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Full Length Research Paper

Agronomic performance of five varieties of annual wormwood (*Artemisia annua* L.) in the humid tropics of Ghana

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The agronomic performance of five varieties of *Artemisia annua* was assessed in the humid tropics of Ghana at the Agriculture Research Station, Anwomaso during the 2008 major growing season. The experiment also examined the effects of variation in the stage of harvest on the plant growth traits and yield. The randomized complete block design was used with five *A. annua* varieties (New hybrid, RC 131, RC 32, UC 2005 and W1) as treatments in three replications. The parameters used for the assessment were plant height, plant 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). All the five varieties of *A. annua* were performed well in comparison with data from other studies. RC 131 recorded the highest vegetative growth among the varieties while RC 32 recorded the lowest values for all the morphological parameters. New hybrid produced the highest artemisinin yield and content at pre-flowering. The highest artemisinin yield at full bloom was recorded by WI. The results showed considerable increase in artemisinin content at full bloom compared to values recorded at pre-flowering. The results showed that the artemisinin yield was positively correlated with leaf yield and stage of harvest. Based on the leaf yield performance, the order of preference recommended for artemisinin production amongst the varieties in the study area is: RC 131, New hybrid, RC 32, W1 and UC 2005.

Key words: Medicinal plants, *Artemisia annua* L., asteracea, artemisinin.

INTRODUCTION

Plants continue to be a major source of medicines in the maintenance of human health throughout the world and notably in the tropics. One of the most important of such medicinal plants is *Artemisia annua*. The plant is currently processed by pharmaceutical firms for the production of artemisinin, which is a major ingredient in the production of artemisinin-based combination therapies (ACTs) that are used in the treatment of malaria. *A. annua* became a valuable agricultural crop after the World Health Organization recommended artemisinin as a component of artemisinin-combination based therapies for malaria in 2004. This recommendation caused the demand for artemisinin to increase, leading to supply shortages (Cyranoski, 2004). Aside from *Plasmodium* species that cause malaria, artemisinin-derived drugs and crude leaf extracts of the plant have also been shown to be effective against a wide variety of other parasites that affect the health of livestock and humans such as *Coccidia* spp., *Babesia* spp. and *Leishmania* spp. (Brisibe et al., 2008; Ferreira et al., 2006; Klayman, 1993; Allen et al., 1997; Utzinger et al., 2001). These parasites hinder economic development when they affect livestock and humans.

The most important benefit to global health and economics is the agricultural expansion of artemisia cultivation to produce artemisinin to treat multidrug-resistant *Plasmodium falciparum*. Malaria-inflicted economic losses in Africa involve loss of labour,

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agricultural losses due to the debilitating effect of the disease and poverty. The disease is a burden and a challenge to human development since it is both a cause and consequence of under development in the continent. Studies into the performance of *A. annua* in the humid tropics will go a long way to boost the efforts of local pharmaceuticals companies at curbing malaria incidence and severity, especially, in Ghana and Africa as a whole. The objective of this study, therefore, was to determine the performance of five varieties of *A. annua* in the humid tropics of Ghana.

MATERIALS AND METHODS

Description of experimental site

The experiment was carried out during the major season in 2008, which began from March to July at the Kwame Nkrumah University of Science and Technology Agricultural Research Station, Anwomaso, Kumasi. Experimental site is located within the semi-deciduous forest zone of Ghana. The sites lie between latitude 06º. 43° North and longitude 01°. 36° West of the Greenwich meridian. The soil at the project site is Ferric Acrisol which is devoid of any hard solid mass that may hinder cultivation and plant root penetration. It belongs to the Asuansi series with about 5 cm thick top layer of dark grey gritty sandy loam (FAO, 1984).

Experimental design and treatments

The randomized complete block design was used for the study. The treatments were RC 131, RC 32, UC 2005, New hybrid and W1 obtained from Rutgers University, Washington, USA. 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 and was replicated three times.

Sowing and crop husbandry practices

The seeds were sown in sterilized topsoil in seed boxes. Thirty days after germination, the seedlings were pricked out into plastic pots. The seedlings were hardened by gradually exposing them to sunlight and transplanted at eighty days after emergence to the field, which had been slashed, ploughed and 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. Supplementary irrigation was provided through 1.5 L Voltic bottles fitted into the soil near the plant. Manual weeding was done with a hoe at three, seven and eleven weeks after transplanting.

Soil samples were taken prior to transplanting with soil auger at random on each experimental plot from the top 0 to 20 cm and 20 to 40 cm depth. A sizeable quantity of the composite soil samples were air-dried and sieved through a 2 mm mesh and subjected to physical and chemical characterization. Parameters determined include particle size, pH, organic carbon, total N, available phosphorus (ppm), exchangeable cations and dry bulk density.

Data collection

Data were collected weekly on plant height, plant spread and stem width from the fourth to the twelfth week after transplanting from each of six non-border plants. Main branches were counted. Three plants from each plot were harvested at two different schedules. The first harvest was done prior to flowering and the second sample was harvested at full bloom. At harvest, fresh and dry leaf weights were obtained by using an electronic weighing scale. Artemisinin yield per hectare was recorded on dry leaf basis. The sampled plants were dried under natural conditions in a shade for seven days. The dried plants were stripped to obtain the leaves and the flowers and these were thoroughly dried to obtain the biomass.

Laboratory analysis and extraction

The dried biomass was milled using a milling machine. Crude extracts from the samples were extracted using batch percolation method with petroleum ether as the solvent. A thimble, sown from gray-baft material was filled with 100.0 g of the milled biomass and placed in the extractor globe. The round bottom flask was filled with 1000 ml of petroleum ether and the temperature noted. The solvent in the round bottom flask was heated with the heating mantle which had its temperature set at 60°C. Water was allowed to flow through the condenser to condense the vapour of the petroleum ether. The condensed petroleum ether flowed down the column and diffused through the thimble and the biomass until it was completely soaked with the solvent. The colour of the solvent changed from colourless to yellow after diffusing through the dried biomass.

Condensation and diffusion of the solvent occurred simultaneously until the extractor globe was completely filled. The solvent in the extractor globe was siphoned back into the round bottom flask. This process was repeated until the extraction process was complete. The extraction was carried out for all the treatments. The solvent for the extraction was recovered by distillation. The crude extract was dried and weighed. The crude extract was obtained through steam distillation. 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 (Wang et al., 2005).

Data analysis

The data were subjected to statistical analysis using Genstat (2000) to obtain the analysis of variance. The least significant difference (LSD) at 5% was used to separate the means.

RESULTS AND DISCUSSION

Table 1 shows the results of physical and chemical characteristics of the soil before imposition of treatments. The pH of the soil was slightly acidic at both depths. Strong leaching of the basic cations out of the top soil may have contributed to the acidic nature of the soil. It is expected that this factor will affect the dynamics of all nutrients (Quang et al., 1996). 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, 1984). The dry bulk density values were high. The fertility status of the soil was low and decreased with depth and response to the major nutrients

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 (%)	Org.carbon (%)	P (ppm)	Exchangeable basic cations (cmol/kg)				Dry bulk density (g/cm)	Particle size (%)		
					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. Mean plant height, spread, stem width (cm) and number of branches per plant of different *A. annua* varieties.

Treatment	Plant height	Plant spread	Stem width	Number of branches/plant
New hybrid	122.7	113.8	6.04	48.67
RC 131	124.9	116.0	5.63	50.67
RC 32	102.3	106.3	4.40	33.83
UC 2005	107.3	109.0	5.33	40.67
W1	120.0	112.7	5.47	45.67
Mean	115.5	111.6	5.37	43.90
CV (%)	5.9	1.0	7.8	8.7
LSD (0.05)	10.21	15.45	1.31	6.17

was expected.

Vegetative growth

Plant height, spread and width

The height, canopy spread and stem width of plants measured from the fourth to the twelfth week after transplanting is presented in Table 2. The tallest plant was produced by RC 131, with a mean height of 124.9 cm. Significant ($p<0.05$) differences were generally observed between the treatments. Similarly, RC 131 produced plants with the maximum spread with mean value of 116.0 cm while RC 32 produced plants with minimum spread. The stem width ranged from 4.40 to 6.04 cm with a mean of 5.37 cm. New hybrid produced the thickest plants at the twelfth week after transplanting.

Number of branches

The results showed significant ($p<0.05$) difference among the treatments (Table 2). RC 131 recorded the highest number of branches per plant (50) while New hybrid, W1 and UC 2005 recorded 48, 45 and 40 numbers of branches per plant**,** respectively. RC 32 recorded the lowest number of branches per plant with a mean value of 33. At 4 weeks after transplanting the differences in plant height, canopy spread, width and number of branches per plant were very small. However, as the plant grew older phenotypic expression of varietal differences became more pronounced. The variation in genotypic characters could be attributed to differences in genetic constitution, a similar situation was observed by Ferreira et al. (2005) and Delabays et al. (2001).

Yield components

Fresh and dry leaf yield per hectare

The fresh and dry leaf yields at both stages of harvesting for each treatment are presented in Table 3. Results showed that RC 131 recorded the highest dry leaf weight of 86.7 and 422.2 kg/ha at pre-flowering and full bloom stages, respectively. This was followed by new hybrid, W1, UC 2005 and RC 32 in decreasing order at both harvest stages. The difference between mean values of dry leaf yields was significant ($p<0.05$) at both stages of harvesting. The trend in differences observed in the leaf yield within

Table 3. Mean fresh and dry leaf weights (kg) of various *A. annua* varieties.

Treatment	Pre-flowering		Full bloom	
	Fresh leaf	Dry leaf	Fresh leaf	Dry leaf
New hybrid	268.00	75.00	1312.00	398.50
RC 131	273.00	86.70	1386.00	422.20
RC 32	154.00	37.60	544.00	212.90
UC 2005	207.00	49.60	630.00	277.50
W1	224.00	49.60	963.00	329.10
Mean	225.0	62.8	967.0	328.0
CV (%)	21.2	16.7	23.4	11.2
LSD (0.05)	89.9	19.70	425.1	69.10

Table 4. Crude extract weight (g) of various *A. annua* varieties.

Treatment	Pre-flowering	Full bloom
	Crude extract	Crude extract
New hybrid	2.37	1.93
RC 131	3.22	3.25
RC 32	1.76	2.67
UC 2005	2.40	1.81
W1	2.82	1.76
Mean	2.51	2.29
CV (%)	6.5	12.4
LSD (0.05)	0.23	0.64

accessions suggests the suitability for selecting plants with suitable architecture when considering artemisinin production (Arul, 2002). The differences in performance observed within accessions may be due to genotypic differences (Ferreira et al., 2005).

Crude extract weight

Table 4 shows the crude extract weight of the various Artemisia varieties. There were significant ($p<0.05$) differences among the accessions at both stages of harvesting. RC 131 recorded the highest crude extract weight with mean values of 3.22 and 3.25 g at both growth stages. The differences in crude extract content may be due to varietal differences as the crude extract content is under genetic control. This in conformity with the findings of Ferreira et al. (2005) who observed that crude extract content was not influenced by environmental but genetic factors.

Artemisinin content and yield

New hybrid produced the highest artemisinin content of 6.84% and artemisinin yield of 5.13 kg/ha at pre-flowering (Table 5). Significant ($p>0.05$) differences in artemisinin content at full bloom were observed among the accessions. RC 32 produced the highest artemisinin content of 9.66% and artemisinin yield of 22.97 kg/ha at full bloom. Differences in artemisinin content are due to genotypic differences. The artemisinin yield depends on the inherent artemisinin content of the various cultivated genotypes and agronomy of cultivation. Similar findings have been reported by Delabays et al. (2001). The higher percentage of artemisinin content (Table 5) from the crude extracts of this study, compared to artemisinin content from other studies confirms the higher potential of crude extracts to produce higher percentage of artemisinin content. Wang et al. (2005) made similar observations and reported of artemisinin content ranging from 3 to 7% in crude extracts and 0.3 to 1.3% in plant leaves of the same Artemisia variety, respectively.

The correlations between pairs of variables are presented in Table 6. The results show that the artemisinin yield in the *A. annua* was positively correlated with leaf yield. These significant positive correlations indicate that indirect selection for high leaf yield will result in high artemisinin yield (Sushil et al., 2004). Besides, the leaf yield had significant positive correlation with plant height, plant spread and number of branches per plant. The significant positive correlation between plant growth traits and biomass yield of artemisia is consistent with the findings of Dharm et al. (1996). The significant positive

Table 5. Artemisinin content and yield of various *A. annua* varieties on dry leaf yield basis.

Treatment	Pre-flowering		Full bloom	
	Artemisinin content (%)	Artemisinin yield kg/ha)	Artemisinin content (%)	Artemisinin yield (kg/ha)
New hybrid	6.84	5.13	4.16	16.57
RC 131	4.89	4.24	3.97	16.76
RC 32	5.34	2.01	9.66	20.56
UC 2005	4.44	2.20	5.96	19.61
W1	4.70	3.07	8.28	22.97
Mean	5.24	3.33	6.40	19.29
CV (%)	7.6	12.7	6.1	0.9
LSD (0.05)	2.76	2.39	3.35	9.76

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

Parameter	1	2	3	4	5	6	7	8	9
Plant height	1								
Plant spread	0.31**	1							
Number of branches	0.95*	0.26*	1						
Leaf yield at pre- flowering	0.67**	0.46*	0.73**	1					
Leaf yield at full bloom	0.82*	0.34*	0.83*	0.83*	1				
Artemisinin content at pre- flowering	0.09	0.18**	0.04	0.41*	0.15	1			
Artemisini content at full bloom	-0.71	-0.17	-0.76	-0.67	-0.82**	-0.40	1		
Artemisinin yield at pre-flowering	0.32	0.31*	0.34	0.67*	0.33*	0.87*	0.45	1	
Artemisinin yield at full bloom	-0.07	-0.01	0.05	0.13*	0.13*	-0.38	0.53*	0.17	1

*Significant at p<0.05; **Significant at p<0.01.

correlation of growth traits with leaf biomass and artemisinin yield indicates the expected influence of these growth parameters on the yielding ability of *A. annua.* The highest significant positive correlation of 0.95 was recorded between plant height and number of branches per plant at pre-flowering. The number of branches per plant, plant height, plant spread and leaf yield were therefore the major determinants, directly and indirectly influencing artemisinin yield in *A. annua*.

Conclusion

The present study has investigated the agronomic performance of five *A. annua* varieties in the humid tropics of Ghana. Comparing the various results it was noticed that RC 131 and New hybrid performed better than the rest of the varieties. However, W1 produced the highest artemisinin yield at full bloom. The results indicate that there are genotypic differences in yield of crude extract and artemisinin content among the five varieties studied. In terms of yield, all the five Artemisia varieties performed well in the humid tropics of Ghana. Recorded mean yield values fell within or were higher than the reported range. For high artemisinin production, it is recommended that RC 131 is considered compared to the other varieties.

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