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Full Length Research Paper

Organic and inorganic fertilizers application on the growth and yield of *Artemisia annua L.* in the humid tropics of Ghana

Yeboah, S.*, Akromah, R. and Quansah, C.

Department of Plant and Soil Sciences, College of Agriculture and Natural Resources, KNUST, Kumasi, Ghana.

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A study was conducted at the Agriculture Research Station, Anwomaso during the 2008 major growing season to evaluate the effect of organic (poultry manure) and inorganic (chemical) fertilizers on the growth and yield of *Artemisia annua*. The experiment also examined the effects of variation in the stage of harvest on artemisinin yield. The experiment was arranged in a randomized complete block design with three replications and six treatments. The treatments were 0, 45 and 90 kg N/ha compound fertilizer and poultry manure at 2, 4 and 6 tons/ha. Data were collected on: plant height, canopy spread, stem width, number of branches per plant, fresh and dry leaf yield (kg/ha), crude extract weight (g), artemisinin content (%) and artemisinin yield (kg/ha). The results showed that 4 t/ha poultry manure treatment was effective and gave the highest fresh and dry leaf yield. The results indicated that the treatments significantly affected most of the parameters when compared to the control. Application of 4 t/ha poultry manure gave the highest artemisinin yield of 9.57 and 37.24 kg/ha at both stages of harvest respectively. Leaf yield positively correlated with artemisinin yield. Artemisinin content recorded at preflowering was higher than values recorded at full bloom. From the results, it is recommended that application of 4 t/ha poultry manure can enhance artemisinin production.

Key words: Medicinal plants, *Artemisia annua* L., Asteracea, Artemisinin, poultry manure, chemical fertilizers.

INTRODUCTION

Medicinal and aromatic plants are economically of paramount importance. This is because of the continuous and increased demand for their products from local and foreign markets (Abbiw et al., 2002; WHO, 2003). There is therefore, need for a new strategy for developing these medicinal plants as commercial plants. Artemisia is one of the most important medicinal plants in this area of research of developing new strategies for medicinal plant production. *Artemisia annua* is a plant for the production of anti-malarial and anti-bacterial agents and natural pesticides. Artemisinin, the secondary compounds of interest in the plant is mostly found in the leaves, inflorescence and the seeds and less in the stem (Ferreira and Janick, 1996). The plant is currently processed by pharmaceutical firms for the production of artemisinin and its derivatives for Artemisinin-Based Combination Therapies (ACTs) in the treatment of malaria (Ferreira et al., 2005). The artemisinin compounds are effective against *Plasmodium falciparum* and *Plasmodium vivax*, including multidrug-resistant strains (Ferreira et al., 2005). Malaria contributes substantially to the poor health situation in Africa. Sub-Saharan Africa accounts for 90% of the world's 247 million cases and 1 million deaths annually and 20% of all childhood deaths (WHO, 2008).

In Ghana, malaria is the major cause of morbidity accounting for 40 to 60% of outpatients (WHO, 2003). The recommendation by WHO for the use of Artemisinin-Based Combination Therapy as the first line treatments for multidrug – resistance strains of malaria has led to an increase in demand for artemisinin. The anti-malaria compound remains expensive and hardly available on the global scale (Yadav et al., 2003). Field production of *A.*

*Corresponding author. E-mail: proyeboah@yahoo.co.uk. Tel: (+233) 0243 263385.

annua is recommended as the only commercially viable method to produce artemisinin since the total chemical synthesis of the molecule is complex and uneconomical (Yadav et al., 2003). Therefore, agronomic practices that seek to increase leaf biomass will influence positively on the yield of the active ingredient per unit area. The artemisinin content is highly concentrated in the leaves (Laughlin et al., 2002). Soil amendments such as nitrogen fertilizers are reported to increase the total leaf biomass and thereby increased artemisinin obtained from *A. annua* (Simon et al., 1990). Applications of poultry manure at different combinations result in significant increase in leaf biomass production (Singh and Rao, 2009), but these have not been evaluated in Ghana. Accurate determination of optimum levels of manure and compound fertilizer is important to avoid low or excessive application. The study determined the effects of different levels of poultry manure and compound fertilizer on the growth and yield of *A. annua* L., and to determine the optimum stage of harvesting for high artemisinin content.

MATERIALS AND METHODS

Description of study site

The study was conducted during the major raining season of 2008 at the Kwame Nkrumah University of Science and Technology, Agricultural Research Station, Anwomaso, in the semi-deciduous forest zone in Kumasi. The site lies between latitude 06°. 43°N and longitude 01°. 36°W of the Greenwich meridian. The soils, ferric acrisol belong to the Asuansi series with about 5 cm thick top layer of dark grey gritty sandy loam (FAO, 1984).

Sowing and plant husbandry practices

One *A. annua* accession obtained from Rutgers University, Washington, USA was grown for field and laboratory evaluation. The seeds were nursed in sterilized topsoil in seed boxes. The seedlings were pricked out into plastic pots filled with sterilized soil at 30 days after germination. The seedlings were moved outdoors for hardening by gradually exposing them to sunlight while reducing watering at 70 days after germination. The seedlings were transplanted at 80 days after emergence to the field, which had been slashed, ploughed, harrowed and demarcated into plots. Twenty seedlings were transplanted per plot. There were four rows per plot and inter and intra row spacing were 1.5 ×1.0 m, respectively.

An auger was used to take soil samples at random on each experimental plot at depths of 0 to 20 and 20 to 40 cm before planting in accordance to Anderson and Ingram (1998). A sizeable quantity of composite soil samples were air-dried and sieved through a 2 mm mesh and subjected to physical and chemical analysis. The soil was analysed for pH, organic carbon, total nitrogen, potassium, phosphorus, exchangeable K, Na and Ca contents, dry bulk density and particle analysis.

Experimental design and treatments

Randomized complete block design (RCBD) with three replications was used for the study. The treatments were compound fertilizer (NPK-15-15-15) applied at a rate of 0, 45 and 90 kg N/ha, and organic fertilizer (poultry manure) applied at a rate of 2, 4 and 6 t/ha. The amendments were applied at 14 days after transplanting. Each treatment was randomly assigned to a plot measuring 5.0 x 4.5 m which was separated by a furrow of 1.8 m. Supplementary irrigation was provided through 1.5 L of Voltic plastic bottles fitted into the soil near the plant.

Data collection

Data were collected weekly on plant height; plant spread and stems width from the 4 to 12th week after transplanting from each of the six non-border plants. Main branches were also counted. Three plants from each plot were harvested at two different schedules. The first harvest was done prior to flowering (104 days after planting) and the second sample was harvested at full bloom (134 days after planting). At harvest, fresh and dry weights of leaf were obtained by using an electronic weighing scale from Mettler-Toledo Ltd of Switzerland. Artemisinin yield per hectare were recorded on dry leaf basis. The sampled plants were dried under shade for 7 days. The harvested plants were stripped to obtain the leaves and the flowers. These were thoroughly dried to obtain the biomass.

Laboratory analysis and extraction

The dried biomass was milled using milling machine from Fritsch Puloerisette of Germany. Crude extracts from the samples were extracted using batch percolation method with petroleum ether as the solvent. A liquid chromatography–mass spectrometry (LC-MS) method with selected ion monitoring (SIM) was developed and validated for the analysis and standardization of artemisinin in the treatments at the laboratory of Rutgers University, USA.

Data analyses

The data were subjected to statistical analysis using Genstat (2005) to obtain the analysis of variance for the growth and yield parameters of *A. annua*. The least significant difference (LSD) test at 5% was used to separate the means.

RESULTS AND DISCUSSION

The results of physical and chemical analyses of the soil at the experimental site before imposition of treatments are presented in Table 1. The soil pH was slightly acidic at both depths. Soil organic carbon, total nitrogen and potassium contents were low and the levels of available phosphorus ranged from medium to high at both depths. Exchangeable K, Na and Ca values were below the critical values of 0.6, 1.0 and 10 cmol/kg respectively (Landon, 1991). The data showed high values for dry bulk density. The slightly low pH values of soil were similar to those reported for some Ghanaian soils by Adu and Tenadu (1979). The soil was deficient in nitrogen and therefore application of nitrogen fertilizer tended to reduce this limiting factor of growth. Khalid and Shafei (2005) reported similar results. The soil property of the study site is attributed mainly to the excessive leaching of the soils caused by high rainfall prevelant in the humid rainforest in Ghana and constant plant nutrient uptake by the plants with no replenishment.

Table 1. Mean chemical and physical properties of soil at the experimental site before application of treatments.

Soil depth (cm)	pH (H_2O)	Total N (%)	Organic carbon (%)	Exchangeable basic cations (cmol/kg)					Dry bulk density (g/cm)	Particle size (%)		
				P (ppm)	Ca	Mg	K	Na		Sand	Silt	Clay
0-20	6.15	0.16	1.78	20.54	4.53	2.93	0.12	0.39	1.49	78.8	8.0	13.2
20-40	5.98	0.12	1.07	16.57	4.26	2.93	0.17	0.49	1.40	76.1	4.66	19.2

Table 2. Means of maximum plant height, plant canopy spread, stem width (cm) and number of branches per plant.

Treatment	Plant height	Plant spread	Stem width	Number of branches/plant
0 kg N/ha	112.0 (6.35)^{b}	93.2 (7.74)^{b}	5.31 (0.64)^{b}	35.4 (0.68)^{b}
45 kg N/ha	142.9 (7.38)^{a}	121.8 (2.63)^{ab}	7.06 (0.43)^{ab}	49.8 (6.77)^{ab}
90 kg N/ha	154.4 (4.27)^{a}	124.7 (4.87)^{ab}	8.20 (0.20)^{a}	52.1 (3.20)^{ab}
2 tons/ha PM	139.2 (4.11)^{a}	108.8 (6.92)^{b}	6.38 (0.36)^{b}	43.8 (6.33)^{b}
4 tons/ha PM	156.6 (6.00)^{a}	128.1 (6.47)^{a}	7.71 (0.35)^{a}	57.6 (2.42)^{a}
6 tons/ha PM	141.8 (5.71)^{a}	117.9 (4.22)^{a}	7.20 (0.55)^{ab}	47.1 (4.42)^{ab}
Mean	141.2	115.8	6.98	47.6
CV (%)	1.2	2.7	6.5	9.7
LSD (0.05)	19.56	18.81	1.26	12.54

Means for each column followed by the same letter(s) are not significantly different from each other according to Genstat.

Growth performance

The effect of treatments on plant height, canopy spread, stems width measured from the fourth to the twelfth week after transplanting is presented in Table 2. Significant differences (P<0.05) existed among treatments studied. The tallest plants were produced from the 4 t/ha PM treatment which recorded mean height of 156.6 cm. The control recorded the shortest plant with mean height of 112.0 cm. The increase in plant height due to PM treatment may be attributed to adequate supply of nutrients that influenced cell division and cell enlargement resulting in better plant height as reported by Gadhi (1996). 4 t/ha poultry manure registered the highest plant spread whilst 0 kg N/ha recorded the least (Table 2). The differences in plant spread were significant (P<0.05). These results conform to the findings of Ferreira et al. (1995) who reported that providing N-fertilizer leads to improved physiological activities of the plant causing the plant to branch out.

The stem width ranged from 5.31 to 8.20 with a mean of 6.98 (Table 2). Statistical differences were observed in the stem width. The fertilizer treatments had significant effect on number of branches per plant (Table 2). Application of 4 t/ha PM and 90 kg N/ha produced the highest number of branches of 58 and 52 per plant respectively. Application of poultry manure increased nitrogen availability that in turn increased production of branches. This observation confirms the findings of Arul (2002) who reported increased number of branches per plant under poultry manure treatments.

Fresh and dry leaf yield

Generally, Artemisia leaf yields were higher under 4 t/ha PM treatment than the other treatments (Table 3). 4 t//ha poultry manure produced the highest fresh and dry leaf yields at both pre-flowering and full bloom stages of harvesting. Statistical differences were observed in the dry leaf yield at both stages of harvest. The results conform to various reports (Khalid and Shafei, 2005) and this may be attributed to the fact that application of poultry manure increased nitrogen availability, which in turn increased production of leaves resulting in increased biomass (Arul, 2002). The control at both harvests recorded the lowest fresh and dry leaf yield due to the elevated nutritional stress of plants from these plots.

Table 4 shows the crude extract weight of *A. annua*. Plots treated with 4 t/ha PM gave the highest crude weight (2.56 g) at pre-flowering but showed no significant difference (P>0.05) from the other treatments. At full bloom, 4 t/ha PM gave the highest value of 2.98 g but was statistically similar to all plots except the control. The control consistently produced the lowest values at both harvests. The failure of the N fertilizer to affect crude extract at both harvests might be due to the biosynthesis of the secondary metabolites, which is under genetic control. This is in conformity with the findings of Ferreira et al. (2005), which contradict the findings of Delabays et

Table 3. Mean fresh and dry leaf yield of *Artemisia annua* evaluated under different treatments and growth stages (kg/ha).

Treatment	Pre-flowering		Full bloom	
	Fresh leaf	Dry leaf	Fresh leaf	Dry leaf
0 kg N/ha	128.90(44.03)[b]	44.40(9.52)[b]	565.00(7.89)[b]	233.00(2.04)[b]
45 kg N/ha	274.3(8.44)[a]	109.30(11.76)[a]	701.00(78.00)[ab]	313.00(28.05)[b]
90 kg N/ha	287.10(11.1)[a]	96.60(7.67)[ab]	927.00(148.20)[ab]	472.00(101.99)[ab]
2 tons/ha PM	231.50(17.49)[a]	69.10(17.97)[b]	787.00(225.35)[ab]	356.00(53.03)[b]
4 tons/ha PM	310.50(9.63)[a]	134.60(3.94)[a]	1087.00(19.58)[a]	582.00(23.53)[a]
6 tons/ha PM	276.10(33.82)[a]	93.90(23.46)[ab]	993.00(146.84)[ab]	480.00(64.76)[ab]
Mean	251.40	91.3	843.00	406.00
CV (%)	7.6	6.8	7.9	14.00
LSD (0.05)	76.48	46.74	444.6	15.63

Table 4. Effect of treatments on crude extract weight of *Artemisia annua* evaluated under different treatments and growth stages (g).

Treatment	Pre-flowering	Full bloom
	Crude extract	Crude extract
0 kg N/ha	2.17 (0.20)[a]	1.93 (0.66)[b]
45 kg N/ha	2.23 (0.13)[a]	2.61 (0.02)[ab]
90 kg N/ha	2.36 (0.25)[a]	2.56 (0.04)[ab]
2 tons/ha PM	2.46 (0.33)[a]	2.72 (0.26)[ab]
4 tons/ha PM	2.56 (0.54)[a]	2.98 (0.08)[a]
6 tons/ha PM	2.35 (0.71)[a]	2.57 (0.27)[ab]
MEAN	2.35	2.56
CV (%)	28.4	21.9
LSD (0.05)	1.219	1.020

al. (2001) who reported that crude extract content and artemisinin content are both influenced by genetic and environmental factors such as nitrogen availability.

Artemisinin content and artemisinin yield

Application of 2 and 4 t/ha PM produced the highest artemisinin content at pre-flowering and full bloom respectively (Table 5). The results also showed that 4 t/ha PM produced the highest artemisinin yield at both harvests due to high leaf biomass production. Artemisinin content and yield at full bloom stage were significantly influenced by the treatments ($P<0.05$). The higher artemisinin content observed on control plots probably suggests that artemisinin content was not influence by soil fertility status even though Sukhmal et al. (2001) have reported the effect of nitrogen on artemisinin content. Values for artemisinin content at pre-flowering were considerably higher than artemisinin content at full bloom. Laughlin et al. (2002) reported similar results. The higher percentage of artemisinin content recorded from the crude extracts of this study, compared to artemisinin content obtained from plant leaves in other studies is a confirmation of the potential of crude extracts to produce higher percentage of artemisinin content. Wang et al. (2005) made similar observations and reported artemisinin content ranging from 3 to 7% in crude extracts and 0.3 to 1.3% in plant leaves of the same plant varieties respectively.

The estimated correlation among traits is presented in Table 6. The highest significant positive correlation was recorded between plant height and plant canopy spread. The correlation analysis of the data shows that plant height, plant canopy spread and number of branches have significant positive correlation with dry leaf yield and artemisinin yield at both harvests. In their study, Dharm et al. (1996) reported positive correlation between plant growth traits and biomass yield of Artemisia. The significant positive correlation recorded between these parameters suggests the influence of growth parameters on the yielding ability of *A. annua.* The result shows a significant positive correlation between leaf biomass yield and artemisinin yield at both harvests. This result could imply that artemisinin yield largely depends on leaf biomass accumulation. Hence, soil amendments that promote biomass yield will have positive influence on artemisinin production.

Conclusion

Organic and inorganic fertilization positively influenced growth and development of *A. annua.* Poultry manure applied at a rate of 4 t/ha promoted good growth and increased the yield of the plant. The higher fresh and dry yields of Artemisia under 4 t/ha poultry recorded in this study was due to sustainable supply of nutrients to the plant from the manure. The least effective treatment was the control, owing to poor nutrient levels in the soil as shown by the soil analysis before imposition of

Table 5. Artemisinin content and yield of *Artemisia annua* on dry leaf yield basis at different growth stages from different treatments.

Treatment	Pre-flowering		Full bloom	
	Artemisinin content (%)	Artemisinin yield (kg/ha)	Artemisinin content (%)	Artemisinin yield (kg/ha)
0 kg N/ha	7.66 (2.33)[a]	3.40 (1.40)[a]	7.26 (0.46)[a]	16.9 (11.16)[b]
45 kg N/ha	7.97 (0.85)[a]	8.71 (1.56)[a]	7.92 (0.32)[a]	24.78 (2.87)[ab]
90 kg N/ha	6.74 (1.74)[a]	6.51 (1.14)[a]	7.77 (0.97)[a]	36.67 (9.54)[a]
2 tons/ha PM	8.43 (0.37)[a]	5.82 (0.28)[a]	5.05 (0.49)[b]	17.97 (4.14)[b]
4 tons/ha PM	7.11 (2.43)[a]	9.57 (3.32)[a]	6.40 (0.45)[ab]	37.24 (0.77)[a]
6 tons/ha PM	7.23 (1.12)[a]	6.78 (2.40)[a]	4.43 (0.00)[b]	21.26 (0.01)[b]
Mean	7.52	6.80	6.47	25.80
CV (%)	10.42	14.4	4.7	11.3
LSD (0.05)	5.526	6.416	1.742	14.21

Table 6. Correlation analysis of quantitative characters of *Artemisia annua* evaluated under different treatments.

Quantitative characters	1	2	3	4	5	6	7	8	9
1. Plant Height	1								
2. Plant Spread	0.79**	1							
3. Number of Branches	0.70**	0.55**	1						
4. Leaf yield at preflowering	0.66**	0.63**	0.66**	1					
5. Leaf yield at full bloom	0.54*	0.53**	0.59**	0.66**	1				
6.Artemisinin content at preflowering	-0.10	-0.31	-0.16	0.12	-0.33	1			
7. Artemisini content at full bloom	-0.05	-0.02	0.005	0.05	-0.20	0.01	1		
8. Artemisinin yield at preflowering	0.51**	0.40*	0.34*	0.73**	0.15*	-0.34	0.04	1	
9. Artemisinin yield at full bloom	0.44*	0.54*	0.49*	0.56*	0.75**	0.04	0.41*	0.17	1

* Significant at P<0.05; ** significant at P<0.01.

treatments. Plants treated with 4 t/ha poultry manure gave the highest artemisinin yield due to their profuse vegetative growth and large biomass yield. The study has also demonstrated that increasing poultry manure from 4 to 6 t/ha and compound fertilizer from 45 to 90 kg N/ha did not significantly affect growth, yield and artemisinin content. The best option for better performance is the application of 4 t/ha poultry manure or 45 kg N/ha compound fertilizer. The study recommends the application of poultry manure at a rate of 4 t/ha for artemisinin production as an alternative to compound fertilizers which are expensive.

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