KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA.

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

DEPARTMENT OF CROP AND SOIL SCIENCES

ORGANIC AND INORGANIC FERTILIZERS APPLICATION ON THE GROWTH, YIELD AND ARTEMISININ CONTENT OF *ARTEMISIA ANNUA L.* IN THE HUMID TROPICS OF GHANA.

BY

STEPHEN YEBOAH

JUNE, 2010

ORGANIC AND INORGANIC FERTILIZERS APPLICATION ON THE GROWTH, YIELD AND ARTEMISININ CONTENT OF *ARTEMISIA ANNUA L.* IN THE HUMID TROPICS OF GHANA.

A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, COLLEGE OF AGRICULTURE AND NATURAL RESOURCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN AGRONOMY (CROP PHYSIOLOGY).

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DECLARATION

I hereby declare that the research work presented in this thesis is my own original research and that, to the best of my knowledge; this thesis has not been presented at any occasion by another person for the award of a degree. References to other people's work have been duly acknowledged.

Stephen Yeboah …… ……
(Student) *Signature* Date

I declare that I have supervised the student in undertaking the research submitted herein and confirm that the student has my permission to present it for assessment.

Certified by:

Prof. R. Akromah …… ……..
(Supervisor) *Signature* Date

Prof. R. Akromah …… ……..
(Head of Department) *Signature* Date

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DEDICATION

To God as my provider.

ABSTRACT

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, yield and artemisinin content of *Artemisia annua*.

The experiment also examined the effects of variation in the stage of harvest on the plant growth and 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 t/ha. During the study, data were collected on: plant height, plant canopy spread, stem width, internodes distance, number of branches per plant, fresh and dry leaf yield (kg/ha), fresh and dry shoot weight (g), 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 at both pre-flowering and full bloom harvests when compared to other treatments. Growth, development and yield response to organic and inorganic fertilization by *Artemisia annua* largely followed the trend 4 t/ha PM> 90 kg N/ha > 6 t/ha PM > 45 kg N/ha > 2 t/ha PM > 0 kg N/ha. The results indicated that application of the treatments significantly affected most of the parameters when compared to the control. There was significant differences ($p<0.05$) between the treatments for plant height, plant canopy spread, stem width, number of branches, fresh and dry leaf yield at pre-flowering, dry leaf yield, fresh and dry shoot weight, artemisinin content and artemisinin yield at full bloom stage. The results of the study also indicated that while the highest crude extract weight was obtained from 4 t/ha poultry manure at pre-flowering, the highest crude extract weight was obtained from 90 kg N/ha compound fertilizer at full bloom. However, no significant differences ($p>0.05$) among the different fertilizer rates were observed. Application of 4 t/ha poultry manure gave the highest artemisinin yield of 9.57 kg/ha and 37.24 kg/ha at both pre-fowering and full bloom stages, respectively. The results also showed considerable increase in crude extract weight and artemisinin content at pre-flowering compared to values recorded at full bloom. The results showed that the artemisinin yield was positively correlated with leaf

yield and stage of harvests. From the results of this study, it is recommended that application of 4 t/ha poultry manure can be used as an alternative to compound fertilizers which are expensive. This will reduce the cost of *Artemisia annua* production.

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CHAPTER ONE

1.0 INTRODUCTION

Plants continue to be a major source of medicines in the maintenance of human health throughout the world and notably in the tropics. Over 50% of prescription drugs are derived from chemicals first identified in plants (Chandrasekhara, 2004). Interest in medicinal plants is becoming more recognized in health care delivery particularly in developing countries because they are affordable, readily accepted by consumers and locally available (Brown, 1994; Abbiw *et al.*, 2002; WHO, 2003). In many African countries, the significance of traditional medical practitioners is now recognized and attempts are being made to integrate western and indigenous medicine (Brown, 1994). It is estimated that 80% of the people worldwide depend on traditional medicine to meet their primary health care needs (WHO, 2003). Medicinal plants are important in poor countries where western medicines remain expensive (Technoserve, 2004). In Ghana, about 75% of the population, in both the urban and the rural areas, depend on medicinal plants for their health-care needs (Abbiw *et al.*, 2002). Since the productivity of a nation is greatly dependent on the health of its citizens, medicines with less or no side effects are highly preferred in low- income economies like Ghana.

Medicinal and aromatic plants occupy an important economic position because of the continuous and increased demand for their products from local and foreign markets. There is therefore, the need for a new strategy for developing these medicinal plants as commercial crops.

This could be both as an alternate crop for larger commercial farms and for small-scale farmers. Artemisia is one of the most important medicinal plants in this area.

It is a plant for the production of anti-malarial, 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 with low levels in the stem and none in the pollen and the roots (Ferreira and Janick, 1996).

The production and trade of artemisia have gained prominence in many developed and under developed countries in recent times. The crop 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 vivix*, including multidrug-resistant strains (Ferreira *et al.*, 2005).

Depending on its genotype and the environment, artemisinin content in *Artemisia annua* varies between 0.01 to 0.4% and some clones produce over 1% (Delabays *et 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 it's derivatives throughout the plant is inconsistent. Artemisinin has been reported to be higher at the top of the plant in some clones (Charles *et al*., 1990) and equally distributed in others (Laughlin, 1995).

The production of leaf biomass yield varies significantly with environment and management (Khalid and Shafei, 2005). Total fresh biomass yield of 275 - 750 g/plant

has been reported (Simon *et al*., 1990). The biomass yield of dry leaf varies between 1 - 40 t/ha (Wright, 2002). To obtain satisfactory yields, it is recommended to apply fertilizer at a rate of 67 kg N/ha at high density planting distance (Simon *et al*., 1990). The most concentrated areas of artemisia production are in Asia, Europe, and USA and recently in East Africa (Klayman, 1993). The increase in artemisia production in these areas is due to favorable climatic conditions and the adoption of improved technologies.

Concerns have been expressed recently about continuing increase in malaria cases and its impact on the socio-economic development of many countries in sub-Saharan Africa. It is reported that the annual economic growth of countries with intensive malaria is 1.3% lower than that of countries without malaria (Sachs and Malaney, 2002). Each year, malaria is estimated to kill an average of 2.7 million people and over 90% of these cases occur in sub-Saharan Africa (Nussenzweig and Long, 1994).

A number of control measures employed against malaria have their weakness. For instance, some strains of the malaria parasite have developed resistance to traditional treatments using quinine and chloroquine, which were previously effective (Snow *et al*., 2005). There is therefore an urgent need for affordable and effective treatment alternatives. The World Health Organization (WHO) has recommended the use of Artemisinin-Based Combination treatments such as artemether-lumefantrine, sulfadoxine/pyrimethamine as the first line treatments for multidrug – resistance strains of malaria (artesunate-mefloquine, artesunate-amodiaquine, and artesunate-sulfadoxine) (WHO, 2004).

Artemisinin accounts for less than 1% in the international anti-malaria medicine market due to the shortage of artemisinin raw materials (Simon *et al*., 1990). This is because the promising anti-malaria compound remains expensive and hardly available on the global scale. However, the demand for artemisinin based therapies is high since the parasite is rapidly developing resistance to all other drugs.

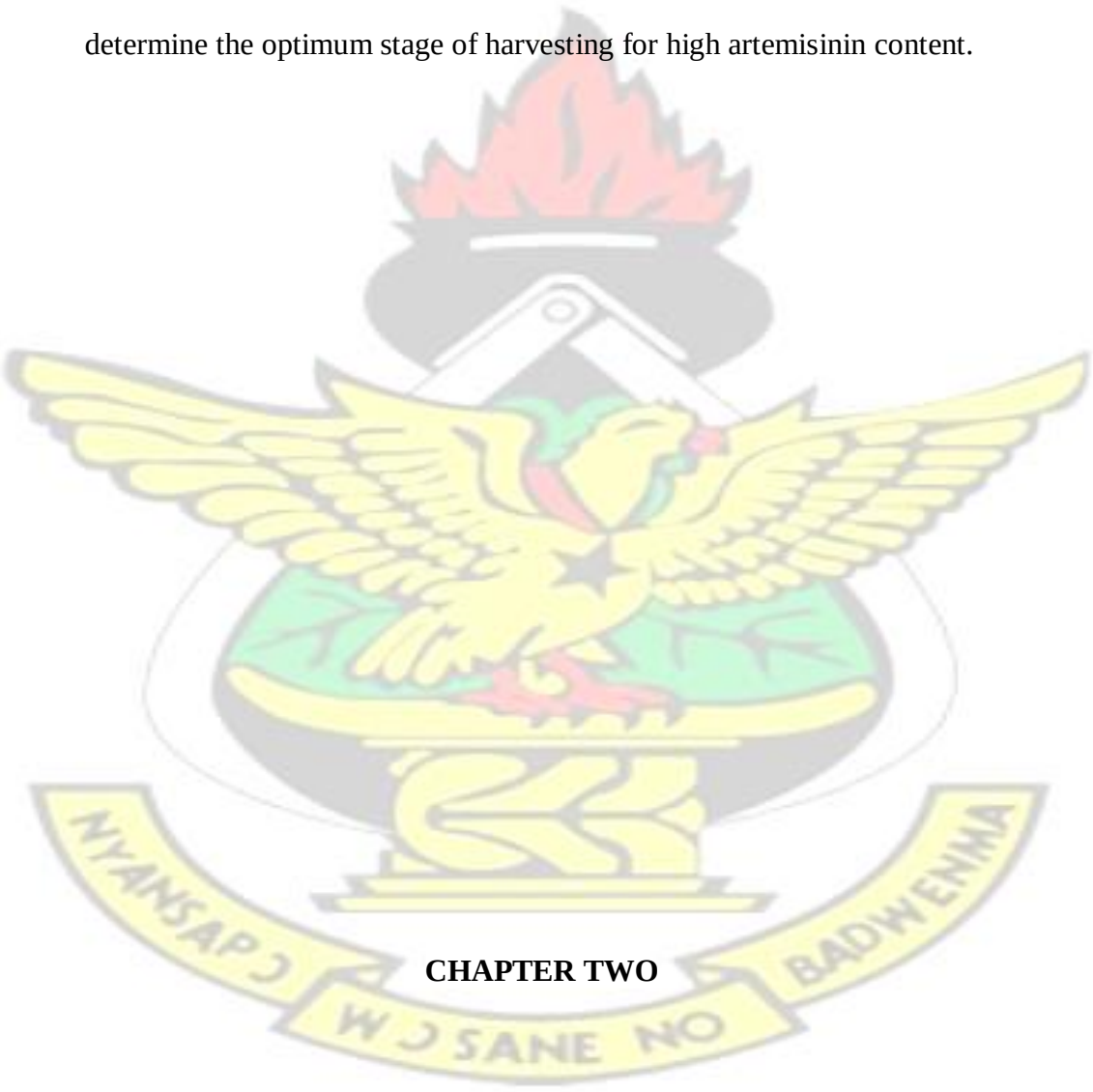
Field production of *Artemisia annua* is therefore; recommended as the only commercially viable method to produce artemisinin since the total chemical synthesis of the molecule is complex and uneconomical (Yadav *et al*., 2003). However, due to the rapid population increase in Ghana and increased pressure on land resulting in reduced fallow period, and continuous cropping, most soils are depleted of their nutrients (Ofori *et al*., 1993).

Soil amendments such as nitrogen fertilizers are reported to increase the total leaf biomass and thereby increased total essential oil and artemisinin obtained from *Artemisia annua* (Simon *et al*., 1990), but these have not been evaluated in Ghana. However, with the withdrawal of subsidies on agricultural inputs, most farmers cannot afford the high prices of compound fertilizers (GGDP, 1991). In view of this, soil fertility is now a problem in most farmers' fields because they either do not apply fertilizer at all or may use inadequate quantities of organic manure. It would therefore be useful to estimate the leaf biomass, crude extract yield, artemisinin content and yield of *Artemisia annua* under different levels of nitrogen fertilizer and poultry manure application rates.

Working on the hypothesis that application of organic and inorganic fertilizers would have significant effect on the growth and yield of artemisia, the objectives of this study were to:

i. determine the effects of different levels of poultry manure and compound fertilizer on the growth and yield of *Artemisia annua* L.

ii. assess the levels of artemisinin in the plant under the different treatments iii. determine the optimum stage of harvesting for high artemisinin content.



CHAPTER TWO

2.0 LITERATURE REVIEW 2.1 ORIGIN AND BOTANY OF *ARTEMISIA ANNUA*

Artemisia (*Artemisia annua* L.) also known as sweet wormwood, originated from China and is the most important and popular herbaceous plant in the daisy family Asteraceae (McVaugh, 1984). The crop grows to a height of 1 – 3 m and 1m in width and it is an annual plant with a growth cycle of about 180 days (80 days in the nursery and 100 days on the field) (Ferreira and Janick, 1995). The plants are generally longer lived, more hardy and aromatic when grown in poor dry soil. *Artemisia annua* is a large shrub with a single-stem and alternate branches and the leaves are aromatic; fern-like and deeply dissected (plate 2.1) and ranges from 2.5cm to 5cm in length (Whipley *et al.*, 1992).

The plant has a short tap root and aggressive fibrous root (Laughlin, 1994). Mitchell (1975) noted that artemisia has flowers which are greenish-yellow and about 2 to 3 mm in diameter. He further found out that the pollen has no spines but is extremely allergic.

The most valuable parts are the leaves and the flowers where artemisinin is concentrated (Ferreira and Janick, 1995). Glandular trichomes are more prominent in the corolla and receptacle florets. There is strong evidence that artemisinin is sequestered in the glandular trichomes (Duke and Paul, 1993).

Plate 2.1: Photographs of *A. annua* foliage (left) and developing florals buds (right)

Source: (Ferreira and Janick, 1995).

2.1.1 Distribution and Geographical Location of *Artemisia annua*

The plant is native to China but is currently found in many countries. *Artemisia annua* occurs naturally as part of vegetation in the northern parts of Chahar and Suiyuan provinces (40N, 109E) in Northern China, at 1000–1500m above sea level (Wang, 1961). The plant grows in many countries, such as Argentina, Bulgaria, France, Hungary, Romania, Italy, Spain, USA and former Yugoslavia (Klayman, 1993). The crop is grown in China and Vietnam as a source of artemisinin and cultivated on small scale in the USA as source of aromatic wreaths (Klayman, 1993).

The geographic range of *Artemisia annua* is paramount in determining areas for potential cultivation. Although *Artemisia annua* originated in relatively temperate latitudes it appears it can grow well at much lower tropical latitudes with lines which are either found

in these areas or which have been adapted by breeding (Magalhaes, *et al*., 1996). The current availability of late-flowering clones makes it possible to cultivate *Artemisia annua* in areas, which were previously considered unsuitable due to their proximity to the equator, and short photoperiod. The high artemisinin concentrations (0.5– 1.5%) in the leaves of some of these clones could allow high artemisinin yields in tropical latitudes, such as Vietnam, Madagascar and sub-Saharan Africa, even though the leaf biomass may not be as high as some strains of *Artemisia annua* grown in temperate latitudes (Delabays *et al.*,1993). The influence that higher altitudes have on the production of *Artemisia annua* at tropical latitudes is a principle that could be applied to parts of tropical Africa and elsewhere. Currently, with the opportunity offered by the availability of late-flowering clones and the world demand for artemisinin, several international agencies are carefully analyzing the possibility of cultivating *Artemisia annua* in tropical countries including Kenya and Tanzania (Technoserve, 2004).

2.1.2 Genotypes

Artemisia (*Artemisia annua* L.) is not an indigenous crop in Africa and most of the germplasm came from China, Vietnam, the Americas and mediplant in Indiana, Center for new crops in Switzerland (Technoserve, 2004). The germsplasm include different populations representing great diversity, which are then further improved in the national breeding programmes to suit local conditions (Ferraira and Janick, 1995). The polymorphism determines a large number of species and different varieties producing artemisinin and essential oil with varying chemical composition (Hussain *et al*., 1988). The genus artemisia includes about 400 species worldwide (Heywood and Humphries,

1977). Some of the species of artemisia includes *Artemisia annua, Artemesia vulgaris, Artemisia arborescens, Artemisia campestries, Artemisia maritima, Artemisia pontica* and *Artemisia verlotiorum (*Heywood *and* Humphries, 1977*).* Harvesting wild *Artemisia annua* before flowering stage has led to depletion of the seeds, which is a threat to the germplasm resources. Therefore, priority has been put on investigation, characterization and collection of *Artemisia annua* germplasm (Ferreira *et al*., 2005).

The genetic base of artemisia is restricted and needs to be broadened. F1 hybrid seeds still remain expensive and F2 populations are still being used for cultivation although yield turns to be low (Delabays *et al*., 2001). Brazil has developed 3 hybrid seeds 2/3 9 x IV, IV x 2/39 and Chx viet 55 in collaboration with Mediplant, Switzerland to increase seed availability (Magalhaes *et al.,* 1996). CIMAP (Central Institute of Medicinal and Aromatic Plants) recently developed a new variety of *Artemisia annua* "Jeevanraksha" which contains high levels of artemisinin (Tandon *et al*., 2003). Anamed (Action for Natural Medicine) coordination in Germany has committed to making hybrid seeds readily available. Hybrid plant named *Artemisia annua* anamed" or"A3" is now widely available for cultivation (Delabays *et al*., 2001). In Turkey three ecotypes; Aduana, samankaya and serinyol that produce high artemisinin yield have been developed compared to other strains (Delabays *et al*., 2001).

CIMAP (Central Institute of Medicinal and Aromatic Plants) is using molecular breeding techniques with *Agrobacterium tumefaciens* to enhance the production of artemisinin. *Agrobacterium* tumefaciens-mediated system of high efficiency of genetic transformation and regeneration of *artemisia annua* has been established (Delabays *et*

al., 1993). The process of identifying a few more genes in *artemisia annua* that, if transplanted into *Escherichia coli* could enable the bacterium to go a few extra steps in the chemical process and produce artemisinic acid, a precursor of artemisinin is being investigated (Srivastava, 2002).

2.2 CONSTRAINTS TO ARTEMISIA PRODUCTION

In many countries where artemisia is grown, production has been affected by numerous factors (biological, physical, climatic and socio-economic) which have led to decreased yields (Technoserve, 2004). In the sub-tropics and the tropics, rainfall is the most important factor. Drought at the early stages of growth induces flowering (Ferreira and Janick, 1995). However, the step to maximize artemisinin yields is to achieve high biomass before the onset of flowering (Laughlin, 1994).

Another constraint to artemisia production is unavailability of enough agro-technology. Laughlin *et al*. (2002) indicated that technical information on ideal planting dates, seed density, harvesting system, post-harvesting and optimum fertilizer application rates under different climatic conditions required for higher yields is not enough. The challenge is to develop a composite variety with stable high artemisinin content. What is most significant to producers is that the artemisinin and essential oil contents are high (Charles *et al*., 1991). A major problem to artemisia production is seed availability and quality. The most productive artemisia seed (Mediplant hybrid, 44CQ App4) is expensive and in short supply (Technoserve, 2004).

Hence, F2 seeds that are cheap and easy to obtain is preferable even though it has lower yield potential. Planting from cuttings may also be an option though difficult to apply on

a large scale (Technoserve, 2004). The unpredictable market is a major risk to artemisia production. The inadequate market availability for raw materials including extraction machines especially in sub-Saharan Africa (EABL, 2005).

2.2.1 Growth Requirements for Artemisia Production

The environment includes all micro-climatological and physical factors such as water, radiation, temperature, evaporation, soil conditions, human management and economic and political considerations. Laughlin (1993) indicated that genetic improvement of the crop plant alone would not meet the world demand for artemisinin. For crop productivity to be increased, the planting of high-yielding cultivars must be combined with improved practices of irrigation, fertilization, pests and disease control. Artemisia is grown in temperate and sub-tropical climates. The plant is not adapted to the tropics because flowering will be induced when the plants are very small but grows easily in temperate areas and tropical areas at higher altitude (Klayman, 1993). On the other hand, it has also been reported that the crop can be grown in the tropics at 1000 – 1500 metres about sea level (Duke and Paul, 1993). It grows well on well-drained sandy loam soils and prefers soils with pH 5.0 – 8.0 with good water holding capacity (Laughlin, 1993). Once established, the plants are drought tolerant. It thrives in temperate to subtropical climates but not very well in the tropics (Ferreira *et al*., 1997). Marchese *et al*., (2002) indicated that depending on genotype and geographical origin, artemisia present variations in the flowering behaviour under the same photoperiod and temperature condition

Water is required at the start of the planting season for good establishment of seedlings but dry weather conditions are needed at harvest for drying. Moisture stress also induces

early flowering and reduce yield. The plant requires an average rainfall between 10001500mm with a minimum rainfall of 600mm during the growth period (Marchese *et al*., 2002). Irrigation is needed to avoid the negative effect of drought (Technoserve, 2004).

Temperature has an important bearing on the productivity of artemisia. A study has revealed that suitable temperature for artemisia cultivation and production ranges between 10 – 17ºC with the optimum temperature between 13 – 29ºC (Liu *et al*., 2003). The plant grows well between longitude 105º-115ºE and latitude 25º-35º N by producing high biomass and artemisinin content (Duke and Paul, 1993). Similar findings have been reported from Vietnam, where the artemisinin content was high in the high-altitude north than in the low-altitude south (WHO, 2003). *Artemisia annua* is a short day plant with a photoperiod requirement of 13.5 hr (Ferreira *et al*., 1995a) and a chromosome number of 2n = 2x= 18 (Bennett *et al*., 1982). The plant is naturally cross– pollinated by insects and wind (McVaugh, 1984). Ferreira *et al*., (1997) reported that self-pollination is not only rare but difficult to achieve which infers the presence of selfincompatibility. The plant at present does not seem to have any particular insect or disease problems.

2.3 ARTEMISININ PRODUCTION

Artemisinin is an odourless, non-volatile compound, which is purified as white crystals with a melting point of 156–157°C (Lin *et al*., 1985). Its molecular weight is 282.1742 kg/mol, with an empirical formula of $C_{15}H_{22}O_5$ (Plate 2.2).The chemical name is 3R, 5aS, 6R, 8aS, 9R, 12S, 12aR-Octahydro-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano (4.3j)-1, 2-benzodioxepin-10(3H)-one (Lin *et al*., 1985),

The structure of artemisinin is represented below:

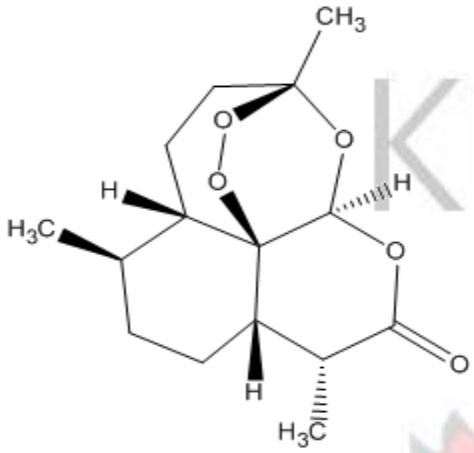


Plate 2.2: Structure of Artemisinin

Source: **Malcolm Cutler**
FSC Development Services Ltd
Alexei Lapkin and Pawel K. Plucinski
Department of Chemical Engineering
University of Bath

Artemisinin is the main active ingredient of *Artemisia annua* effective against malaria. Derivatives of artemisinin include arteether, artemetther and sodium artesunate. Among the artemisinin derivatives, the compounds arteether artisunate, artinulate and di-hydro-artemisinin have been found to be highly potent anti-malarials (Jains *et al*., 2000). At present, a number of formulation of artemisinin and its derivatives are being marketed. The most widely available preparations are artemether, artesunate+amodiaquine, artesunate+ pyrimethamine (WHO, 1994).

The compound is isolated from the shrub *Artemisia annua* which is used in traditional

Chinese medicine. Artemisinin, used in the semi-synthesis of related compounds in Artemisinin-Based Combination therapies (ACTs), are found mainly in the leaves and flowers of *Artemisia annua,* little artemisinin is found in the stems, and none is found in seeds or roots(Acton *et al.*,1985). Not all shrubs of this species contain artemisinin. Apparently, it is only produced when the plant is subjected to certain conditions (Charles *et al*., 1990). Artemisinin compounds have been predominately found in the upper parts of the *Artemisia annua* plant, with the concentration of artemisinin said to peak just before or during full flowering, the difference being attributed to climatic conditions, plant variety, or other, yet undetermined factors (Charles *et al*.,1990). The leaves from the same plant have different artemisinin contents according to their localization along the stem with upper leaves containing significantly more artemisinin than middle and lower ones (Charles *et al*., 1990; Laughlin, 1995). The plant content of artemisinin also varies during the season (Delabays *et al*., 2001).

Artemisinin and its precursor, artemisinic acid, have been shown to be localised in the glandular trichomes on the leaf surface. The main consequences of this are that; it may not be necessary to mechanically crush the plants prior to extraction for reasons other than to increase the packing density and the artemisinin content depends on the age of the leaf, since in older leaves the glands often ruptured (Mehrotra *et al*., 1990).

The demand for ACTs is increasing and increased from a million in 2003 to 30 million in 2004 (WHO, 2004). The world market price for artemisinin was USD 200-300/kg in 2002, increased to USD 400 – 500/kg and USD 600 – 800/kg in 2003 and 2004 respectively, with the price expected to stabilize around 250/kg (Technoserve, 2004).

EABL (2005) compared yield of artemisia and noted that artemisinin content is highly variable; natural population may be as low as 0.1% and improved cultivars as high as 1.3% with the range in East Africa by assay between 0.85% - 1.2%. Artemisinin can also be obtained from artemisinic acid which occurs in concentration as much as 10fold higher than artemisinin (Acton *et al*., 1985).

The essential oils of *Artemisia annua* contain at least 40 volatile compounds and several non-volatile sesquiterpenes, of which artemisinin and related compounds are the ones of most interest due to their anti-malarial properties (Charles *et al*., 1991). Some of the major constituents of the essential oils include (in relative % of total essential oil) alphapinene (0.032%), camphene (0.047%), ß-pinene (0.882%), myrcene (3.8%), 1, 8-cineole (5.5%), artemisia ketone (66.7%), linalool (3.4%), camphor (0.6%), borneol (0.2%), and ß-caryophyllene (1.2%) (Chen *et al*., 1991). The plant also yields 0.3% essential oil used in the perfumery and flavouring industries (Chen *et al*., 1991). Artemisinin also has phytotoxic activiy and a candidate as a natural pesticide (Chen *et al*., 1991).

The screening of *Artemisia annua* germplasm for both artemisinin and essential oil content in one operation is a useful strategy to increase cost-benefit ratio of the extraction (Laughlin, 1994). This is confirmed by Vonwiller *et al*. (1993) who reported that the extraction method which makes possible the extraction of both compounds from the same plant material increases the final production of artemisinin. Vries *et al*. (1998) found out that substances in the aqueous extract protect the extract against resistances from the malaria strains. Clinical trials have shown artemisinin to be 90% effective than standard drugs (Blanke *et al*., 2008).

Simon *et al.* (1990) indicated that the levels of artemisinin and its derivatives achieved are linked to inherent genetic factors and agronomy of cultivation. Commercial production of artemisinin in Africa has largely been limited to Kenya and Tanzania. However, current demand for artemisinin has led to a significant increase in the commercial production, both in established as well as new areas (EABL, 2005).

2.3.1 Glandular Trichomes as Sites of Artemisinin Accumulation

The glandular trichomes are more prominent in the corolla and receptacle florets than in leaves, stems, or bracts (Mehrotra *et al.*, 1990). Although these glands are present since the early stage of development on both leaves and inflorescences (Plate 2.3), artemisinin increases at anthesis, suggesting that it accumulates as the glands reach physiological maturity, a stage, which coincides with the end of cell expansion in floret development (El-Sohly *et al*., 1990).

As glands approach maturity, there appears to be a cellular discharge into the subcuticular space around the apical cells and the contents are spread over the epidermis when the glands burst. After anthesis, artemisinin decreases and so does the number of intact glands. The association of artemisinin with glandular trichomes sequestration explains why artemisinin was not detected in parts of the plant that do not bear glands (Plate 2.4) such as pollen or roots (Ferreira *et al.*, 1995a).

Glandular trichomes are observed in leaves and stems of differentiated shoot cultures and artemisinin content of in-vitro shoot cultures was similar to artemisinin content in

vegetative clones grown in the greenhouse (Ferreira *et al.*, 1995b). Nicely grown-up plants may be devoid of artemisinin due to the rupturing of the glandular trichomes (Duke *et al.*, 1994). In order that this product is synthesized by the plant, special agricultural conditions must be respected (Ferreira *et al.*, 1995a). The plant can grow in many places but it may not contain artemisinin (Duke *et al*., 1994).

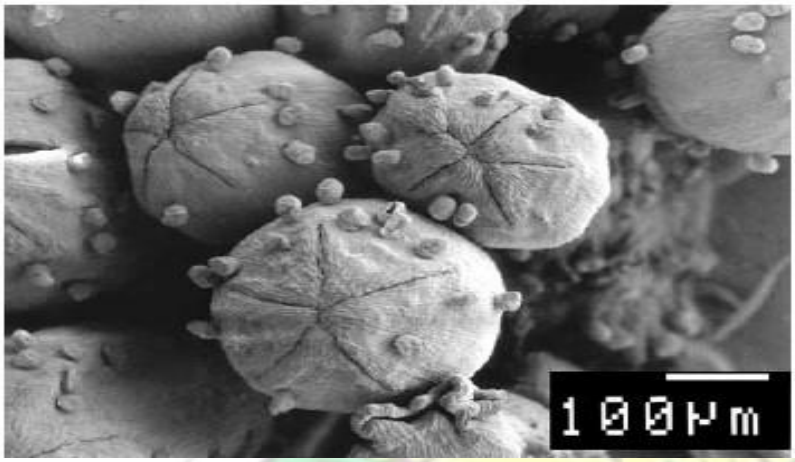


Plate 2.3: Scanning electron micrograph of *Artemisa annua* with biseriate glandular trichomes in leaves and flowers in normal biotypes

Source: (Ferreira and Janick, 1996).

Plate 2.4: Scanning electron micrograph of glandless biotypes

Source: (Ferreira and Janick, 1996).

2.3.2 Mechanism of Action of Artemisinin

Artemisinin contains two oxygen atoms linked together in what is known as an 'endoperoxide bridge', which react with iron atoms to form free radicals and the artemisinin becomes toxic to malaria parasites. When it reacts with the high iron content of the parasites, it generates free radicals, which leads to damage to the parasite (Luo and Shen, 1987).

By this same mechanism, artemisinin becomes toxic to cancer cells, which sequester relatively large amounts of iron compared to normal, healthy human cells. Tests conducted show that artemisinin causes rapid and extensive damage and death in cancer cells and yet has relatively low toxicity to normal cells (Luo and Shen, 1987).

Artemisinin from the genus *Artemisia* has been shown to regulate plant growth by inhibiting lateral root growth (Duke *et al.*, 1988). Artemisinin has been more effective

than glyphosate, when tested as a herbicide in the mung bean (*Vigna radiata*) (Chen *et al*., 1991). However, its herbicidal mode of action has not been elucidated.

2.4 METHODS OF EXTRACTION OF *ARTEMISIA ANNUA*

With regard to artemisinin production, the *Artemisia annua* has proved amenable to domestication and cultivation (Gupta *et al*., 2002) and several procedures for the extraction of artemisinin from *Artemisia annua* herbage have been described (Jain *et al*., 1999). However, most of them are either not sufficiently sensitive and do not offer reliable results, or are difficult to apply in routine analyses (Christen and Veuthey, 2001). Therefore, new methods for the determination of these compounds are being investigated (Wang *et al*., 2005)

Traditionally the majority of *Artemisia annua* grown worldwide is processed through solvent extraction, using hexane and petroleum ether (Vonwiller *et al*., 1993).

Extraction of artemisinin from *Artemisia annua* is currently mainly performed using hydrocarbon extraction processes (El-Sohly *et al*., 1990). Currently there are other solvents that have been considered for extraction of artemisinin. These are supercritical carbon dioxide, ethanol, ionic liquids and hydro fluorocarbon HFC-134a and it has been observed that extraction of artemisinin using these solvents has proven to be better than the traditional hydrocarbons (Vonwiller *et al*., 1993). Even though the extraction with these solvent has shown high process efficiency, it is very expensive to run an extraction plant. Therefore, the traditional method of using hexane and petroleum ether is employed (Vonwiller *et al*., 1993).

2.4.1 Extraction with Petroleum Ether/Hexane Ethyl Acetate Mixture

In the simple batch percolation extraction, the dried crushed leaf is soaked three – four times in fresh portions of warm (45 – 46 ˚C) petroleum ether (El-Sohly *et al*., 1990). Each extraction cycle takes 10 to 48 hours (El-Sohly *et al*., 1990) in order to improve efficiency of extraction; a small amount of co- solvent ethyl acetate can be added to the main non-polar hydrocarbon solvent (El-Sohly *et al*., 1990). This increases the solubility of artemisinin in the solvent mixture by about two orders of magnitude (Vries *et al*., 1998). Following extraction, the solvent is drain and spent biomass must be stripped of the residual solvent. Stripping of the solvent can be achieved by simple evaporation in air under natural convection, which is potentially hazardous and leads to the release of significant quantities of environmentally harmful volatile hydrocarbon, or more efficiently by steam stripping followed by condensation and recovery of solvent (Vries *et al*., 1998). The recovery and reuse of the solvent reduce the environmental impact and improves the cost-effectiveness of the process.

Vacuum stripping may also be used to avoid potential biomass decomposition under steam and to avoid downstream water-solvent separation (Reitz and Hill, 2004). The obtained crude extract is flash-evaporated to 10% of its initial volume and the remaining liquor is left to stand at ambient temperature (Vries and Chan, 1998). Crude artemisinin is washed with warm hexane to remove the waxes and other precipitated impurities (El-Feraly *et al*., 1990). In order to remove the waxes artemisinin is recrystallized several times from ethanol-water azeotrope (95% ethanol) in the presence of activated carbon adsorbent, followed by vacuum evaporation (El-Feraly *et al*., 1990). An alternative method of separating artemisinin from the initial hexane extraction involves liquid-liquid

extraction of artemisinin related compounds from hexane into acetonitrile (El-Sohly *et al*., 1990). This method is not considered due to the hazardous nature of acetonitrile to the environment and human health, rendering its large-scale use unacceptable (Haynes, 2006).

2.4.2 Extraction of Artemisinin by Supercritical CO_2

Extraction of artemisinin by $scCO_2$ or sub-critical liquid CO_2 has been described in the literature and large-scale trials are currently being undertaken (Wheatley *et al*., 2001). The efficiency of extraction of artemisinin from biomass is reported to be quantitative, rapid and with higher selectivity compared with the hydrocarbon solvents extraction, based on the gram scale laboratory tests (Kohler *et al*., 1997). However, there is wide variability in the efficiencies of extraction with $scCO_2$ dependent on the scale of extraction, use of co-solvents, temperature and pressure of extraction, and superficial velocity of the solvent in the extractor (Quipe-Condori *et al*., 2005). The duration of extraction cycle depends greatly on the scale of extraction, use of co-solvents as well as more specific aspects of extractor design that influence optimal solvent mass flow rate (Christen and Veuthey, 2001). Thus, 20 minutes extraction cycle was quoted for *ca*. 1 L scale in the case of $scCO_2$-ethanol system, whereas detailed kinetic study of artemisinin extraction with $scCO_2$ without co-solvents showed extraction times up to 2 h on a 0.2 L scale (Chester *et al*., 1994).

2.4.3 Extraction of Artemisinin Using Hydrofluorocarbon HFC-134a

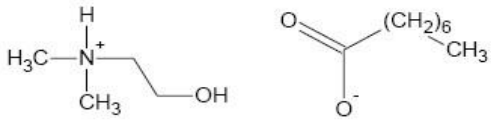
Hydro fluorocarbon HFC-134a (1, 1, 1, 2-tetrafluoroethane) is classed as nonflammable and has zero ozone depleting potential (McCulloch and Lindley, 2003). It is amongst the

most studied and utilised materials for which there are a life-cycle impact study. In Europe, Japan and USA, HFC-134a is accepted by regulatory bodies for use as a solvent in the extraction of food flavorings (Wilde, 1996). One drawback of the solvent is its high global warming potency factor – 1300 times larger than that of carbon dioxide (Wilde *et al*., 2002). Therefore, complete recycle and capture of the solvent within a process is of significant importance. Hydrofluorocarbons are gases under normal conditions and are liquefied at relatively low pressures. Therefore, these solvents are ideally suited for continuous extraction processes, when depressurization of the solvent results in a rapid separation of the extracted material (Corr, 2002). Because of the modest pressures and low operating temperatures, the energy required for continuous depressurisation/pressurisation cycle is not high, resulting in low energy costs, low operating costs, and low greenhouse gas emission due to energy duty (Klayman *et al*., 1984). Furthermore, re-circulation of solvent can be achieved without pumps, by establishing a condensation-evaporation cycle, thus avoiding the need for expensive capital investment in the pump and the compressor (Wilde, 1996). In this case, the flow-rate of solvent depends on the efficiencies of condenser and evaporator, as well as percolation properties of the packed biomass (Wilde, 1996).

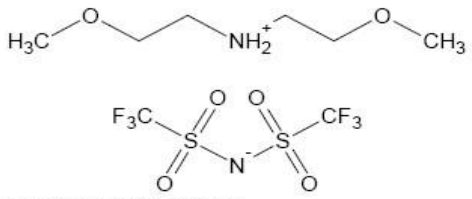
2.4.4 Extraction Using Organic Ionic Liquids

Organic ionic liquids (organic equivalent of molten salts) is a new class of solvents, characterized by negligible vapour pressure, nonflammability and possibility to tune solvation properties over a very broad range (Rodriques *et al*., 2006). Since ionic liquids lack two major drawbacks of the hydrocarbon solvents: vapour pressure and flammability, these solvents are often cited as a 'green alternative' (Rodriques *et al*., 2006). Two out of

five screened ionic liquids showed promising performance: N, Ndimethylethanolammonium octanoate (DMEA oct, **1**) and bis (2-methoxyethyl) ammonium bis (trifluoromethylsulfonyl) imide (BMOEAbst, **2**) (Chester *et al*., 1994). The extraction process is similar to a standard liquid-solid extraction and was performed in a batch regime at 25 ºC. Extraction with the DMEA octanoate solvent reached maximum solute concentration after 30 minutes of extraction (Zhuo *et al*., 1988).



1 N,N-dimethylethanolammonium octanoate



2 bis(2-methoxyethyl)ammonium bis(trifluoromethylsulfonyl)imide

In the case of the solvent the rate of extraction is considerably slower than that with. However, the maximum concentration of artemisinin in solution was higher by 23 % (Rodriques *et al*., 2006) .The obtained rate of extraction with is similar to the rate of extraction with n-hexane at same temperature (Rodriques *et al*., 2006). Thus, in comparison with hexane, ionic liquid gave a similar efficiency of extraction at a considerably faster rate, whereas ionic liquid gave higher extraction efficiency at the same rate (Rodriques *et al*., 2006).

The process of separation of artemisinin from the raw extract involved partitioning with water at ambient temperature (Chester *et al*., 1994). This causes simultaneous separation of the oil fraction and crystallisation of artemisinin. Crystallisation allows a separation of 82 % of the total extracted amount of artemisinin; the remainder is assumed to be lost with the oil phase (Chester *et al*., 1994). The crystals are 95 % artemisinin and are

essentially free of solvent. Separation was achieved in about 10 minutes (Chester *et al*., 1994).

2.4.5 Extraction of Artemisinin Using Ethanol

Ethanol is potentially an attractive solvent due to its wide spread availability from renewable feedstock. Extraction by ethanol aqueous azeotrope at room temperature claimed high efficiency of extraction (Rodriques *et al*., 2006). This is especially important for processes that are predominantly focusing on locally sourced materials to improve the overall process sustainability. A potential constraint on the use of ethanol as a process solvent is its use as a spirit (Rodriques *et al*., 2006). This can be resolved by using a spiked solvent. However, there are similar concerns with the use of ethanol as in the case of hexane: it is a flammable solvent, with high toxicity and high risk in use (Chester *et al*., 1994).

The process based on ethanol extraction involves three sequential extractions with fresh solvent portions followed by flash evaporation of solvent to reduce the volume of the primary extract (Rodriques *et al*., 2006). Some process optimization is possible to reduce the ratio of solvent to biomass. Ethanol extraction of artemisinin in a pressurized percolator extractor has been unable to replicate high extraction efficiency (Kohler *et al*., 1997).

2.5 PURIFICATION OF ARTEMISININ

The crude extract is washed with hexane to get rid of the waxes, oils and chlorophyll in it, subsequently crystallized, and recrystallised in ethanol to obtain the pure artemisinin.

The product obtained is assayed by high performance liquid chromatography (Zhuo, 1986) or thin layer chromatography (Roth and Acton, 1988). It is recommended to remove the extraction solvent under gentle conditions. Crude artemisinin will crystallize at ambient temperature in approximately 48 hours (Roth and Acton, 1988). The mother liquor is partly decanted and the crystals are washed with rather warm solvent to remove the precipitated waxes. Separation of these waxes is essential. Further recrystallization in ethanol/extraction solvent mixtures are often done to remove all waxes. The quantity of waxes in the raw material depends on several factors, among which is the characteristic of the cultivation soil.

The crude and/or recrystallised crude artemisinin is dissolved in hot ethanol 96% 1:6 (w/v) to which some active carbon has been added for decolourisation. Pure artemisinin will crystallize after filtration and cooling to ambient temperature. The pure artemisinin crystals are carefully washed with fresh ethanol at 96%. Pure artemisinin is dried under vacuum at low temperature until most of the ethanol has been removed. Drying is completed at 55-60°C.

2.6 MODE OF HARVESTING OF *ARTEMISIA ANNUA*

The concept of harvesting for both essential oils and artemisinin and its derivatives warrants exploration. The optimum time of harvest must take into consideration the maximum yields of artemisinin per unit area, balanced against production and extraction cost (Technoserve, 2004).

Woerdenbag *et al*. (1994) compared yield response of artemisia to different harvesting stages and noted that harvesting at pre-flowering stages promotes the production of high artemisinin contents. Laughlin *et al*. (2002) also confirmed that the optimum time to harvest artemisia is just before flowering; this is also important because of the allergic reaction of the pollen. It was further observed that time of harvesting at different ecological zones for high artemisinin content remains a technological problem.

On the other hand, it has been reported that artemisinin reaches its peak during flowering. The association of peak artemisinin with flowering is related to the abundance of glandular trichomes in the inflorescence particularly florets and receptacle (Ferreira *et al*., 1995a). Morales *et al*. (1993) also confirmed that peak artemisinin was achieved during full bloom in a number of greenhouses and field trials. This is confirmed by Ferreira *et al*.(1995b) who found that artemisinin reaches its peak during full flowering in a Chinese clone for both greenhouse and field conditions. EABL (2005) also reports that when half to three quarters of the plants shows signs of bud initiation, artemisinin content will be at maximum.

Artemisinin content is relatively low as such harvesting is carried out when artemisinin content per unit area is at a maximum in order to reduce extraction and processing cost. Thus, the optimum time of harvest must take into consideration maximum artemisinin content and biomass yield.

Wright (2002) however, observed that the optimum time of harvest depends on the target compound desired and on the variety grown. All these have a strong genotype and

environment interaction and the optimum time of harvest will have to be established locally (Laughlin *et al*., 2002).

2.7 BIOTECHNOLOGICAL PRODUCTION OF ARTEMISININ

Due to relatively low level of artemisinin in *A. annua* (0.01-0.8 %) (Ferreira *et al.*, 1997; Bhakuni *et al.*, 2001), several strategies have been adopted to increase artemisinin production to meet the demand in the medical market. This includes plant tissue culture, chemical synthesis, biotransformation and genetic engineering of *A. annua* (Abdin *et al.*, 2003). Tissue culture of shoots, callus suspension culture and hairy roots have been investigated (Weathers *et al.*, 1994; Liu *et al*., 2004). However, the artemisinin content from tissue cultures is inconsistent and non-reproducible in some cases (Jaziri *et al*., 1995).

Attempts to increase artemisinin production by adjusting the nutrients, hormones, growth condition, elicitors, and stresses were also reported (Wang and Tan., 2002., Liu, 2003). Chemical synthesis of artemisinin is possible but complicated and not feasible for large-scale production due to the complex structure of artemisinin (Jung *et al.*, 2004; O'Neill, 2005; Jefford, 2007). Heterologous expression of artemisinin metabolic pathway in microorganisms, such as *Escherichia coli* and *Saccaromyces cerevisiae* is an attractive way due to the low cost carbon source and feasibility of large scale preparation (Chang and Keasling, 2006; Ro *et al.*, 2006). Genetic engineering of *A. annua* has become attractive since the transformation of *A. annua* was established (Banerjee *et al.*, 1997; Ghosh *et al;* 1997; Hans *et al.*, 2005), but both methods are reported to be complex (Hans *et al*., 2005).

Singh (2001) however indicated that to improve the economics and commercial production of artemisinin and anti-malarials, there is the need to increase the yields of artemisinin from the field grown *Artemisia annua* through the application of fertilizers. The application of fertilizers has been reported to increased leaf yield and subsequently increased the amount of artemisinin obtained (Singh, 2001).

2.8 RESPONSE OF *ARTEMISIA ANNUA* TO NITROGEN FERTILIZER

The artemisinin yield from *Artemisia annua* crops is expected to depend on the inherent artemisinin content of the cultivated genotype and agronomy of cultivation (Kawamoto *et al*., 1999). The large scale availability and cost of artemisinin-related anti-malarials will be determined by the economics of artemisinin yield from *Artemisia annua* crops (Ferraira *et al*., 1997). The kind and amount of fertilizer to be applied is important not only for the economic returns the producer expects but for environmental reasons too. Therefore, the need for soil test analysis has been recommended for predicting fertilizer needs (Sallah, 1991).

Plants usually respond positively to N application, but this is not always true depending on the agro-ecology and the cropping history of the field (GGDP, 1996). Adequate and balanced nutrients are necessary to obtain high yields. Rodriquez (1986) and Agboola (1986) in their report of improving maize grain yield suggested that response were found with up to 100 kg N/ha in the Sudan savannah and 150 kg N/ ha in the guinea savannah and even higher doses for the forest region. Thus, optimum application of the fertilizer is important in crop production but may vary from one agro-ecology to the other. Dennis

(1990) observed that optimum application of fertilizer may be around 80 kg N/ha but higher rates are needed on Savannah soils.

Simon *et al*. (1990) reported that both optimum essential oil of 85 kg/ha and fresh whole plant biomass yield of 35 t/ha were achieved at 67 kg N/ha at high plant density. However, the plants had a lower leaf - to- stem ratio. It was further observed that fresh whole plant biomass of 270-750 g/plant was produced. Nitrogen deficiency was associated with a large decrease in artemisinin and leaf biomass yield (Figuera, 1996). Figuera (1996) observed that the omission of nitrogen or phosphorus drastically reduced plant growth and dry matter production in hydroponic studies in Brazil.

Magalhaes *et al*. (1996) conducted a study with four levels of nitrogen (0, 32, 64 and 97 kg N/ha) applied as urea. The results revealed that the highest fresh whole biomass yield of 3880 kg/ha and artemisinin yield of 40.4 kg/ha was recorded. In the same study, no significant differences were found for leaf biomass and artemisinin yield when ammonium sulphate and ammonium nitrate were compared as a source of nitrogen.

To obtain high efficiency of applied nitrogen, Rodriquez (1986) reported that the nitrogen be applied in split and banded or side-dressed. Moll *et al*. (1982) defined nitrogen use efficiency (NUE) of a cultivar as yield produced per unit of available N (soil + compound fertilizer N). EABL (2005) reported that 50 – 50 split application of N, 50% at planting and 50% when the crop reaches 50 cm tall increases leaf biomass and artemisinin content.

According to Ferreira *et al.* (1995a) flowering could be delayed by cutting the apical meristem and providing nitrogen fertilizer causes the plant to branch out and potentially increase leaf biomass.

WHO (1988) conducted a study on the vegetative growth response of *Artemisia annua* to specific micronutrients; nitrogen, phosphorus and potassium. They reported that significant increase of total plant and leaf dry matter was obtained where a complete fertilizer mixture containing 100 kg N, 100 kg P and 100 kg K /ha was applied. Dry leaf yields of Artemisia between 6 – 12 t/ha were obtained in a mixed fertilizer containing 60 kg N, 60 kg P and 50 kg K/ha (Laughlin, 1994). Muchow (1988) reported that nitrogen supply to plants increase leaf area development and delays leaf senescence and increases the photosynthetic capacity of the leaf canopy. GGDP (1982) noted that maximum yield was obtained by the application of 83 and 95 kg N/ha in the forest and transition zones respectively. Response to P was non-significant.

Wright (2002) noted that nitrogen is a very mobile element and easily leached out of the root zone in tropical and sub-tropical conditions. He further observed that the yield of dry leaf per hectare varies from 1-40 t\ha.

Artemisinin responds well to balanced fertilizers, and appears to be responsive to nitrogen but only a few data is available on the accumulation of artemisinin relative to fertility. It has also been noted that nitrogen is the main constituent of protein and nucleic acid, which influences cell division, cell enlargement which might cause proliferation of roots and increase in shoot length resulting in better plant height (Gandhi Kumar, P. 1996)

Veldkamp (1992) reported that increase in shoot weight due to increase in N-levels may be attributed to adequate supply of plant nutrient needed for protein, amino acid, energy synthesis and improved metabolic activities. The number of branches increased substantially in treatments containing average level of manure or compound fertilizer additions (Ming, 1994). The number of branches per plant was significantly enhanced by the greater availability of chemical or organic nutrients due to their positive effect on shoot biomass production (Rao *et al.*, 1985). Nagalhaes *et al.*, (1996) reported that nitrogen application increases the leaf dry yield about two fold. Martinez and Staba (1988) also observed that N-fertilization stimulates the vegetative development of the plant, the greater the application of N, the greater the height of the plant. Plants with no manure or compound fertilizers additions were generally shorter and produced fewer nodes (Silva *et al.*, 1971)

2.9 RESPONSE OF *ARTEMISIA ANNUA* TO ORGANIC MANURE

The benefits of using manures and compost as soil additives are a well established and ancient agricultural practice used by small and large scale farmers alike. Since poultry manure is organic, it is able to have good effect on the physical and chemical properties of the soil. Soil fertility regeneration and maintenance appear to be the most serious agronomic challenge to crop productivity in Ghana (Asafu-Agyei *et al.*, 1997). The purpose of organic fertilization besides the addition of nutrients to the soil is to improve the soil organic matter content. It is also known to improve soil aeration, water holding capacity, permeability and soil resistance to erosion (Muchena, 1986). The importance of

using manures and compost as soil additives is a well established and traditional agricultural practice used by both small and large scale farmers.

Miller and Turk (1991) reported that poultry manure promotes the growth of plants as a result of the presence of microorganisms, micronutrients, organic matter and regulating substances. According to Bandel *et al*. (1972), poultry manure contains appreciable amount of N, P and K based on the following percentages; 45% nitrogen, 2.5% phosphorus and 2% potassium. Ahn (1993) who found out that poultry manure is rich in nitrogen and the most concentrated farmyard manure and thereby increased yield also confirms this.

Kallah and Adamu (1988) conducted a study involving the use of different organic manures. It was reported that the relative efficiency of organic manure in improving soil fertility followed the order; poultry manure > pig manure > farmyard manure. De Ridder (1990) reported significant yield responses from the application between 2.5 - 5.0 tons/ha of manure. The manure tonnage is high because the concentration of nitrogen in animal manure varies for as much as four folds, depending on the type of feed fed to the animals. This is confirmed by Ofori *et al*. (1997) who reported a variation from 0.5 to 2.0% nitrogen in manure. Liebhardt (1976) reported that the addition of 5 t/ha of poultry manure doubled yield of artemisia as compared to the control. Hileman (1971) also reported that poultry manure enhances rapid releases of ammonia. Application of organic fertilizer increased the biomass yield and total essential oil yields of artemisia (Parakasa, 1997).

Poultry manure have good effects on the physical and chemical properties of the soil through increase in water infiltration rate, water holding capacity, cation exchange capacity and structure stability of the soil (Moore *et al.,* 1995). Bationo and Mokwunye (1992) also confirmed that the addition and incorporation of organic materials either in the form of manures or crop residues have beneficial effects on the soil chemical, physical and biological properties.

According to Khalid and Shafei (2005) treating plants with different combinations of organic fertilizers and its rates resulted in a significant increase in growth, yield characters and artemisinin content extracted from the plants. Marculescu *et al.,* (2002) observed that a soil rich in macro and microelements enhanced by the use of organic fertilizers plays an essential role in plants growth and development and biosynthesis of the organic substances at all levels. The reason for increase in crude extract may be due to the influence of poultry manure in promoting vegetative growth, which resulted in increased herbage production, consequently the crude extract yield increased to a greater extent (Singh, 2001; Singh *et al.,* 2004)

The possible reason for increased in shoot weight may be due to increase in length of shoot when poultry manure was applied due to its effect on photosynthetic efficiency of a crop resulting in the production of more number of leaves and stems or increased vegetative growth (Majbur Ragman *et al*., 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS 3.1 EXPERIMENTAL SITE

The study was conducted during the major season of 2008 at the Kwame Nkrumah University of Science and Technology, Agricultural Research Station, Anwomaso in the semi-deciduous forest zone. It lies between latitude 06º. 43º North and longitude 01º. 36º West of the Greenwich meridian. The soils, Ferric Acrisol belong to the Asuansi series with about 5cm thick top layer of dark grey gritty sandy loam (FAO/UNESCO, 1964). The soil pH was slightly acidic and ranges from 5-7. The site is characterized by a bimodal rainfall pattern. The experimental fields were not planted to any crop in the previous season.

3.2 EXPERIMENTAL MATERIALS

The field evaluation and laboratory analysis to determine the percent artemisinin content involved one variety of *Artemisia annua*. The genotype comprises seeds supplied by Professor J.E Simon of Rutgers University, Washington, USA.

3.3 SOIL STERILIZATION

The soil mixture was sterilized by steam using a steel barrel sterilizer at KNUST. The steam sterilizer was filled with soil and covered with a jute sack to prevent steam from escaping. The sterilizer had two chambers filled with soil on platform spread with jute sack. Heat was supplied from pieces of firewood set up under the steam sterilizer supported by three metal stands. The soil was sterilized at a temperature 103 °C for a period of two hours. It was allowed to remain on the fire for approximately 24 hours.

3.4 NURSING OF SEEDS AND SEEDLINGS

The artemisia seeds were sown in seed boxes containing sterilized soil. The seeds were not covered with soil after sowing to avoid germination failure (Ferreira *et al*., 1997). The soil used for the nursing was composed of 50% river sand and 50% loam to facilitate drainage. The seedlings were pricked out into plastic pots filled with sterilized soil after thirty days of germination. After seventy days in the greenhouse, the plants were moved outdoors to harden by gradually exposing them to sunlight whiles reducing watering, prior to transplanting.

3.5 LAND PREPERATION AND FIELD LAYOUT

The field measuring 41.0 x 18.5m (758.5m^2) was slashed, ploughed and harrowed to a fine tilth for the experiment. Eighteen plots, each measuring 5.0 x 4.5m was marked out. The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replications. Spacing between replication (block) was 2m apart and 1.8m between plots.

3.5.1 Transplanting

Eighty days old and healthy artemisia seedlings were transplanted into the field. Twenty seedlings were transplanted per plot. There were four rows per plot and inter and intra row spacing were 1.5 ×1.0m respectively.

3.6 INITIAL SOIL SAMPLING AND ANALYSIS

An auger was used to take soil samples at random on each experimental plot at depths of 0-20 cm and 20-40 cm. The soil samples from each depth per replication were mixed thoroughly to form a composite sample. A sizeable quantity of 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 KNUST.

3.6.1 Soil Physical Analyses

The soil physical properties determined were particle size and bulk density.

3.6.1.1 Particle size analysis

The hydrometer method as described by Bouyoucos (1963) was used. This method is based on the effects of particle size on the differential settling velocities within a water

column. The settling velocity is also a function of liquid temperature, viscosity and specific gravity of the falling particle (Okalebo *et al*., 1993).

A 51 g soil sample was weighed into a 'milkshake' mix cup. To this 50.0 ml of 10% sodium hexametaphosphate and 100 ml distilled water were added. The mixture was shaken for 15 minutes after which the suspension was transferred from the cup into a 1000 ml measuring cylinder. With a hydrometer in the suspension, distilled water was added to reach the 1000 ml mark. The mixture was inverted several times until all soil was in suspension. The cylinder was placed on a flat surface and the times for the readings were noted. The first hydrometer and temperature readings were taken at 40 seconds. After the first readings, the suspension was allowed to stand for 3 hours and the second hydrometer and temperature readings were taken. The first reading indicated the percentage of sand and the second reading percentage clay. The percentage of silt was determined by difference.

Calculations:

$$\% \text{ Sand} = 100 - (H_1 + 0.2\,(T_1 - 20) - 2.0) \times 2$$

$$\% \text{ Clay} = (H_2 + 0.2\,(T_2 - 20) - 2.0) \times 2$$

$$\% \text{ Silt} = 100 - (\% \text{ sand} + \text{clay})$$ Where:

H_1 = Hydrometer reading at 40 seconds

T_1 = Temperature at 40 seconds

H_2 = Hydrometer reading at 3 hours

T_2 = Temperature at 3 hours

$0.2\,(T - 20)$ = Temperature correction to be added to hydrometer reading

- 2.0 = Salt correction to be added to hydrometer reading.

3.6.1.2 Soil bulk density (ℓb)

Soil bulk density is the ratio of the mass of dry soil to the bulk volume of the soil. This was determined using the metal core sampler method (Blake and Harte, 1986). The core sampler was driven into the soil with the aid of a mallet. Soil at both ends of the tubes was trimmed and the end flushed with a straight-edged knife. The core sampler with its content was dried in the oven at 105^0C to a constant weight, removed, allowed to cool and its weight taken. The weight of the core cylinder and its volume was determined.

Calculation:

$$\text{Dry bulk density } \ell_b \text{ (g cm}^{-3}) = \frac{W_2 - W_1}{V}$$

Where:

W_2 = Weight of core cylinder + oven-dried soil

W_1 = Weight of empty core cylinder

V = Volume of core cylinder ($\pi r^2 h$), where:

π = 3.142 r = radius of the core cylinder h = height of the core cylinder

3.6.2 Soil Chemical Analyses

The soil chemical properties determined were pH, organic carbon, total nitrogen, available phosphorus and exchangeable bases (calcium, magnesium, potassium and sodium).

3.6.2.1 Soil pH

The pH of the soil was determined using a Suntex pH (mv) Sp meter (701) at soil: water ratio of 1:2.5 as described by McLean (1982). A 20 g soil sample was weighed into a 100 ml beaker. To this 50 ml distilled water was added and the suspension was stirred continuously for 20 minutes and allowed to stand for 15 minutes. After calibrating the pH meter with buffer solutions of pH 4.0 and 7.0, the pH was read by immersing the electrode into the upper part of the suspension.

3.6.2.2 Soil organic carbon

Organic carbon was determined by a modified Walkley-Black wet oxidation method as described by Nelson and Sommers (1982). Two grams of soil sample was weighed into 500 ml erlenmeyer flask. A blank sample was also included. Ten millilitres of 1.0 *N* $K_2Cr_20_7$ solution was added to the soil and the blank flask. To this, 20 ml of concentrated sulphuric acid was added and the mixture allowed to stand for 30 minutes on an asbestos sheet. Distilled water (200 ml) and 10 ml of concentrated orthophosphoric acid were added and allowed to cool. The excess dichromate ion ($Cr_20_7^{-2}$) in the mixture was back titrated with 1.0 *M* ferrous sulphate solution using diphenylamine as an indicator.

Calculation:

$$\% \text{ Organic C} = \frac{(\text{m.e } K_2Cr_20_7 - \text{m.e. } FeS0_4) \times (1.32) \times 0.003 \times 100}{w}$$

Where:

m.e. = normality of solution x ml of solution used

0.003 = m.e. wt of C in grams (12/4000)

1.32 = correction factor w = weight of soil (g)

3.6.2.3 Total nitrogen

The total nitrogen content of the soil was determined using the Kjeldahl digestion and distillation procedure as described by Bremner and Mulvaney (1982). A 10 g soil sample was put into a Kjeldahl digestion flask and 10 ml distilled water added to it. Concentrated sulphuric acid and selenium mixture were added and mixed carefully. The sample was digested on a Kjeldahl apparatus for 3 hours until a clear and colourless digest was obtained. The volume of the solution was made to 100 ml with distilled water. A 10 ml aliquot of the solution was transferred to the reaction chamber and 10 ml of 0.1 ml NaOH solution was added followed by distillation. The distillate was collected in boric acid and titrated with 0.1*N* HCl solution with bromocresol green as indicator. Traces of nitrogen in the reagents and water used were taken care of by carrying out a blank distillation and titration.

Calculation:

$$\% N = \frac{14 \times (A - B) \times N \times 100}{1000 \times w}$$

where:

N = concentration of HCl used in titration.

A = ml HCl used in sample titration B = ml HCl used in blank titration 14 = atomic

weight of nitrogen w = wt. of soil sample

in gram

3.6.2.4 Available phosphorus

This was determined using the Bray P_1 method (Olsen and Sommers, 1982). The method is based on the production of a blue complex of molybdate and orthophosphate in an acid solution. A standard series of 0, 0.8, 1.6, 2.4, 3.2, and 4.0 µgP/ml were prepared by diluting appropriate volumes of the 10 µgP/ml standard sub- stock solution. These standards were subjected to colour development and their respective transmittances read on a 21 D spectrophotometer at a wavelength of 520 nm. A standard curve was constructed using the readings.

A 2.0 g soil sample was weighed into a 50 ml shaking bottle and 20 ml of Bray- 1 extracting solution was added. The sample was shaken for 10 minutes and then filtered through No. 42 Whatman filter paper. Ten millilitres of the filtrate was pipetted into a 25 ml volumetric flask and 1 ml each of molybdate reagent and reducing agent were added for colour development. The percent transmission was measured at 520 nm wavelength on a 21 D spectrophotometer. The concentration of P in the extract was obtained by comparison of the results with a standard curve.

Calculations:

$$P\ (mgkg^{-1}) = \frac{\text{Graph reading} \times 20 \times 25\ w}{\times 10}$$

where:

w = sample weight in grams

20 = ml extracting solution

25 = ml final sample solution

10 = ml initial sample solution

3.6.3 Exchangeable Cations Determination

Exchangeable bases (calcium, magnesium, potassium and sodium) content in the soil were determined in 1.0 *M* ammonium acetate (NH_4OAc) extract (Black, 1965) and the exchangeable acidity (hydrogen and aluminum) was determined in 1.0 *M* KCl extract (McLean, 1965).

3.6.3.1 Extraction of the exchangeable bases

A 10 g soil sample was weighed into an extraction bottle and 100 ml of 1.0 *M* ammonium acetate solution was added. The bottle with its contents was shaken for one hour and the supernatant solution was filtered through No. 42 Whatman filter paper.

3.6.3.2 Determination of calcium

For the determination of calcium, a 10 ml portion of the extract was transferred into an erlenmeyer flask. To this, 10 ml of potassium hydroxide solution was added followed by 1 ml of triethanolamine. Few drops of potassium cyanide solution and few crystals of cal-red indicator were then added. The mixture was titrated with 0.02*N* EDTA (ethylene diamine tetraacetic acid) solution from a red to a blue end point.

3.6.3.3 Determination of calcium and magnesium

A 10 ml portion of the extract was transferred to an erlenmeyer flask and 5 ml of ammonium chloride-ammonium hydroxide buffer solution was added followed by 1 ml of trietnanolamine. Few drops of potassium cyanide and Eriochrome Black T solutions were then added. The mixture was titrated with 0.02*N* EDTA solution from a red to a blue end point.

Calculations:

$$\text{Ca + Mg (or Ca) (cmol/kg soil)} = \frac{0.02 \times V \times 1000}{W}$$

where:

W = weight in grams of soil extracted

V = ml of 0.02 *N* EDTA used in the titration

0.02 = concentration of EDTA used

3.6.3.4 Determination of exchangeable potassium and sodium

Potassium and sodium in the soil extract were determined by flame photometry. Standard solutions of 0, 2, 4, 6, 8 and 10 ppm K and Na were prepared by diluting appropriate volumes of 100 ppm K and Na solution to 100 ml in volumetric flask using distilled water. Photometer readings for the standard solutions were determined and a standard curve constructed. Potassium and sodium concentrations were read from the standard curve.

Calculations:

$$\text{Exchangeable K (cmol/kg soil)} = \frac{\text{Graph reading} \times 100}{39.1 \times w \times 10}$$

$$\text{Exchangeable Na (cmol/kg soil)} = \frac{\text{Graph reading} \times 100}{23 \times w \times 10}$$

Where:

w = air –dried sample weight of soil in grams

39.1 = atomic weight of potassium

23 = atomic weight of sodium

3.7 TREATMENT APPLICATION

Organic (poultry manure) and inorganic (chemical) fertilizers were applied. There were six treatments and are listed below:

T1 - 0 kg N/ha

T2 - 45 kg N/ha

T3 - 90 kg N/ha

T4 - 2 t/ha poultry manure

T5 - 4 t/ha poultry manure

T6 - 6 t/ha poultry manure

Water was sprinkled on the poultry (layer) manure and covered with black polythene sheet for rapid decomposition. Compound fertilizer (NPK-15-15-15) was applied at a rate of 0, 45 and 90 kg N/ha. The fertilizer was applied 5 cm away from the plant at a depth of 2-3cm using the ring method. The organic fertilizer (poultry manure) was also similarly applied at a rate of 2, 4 and 6 t/ ha. The amendments were applied at fourteen days after transplanting.

3.8 CULTURAL PRACTICES

3.8.1 Watering

Since the work was carried out under rainfed condition, supplementary irrigation was done up to harvesting when moisture levels of the soil falls. The same volume of water was provided to all the plants through 1.5 litres Voltic bottles fitted into the soil near the plant (Plate 3.1). A small hole was perforated under the bottles to provide water in a form of drip irrigation.

Plate 3.1: Field experiment showing watering system of *Artemisia annua*

3.8.2 Weeding

Weeds control was by hand hoeing at three, seven and eleven weeks after transplanting (WAP).

3.9 PARAMETERS MEASURED

The following parameters of *Artemisia annua* were measured,

i. Plant height

ii. Stem width iii. Plant canopy spread iv. Internodes distance

v. Number of branches per plant

vi. Fresh and dry shoot weight

vii. Fresh and dry leaf yield

viii. Crude extract weight

xi. Percent artemisinin content

3.10 DATA COLLECTION

Four weeks after transplanting, plant height, canopy spread, stem width and internodes distance were recorded and continued at two-weeks interval until flowering. Data from samples of 6 plants per plot were taken at the various growth stages.

A 5m measuring tape was used to measure the height of the plant from the level of the soil to the top of the highest leaf. The plant canopy spread was measured by stretching measuring tape horizontally across the center of the plant. The stem width, 5cm above the ground was measured by means of a thread. The internodes distance was also measured by using thread. The exact measure was determined by stretching the thread along a 30cm rule.

At harvest, fresh and dry weights of leaf and shoot were obtained by using an electronic weighing scale. Samples were dried under shade for seven-days and the dry weights taken using electronic weighing scale.

3.11 HARVESTING FOR EXTRACTION

Three plants from each plot were harvested at two different schedules. The first harvest was done prior to flowering (104 days after planting). The second sample was harvested at full bloom (134 days after planting). The sampled plants were dried under shade for seven days. The harvested plants were prunned to obtain the leaves and the flowers. These were thoroughly dried to obtain the biomass. The dried biomass was size reduced by cutting using a cutlass after which it was milled using the milling machine.

3.12 EXTRACTION

Crude extracts from the samples from the plots were prepared at Chemical Engineering Department, KNUST. The method used for extraction was batch percolation method using petroleum ether as the solvent. The following were some of the major equipment used for the extraction: Soxhlet extractor, cutting mill, analytical balance/ weighing scale, heating mantle, stop watch, thimble, beakers, distillation apparatus and evaporator. The Soxhlet apparatus is the main solvent extraction set-up that was used in this experiment (Plate 3.2). It consisted of four main parts, namely: the heating source, round bottom flask, the soxhlet or thimble holder and the condenser.

A thimble, sown from gray-baft material was filled with 100.0g of the milled biomass and placed in the extractor globe. The round bottom flask was filled with 1000ml 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. The time taken for the solvent to fill the globe and drain out of the globe was noted. The temperature of the extract after the draining time was noted. 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.

Plate 3.2: Experiment Setup for Batch Percolation

3.12.1 Purification

The crude extract obtained was flash-evaporated to 10% of its initial volume. The remaining liquor was left to stand at ambient temperature for 48h to crystallize crude artemisinin. The liquor was decanted to obtain crude artemisinin. The crude artemisinin was washed with warm hexane to remove the waxes and other precipitated impurities. Artemisinin was recrystallized several times from ethanol-water azeotrope (96 % w/v ethanol) in the presence of activated carbon adsorbent in order to remove the waxes. It was then followed by vacuum filtration to obtain crystals of artemisinin in ethanol.

3.12.2 Detection of Artemisinin in Crude Extract by Liquid ChromatographyMass Spectromatry

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.

3.13 STATISTICAL ANALYSIS

All data collected were subjected to analysis of variance (ANOVA). Genstat (2000) statistical package was used to analyze the data collected. The least significant difference (LSD) test at 5% was used to compare treatment means.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 INITIAL SOIL PROPERTIES AND TOTAL NUTRIENT CONTENT OF POULTRY MANURE

4.1.1 Results

4.1.1.1 Soil characteristics

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, 1984). The data showed high values for dry bulk density. Generally, the fertility status of the soil was low and decreased with depth and response to the major nutrients was expected. From the results of particle size analysis, the content of sand, clay and silt were high, low and moderately low respectively at both depths. Hence, the soil was classified as sandy loam at both depths (Landon, 1984).

4.1.1.2 Nutrient content of poultry manure

Nitrogen and potassium contents of the poultry manure were high (Table 2). However, phosphorus and calcium contents were low, whilst pH value indicated alkalinity (Landon, 1984)

Table 4.1: Mean chemical and physical properties of soil at the experimental site before application of treatments

Soil Property	Soil Depth (cm)	
	0-20	**20-40**
PH (1:2.5 H_20)	6.15	5.94
Org. Carbon (%)	1.78	1.07
Total N (%)	0.163	0.12
P (ppm)	20.54	16.57
Exchangeable Cations (cmol/kg)		
Ca	4.53	4.26
Mg	2.93	2.93
K	0.12	0.17
Na	0.39	0.49
Dry Bulk Density (g/cm)	1.49	1.40
Particle Size (%)		
Sand	78.8	76.1
Silt	8.0	4.66
Clay	13.2	19.2

Table 4.2: Total nutrients content of poultry manure

Physico-chemical Property	Mean (%)
N	2.71
P	0.105
Ca	4.20
Mg	5.70
K	26.80
Na	7.0
pH	8.05

4.1.2 DISCUSSION

Soil characteristics before imposition of treatments

The slightly low pH values of soil (Table 4.1) were similar to those reported for some Ghanaian soils by Adu and Tenadu (1979). Strong leaching of the basic cations out of the top soil contributed to the acidic nature of the soil. It is expected that this factor will affect the dynamics of all nutrients and especially phosphate because the available P depends to a large extent on interactions with constituents carrying a variable charge (Quang *et al*., 1996).

The soil for the study 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. In their study, they observed an increase in yield of artemisia after application of nitrogen fertilizers. The low soil organic carbon and total N contents recorded were by virtue of high temperatures resulting in rapid organic carbon decomposition. According to Metson (1961), a productive soil should have an organic carbon content of 2.32 %. The low organic content and low exchangeable bases reflected the generally weathered soils in the humid rainforest agro-ecological zone of the country (Owusu-Bennoah *et al*., 2000). This soil property is attributed mainly to the excessive leaching of the soils caused by high rainfall associated with humid rainforest in Ghana and constant plant nutrient uptake by the crops. The high build up of available K and Na in the 20-40cm depth could be attributed to movement of the minerals due to leaching.

4.2 GROWTH AND DEVELOPMENT OF *ARTEMISIA ANNUA*

4.2.1 Results

4.2.1.1 Plant height (cm)

Figure 4.1 shows the effect of treatments on plant height. Treatment significantly affected plant final height. Over the period, the highest and the lowest plant height values of 156.7 cm and 45.3 cm were recorded by 4 t/ha poultry manure and 0 kg N/ha treatments respectively. Mean plant height in the 4th week was 47.1cm. This increased to 67.5, 105.4, 126.9 and 143.8 cm at 6, 8, 10 and 12th week respectively.

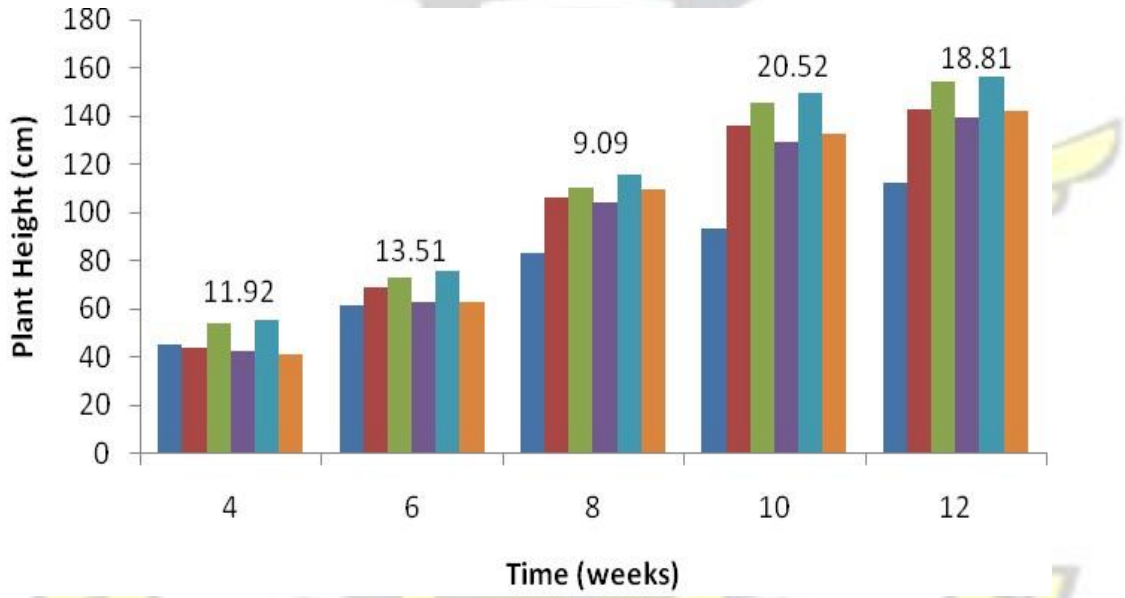


Figure 4.1: Effect of treatments on plant height of *Artemisia annua*

Values on bars represent lsd values at $p<0.05$

4.2.1.2 Plant canopy spread (cm)

Application of 4 t/ha poultry manure registered the highest plant spread whilst 0 kg

N/ha recorded the least (Fig.4.2). The difference in plant spread were however, significant (P<0.05). The pattern of growth of *Artemisia annua* for the different treatments from 4th to 12th week was the same, an indication that the growth was mirrored in the subsequent years.

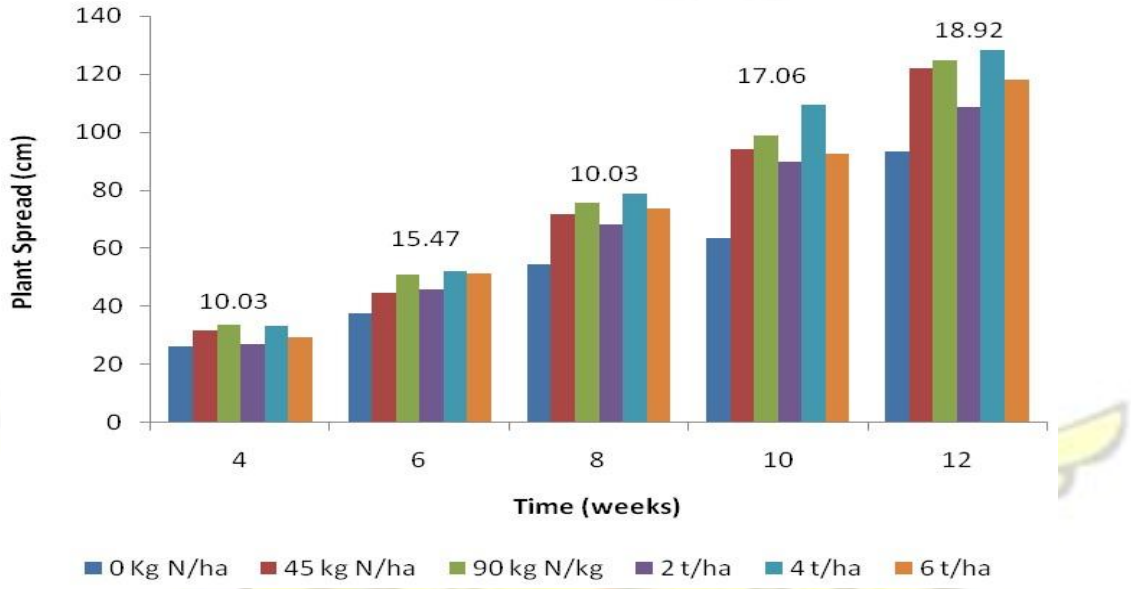


Figure 4.2: Effect of treatments on plant canopy spread of *Artemisia annua.*

Values on bars represent lsd values at p<0.05

4.2.1.3 Stem width (cm)

Fig 4.3 shows growth pattern as measured by mean stem width for artemisia with application of treatments. The plant responded positively to increase in fertilizer levels. *Artemisia annua* plants treated with 90 kg N/ha recorded higher stem width compared to the other treatments. The treatments produced significant differences (p< 0.05) in stem width at the final sampling period.

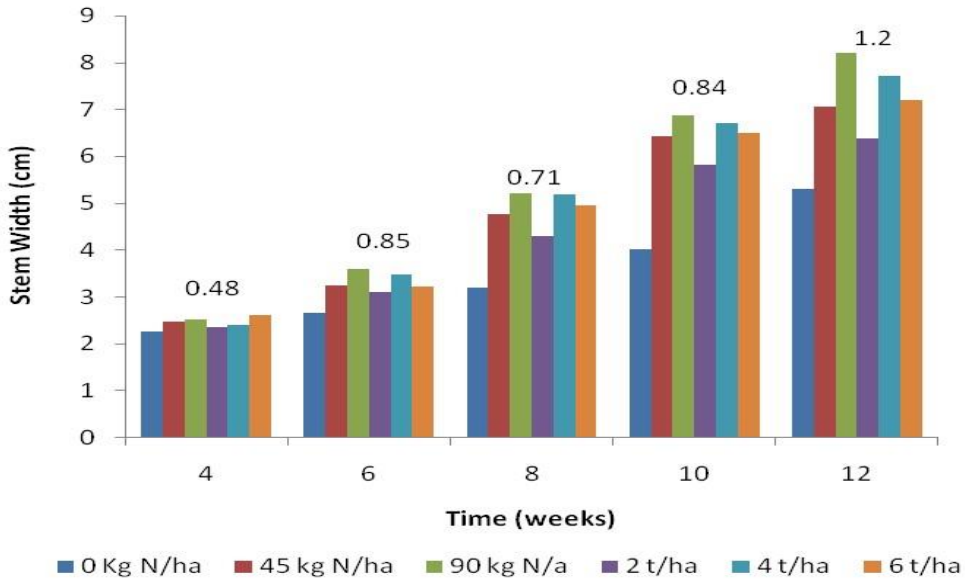


Figure 4.3: Effect of treatments on stem width of *Artemisia annua*

Values on bars represent lsd values at $p<0.05$

4.2.1.4 Internodes distance (cm)

Application of 4 t/ha poultry manure produced plants with longer internodes with 0 kg N/ha recording the lowest internodes distance (Fig. 4.4). Treatments showed consistent growth pattern in this trait but differences were not significant ($P>0.05$) between treatments.

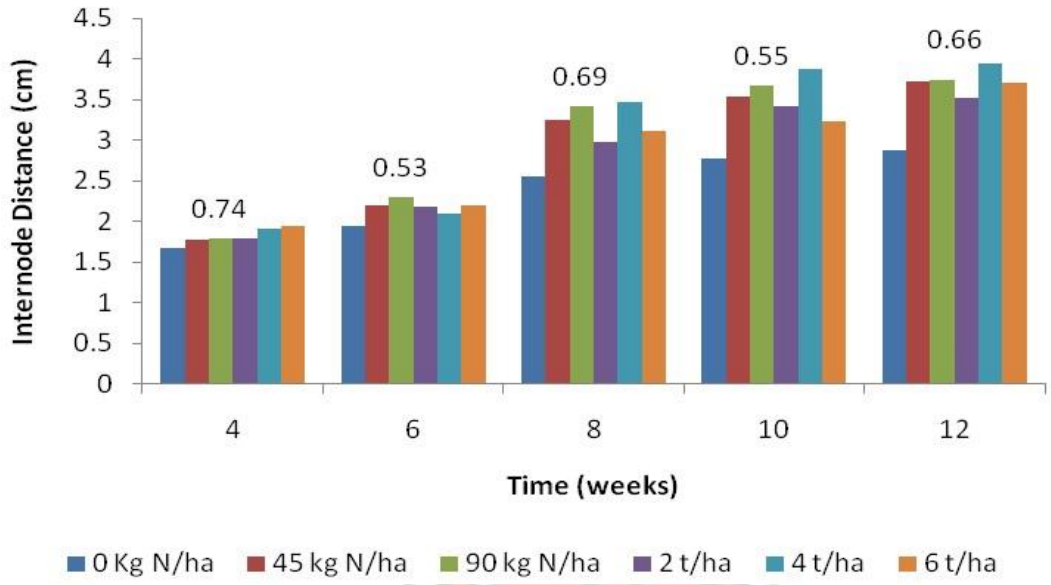


Figure 4.4: Effect of treatments on internodes distance of *Artemisia annua*

Values on bars represent lsd values at $p<0.05$

4.2.1.5 Number of branches per plant

Figure 4.5 shows the mean number of branches or laterals as affected by the treatments. The number of branches per plant produced varied significantly ($P<0.05$) among treatment means for 4 t/ha PM, 90 kg N/ha, 45 kg N/ha, 6 t/ha PM and the control. The results indicated that 4 t/ha PM and 90 kg N/ha produced the highest number of branches of 58 and 52 per plant respectively. This was followed by 45 kg N/ha, 6 t/ha PM and 2 t/ha PM with 0 kg N/ha producing the least number of branches. The control recorded the lowest mean number of branches (35) per plant.

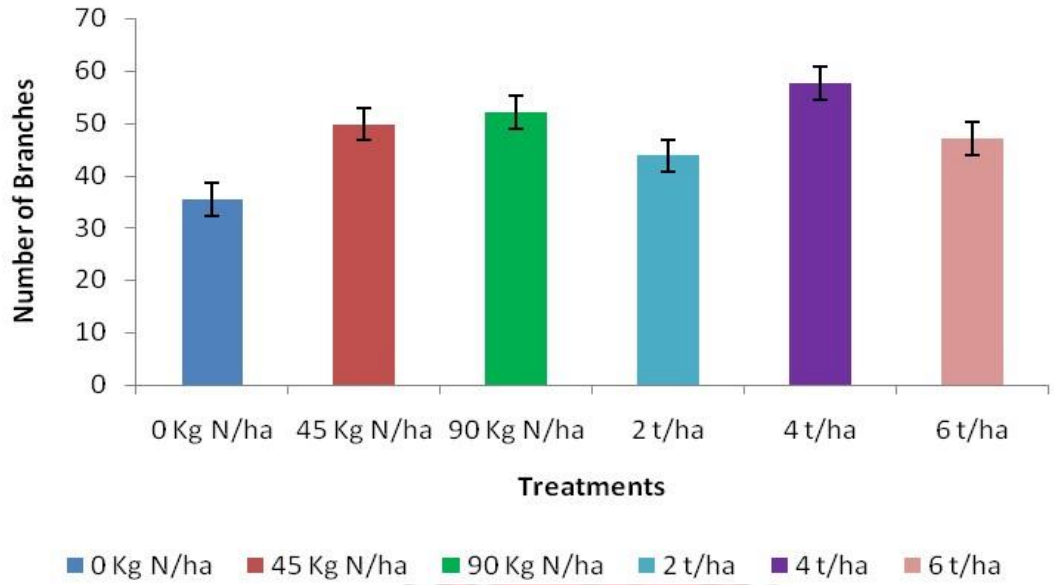


Figure 4.5: Effect of treatments on number of branches per plant of *Artemisia annua*

The bars represent lsd values at $p<0.05$

4.2.2 Discussion

Influence of nitrogen fertilizer on growth and development of *Artemisia annua*

Variations in vegetative growth parameters (plant height, plant spread, stem width, internodes distance and number of branches per plant) during the period of study were due to differences in rates of organic and inorganic fertilizers applied.

Results of plant height showed consistent increase with 4 t/ha producing the highest height (Fig.4.1). Significant differences existed among treatments studied. The increased in plant height due to increase in N- fertilizer application (Fig.4.1) may be attributed to adequate supply of nutrients that influenced cell division and cell enlargement resulting in better plant height as suggested by Martinez and Staba (1988), and Gadhi Kumar (1996). Plants with no manure or compound fertilizer additions were generally shorter and produced

least growth indicating least plant nutrients availability in the soil to support artemisia growth, a similar situation observed by Silva *et al.*(1971).

Application of N-fertilizer at a rate 90 kg N/ha did not produce significantly taller plants than those of 45 kg N/ha treated plots. This suggests that application at a rate of 45 kg N/ha is probably the optimum rate above which artemisia does not respond. Poultry manure at a rate of 4 t/ha produced generally higher plants, which did not differ from that of 6 t/ha. This indicates that excessive application of plant nutrients results in inefficient use by plants as high total dissolved salt levels accumulate in the soil making nutrients unavailable (Hileman,1971).

Results of plant canopy spread (Fig.4.2) lend credence to the findings by Ferreira *et al*. *(*1995a) who reported that providing N-fertilizer causes the plant to branch out and substantially increased leaf biomass. The significant increase in plant spread with increase in N-levels especially 4 t/ha poultry manure was possible because higher N levels probably led to improved physiological activity. Good nutrition enabled proper seedling establishment, active vegetative growth, enhanced dry matter accumulation and increased crop yield a similar trend observed by Miller and Turk (1991). The maximum plant height and plant spread produced greatly influenced the yield of leaf biomass recorded in this study.

The mean stem width of *Artemisia annua* recorded for the various poultry manure treatments were significantly different from each other (Fig.4.3). The results corroborate with the findings of Ferraira *et al*. (1995b) 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 (Bandel *et al*., 1972; Kallah and Adamu, 1998).

The results for internode distances (Fig.4.4) corroborate the observation made by Bandel *et al*. (1972) that poultry manure contains appreciable amount of nitrogen that results in the production of longer internodes. Similarly, Kallah and Adamu (1998) observed that organic soil amendments promote vigorous vegetative growth, development and produced long nodes.

The results for number of branches per plant (Fig.4.5) showed significant differences between treatments. This is in agreement with Rao *et al.* (1985) who studied the effect of organic and inorganic fertilizers on artemisia and found out that the number of branches per plant was significantly enhanced by the greater availability of organic nutrients. An obvious reason for this observation was due to their positive effect on shoot biomass production. The higher number of branches recorded on the poultry manure treated plots was expected since application of poultry manure increased nitrogen availability that in turn increased production of laterals, a similar observation made by Ming (1994) and Arul (2002). Application of biofertilizers increased the number of laterals of artemisia due to the arrest of the apical dominance resulting in increase in laterals (Krishnamoorthy and Madalageri, 2002).

The 4 t/ha poultry manure and 45 kg N/ha had 66.6% and 48.1% yield advantage over the control, respectively. However, 4 t/ha poultry manure was higher than 45 kg N/ha by 12.4% (Fig. 4.5).

Table 4.3 - 4.6 show mean squares of quantitative characters measured in *Artemisia annua*. Plant height, plant spread, stem width and number of branches per plant showed significant variation. Yield traits such as fresh and dry leaf yield at pre-flowering, dry leaf yield at full bloom, fresh and dry shoot weight, artemisinin content and artemisinin yield at full bloom showed significant variation between the treatments.

Table 4.3: Mean Squares of Quantitative Characters in *Artemisia annua* evaluated under different treatments and growth stages

Sources of Variation	Degrees of freedom	plant height (cm)	plant spread (cm)	Stem width (cm)
Replication	2	16.8	60.8	1.2329
Treatment	5	761.4**	498.7*	3.1337**
Residual	10	115.6	106.9	0.4809

*, ** Significant at 5% and 1% respectively

Table 4.4: Mean Squares of Quantitative Characters in *Artemisia annua* evaluated under different treatments and growth stages

Sources of Variation	Degrees of freedom	Fresh leaf yield (kg/ha) at pre- flow.	Dry leaf yield (kg/ha) at pre-flow.	Dry leaf yield (kg/ha) at full bloom
Replication	2	1779	231.5	19352
Treatment	5	10352**	2952.8*	49173**
Residual	10	1432	660.0	7382

*, ** Significant at 5% and 1% respectively

Table 4.5: Mean Squares of Quantitative Characters in *Artemisia annua* evaluated under different treatments and growth stages

Sources of Variation	Degrees of freedom	Fresh shoot weight (g) at full bloom	Dry shoot weight (g) at full bloom	Artemisinin content (%) at full bloom
Replication	2	8668	726.6	0.5481
Treatment	5	3826*	6000.3**	6.3886**
Residual	10	15787	950.2	0.916

*, ** Significant at 5% and 1% respectively

Table 4.6: Mean Squares of Quantitative Characters in *Artemisia annua* evaluated under different treatments and growth stages

Sources of Variation	Degrees of freedom	Artemisinin yield (kg/ha) at full bloom	Number of branches/ plant
Replication	2	50.93	129.35
Treatment	5	238.79*	172.49*
Residual	10	60.98	47.50

*, ** Significant at 5% and 1% respectively

4.3 YIELD OF *ARTEMISIA ANNUA*

4.3.1 Results

4.3.1.1 Fresh and dry leaf yield of *Artemisia annua*

4 t//ha poultry manure produced the highest fresh and dry leaf yields at both preflowering and full bloom stages of harvesting (Table 4.7). Mean values for 4 t/ha PM, 90 kg N/ha, 6 t/ha PM, and 45 kg N/ha of fresh and dry leaf yields at pre-flowering were significantly

higher (P<0.05) than 0 kg N/ha. Significant differences (P<0.05) in fresh leaf yield at full bloom were observed between 4 t/ha PM and 0 kg N/ha. However, differences between 45 kg N/ha, 90 kg N/ha, 2 t/ha PM and 6 t/ha PM were statistically the same. Dry leaf yield at full bloom for the treatments 90 kg N/ha, 4 t/ha PM and 6 t/ha PM were significantly (P<0.05) higher than the control. Differences between 90 kg N/ha, 4 t/ha PM and 6 t/ha PM were statistically the same at full bloom. 4 t/ha PM recorded 140.8 % and 203.2 % fresh and dry leaf yield advantage over the control at pre-flowering and 92.4 % and 149.8 % yield advantage over the control at full bloom. 4 t/ha PM had 8.2% and 17.3% superiority over 90 kg N/ha fresh leaf yield at both pre-flowering and full bloom harvests respectively, whilst 90 kg N/ha had 4.7% and 32.2% yield advantage over 45 kg N/ha at full bloom.

Table 4.7: 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	44.40	565.00	233.00
45 kg N/ha	274.30	109.30	701.00	313.00
90 kg N/ha	287.10	96.60	927.00	472.00
2 tons/ha PM	231.50	69.10	787.00	356.00
4 tons/ha PM	310.50	134.60	1087.00	582.00
6 tons/ha PM	276.10	93.90	993.00	480.00
MEAN	251.40	91.3	843.00	406.00
CV (%)	7.6	6.8	7.9	14.0
LSD (0.05)	76.48	46.74	444.6	156.3

4.3.1.2 Fresh and dry shoot weight (g)

Significant differences (P<0.05) in fresh and dry shoot weight at full bloom were observed between treatments (Table 4.8). However, differences between 90 kg N/ha, 4 t/ha PM and 6 t/ha PM were statistically the same. Mean value of 4 t/ha PM for Fresh shoot weight at pre-flowering were statistically similar to all plots except the control. However, mean values for dry shoot weight at pre-flowering did not record any significant difference (P<0.05) between the treatments. Plots treated with 4 t/ha PM registered the highest fresh and dry shoot weight at both harvests whilst the control recorded the least. There were considerable increases in fresh and dry shoot weight at full bloom over values recorded at pre-flowering. The control recorded fresh and dry shoot weights increases of 390.5% and 259.1% at both harvesting stages respectively whilst 6 t/ha poultry manure recorded increases of 413% and 397.5% in fresh and dry shoot weights at both stages of harvesting respectively.

Table 4.8: Mean fresh and dry shoot weight of *Artemisia annua* evaluated under different treatment and growth stages (g)

Treatment	Pre-flowering		Full bloom	
	Fresh shoot	Dry shoot	Fresh shoot	Dry shoot
0 kg N/ha	57.9	19.8	284.0	71.1
45 kg N/ha	81.1	29.3	510.0	127.4
90 kg N/ha	118.1	31.1	678.0	144.8
2 tons/ha PM	84.0	21.1	446.0	111.4
4 tons/ha PM	143.2	34.6	751.0	207.1
6 tons/ha PM	111.5	27.9	572.0	138.8
MEAN	99.3	27.3	540.0	133.4
CV (%)	18.4	25.6	7.0	8.2

LSD (0.05)	67.37	18.9	228.6	56.08

4.3.1.3 Crude Extract content of *Artemisia annua* (g)

Results indicated significant effects of treatments on crude extract weights at both preflowering and full bloom (Table 4.9). The control consistently produced the lowest values at both harvests. Plots treated with 4 t/ha PM gave the highest crude weight (2.56g) at pre-flowering which showed no significance (P >0.05) from the other treatments. At full bloom, 90 kg N/ha gave the highest value of 2.98g which was statistically similar to all plots except the control. Crude extract weight at pre-flowering showed higher variability (CV=28.4%) among treatments than at full bloom (CV=21.1%). There were considerable increased in crude extract weights at preflowering compared to values obtained at full bloom. In calculating the relative yield increase (RI) of treated plots, control plots were used as standard treatment. The RI values obtained during pre-flowering for 45 kg N/ha, 90 kg N/ha, 2 t/ha PM, 4 t/ha PM and 6 t/ha PM were 2.7, 8.7, 13.3, 17.9 and 8.2 % respectively. However, at full bloom the values had increased to 35.2, 54.4, 40.9, 32.6 and 33.1 % for 45 kg N/ha, 90 kg N/ha, 2 t/ha PM, 4 t/ha PM and 6 t/ha PM respectively.

Table 4.9: Effect of treatments on crude extract weight of *Artemisia annua* evaluated under different treatments and growth stages (g)

	Pre-flowering	Full bloom
Treatment	**Crude extract**	**Crude extract**
0 kg N/ha	2.17	1.93
45 kg N/ha	2.23	2.61
90 kg N/ha	2.36	2.98
2 tons/ha PM	2.46	2.72
4 tons/ha PM	2.56	2.56
6 tons/ha PM	2.35	2.57

MEAN	2.35	2.56
CV (%)	28.4	21.9
LSD (0.05)	1.219	1.020

4.3.1.4 Artemisinin content and yield

Table 4.10 shows the results of artemisinin content and yield taken at both harvests. 2 t/ha PM and 45 kg N/ha produced the highest artemisinin content at pre-flowering and full bloom respectively. The treatments effect on artemisinin content followed the order of 2 t/ha PM>45 kg N/ha>0 kg N/ha>6 t/ha PM>4 t/ha PM>90 kg N/ha at pre-flowering and 45 kg N/ha>90 kg N/ha>0 kg N/ha>4 t/ha PM>2 t/ha PM>6 t/ha PM at full bloom in a decreasing order. The results indicate that mean values at pre- flowering were not significantly different from the control ($p>0.05$). Mean values for artemisinin content at full bloom for 45 kg N/ha and 90 kg N/ha were significantly different from 6 t/ha PM, 2 t/ha PM and 4 t/ha PM ($P<0.05$). The results also showed that 4 t/ha PM produced the highest artemisinin yield at both harvests. Artemisinin yield at pre-flowering were not significantly different ($p>0.05$). However, values of 4 t/ha PM and 90 kg N/ha for artemisinin yield at full bloom were significantly different ($p<0.05$) from 0 kg N/ha and 2 t/ha PM.

Table 4.10: 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	3.40	7.26	16.91
45 kg N/ha	7.97	8.71	7.92	24.78

90 kg N/ha	6.74	6.51	7.77	36.67
2 tons/ha PM	8.43	5.82	5.05	17.97
4 tons/ha PM	7.11	9.57	6.40	37.24
6 tons/ha PM	7.23	6.78	4.43	21.26
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

4.3.2 Discussion

Effect of nitrogen fertilizer on yield of *Artemisia annua*

As found in this trial, increasing poultry manure treatments from 4 to 6 t/ha on fresh and dry leaf yield did not show significant differences (Table 4.7). The first increments of N added to the soil were effective in increasing dry matter yield and secondary product accumulation in herbs. Further increase in N-application generally does not result in large yield increase. Such a relationship between N-levels and plant response has been observed in artemisia (Laughlin, 1993). This indicates that for organic fertilizers application, it would be reasonable to apply 4 t/ha poultry manure instead of 6 t/ha, because the additional 2 t/ha did not cause any significant gain. The mean values obtained for fresh and dry leaf yields in this study were similar for 45 kg N/ha and 90 kg N/ha but higher than the control. Fresh and dry leaf yields from the manure-treated plots were similar to those from the compound fertilizer-treated plots, suggesting that manure at this concentration may replace chemical fertilization.

The results reported here are similar to those established by several investigators (Snedecar and Cochran, 1990; Parakasa, 1997) that applications of poultry manure at the rate of 3.0 and 4.5 t/ha increased biomass yield and total essential oil of artemisia. Field

trial results have indicated that between 3.0 and 4 t/ha, poultry manure was beneficial in artemisia production (Liebhardt, 1976; De Ridder, 1990). The data obtained in this study confirms previous reports that poultry manure yielded higher than compound fertilizer treatments (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 fresh herbage yield (Arul, 2002; Krishnamoorthy and Madalageri, 2002). A soil rich in nutrients enhanced by the use of organic fertilizers plays an essential role in plant growth and development, biosynthesis of the organic substance at all levels and growth yield characters such as biomass yield as reported by Marculescu *et al. (*2002).

The data indicated that 90 kg N/ha produced higher fresh and dry herbage yields compared to the 45 kg N/ha. This observation corroborates with findings of Simon *et al.* (1990) and Magalhaes *et al. (*1996) who observed that a high whole plant biomass was achieved when N was applied at 64 kg N/ha but not 32 kg N/ha. This, however, contrasts the findings of Wright (2002) who noted an increased in the yield of a whole plant biomass when N was applied at a rate of 40 kg N/ha but not 80 kg N/ha.

The control at both harvests had the lowest fresh and dry leaf yield (Table 4.7). The lowest yield recorded by the control was possibly due to the elevated nutritional stress of plants from these plots. Results from this study were consistent with the findings of Cox (1992) who reported that the largest growth and yield response in artemisia generally results from N-application. Reduced nutrient uptake or stress has a great influence on photosynthesis. Since N is a constituent of chlorophyll, if it is limited, chlorophyll may not be formed and

yield reduction or complete crop failure may occur. Evaluation of leaf biomass is important since it is the precursor for higher artemisinin yield. The lowest yield recorded by the control is a major agronomic limitation since leaf biomass is an important attribute in artemisia, which influences final artemisinin yield.

The results of the shoot weight (Table 4.8) confirm the findings of Dixit (1997) and Mathias (1997) who reported that nitrogen in poultry manure increased plant shoots produced. The possible reason for increased in shoot weight may be due to increased length of shoot when poultry manure was applied due to its effect on photosynthetic efficiency of a crop resulting in the production of more number of leaves and stems. It was observed that plants without poultry manure treatment produced the lowest shoots (Table 4.8) due to the low soil nutrients status as shown by the soil physico-chemical analysis. This is in agreement with the observations of Agyenim Boateng (1999) who, working on maize found that the total weight of dry matter was higher in plants treated with poultry manure.

The results indicated that the crude extract at both harvests did not show any significant difference due to the application of the fertilizers (Table 4.9). 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 Singh (2001) who observed that crude extract contents was not influenced by the application of different levels of fertilizers. This observation however, is in contrast with a report by Delabays *et al*. (2001) that crude extract content and artemisinin content are both influenced by genetic and environmental factors such as nitrogen availability. The

increase in crude extract content by the application of organic and inorganic fertilizers compared to the control might also be due to increased number of laterals that increased the number of flower heads, which eventually enhanced the level of crude extract; similar situation was observed by Singh *et al*. (2004). 4 t/ha of poultry manure and 90 kg N/ha compound fertilizer which gave higher concentration of crude extract content at pre-flowering and full bloom (Table 4.9) possibly had higher concentration of waxes, chlorophyll, essential oils and artemisinin (Munnu and Ramesh, 2000).

The higher amount of artemisinin content (Table 4.10) from the crude extracts from this study, compared to artemisinin content from plant samples of previous studies is a confirmation of the higher potential of crude extracts to produce higher artemisinin content due to the presence of other metabolites such as waxes and chlorophyll. 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 samples respectively.

Judging from the results, it appears that N-fertilization did not affect artemisinin content (Table 4.10) even though the effect of nitrogen on artemisinin content has been reported by Sukhmal *et al*. (2001). The higher artemisinin content observed on control plots probably suggests that artemisinin content was not influenced by soil fertility status. This result is consistent with the findings of Cox (1992) and Singh (2001) who indicated that artemisinin content is influenced by genetic factors. The pathway of artemisinin synthesis and the mechanism for its accumulation in leaves and inflorescences most probably determined the final artemisinin content in *Artemisia annua* and the effect of other

environmental factors such as nutrient availability might be incidental; a situation observed by Chalapathi *et al*. (2004).

The artemisinin content and artemisinin yield at full bloom stage were significantly influenced by the treatments ($P<0.05$). This observation confirms reports by other investigators (Parakasa, 1997; Sukhmal *et al.*, 2001) who claimed that organic and inorganic fertilizers increased leaf biomass production and subsequently led to an increase in artemisinin yield. This explains why evaluation of leaf biomass accumulation or production by *Artemisia annua* was more important, since it is a precursor for higher artemisinin yield (EABL, 2005). The study also reveals that the pattern of plant height, plant spread, number of branches per plant and dry leaf yield largely correspond to that of artemisinin yield obtained. This corroborates findings by Laughlin (1994) who stated that to achieve a commercially viable yield of artemisinin from *Artemisia annua*, the evaluation of growth and yield components is important. This implies that treatments that yield high morphological traits should be considered for higher artemisinin production. The lowest artemisinin yields of 3.40 kg/ha and 16.91 kg/ha at both harvests were obtained from the control plots. This was probably due to low leaf biomass production from these plots.

Decrease in yield in the control plots can be attributed to the low inherent nutrient content of the soil as shown by the soil chemical analysis (Table 4.1). Though, it was evident that the control plots had the potential to give high artemisinin content (Table 4.10), their ability to produce artemisinin yield on commercial basis would be lower on account of their low leaf biomass production. Application of 4 t/ha poultry manure

produced highest artemisinin yield at pre-flowering and full bloom due to its influence on profuse leaf biomass production.

Values for artemisinin content at pre-flowering were considerably higher than artemisinin content at full bloom (Table 4.10). Several investigators, (Laughlin 1994, Wordanbag *et al*., 1994, Laughlin *et al*., 2002) have reported similar results of higher artemisinin content at pre-flowering. The slight decline in artemisinin content at full bloom is probably due to the partition of artemisinin or its precursors into flowering tops or due to its catabolism as suggested by (Laughlin *et al*., 2002).

4.4 Correlation Analysis of Morphological Traits of *Artemisia Annua*

4.4.1 Results

4.4.1.1 Correlation among traits

The estimated correlation among traits is presented in Table 4:11. Significant positive correlations were observed between plant height, plant canopy spread and number of branches per plant and dry leaf yield at pre-flowering and full bloom. Artemisinin yield at both harvests ranges from 0.15 to 0.79.

The lowest correlations were observed between maximum plant height and crude extract at full bloom while the highest were recorded between maximum plant height and plant canopy spread. The number of branches per plant had high positive significant correlation with all the parameters measured, except for crude extracts and artemisinin content at both harvests (Table 4.11). Crude extract content at pre-flowering and full bloom was mostly not significant with all parameters, except between crude extract content at pre-

flowering and plant spread. The correlations between all parameters for artemisinin content at both harvests were mostly not significant except in the artemisinin yields at both harvests. The correlation analysis of the data also shows that plant height and plant spread have significant positive correlation with dry leaf yield and artemisinin yield at both harvests.

Table 4.11: Correlation analysis of quantitative characters of *Artemisia annua* evaluated under different treatments

	1	2	3	4	5	6	7	8	9	10	11
1	1										
2	0.01	1									
3	0.005	-0.16	1								
4	-0.14	0.18	0.13	1							
5	-0.19	0.007	0.32	0.39	1						
6	0.41*	-0.34	0.49*	0.06	0.19	1					
7	0.04	0.65**		0.34*	0.07	0.13	0.17	1			
8	-0.20	-0.33	0.59**		0.30	0.30	0.75**		0.15*	1	
9	0.05	0.12	0.66**		-0.02	0.16	0.56*	0.73**		0.56*	1
10	-0.05	-0.10	0.70**		-0.01	0.33	0.44*	0.51**		0.54*	0.66**
11	-0.02	-0.31	0.55**		-0.18	0.49*	0.54*	0.40*	0.53*	0.63**	

											1
										0.79**	

* Significant at p=0.05; ** Significant at p=0.01

1. Artemisinin content at full bloom
2. Artemisinin content at pre-flowering
3. Number of branches per plant
4. Crude extracts weight at full bloom
5. Crude extracts weight at pre-flowering
6. Artemisinin yield at full bloom
7. Artemisinin yield at pre-flowering
8. Dry leaf yield at full bloom
9. Dry leaf yield at pre-flowering
10. Plant height
11. Plant canopy spread

4.4.2 Discussion

Associations among quantitative traits in *Artemisia annua*

The correlation analysis revealed a significant positive correlation between plant height, plant spread, number of branches and dry leaf biomass and artemisinin yield at preflowering and full bloom stage (Table 4.11). This trend was expected since the major organ in artemisinin production is the foliage. The results suggested that the rate of leaf biomass yield kept pace with the rate of crop growth parameters. An experiment has confirmed positive correlation between plant growth traits and biomass yield of artemisia (Dharm *et al*., 1996).

The significant positive correlation of plant height, plant spread, number of branches with leaf biomass and artemisinin yield indicates the expected influence of these growth parameters on the yielding ability of *Artemisia annua*. It can therefore be inferred that leaf biomass yield and artemisinin yield are largely dependent on the effect of the growth traits such as plant height and plant spread. There is a positive correlation between leaf biomass yield and artemisinin yield at pre-flowering and full bloom stage. The results could imply that artemisinin yield largely depends on leaf biomass accumulation. The linear relationship between leaf biomass accumulation and artemisinin yield indicates that indirect selection via high leaf biomass production would generally be effective for high artemisinin yield (Sushil, *et al*., 2004). The correlation between the artemisinin yield and foliage production confirms this trend. A similar observation was made by Sushil *et al*. (2004) who reported positive correlation between artemisinin in leaves and artemisinin yield harvested at different stages. The positive correlation is an indication of a high linear

correlation between artemisinin content and artemisinin yield. Hence, soil amendments that promote biomass yield will have positive influence on artemisinin production.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

The main purpose of investigating the effect of organic and inorganic fertilizers on the growth, development and yield of *Artemisia annua* was to estimate the leaf biomass and artemisinin content under different rates of application of compound fertilizers and poultry manure.

From the results of the study, the following conclusions can be drawn:

Organic and inorganic fertilization did positively influence growth and development of *Artemisia annua*. Poultry manure applied at a rate of 4 t/ha promoted good growth and increased the yield of *Artemisia annua*. 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 crop 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 treatments.

The study has revealed that poultry manure applied at a rate of 4 t/ha produced more crude extract at full bloom, whilst application of 90 kg N/ha produced more crude extract at pre-flowering. However, there were no significant differences ($P \geq 0.05$) between the treatments at both harvests. The research has indicated that, poultry manure applied at a rate 4 t/ha produced high artemisinin yield at both pre-flowering and full bloom stage. Unlike artemisinin yield at pre-flowering, artemisinin yield at full bloom showed observable differences among treatments.

The study has clearly shown that, the mean crude extract weight and artemisinin content were higher when the plants were harvested at pre-flowering stage compared to full bloom stage. Plants treated with 4 t/ha poultry manure and harvested at full bloom gave the highest crude extract and artemisinin yield content 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 N kg/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.

Generally, the results of the study indicated significant effect of treatments on plant height, plant canopy spread, number of branches per plant, fresh and dry leaf yield at pre-flowering, dry leaf yield at full bloom, fresh and dry shoot weight at full bloom and artemisinin content and yield at full bloom. However, internodes distance, fresh leaf yield at full bloom, fresh and dry shoot weight at full bloom and artemisinin content and yield at pre-flowering did not show any significant treatment effect. Based on these observations the hypothesis of this study could be accepted.

5.1 AREAS FOR FUTURE INVESTIGATION

For future research work, it is recommended that the following aspects of poultry manure and compound fertilizer in artemisinin production be considered:

- long-term evaluation of poultry manure and compound fertilizers to determine their agronomic efficacy in the production of artemisia.
- future studies need to consider the socio-economic implication of poultry manure and compound fertilizers in the production of artemisinin.
- further studies need to be conducted to determine the complementary effect of organic and inorganic fertilizers on artemisinin production.

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APPENDIX

Appendix 1: Mean climatic data of experimental location during the period of Study

Month & Year	Temperature (°C) Min.	Max.	Relative Humidity (%)	Rainfall (mm)	Sunshine Hours
April, 2008	22.9	33.3	83	117.1	5.5
May, 2008	22.8	33.0	82	185.8	5.3
June, 2008	22.5	31.4	85	279.8	4.6
July, 2008	22.3	29.8	88	145.0	3.3
August, 2008	20.8	29.5	88	164.5	3.4
September, 2008	21.3	30.0	87	148.9	3.3
October, 2008	21.6	31.3	85	95.8	5.7
November, 2008	22.2	32.7	84.20	30.7	4.8
December, 2008	21.1	32.6	84.0	47.5	5.6