Trop J Nat Prod Res, June 2019; 3(6):184-189
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effects of Organic and Inorganic Fertilizers on The Vegetative Growth of Worm Wood (*Artemisia annua* Linn.)

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ARTICLE INFO

Article history:
Received 06 September 2017
Revised 02 July 2019
Accepted 03 July 2019
Published online 09 July 2019

ABSTRACT

The propagation of *Artemisia annua* is hindered by shortage of seeds, germination problem and effective protocol. Field experiments were conducted during both rainy and dry seasons to determine the effect of levels of organic and inorganic manure on the vegetative growth of *A. annua*. Results of Analysis of Variance (ANOVA) indicated no significant difference between the seasons with respect to the parameters studied excerpt the canopy spread and leaf fresh weight before flowering ($P \geq 0.05$). Similarly, no significant difference was observed during the irrigation trial except in 25 kg/ha NPK on the canopy spread, 50 kg NPK on whole fresh weight after flowering and 4 tone/ha poultry fertilizer on leaf fresh weight after flowering. However, a significant difference was observed under the rainy season experiment except in whole fresh weight before flowering, leaf dry weight before flowering and leaf dry weight after flowering. Application of 6 t/ha of poultry manure during the raining season had the highest plant height followed by 4 t/ha of poultry manure during the irrigation trial. Similarly, rainy season trial had the highest number of branches in 50 kg/ha of NPK. Rain trial had the best leaf fresh weight after flowering at 6 t/ha of poultry manure and the best leaf dry weight before flowering was observed at 25 kg/ha of NPK during the rain trial.

Keywords: *Artemisia annua*, Plant height, Canopy spread, Fertilizer, Vegetative growth.

Introduction

Artemisia annua L (Wormwood) belongs to the family Asteraceae[1], which consist of about 400 species.[2-7] The family is characterized by extreme bitterness of all parts of the plant.[8-10] Cultivation of *A. annua* spread from China to Africa, mainly Kenya, Tanzania and Nigeria in response to the call by the World Health Organization for the use of Artemisinin-Combination Therapy (ACT). The most concentrated areas of *Artemisia* production are Asia, Europe, and USA and recently East Africa.[6] However, global estimates for *A. annua* revealed a gradual increase in production area of 2,000 ha in 2003, 3000 ha in 2004, 9,500 ha in 2005, 26,000 ha in 2006, 14,500 ha in 2007, 4,500-5,000 ha in 2008, 6,000 ha in 2009, 15,000 ha in 2010, 17,500 ha in 2011 and 16,500 ha in 2012.[11-13] Production in 2013 was very uncertain due to labour shortages, competition from other crops and currency devaluation, but above all, due to the reductions in artemisinin prices, market uncertainties and the introduction of semi-synthetic artemisinin.[14-16] In China, production rate of 2,000 ha in 2004, 6,000 ha in 2005 and 9,000 ha in 2006 were reported.[17] Vietnam is also a major producer but was reported to have dropped from 10,000 ha in 2006 to 3,000 ha in 2007, 1,000 ha in 2008, 700 in 2009, 500-700 in 2010, and then expanded to 1,500 ha in 2011.[11-13] India has the potential for *Artemisia* cultivation but no estimates of production is available.[18,19] In 2005, Kenya, Uganda, and Tanzania recorded an annual production of 1,650 ha. The yield rose in 2007 to 3,500 and 4,000 ha.[20]

Artemisia grows very well in slightly alkaline loamy and well drained soils. The plant grows well in places exposed to good sunlight throughout the day. *Artemisia* is quite tolerant to drought and low moisture conditions.[10] In fact, plants grown in poor and dry soils do produce a high aromatic quality. However, *Artemisia* tolerates temperatures that are as low as -5°C.[21]

In the year 2013, 123-283 million malaria infections and deaths in the range of 367,000 – 755,000 were reported globally, while 437,000 African children were reported dying annually due to malaria infection. In 2014, malaria cases were reported in 97 countries and territories. An ‘estimated 3.3 billion people were at risk of malaria, out of which 1.2 billion are at high risk.[22]

Conventional means of production of *A. annua* is presently the most commercially feasible approach to produce artemisinin and related compounds, but farmers face the challenge of access to good-quality seeds, germination inhibition and dormancy of the plant, decline in the natural habitats due to destruction of plant resources as a result of Man’s economic and social activities, lack of sufficient training, difficulty to meet quality standards for processing and extraction, steady market and profitable price to attract farmers, weed control, harvesting and drying techniques.[23]

The aim of this work is to study the effect of varying rates of both organic and inorganic fertilizers on some growth parameters of *A. annua*.

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Citation: Tahir MS and Sufiyanu S. Effects of Organic and Inorganic Fertilizers on The Vegetative Growth of Worm Wood (*Artemisia annua* Linn.). Trop J Nat Prod Res. 2019; 3(6):184-189. doi.org/10.26538/tjnpr/v3i6.1

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Study Area
Field experiments were conducted on the farm of Institute for Agricultural Research (IAR) during the 2012 rainy season and 2013 dry season in Ahmadu Bello University, Zaria, latitude 11º 11 N and 07º38 E, altitude 670 m above sea level.

Planting Materials
Seedlings of *Artemisia annua* var. Chiyong were obtained from the Artemisia programme unit of IAR for the experiment (Plate i).

Soil Analysis
Soil samples were taken at random on each experimental plot at depths of 0-15 cm, 15-30 and 30-40 cm. The soil samples from each depth per replication were mixed thoroughly to form a composite sample. The composite soil samples were air-dried and sieved through a 2mm mesh and subjected to physical and chemical analysis at the Soil science laboratory of IAR, Ahmadu Bello University, Samaru, Zaria.[24]

Experimental design
The experimental materials were laid in a 4x4 factorial design in RCBD with 3 replications. A poultry manure of 2.9% nitrogen content was applied at the rate of 2, 4, 6 and 8 ton/ha and NPK fertilizer (15-15-15) at the rate of 25, 50, 75 and 100 kg/ha was applied with a control.

Ridge Preparation
The ridges were prepared after ploughing and harrowing the sandy loam soil using a tractor. This provide a good drainage and moist but damp soil condition. Twenty seven plots, each measuring 5.0 x 4.5m were marked out.[25]

Transplanting
Prior to transplanting, two (2) months old seedlings were hardened gradually by exposing them to sunlight while reducing watering. Twenty seedlings were transplanted per plot of 5.0 x 4.5 m. There were four rows per plot and inter and intra row spacing of 1.5 ×1.0m respectively. Ridges were labeled and irrigated during the dry season experiment and rain was the sources of watering during the raining season.

Weeding
Weeds were controlled by hoeing at two weeks interval (WAP) and seven days after transplanting, the following parameters were studied using samples of 6 plants per plot at various growth stages:
Plant height was determined using measuring tape was used to measure the height of the plant from the level of the soil to the top of the shoot apex. Number of branches were determined by counting the total number of branches on each plant. The plant canopy spread was determined by stretching measuring tape horizontally across the plant. Fresh leaf weight before flowering, whole fresh weight before flowering, leaf dry weight before flowering, leaf dry weight after flowering, leaf fresh weight after flowering and whole fresh weight after flowering, were obtained by using an electronic weighing scale.

Data Analysis
The data was analyzed using Analysis of Variance (ANOVA), SAS (2013) statistical package. Least significant difference (LSD) was used to compare treatment means ($P < 0.05$) when F was significant.[26]

Results and Discussion

Results of soil analysis indicated that the soil sample was a sandy loam soil which is ideal for the propagation of *A. annua*. The soil was also found to contain 4.0% carbon (C), 4.3% Phosphorous (P) and 0.05% Nitrogen (N). Similarly, the poultry manure was found to contain 3.0% Nitrogen. The Artemisia seedlings were established on the field, 3 weeks after transplanting (Plate ii). Application of 50 kg/ha of NPK during the rainy season trial, had the highest number of branches (Table 2). This is similar to the findings of Ming (1994)[27] who observed substantial number of branches with application of average level of compound fertilizer. However, this is contrary to the findings of Arul (2002)[28] and Rao *et al*. (1985)[29] who recorded highest number of branches with poultry manure.
Six (6) t/ha of poultry manure during the rainy season had the highest plant height (Table 2). Rain trial had the best leaf fresh weight after flowering with 6 t/ha of poultry manure (Table 2 and Plate iii). This corresponds with the findings of MajburRahman and Osman (2003)[30] who attributed that to the photosynthetic efficiency of poultry manure on plant which yields high number of leaves and stems and increased vegetative growth. Similarly, Arul (2002),[28] Khalid and Shafei (2005)[31]observed that application of poultry manure increases nitrogen level of a soil, which in turn increase production of leaves resulting in increased fresh herbage yield. The use of organic fertilizers play an essential role in plant growth and development, biosynthesis of the organic substance at all levels and growth yield characters such as biomass yield.[32] This also, suggests that application of poultry manure at a rate of 6 t/ha may be considered as the optimum rate above which Artemisia may not respond very well. Application of 8 t/ha of Poultry manure may be considered as excessive and is not efficiently utilized by plants. Consequently, high quantities of dissolved salt accumulate in the soil making nutrients unavailable.[42]
On the other hand, the above results, contradicts what was reported by Martinez and Staba (1988)[37] that N-fertilization stimulates the vegetative development of the plant, the greater the application of N, the greater the height of the plant. This may also be attributed to adequate supply of nutrients that influenced cell division and cell enlargement resulting in better plant height as.[36,38]
The best leaf dry weight before flowering was observed with 25 kg/ha during the rain trial. It was followed by the same quantity of NPK during the irrigation trial (Table 1). Similarly, rain had the highest leaf fresh weight before flowering with 2 t/ha of poultry manure. Also, the rainy season had the best result with respect to leaf dry weight after flowering with 8 t/ha of poultry manure (Table 2). This agreed with the findings of Dixit (1997)[33] and Mathias (1997)[34] who reported that, poultry manure increased plant shoots. This was attributed to probably low soil nutrients status.[35] Similar results were also reported by Agyenim (1999)[36] working on maize and found that the total weight of dry matter was higher in plants treated with poultry manure.
No significant difference was observed between the two seasons with respect to canopy spread (Table 3). These results corroborate with the findings of Ferreira *et al*. (1995)[39] who reported that organic amendments promoted vigorous growth and development, strong root system and stem development of Artemisia. Poultry manure contains appreciable amount of nitrogen that results in the production of larger stem width and longer internodes.[40,41]
Rainy season had the highest plant height and leaf weights compared with the irrigation trial, this might not be unconnected with the fact that water constitutes 80-95% of the mass of growing plant tissues and plays a crucial role for plant growth by acting as base material for all metabolic activities and helps to keep the plant erect by maintaining plant's turgidity.[43,44]
Water is a constituent of protoplasm, maintaining cell turgidity for structure and growth. Growth occurs as water enters the cell in response to an osmotic driving force, and the pressure of the intracellular water inflates the cellulosic wall which bounds the cell. The primary process of volume expansion is driven by water uptake. The structural rigidity of the soft plant tissues is also conferred by water. This can be seen in the wilting of leaves of plants subjected to sudden drought.[44,45]
Rainy season trial had the highest number of branches with 50 kg/ha of NPK followed by same quantity of NPK during the irrigation trial. The best leaf dry weight before flowering was observed at 25 kg/ha during the rain trial. It was followed by the same quantity of NPK during the irrigation trial (Table 1). This is similar to the findings of Zhongguo and Yao (2009)[46] who studied the effects of NPK and plant density on the growth of *A. annua*. They reported that, N, P and K in moderate supplies, significantly increased total biomass, leaf yield and artemisinin contents in *A. annua*.

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Effects of Organic and Inorganic Manure on the Growth of *A. annua* During the Irrigation.

	Growth Parameters								
Treatment	**PH (cm)**	**NB**	**CS (cm)**	**LFWBF (g)**	**WFWBF (g)**	**LDWBF (g)**	**LDWAF (g)**	**LFWAF (g)**	**WFWAF (g)**
NPK									
25 kg/ha	42.26a	25.67a	39.62a	63.72a	208.97a	36.97a	15.13a	25.30bcd	265.92ab
50 kg/ha	49.05a	26.00a	32.24ab	42.67a	262.67a	26.87a	14.57a	16.38bcd	363.80a
75 kg/ha	42.31a	24.17a	25.61b	33.98a	187.55a	29.77a	15.07a	19.73bcd	199.65b
100 kg/ha	46.45a	23.83a	30.49b	39.65a	246.93a	32.75a	13.83a	31.02ab	162.90b
Poultry manure									
2 t/ha	47.23a	24.00a	28.25b	63.77a	250.70a	30.40a	13.72a	18.42bcd	256.77ab
4 t/ha	50.00a	24.00a	29.57b	46.13a	214.87a	25.50a	18.70a	44.68a	181.00b
6 t/ha	46.89a	25.67a	27.58b	47.87a	248.40a	26.10a	17.48a	12.30d	177.55b
8 t/ha	46.22a	24.83a	30.13b	53.08a	107.53a	18.37b	19.28a	26.88bc	234.63b
Control.	41.86a	26.67a	25.28b	44.07a	259.95a	27.13a	21.43a	21.783bcd	248.13ab

Means with same letters are not significantly different using LSD at 0.05%. PH = Plant height. NB = No. of Branches. CS= Canopy spread. LFWBF = leaf fresh weight before flowering. WFWBF = Whole fresh weight before flowering. LDWBF = Leaf dry weight before flowering. LDWAF = leaf dry weight after flowering. LFWAF = Leaf whole fresh weight after flowering. WFWAF = Whole fresh weight after flowering.

Table 2: Effects of Organic and Inorganic Manure on the Growth of *A. annua* During the Rainy Season.

	Growth Parameters								
Treatment	**PH (cm)**	**NB**	**CS (cm)**	**LFWBF (g)**	**WFWBF (g)**	**LDWBF (g)**	**LDWAF (g)**	**LFWAF (g)**	**WFWAF (g)**
NPK									
25 kg/ha	71.17c	39.33ab	40.00b	125.80a	758.20a	36.97a	16.32a	174.88ab	4455.00a
50 kg/ha	80.50ab	41.50a	43.00ab	122.46a	705.10a	26.87a	15.67a	179.93ab	3892.50a
75 kg/ha	77.00ac	35.50b	44.83ab	120.38a	606.70a	29.77a	16.20a	200.60ab	3384.20a
100 kg/ha	74.00c	38.167ab	46.33a	188.67a	571.30a	32.75a	14.75a	261.60a	4039.50a
Poultry Manure									
2 t/ha	73.67c	39.178ab	44.50ab	179.87a	502.50a	30.40a	14.75a	231.83ab	4189.20a
4 t/ha	76.00c	27.33ab	44.83ab	149.54b	598.10a	25.50a	19.68a	209.35ab	4333.80a
6 t/ha	84.5a	35.00b	44.50ab	145.67b	672.10a	26.10a	17.78a	258.58a	4150.20a
8 t/ha	76.92c	36.83ab	45.17ab	147.55b	649.40a	18.37b	20.38a	152.77b	3619.40a
Control	80.67ab	38.500ab	46.00a	139.40b	673.30a	27.13a	25.55a	202.20ab	4457.60a

Means with same letters are not significantly different using LSD at 0.05%. PH = Plant height. NB = No. of Branches. CS = Canopy spread. LFWBF = leaf fresh weight before flowering. WFWBF = Whole fresh weight before flowering. LDWBF = Leaf dry weight before flowering. LDWAF = leaf dry weight after flowering. LFWAF = Leaf whole fresh weight after flowering. WFWAF = Whole fresh weight after flowering.

Table 3: Mean Square For Effects of NPK and Poultry Manure on the Growth of *A. annua* Under Rain and Irrigation Season.

Source	**DF**	**PH (cm)**	**NB**	**CS**	**LFWBF**	**LDWBF**	**WFWBF**	**LDWAF**	**LFWAF**	**WFWAF**
Treatments	8	166.0**	17.3	21.2	1418.2	1993.8**	36111.59	2638.44	4316.7	423647
Season	1	2974.1**	4880.3**	154.8	3997.8	228086.6**	4673178**	538397**	1019523**	441209769.2**
Trt *Season	8	90.7	14	154.8	44.9	685.32	8630.13	3318.89	3985.41	414798.6
Error	31	41.5	13.45	17.8	887.9	582.56	25159.5	1958.8	2813.72	1322164.9
Grand Mean		75.5	31.26	43.7	54.29	70.12	440.49	85.91	110.81	2040.07

** Highly significant. PH = Plant height. NB = No. of Branches. CS = Canopy spread. LFWBF = leaf fresh weight before flowering. WFWBF = Whole fresh weight before flowering. LDWBF = Leaf dry weight before flowering. LDWAF = leaf dry weight after flowering. LFWAF = Leaf whole fresh weight after flowering. WFWAF = Whole fresh weight after flowering.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Plate i: *Artemisia annua* seedlings at the Nursery.

Plate ii: Three weeks old *Artemisia annua*.

Plate iii: *In situ Artemisia annua* at the flowering stage.

Conclusion

Artemisia production is possible in Nigeria. Leaf biomass harvested from the application of 6 t/h of poultry manure and 75 kg/h of NPK during the in situ experiment produced the highest leaf biomass which suggests that, in situ regenerated plantlets can produce high quantity of the Artemisinin needed by the Pharmaceutical Companies for the production of anti-malarial drugs.

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this work will be borne by them.

Acknowledgement

We thank the Artemisia research programme of the Institute for Agricultural Research (IAR). Ahmadu Bello University, Zaria, Nigeria for their support. We also thank Mal Muhammad Sani of the Horticultural garden of the Institute.

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