INFLUENCE OF SOIL TYPE ON GROWTH AND ARTEMISININ CONTENT OF WORMWOOD (*ARTEMISIA ANNUA* L.) CHEN YOUNG VARIETY IN SOKOTO

Lawal Gandi ABDULKADIR[1*], Adamu Aliyu ALIERO[1],
Hassan Muhammad MAISHANU[1], Abdullahi Yahaya ABBAS[2]

[1] Department of Plant Science, Usmanu Danfodiyo University, Sokoto – Nigeria.
[2] Department of Biochemistry, Usmanu Danfodiyo University, Sokoto – Nigeria.
* Corresponding author. E-mail: gandiology@gmail.com

Abstract: *Artemisia annua* L. produce an array of complex secondary plant metabolite including artemisinin (ART), which kills the principal malarial parasite, *Plasmodium falciparum*, a compound of current interest in the treatments of drug resistant malaria. However, this compound remain expensive and hardy available on global scale. Synthesis of ART has been proved to be economically impossible. Therefore, increase in yield of natural occurring ART is necessary. The study evaluated the influence of soil types on germination, growth and artemisinin content of *A. annua* of Chen Young variety in Sokoto agro ecological region of Nigeria and. A greenhouse experiment was conducted in 2017 at Botanical garden of Usmanu Danfodiyo University Sokoto. The seeds were sown in plastic pots containing clay, sandy and loamy soils in completely randomized design with 4 replications. Germination percentage (G), Mean germination time (MT), Coefficient of variation of the germination time (CV_t), Mean germination rate (MR), Uncertainty of germination (U) and Synchrony of germination (Z) were evaluated. Growth parameter and Artemisinin content were also determined. ART was determined and quantified with high–performance liquid chromatography (HPLC) using calibration curve constructed by plotting the peak area against the concentration (5, 10, 15, 20, 25 µg/ml) of ART standard solutions. The results revealed that soil types had no significant effect on germination and growth parameters evaluated (P<0.05). However, clay had the highest G, MT and CV_t than sandy and loamy soils. The highest speed of germination per day was observed on sandy soil. Clay soil had the least values of U (2.277±0.2 bit) while the degree of overlapping germination was frequent in the clay (Z). The result obtained shows that treatment had no significant effect on the growth parameters evaluated (P<0.05). Artemisinin content was significantly affected by soil type with the higher content in sandy soil (37.73 µg/ml) follow by clay and loamy soils with 17.90 and 15.70 µg/ml respectively. This study concludes that *A. annua* seeds can germinate and survived on different soils type in Savanna region of Nigeria. The study suggested the use of sandy soil in cultivation of the plant for its influence on high artemisinin content.

Keywords: *Artemisia annua*, artemisinin, germination, high performance liquid chromatography, soil type.

Introduction

Artemisia annua belongs to the family Asteraceae (Compositae) and is the second largest family of the flowering plant in the world. *Artemisia* is a large, diverse genus with between 200 and 400 species and comprises of hardy herbs and shrubs. *A. annua* is an annual shrub of 50-150 cm in height. It grows in temperate climate, and is most widespread in China and Vietnam, but it is also cultivated in east Africa, United States, Russia, India, Brazil and some other countries [BHAKUNI & al. 1988; 1990; LESTARI & al. 2011]. It is characterized with extreme bitterness and has been used in China for over 2000 years to treat fever [The State Pharmacopoeia of people Republic of China, 1985]. *A. annua* contains a natural chemical called

artemisinin, which was isolated in 1972 [HIEN & WHITE, 1993] and kills the principal malaria parasite, *Plasmodium falciparum*.

Artemisinin is a complex secondary plant metabolite which cannot be economically synthesized *de novo*. The unique configuration of the oxygen atoms in this molecule make it difficult to synthesize and this is key to its potent anti-malarial activity. Artemisinin is thought to work through generation of a carbon-centered free radical, which interferes with more than one biochemical pathway important in the growth and infection process of *P. falciparum* parasites. This complex mode of action, which could be said to 'starve' the parasite, may also help to limit the buildup of resistance. The World Health Organization (WHO) has recommended artemisinin-based therapies in combination with another effective blood schizontocide (such as mefloquine) to reduce recrudescence and to slow the development of resistance. More than fifty countries have now adopted *A. annua* – based anti-malaria as the front line treatment for multi-drug resistant malaria.

Artemisinin is a sesquiterpene lactone that is produced and stored in the glandular trichomes that are mainly on the leaves and floral buds of *A. annua* [DUKE & al. 1994; DUKE, 2001; FERREIRA & al. 2005]. The plant also produces more than forty flavonoids [FERREIRA & al. 2010], many polyphenols and a variety of other terpenes including mono-, sesqui-, di-, and triterpenes [BHAKUNI & al. 2001]. Many of these have weak anti-malarial activity, and, based on transcriptome analyses, many also seem to be produced and/or stored in the glandular trichomes that also contain artemisinin [WANG & al. 2009].

In low income and developing nations, malaria is the fifth most prevalent infectious disease and the tenth overall cause of death, and is projected to remain at that level until at least 2030 [MATHERS & LONCAR, 2006]. The WHO estimated that more than 229 million cases of malaria occurred in 2019 and accounting for more than 386,000 deaths in Africa [WHO, 2020]. The development of resistance by *P. falciparum* to many classes of anti-malarial drugs made the World Health Organization (WHO) to recommend artemisinin-based combination therapy (ACT) as first-line treatment of Malaria [WHO, 2018].

A. annua is a medicinal herb (Asteraceae) used for the production of artemisinin, a sesquiterpene lactone with anti-malarial effects against susceptible and multi-drug resistant *Plasmodium* species [TIRUNEH & al. 2010]. Although, artemisinin can be synthesized chemically, the analogues are unlikely to be economically competitive with that produced in plant due to the high cost and complexity of the process [FERREIRA & al. 2005]. In spite of the huge economic burden borne by many countries in the tropics, especially those within sub-Saharan Africa as a result of malaria, little is known about the agronomic performance of *A. annua* in most of these areas. Empirical reports indicating its introduction in a few countries in Africa such as Cameroon, Ethiopia, Kenya, Mozambique, Tanzania, Uganda and Zambia are available all in high-altitude regions and/or regions with a pronounced cool period [MAGALHÃES & al. 1997, MUELLER & al. 2004; EABL, 2005; FERREIRA & al. 2005]. In Nigeria, successful propagation of *A. annua* has been reported by EBIAMADON & al. (2012) in Cross River, south-eastern Nigeria. Consequently, the drug is in short supply leading to the scarcity of ACTs (presently the most effective treatment for malaria) and needed in countries where the disease is endemic [BRISIBE & al. 2008].

For these reasons, the availability, accessibility and affordability of *A. annua* remain a problem especially in sub-Sahara Africa where malaria is endemic. Therefore, the improvement in naturally occurring artemisinin through good agricultural practice of *A. annua* is the only possible alternative at a moment. Despite this limitation to fulfil the growing demand of the drug worldwide, there is no information on the germination, growth and artemisinin content of *A. annua* grown in Savanna region of Nigeria.

Combinations of artemisinin and other anti-malarial drugs, such as mefloquine or lumefantrine, have been proven to be highly effective against the multidrug-resistant *Plasmodium falciparum* [PRICE & al. 1996; VAN VUGT & al. 2000]. In addition to this, artemisinin and its derivatives have been shown to be effective against a number of viruses, *Pneumocystis carinii*, *Toxoplasma gondii*, a number of human cancer cell lines [EFFERTH, 2007], tuberculosis [ZHENG & al. 2017], diabetes [LI & al. 2017] and a variety of other parasitic tropical diseases including schistosomiasis [UTZINGER & al. 2001], leishmaniasis [SEN & al. 2007], Chagas disease and African sleeping sickness [MISHINA & al. 2007]. Recently Madagaska's Institute of Applied research has produced Artemisia-containing tonic that supposedly prevent and treat COVID 19 [RAJOELINA, 2020]. All these diseases probably can be successfully treated with artemisinin and Artemisia plant if enough of the drug is made available and also affordable for developing countries. There is still a worldwide shortage of the drug just for treating malaria let alone other diseases against which artemisinin is promising [DE RIDDER & al. 2008]. It is estimated that the worldwide area needed to meet the current Boston Consulting Group (BCG) estimated demand for 275 million ACTs for 2015 is 23,000 hectares, based on the estimate that 1 ha^{-1} of *Artemisia* produces enough artemisinin for approximately 25,000 adult courses of ACT [NAS, 2004]. Producing artemisinin from *A. annua* is currently the only economical alternative, thus its availability is limited by low plantation. To meet the artemisinin demand in Africa, African Botanicals have to expand area and region of planting the plant. As a result, the expansion in regions under cultivation of the plant for the production of naturally occurring artemisinin through the good agricultural practice is desirable. Information on the adaptability of *A. annua* to Sokoto agro ecological zone of Nigeria is not available and hence the important for the current study.

The aim of this study is to evaluate the influence of soil types on growth and artemisinin content of *Artemisia annua* in Sokoto, Nigeria.

Material and methods

Study area

The study was conducted in the Department of Biological Science of Usmanu Danfodiyo University Sokoto. The main campus lies between latitude 13º06'-13º08' N, longitude 5º11'-5º12' E and altitude of 351.0 m above the sea level. It has about 70-125 days of rainy season and long dry season throughout the year. The mean annual rainfall is 700 mm per annum (SERC - Sokoto Energy Research Centre, 2016).

Collection of plant materials and soil

Seeds of *Artemisia annua* Chen Young variety were sourced from Artemisia Programme Unit at the Institute for Agricultural Research (I.A.R.) Ahmadu Bello University, Zaria. Three different soil types used in this research were; clay, sandy and loamy soils. Clay was obtained from University research farm in Kwalkwalawa while sandy and loamy were obtained from Biological Sciences garden of Usmanu Danfodiyo University and Nakasari area of Sokoto state respectively.

Experimental design and germination studies

The experiment was designed to investigate the influence of three different soils on germination, growth and Artemisinin contain of *A. annua*. Treatment consisted of three soils types replicated four times in a completely randomized design. The soil was potted and

replicated four times and in each pot one hundred and fifty seeds were sown (150). Random sampling was applied to both the experimental media and test plants. Transparent wire cage was used to cover the germinating media (sands) against pest and the cage was covered with transparent polythene bag to maintain adequate moisture, temperature and humidity levels in the soil which are essential for Artemisia seeds germination. The physical and chemical properties of the soil used in the experiment are presented in Table 4.

Germination studies

The seeds obtained from pilot test were collected and sown on March 20, 2017 and observation was made at 24 hours interval (a day) after sowing. Data collected include the time in days (t_i) of sowing, number of seeds germinated at each observation (n_i). The expressions for the most important germination parameters according to RANAL & al. (2009) were considered, these include; germination capacity (germinability or seedling emergence), time spent to germinate or emerge (mean germination time), speed (mean germination rate), homogeneity (coefficient of variation of the germination time), uncertainty and synchrony.

Germinability was calculated according to the formulae cited by LABOURIAU & VALADARES (1976):

Percentage germination:

$$G = \left(\frac{N}{A}\right) \cdot 100$$

where, G = percentage of germination; N = number of germinated seeds; A = total number of seeds sowed.

Mean germination time was calculated by the expression:

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

where, t_i: time from the start of the experiment to the ith observation (day for the example); n_i: number of seeds germinated in the ith time (not the accumulated number, but the number correspondent to the ith observation), and k: last time of germination.

Variance of germination time was calculated by the expression:

$$s_t^2 = \frac{\sum_{i=1}^{k} n_i (t_i - \bar{t})^2}{\sum_{i=1}^{k} n_i - 1}$$

where, t: mean germination time; t_i: time between the start of the experiment and the ith observation (day for the example); n_i: number of seeds germinated in the ith time, and k: last time of germination. The variance value will be used to calculate the CV_t.

Coefficient of variation of the germination time is calculated by the expression:

$$CV_t = \frac{s_t}{\bar{t}} 100,$$

where, s_t: standard deviation of the germination time and t: mean germination time. Replications or samples with only one seed germinated do not have the value of this measurement because the divisor of the variance of the germination time is zero.

Mean germination rate is the average speed of germination was calculated as the reciprocal of the mean germination time:

$$V = \frac{1}{t}$$

where, V = average speed of germination; t = mean germination time.

Uncertainty of germination was calculated by the expression:

$$U = -\sum_{i=1}^{k} f_i \log_2 f_i, \text{ being } f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$$

where, n_i: number of seeds germinated on the ith time, and k: last day of observation.

Synchrony of germination was calculated by the expression:

$$Z = \frac{\sum_{i=1}^{k} C_{n_i,2}}{C_{\sum n_i,2}}, \text{ being } C_{n_i,2} = n_i(n_i-1)/2$$

being $C_{ni} = n_i(n_i-1)/2$, where $C_{ni,2}$: combination of the seeds germinated in the ith time, two by two, and n_i: number of seeds germinated in the ith time.

Z is the quotient between the sum of the partial combinations of the number of seeds germinated in each t_i, two by two and the two by two combination of the total number of seeds germinated at the end of the experiment, assuming that all seeds that germinated did so simultaneously. While waiting for the seeds to germinate, the soils were kept moist and damp by regular watering.

Effects of soil type on growth and artemisinin content in *A. annua*

To evaluate the effects of soil type on growth and artemisinin content, three different soils of the same quantity (15 kg) were potted namely loamy, sandy and clay. The three treatments were replicated four times and laid out in Completely Randomized Design (CRD). Four weeks old seedling of 12 cm height were transplanted to the experimental pots and observed for seven months. The following morphological characters were measured: plant height, length of the longest branch, branch numbers and days to flowering.

Plant height and length of the longest branch were measured in centimeter using thread and meter ruler. Branch number were numerically counted, days to flowering was taken by counting the days taken for the plant to flowers.

After harvesting, root length and fresh weight are measured. Fresh weight was measured using weighing balance (L.P 202) and the biomass was sun dried until constant weight was obtained, as experience shown that natural sun drying produces high artemisinin content [ZHANG & YU, 1997]. The dry weight of the sample was also measured. The sun dried plant sample was divided into leaves, stem and roots which were grinded using mortar and pestle, and the ground powder was used for Soxhlet extraction.

Extracts preparation

Fresh leaves of *A. annua* L. were dried for two weeks, pulverized into powder using mortar and pestle and homogenized. Adopting the method of CHRISTEN & VEUTHEY (2001), 5 g of pulverized sample was extracted with 200 mL of *n*-hexane at 60 °C in Soxhlet apparatus. The hexane was then evaporated under a vacuum and the samples reconstituted in 10 ml acetonitrile then filtered through whatman filter paper.

Preparation of standards and high performance liquid chromatography

Standard artemisinin was purchased from Sigma-Aldrich (Germany). Artemisinin solution was obtained by dissolving 10 mlg in 100 ml of acetonitrile to form the stock solution, from the stock solution, five different concentration of 5, 10, 15, 20 and 25 µg/ml were formed. Each was run 3 times in HPLC, from the result, Standard calibration curve was obtained by plotting the peak area against the concentration of ART standard solutions.

The HPLC analyses were performed with Agilent Technologies 1200 series on Eclipse XBD-C18 (4.6 x 150 mm), column and detection was conducted at 214 nm wavelength. The acetonitrile was used as a mobile phase with 0.8 ml/min flow rate [LAPKIN & al. 2009]. Injection column was 10 µL; run time of 20 min, temperature 30 °C. The gradient HPLC-UV method is widely recommended and used for quantification of artemisinin purity and amount in plant material.

Calibration curve

The calibration curve was constructed by plotting the peak area against the concentration. The Concentrations ranging between 5 and 25 µg/ml of artemisinin were prepared from the standard stock solution by serial dilution with acetonitrile for the calibration data. The calibration curves were obtained by the external standard method on five levels of concentration of standard (5, 10, 15, 20, and 25 µg/ml), with three injections per level. Linear regression was used to establish the calibration curve. Results were calculated using the peak areas with determination coefficient (R^2) was 0.951.

Soil analyses

Some of the physico-chemical properties of soil samples analysed include particle size distribution, organic C, total N, available P, exchangeable bases, soil pH and CEC. Particle size analysis was carried out using Boyoucos hydrometer method as described by GEE & BAUDER (1986). The textural class was determined using USDA soil textural triangle. Soil pH was determined with a glass electrode pH meter 1:2 soil:water ratio [BATES, 1954]. Total N was determined using micro-Kjedahl method as described by NELSON & SOMMERS (1982). Available P by Bray No. 1 Method [BRAY & KURTZ, 1945] and exchangeable bases were determined for calcium and magnesium using EDTA titration method as described by DEVIS & FREITAS (1970) and potassium using flame photometry [RICH, 1965].

Data analysis

The data obtained were analyzed using one-way analysis of variance with Minitab Statistical Software vision 17. Significant means were compared using Turkey simultaneous test at P<0.05.

Results and discussion

Germination of *A. annua* seeds on all the soil types tested was successful as they germinated promptly when subjected to conditions which are normally regarded as suitable for germination indicating that they are not dormant. The seeds do not require to be artificially subjected to some physical and chemical pre-sowing treatment in order to overcome the dormancy and prematured period as uniform, rapid and synchronous germination was observed in this study with 73.6, 56.6 and 56.33% germination on clay, loamy and sandy soils respectively (Table 1). This finding was in disagreement with previous report that chemical dormancy due to phenolic secretion on the seed of *A. annua* was overcome by the use of Gibberellic acid [BEWLEY & MICHAEL, 1994; NICOLAS, 2003; FAROUK & al. 2008].

Twenty four (24) hours after sowing there was no record of germination. However, germination commenced 48 hours after sowing on all the soil types at the same time. This confirms the result of MÜLLER & BRANDES (1997) that seeds of *A. annua* germinated at the same time on different soil types tested. JAMALEDDINE & al. (2011) reported that 80-90% germination of *A. annua* started 5th to 7th days after seeds inoculation and addition of hormones (BA and NAA) do not alter the duration of germination (Info net biodivision, 2010). At Zaria, Kaduna State, research by TAHIR & al. (2013) showed that germination of *A. annua* seeds commenced 3 days after inoculation and 50 to 60% seeds germinated to plantlets.

The early germination observed may be attributed to the high temperature of the state (average 29 °C and relative humidity 14% in January, 2017), which is essential for *Artemisia* seeds germination. Similarly, HARTMANN & al. (1997) reported that temperature is the single most important factor in the regulation of the timing of germination, because of its role in dormancy control and/or release, or climate adaptation.

The highest germination count was observed on day 4 with 33.5, 37.5 and 16.6% seedlings on loamy, clay and sandy soils respectively. Germination was recorded last on day 9 on loamy and day 10 on clay and sandy soils respectively. The first pair of leaves appeared or opened between 4 to 5 days after germination. This agreed with the WHO report that appearance of the first pair of leaves commenced between 4 to 5 days in Kenya and Tanzania. Two weeks after germination, the second set of leaves began to appear. Five days after the appearance of the second set of leaves, the third set appeared.

Germination measurements of *Artemisia annua* seeds

The results of germinability (G), Mean germination time (MT), Coefficient of variation of the germination time (CV_t), Mean germination rate (MR), Uncertainty (U) and synchrony (Z) of the germination of *A. annua* are presented in Table 1. The results showed that soil type has no significant (P>0.05) effect on all the parameter evaluated (G, MT, CV, MR, U and Z). However, clay soil was the most effective treatment in enhancing germinability with 73% seeds germinated, followed by 56 and 55% on loamy and sandy soils respectively. This may be attributed to the ability of clay soil to support the plants by its favorable water retention and its available macro and micro nutrients compared to loamy and sandy soils and neutral soil pH as earlier reported by ABOU HUSSIEN & al. (2010) (Table 5). Artemisia seeds require light and uniformly high level of moisture at temperature of 18-20 °C. This discovery contrasts the finding of SCHÜTZ & al. (2002) that observed germination to commence earlier and faster in sandy soil than loamy. However, MÜLLER & BRANDES (1997) stated that germination and growth of *A. annua* was limited in sandy soil as it reaches the average height of 24 to 66.5 cm. Seeds sown in clay soil generally had the highest emergence percentage and mean germination time

as compared with the seeds sown in sandy and loamy soils (Table 1) but the difference in germination percentage was not significant (P>0.05). The coefficient of variation of the germination time was proposed to evaluate the germination uniformity or variability in relation to the mean germination time and was applied to seeds of *Myracrodruon urundeuva* Allemão [DORNELES & al. 2005] and *Anacardium humile* A. St.-Hil. [CARVALHO & al. 2005]. Sandy soil had the highest coefficient of variation of germination time, a direct interpretation on the sense that high values would be associated with concentrated germination in time (seedlings weremore uniform in size). Thus, the sandy soil had more frequent germination over time than clay and loamy but means comparison showed that they were not significantly different (P>0.05). The rate of germination is also quite higher on sandy soil than clay and loamy soil with means values of 0.2264, 0.2102 and 0.1839 (Table 1). The results revealed that the rate at which seeds germinated was significantly the same (P<0.05) when compared with other soil types.

Uncertainty of germination(U)measures the degree of spreading of germination through time and can be used, by inference, to measure the synchrony of germination. Low values of U indicate frequencies with few peaks, that is, germination more concentrated in time. Only one seed germinating changes the value of U. Germination was more uncertain in clay soil than loamy and sandy soil because it had the least mean values of 2.277 bit and high value of mean germinated time. Frequency with high peaks in germination occurs most in sandy soil hence it had the highest value of U (2.424 bit). Statistically however, U values appear to be the same when compared between the treatments.

Synchrony of germination (Z) produced a number if and only if there are two seeds finishing the germination process at the same time. Thus, itmeasuresthe degree of germination overlapping). Z is zero when no overlapping was observed for n germinated seeds and will be null when no seed could complete the germination process [RANAL & al. 2009]. Clay soil had the highest overlapping emergence among the treatment followed by loamy and sandy soil. Mean comparison showed that degree of germination overlapping was thesame among the treatment (P<0.05) using Turkey simultaneous test.

Table1. Germination measurements (mean ± standard deviation) of *Artemisia annua* seed on different soil types

Soil type	G (%)	MT(day)	CV$_t$ (%)	MR (day^{-1})	U (bit)	Z
Clay	73.8±21.3 [a]	4.803±0.6 [a]	35.44±1.8 [a]	0.2102±0.02 [a]	2.2777±0.24[a]	0.2474±0.06 [a]
Sandy	56.0±10.3 [a]	4.454±0.5 [a]	34.68±2.9 [a]	0.2264±0.02 [a]	2.4246±0.20[a]	0.2080±0.04 [a]
Loamy	55.5±21.8 [a]	4.413±0.1 [a]	24.37±12.4 [a]	0.1839±0.08 [a]	2.2909±0.14[a]	0.2340±0.04 [a]

G – germinability, MT – Mean germination time, CV$_t$ – Coefficient of variation of the germination time, MR – Mean germination rate, U – Uncertainty and synchrony of the germination (Z). Means followed by the same superscript in each column are not significantly (P>0.05) different based on the Tukey test.

Effect of soil type on growth of *Artemisia a*nnua

The effect of soil type on plant height of *A*. *annua* is presented in Figure 1. The result revealed that soil types do not shows any significantly (P>0.05) affect on plant height. However, height of *A*. *annua* grown in sandy soil was higher than those grown in clay and loamy soils respectively during the first eight weeks after planting (WAP). At the tenth week, loamy soil had the highest plant height with 50.40 cm followed by sandy and clay with 49.20 and 48.60 cm soil respectively. This result corroborates with the finding of OMER & al. (2013) who reported that

A. annua grown under clay loamy soil had the highest vegetative growth parameters than those in sandy loamy soil. Clay soil had the highest plant height at 14 and 16 WAP while the lowest mean value was observed in sandy (88.80) at 16 WAP. In general, sandy soil had the best of plant height with the highest mean values of 22.60, 30.20, 35.80, 40.00, 60.80 and 71.40 at 2, 4, 6, 8 and 12 weeks respectively. Plant height in all the soil types significantly increased with the plant age to reach its maximum at flowering after 150 days and recorded 114, 108 and 97 cm in clay, loamy and sandy respectively. These values obtained were lower than those recorded by OMER & al. (2013) at 210 days (371 and 247 cm in clay loamy and sandy loamy soil). Similarly, MUELLER & al. (2000) stated that *A. annua* grow rapidly into a larger plant at higher altitudes (2000 m) where they reach the average height of 2.5 m in 7-8 month after germination.

Mean number of branches of *A. annua* grown in three different soils is presented in Table 2. The result shows that soil types had no significant (P>0.05) effect on the number of branches. This finding contradict the report of OMER & al. (2013) who noted that loamy soil produces the highest number of branches (58) than those grown in sandy loamy soil with 51 branches. The numbers of branches increased significantly between first and second month and remain the same in third and fourth month on clay and loamy soil.

The result of the longest branch length of *A. annua* plant is presented in Table 2. The result revealed that no significant (P>0.05) differences were observed between the experimental groups and length of the longest branch of *A. annua* in October and November. However, loamy soil had the highest branch length than clay and sandy in third and fourth month respectively, means comparison shows that treatment has significantly (P>0.05) effect on the longest branch length of *A. annua* plant with the highest mean on loamy (43.0), clay (34.0) and sandy (28.7) respectively. The longest branch length of *A. annua* increased significantly with the progress of plant age.
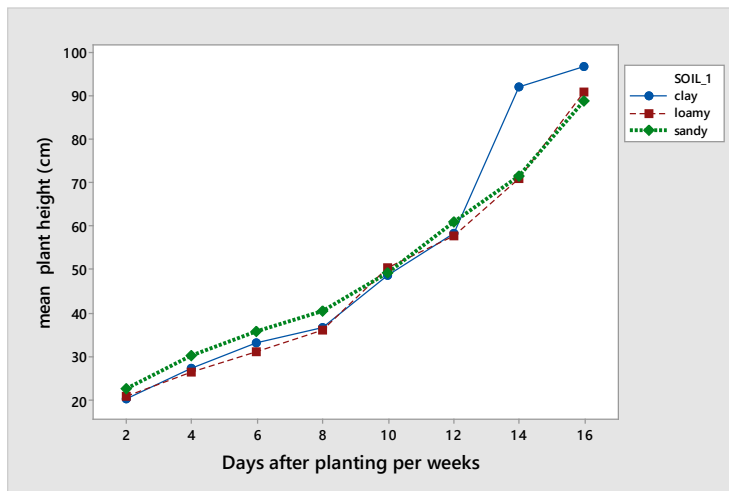

Figure 1. Effect of soil types on plant height of *A. annua*

Table 2. Effect of soil types on the number of branches and longest branch in *A. annua*.

Parameters	Treatment	Sampling period (Month)			
		1	**2**	**3**	**4**
No. of Branch	Clay	18.00 ± 4.12	23.20 ± 4.21	24.80 ± 4.02	26.20 ± 3.27
	Loamy	22.20 ± 7.82	27.00 ± 7.62	27.40 ± 6.07	29.40 ± 6.23
	Sandy	23.80 ± 11.90	27.80 ± 9.81	28.40 ± 9.10	31.00 ± 9.59
	SE	5.41	5.08	4.26	4.40
Branch length	Clay	16.20 ± 6.10	23.60 ± 3.21	29.00 ± 4.53[ab]	34.80 ± 6.69[ab]
	Loamy	17.60 ± 7.44	25.50 ± 7.72	34.80 ± 6.30 [a]	43.00 ± 11.75[a]
	Sandy	13.40 ± 4.22	25.60 ± 1.52	26.80 ± 2.17[b]	28.75 ± 0.957[b]
	SE	3.83	3.06	2.92	5.48

Replication × 5. Means followed by same superscript in a column are not significantly different (P>0.05). SE; Standard error.

Effects of soil types on days to flowering

The number of days to flowering is presented in Table 3, the result shows that soil types had no significant effect on days to flowering in *A. annua* plant (P>0.05). However, sandy soil had the longest period to flower followed by clay and loamy with 150, 147 and 145 days respectively, in respect to days to flowering, sandy soil will produces the highest biomass than clay and loamy soils since the longer the days the better the leaves biomass and artemisinin content. *A. annua* grown in savanna region had the longest time to flowering than those planted in Lucknow (26º51' N) that flower earlier with life span of 75 days [SINGH & al. 1988]. This finding totally disagreed with MARCHESE & al. (2005) finding who proposed that *A. annua* is not suited to African continent because of short photoperiod that will make it flower early. Report shows that fertilizer types had significant effect on flowering time. The longest flowering time was observed in treatment with cow manure compost with medium proportion of 1:4, which is 77 days, while treatment with horse manure as a medium treatment showed the fastest flowering time of 42 days [PAMBUDI & al. 2017].

Effect of soil types on plant height at flowering

Loamy soil significantly (P<0.05) enhanced plant height parameter at the flowering stage of *A. annua* than those grown in sandy and clay soils (Table 3). The difference between the soils at this stage (plant height at flowering) may be ascribed to slightly alkalinity of the soil (7.8) (Table 5). This finding disagreed with OMER & al. (2013) that revealed that at flowering and maturing stage the differences in plant height between clay loamy and sandy loamy were insignificant (between 180 and 210 days after planting).

Effect of soil types on stem diametre of *A. annua*

Plants cultivated in clay soil showed a positive increase in the stem diameter than those cultivated in sandy and loamy soils. This may be attributed to present of high organic carbon, organic manure, Nitrogen, Phosphorus, Potassium, Calcium, Sodium and cation exchange capacity content than those found in loamy and sandy soils (Table 4). This goes in line with previous report that diameter of *A. annua* cultivated in clay loamy soil was higher than those cultivated in sandy loamy soil and it was true in the two seasons in all plant stages [OMER & al. 2013].

Table 3. Effect of soil types on days to flowering, plant height at flowering
and stem diameter of *A. annua*

Treatment	Days to flowering	Plant height at flowering (cm)	Stem diametre (cm)
Sandy soil	150.75 ± 13.60	97.33 ± 7.57[b]	0.767 ± 0.611
Clay soil	147.50 ± 4.04	88.67± 1.528[b]	0.827 ± 0.433
Loamy soil	145.33 ± 3.79	118.00 ± 9.54[a]	0.593 ± 0.222
S.E	6.79	5.79	0.67

Replication × 5. Means followed by same superscript in a column are not significantly different (p>00.5).
SE Standard error.

Table 4. Physical and chemical properties of soils used in the experiment

Treatment	pH	Org. C (%)	Org. M (%)	N (%)	P (mg/kg)	Ca	Mg	K (cmol kg⁻)	Na	C.E.C.	% sand	% silt	% clay
Clay	7.0	0.74	1.28	0.116	0.88	0.85	0.70	0.85	0.65	8.6	38.4	17.1	44.5
Loamy	6.8	0.12	0.21	0.070	0.79	0.65	0.45	0.72	0.39	7.8	79.8	10.1	10.1
Sandy	7.6	0.38	0.66	0.039	0.65	0.50	0.25	0.23	0.22	5.6	89.60	6.59	3.81

pH: degree of acidity or alkalinity of a substances, Organic carbon (Org. C), Organic Mineral (Org. M), Nitrogen (N), Phosporus (P), Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), Cation Exchange Capacity (C.E.C.).

Effect of soil type on artemisinin content in Artemisia *annua*

The results of HPLC analysis of artemisinin content of *A. annua* are presented in Table 5. The result revealed that sandy soil significantly ($P \leq 0.05$) had the highest artemisinin content in *A. annua* than clay and loamy soils. This is follow by clay and loamy soils with 17.9 and 15.70 µg/ml respectively. This difference may be attributed to slightly alkalinity of soil ph (7.6), fertility stress and the poor water retention property (Table 4). Similar report were made by OMER & al. (2013), that, *A. annua* plants growing in the sand loamy soil, showed a positive increase in artemisinin percentage comparing to those growing in clay soil. This result confirmed the previous works of GUPTA & al. (2002) and KUMAR & al. (2004). JESSING & al. (2013) revealed that, the largest contributor of artemisinin lost in *A. annua* was allelophatic effect of dead leaves in the soil environment. PRASAD & al. (1998) reported that, artemisinin content in the vegetative tissue was not influenced with a salinity stress of 10.4 dS/m. Variations in artemisinin content were reported in different plant parts, different stages of vegetative growth and strain origins [LAUGHLIN & al. 2002]. *A. annua* from Italy was reported to contain only 0.04% to 0.05% artemisinin dry weight. Artemisinin contents from other European origins ranged from 0.03% to 0.22% [CHARLES & al. 1991], while those obtained from China varied from 0.01% to 0.50% dry weight [KLAYMAN, 1985]. BUI THI & al. (2011) concluded that, the growth of the *A. annua* and the variations in artemisinin contents were attributed more strongly to environmental factors than to genetic factors because two similar clones planted at the two different study sites gives different artemisinin content.

Genotype and the environment are two factors that alter artemisinin content in *A. annua*. Artemisininvaries between 0.01 to 0.4% and some clones produce over 1% [DELABAYS & al. 1993]. Estimate of yield per hectare varies significantly, but reports indicate a yield of 10-15 kg/ha from well-managed plantations in Africa [WRIGHT, 2002]. Reports on the distribution of artemisinin and its derivatives throughout the plant is inconsistent. Artemisinin has been reported to be higher at the top of the plant in some clones [CHARLES & al. 1991] and equally distributed in others [LAUGHLIN, 1995].

Table 5. Artemisinin content of *A. annua* grown on different soils in Sokoto (µg/ml)

Soil type	Ret time (min)	Area (mAU*s)	Conc. (µg/ml)
Sandy	2.518 ± 0.007	22408 ± 4524[a]	37.73[a]
Loamy	2.498 ± 0.018	9322 ± 2872[b]	15.70[b]
Clay	2.494 ± 0.012	10616 ± 1289[b]	17.90[b]

means followed by same superscript in a column are not significantly different (p>00.5).

Conclusion

This study concludes that *A. annua* seeds could germinate and grow on different soil types in Sokoto geo-ecological environment andprobably all the surrounding areas that shares the same environmental, ecological and geographical factors for the first time as a promising cultivation area for *Artemisia annua* with artemisinin content. This new promising cultivation area may be used in a large scale in order to improve the overall supply of artemisinin. Soil types have no significant effect on germination, plant height, number of leaves, days to flowering and stem diametre. However, loamy soil significantly enhances plant height at flowering stage and length of the longest branch of *A. annua*. Artemisinin content of *A. annua* grown in sandy soil was significantly higher than that produced by clay and loamy soils. Pretreatment of *A. annua* stem cuttings with IBA 400 and 600 ppm significantly increased percentage survival and reduce mean regenation time when compared with control. The overall growth performance parameters indicated that 600 ppm on apical stem cuttings were significantly superior to all other stem cuttings in term of percentage survival and mean regeneration time.

Recommendations
This study recommends the use of Sandy soil in cultivation of *A. annua* plant in savanna region of the country, for its influence artemisinin content accumulation to the farmers and pharmaceutical companies.

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