheep dropping manure over the poultry droppings was first indicated in the number of days between transplanting and date of first flowering Table 7. The mean number of days after transplanting to first flowering for poultry droppings was 97.2 as against that of sheep which was 102.7; while that of control was 78.3. *Artemisia annua* has been shown to delay flowering for a longer period where it receives good nourishment [12]. Secondly the results of fresh and dry weight of leaves where the sheep droppings had higher weight than the poultry droppings as shown in Table 8. These slight superiorities may be because of the pellet-like nature of the sheep droppings which makes the release of the nutrients from them to be slower than those of poultry droppings which are granular.

Effects of Manure Levels

The performance of 20t/ha level of manure recorded the highest plant height at all stages of growth in the experiment. This could be because of the high level of nutrient available to the plant that promotes cell division and cell enlargement resulting in better plant height as reported by Gandhi [26].

The number of branches and foliage diameter at all stages of the experiment increased from 0t/ha, to 10t/ha, 15t/ha and 20t/ha in that order. These results conform to the findings of Ferreira et al. [27] who reported that providing nitrogen fertilizer leads to improved physiological activities of the plant causing the plant to branch out.

The mean stem girth per plant within the different manure levels was significantly different only at the early stages of growth Table 3. This is because increase in stem girth is a very slow aspect of plant growth that involves cell division and enlargement of the vascular bundles and that could be why the mean differences at the later growth stages which are likely to be much slower were not significantly different. In spite of this however, the 20t/ha application produced the thickest stems at all stages of growth likely because of the availability of more nitrogen to the plant.

Data on foliage volume, Table 6 showed that the 20t/ha manure level produced plants with the largest mean volumes per plant. This is to be expected because, the tallest plant, the highest number of branches and the widest plant diameter were recorded at this 20t/ha level. Ordinarily, plants with the largest volume would probably have been expected to produce the heaviest fresh and dry weight of leaves. This is not so in this experiment because, the voluminous plants which were tall, and had wide and high number of branches also had air trappings within the branches and leaves. This is the plausible reason why, though the 20t/ha level had the most voluminous plants, they did not produce the heaviest fresh and dry weight of Leaves.

The heaviest fresh and dry weight of leaves was recorded at 10t/ha level which is the lowest of all the three levels. This is in agreement with the work of Yeboah et al. [8] who found that moderate application of poultry manure gives high leaf yield. In addition, this result also conforms to the work of Khalid and Shafei [28].

Correlations

The highest positive correlation as presented in Table 9 was recorded between fresh weight of leaves and dry weight of leaves. Other correlation coefficients show positive correlation between dry weight of leaves, number of branches, stem girth, diameter of plant foliage, Volume of plant foliage and plant height. The above results are in agreement with work of Dharm *et al.* [29] who reported that positive correlation existed between plant growth traits and dry leaf yield of *Artemisia*. Analysis of the data also show that fresh weight of leaves, dry weight of leaves, plant height, stem girth, diameter of plant foliage, and volume of plant foliage have positive correlation with number of days to first flowering; this agrees with the work of Utoblo and Ajala [12] who reported that providing good nourishment enhances growth and delays flowering for a longer period in *Artemisia annua*. The positive correlations recorded between these parameters in this work suggest the influence of growth parameters on the yielding ability of *A. annua*.

Phytochemical Screening of *A.annua* leaves

There was no remarkable difference in the phytochemical analysis of the leaves of plants cultivated with varying levels of both manure types and control Table 9; probably because, the *A. annua* seeds which were sewn on the nursery bed are of the same species and source which implies that the presence or absence of phytochemicals is heritable.

Interactions

There were a very large number of interactions that were not significant between the two main variables (manure and level) in this research. This indicates that there were no differences in the combined forces of the data collected. This agrees with the report of Dhaliwal and Sahota [30] that interactions are the tendency of one factor to have a different effect at different levels of another factor. The only instance of a significant interaction was at the 5WAT stage of foliage diameter parameter. A detailed display of this interaction (Table 5) exposes how incomplete the interpretation of the main effect. It can be concluded that, from the performance of the various parameters measured in this study, either the poultry droppings or sheep droppings will give a high foliage yield when used for the cultivation of *Artemisia annua*. Sheep droppings are slightly better suited for use than poultry droppings and the best level for use in this research is the 10t/ha level for either of them. Poultry droppings or sheep droppings are therefore recommended for use in the cultivation of *Artemisia annua* in this ecological region.

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