Chapter one

Introduction and Objectives

1.1 Introduction

Aloe vera (L.) Burm. is a multifarious and enormously adaptable plant having botanical name as *Aloe barbadensis* Miller, it is a well known for its health, beauty, medicinal and skin care properties (Mukesh *et al.*, 2010; Dagne *et al*.2000). The name aloe was derived from the Arabic word Alloeh means "shining bitter substance," while vera in Latin means "true." It belongs to Asphodelaceae, Liliaceae, or Aloaceae families, and its legitimate name according to the international rules of botanical nomenclature is *Aloe vera L.* Burm. (Grindlay and Reynolds, 1986). It is a shrubby or arborescent, perennial, xerophytic, succulent, pea- green color plant. It can grow from 80 cm to 100 cm tall. The leaves are 40 to 60 cm long with thorns on both edges, with a width at the base as 6 cm to 15 cm. It grows mainly in the dry regions of Africa, Asia, Europe, and America (Dagne *et al*.2000). So, there are over 400 species of *Aloe vera* in the Lily family. It is famous by the name Lilly of the desert (Mukesh *et al.*, 2010). The plant has triangular, ample leaves with saw-like edges, blonde tubular flowers and fruits that contain several seeds. Each leaf has a three layers composition, (i) Aloe Rind- the protective, green, outer leaf skin. This layer does not contain any significant nutritional value. (ii) Aloe Latex- the pungent, yellowish, deplete fluid which has strong odor and flows in between the leaf ring and the inner fleshy part of leaf. Aloe latex is not recommended for consumption and (iii) Inner Leaf Juice- the lucid, inner fleshy portion of leaf has a tremendous nutritional value for the health and can be consumed both internally and externally. *Aloe vera L.* has seventy five active constituents with the most documented are: 20 minerals, 20 amino acids, 12 vitamins, and water (Thiruppathi *et al.,* 2010). The vitamins present in Aloe plants are vitamin A (beta carotene), vitamin B (thiamine), B2 (riboflavin), B3 (niacin), B5, B6 (pyridoxine), B12, Vitamin C, Vitamin E, and Folic Acid. Vitamin B complex and vitamin C play an important role in reducing stress and inflammation. Besides vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids etc.. are also present (Rodriguez Garcia *et al.*, 2007; Andreas *et al*., 2001). Minerals found in the *Aloe vera* are calcium, sodium, zinc, chromium, potassium, magnesium, copper, manganese, and selenium as reported by Andreas *et al.*, (2001). It provides 20

out of 22 required amino acids required for human being and 7 of the 8 essential amino acids. Essential amino acids present are alanine, arginine, asparagine, cysteine, glutamic acid, glycine, histidine, proline, serine, tyrosine, glutamine, and aspartic acid. Moreover, it contains salicylic acid that possesses anti-inflammatory and antibacterial properties (Josias, 2008). Lignin, an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients into the skin. Saponins that are the soapy substances form about 3% of the gel and have cleansing and antiseptic properties. As with age, the level of collagen in human bodies naturally decreases and is one of the reason for older people to heal up the wound as compared to the children (Josias, 2008). *Aloe vera* contains the bulk of the required amino acids and vitamins that the human skin needs to heal. The glue-like substance keeps out any bacteria or agents that could cause healing to slow or cease completely (Josias, 2008). Several researchers had reported different hypothesis that attest to the effects of Aloe. Still the researchers are reporting latest information as every person has a different genetic structure that requires different amounts of nutrients depending on the particular situation (Andreas *et al*., 2001 and Josias, 2008). Thus, *Aloe vera* is an excellent source of nutrients that can help our body in a multitude of ways. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or an other were used for medicinal purposes (Grindlay and Reynolds, 1986). It has been estimated that in developed countries such as the United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80% (FAO, 2012).These countries provide two thirds of the plants used in modern system of medicine and the health care system of rural population depends on indigenous systems of medicine (FAO, 2012).

Although biosynthesis of plant metabolites (e.g. alkaloids, terpenoids, phenolic compounds, etc.) is primarily controlled genetically, environmental factors such as stresses and nutrient elements affect the production of metabolites in plants. Many

biological activities have been ascribed to these metabolites. In addition to their therapeutic effects, they play a role as chemical defense agents against microorganisms and herbivores. Therapeutic effects of medicinal plants are associated with their chemical peculiarities. Chemical features of these plants serve as integral determinant of their species specificity and pharmacological properties and facilitate their wide use in medical practice and other uses (Vio *et al*, 2005). The relationship between the synthesis of physiologically active substances and accumulation of elements is mediated by several levels of molecular regulation. There is general agreement that various mineral nutrients increase the growth of individual plants and, consequently, enhance the total plant biomass yield. However, the effects of certain macro- and micro-nutrients on the production of secondary metabolites are also modulated by environmental conditions and depend on plant species. Incorporation of one or more of the trace elements could increase or decrease the production of secondary metabolites depending on the plant species as well as on the concentrations of these elements (FAO, 2012).

Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as source materials for various scientific investigations. Many secondary metabolites of plants are commercially important and find use in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost and selection of the superior plant stock and over exploitation by pharmaceutical industry (FAO, 2012). However, some plant products either sprayed in extract forms or direct addition to the soil had been shown to enhance growth and yield parameters as well as having pesticidal properties. The geographic origin of *Aloe vera* L. is believed to be in Sudan, with the plant subsequently being introduced in the Mediterranean region and most other warm areas of the world (Grindlay and Reynolds, 1986). Aloe (*Aloe vera*) is an important traditional medicinal plant of the family Liliaceae and it is indigenous to Africa and Mediterranean countries. The species does not have any naturally occurring populations, although closely related Aloes do occur in northern Africa (Boudreau and Beland, 2006). However, it was reported to grow wild on islands of Cyprus, Malta, Sicily, Canary Islands, Cape Verde and arid tracts of India. It is a hardy perennial tropical plant that can be cultivated in drought areas, but its potential is yet to be exploited (Lans, 2006). Aloe, despite being identified as ‘a new plant

resource with the most promising prospects in the world, remains a disregarded plant. It is scattered in the wild, along the coast of southern India, China, U.S.A., Mexico and Australia. Some of the Latin American countries are the major producers and exporters of Aloe products. These countries are exploiting the plant potential with the growing cosmetic and neutraceutical market (Danhof, 1987). Aloe was officially listed as a purgative and skin protectant by the U.S. pharmacopoeia in 1820 (Park and Lee, 2006) and was clinically used in the 1930s for the treatment of radiotherapy burns to the skin and mucous membranes (Collins and Collins, 1935; Manderville 1939). Until today, *Aloe* is an important traditional medicine in many countries, including China, India, the West Indies, South Africa, and Japan (Grindlay and Reynolds, 1986). Application of chemicals in intensive agriculture has not been successful and has resulted in soil degradation, reduction of soil organic matter content, imbalance condition of soil acidity and mineral nutrient content. The mineral nutrients of non-chemical fertilizers are released more slowly; however, are more persistent in soil and supports plants in longer period. On the other hand, improvement of environmental and health conditions, in addition to the reduction of fertilizers costs are the reason why non-chemical fertilizers are more under attention today (Nejatzadeh *et al* 2011).

Potassium (K), nitrogen (N), phosphorous (P), sulfur (S), magnesium (Mg) and calcium (Ca) are the macro-nutrient elements, which have some important functions in living organisms. These elements are known to be essential and necessary for plants (Yaronskay *et al.*, 2007). Plants and other living organisms utilize the following three elements: potassium (K), sodium (Na) and calcium (Ca) for regulation of cell membrane potential and turgor. The uptake of K+, Na+ and Ca2+ occurs through ion channels in the plasma membrane of the cells. The concentrations of potassium, sodium and calcium induced changes of membrane permeability that facilitates solute diffusion into plant tissues. However, the buildup of cell structures and the capacity for cell elongation and cell division are dependent on the accessibility of calcium and the influence of Ca2+ on carbohydrate metabolism is known, too, (Stirk *et al.*(2008).

Magnesium (Mg) is one of the key nutrients for plant. Mg2+ influences the organization of protoplasmatic structures in cells, growth of plants, on plant pigments synthesis, activates many enzymatic systems of photosynthesis and respiration and acts on phosphorus management in plants and Mg is constituent of chlorophyll

molecules. Magnesium deficiency induces chlorosis and necrosis of the leaves and easy reutilization (Hamman, 2008).

Iron (Fe) also plays an important role in cell function and is an essential metal for some metabolic processes in plants. Iron enters many plant enzymes which play the dominant role in oxidoredox reactions of photosynthesis and respiration. It also takes part in chlorophyll pigment synthesis, synthesis of some proteins and in the accumulation of nitrogen in plants (Strnad *et al*, 1997). Iron participates in content of many enzymes: Cytochromes, ferredoxine, SOD (superoxide dismutase), catalases, peroxidases and nitratereductases. The deficiency of Fe^{2+} in plants causes significant changes in the plant metabolism and induces chlorosis. One of the most important factors determining uptake of this element is the form and concentration of Fe in the soil. Iron in low concentrations influences in a negative way on plants induces toxicity of manganese but the higher concentrations of iron are toxic and induce deficiency of Mn^{2+}. At high concentrations Fe is toxic to plants (Hamman, 2008). Manganese (Mn), in turn, is regarded as an activator of many different enzymatic reactions and takes part in photosynthesis. Mn^{2+} activates decarboxylases, dehydrogenases and is a constituent of the complex PSII-protein, SOD and phosphatase. Deficiency of Mn induces inhibition of growth, chlorosis and necrosis, falling the leaves, and low reutilization (Strnad *et al*, 1997).

Copper (Cu) is an essential micronutrient for plant metabolism, enters the oxidoredox enzymes, acts as a component of several enzymes, involved in carbohydrate, nitrogen (N) and cell wall metabolism. Copper takes part in protein synthesis and in the synthesis of some amino acids. Cu^{2+} is important to seed production, disease resistance and the water relations in the plant. Visible symptoms of Cu toxicity are small chlorotic leaves and early leaf fall, the growth stunted and poor initiation of roots and development of root laterals are also poor. Cu^{2+} ions inhibit photosynthesis and respiration and copper also decreased the chlorophyll content in the leaf cells. Copper plays an important role in cell function and is essential in structural stability of chromosomes and energy transfer. Deficiency of Cu induces chlorosis of leaves, (Hamman, 2008).

Zinc (Zn) is an essential trace element for every living organism. About two hundred enzymes and transcription factors require Zn^{2+} as a functional component. Therefore, zinc affects major metabolic processes, as well as regulation of the cell cycle and cell division. This metal plays an important role in the protein synthesis, as in the

carbohydrate, takes part in metabolism regulation of saccharides, nucleic acid and lipid metabolism. One of the first symptoms of zinc deficiency is an inhibition of cell growth and proliferation. The toxic concentrations of zinc negatively affect photosynthetic electron transport and photophosphorylation and have an effect on the photosynthetic enzymes. Zinc affects the biosynthesis of chlorophyll. One of the primary mechanisms of Zn toxicity may be an increased permeability of root membranes, which will cause nutrients to leak out from the roots, Dagne *et al.* (2000).

1.2 Objectives:

In effort to establish a scientific base that enables enhanced production of *Aloe vera* under Sudan conditions, this study aimed to fulfill the following objectives:

1. To investigate the influence of nutrient elements namely: nitrogen, phosphorus, sulfur and the compound fertilizer NPK on growth and gel yield.
2. To test the influence of various concentrations of 6- Benzyl adenine on growth attributes and gel content of *Aloe Vera* plants.
3. To test the bio-stimulative effects of two Sudanese indigenous plants, namely Argel and Haza, on growth and gel yield as a step towards organic production of *Aloe Vera* plants.

Chapter Two

General Literature Review

2.1. Origin and distribution:

Aloe vera L. is an important medicinal plant from the Liliaceae family with African origin. Among 300 species, it is considered as an important medicinal plant in many countries (Hasanuzzaman *et al*., 2008). The natural range of *Aloe vera* is unclear, as the species has been widely cultivated throughout the world. Naturalized stands of the species occur in the southern half of the Arabian Peninsula, through North Africa (Morocco, Mauritania, and Egypt) as well as Sudan and neighboring countries, along with the Canary, Cape Verde, and Madeira Islands (Reynolds, 2004). It is originated in South Africa or Sudan, but have been indigenous to dry subtropical and tropical climates, including the southern USA. This distribution is somewhat similar to the one of *Euphorbia balsamiferous*, *Pistacia atlantica*, and a few others, suggesting that a dry sclerophyl forest once covered large areas, but has been dramatically reduced due to desertification in the Sahara, leaving these few patches isolated Reynolds and Dweck (1999). Several closely related (or sometimes identical) species can be found on the two extreme sides of the Sahara: Dragon trees (Dracaena) and Aeonium being two of the most representative examples as the species were introduced to China and various parts of southern Europe in the 17th century. The species is widely naturalized elsewhere, occurring in temperate and tropical regions of Australia, Barbados, Belize, Nigeria, Paraguay and the United States It has been suggested that the actual species' distribution is the result of human cultivation (Reynolds, 2004). Recently, only a few species of Aloe have been considered for commercial importance, of which *Aloe vera* is considered the most potent and, thereby, the most popular plant in the research field (Eshun and Qian, 2004). However, over the last decade, various Aloe species have gained popularity as therapeutic botanicals.

2.2. Botany:

A.vera is a succulent plant. Succulents are xerophytes, which are adapted to areas of low water availability and are characterized by possessing a large water storage tissue (Reynolds and Dweck, 1999). *Aloe vera* is perennial succulent xerophytes; with elongated and pointed leaves that are joined at the stem in a rosette pattern and grow

to about 30–50 cm in length and 10 cm in breadth at the base in the adult plant (WHO, 1999). The leaf is protected by a thick, green epidermis layer (skin or rind), which surrounds the mesophyll. Immediately beneath the rind are located the vascular bundles, which are composed of three types of tubular structures; the xylem (transports water and minerals from roots to leaves), the phloem (transports starch and other synthesized products to the roots), and the large pericyclic tubules (contains the yellow leaf exudates commonly referred to as “aloes,” “sap,” or “latex”; (Boudreau and Beland, 2006). The pericyclic portion of the vascular bundle is adherent to the rind, whereas the remainder of the vascular bundle protrudes into the mesophyll layer (Danhof, 1987). The mesophyll can be differentiated into chlorenchyma cells and thinner-walled parenchyma cells.

Aloe (*Aloe vera*) is an important and traditional medicinal plant belonging to the family Liliaceae (WHO, 1999). It is indigenous to Africa and Mediterranean countries. The species does not have any naturally occurring populations, although closely related aloes do occur in northern Africa. However, it is reported to grow wild on islands of Cyprus, Malta, Sicily, Canary cape, Cape Verde and arid tracts of India. This is a hardy perennial tropical plant that can be cultivated in drought areas. But its potential is yet to be exploited (Danhof, 1987).

2.3 *Aloe vera* Varieties:

Commercially important sub-species are *Aloe barbadensis*, *A. chinensis*, *A. perfoliata*, *A. vulgaris*, *A indica*, *A. littoralis* and *A. abyssinica*. National Botanical and Plant Genetic Resource, ICAR, has released varieties like IC111271, IC111269, and IC111280 etc. Central Institute of Medicinal and Aromatic Plants, Luck now, has also released the variety *AL*-1 for cultivation (Reynolds, 2004).

2.4 Environmental requirements:

2.4.1 Soils:

According to Lans (2006), the plant can be grown in a variety of soils ranging from sandy coastal soils to loamy soils of plains. It is sensitive to water logged conditions. The crop also comes up well in light soils. It can tolerate higher pH and high Na and K salts. Growth is faster under medium fertile, heavy soils such as black cotton soils.

In well drained, loam to coarse sandy loam in a pH range up to 8.5, it grows well with higher foliage.

2.4.2 Climate:

As reported by Reynolds (2004), Aloe has wide adaptability and can be grown in various climatic conditions. It can be seen growing equally good in warm humid or dry climate. However, it is intolerant to extreme cool conditions. The plant flourishes well on dry sandy soils at localities with lower annual rainfall of 50 to 300mm. It needs protection against frost and low winter temperature. The species is popular with modern gardeners as a putatively medicinal plant and due to its interesting flowers, form, and succulence. This succulence enables the species to survive in areas of low natural rainfall, making it ideal for rockeries and other low-water use gardens. The species is hardy in warm zones of the world and intolerant to very heavy frost or snow (Eshun and Qian (2004).

2.5 Cultivation:

According to IJAS (2012), *Aloe vera* has been widely grown as an ornamental plant. The species is relatively resistant to most insect pests, though spider mites, mealy bugs, scale insects, and aphid species may cause a decline in plant health. In pots, the species requires well-drained sandy potting soil and bright sunny conditions; however, aloe plants can burn under too much sun or shrivel when the pot does not drain the rain (Pichgram, 1987). The use of a good-quality commercial propagation mix or pre-packaged “cacti and succulent mix” is recommended, as they allow good drainage (Lans, 2006). Terracotta pots are preferable as they are porous. Potted plants should be allowed to completely dry prior to re-watering. When potted aloes become crowded with “pups” growing from the sides of the “mother plant,” they should be divided and re-potted to allow room for further growth and help prevent pest infestations. During winter, *Aloe vera* may become dormant, during which little moisture is required (Reynolds, 2004). In areas that receive frost or snow, the species is best kept indoors or in heated glasshouses. Large scale agricultural production of *Aloe vera* is undertaken in Australia, Bangladesh, Cuba, the Dominican Republic, China, Mexico, India, Jamaica, Kenya, Tanzania and South Africa, along with the USA to supply the cosmetics industry with *Aloe vera* gel (IJAS, 2012).

2.6 Propagation:

It is generally propagated by root suckers or rhizome cuttings. For this purpose, medium sized root suckers are chosen and carefully dug out without damaging the parent plant at the base and directly planted in the main field (Lans, 2006). It can also be propagated through rhizome cuttings. In this case, after the harvest of the crop, the underground rhizome is also dug out and made in 5-6 cm length cuttings which should have a minimum of 2-3 nodes on them. It is rooted in specially prepared sand beds or containers and after starting sprouting, it is ready for transplanting. On an average, about 36500 suckers are required for a nursery of 1 ha size (14550 for 1 acre nursery) (IJAS, 2012).

Aloe vera (Liliaceae) is a succulent plant indigenous to Northern Africa and Mediterranean countries and has become naturalized almost in all parts of India (Klein and Penneys, 1988). The plant has stiff gray-green lance-shaped leaves containing clear gel in a central mucilaginous pulp. *Aloe Vera* has been used for several thousands of years in folk medicine in many cultures from ancient Egypt, Greece, and Rome to China and India (Kemper and Chiou, 1999). *Aloe vera* propagates vegetatively in its natural state. However, propagation rate is very slow because a single plant can produce only three to four pronounced lateral shoots in a year. Moreover, the production of Aloe leaves is insufficient to meet the industry demand in India (Aggarwal and Barna, 2004). The production of cosmetics, foods and pharmaceuticals containing *Aloe vera* has experienced a slow increase due to limited availability of raw material with high quality (Campestrin *et al.*, 2006). Therefore, there is a need to develop suitable, an alternative method for traditional propagation of *Aloe vera*. *In vitro* techniques using micro propagation and tissue culture offer a great possibility to overcome this problem. Micro propagation using stem and lateral shoot pieces of *Aloe vera* has already been proved successful (Natali *et al.* 1990; Roy and Sarkar, 1991; Meyer and Staden, 1991; Aggarwal and Barna, 2004). However, source of explants, their sterilization procedure, media composition, culture conditions, phenolic browning of explants and media discoloration greatly affect shoot regeneration from different genotypes of the same species. *Aloe vera* exudes lot of phenolic substances into the culture media which could decrease the survival of explants (Roy and Sarkar, 1991). Concentration of phenolic compounds may vary in different genotypes of the same species (Glynn, *et al.*, 2004), and also those were

grown under different climatic conditions (Kjaer *et al.*, 2001). Hence culture conditions are needed to be modified accordingly to achieve the desirable targets. Thus it is important develop a rapid and high frequency shoot regeneration protocol for elite plants of *Aloe vera* suitable for mass propagation by improving culture media while controlling phenolic browning of ex-plants.

2.7 Spacing and plant population:

Normally a spacing of 40cm x 45cm or 60cm x 30cm is followed. This accommodates about 55000 plants per hectare (Reynolds, 2004; Pichgram, 1987).

2.8 Land preparation and planting:

The land is ploughed and cross ploughed thoroughly. Farm yard manure is added at 15 t/ha during the last ploughing. Ridges and furrows are formed at 45 or 60cm apart. The plot may be irrigated if necessary. The suckers are planted at 40 or 30cm apart, maintaining the spacing suggested (IJAS, 2012).

2.9 Manures and fertilizers:

The crop responds well to the application of farm yard manure and compost. In the first year of plantation, (FYM) at 15 t/ha is applied during the land preparation. During the subsequent years, the same dose of FYM is applied every year. Besides 50:50:50 kg/ha of N: P: K is applied as basal dose (Steenkamp and Stewart, 2007; Pichgram, 1987).

2.10 Irrigation:

According to (IJAS, 2012), Aloe can be successfully cultivated both under irrigated and rain fed conditions. Provision of irrigation immediately after planting and during summer season will ensure good yields. However, the plants are sensitive to water logged conditions.

2.11 Plant protection:

Aloe is known to be infected by fungus causing leaf spot disease. This affects yield and quality of the gel adversely. The disease can be controlled by spraying recommended fungicides (Lans, 2006).

2.12 Chemical constituents:

The main feature of the *A.vera* plant is its high water content, ranging from 99-99.5%. (Hamman, 2008). the remaining 0.5-1.0% solid material is reported to contain over 75

different potentially active compounds including water- and fat-soluble vitamins, minerals, enzymes, simple/complex polysaccharides, phenolic compounds, and organic acids. In compositional studies on the structural components of the *Aloe vera* plant leaf portions, the rind was found to be 20-30% and the pulp 70-80% of the whole leaf weight.

On a dry weight basis, the percentages of the rind and pulp represented as lipids (2.7% and 4.2%) and that as proteins (6.3% and 7.3%) only accounted for a minor fraction (Femenia *et al*., 1999). However, the non starch polysaccharides and lignin represented the bulk of each leaf fraction and were found to be 62.3% and 57.6% of the dry weight of the rind and pulp, respectively.

Consist of linear chains of glucose and mannose molecules; of which mannose is more concentrated than glucose, thereby the molecules are referred to as polymannans (Ni *et al*., 2004). These are linear chains ranging in size from a few to several thousand molecules (Hutter *et al*., 1996). The major polysaccharide, acemannan, is composed of one or more polymers of various chain lengths with molecular weights ranging from 30 kDa to 40 kDa or greater, and consisting of repeating units of glucose and mannose in a 1:3 ratio (Femenia *et al*., 1999; Chow *et al*.,2005).. In western societies, especially in the USA, *Aloe vera* has been grown mainly to supply the latex component of the leaf to the pharmaceutical industry (Lee *et al*., 2000).

The ten main areas of chemical constituents of *Aloe vera* include: amino acids, anthraquinones, enzymes, minerals, vitamins, lignins, monosaccharide, polysaccharides, salicylic acid, saponins, and sterols, (Boyer, 2012). The amino acids in *Aloe vera* are the building blocks of protein and influence brain function (AOAC, 1990). Humans require 22 amino acids and the body will make all of them except for eight essential amino acids which our body gets from the food/drinks that we take in. Every one of the essential amino acids is available in *Aloe vera* and they include isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, and tryptophan. Some of the other non-essential amino acids found in *Aloe vera* include alanine, arginine, asparagine, cysteine, glutamic acid, glycine, histidine, proline, serine, tyrosine, glutamine, and aspartic acid, AOAC (1990).

Located in the sap of the leaves, are phenolic compounds that has stimulating effects on the bowels and antibiotic properties. In small amounts the anthraquinones do not have a purgative effect. They help with absorption from the gastro intestinal tract and have anti-microbial and pain killing effects (Rajasekaran *et al*., 2006). Too many

anthraquinones can produce abdominal pain and diarrhea. The most important anthraquinones are aloin and emodin. They are anti-bacterial, anti-viral, and analgesic Steenkamp and Stewart (2007).

Enzymes act as biochemical catalysts that break down the proteins into amino acids. The enzymes turn the food we eat into fuel for every cell in our body, enabling the cells to function and work efficiently. The main enzymes found in *Aloe vera* include Amylase (breaks down sugars and starches), Bradykinase (stimulates immune system, analgesic, anti-inflammatory), Catalases (prevents accumulation of water in the body), Cellulase (aids digestion - cellulose), Lipase (aids digestion - fats), Oxidase, Alkaline Phosphatase, Proteolytiase (hydrolyses proteins into their constituent elements), Creatine Phosphokinase (aids metabolism), and Carboxypeptidase (Chandan *et al.*, 2007).

Aloe vera contains salicylic acid which is an aspirin-like compound with anti - inflammatory, analgesic, and anti-bacterial properties. It has anti-pyretic properties for reducing fevers.

Other constituents of *Aloe vera* would include prostaglandins, tannins, magnesium lactate, resins, mannins, proteins such as lectins, monosulfonic acid and gibberellins (Aro *et al.*, 2012). Another constituent of *Aloe vera* includes saponins. These are soapy substances from the gel that is capable of cleansing and having antiseptic properties.

Saponins perform strongly as anti-microbial against bacteria, viruses, fungi, and yeasts (Aro *et al.*, 2012). The plant sterols or phyto-steroids in *Aloe vera* include Cholesterol, Campesterol, Lupeol, and B (Beta sign) Sitosterol. The plant steroids have fatty acids in them that have antiseptic, analgesic, and anti-inflammatory properties (Chandan *et al.*, 2007).

Aloe gel: The parenchyma (filet or pulp), which is the major part of the leaf by volume, contains a clear mucilaginous gel known as *Aloe vera* gel (Femenia *et al*., 1999; Femenia *et al*., 2003).

Other medicinal constituents: *Aloe vera* has marvelous medicinal properties. Scientists have discovered over 150 nutritional ingredients in *Aloe vera*. There seems to be no single magic ingredient. They all work together in a synergistic way to create healing and health giving benefits (Femenia *et al.*, 1999).

It also contains Vitamins B1, B2, B3, B5, B6, and B12 along with choline, calcium (teeth and bone formation, muscle contractions and heart health),

magnesium(strengthens teeth and bones, maintains healthy muscles and nervous system, activates enzymes), zinc (speeds up wound healing, mental quickness assists with healthy teeth, bones, skin, immune system, and digestive aid), manganese (activates enzymes, builds healthy bones, nerves and tissues), chromium (assists with protein metabolism and balancing of blood sugars), selenium which all influence brain performance, Steenkamp and Stewart *et al*.,(2007). Additional minerals found in *Aloe vera* include copper (important for red blood cells, skin and hair pigment, iron (involved in oxygen transportation and making of hemoglobin in red blood cells, potassium (helps with fluid balance), phosphorus (helps build bones and teeth, assists with metabolism and body pH, and sodium regulates body liquids, helps with nerve and muscle performance, and helps deliver nutrients into body cells (Aro *et al.*, 2012). *Aloe vera* also contains the trace minerals of rhodium and iridium used in cancer and tumor research experiments Chandan *et al.* (2007). Another component of *Aloe vera* consists of the lignins, a major structural material of cellulose content that allows for penetrative properties, (AOAC, 1990). *Aloe vera* can soak into the skin up to seven layers deep. Lignins penetrate the toughened areas of the skin being beneficial for skin problems such as eczema and psoriasis Steenkamp and Stewart *et al.* (2007).

2.13 Uses of *Aloe vera*:

2.13.1 Traditional uses:

Aloe vera has a long history of popular and traditional use. It is used in traditional Indian medicine for constipation, colic, skin diseases, worm infestation, and infections (Heber, 2007). It is also used in Trinidad and Tobago for hypertension Lans (2006) and among Mexican Americans for the treatment of type 2 diabetes mellitus (Coronado *et al.*, 2004). In Chinese medicine, it is often recommended in the treatment of fungal diseases, while in Western society, *Aloe vera* is one of the few herbal medicines in common usage, and it has found widespread use in the cosmetic (Heber, 2007).

2.13.2 Medical uses:

A.vera has been used in folk medicine for over 2000 years, and has remained an important component in the traditional medicine of many contemporary cultures, such as China, India, the West Indies, and Japan. It is also used in Trinidad and Tobago for

hypertension, pharmaceutical and food industries (Lans, 2006). In the case of health, the therapeutic claims for the topical and oral application of *Aloe vera* cover a wide range of conditions, but few claims have been the subject of robust clinical investigation (Heber, 2007). The conditions for which clinical trials of *Aloe vera* have been conducted include skin conditions, management of burn and wound healing (Ashley *et al.*, 1957), constipation and gastrointestinal disorders (Coronado *et al*., 2004).

Aloe has been used extensively by the Egyptians, Assyrians, and Mediterranean civilizations and in Biblical times (Grindlay and Reynolds, 1986). *Aloe vera* is one of the few herbal medicines widely used in Western society, with the manufacturing of *Aloe vera* extracts being one of the largest botanical industries worldwide (Eshun and He, 2004; Grindlay and Reynolds, 1986). In 2004, the value of the *Aloe* industry was estimated to be US$125 million for the cost of the raw *Aloe* material and US$110 billion for finished *Aloe*-containing products (IASC, 2009). *Aloe vera* is used in the cosmetic, food, and pharmaceutical industries. In the cosmetic and toilet industry, it is used as a base material for skin moisturizers, soaps, shampoos, sun lotions, makeup creams, perfumes, shaving creams, bath aids, and many other products (Boudreau and Beland, 2006; Eshun and He, 2004). The food industry uses *Aloe* in the manufacture of functional foods, especially health drinks, and as a bitter agent (Saccu *et al.*, 2001). Pharmaceutical products are available for topical applications (gels and ointments) and oral use (tablets and capsules; Hamman 2008). *A.vera* has been used in folk medicine for over 2000 years, and has remained an important component in the traditional medicine of many contemporary cultures, such as China, India, the West Indies, and Japan. (AOAC,1990). Some of the most important pharmacological activities of *A. vera* are antiseptic (Capasso *et al.*,1998), antitumor (Winter, *et al.*,1981), antiinflammatory (Yagi *et al.*,1998), wound and burn healing effect (Heggers *et al.*, 1993), anti diabetic (Rajasekaran *et al.*, 2006) and as an adjunct to current AIDS therapy (Mc Daniel *et al.*, 1990). Because of the healing properties of *Aloe vera* and its synergistic action, the body receives what it needs to work properly.

Aloe vera, an anti-oxidant rich plant, contains vitamins such as A, C, and E plus the minerals, zinc, and selenium. Anti-oxidants help boost the immune system and combat free radicals in the body (AOAC, 1990).

2.14 The scientific evidence:

The therapeutic claims for *Aloe vera* cover a broad range of conditions. It is commonly used topically in the treatment of dermatological and wound healing conditions, (t'Hart *et al*. 1990). The oral application of the *Aloe vera* latex is promoted as a laxative, whereas gel and whole-leaf oral preparations have been variously recommended for use as an adjunct to chemotherapy treatment and to ameliorate diverse disorders such as DM, infectious diseases, metastatic cancer, and ulcerative colitis (t'Hart *et al*. 1990). The clinical use of *Aloe vera* is supported primarily by anecdotal evidence and case reports. The number of clinical trials exploring its effectiveness has begun to increase; however, a standardization of methodological trial quality has yet to be achieved, (Samman, 1998).

Topical Applications: The first case report of the beneficial effects of *Aloe vera* in the treatment of skin and wound healing was published in 1935, with fresh whole-leaf extract reported to provide rapid relief from the itching and burning associated with severe roentgen (radiation) dermatitis and complete skin regeneration (Collins and Collins, 1935). Numerous subsequent reports have explored the role of topical *Aloe vera* administration in skin conditions and wound healing management, including psoriasis, dermatitis, oral mucositis, burn injuries, and surgical wounds.

Safety and efficacy: Determining the safety and efficacy of *Aloe vera* is difficult due to the lack of standardization of commercially available *Aloe vera* preparations. Similarly, the need for a more detailed understanding of the plant's active components makes it difficult to evaluate the optimal doses of particular *Aloe vera* preparations for the treatment of specific disorders (Collins and Collins, 1935). Despite these challenges, a recent systematic review of *Aloe vera* by the Natural Standard Research Collaboration concluded that topical application of *Aloe vera* gel or extract is safe for the treatment of mild to moderate skin conditions, burns, wounds, and inflammation (Ulbricht *et al*. 2008). In terms of efficacy, reasonable evidence in humans supports the topical use of *Aloe vera* for the treatment of burn wounds. Evidence for its use in psoriasis, dermatitis, and surgical wound healing is conflicting. Regarding the increasing significance of *Aloe vera* in human health and its application in development of new food and cosmetic products, it is important to study the methods of increasing its yield and quality (Manderville, 1939).

2.15 Effect of cultivation and processing:

The composition of *Aloe vera* extracts differs according to the plant variety, climatic and seasonal variations, and the age of the plant (Eshun and He, 2004). However, the processing method has the largest effect on the number and amount of active ingredients in a product (Wang and Strong, 1995). The commercial production process of *Aloe vera* products typically involves crushing, grinding, or pressing of the whole *Aloe vera* leaf to produce juice, followed by various steps of filtration and stabilization to achieve the desired extract (Eshun and He, 2004). This method provides ease of processing and higher efficiency in the recovery of the solids (Agarwala, 1997), but it can result in a product that contains little or no active ingredients (Eshun and He, 2004).

2.16 The role of macro-nutrient elements on plant growth:

The role of macronutrients, some plant extracts and growth regulators in various physiological and biochemical processes in plant is well known, which enables a rapid change in the physiology of plant within one season to achieve desirable results. The essential mineral elements which are required in higher concentrations by the plant have a major role in determining the growth and development of Aloes, often produces more vegetative growth, than needed for maximum latex production especially when climatic conditions favor vegetative growth by directing the nutrients and photo assimilates towards vegetative growth at the expense of latex and gel production (Hazrati, 2012); Ji-Dong *et al.*, 2006).

Minerals have a diversified role in medicinal plant metabolism. Severity or scarcity of these causes multifarious effects in plant metabolism. Each and every aspect of plant biochemistry, physiology, anatomy, etc. is affected due to mineral nutrient composition of soils. Medicinal plants inherit resistance due to biosynthesis of bioactive substances (secondary metabolites) against various types of diseases caused due to fungus, bacteria, viruses, mycoplasmas, insects and pests. The concentration of these minerals of both group i.e. activators or inhibitors present in the soil play a vital role in secondary plant metabolism. Minerals also play a major role in the reproduction of these medicinally important plants. Bioactive molecules of medical relevancy such as alkaloids, flavonoids, lignins, lipids, carbohydrates, resins, glycosides, phenolic compounds, volatile oils, vitamins, tannins etc. produced through various biosynthetic pathways of plants are a boon to urban, hilly and remote

population of each nation. However, soils with different compositions of mineral elements adversely influence the metabolic activities of such valuable medicinal plants (Saradhi *et al*., 2007). Various physiological activities are governed through important mineral elements present in soils from where these are transferred to area where their need arises. So, accumulation and biosynthesis of these bioactive molecules in plant system are widely dependent on the availability of mineral elements in the soil. Different developmental stages of the medicinal plants need supplementation of different macro- and micro- elements during its various growth and biosynthesis steps Babatunde and Yongabi (2008).

2.17 The effect of nitrogen fertilizer (soil application) on growth and yield parameters:

The largest natural source of nitrogen is the Earth's atmosphere, which is roughly 78% gaseous nitrogen, an inert and essentially biologically unavailable form of the element for natural use. Its biological unavailability is because the two nitrogen atoms form an extremely stable bond, which is not easily broken. Apart from human industrial processes that fix nitrogen gas to solid or liquid forms, the primary means of nitrogen fixation are through the high temperature and energy of lightning strikes and biological nitrogen fixation by bacteria. These processes produce nitrogen in three main forms, each of which is available to plants: nitrate, nitrite, and ammonium (Wiedenhoeft *et al.*, 2006).

Hernández-Cruz, *et al*. (2002), reported that, Nitrogen is the one nutrient most often limiting plant growth. The needs of the plants for nitrogen depend on plant species. However, tomatoes and vine crops (cucumbers, squash, and melons) develop excessive vine growth at the expense of fruiting with excess nitrogen. Potatoes, corn and Cole crops (cabbage, broccoli, and cauliflower) are heavy feeders and highly benefit from soil nitrogen. Trees and shrubs have a low relative need for soil nitrogen, as reported by Ji-Dong *et al*. (2006). Hazrati (2012) reported that the application of nitrogen had positive effect on the growth and aloin concentration of *Aloe vera* plants. Mengel and Kirkby (1987) reported that, inadequate level of nitrogen, shortens the plants life cycle, plant matures early and economic yield is generally poor. Also it was found that, the reason for the significant increase in yield parameters of *Aloe vera* is recognized to be the fact that nitrogen is often regarded as limiting for biomass production in natural ecosystems (Babatunde and Yongabi, 2008). (Hazrati, 2012)

found that, the application of nitrogen on *Aloe vera* plant has increased the growth parameters as the best growth parameters were obtained in the plants treated with 1000 mg nitrogen. In this regard, Tawaraya *et al*., (2007) showed that, shoot fresh weight was increased by raising the dose from 500-1500mg/ plant and similar results were obtained by Babatunde and Yongabi (2008), as they reported that, the growth parameter values were increased by increase of nitrogen levels.

(Hazrati, 2012), reported that, the volume of leaf increased as a result of increase in length and thickness of leaves. Thus, leaf volume can be an important factor for the determination of leaf yield and leaf fresh weight (Hernández-Cruz *et al*., 2002). The leaf is an important factor in yield determining in *Aloe vera* plants (Eshun and He, 2004). The application of 1000 mg nitrogen per pot had significantly increased the yield. This result was confirmed by (Khandelwal *et al*., 2009). Increased nitrogen uptake from the soil by the root system of *Aloe vera* plant could be the reason for its higher gel content (Ray, 1999). (Ji-Dong *et al.,* 2006) reported that, the nitrogen application increased leaf fresh weight and total biomass.

Phenolic compounds are considered to be secondary metabolites synthesized in plants through the phenylpropanoid pathway. These make a defense mechanism that reacts to different biotic and a biotic stress conditions (Dixon and Paiva, 1995). Aloin is an important phenolic compound in *Aloe vera* plants and the application of nitrogen caused an increase in aloin concentrations (Hazrati, 2012). Similar results were obtained by (Ji-Dong *et al.,* 2006), as they showed that, the amount of aloin was enhanced in *Aloe vera* with nitrogen increases. In another study, the results recorded showed that, the application of nitrogen increased the phenolic compounds (aloin, barbaloin) in leaf latex (Saradhi *et al*., 2007).

Nitrogen is one of the most important elements of the chlorophyll structure; low rate of photosynthesis under conditions of nitrogen limitation can too often be attributed to the reduction of chlorophyll content (Toth *et al*., 2002), As they observed that, the application of nitrogen increased the chlorophyll content in leaves of the *Aloe vera* plants. Generally, the highest levels of chlorophyll 'a' and 'b' were obtained in the highest level of nitrogen.

(Moradkhani *et al*., 2010) reported in (*Mellissa officinalis* L), the best plant height was obtained under application of 90 kg N ha^{-1}. Suresh (1980) revealed that, application of nitrogen at 100 kg per h^{-1} was optimum for increasing stem diameter,

while nitrogen at 150 kg per h^{-1} it was optimum for increasing plant height of *Catharanthus roseus.*

(Garnayak *et al.,* 2000) studied the effect of varied levels of nitrogen on plant height of mustard and reported significant increase in the plant height with the increase in the level of nitrogen application from 0 to 120 kg N per h^{-1}. The highest was recorded in the treatment supplied with 120 kg N per h^{-1} which was 200 cm. Okut and Yidirmi (2005) concluded that, application of 90 kg N ha^{-1} in coriander resulted in significantly higher plant height.

Attoe and Osodeke (2009) reported significantly higher plant height of ginger (*Zingiber officinale Roscoe*) at 200 kg N per h^{-1} compared to other levels of nitrogen. Katarzyna and Jarosz (2006) reported significant increase in essential oil content of *Satureja hortensis* L upon treatment of the plants with different levels of nitrogen. (Hocking *et al*., 1997) assessed the influence of nitrogen on oil content of canola in a two year field experiment and reported that, nitrogen application up to 75 kg per h^{-1} increased the oil content. Application of 120 kg N per h^{-1} recorded significantly higher oil content of mustard, which was superior over all other N levels tested (Anilkumar *et al*., 2001). Similarly, Shishu and Vinay (2005) recorded significantly higher oil content of mustard at 80 kg N ha-1 which was superior over all other N levels including control.

In fact, the unique role of nitrogen in photosynthesis, energy compounds and important physiological processes increases the content of chlorophyll, and is an indirect cause of growth and yield enhancing and chemical compound (aloin) in *Aloe Vera* plant (Nahed and Aziz, 2007). Similarly, Hazrati (2012) showed that, the nitrogen increased growth, yield and aloin concentration in *Aloe vera* plants.

2.18 The effect of soil application of NPK fertilizer on growth and Yield parameters of *Aloe vera* plants:

Merestala (1996) reported that, the soil application of (NPK) to *Aloe vera* plants increased the yield by 25% under $N_{120}P_{60}K_{120}$ treatment compared to the control. Growth rate over a period of four months showed a maximum increase of 43% in $N_{120}P_{60}K_{120}$ and minimum of 23% in control and the maximum average number of leaves per plant was obtained at highest concentration of chemical fertilizer (Merestala, 1996).

Chlorophyll content in leaves was significantly higher under fertilizer treatments as compared to control (Merestala, 1996), and also stated that, the maximum chlorophyll content of 39.13 mg/g was recorded under treatments supplied with the highest dose of chemical fertilizers at $N_{120}P_{60}K_{120}$ level. The above findings showed that, the *Aloe vera* responded to fertilizer dose in increasing the growth parameters.

The biological yield and gel yield of *Aloe vera* (fresh and dry weights) were significantly increased under different fertilizer treatments as compared to control, as reported by (Saradhi *et al*., 2007). Maximum biological yield of 130 g/plant and gel yield of 61.4 g/plant were obtained under chemical fertilizer treatment at $N_{120}P_{60}K_{120}$ level (Saradhi *et al*., 2007).

(Merestala, 1996) reported that, The growth and weight of *Aloe vera* root (fresh weight) attained maximum weight of 9.8 g/plant when the crop received chemical fertilizer at $N_{120}P_{60}K_{120}$ compared to the control. Higher dose of chemical fertilizer is expected to release greater quantity of nutrients particularly N, P and K at a faster rate and higher level and there by greater uptake by the plants which resulted in higher growth and yield parameters of *Aloe vera* plants (Saradhi *et al*., 2007).

2.19 The effect of soil application of Phosphorus fertilizer on growth and Yield parameters of *Aloe vera* plants:

Phosphorus has many important functions in plants and medicinal plants, the primary one being the storage and transfer of energy through the plant. Adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are high-energy phosphate compounds that control most processes in plants including photosynthesis, respiration, protein and nucleic acid synthesis, and nutrient transport through the plant's cells (Sharpley *et al*., 1994).

Phosphorus is frequently a limiting nutrient, particularly in tropical regions, where the soil chemistry differs from temperate soils, or in highly weathered soils, where phosphorus has long since been leached away. Phosphorus is one of the three main elements in commercial lawn fertilizers, though there is mounting evidence that many lawns and green areas already have ample phosphorus, and thus it is being phased out of some commercial fertilizers. The ultimate source of virtually all terrestrial phosphorus is from the weathering of minerals and soils in the Earth's crust. Phosphorus is generally available as phosphate, an anion that is not bindable by the

cation exchange complex and thus can be easily leached from the soil by rain or run off (Wiedenhoeft, 2006).

Literature review shows that, although there are large amount of information on medicinal and cosmetic functions of *Aloe vera*; however, we lack information about cultivation and production of the plant (Merestala, 1996).

Nematian *et al,* 2011) reported that, Aloe plants treated with Phosphorus fertilizer at once 0, 5, 10, 15 and 20 g/plant during the growth period of six month, showed significant increase in leaf number, as all treated plants were better than those of control with the average mean of (9 , 12 ,13 , 15 , 18) respectively. Regarding the role of phosphorus in plants vegetative growth, high phosphorus content in soil would probably increase the number of leaves, leaf weight, leaf diameter and leaf chlorophyll content, Nematian *et al*., 2011).

In an experiment on the effect of phosphorus fertilizer on *Aloe vera*, it was indicated that, higher phosphorus application rates increased the number of leaves, leaf fresh weight, leaf length and leaf diameter (Luis Rodolfo *et al*, 2002). In another experiment it was indicated that, the highest number of leaves was achieved in 5 % phosphorus fertilizer + 95% soil, and the highest leaf length was also achieved in 5 % P fertilizer + 95% soil (Nejatzadeh *et al*, 2011).

Boroomand *et al*. (2011) found that, application of 100 kg P_2O_5 / ha^{-1} increased leaf number and leaf length in *Aloe vera* plants and reported significantly higher plant height of Basil at 150 kg P_2O_5 / ha^{-1} which was superior over all other phosphorus levels tested. (Saraf *et al*., 2002), studied the effect of two levels of P_2O_5 25 and 50 kg ha^{-1}) on the yield of Danti (*Baliospermum montanum*) crop, as the effect of phosphorus was significant and the highest plant height, number of leaves per plant, branches per plant and plant spread were observed with the application of 50 kg P_2O_5 per ha^{-1} as compared to 25 kg P_2O_5 per ha^{-1}.

The effect of phosphorus fertilizers on fresh leaf weight of *Aloe vera* was also revealed in the experiment of Faryabi and Ghazanchi (2005), as they reported that, the higher doses of phosphorus significantly increased the fresh leaf weight, while medium doses of nitrogen induced significances. (Mirza *et al*., 2008), also reported that, mixing 5 % phosphorus with 95% soil resulted in the highest leaf growth rate, number of leaves/plant, leaf fresh weight, leaf area index and leaf width.

Regarding the effect of phosphorus application on leaf chlorophyll content, (Kawther *et al*., 2001) found that, application of phosphorus increased *Aloe vera* leaf

chlorophyll content and they reported that leaf chlorophyll content is directly correlated with phosphorus rate. Application of 100 kg P_2O_5 / h^{-1} to Basil resulted in significantly higher oil content (2.40%) which was superior over all other phosphorus levels tested (Boroomand *et al.,* 2011). Harendra and Yadav (2007) reported significant increase in the oil content of mustard with increase in phosphorus level from 0 to 39.3 kg P_2O_5 /ha^{-1} as (30%) of oil content was recorded at 39.3 kg P_2O_5 /ha^{-1} which were significant over all other levels including their control. (Boroomand *et al*., 2011) found that application of 100 kg P_2O_5 /ha^{-1} increased leaf number, plant height and leaf length in *Aloe vera*.

One of the earliest and most pronounced responses to phosphorus deficiency is reduction in shoot growth, specifically reduction in leaf number and leaf size (Lynch *et al.,* 1991) where the Shoot development requires the production of leaves by shoot apical meristem and their subsequent expansion. Decreased number of leaves with phosphorus deficiency implies changes in leaf initiation and activity of shoot apical meristem, while plant height of *Phaseolus trilobus* was increased due to increased phosphorus fertilization at 80 kg P_2O_5 /ha^{-1} (Kumar *et al.,* 2002). Anilkumar *et al*. (2001) reported significantly higher plant height of mustard at 37.35 kg P_2O_5 /ha^{-1} (209.4 and 208.0 cm during 1998 and 1999, respectively which was superior over all other phosphorus levels tested. Panwar and Singh (2003) also recorded significantly higher plant height of groundnut at 60 kg P_2O_5 /ha^{-1} (49.80 cm) over control.

Field investigation carried out by Pareek *et al*. (2002), to study the effect of P_2O_5 levels on growth of Panchpatri (*Ipomoea pestigrides*), showed that, maximum plant height and number of leaves per plant was obtained by application of 25 kg P_2O_5 /ha^{-1}, but increased levels of phosphorus over 25 kg decreased these parameters. Harendra and Yadav (2007) reported that, plant height of mustard increased significantly with each increment in the dose of phosphorus up to 13.1 kg P_2O_5 /ha^{-1} (165.7 cm). However, the differences in plant height due to further increase in the dose of phosphorus to 39.3 kg P_2O_5 /ha^{-1} were not significant.

Pareek *et al*. (2002) reported significantly higher number of branches of Panchpatri plant at 25 kg P_2O_5 /ha^{-1}. Harendra and Yadav (2007), reported significant increase in the number of primary branches of mustard with an increase in the phosphorus levels from 0 to 13.1 kg P_2O_5 /ha^{-1} which was 5.3 and 6.2 branch per plant respectively. However, there was no significant increase in the number of primary branches with further increase in the phosphorus level.

Plants ultimately depend on green leaf area for dry matter accumulation as the leaves intercept solar radiation and produce photosynthates through photosynthesis (Saraf *et al.*,2002). The production, expansion and survival of green leaves are the important determinants of crop productivity. The primary symptoms of nutrient deficiency are reduction in leaf expansion.

In *Aloe vera;* application of 150 Kg P_2O_5 /ha^{-1} caused an increase in leaf area (Boroomand *et al*., 2011). Lewis, *et al.* (1987) examined the response of oil seed rape to phosphorus fertilization at 21 sites and significant increase in oil content to the tune of 0.7 per cent was recorded in only one site.

Boroomand *et al*. (2011) recorded significantly higher gel content of *Aloe vera* at 150 Kg P_2O_5 /ha^{-1} which was superior over all other phosphorus levels including control. Response of various crops to phosphorus application indicated that yield and growth attributing parameters increased with increase in levels of phosphorus and the response varied from 25 to 80 kg P_2O_5 /ha^{-1}.

These results indicated that the *Aloe vera* responds differently to inorganic sources. At equivalent nutrient level of $N_{80}P_{40}K_{80}$ organic sources of fertilizer treatments was superior to inorganic source. The information thus generated shows prospect of growing organic *Aloe vera* in a commercial cultivation as a better marketable product, Lewis *et al*. (1987).

2.20 The effect of sulfur fertilizer (soil application) on growth& yield of *Aloe vera* plants:

Lalitha and Gopala (2004) reported that, sulfur is the one nutrient most often limiting plant growth. The need for sulfur and nitrogen varies from plant to plant. For example, tomatoes and vine crops grow vegetatively and develop excessive shoot growth at the expense of fruiting with excess sulfur and nitrogen Korikanthimath (1994). (Ross *et al.,* 2010); Ross, 2004) reported that, the application of sulfur had positive effect on the growth and aloin concentration of *Aloe vera* plants. Korikanthimath (1994) also reported that, inadequate level of sulfur, prolongs the plants life cycle, plant matures relatively late and economic yield is generally poor. Also it was found that, the reason for the significant increase in yield parameters of *Aloe vera* is recognized to be the fact that sulfur is often regarded as limiting for biomass production in natural ecosystems (Minard, 1978). Hazrati (2012) found that, the application of sulfur on *Aloe vera* plant has increased the growth parameters, as

the best growth parameters were obtained in the plants treated with 2000 mg/ plant sulfur. In this regard, Tawaraya *et al.* (2007) showed that, shoot fresh weight was increased by bacterial colonization and can increase the nutrient up to 3000 mg / plant.

Lynch *et al.* (1991) reported that, the growth parameter values were increased by enhancement of nitrogen and sulfur levels applied. (Hazrati, 2012), reported that, the volume of leaf increased as a result of increase in length and thickness of leaves. Thus, leaf volume can be an important factor for the determination of leaf yield and leaf fresh weight (Sadanandan and Hamza (1998). A leaf is an important factor in yield determining in *Aloe vera* plants (Eshun and He, 2005). The application of 2000 mg sulfur per pot had significantly increased the yield. This result was confirmed with that obtained by Khandelwal *et al.* (2009) in their study aimed towards investigating the effect of sulfur fertilizer on growth and yield of sun flower (*Allianthus annus*). Increased sulfur uptake from the soil by the root system of *Aloe vera* plant could be the reason for its higher gel content (Ray, 1999).

Ji-Dong *et al.* (2006) reported that, the sulfur application increased leaf fresh weight and total biomass. Ross (2005) Showed that, the yield of aloe gel was better with a low frequency of watering and a high amount of fertilizer (2000 -3000 mg) sulfur per plant.

Wang and Strong (1990) reported that, there was a significant response when aloe plants were fertilized with sulfur, the best results were obtained with the increase in dose (0, 50, 100, 150 and 200 gm / h^{-1}), as all the growth characters (number of leaves, plant height, fresh leaf weight, number of offsets) were higher than the controlled ones. Muralidharan (1973) and Muralidharan *et al.* (1976) reported a slight increase in the yield of fresh ginger rhizome when sulfur was raised from 50 to 100 kg per ha^{-1}. Further increase in sulfur levels showed a decline in the yield.

A field experiment conducted by Ray (1999) with two levels of sulfur at 5 and 10 kg per ha^{-1} revealed that, the plant characters studied did not show any significant differences due to the application of sulfur to Danti crop. These results were confirmed with those obtained by (Saraf *et al.*, 2002) on their study on Danti crop investigating the effect of sulfur fertilization on growth and yield parameters. Ashokan *et al.* (1984) reported that, application of 10 kg sulfur per ha^{-1} produced maximum marketable tuber yield of sweet potato (13.8 ton per ha^{-1}), dry matter content in vine (11.9 %) and tuber (30.7 %) as compared to 7.5 kg sulfur per ha^{-1}.

Marlatt (1974) found that, *Sensevieria trifasciata* developed chilling injury (CI) symptoms of white lesions after exposure to 2 to 8 °C and found symptoms of chilling in *Sensevieria trifasciata* to be more severe with increasing levels of nitrogen and sulfur applications.

Conover and Poole (1976) determined that, increased tissue nitrogen and decreased levels of sulfur were mostly closely associated with increased CI. These observations are consistent with the more severe CI found on the fertilized aloe plants. The results of the second experiment suggest that, there is no apparent benefit of applying powdered sulfur and micronutrients, whether or not plants are fertilized Ashokan *et al.* (1984).

In the experiment conducted by Kawther *et al.,* (2001), regarding the effect of sulfur application on leaf chlorophyll content, they found that, application of sulfur increased *Aloe vera* leaf chlorophyll content with the raise in level of sulfur and they concluded that, leaf chlorophyll content is directly correlated with sulfur rate.

2.21 The role of biostimulants on plant growth and development:

Growth is a multifactor dependent rhythmic phenomenon. Besides the availability of nutrients, plants require growth regulators for their growth and development and these are available/ synthesized endogenously in the plant system. Under favorable conditions, the levels of endogenous growth promoting substances are higher than that of inhibiting substances which, indeed, accelerate growth and development of the plant (Thomas *et al.,* 2009; Gensheng, 1991). The plant growth regulators, as reported by (Thomas *et al.,* 2009) include: a. Auxins: 1-naphthalenacetic acid (NAA), 2,4-D, 3-indoleacetaldehyde acid, 3-indoleacetic acid (IAA), 3-indolepyruvic acid (IPA) and indolebutyric acid (IBA) b. Gibberellins (GA): GA4, GA7 and GA3 c. Cytokinins: CPPU and kinetin. d. Ethylene and Ethylene releasers (ethephon / ethylene). e. Inhibitors/Retardants: Abscisic acid (ABA), ancymidol, carbaryl, chlormequat, chloro IPC, daminozide, flurprimidol, hydrogen cyanamide (H2CN2), mefluidide, mepiquat chloride, paclobutrozol, prohexadione calcium and succinic acid (SADH). Several organic products called “biostimulants” are now available in the market to make agriculture more sustainable Collal *et al.* (2014). Agricultural biostimulants include diverse formulations of compounds, substances and micro organisms that are applied to plants or soils to improve crop vigour, yields and quality (Khan *et al.,* 2009). The crop life cycle from seed germination to plant maturity can be influenced

by biostimulants in some certain ways, that include increase plant metabolism efficiency, improve plant tolerance to a biotic stresses, facilitating nutrient assimilation, translocation and use, enhancing quality attributes of produce, including sugar content, color, fruit seeding, etc., increasing water use efficient, enhancing certain physicochemical properties of the soil and support to the development of complementary soil microorganisms. Commonly used biostimulants are amino acid formulations, mixtures of nutrients, hydrolyzed proteins, triacontanol, humic acids, seaweed extracts and brasinolides (Calvo *et al.,* 2014; Thomas *et al.,* 2009; Mandal *et al.,* 2007). Some studies expressed that foliar application of these chemicals, are able to enhance plant productivity (Khan *et al.,* 2002; Biles and Cothren (2001). Cerdan *et al.* (2009) and Ertani *et al*. (2009) reported nutrient uptake in particular nitrogen and iron increased in corn and tomato plants with using of protein hydrolysates. Also they stated that increasing nitrogen and iron uptake, led to enhancing activities of nitrate reductase and glutamine synthetase, and Fe (III)-chelate reductase activity, respectively. Amino acids are biologically important organic compounds which are composed of amine and carboxylic acid functional groups. The amino acids have various functions in plants. So they are vital for the synthesis of proteins or used as precursors for various metabolites with multiple functions in plant growth and development such as hormones, cell wall components, and a large group of secondary metabolites. For example methionine is a precursor for ethylene and polyamines such as spermine and spermidine, which synthesized from *S*–adenosyl methionine. Tryptophan is a precursor for auxin synthesis. Glycine and glutamic acid are fundamental for chlorophyll synthesis. Proline helps in pollen fertility and lysine, methionine; glutamic acids are essential amino acids for pollination. Also, amino acids have a chelating effect on micronutrients when applied together with micronutrients. Amino acids exudated from the plant's root help in improving the micro flora of the soil, thereby facilitating nutrient uptake (Less and Galili, 2008; Tempone *et al.,* 2007; Sharma and Dietz (2006). Nutritional spray on plants can decrease the delay between absorption and consumption of elements of plant that is very important for fast growth stage of plant (Taiz, and Zeiger, 2002). Follet *et al*., (1981) reported that chlorophyll content is related to the amount of nutrient uptake by plants from the soil. Follet *et al*., (1981) and Khan *et al*., (2002) reported foliar application of chitosan enhanced physiological efficiency of plants. Mustafa *et al.* (2009) studied the effect of plant growth regulators for improvement of ornamental

plants. There are several reports on the effect of growth promoters on crop with particular reference to increased vegetative growth characters; the quantity and quality yield (Hampton *et al.,* 1994). Increased values of biochemical constituents strengthened the role of biologically active amino acids in the metabolism of tea plants (*Camellia* sp.) (Thomas *et al.,* 2009).

2.22 The effect of foliar application of 6-Benzyl Adenine (BA) on growth and yield parameters of *Aloe vera* plants:

(Hazrati, 2012) reported that, application of BA had positive effect on the growth and aloin concentration of *Aloe vera* plants. Similar results were obtained by (Toth *et al*., 2002), as they found that, BA sprayed on *Aloe vera* plants has increased the growth parameters and the best growth parameters were obtained in the plants treated with 1000 ppm BA foliar spray. However, a number of studies showed that, an increase in BA, led to increase in photosynthesis, chlorophyll content and cell division in apex meristem and cambium that caused an increase in the leaf yield and growth parameters in *Aloe vera* plants (Sakakibara, 2006; Hamman, 1990). Also, by increase in the levels of BA, growth parameters were increased, while in the plants untreated with BA increase in the growth parameters was less observable (Toth *et al*., 2002).These results were similar with other studies obtained by (Nahed and Aziz, 2007).

The volume of leaf increased as a result of increase in length and thickness of leaves. Thus, leaf volume can be an important factor for the determination of leaf yield and leaf fresh weight (Hernández-Cruz *et al*., 2002). A leaf of *Aloe vera* is an important factor in yield determination in *Aloe vera* plants (Eshun and He, 2005) as he reported that, the application of 1000 ppm BA per pot had significantly increased the yield. Similar results on aloe plants were obtained by Khandelwal *et al*. (2009). On the other hand, the increased in BA intake from the leaves by the shoot system of *Aloe vera* plant could be the reason for its higher gel content (Ray, 1999). Ji-Dong *et al*., (2006) reported that, the BA application increased leaf fresh weight and total biomass. However, cytokinin can increase cell division, cell enlargement and distribution of assimilates in succulent plants and thus cause better development of the leaves and increase in gel weight (Carey, 2000). It was observed that, the BA increased the number of offsets in *Aloe vera* plants that might be due to the release apical dominance and stimulation of branches (Duck *et al*., 2004). Similar results were obtained by Carey *et al.* (2008) on *Echeveria spp* and *Sempervivum juncea* plants that

belong to the Liliaceae family. Thus, BA may be used for increasing the number of offsets and the number of propagules or for reducing the apical dominance of the *Aloe vera* plants (Sakakibara, 2006). Aloin is an important phenolic compound in aloe vera plants. (Carey, 2000) reported that, the application of BA caused an increase in aloin concentrations. Similar results were obtained by Ji-Dong *et al*. (2006), as they showed that, the amount of aloin was enhanced in *aloe vera* with BA increases. In another study carried on Aloe plants, it was revealed that, the application of BA increased the phenolic compounds (aloin and Barbaloin) in latex leaves (Saradhi *et al*., 2007), as the treatment that had the highest level of yields had also the highest aloin concentration. (Toth *et al*., 2002) reported that, low levels of BA resulted in low rate of photosynthesis and under conditions of N limitation can too often be attributed to the reduction of chlorophyll content. Exogenous application of cytokinin increased the chlorophyll content in the chloroplast (Davies, 2004). Also it was observed that, the application of BA increased the chlorophyll content in leaves of the aloe *vera* plants.

2.23 The effect of Argel and Haza extracts on growth and yield parameters:

Sandra (2011), reported that, some plants extracts as: onion (*Allium ascalonicum* L.), coconut water (*Cocos nucifera* L), snaps (*Phaseolus vulgaris* L), banana (*Musa paradisiaca* L.), mung bean sprouts (*Vigna radiata*), corn grain (*Zea mays* L.) are expected to contain bioactive compound to be used as plant growth regulators.

Sandra (2011) also, suggested that, mung bean sprouts (*Vigna radiata*), bananas (*Musa paradisiacal* L.), onions (*Allium ascalonicum* L.) contain auxin; snaps (*Phaseolus vulgaris* L) contain cytokinins; coconut water (*Cocos nucifera* L.) contains auxin, cytokinins and gibberellins. Whereas (Anonymous, 2011) stated that, corn grain (*Zea mays* L.) contains giberellin and cytokinin.

Lethan and Miller, 1963 in Wijaya (2002), Zamroni and Darini (2009) studied the effects of coconut water on growth of chilli (*Capsicum annuum* L.). The results obtained showed that, the use of 25% coconut water (*Cocos nucifera* L.) significantly enhanced the growth of chilli plants compared to the untreated.

Mahanani (2003) investigated the effect of various sources of natural plant growth regulator and frequency of application of the growth and yield of *Aloe vera* plants, in which coconut water (*Cocos nucifera* L.), mung bean sprouts (*Vigna radiata*), soy bean sprouts (*Glycine max*) and cow urine were used. The result showed the

Application of mung bean sprouts (*Vigna radiata*) extract on aloe varieties twice gave the best growth and yield compared with control.

Beneficial effects were recorded by (Idris *et al,* 2011), as they stated that, the addition of dried argel leaves to the soil significantly affected the growth and yield of palm trees compared to the control, where the Barakawi date palm cultivar was planted. The addition of argel in split doses resulted in doubling of the yield in the first season. However, all the treated trees showed significant increase in yield in both seasons compared to the control.

In the experiment conducted by (Idris *et al*, 2011) on the effect of argel leaf extract as antifedant against cotton leaf worm, showed that, all the treated plants showed healthy growth and significant increase in yield compared to the untreated ones.

(Idris *et al*, 2011), reported that, the application of high doses in two successive seasons should be avoided because of the suppressive effect of argel in high doses. The significant effect of argel was also shown in the increment of the fruit length of Barakawi which is considered as one of the market preferences.

The overall argel benefits may be owed to either of the following effects: an insecticidal effect as proposed by Sidahmed *et al.* (2011) who reported an insecticidal effect on white scale insects upon treatment of date palms with argel. Elkamali (2001) reported larvicidal effects of argel's crude aqueous extract against mosquito larvae. Moreover, an argel insecticidal effect on adult beetles of faba beans was also reported by Bakhiet and Taha (2009). Similarly, the beneficial effect was also reported by (Idris *et al*, 2011) as the application of argel resulted in reduction of termites and ants populations after the treatment.

In addition, the application of argel on palm trees resulted in antimicrobial effects as proposed by Elhady *et al.,* (1994) had improved the health status of the palms. Moreover, the speculation of a flowering promoting effect seems valid and fortified by the increments in number of bunches and spike length (Idris *et al*, 2011). A nutritive effect cannot result from the low amounts of argel leaves used in the study. A date palm is normally fertilized by 20-40 kg of farm yard manure in Sudan. Argel action seems to be systemic, absorbed by roots and translocated upwards where it imposes its effects on foliage and flowering initials. This assumption needs further biochemical studies to bio-assay the chemical constituents responsible for growth and flowering enhancements (Idris *et al*, 2011). Significant yield gains were obtained in

this study upon addition of low quantities of non-costly argel leaves to the soil of the date palms. This study attests to the practical potential of argel and Hazza which might be extended to other horticultural crops. As these plants did not receive agronomic research attention, such a move might be needed in the near future. Different plants extracts contain varied types of growth regulators therefore have different effect on the improvement of the plant seedlings growth, hence quality of the whole plant.

According to Sandra (2011), bioactive compounds such as auxin, cytokinin and gibberellins could be extracted from coconut water and corn grain, while auxin is found in mung bean sprouts, banana and onion extracts. In addition, cytokinin was found in snaps extract. These plants extract have potential to be used as exogenous growth regulator as reported by Sandra (2011).

Application of exogenous growth regulators from plant extracts can stimulate growth which is mostly related to the mechanism played by the growth regulators. Increased growth such as longer shoot and root or higher leaf numbers (Mahanani, 2003).

Hartati (2009) showed that, the effect of maize grain extract application as a natural plant growth regulator was significant in *Vigna radiata* seedlings at the time of root emergence and number of roots produced. As natural plant extracts contain different growth regulators, Hayashi (1961) and Arteca (1996) reported that, administration of giberellin (GA3) through the leaves may increase growth due to an increase in the effective leaf area, hence photosynthesis increases.

Other than gibberellins, extract of corn grain also contain cytokinin. Cytokinins were also reported as one of important factors for the regulation of root growth, especially cell differentiation in the elongation zone (Dello *et al.*, 2007).

Auxins are formed from the amino acid tryptophan which acts to stimulate stem elongation, leaf growth, and influence the development of roots in plants. Tryptophan is an auxin-forming compound (Abidin, 1983). According to Lathan and Miller (1963) in Wijaya (2002), there are natural cytokines in the immature corn kernels called zeatin. Cytokinins are compounds that can increase cell division in plant tissues and regulate plant growth and development, as well as kinetin (6-furfurylaminopurine) (Zulkarnain, 2009).

The work of Arditti and Ernts (1992) showed auxin and gibberellins were contained in banana. Similarly, use of maize seed extract as growth regulator has been investigated by Hartati (2009) to study its effect on orchid plantlets and the results

showed that, the extract application significantly affected early root emergence and the number of roots produced. Although researches on the use of plant growth regulators naturally in plants has been done, but its use is limited to the production of crops, instead of improving the quality of cuttings (Sujal *et al.*, 2012). In addition, the type of plants used as plant growth regulators is still limited. Thus research needs to be done on a wider variety of crops to determine the concentration of growth regulators containing compounds and see its effect on the growth of plant growth and development.

Chapter Three

General References

Abidin, Z. (**1983).** Knowledge Base Materials Growth Regulator. Space. *Journal of Agric. Sci.*, 7 (16): 85-88. Bandung (*Indonesian*).

Agarwala, O.M. (1997). Whole leaf Aloe gel vs. standard Aloe gel. *Journal of Plant Biochemistry & Biotechnology*; 60 : 22–8.

Aggarwal, D. and Barna, K.S. (2004). Tissue culture propagation of elite plant of *Aloe vera* Linn. *J. Plant Biochemistry & Biotechnology*. 13: 77-79.

Andreas, A., Schaller, D. and Frasson, D. (2001). Induction of wound response gene expression in tomato leaves by ionospheres," *Planta,* 212: 431-435.

Anilkumar, B., Yadav, Y. and Dhankhar, O.P. (2001). Performance of four-rowed Indian mustard (*Brassica juncea*) mutant under different levels of nitrogen and phosphorus. *Indian J. Agric. Sci.*, 71(6): 375-377.

Anonymous. S. (**2011)**. Extraction Technique Hormone Auxin, Gibberellins. *Journal of Biotechnology,* Dakar; 2(17): 54-65.

AOAC. (1990). Official methods of analysis of the Association of Official Analytical Chemists. 15th Ed. Association of Official Analytical Chemists Inc. Suite 400 2200 Wilson Boulevard, Arlington, Virginia 22201, USA. pp. 69-81.

Arditti, J. and Ernts, R. (1992). Micro propagation of Aloe plants. *Indian Journal of Agric. Sci.*, 71(6):102-107.

Aro, A.A., Nishan, U., Perez, M.O., Rodrigues, R.A., Foglio, A., Carvalho, F., Gomes, L., Vidal, B.C. and Pimentel, E. (2012). Structural and biochemical alterations during the healing process of tendons treated with *Aloe vera*. *Life Science*; 91(17-18):885-893.

Arteca, R. (1996). Plant Growth Substances: Principles and Applications. Chapman & Hal. New York.

Ashley, F.L., O'Loughlin, B.J., Peterson, R., Fernandez, L., Stein, H., Schwartz,_A.N. (1957). The use of *Aloe vera* in the treatment of thermal and irradiation burns in laboratory animals and humans. Plast Reconstr Surg; 20:383–96.

Ashokan, P.K., Vikramannari, R. and Kurian, T.M. (1984). Nitrogen and potassium requirements of rainfed sweet potato (*Ipomoea batatas* L.). *J. Root Crop*, 10(12): 55-57.

Attoe, Z. and Osodeke, M. (2009). Studies on rhizosphere microbial diversity of some commercially important medicinal plants. Colloids and Surfaces B: Biointerfaces. In Press; 74:214- 222.

Babatunde, F,E., Yongabi, K.A. (**2008)**. Effect of N on productivity of *Aloe barbadensis* L. and its inhibitory effect on *Trichophyton rubrum*. *Adv. Hort. Sci*., 22(3): 187-190.

Bakhiet. H. and Taha, R.O. (2009). The insecticidal effect of argel on adult beetles of *Faba bean*. Journal of Environmental Science and Health Council;17:87–85.

Biles, S.P. and Cothren, J.T. (2001). Flowering and yield response of cotton to application of mepiquat chloride and PGR-IV. *Crop Sci*.; 41: 1834 - 1837.

Boroomand, N., Nakhaei, M. and Sadat, H.G.M. (2011). Effect of potassium and phosphorous on growth and yield of *Aloe Vera* L. *7th Iranian Congress of Horticultural Science*, Isfahan, Iran. Pp. 375-381. September, 2011.

Boudreau, M.D. and Beland, F.A. (2006). An evaluation of the biological and toxicological properties of *Aloe barbadensis* (Miller), *Aloe vera*. Journal of Environmental Science and Health Council; 24:103–54.

Boudreau, M.D., Mellick, P., Olson, G., Felton, R., Thorn, B. and Beland, F. (2012). Clear evidence of carcinogenic activity by a whole leaf extract of *Aloe barbadenis* Miller (*Aloe vera*) in F344/N Rats. *Toxicological Sciences*. 131(1)26-39.

Boyer, F.R.(2012). Modern Experimental Biochemistry 2nd.ed. *The Benjamin/Cummings Company*, Inc 390 Bridge Parkway, Redwood *City, California, USA. pp. 433-439.*

Calvo, P., Nelson, L. and Kloepper, J.W. **(2014**). Agricultural uses of plant biostimulants. *Journal of Plant Soil*. 2014; 383: 3 - 41.

Campestrini, L.H., Kuhnen, S., Lemos, P.M.M. Bach, D.B., Dias, P.F. and Maraschin, M. (2006). Cloning protocol of *Aloe vera* as a study-case for "Tailor-Made" biotechnology to small farmers. *Journal of Technology; Management and Innovation*. 1(5):76-79.

Capasso, F. Borrelli, F. and Capasso, R. (1998). Aloe and its therapeutic use. *Phytotherapy Res.* 12: 121-127.

Carey, D. (2000). The effect of BA application on *Aloe vera* leaf gel. *Journal of Horticultural Science;* 22: 57-61.

Carey, D., Whipker, B., McCall, I. and Buhler, W. (2008). Benzyl adenine foliar sprays increase offsets in *Sempervivum* and *Echeveria*. *J. Hort. Sci*., 53: 19-21.

Cerdan, M., Sanchez-Sanchez, A., Oliver, M., Juarez, M. and Sanchez-Andreu, J.J. (2009). Effect of foliar and root applications of amino acids on iron uptake by tomato plants. *Acta Hortic*.; 830: 481 - 488.

Chandan, B..K., Saxena, A.K., Shukla, S., Sharma, N., Gupta, D.K., Suri, K.A., Suri, J., Bhadauria, M. and Singh, B. (2007). Hepatoprotective potential of *Aloe barbadensis* Mill. Against carbon tetrachloride induced hepatotoxicity. *Journal of Ethnopharmacology;* 111(3) 560-566.

Chow, D., Yates, K.M. and Tizard, I. (2005) Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. *International Immunopharmacology*; 4 (14):45-55.

Colla1, G., Rouphael, Y., Canaguier, R., Svecova, E. and Cardarelli, M. (2014). Biostimulant action of plant-derived protein hydrolysates produced through enzymatic hydrolysis. *Front Plant Sci*.; 5: 44- 48.

Collins, E. and Collins, C. (1935). Roentgen dermatitis treated with fresh whole leaf of *Aloe vera*. American Journal of Roentgenol; 33:396–397.

Conover, D. and Poole, E. (1976). The Mineral Nutrition of Higher Plants." *Annual Review of Plant Physiology*, 31: 239–298.

Coronado, G.D., Thompson, B., Tejeda, S. and Godina, R. (2004). Attitudes and beliefs among Mexican Americans about type 2 diabetes. Journal of Health Care Poor Underserved; 15:576–588.

Dagne, E., Bisrat, D., Viljoen, A. and Van Wyk, B.E. (2000). Chemistry of Aloe species. Curr Org Chem.; 4:1055–1078.

Danhof, I.E. (1987). Remarkable Aloe: Aloe through the Ages. Grand Prairie, TX: Omnimedicus Press. American Journal of Roentgenol; 3(6): 574- 587.

Davies, P.J. (2004). The plant hormones: their nature, occurrence and function. In Davies P.J. (Eds). Plant hormones biosynthesis, signal transduction, action, *Kluwer Academic Publishers*, Dordrecht, Boston, London, pp. 471-492.

Dello, I.R., Linhares, F.S., Scacchi, E., Casamitjana-martinez, E., Heidstra, R., Costantino, P. and Sabatini, S. (2007). Cytokinins determine arabidopsis root-meristem size by controlling cell differentiation. *current biology;* 17: 678-682.

Dixon, R.A. and Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell*, 7(7): 1085-1097.

Duck, M.W., Gregg, B.M., Fernandez, R.T., Royal, D.H. and Cardoso, F.F. (2004). Height control of *Picea spp* and *Chamaecyparis lawsoniana* with uniconazole and 6-benzyladenine. *Journal of Environmental Horticulture;* 22 (3): 165-169.

Elhady, A., **Musa, F.A. and Mardi, H.G. (1994).** Improved date palm status through Argel treatments. *Emirate Journal of Agriculture,* 2(4): 30-35.

Elkamali, H. H. (2001). Larvicidal activity of crude aqueous extracts of *Solenostemma argel* against mosquito and cotton leaf worm larvae; J*ournal of herbs spices & medicinal plants*; vol. 8 (4); 83 – 86.

Ertani, A., Cavani, L., Pizzeghello, D., Brandellero, E., Altissimo, A., Ciavatta, C. and Nardi, S. (2009). Biostimulant activity of two protein hydrolyzates in the growth and nitrogen metabolism of maize seedlings. *J. Plant Nutr.Soil Sci.*; 172: 237 - 244.

Eshun, K. and He, Q. (2004). *Aloe vera*: A valuable ingredient for the food, pharmaceutical and cosmetic industries-A Review. *Critical Reviews in Food Science and Nutrition.* 44 (2) 91-96.

Eshun, K. and He, Q. (2005). *Aloe vera*: A valuable ingredient for the food, pharmaceutical and cosmetic industries a review. *Crit. Rev. Food Sci. Nutr.,* 44: 91-96.

FAO, Food and Agriculture Organization (2012). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany*; 57 (4): 711 - 726.

Faryabi, A. and Ghazanchi, R. (2005). Study of the Effect of Chemical Fertilizers, Compost and their Combination on *Aloe vera* Growth and Yield. Science and Research Branch of Islamic Azad University Publications, Iran.

Femenia, A., Garcia-Pascual, P., Simal, S. and Rosello, C. (2003). Effects of heat treatment and dehydration on bioactive polysaccharide acemannan and cell wall polymers from Aloe barbadensis Miller. Carbohydr Polym.; 51: 397–405.

Femenia, A., Sànchez, E.S., Simal, S., Rossello, C.(1999). Compositional features of polysaccharides from *Aloe vera* (*Aloe barbadensis* Miller) plant tissues. Carbohydr Polym.; 39:109–117.

Follet, R.H., Murphy, L.S. and Donalua, R.L. (1981). Soil-fertilizer-plant relationship. *Fertilizer Soil;* 16: 478 - 481.

Garnayak, H., Hord, D. and Reynaldo, F. (2000). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* 17: 319–339.

Gensheng, P. (1991). Study on the relations between growth of tea and endogenous hormones IAA and ABA. *J. Tea Sci.*; 11: 25 - 28.

Glynn, C., Ronnberg, W., A., Julkunen-Titti, R. and Weih, M. (2004). Willow genotype, but not drought treatment, affects foliar phenolic con-centrations and leaf-beetle resistance. *Ento-mologia Experimentalis et Applicata*. 113 (1): 1-14.

Grindlay, D. and Reynolds, T. (1986). The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. Journal of Ethnopharmacology; 16:117–151.

Hamman, J.H. (2008). Composition and applications of *Aloe vera* leaf gel. Molecules. *Indian J. Agron*., 23(2): 15-17.

Hamman, M. (1990). Synthetic plant growth regulators. *Adv. Agron*., 43: 47-55.

Hamman. J.H. (1990). Composition and Applications of *Aloe vera* Leaf Gel. Molecules, *Indian J. Agron.,* 13: 25-30.

Hampton, M.O. Schaffer, B. and Bryan, H. (1994). Nutrient concentration, growth and yield of tomato and squash in municipal solid-waste amended soil. *Horticultural Science*; 29: 785 - 788.

Harendra, K. and Yadav, D.S. (2007). Effect of phosphorus and sulphur levels on growth, yield and quality of Indian mustard (*Brassica juncea*) cultivars. *Ind. J. Agron*., 52(2): 1541-1557.

Hartati, S. (2009). Effect of Various Extracts of Organic Materials and PGR against orchid plantlets Growth Crosses Results on Culture Media. UNS Faculty of agriculture, Semarang. (Indonesian).

Hasanuzzaman, M., Ahamed, K.U., Khalequzzaman, K.M., Shamsuzzaman, A.M.M. and Nahar, K. (2008). Plant characteristics, growth and leaf yield of *Aloe vera* L. as affected by organic manure in pot culture. *Aust. J. Crop. Sci*., 2(3): 158-163.

Hayashi, G.F. (1961). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol. Asp. Med*., 27: 1-93.

Hazrati, S. (2012). Effects of various levels of N on productivity of *Aloe barbadensis* L. and its inhibitory effect on *Trichophyton rubrum*. *Advances in Horticultural Science*; 24 (4): 187-190.

Heber, D. (2007). Physicians' Desk Reference for Herbal Medicines. 4th. Montvale, NJ: Thomson.

Heggers, J., Pelley, R. and Robson, M. (1993). Beneficial effects of *Aloe vera* in wound healing. *Phytotherapy Research.* 7: S48-S52.

Hernández-Cruz, L.R., Rodríguez-García, R., Rodríguez, D.J. and Angulo-Sánchez, J.L. (2002). *Aloe vera* Response to Plastic Mulch and N. *In En Janik J, Whipkey A* (Eds). Trends in new crops and new uses, ASHS Press. Alexandria: VA, EEUU, pp. 570-574.

Hocking, F.D., Siag, R.K. and Mann, P.S. (1997). Response of Indian mustard (*Brassica juncea*) to nitrogen and phosphorus on Torripsamments of north-western Rajasthan. *Indian Journal of Agronomy,* 45(4): 746-751.

Hutter, J.A., Salman, M., Stavinoha, W.B. (1996). Antiinflammatory C-glucosyl chromone from *Aloe barbadensis. Journal of Natural Products,* 59:541- 543.

IASC (2009) International Aloe Science Council. How large is the Aloe Market? (Accessed August 18, 2009).

Idris, T.I.M., Ibrahim, A. M.A., Mahdi, E. M., and Taha A. K. (2011). Influence of argel (*Solenostemma argel* Del. Hayne*)* soil applications on flowering and yield of date palm (*Phoenix dactylifera* L.*). .Agriculture and Biology Journal of North America,* 2(3):538-542.

IJAS (2012) International Journal of Agriculture Systems. Propagation of Orchids. Irvine: Department of Developmental and Cell Biology, University of California.

Ji-Dong, W., Zaho-pu, L., Qing-song, Z., Ling, L. and Feng- zhi, P. (2006). Effects of different N levels on seedling growth, nitrat and its secondary metabolites in *Aloe vera* seeding. *Plant Nutr. Fertil. Sci*., 12(6): 864-868.

Josias, H. (2008). Composition and applications of *Aloe vera* leaf gel, *Molecules*, vol. 13, pp. 1599-1616.

Katarzyna, D. and Zbigniew, J. (2006). Influence potassium fertilization on the yield and on the nutrients content in *Satureja Hortensis* L. Acta Agrophys., 7: 879-884.

Kawther, M., Tawfik, A., Soad, W. and Ahmed, Z.E. (2001). The effect of inorganic fertilizer applications on gel characters in *Aloe vera. Egypt J. Biol, 3, 149-159.*

Kemper, K.J. and Chiou, V. (1999). *Aloe vera*: The Longwood Herbal Task Force and The Center for Holistic Pediatric Education and Research. 11-29.

Khan, W., Rayirath, U.P., Subramanian, S., Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J., Norrie, J. and Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regulation*; 28: 386 - 399.

Khan, W.M., Prithiviraj, B. and Smith, D.L. (2002). Effect of foliar application of chitin oligosaccharides on photosynthesis of maize and soybean. *Photosynthetica;* 40: 621 - 624.

Khandelwal, S.K., Meenakshi, J., Choudhary, M.R. and Gupta, K.N. (2009). Effect of N and spacing on growth and yield of Indian aloe (*Aloe barbadensis* L.). *J. Med. Aromat. Plant Sci*., 31(3): 203-205.

Kjaer, C., Elmegaard, N., Pedersen, .B.M., Damgaard, R. and Nielson, J.K. (2001). *International journal of Pesticide Research*; 2 (41): 55- 65.

Klein, A.D. and Penneys, N.S. (1988). *Aloe vera. J. Am. Acad. Dermatol*. 18:714-720.

Korikanthimath, D.G. (1994). Nutrition of cardamom. In: *Advances in Horticulture (Eds.)* Chadha, K.L. and Rethinam, P. Malhotra Publishing House, New Delhi, 9(1): 467-476.

Kumar, P., Adityasharma Sing, P.K. and Hord D.K. (2002). Integrated nutrient management in mudgaparni (*Phaseolus trilobus*). *Extended summaries 2nd Int. Agron. Cong*., New Delhi, 1(26-30): 317- 318.

Lalitha, S.G. and Gopala, R.P. (2004). Influence of plant population and nitrogen levels on growth characters and seed yield of sunflower (*Helianthus annus* L.). *The Andhra Agric. J*., 51(3&4): 281-283.

Lans, C.A. (2006). Ethno medicines used in Trinidad and Tobago for urinary problems and *Diabetes mellitus*. J. Ethnobiol Ethnomed. ; 2:45–55.

Lee, H.S., Struik, P.C. and Damian, G. (2000). Effects of irrigation and N on the vegetative growth of *Aloe barbadensis* Mill. *Trop. Agric*., 74(2): 82-91.

Less, H. and Galili, G. (**2008**). Principal transcriptional programs regulating plant amino acid metabolism in response to abiotic stresses. *Plant Physiol.* ; 147: 316 - 330.

Lethan, A. and Miller, G.R. (1996). Antidiabetic activity of *Aloe vera* L juice. II. Clinical trial in diabetes mellitus patients in combination with glibenclamide. Phytomedicine ; 3:245–248.

Lewis, D.C., Potter, T.D., Weckert, S.E. and Grant, I.L. (1987). Effect of nitrogen and phosphorus fertilizers on the seed yield and oil concentration of oil seed rape and the prediction of responses by soil tests and past paddock use. *Austr. J. Exp. Agric*., 27: 713-720.

Lewis, J.E. McDaniel, H.R., Agronin, M.E., Loewenstein, D.A., Riveros, J., Mestre, R., Martinez, M., Colina, N., Abreu, D., Konefal., J., Woolger, J.M. and Ali, K.H. (2011). The effect of an Aloe polymannose multinutrient complex on cognitive and immune functioning in Alzheimer's disease. *Journal of Alzheimer's Disease*. 33(2) 393-406.

Luis Rodolfo, H.C., Ranl, R.G., Diana, J.R. and Jose, A.S. (2002). *Trends in New Crops and New Uses*; 3(1): 125-131.

Lynch, J., Lauchli, A. and Epstein, E. (1991). Vegetative growth of the common bean in response to phosphorus nutrition. *Crop Sci*., 31: 380-382.

Mahanani, A. (2003). Effect of various sources of natural PGR and Frequency of Application of the Growth and yield of Potato (*Solanum tuberosum* L.) Granola varieties. Muhammadiyah University, Malang. (*in Indonesian*).

Mandal, A.K.A., Kumar, R. and Thomas, J. (2007). An overview of PGR trials in UPASI TRF. *Planters Chron*. 2007; 103: 12 - 16.

Manderville, F. (1939). Aloe vera in the treatment of radiation ulcers and mucous membranes. Radiology; 32:598–599.

Marlatt, J.G. (1974). Nitrogen nutrition of garlic (*Allium sativum* L.) under irrigation components of yield and indices of crop nitrogen status. *Sci. Hortic*., 29: 69-76.

Mc Daniel, H., Carpenter, R., Kemp, M., Kahlon, J. and Mc Analley, B. (1990). Extended survival and prognostic criteria for Acemannan (ACE-M) treated HIV Patients. *Antiviral Res.* Suppl. 1: 117.

.

Mengel, K. and Kirby, E.A. (1987). Principles of Plant Nutrition. 4ed. Bern: International Potash Institute, pp 687.

Merestala, T. (1996). Abstract Bibliography of researches of Bio and Organic Fertilizers at Benguet State University 1975-1996. La Trinidad, Benguet. 53p.

Meyer, H.J. and Staden, J.V. (1991). Rapid *in vitro* propagation of *Aloe barbadensis* Mill. *Plant Cell Tissue and organ Culture*. 76(1): 83-86.

Minard, H.R.G. (1978). Effect of clove size, spacing, fertilizer and lime on yield and nutrient contens of garlic (*Allium Sativum* L) Newzeland. *J. Exp. Agric.*, 6: 139-143.

Mirza, H.U., Ahamed, K., Uzzaman, K.M., Shams, U. and Kamran, N. (2008). The Effects of Chemical and Organic Fertilizers on Chemical Constituents of Medicinal Plants. *Australian Journal of Crop Science*; 20 (3):158-163.

Moradkhani, H., Sargsyan, E., Bibak, H., Naseri, B., Sadat-Hosseini, M., Fayazi-Barjin, A. and Meftahizade, H. (2010). *Melissa officinalis* L., a valuable medicine plant: **A *revie*w.** *J. Med. Plants Res*., 4: 2753-2759.

Mukesh, S. Sikarwar, M. B., Patil, S. S. and Vishnu, B. (2010). *Aloe vera*: Plant of Immorality, *International journal of Pharma Sciences and Research*, vol. 1, no. 1, 7-10.

Muralidharan, A. (1973). Varietal performance of ginger in Waynad (Kerala). *J. Plant. Crop*, 1: 19-20

.

Muralidharan, G., Sadandan N. and Vikraman N.N. (1976). Effect of time of application of nitrogen at different levels on the uptake of nitrogen by sweet potato varieties. *J. Root Crop*., 2(2): 15-12.

Mustafa, S.G., Kaukaband, M. and Ahmad, A. (2009). Foliar application of plant growth regulators (PGRs) and nutrients for improvement of lily flowers. *Pakistan. J. Botany;* 41: 233 - 7.

Nahed, G. and Aziz, E. (2007). Stimuatory Effect of NPK Fertilizer and Benzyladenine on Growth and Chemical Constituents of *Codiaeum variegatum* L. Plant. Am-Eurasian. *Journal of Environmental Science*, 2(6): 711-719.

Natali, L., Sanchez, I.C. and Cavallini, A. (1990). *In vitro* culture of *Aloe barbadensis* Mill. Micropropa-gation from vegetative meristems. *Plant Cell, Tissue and Organ culture*, 20:71-74.

Nejatzadeh, F., Tahmasebi Enferadi, S., Naghavi, M., Hasani, Y., Mostoufi, D. and Mousavi, H. (2011). *Iranian Journal of Medical and Aromatic Plants*; 5 (18): 4537-4544.

Nematian, A. Ghoushchi, F. Farnia, A., Arianpour, J. and Mashhadi, A. (2011). The Effects of Chemical and Organic Fertilizers on Chemical Constituents of Medicinal Plants. *Iranian Journal of Medical and Aromatic Plants*;27 (2): 347-388.

Ni, Y., Turner, D., Yates, K.M. and Tizard, I. (2004). Isolation and characterization of structural components of Aloe vera L. leaf pulp. Int immunopharmacol ; 4:1745–1755.

Okut, N. and Yidirmi, B. (2005). Effect of different row spacing and nitrogen doses on certain agronomic characteristics of coriander (*Corianderum sativum* L.). *Prod. J. Biol. Sci*., 8: 901-904.

Panwar, A.S. and Singh, N.P. (2003). Effect of conjunctive use of phosphorus and bioorganics on growth and yield of groundnut (*Arachis hypogae*). *Indian J. Agron*., 48: 214-216.

Pareek, S.K., Virendersingh, S.A., Pilania, D.S. and Ashok, K. (2002). Effect of method of sowing, time of harvesting and nitrogen on dry root yield of Aloe plants. *Sci. Hortic*., 32: 42-47.

Park, Y. and Lee, S. (2006). New Perspectives on Aloe. New York: Springer Verlag. *Indian Journal of medicinal plants; 22*(48): 114-126.

Pichgram, N. (1987) Investigation on plant spacing and fertilizer effect on growth of aloe (*Aloe barbadensis* Mill.). Ph.D. Thesis., Dept. of Horticulture, Graduate School. Faculty of Agriculture, Kasetsart Univ., Bangkok (Thailand).

Rajasekaran, S., Ravi K., Sivagnanam, K. and Subramanian, S. (2006). Beneficial effects of *Aloe vera* leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clin Exp Pharmacol Physiol.*;* 33: 232–237.

Ray, H.N. (1999). Benzyl adenine foliar sprays increase offsets in *Sempervivum* and *Echeveria*. *J. Hort. Sci*., 53: 19-21.

Reynolds, T. (2004). Aloe chemistry. In Reynolds T. (Ed). Aloes: the genus Aloe, *CRC* Press, Boca Raton. Florida, USA, pp. 39-74.

Reynolds, T. and Dweck, A.C. (1999). *Aloe vera* gel leaf: a review update *Journal of Ethnopharmacol*; 68:3-37.

Rodriguez Garcia, D., jasso de Rodriguez, J. A., Gil-Marin, J. L. and Angulo Sanchez, R. H. (2007). Lira- Saldivar. *Industrial crops and Products*; 6(25): 123-128.

Ross, S.A., Elsohly, M.A. and Wilkins, S.P. (2010). Quantitative analysis of *Aloe vera* mucilaginous polysaccharide in commercial *Aloe vera* products. *Journal of AOAC Int*.; 80:455–457.

Roy, S.C. and Sarkar, A. (1991). *In vitro* regeneration and micro propagation of *Aloe vera* L. *Scientia Horticulturae*. 47: 107-113.

Saccu, D., Bogoni, P. and Procida, G. (2001). Aloe exudates: Characterization by reversed phase HPLC and head-space GC-MS. Journal of Agricultural Food Chem.; 49:4526–30.

Sadanandan, A.K. and Hamza, S. (1998). Organic farming on yield and quality of spices in India (Abs.) 16th World Congress on Soil Science; Montpellier, France during 20-26. P. 738.

Sakakibara, H. (2006). Cytokinins: activity, biosynthesis and translocation, *Annual Review of Plant Biology;* 57, 431-449.

Samman, S. (1998). Lipid metabolism. In Schaum's Outlines of Theory and Problems of Biochemistry. New York: McGraw Hill Book Company;. pp. 362–401.

SanchezGovin, E., Rodriguez Gonzales, H., Carballo Guerra, C. and Milanes Figueredo, M. (2005). Influencia de los abonosorgánicos y biofertilizantes en la calidad de lasespecies medicinales *Calendula officinalis* L. *Matricaria recutita* L. *Revista Cubana de Plantas Medicinales.* 10 (1): 1 - 5.

Sandra, E. (2011). Plant hormones and growth, accessed October 25, Bogor (*in Indonesian*).

Saradhi, V.S.P., Khanam, S., Shivananda, B.G., Vasantha, K.T. and Shivananda, T.N. (2007). Effect of NPK fertilizers on chemical constituents of *Aloe vera* leaves. *J. Nat. Rem*., 7(2): 258-262.

Saraf, G., Alfaro, U. and Singh, H. (2002). Two trails on the effect of nitrogen and phosphorus on garlic. *Agric. Technol*., 42: 143-147.

Sharma, S.S. and Dietz, K.J. (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot*.; 57 (4): 711 - 726.

Sharpley, S.K., Virendersingh, S.A., Pilania, D.S. and Ashok, K. (1994). Effect of method of sowing, time of harvesting and nitrogen on dry root yield of *Aloe vera* plants.

Shishu, J. and LVinary, A. (2005). Vegetative growth and oil content of Mustard in response to nitrogen and phosphorus nutrition. Crop Sci., 31: 311-322.

Shudo, K. (1994). Chemistry of phenylurea cytokinins. In Mokk D.V., Mok Mc. (Ed) Cytokinins: Chemistry, activity and function, CRC Press, Boca Raton, pp. 35-42.

Sidahmed, O. A., Musa, F.A. and Mardi, H.G.(2011) .Application of plant based extracts for control of the green pit scale insect on date palm. *Emirate Journal of Agriculture,* 23(5): 387-403.

Steenkamp, V. and Stewart, M.J. (2007). Medicinal applications and toxicological activities of Aloe products. Pharm Biol.;45:411–20.

Stirk, W.A., Novak, O., Vaclavikova, K., Tarkowski, P., Strnad, M., van Staden J. **(2008).** Spatial and temporial changes in endogenous cytokinins in developing pea roots, *Planta* , 227, 1279 -1289.

Strnad, M., Hanus, J., Vanek, T., Kaminek, M., Ballantine, J., Fussel, B. and Hanke, D.E. (1997). Meta- Topolin, a highly active aromatic cytokinin from poplar leaves (*Populus canadensis* Moench. cv. *Robusta*), *Phytochemistry*; 45, 213-218.

Strnad, M.C., Mead, A., Harriman, M., Trueman, L.J., Smith, B.M., Thomas, B. and Broadley, M.R. (2004). Interactions between selenium and sulphur nutrition in Arabidopsis thaliana, *Journal of Experimental Botany* , 55, 1927-1937.

Sujal, D.A., Virendersingh, S.A. and Pilania, D.S. (2012). Effects of plant growth regulators on growth, yield and quality of Indian mustard (*Brassica juncea*) cultivars. *Ind. Journal of Agronomy;* 72(2): 41-45.

Suresh, R. (1980). British Herbal Compendium, British Herbal Medicine Association: Vol. 1.l. Bournemouth, UK.

Taiz, L. and Zeiger, E. (2002). Plant physiology. 4th Edition. Sinauer Associates. Sunderland; pp: 690 - 691.

Tawaraya, K., Turjaman, M. and Ekamawanti, H.A. (2007). Effect of Arbuscular Mycorrhizal Colonization on Nitrogen and Phosphorus uptake and Growth of *Aloe vera* L. *Hort Science.* 42(7):1737–1739.

Tempone, A.G., Sartorelli P., Mady C. and Fernandes, F. (2007). Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. *Cell Mol. Biol.* ; 15: 15 – 25.

t'Hart, L.A., Nibbering, P.H., van den Barselaar, M.T., van Dijk, H., van den Burg, A.J. and Labadie, R.P. (**1990**). Effects of low molecular constituents from aloe vera gel on oxidative metabolism and cytotoxic and bactericidal activities of human neutrophils. *Int J Immunopharmacology*;12:427–434.

Thiruppathi, S., Samasubramaniam, V., Sivakumar, A. and Thirumalai, A. V. (2010). Antimicrobial activity of *aloe vera* (L) Burm. F. against pathogenic microorganisms, *Journal of Biosciences Research,* vol. 1, no. 4, pp. 251-258.

Thomas, J., Mandal, A.K.A., Raj Kumar, R. and Chordia, A. (2009). Role of biologically active aminoacid formulations on quality and crop productivity of tea (*Camellia* sp.). *International Journal of Agricultural Research* ; 4: 228 - 236.

Thomas, P. and Ravindra, M.B. (1997). Shoot tip culture in mango: influence of medium, genotype, ex-plant factors, season and decontamination treat-ment on phenolic exudation, explant survival and axenic culture establishment. *Journal of Horticultural Science.* 72:(5) 713-722.

Toth, V.R., Meszkaros, I., Veres S. and Nagy, J. (2002). Effects of the available N on the photosynthetic activity and xanthophyll cycle pool of maize in field. *J. Plant. Physiol*., 159: 627-634.

Ulbricht, C., Armstrong, J. and Basch, E. (2008). An evidence-based systematic review of Aloe vera by the Natural Standard Research Collaboration. Journal of Herbal Pharmacology; 7: 279–323.

Vio, Z., Farahnejad, T., Ghazanfari, H.G. and Yaraee, R. (2005). Immunomodulatory effects of *Aloe vera* and its fractions on response of macrophages against Candida albicans. *Immunopharmacology and Immunotoxicology*. 33(4) 676-681.

Wang Y. and Strong, K. A. (1995). Two-year study monitoring several physical and chemical properties of field-grown Aloe barbadensis Miller leaves. Subtropical Plant Science ; 47: 34–38.

Wang, W., Cuyckens, F. and Van den Heuvel, H. (2003). Structural characterization of chromone C-glucosides in a toxic herbal remedy. Rapid Commun Mass Spectrom. ;17:49–55.

Wang, Y. and Strong, K. (1990).A two-year study monitoring several physical and chemical properties of field-grown Aloe barbadensis Miller leaves. Subtropical Plant Sci. ;47:34–8.

WHO, **World Health Organization** (1999). Monographs on Selected Medicinal Plants. Vol. 1. Geneva: World Health Organization.

Wiedenhoeft, R., Mangiafico, S. and Ritchey, C.R. (2006). A chemical investigation of *Aloe barbadensis* Miller. Proc. *Okla. Acad. Sci*., 58: 60-70.

Wijaya, H. (2002). Shoots response interfascicular pinus jung et de vries against cuts and giving hormone treatment 6 -bap. faculty of forestry, hasanuddin university, makassar.

Winter, W.D., Benavides, R. and Clouse, W.J. (1981). Effects of *Aloe* extracts on human normal and tumor cells *in vitro*. *Economic Botany*. 35: 89-95.

Yagi, A., Tsunoda, M., Egusa, T., Akasaki, K. and Tsuji, H. (1998). Immunochemical distinction of *Aloe vera, A. arborescens,* and *A. chinensis* gels. *Planta Med* 64: 277-278.

Yaronskay, E.B., Gritskevich, E.R., Trukhanovets, N.L. and Averina, N.G. (2007). Effect of macro-nutrient elements on early stages of chlorophyll biosynthesis in streptomycin-treated barley seedlings, *Russian Journal of Plant Physiology*; 54:388-395.

Zamroni, W. and Darini, G. (2009). Growing an organizing influence Defolasi Natural and Herbal Leaves on Growth Cuttings. Faculty of Agriculture. Tamansiswa Sarjawinaya University of Yogyakarta. Info@fp.ustjogja.ac.id, (*Indonesian*).

Zulkarnain, G.G. (2009). Plant Tissue Culture. Cultivation Plant Propagation Solutions. Earth Literacy, Jakarta. (*Indonesian*).

Chapter Four

Impact of urea applications on growth attributes and gel yield of *Aloe vera* L. plant.

Abstract:

Although *Aloe vera* is an indigenous plant of Sudan with numerous applications in health, cosmetics and food industries in global markets, agricultural research to exploit the potential of this plant is almost lacking. This study aimed to investigate the impact of urea applications at various rates on the growth attributes and quality of *Aloe vera* plants under nursery conditions, at Shambat, Khartoum North, Sudan. Five levels of urea (0, 1.5, 3, 4.5 and 6 g/plant) were tested as soil dressings in 25X30 cm plastic bags. The study was arranged in a complete randomized design where each treatment was replicated eight times. Data were collected after 12 months. The 1.5 g urea treatment enhanced growth, leaf gel and chlorophyll contents, whereas the higher levels of urea were suppressive. The encouraging results of this study elucidated an economical potential for possible large scale production of the plant under Sudan conditions.

Key words: *Aloe vera;* Urea, Growth, Chlorophyll, Gel Content.

Introduction:

Aloe vera is a medicinal plant belonging to the family Liliaceae (Hasanuzzaman, *et al*., 2008). It is indigenous to Sudan and other African countries (Grindlay and Reynolds 1986). The exploitation of the potential of the plant is increasing with growth of cosmetic and neutraceutical markets (Danhof, 1987). This perennial succulent is drought tolerant and can thrive well under xerophytic conditions (Heber, 2007). It was officially listed as a purgative and skin protectant by U.S. pharmacopoeia in 1930 (Park and Lee, 2006). The two major products of *Aloe vera* are yellow latex and clear gel, which are obtained from the large parenchymatic cells of the leaf (Ni, *et al*., 2004). The main constituents of the latex are anthraquinones including the hydroxyanthracene derivatives, aloin A and B, barbaloin, isobarbaloin and aloeamedin (Bradley, 1992). *Aloe vera* possesses several biological and physiological activities, such as wound healing, anti-inflammation, anti-

bacterial, anti-viral, anti-fungal, anti-diabetic and anti-neoplastic (Hamman, 2008; Eshun and He, 2005; Reynolds, 2004).

The uses of *Aloe vera* are diverse in Sudan. In ethno medicine it is used for skin moisturing, treatment of burns and a purgative especially for injuries in diabetic patients. Besides, it is a component of the drought tolerant ornamentals of rocky gardens. Research aiming at economic exploitation of this plant under Sudan conditions is almost lacking. In response to the international awareness about the significance of *Aloe vera* in human health and its applications in development of cosmetic products, it is important to study the means of increasing its production and quality. Hence, this study aimed to investigate the impact of urea applications on growth attributes of *Aloe vera*.

Materials and method:

This test was conducted in complete randomized design in the nursery of Sudan University of Science and Technology, at Shambat, Khartoum North, Sudan, for determination of the impact of urea applications on growth attributes of *Aloe vera*. Tillers, 12-15 cm long were planted in 25X30 cm plastic bags containing river Nile sedimentary silt soil (Gureira). A month after establishment, they were used as test plant material. Urea (46% N) treatments were applied in rates of: 0.0, 1.5, 3.0, 4.5 and 6.0 g/plant as soil dressing. Each treatment was replicated 8 times and each plant in a bag was considered a replicate. Urea applications were repeated every 3 months and irrigation was applied according to need. Final data were collected after 12 months for number of leaves, leaf length, leaf width, leaf thickness, number of tillers, number of roots, root length, shoot fresh and dry weights, root fresh and dry weights, leaf fresh weight, leaf gel weight, leaf peel weight, and chlorophyll 'a' and 'b' contents. For dry weights, the harvested shoots and roots were subjected to sun drying for 30 days, prior to oven drying for a week at 70° C until weights were constant. For determination of leaf gel content, the leaves were cut into several portions with scalpel blades to ease gel extraction after weighing the leaves. The remaining peels were weighed separately with a portable digital balance. Determination of chlorophyll content was performed according to the method of Arnon (1949) by using the chlorophyll flourometer (Li-Cor, Lincoln, NE, USA). Two hundred milligrams of fresh leaf samples were ground with 10 ml of 80% acetone at 4^{o}C and centrifuged at 2500 rpm for 10 minutes at 4^{o}C. Three milliliters aliquots of the

extract were transferred to a cuvette and the absorbance was read at 665 and 649 nm with spectrophotometer after which the chlorophyll (a) and (b) were determined by Vernon's models. The collected data were subjected to analysis of variance and means were separated by Duncan's Multiple Range Tests with the aid of MstatC computer program.

Results:

According to Table (1) no significant differences were observed among treatments in number of leaves. The leaf length was not significantly different between the control and urea treatments 1.5- 4.5 g/plant, although the value of the control was slightly higher. However, the highest dose of urea resulted in significant decrease in leaf length compared to the control (Table 1). The leaf width was equally increased by the different urea treatments with significant difference from the control (Table 1). On the other hand, the results of leaf thickness showed no significant differences between the treatments and the control (Table 1).

Significant differences were observed among treatments in the number of tillers/plant. The highest values were recorded for the 1.5 g urea treatment. Above that, the 3 g urea treated tillers showed no significant difference from the control. However, the 4.5 and 6 g urea treatments were inferior compared to the control (Table 2). The 1.5 g urea treatment resulted in increase in the number of roots significantly compared to other treatments. With increase in urea level the number of roots decreased steadily (Table 2). However, the least root length resulted from the 1.5 and 6 g urea treatments, while the other urea treatments performed similar to the control and ranked top (Table 2).

Table (3) illustrates the impact of urea treatment on fresh and dry weights of shoots and roots. Except the 3 g/plant urea treatment that was equal to the control, the other urea levels resulted in significant decrease in shoot fresh and dry weights. The 1.5 g urea treatment resulted in significant increase in the root fresh weight compared to other treatments except the 3 g urea treatment. Again the 1.5 g urea treatment increased root dry weight significantly over the 4.5 and 6 g urea treatment but without difference from the control and the 3 g urea treatment.

Table (4) demonstrates the impact of urea treatments on leaf fresh weight, gel weight, peel weight and chlorophyll content. The highest leaf fresh weight was obtained from the

1.5 g urea treatment followed by the 3 g treatment. Regarding gel weight, the 1.5 g urea treatment also excelled other treatments that performed alike with significant decrease compared to the control. The 1.5 urea treatment also resulted in significant increase in peel weight compared to other treatment except the 3 g treatment. The 1.5 g urea treatment also increased leaf chlorophyll 'a'content significantly compared to other treatments. The highest urea dose resulted in the least chlorophyll 'a'content. However, chlorophyll 'b' content was equally enhanced by the 1.5- 4.5 g urea treatments with significant difference from the other treatments.

Discussion:

Fertilizers are sources of plant nutrients that can be added to the soil or foliarly applied to maintain optimum productivity. There is a usually dramatic improvement in both quantity and quality of plant growth when appropriate fertilizers are added (Sakakibara, *et al*., 2006). According to Hernández-Cruz, *et al.* (2002), nitrogen is one of the nutrients elements that often limit growth of most plants and the need for nitrogen varies from plant to plant. Mengel and Kirkby (1987) reported that, inadequate level of nitrogen shortens plant life cycle, and decrease economic yield. Toth, *et al*., (2002) reported that nitrogen is one of the most important elements of the chlorophyll structure and low rate of photosynthesis under conditions of nitrogen limitation can too often be attributed to the reduction of chlorophyll content. Besides, they observed that, the application of nitrogen increased the chlorophyll content in leaves of the *Aloe vera* plants and the highest levels of chlorophyll 'a' and 'b' were obtained in the highest levels of nitrogen. Soil fertility management may be one of the strategies to increase yield of *Aloe vera* (Hasanuzzaman, *et al*., 2008). Urea is an accessible source of nitrogen which is the basic unit for amino acids and subsequently the biosynthesis of proteins. However, the results of this study revealed the benefits of urea addition to the potting soil of *Aloe vera* plants at low concentration as it enhanced most of growth attributes significantly. The results are in line with preceding findings as it had been reported that the application of N fertilizer enhanced the growth and yields of *A. vera* (Khandelwal *et al*., 2009; Van Schaik *et al*., 1997). Besides, Hazrati, (2012), reported that the application of nitrogen had positive effect on the growth and aloin concentration of *Aloe vera* plants. Nevertheless, the result is in contrast with that of Babatunde and Yongabi (2008) who observed an

increase in growth parameters of *Aloe vera* plants with increase in N levels. The river Nile sedimentary silt soil used in this study seems fertile enough to meet the nutritional requirement of the plants without need for higher urea concentrations. The increase in tillers obtained by the low urea level is advantageous for propagators as tillers are the major propagation means of this plant. The increase in gel weight by the lowest urea treatment means increase in marketable yield. This enhancement may owe to the increase in chlorophyll content of leaves. This result is supported by the findings of Nahed and Aziz, (2007) who stated that, the unique role of nitrogen in photosynthesis and other important physiological processes had been related to increase in chlorophyll content, and the indirect effect on enhanced yield and concentration of aloin which is the active ingredient in *Aloe* v*era* plant. Similarly, Hazrati, (2012) showed that, the nitrogen increased growth, yield and aloin concentration in *Aloe vera* plants. The increments obtained in other parameters in this study were also supported by the findings of preceding studies. As the leaf is an important factor in yield determining in *Aloe vera* plants (Tawaraya, *et al.,* (2007), Eshun and He, 2005), showed that, shoot fresh weight of *Aloe vera* was increased by application of nitrogen in the range of 0.5-1.0 g/ plant. Besides, Hazrati, (2012), reported that, the volume of leaf increased as a result of increase in length and thickness of leaves upon nitrogen application to *Aloe vera* plants. Ji-Dong, *et al*., (2006) reported that, the nitrogen application increased leaf fresh weight and total biomass. Inasmuch as, Hernández-Cruz, *et al*., 2002), showed that, the yield of aloe gel was better with a low frequency of watering and a adequate amount of fertilizer. **In conclusion** the results of this study confirmed the need of *Aloe vera* plants to nitrogen nutrition for enhanced growth attributes and gel yield. Besides, the encouraging results of this study elucidated an economical potential for possible large scale production of the plant under Sudan conditions. Yet, further biochemical studies are needed to determine the impact of nitrogen levels on active ingredients contents, which are the critical quality determining factors.

References:

Arnon, DI. **(1949).** Enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris. Plant Physiol*ogy, 24: 1-15.

Babatunde, F. E. and Yongabi, K.A. **(2008).** Effect of N on productivity of *Aloe barbadensis* L. and its inhibitory effect on *Trichophyton rubrum*. *Advances in Horticultural Science*, 22 (3): 187-190.

Bradley, P.R. (1992). *British Herbal Compendium*, British Herbal Medicine Association: Vol. .l, Bournemouth, UK.

Danhof, I.E. (1987). Remarkable Aloe; Aloe Through the Ages. Omnimedicus Press. Grand Prairie, TX.

Eshun, K. and He, Q. (2005). *Aloe vera*: A valuable ingredient for the food, pharmaceutical and cosmetic industries a review. *Critical Reviews of Food Science and Nutrition*, 44: 91-96.

Grindlay, D. and Reynolds, T. (1986). The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. *Journal of Ethnopharmacology*, 16: 117–151.

Hazrati, S. (2012). Effects of various levels of N on productivity of *Aloe barbadensis* L. and its inhibitory effect on *Trichophyton rubrum*. *Advances in Horticultural Science*, 24 (4): 187-190.

Hamman, J.H. **(2008).** Composition and Applications of *Aloe vera* Leaf Gel. *Molecules*, 13: 1599-1616.

Hasanuzzaman, M., Ahamed, K.U., Khalequzzaman, K.M., Shamsuzzaman, A.M.M. and Nahar, K. (2008). Plant characteristics, growth and leaf yield of (*Aloe vera* L.) as affected by organic manure in pot culture. *Australian Journal of Crop Science*, 2 (3): 158-163.

Heber, D. (2007). Physicians' Desk Reference for Herbal Medicines. 4th.ed. p. (114-123) Thomson; Montvale, N.J.

Hernández-Cruz, L.R., Rodríguez-García R, Rodríguez, D.J. and Angulo-Sánchez, J.L. (2002). *Aloe vera r*esponse to plastic mulch and N. In Janik, J. and Whipkey A. (Eds). *Trends in New Crops and New Uses*, ASHS Press. Alexandria; VA, EEUU. pp. 570-574.

Ji-Dong, W., Zaho-pu, L., Qing-song, Z., Ling, L. and Feng-zhi, P. **(2006).** Effects of different N levels on seedling growth, nitrates and its secondary metabolites in *Aloe vera* seedlings. *Plant Nutrition and Fertilizer Science*, 12(6): 864-868.

Khandelwal, S.K., Meenakshi, J., Choudhary, M.R. and Gupta, K.N. (2009). Effect of N and spacing on growth and yield of Indian aloe (*Aloe barbadensis* L.). *Journal of Medicinal and Aromatic Plant Science*,31(3): 203-205.

Mengel, K. and Kirkby, E.A. (1987), *Principles of Plant Nutrition*. 4th. ed.: International Potash Institute, Bern; pp 687.

Nahed, G. and Aziz, E. (2007). Stimuatory effect of NPK fertilizer and benzyladenine on growth and chemical constituents of *Aloe vera* and *Codiaeum variegatum* L. Plant. *American-Eurasian Journal of Environmental Science*, 2(6): 711-719.

Ni, Y., Turner, D., Yates, K.M. and Tizard, I. (2004). Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. *International Immunopharmacology*, 4(14): 1745-1755.

Park, Y. and Lee, S. (2006). *New Perspectives on Aloe*. Springer Verlag, New York.

Reynolds, T. **(2004)**. Aloe chemistry. In Reynolds T (Ed). *Aloes: the genus Aloe*, CRC Press, Boca Raton. Florida, USA, pp. 39-74.

Sakakibara, H., Takei, K. and Hirose, N. (2006). Interactions between N and cytokinin in the regulation of metabolism and development. *Trends in Plant Sci*ence, 11: 440-448.

Tawaraya, K., Turjaman, M. and Ekamawanti, H.A. (2007). Effect of arbuscular mycorrhizal colonization on nitrogen and phosphorus uptake and growth of *Aloe vera* L. *Journal of Horticultural Science*, 42 (7):1737–1739.

Toth, V.R., Meszkaros, I., Veres, S. and Nagy, J. (2002). Effects of the available N on the photosynthetic activity and xanthophyll cycle pool of maize in field. *Journal of Plant Physiology*, 159: 627-634.

Van Schaik, H.S., Struik, P.C. and Damian, G. (1997). Effects of irrigation and N on the vegetative growth of *Aloe barbadensis Mill. Tropical Agriculture*, 74 (2): 104-109.

Table 1: Impact of urea levels on number, length, width and thickness of leaves of *Aloe vera* L. plants.

Urea level (g/plant)	No. of leaves	Leaf length (cm)	Leaf width (mm)	Leaf thickness (cm)
0.0	14.6 a	41.10a	3.10 b	0.94 a
1.5	14.8 a	38.36ab	4.12a	1.06 a
3.0	15.0 a	38.34ab	3.76a	1.10 a
4.5	14.4 a	40.21ab	4.02a	1.16 a
6.0	14.8 a	36.36b	4.12a	1.04 a
C.V.	8.14	9.20	10.79	19.10

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limit according to DMRT.

Table 2: Impact of urea levels on number of tillers, number and length of roots of *Aloe vera* L plants

Urea level (g/plant)	No. of tillers per plant	No. of roots per plant	Root length (cm)
0.0	13.8b	24.8bc	21.08a
1.5	17.6a	36.8a	14.88b
3.0	13.2b	28.0b	21.48a
4.5	10.8c	27.8b	21.48a
6.0	10.6c	22.4c	14.82b
C.V.	13.39	12.22	10.84

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limit according to DMRT.

Table 3: Impacts of urea levels on shoot and roots fresh and dry weights of *Aloe vera* L. plants.

Urea level (g/plant)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
0.0	738.4a	73.96a	29.54b	2.98ab
1.5	632.8b	63.34b	33.96a	3.36a
3.0	777.3a	77.66a	31.40ab	3.12ab
4.5	527.2c	52.76c	27.82b	2.78b
6.0	558.8c	55.98c	20.40c	2.02c
C.V.	4.93	4.57	11.37	11.12

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limit according to DMRT.

Table 4: Impacts of urea levels on leaf, gel and peel weights and chlorophyll 'a' and 'b' contents of *Aloe vera* L. plants

Urea level (g/plant)	Leaf fresh weight (g)	Gel weight (g)	Peel Wt (g)	Chlorophyll (mg/g) a	b
0.0	196.2c	113.0 b	082.2c	17.96b	08.24 b
1.5	228.2a	131.2 a	103.8a	24.38a	12.66 a
3.0	211.2b	111.0 b	100.2ab	19.64b	12.12 a
4.5	188.0c	101.6 b	089.6bc	18.58b	10.96 a
6.0	171.8d	085.8 c	079.4 c	11.76c	07.34 b
C.V.	5.16	8.25	10.16	10.84	18.24

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limit according to DMRT.

Chapter Five

Response of *Aloe vera* plants to phosphorous nutrition

Abstract:

This study aimed to study the impact of phosphorus applications at various rates on growth attributes of *A.vera* plants under nursery conditions, at Shambat, Khartoum North- Sudan. Triple super phosphate (46% P_2O_5) was tested at 0, 2.5, 5, 7.5 and 10 g/plant as soil dressings in 25X30 cm plastic bags containing (Gureira). The complete randomized design was used and each treatment was replicated eight times. Data were collected 12 months after commence of the test. The analysis revealed a general increase in growth parameters in P-treated plants compared to the control. Except for the peel weight, the highest values of all growth parameters were obtained from the 10 g P treatment. The improvements in growth and gel content are indicators of the benefit of P application.

Key words: phosphorus, growth, chlorophyll, gel content.

Introduction:

Aloe vera is one of approximately 300 species of the genus *Aloe*, which is variously classified as belonging to the Asphodelaceae, Liliaceae, or Aloaceae families (Dagne, *et al*., 2006, Hasanuzzaman, *et al*., 2008; Reynolds, 2004). Commonly known as *Aloe barbadensis* Miller, its legitimate name according to the international rules of botanical nomenclature is *Aloe vera* L. (Grindlay and Reynolds, 1986). The geographic origin of *Aloe vera* is believed to be in Sudan, with the plant subsequently being introduced in the Mediterranean regions and most other warm areas of the world (Grindlay and Reynolds, 1986). This perennial succulent is drought tolerant and can thrive well under xerophytic conditions (Heber, 2007). It was officially listed as a purgative and skin protectant by U.S. pharmacopoeia in 1930 (Park and Lee, 2006). The two major products of *Aloe vera* are yellow latex and clear gel, which are obtained from the large parenchymatic cells of the leaf (Ni, *et al*., 2004). The main constituents of the latex are anthraquinones (Bradley, 1992). The most important anthraquinones are aloin and emodin which are anti-bacterial, anti-viral, anti-fungal, anti-diabetic and analgesic (Eshun and He, 2005; Hamman, 2008;

Reynolds, 2004 and Steenkamp, *et. al.*, 2007). The uses of *Aloe vera* are diverse in Sudan. In ethno medicine it is used for skin moisturing, treatment of burns and a purgative especially for injuries in diabetic patients. Moreover, it is among the drought tolerant ornamentals used in rocky gardens.

The exploitation of the potential of the plant is increasing with growth of cosmetic and neutraceutical markets (Danhof, 1987). According to Ndhlala, *et al.,* (2016), the global market of aloe products is currently estimated to be USD 13 billion and it has appeared in more than 50 different product categories globally since 2012. Research aiming to the economic exploitation of this plant under Sudan conditions is almost lacking. In response to the international awareness about the significance of *Aloe vera* in human health and its applications in development of cosmetic products, it is important to study the means of increasing its production and quality. Hence, this study aimed to investigate the impact of phosphorous applications on growth attributes of *Aloe vera* plants.

Materials and methods:

This test was conducted in complete randomized design in the nursery of Sudan University of Science and Technology, at Shambat, Khartoum North, Sudan, for determination of the impact of phosphorous applications on growth attributes of *Aloe vera*. Tillers, 12-15 cm long were planted in 25X30 cm plastic bags containing river Nile sedimentary silt soil (Gureira). A month after establishment, they were used as test plant material. Triple super phosphate (46% p_2o_5) was tested in rates of: 0.0, 2.5, 5.0, 7.5 and 10.0 g/plant as soil dressing. Each treatment was replicated 8 times and each plant in a bag was considered a replicate. All tested plants received a dose of 2 g urea once at the beginning of the study. The P applications were repeated every 3 months and irrigation was applied according to need. Final data were collected after 12 months for number of leaves, leaf length, leaf width, leaf thickness, number of tillers, number of roots, root length, shoot fresh and dry weights, root fresh and dry weights, leaf fresh weight, leaf gel weight, leaf peel weight, and chlorophyll 'a' and 'b' contents. For dry weights, the harvested shoots and roots were subjected to sun drying for 30 days, prior to oven drying for a week at 70° C until weights were constant. For determination of leaf gel content, the leaves were cut into several portions with scalpel blades to ease gel extraction after weighing the leaves. The remaining peels were weighed separately with a portable digital

balance. Determination of chlorophyll content was performed according to the method of Arnon (1949) by using the chlorophyll flourometer (Li-Cor, Lincoln, NE, USA). Two hundred milligrams of fresh leaf samples were ground with 10 ml of 80% acetone at 4^{o}C and centrifuged at 2500 rpm for 10 minutes at 4^{o}C. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 665 and 649 nm with spectrophotometer after which the chlorophyll (a) and (b) were determined by Vernon's models. The collected data were subjected to analysis of variance and means were separated by Duncan's Multiple Range Tests with the aid of MstatC computer program.

Results:

The number of leaves was equally affected by various levels of phosphorus with significant increase compared to the control (Table 1). All P treatment resulted in significant increase in leaf length compared to the control. The 10 g P treatment ranked top with significant difference from other P treatments that shared a second position (Table 1). Likewise, all P treatments excelled the control for leaf width. The highest dose of P ranked top but without significant difference from the 7.5 g P treatment (Table 1). Regarding leaf thickness, all P treatment increased this parameter significantly compared to the control. The highest value was recorded for the 10 g P treatment without significant difference from the 7.5 and 5 g P treatments (Table 1).

According to Table (2), the number of tillers was significantly increased by all P treatments. The highest values were equally obtained from the 7.5 and 10 g P treatments. The number of roots and root length were affected by the different P treatments in a similar pattern. The 7.5 and 10 g P treatments shared the top rank with significant difference from other treatments, while the 5 g treatment ranked second with significant difference from the control (Table 2).

The shoot fresh weight was significantly enhanced by P treatments except the 2.5 g P treatment that resembled the control (Table 3). The shoot dry weight was also increased significantly by all P treatments, and the increase was positively correlated to dose increase. The root fresh weight was enhanced by all P treatments. The higher the dose, the higher the increase, but the difference between the 2.5 and 5 g P treatments was not significant. Similarly, the root dry weight was significantly increased by P treatments compared to the control and the increase was progressive with increase of P dose (Table 3).

According to Table (4), progressive increase was obtained in leaf fresh weight with increase of P dose and all P levels increased this parameter significantly compared to the control. The same pattern was obtained for gel weight. However, the highest peel weight resulted from the 5 g P treatment with significant difference from all treatments. Leaf chlorophyll 'a' content was significantly increased by all P treatments. The 10 g P treatment almost tripled this parameter in comparison to the control. As for chlorophyll 'b' content, the 5, the 7.5 and the 10 g P treatments performed equally and induced significant increase over the control which equaled the 2.5 g P treatment statistically.

Discussion:

Fertilizers are sources of plant nutrients that can be added to the soil or foliarly applied to maintain optimum productivity. There are usually dramatic improvements in both quantity and quality of plant growth when appropriate fertilizers are added (Sakakibara, *et al*., 2006). Phosphorous is a vital element in plant biochemistry. It is a structural component of nucleic acids, phosphor-lipids and co-enzymes and it functions in energy transfer via the phosphate bond in ATP. Phosphate groups attached to different sugars provide energy in respiration, photosynthesis while phosphate bound to proteins regulate their activities (George *et al.,* (2008). According to (Saradhi, *et al*., 2007), the primary function of phosphorous is the storage and transfer of energy through the plant and adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are high-energy phosphate compounds that control most processes in plants including photosynthesis, respiration, protein and nucleic acid synthesis, and nutrient transport through plant cells. The results of this study revealed improvements in growth attributes upon phosphorous application and the best results were obtained with the highest dose of P. The result is in

agreement with the findings of Nematian, *et al*., (2011), who reported that, Aloe plants treated with various levels of phosphorus fertilizer, showed significant increase in number of leaves and the growth of all P treated plants was better than that of the control. Besides, Luis, *et al*, (2002), reported enhancements in the number of leaves, leaf fresh weight, leaf and root length and leaf diameter of *aloe vera* plants with the higher phosphorus application rates. Similar results were also reported by Boroomand, *et al*., (2011) who reported that, the application of P fertilizer enhanced the growth attributes of *Aloe vera* plants. The results are also matching those of Kumar, *et al*. (2002), Babatunde and Yongabi (2008) who observed an increase in growth parameters of *Aloe vera* plants with increase in P levels. However, the results of this study revealed the benefits of phosphorus addition to the potting soil of *Aloe vera* plants as it enhanced most of growth attributes significantly. The river Nile sedimentary silt soil used in this study seems deficient to meet the ultimate P nutritional requirement of the plants, and therefore plant growth increase with increase in P concentration. The increase in number of tiller upon P application is advantageous for propagators as tillers are the conventional means of the vegetative propagation of aloes. The increase in gel weight with increase in P dose means an enhanced marketable yield. The enhanced gel yield may owe to the increase of chlorophyll content of leaves in P treated plants. This result is supported by Faryabi and Ghazanchi (2005) who reported that, the higher doses of phosphorus significantly increased the fresh leaf weight, aloin content and chlorophyll content in *Aloe vera* plants. Inasmuch as, Harendra and Yadav (2007) reported significant increase in the gel content in P treated *Aloe vera* plants. In conclusion, the results of this study indicated the need of *Aloe vera* plants to phosphorus nutrition for enhanced quantitative growth attributes. Yet, further biochemical studies are needed to determine the impact of phosphorus levels on active ingredients contents, which are the critical quality determining factors.

References:

Arnon, DI. (1949). Enzymes in isolated chloroplast. Polyphenoloxidase in *beta vulgaris. Plant. Physiol*., 24: 1-15.

Babatunde, F. E. and Yongabi, K.A. (2008). Effect of P on productivity of *Aloe barbadensis* L. and its inhibitory effect on *Trichophyton rubrum*. *Adv. Hort. Sci*., 22 (3): 187-190.

Boroomand, N., Nakhaei, M. and Sadat, H.G.M. (2011). Effect of potassium and phosphorous on growth and yield of *Aloe Vera* L. *7th Iranian Congress of Horticultural Science;* Isfahan, Iran. Pp. 375-381.

Bradley, P.R. (1992). British Herbal Compendium, *British Herbal Medicine Association:* Vol. l: 122- 127, Bournemouth, UK.

Dagne, E., Bisrat, D., Viljoen, A. and Van Wyk B.E. (2006). *Chemistry of Aloe species.* Curr Org Chem.; 4th ed. pp (72–78).

Danhof, I.E. (1987). Remarkable Aloe; Aloe Through the Ages. *Omnimedicus Press*, Grand Prairie, TX. Pp 720- 729.

Eshun, K. and He, Q. (2005). *Aloe vera*: A valuable ingredient for the food, pharmaceutical and cosmetic industries a review. *Crit. Rev. Food Sci. Nutr*., 44: 91-96.

Faryabi, A. and Ghazanchi, R. (2005). Study of the effect of chemical fertilizers, compost and their combination on *Aloe vera* growth and yield. *Science and Research Branch of Islamic Azad University Publications*, Iran. Vol. (5)2nd ed.(654- 660).

George, E.F., Hall, M.A. and Deklerk, G. (2008). *Plant Propagation by Tissue Culture*, Vol. (1). 3rd ed., Springer, Dordrecht, Netherlands.

Grindlay, D. and Reynolds, T. (1986). The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. *J. Ethnopharmacol*, 16:115–117.

Hamman, J.H. **(2008).** Composition and applications of *Aloe vera* leaf gel. *Molecules*, 13: 1599-1616.

Harendra, K. and Yadav, D.S. (2007). Effect of phosphorus and sulphur levels on growth, yield and quality of Indian mustard (*Brassica juncea*) cultivars. *Ind. J. Agron*., 52(2): 154-157.

Hasanuzzaman, M., Ahamed, K.U., Khalequzzaman, K.M., Shamsuzzaman, A.M.M. and Nahar, K. (2008). Plant characteristics, growth and leaf yield of (*Aloe vera* L.) as affected by organic manure in pot culture. *Aust. J. Crop. Sci*., 2(3): 158-163.

Heber, D. **(2007).** *Physicians' Desk Reference for Herbal Medicines*. 4th. ed. p. (114-123), Thomson, Montvale, N.J.

Kumar, P., Adityasharma, S.P.K. and Hord, D.K. **(2002).** Integrated nutrient management in mudgaparni (*Phaseolus trilobus*). *Extended summaries, 2nd Int. Agron. Cong*., New Delhi, pp. 317- 318.

Luis, H.C., Rodolfo, R.G., Ranl, J.R., and Jose, A.S. (2002). *Trends in New Crops and New Uses,* vol. 1, 3rd. ed., pp (125-131).

Ndhlala, A. R., du Plooy, C. P. and Abdelgadir, H. A. **(2016).** The potential of *Aloes* as medicine and cosmetic ingredients in South Africa. Abstracts of workshop: (*Linking Sudanese Medicinal and Aromatic Plants Producers to the Global Markets*), Coral Hotel, Khartoum, Sudan, 22-24 March, 2016. Pp. 25.

Nematian, A., Ghoushchi, F., Farnia, A., Arianpour, A. and Mashhadi, A. and Boujar, M.(2011). An anti-complementary poly-saccharide with immunological adjuvant activity from the leaf parenchyma gel of *Aloe vera*. *Iranian Journal of Medical and Aromatic Plants*, 27 (2), (347-388).

Ni, Y., Turner, D., Yates, K.M. and Tizard, I. (2004). Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. *Int. Immunopharmacol*, 4(14): 45- 55.
Park, Y. and Lee, S. (2006). *New Perspectives on Aloe*: Springer Verlag, New York. 2 (12): 174-175.

Reynolds, T. (2004). Aloe Chemistry. In: Reynolds, T. (ed). Aloes: the Genus *Aloe*, *CRC Press*, Boca Raton. Florida, USA, pp. 39-74.

Sakakibara, H., Takei, K. and Hirose, N. (2006). Interactions between N, P and cytokinin in the regulation of metabolism and development. *Trends Plant Sci*., 11: 440-448.

Saradhi, V.S.P., Khanam, S., Shivananda, B.G., Vasantha, K.T. and Shivananda, T.N. (2007). Effect of NPK fertilizers on chemical constituents of *Aloe vera* leaves. *J. Nat. Rem*., 7(2): 258-262.

Steenkamp, V. and Stewart, M.J. (2007). Medicinal applications and toxicological activities of *Aloe* products. *Pharmaceutical Biology,* 45(5): 411-420.

Table 1: Impact of phosphorous levels on number of leaves, leaf length, width and thickness of (*Aloe vera* L.) plants

P levels (g/ plant)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)
00.0	14.0b	34.64c	3.72d	0.72c
02.5	17.2a	39.82b	4.32cd	1.08b
05.0	18.0a	40.14b	4.60bc	1.14ab
07.5	17.8a	41.02b	5.02ab	1.18ab
10.0	18.8a	44.80a	5.50a	1.36a
C.V.	6.23	4.33	10.05	17.41

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 2: Impact of phosphorous levels on number of tillers, root number and root length of (*Aloe vera* L.) plants.

P levels (g/ plant)	No. of tillers	No. of roots	Root length (cm)
00.0	19.0 d	26.8c	18.4 c
02.5	21.8c	28.4bc	20.8bc
0 5.0	24.8b	33.2b	22.2 b
07.5	27.6a	38.4a	25.6 a
10.0	27.6a	40.6a	26.1 a
C.V.	6.77	11.44	8.52

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 3: Impact of phosphorous levels on shoot fresh and dry weights, root fresh and dry weights of (*Aloe vera* L.) plants

P levels (g/ plant)	Shoot fwt (g)	Shoot dwt (g)	Root fwt (g)	Root dwt (g)
00.0	337.3 d	23.71 e	15.56 d	1.546 d
02.5	365.0 d	36.34 d	19.04 c	1.894 c
05.0	456.3 c	45.82 c	20.54 c	2.096 bc
07.5	862.1 b	86.35 b	23.04 b	2.294 b
10.0	956.4 a	95.6 0 a	27.94 a	2.856 a
C.V.	9.12	9.46	6.49	8.06

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 4: Impact of phosphorous levels on leaf fresh weight, gel and peel weights and chlorophyll 'a' and 'b' contents of (*Aloe vera* L.) plants

P levels (g/ plant)	Leaf fwt (g)	Gel wt (g)	Peel wt (g)	Chlorophyll (mg/g) a	b
00.0	215.4d	101.2 d	114.2 c	08.16d	08.04b
02.5	233.6c	112.8 c	120.8 bc	19.70c	08.72b
05.0	258.2b	126.4 b	132.0 a	21.74b	10.94a
07.5	258.6ab	134.8 ab	123.4 b	22.32b	11.10a
10.0	263.6a	140.2 a	123.4 b	24.10a	11.48a
C.V.	1.63	5.85	4.94	5.72	10.92

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Chapter Six

Influence of NPK on growth and yield of *Aloe vera* plants

Abstract:

This study aimed to investigate the impact of the application of NPK fertilizer at various rates on growth and gel yield of *Aloe vera* plants under nursery conditions, at Shambat, Khartoum North, Sudan. Commercial NPK (15-10-15) was tested at 0, 2.5, 5, 7.5 and 10 g/plant as soil dressings on 12-15 cm long tillers of the plant grown in 25X30 cm plastic bags containing (Gureira). The study was conducted in a complete randomized design where each treatment was replicated 8 times. Data were collected 12 months after commence of the study. The analysis revealed no significant differences in parameters of number of leaf and root length, while limited enhancement was observed in other parameters in NPK-treated plants compared to the control. Except for the number of leaves and the root length, the highest values of growth parameters were obtained from the 2.5 g NPK treatment. The improvements in growth and gel content at the low rates of the fertilizer are indicators of the benefit of NPK application.

Key words: *Aloe vera*; NPK fertilizer; Growth; Chlorophyll; Gel content.

Introduction:

The genus *Aloe* (family Liliaceae) consists of at least 600 known species, many of which have been used as botanical medicines in many countries for centuries (Okamura *et al.*, 1996). Species of *Aloe* which have been used as folk medicine includes: Curacao Aloe (*Aloe barbadensis* or *Aloe vera*), Cape Aloe (*Aloe ferox*), and Socotra Aloe (*Aloe perryi*). *Aloe vera* is perennial, succulent evergreen plant of the family Liliaceae (Omidbeigi *et al.*, 2009). The geographic origin of *Aloe vera* was believed to be in Sudan, with the plant subsequently being introduced into the Mediterranean regions and most other warm areas of the world (Grindlay and Reynolds, 1986). This perennial succulent plant is drought tolerant and can thrive well under xerophytic conditions (Heber, 2007).The useful parts of the plant are leaves which contain the gel. This plant species can be easily propagated from tillers and is probably the most widely cultivated species of the genus in the world. It is widely used in world, not only as a folk remedy for gastrointestinal complaints, skin injuries and burns, but also as an ingredient in health foods and cosmetics (Capasso *et al.*, 1998). It is beneficial in curing wounds, scorches, blisters; and it has shown to have some

effects on AIDS, cancers and diabetes (Yazdani *et al*. 2006). The gel of *Aloe vera* contains an anthraquinone compound, which is why this gel is important in traditional medicine. The most important Active ingredients in aloe are in the group of anthraquinone glycosides. Aloe emodin, aloenoside and aloein are among these compounds (Faryabi and Ghazanchi 2005). Aloe gel, among other things, enhances immunity, improves liver function, prevents asthma and has antiinflammatory, anti-ulcerous, anti-diabetic and antihypertensive properties (Dagne *et al*., 2000). Besides, epidemiological data suggest that, the intake of *A. vera* gel prevents human lung cancer (Sakai, 1989). Regarding the increasing significance of *Aloe vera* in human health and its application in development of new food and cosmetic products, it is important to study the methods of increasing its yield and quality.

The cultivation of *A. vera* has acquired great commercial importance for medicinal products and cosmetics processing but information are scarce about agronomic management of this crop (Nasrin *et al*., 2013). Cultivation of *A. vera* is expanding day by day in the world as it provides quick and regular income to the farmers. Fertility management in *A. vera* field may be one of the strategies for increasing of the yield of *A. vera* (Saha *et al*., 2005).

The role of macro and micro nutrient elements in various metabolic and biochemical processes in plant is well recognized and subsequently used in plant nutrition to enhance rapid growth changes to increase the productivity of various crop species. The compound fertilizer 'NPK' is composed of 3 essential macro-nutrient elements which are generally required in high concentrations by plants due to their diverse roles in plant structure and physiology and are growth limiting factors. In frequent cases, growth and yield responses upon use of compound fertilizers had been reported better in various crops than the use of a nutritional regime based on a single element of the compound (Faryabi and Ghazanchi 2004). The exploitation of the potential of the plant is increasing with growth of cosmetic and neutraceutical markets (Danhof, 1987). According to Ndhlala *et al.,* (2016), the global market of aloe products is currently estimated to be USD 13 billion and it has appeared in more than 50 different product categories globally since 2012. Research aiming to the economic exploitation of this plant under Sudan conditions is almost lacking. In response to the international awareness about the significance of *Aloe vera*

plant in human health and its applications in development of cosmetic products, it is important to study the means of increasing its production and quality. Hence, this study aimed to investigate the impact of fertilizing the *Aloe vera* plants with different rates of the compound fertilizer (NPK) on their growth and gel yield.

Materials and methods:

This study was conducted in the nursery of Sudan University of Science and Technology, at Shambat, Khartoum North, Sudan. *Aloe vera* tillers, 12-15 cm long, were planted in 25X30 cm plastic bags containing river Nile sedimentary silt soil (Gureira). A month after establishment, they were used as test plant material. Commercial NPK (15-10-15) fertilizer was tested in rates of: 0.0, 2.5, 5.0, 7.5 and 10.0 g/plant as soil dressing. Each treatment was replicated 8 times and each plant in a plastic bag was considered a replicate. All tested plants received a dose of 2 g urea once at the beginning of the study. The NPK applications were repeated every 3 months and irrigation was applied according to need. Treatments were arranged in randomized complete block design. Final data were collected 12 months after commence of applications for number of leaves, leaf length, width and thickness, number of tillers, number of roots, root length, shoot and root fresh and dry weights, leaf weight, leaf gel weight, leaf peel weight, and chlorophyll 'a' and 'b' contents. For dry weights, the harvested shoots and roots were subjected to sun drying for 30 days, prior to oven drying for a week at 70° C until weights were constant. For determination of leaf gel content, the leaves were cut into several portions with scalpel blades to ease gel extraction after weighing the leaves. The remaining peels were weighed separately with a portable digital balance. Determination of chlorophyll content was performed according to the method of Arnon (1949) by using the chlorophyll flourometer (Li-Cor, Lincoln, NE, USA). Two hundred milligrams of fresh leaf samples were ground with 10 ml of 80% acetone at 4^{o}C and centrifuged at 2500 rpm for 10 minutes at 4^{o}C. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 665 and 649 nm with spectrophotometer after which the chlorophyll (a) and (b) were determined by Vernon's models. The collected data were subjected to analysis of variance and means were separated by Duncan's Multiple Range Tests with the aid of MstatC computer program.

Results:

Result of the impact of NPK applications on number of leaves, leaf length, width and thickness of *Aloe vera* plants is presented in Table (1). The number of leaves was not affected by NPK treatments. The longest leaves were obtained from the control, while NPK treatments resulted in lesser values with successive decrease upon the increase of NPK dose. However, the 2.5 and 5 g/plant NPK doses did not differ significantly from the control. The best leaf width was recorded for the 2.5 g/plant NPK treatment that ranked top without significant difference from the control, but with significant difference from the other treatments. The increases in NPK doses resulted in decreases of this parameter. Likewise, the best leaf thickness was obtained from the 2.5 g/plant NPK treatment with significant difference from the 7.5 and 10 g/plant NPK treatments, but without significant difference from the control and the 5 g treatments.

Data regarding the impact of NPK treatments on the number of tillers, number of roots and root length of *Aloe vera* plants are shown in Table (2). The 2.5 g of NPK per plant enhanced the formation of tillers significantly compared to other treatments. The 5 and 7.5 g treatments also excelled the control for this parameter, while the 10 g NPK treatment equaled the control and shared the bottom rank for number of tiller. Data revealed a general decrease in the number of roots with the application of NPK. The best number of roots was achieved from the control which was significantly different from all NPK treatments except the 2.5 g dose. However, regarding root length, no significant difference was obtained from NPK applications which were statistically equal to the control.

Results regarding the impact of NPK treatments on fresh and dry weights of shoots and roots of *Aloe vera* plants are illustrated in Table (3). The control and the 2.5 g NPK treatments ranked top with significant difference from the other treatment for the fresh and dry weights of the shoot, beside the root dry weight. The best root fresh weight was obtained from the control with significant difference from all NPK treatments.

Table (4) demonstrates the impact of NPK on leaf, gel and peel weights beside leaf chlorophyll content of *Aloe vera* plants. The highest leaf weight was recorded for the 2.5 and 5 g NPK treatments with significant difference from the other treatments. The gel was increased significantly by the 2.5 g treatment that ranked top, while the 5 g treatment

ranked second with significant difference from the other treatments. The control, the 7.5 and 10 g treatments resulted in the least peel weight, while the 2.5 g treatment ranked top for this parameter. The highest chlorophyll 'a' content resulted from the 2.5 g, followed by the 5 g NPK treatment and the least values were recorded for the control and the 10 g treatments that shared the bottom rank. Regarding chlorophyll 'b' content, the 2.5 and the 5 g treatments resulted in the highest values with significant difference from other treatments while the control and the 10 g treatments recorded the least values and were lower than other treatments.

Discussion:

Fertilizers are sources of nutrients that can be added to the soil or foliarly to plants to maintain optimum growth and productivity. Their application is generally followed by considerable improvements in growth and yield of different plant species (Sakakibara, *et al.*, 2006). However, the results of this study revealed no or limited gains in growth attributes from the various levels of NPK, while the yield was enhanced upon treating *Aloe vera* plants with low levels of the fertilizer. Except for the number of tillers, this result is contradictory to the findings of Nematian, *et al.*, (2011), who reported significant increases in number of leaves, tillers and leaf length in NPK treated *Aloe vera* plants. The result also differ from those reported by Boroomand, *et al.*, (2012) who obtained enhanced growth attributes of *Aloe vera* plants treated with NPK. However, this result is in agreement with Faryabi and Ghazanchi (2005) who reported that, the higher doses of NPK significantly decreased the leaf weight, aloin and chlorophyll contents in *Aloe vera* plants. It is worthy to recognize the benefit of the low NPK dose (2.5 g) that matched the control in most measured growth parameters and excelled it in gel and chlorophyll contents. This result might be indicative to a positive linkage between gel yield and high chlorophyll content. The enhanced formation of tillers obtained from the low dose (2.5 g) is advantageous for propagators. The use of a low fertilizer dose that satisfies optimum growth and yield is of economical advantage. In conclusion, the results of this study are indicative to the possibility of a large scale production of *Aloe vera* plants under Sudan conditions with very low fertilizer input.

REFERENCES:

Arnon, D.I. (1949). Enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris. Plant Physiology,* 24: 1-15.

Boroomand, N., Nakhaei, M. and Sadat, H.G.M. (2012). Effect of NPK on growth and yield of *Aloe Vera* L. *8th Iranian Congress of Horticultural Science;* Isfahan, Iran. 7(5): 75-81.

Capasso, F., Mascolo, N., Autore, G. and Duraccio, M.R. (1998). Effect of indomethacin on aloin and 1.8 dioxianthraquinone-induced productions of prostaglandins in rat isolated colon. Prostaglandins, 26: 57–62.

Dagne, E., Bisrat, D., Viljoen, A., Van Wyk, B.E. (2006). *Chemistry of Aloe species.* Current Organic Chemistry, 4:1055–78.

Danhof, I.E. (1987). Remarkable Aloe: Aloe through the Ages. Grand Prairie, TX: *Omnimedicus Press.*

Faryabi, A. and Ghazanchi, R. (2005). Study of the effect of chemical fertilizers, compost and their combination on *Aloe vera* growth and yield, *Science and Research Branch of Islamic Azad University Publications,* Iran.

Faryabi, A. and Ghazanchi, R. (2004). Study of the effect of chemical fertilizers and their combinations on Aloe vera growth and gel yield, *Science and Research Branch of Islamic Azad University Publications,* Iran.

Grindlay, D., Reynolds, T. (1986). The Aloe vera phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. Journal of Ethnopharmacology, 16:117–51.

Heber, D. (2007). Physicians' Desk Reference for Herbal Medicines. 4th. Montvale, N.J., Thomson.

Nasrin, A. H., Ahmad, G. and Jafar Mohammadi1 (2013). The effect of organic fertilizers and organic wastes on *Aloe vera* growth and development. *Annuals of Biological Research,* 4 (8): 90-95.

Ndhlala, A. R., du Plooy, C. P. and Abdelgadir, H. A. (2016). The potential of *Aloes* as medicine and cosmetic ingredients in South Africa. Abstracts of workshop: (*Linking Sudanese Medicinal and Aromatic Plants Producers to the Global Markets*), Coral Hotel, Khartoum, Sudan, 22-24 March, 2016. Pp. 25.

Nematian, A., Ghoushchi, F., Farnia, A., Arianpour, A. and Mashhadi Akbar Boujar, M. (2011). Effect of NPK fertilizer on growth characters of *Aloe vera* plants. *Iranian Journal of Medical and Aromatic Plants,* 27 (2), 347-388.

Okamura, P., Adityasharma, S.P.K. and Hord, D.K. (2009). Integrated nutrient management in mudgaparni *(Phaseolus trilobus*). *Extended summaries, 2nd Int. Agron. Cong.*, New Delhi, pp. 117- 128.

Omidbeigi, R. (2009). Production and Processing of Medicinal Plants, vol. 4, *Astan-e-Ghods Publications*, Iran.

Reynolds, T. (2004). Aloe chemistry. In Reynolds T. (Ed). *Aloes: the genus Aloe, CRC Press*, Boca Raton. Florida, USA, pp. 39-74.

Saha, S., Fekri, M., Mahmoudabadi, M. and Boroomand, N. (2011). Effect of inorganic fertilizers on Aloe vera leaf gel content. *Iranian Journal of Medicinal Plants*, 3: 11-18.

Sakakibara, H., Takei, K., Hirose, N. (2006). Interactions between N and cytokinin in the regulation of metabolism and development. *Trends Plant Sci*., 11: 440-448.

Sakai, M.C., Ranl, R.G., Diana, J.R. and Jose, A.S. (1989). *Trends in New Crops and New Uses*, 1: 125-131.

Yazdani, D., Rezaei, M.B., Kianbakht, S. and Khosravani, S. (2006). A valuable ingredient for the food, pharmaceutical and cosmetic industries. *Iranian Journal of Medicinal Plants*, 5 (19): 1-8.

Table 1: Impact of NPK levels on number of leaves, leaf length, leaf width and leaf thickness of (*Aloe vera* L.) plants

NPK levels (g/plant)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)
00.0	16.0a	39.0a	4.80ab	1.18ab
02.5	17.6a	38.2ab	5.54a	1.30a
05.0	17.2a	37.2ab	4.66b	1.14ab
07.5	16.0a	35.9b	3.78c	0.94bc
10.0	16.2a	32.3c	3.68c	0.72c
C.V.	8.19	6.47	13.24	19.38

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 2: Impact of NPK levels on number of tillers, number of roots and root length of *Aloe vera* plants.

NPK levels (g/plant)	No. of tillers	No. of roots	Root length (cm)
00.0	15.8d	39.6a	20.6a
02.5	27.8a	34.4ab	20.6a
05.0	21.0b	30.4b	18.4a
07.5	19.2c	30.2b	20.9a
10.0	17.2d	31.0b	20.1a
C.V.	6.78	12.31	10.30

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 3: Impact of NPK levels on *Aloe vera* shoot fresh and dry weights, root fresh and dry weights.

NPK levels (g/plant)	**Shoot fwt (g)**	**Shoot dwt (g)**	**Root fwt (g)**	**Root dwt (g)**
00.0	954.5a	97.68a	25.71a	2.58a
02.5	947.1a	94.82a	22.76b	2.65a
05.0	568.9b	56.70c	17.04c	1.68b
07.5	624.3b	64.34b	19.57c	1.95b
10.0	587.7b	66.72b	12.59d	1.25c
C.V.	6.07	5.08	10.42	11.08

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 4: Impact of NPK levels on *Aloe vera* leaf fresh weight, gel weight, peel weight and chlorophyll content

NPK levels (g/plant)	**Leaf wt** (g)	**Gel Wt** (g)	**Peel Wt** (g)	**Chlorophyll** (mg/g) a	b
00.0	203.2c	103.2d	100.0c	11.48d	09.08b
02.5	263.4a	136.8a	126.6a	23.40a	12.60a
05.0	249.4a	130.8b	118.6b	20.54b	11.84a
07.5	220.2b	117.4c	102.8c	18.00c	09.19b
10.0	194.4c	100.6d	099.8c	12.44d	07.22c
C.V.	5.89	10.02	11.14	11.0	13.98

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Chapter Seven

Influence of sulfur fertilizer on growth and yield of *Aloe vera* plants

Abstract:

This study aimed to investigate the influence of sulfur fertilizer on growth and yield of *Aloe vera* plants under nursery conditions, at Shambat, Khartoum North, Sudan. The experimental materials were tillers of the plant 12-15 cm long, planted in 25X30 cm plastic bags containing river Nile sedimentary soil (Gureira). Prior to test, each plant received a dose of 2 g urea. Elemental sulfur was tested as soil application to the potting media at rates of 0.0, 0.5, 1.0, 2.0 and 4.0 g/plant. The study was accomplished in complete randomized design and each treatment was replicated 8 times. Treatments were repeated every 3 month. Data were collected 12 months after the commencement of the study. Data analysis revealed a general increase in growth parameters in sulfur treated plants compared to the control. Except for the peel weight and chlorophyll content, the highest values of measured parameters were obtained from the 4.0 g sulfur treatment / plant. Under the conditions of the study, these results elucidated the benefit of sulfur fertilizer as a tool for enhanced production of *Aloe vera*.

Key words: *Aloe vera;* Sulfur; Growth; Chlorophyll; Gel content.

Introduction:

Aloes are xerophytes in the *Aloeaceae* family that are cultivated for ornamental, medicinal, vegetable, and cosmetic purposes in Africa, North America, Europe, and Southeast Asia (Tawaraya, *et al.*, 2007). Approximately 500 species had been described in the genus *Aloe*, ranging from diminutive shrubs to large tree-like forms and it is represented in several biodiversity hotspots (Zapata, *et al.*, 2013). Basically, all the *Aloe* species have similar constituents; however, *Aloe barbadensis* Miller (often called *Aloe vera* L.) and *Aloe arborescens* Miller are the most extensively cultivated in the world (Liao, *et al*., 2006). The chemical composition of the leaf gel is very complex, composed mainly of polysaccharides and soluble sugars followed by proteins, many of which are enzymes, amino acids, vitamins, and anthraquinones (Liu, *et al*., 2007). It had been shown that polysaccharides derived from *Aloe vera* enhance immunity activity and exert

antioxidant effects (Zhanhai, *et al.*, 2009) and most of the activity was attributed to β-polysaccharides (Ramachandra and Srinivasa Rao, 2008). Anthraquinones are the second class of bioactive metabolites, including C-glucosyl derivatives such as barbaloin (10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9–10H-anthracenone), a mixture of the two diastereoisomers aloin A and B, as well as glucose-free compounds such as aloe-emodin (Fanali, *et al*., 2010). Anthraquinones were reported to have cathartic effects, anti-inflammatory effects *in vivo* as well as anti- (bacterial, viral and cancer) effects (Park, *et al.*, 2009; Pellizzoni, *et al.*, 2012). The demand for the aloe products is increasing with growth of cosmetic and neutraceutical markets (Danhof, 1987). According to Ndhlala, *et al.*, (2016), the global market of aloe products is currently estimated to be USD 13 billion and it has appeared in more than 50 categories of products since 2012. Research aiming to the economic exploitation of this plant under Sudan conditions is almost lacking, but badly needed.

Sulfur is among the essential macro nutrient elements that limit plant growth. Its deficiency has deleterious effects on plant growth. In *Aloe vera* plants, sulfur deficiency resulted in reduced leaf size, retarded growth and caused chlorosis (Ergle, *et al.,* 2005). The functional chloroplasts are normally rich in sulfur (Hanson, *et al*., 2011) and the chloroplast morphology is considerably affected by sulfur deficiency (Hall, *et al.,* 2002; Repica, *et al*., 2001). According to Pirson (1955), sulfur deficiency upsets photosynthesis in a profound way which, after readdition of external sulfate, can only be corrected slowly through the synthesis of new protein and chloroplasts. However, beneficial responses to sulfur fertilization had been reported in lime (*Citrus aurantifolia* Swingle) (Idris *et al.*, 2012) and banana (*Musa* AAA) (Idris *et al.*, 2014) under Sudan conditions. Based on these considerations, this study aimed to investigate the influence of sulfur fertilizer on growth and yield of *Aloe vera* plants.

Materials and methods:

This test was conducted in complete randomized design in the nursery of Sudan University of Science and Technology, at Shambat, Khartoum North, Sudan, to determine the impact of sulfur fertilization on growth attributes and yield of *Aloe vera* plants. Tillers, 12-15 cm long were planted in 25X30 cm plastic bags containing river Nile sedimentary silt soil (Gureira). A month after establishment, they were used as test

plant material. Elemental sulfur was tested in rates of: 0.0, 0.5, 1.0, 2.0 and 4.0 g/plant as soil dressings in 25X30 cm plastic bags. Each treatment was replicated 8 times and each plant in a bag was considered a replicate. All tested plants received a dose of 2 g urea once, at the beginning of the study. The S applications were repeated every 3 months and irrigation was applied according to need. Final data were collected after 12 months for number of leaves, leaf length, leaf width, leaf thickness, number of tillers, number of roots, root length, shoot fresh and dry weights, root fresh and dry weights, leaf weight, leaf gel weight, leaf peel weight, and chlorophyll 'a' and 'b' contents. For dry weights, the harvested shoots and roots were subjected to sun drying for 30 days, prior to oven drying for a week at 70° C until weights were constant. For determination of leaf gel content, the leaves were cut into several portions with scalpel blades to ease gel extraction after weighing the leaves. The remaining peels were weighed separately with a portable digital balance. Determination of chlorophyll content was performed according to the method of Arnon (1949) by using the chlorophyll flourometer (Li-Cor, Lincoln, NE, USA). Two hundred milligrams of fresh leaf samples were ground with 10 ml of 80% acetone at 4^{o}C and centrifuged at 2500 rpm for 10 minutes at 4^{o}C. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 665 and 649 nm with spectrophotometer after which the chlorophyll (a) and (b) were determined by Vernon's models. The collected data were subjected to analysis of variance and means were separated by Duncan's Multiple Range Tests with the aid of MstatC computer program.

Results:

The result of the influence of sulfur fertilizer treatments on number, length, width and thickness of leaves is indicated in Table 1. Compared to the control, no significant differences were observed for number of leaves, leaf width and leaf thickness. However, longest leaves were obtained from the 4 g sulfur treatment with significant difference from the 2 g sulfur treatment, but without significant difference from the other treatments.

Table (2) presents the results of the impact of sulfur fertilizer treatments on number of tillers, number of roots and root length. The number of tillers was best increased by the 4

g sulfur treatment, with significant difference from the control and the 0.5 g sulfur treatments, while the other treatments ranked intermediate. All sulfur treatments increased the number of roots and root length significantly in comparison to the control. The highest values for number of roots were recorded for the 4 and 2 g sulfur treatments which were significantly higher than the 1.0 and 0.5 g sulfur treatments. Likewise, all sulfur treatments increased root length significantly compared to the control. The 4 g treatment was best although it did not differ significantly from the other sulfur treatments. Results regarding the impact of sulfur applications on shoot and root fresh and dry weights are compiled in Table 3. Except the shoot fresh weight, all sulfur treatments enhanced these parameters significantly compared to the control. The 4.0 g treatment resulted in best increase in shoot fresh and dry weights. The increase was significant compared to other treatments. The 2 g sulfur treatment enhanced root fresh and dry weights significantly and ranked top, sharing this position with the 4 g sulfur treatment for the root dry weight.

The statistical analysis of data concerning leaf, gel and peel weights and chlorophyll content is illustrated in Table 4. Except for the peel weight, all sulfur treatments enhanced these parameters significantly compared to the control. The 4 g sulfur treatment resulted in best leaf and gel weights but without significant difference from the 1.0 and 2.0 g treatments for the gel weight. The peel weight decreased in all sulfur treatments significantly compared to the control and the least value was recorded for the 4 g sulfur treatment. The highest chlorophyll 'a' and 'b' contents were recorded for the 0.5 g sulfur treatment. However, the least value for chlorophyll 'a' was obtained from the control, while the least chlorophyll 'b' content was obtained from the 4 g sulfur treatment.

Discussion:

Manipulation of target compounds in plants such as phytochemicals and antioxidants by the management of the mineral nutrition had been recognized as a research area attracting applied scientists interest (Fallovo, *et al*., 2009a, 2009b; Fanasca, *et al*., 2006a, 2006b). Proper management of the fertilization and the use of suitable irrigation water can provide an effective tool to improve the target compounds in plants such as phytochemicals and antioxidants (Rouphael, *et al.*, 2012a). Sulfur is one of the most

essential macro-nutrient minerals that play different structural and physiological roles in plant. It is a constituent of some amino acids such as cysteine, cysteine, and methionine and thus contributes to protein synthesis and buildup of plant mass. In this study the *Aloe vera* plant responded positively to the low doses of sulfur fertilizer as reflected in most measured growth and yield parameters. This growth and yield enhancements obtained from sulfur application in this study is in line with preceding research findings as Ross (2005) reported positive effects from sulfur application on the growth and gel content of *Aloe vera* plants. The result also agreed with the findings of Korikanthimath (1994) and Lalitha (2004), who reported significant increase in number of leaves and other growth parameters in sulfur treated *Aloe vera* plants compared to the control. The improvements obtained from sulfur applications might also be interpreted by efficient utilization of nutrient elements as suggested by Kumar and Singh (1994), who attributed low yield of onions under conditions of sulfur deficiency to poor utilization of macro and micronutrients.

According to Minard, (1978), sulfur is often regarded a limiting factor for biomass production in natural ecosystems. Besides, Korikanthimath (1994) reported that inadequate level of sulfur prolongs the life cycle of *Aloe vera* plant, delays maturity and decreases its economical yield. The improvements obtained in this study might be interpreted by efficient uptake and metabolism of nitrogen under conditions of sulfur availability. According to Nasreen *et al.,* (2005), absorption of nitrogen is disrupted in soils deficient in sulfur. Improved growth upon use of a combination of nitrogen and sulfur fertilizers had been reported by Smriti *et al.,* (2002) for onion, Idris *et al.,* (2012) for lime and Idris *et al.,* (2014) for banana. Regarding chlorophyll content, the application of sulfur at 0.5-1.0 g /plant was associated with increases in both chlorophyll ‘a’ and ‘b’ compared to the control. This result is partially contradictory to the findings of Hanson, *et al,* (2011), who reported increase in leaf chlorophyll content with sulfur application, but the increase was steady with the increase of sulfur dose. This might be explained by differences in soils sulfur content. Nevertheless, the 4 g/plant sulfur treatment was found most enhancive for leaf weight and gel content which are the most important yield parameters of this plant and the result is matching that of Idris *et al.,* (2014) and Salih (2013) who reported growth enhancements in banana propagules

planted in Gureira soil under the same conditions of this study they found that, application of sulfur increased *Aloe vera* leaf chlorophyll content with the raise in level and they concluded that, leaf chlorophyll content is directly correlated with sulfur rate. The results of this study disagreed with the findings of Hanson, *et al.*, (2011) and Zheng *et al.,* (2009) who claimed a positive correlation between sulfur concentration and leaf chlorophyll content; the disagreement may owe to differences in soils used in the two studies. The enhanced formation of tillers due to sulfur treatments is an advantage to propagators as tillers are the major propagation means of the plant. The enhanced gel formation by 1-4 g sulfur treatments is a privilege as the gel is the economically valued harvest, and therefore the 1 g sulfur treatment might seem more attractive on cost reduction bases. In conclusion, regarding commercial production of *Aloe vera* under Sudan conditions, the overall results of this study may seem encouraging enough for adoption of a pilot project to found the basis for future expansions. The sulfur nutrition should be accompanied as a cultural due to its significant impact on growth and gel yield of the plant. Besides, the positive result obtained here from sulfur fertilization, fortifies the limited preceding reports on the positive impact of sulfur fertilization and may justify further research on sulfur nutrition of other plant genera and species under Sudan conditions.

References:

Arnon, D.I. (1949). Enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris. Plant. Physiol*., 24: 1-15.

Danhof, I.E. (1987). Remarkable Aloe: Aloe Through the Ages. Grand Prairie, TX: *Omnimedicus Press.*

Ergle, D. R., And, M. and Eaton (2005). Sulphur nutrition of *Aloe vera* plants. *Plant Physiology,* 26: 639- 654.

Fallovo, C., Rouphael, Y., Cardarelli, M., Rea, E., Battistelli, A., Colla, G. (2009a). Yield and quality of leafy lettuce in response to nutrient solution composition and growing season. *J. Food Agr. Environ., 7:*456*–462.*

Fallovo, C., Rouphael, Y., Rea, E., Battistelli, A., Colla, G. (2009b) Nutrient solution concentration and growing season affect yield and quality of *Lactuca sativa L. var. acephala* in floating raft culture. *J. Sci. Food Agr., 89:*1682*–1689.*

Fanali, S., Zeineb, A., D'Orazio, G., Rocco, A., Ferranti, A., Mercolini, L., Raggi, M.A. (**2010)**. Analysis of Aloe based phytotherapeutic products by using nano-LC-MS. *J. Sep. Sci., 33:*663*–670.*

Fanasca, S., Colla, G., Maiani, G., Venneria, E., Rouphael, Y., Azzini, E., Saccardo, F. (2006a). Changes in antioxidant content of tomato fruits in response to cultivar and nutrient solution composition. *J. Agr. Food Chem., 54:*4319*–4325.*

Fanasca, S., Colla, G., Rouphael, Y., Saccardo, F., Maiani, G., Venneria, E., Azzini, E. (2006b). Evolution of nutritional value of two tomato genotypes grown in soilless culture as affected by macrocation proportions. *Hort. Science, 41:*1584*–1588.*

Hanson, E. A., Barrien, B. S. and Wood, J. G. **(2011).** Relations between protein-nitrogen, protein-sulfur, and chlorophyll in leaves of Sudan grass. *Aust. J. Exp. Biol*., 19: 231-234.

Idris, T. I.M., Samia E.S. and Farouq H.E. (2014). Response of micro propagated banana to fertilization. *Submitted to Sudan Journal of science and technology*, (March2014).

Idris, T.I.M., Abdelazim A.K. and Mohamed A.H. **(2012).** Growth and yield responses of a dry date palm *(Phoenix dactylifera* L.) cultivar to foliar and soil fertilizers. *Intl. Research Journal of agriculture and soil science*, 2(9): 390-394.

Ismael, H. S.E. (2013). Influence of argel, micro-elements and growth regulators on flowering and fruit set of Mango. *A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Horticulture.*

Korikanthimath, V.S. (1994). Nutrition of cardamom. In: *Advances in Horticulture* (Eds.) Chadha, K.L. and Rethinam, P. *Malhotra Publishing House*, New Delhi, 9(1): 467-476.

Kumar, A., Singh, O. (1994). Role of sulphur in nutrient utilization and catalase activity in onion crop. *Indian Journal of Agricultural Research,* 28:15-19

Lalitha, S.G. and Gopala, R.P. (2004). Influence of plant population and sulfur levels on growth characters and seed yield of sunflower (*Helianthus annus* L.). *The Andhra Agric. J.,* 51(3&4): 281-283.

Liao, H.M., Sheng, X., Hu, Z.H. (2006). Ultra structural studies on the process of aloin production and accumulation in *Aloe arborescens* (*Asphodelaceae*) leaves. *Bot. J. Linn. Soc., 150:*241*–247.*

Liu, C.H., Wang, C.H., Xu, Z.Y., Wang, Y. (2007). Isolation, chemical characterization and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with sea water. *Process Biochem., 42:*61*–970.*

Minard, H.R.G. (1978). Effect of clove size, spacing, fertilizer and lime on yield and nutrient contents of garlic (*Allium Sativum* L) Newzeland. J. *Exp. Agric*., 6: 139-143.

Miranda, M., Maureira, H., Rodríguez, K., Vega-Galvez, A. **(2009*).*** Influence of temperature on the drying kinetics, physicochemical properties, and antioxidant capacity of *Aloe vera* (*Aloe barbadensis* Miller) gel. *J. Food Eng., 91:*297*–304.*

Nasreen S., Haq O. and Dhalyn S.M.I. (2005). Effect of sulphur fertilization on yield, sulphur content and uptake by onion. Ind. *Journal of Agricultural Research,* 39:122-127

Ndhlala A. R., du Plooy, C. P. and Abdelgadir, H. A. (2016). The potential of *Aloes* as medicine and cosmetic ingredients in South Africa. Abstracts of workshop: (*Linking Sudanese Medicinal and Aromatic Plants Producers to the Global Markets*), Coral Hotel, Khartoum, Sudan, 22-24 March, 2016. Pp. 25.

Park M.Y., Kwon, H.J., Sung, M.K. (2009). Evaluation of aloin and aloe-emodin as antiinflammatory agents in aloe by using murine macrophages. *Biosci. Biotechnol. Biochem., 73:*828*–832.*

Pellizzoni, M., Ruzickova, G., Kalhotka, L., Lucini, L. **(2012).** Antimicrobial activity of different *Aloe barbadensis Mill. and Aloe arborescens* Mill. Leaf fractions. *J. Med. Plant Res., 6:*1975*–1981*.

Ramachandra, C.T., Srinivasa, Rao, P. (2008). *Processing of Aloe vera leaf gel: A review. American Journal of Agricultural Biological Science, 3:*502*–510.*

Repica, J., M., Saric, J., Marek, and Zima, A. (1971). Effects of macro element deficiency on structure of chloroplast and productivity of photosynthesis in maize plants. *Soviet Plant Physiol*. 18: 938-943.

Ross, A.I. (2005). Medicinal plants of the world. (eds). Chemical *Constituents*.

Rouphael, Y., Cardarelli, M., Bassal, A., Leonardi, C., Giuffrida, F., Colla, G. **(2012a).** Vegetable quality as affected by genetic, agronomic and environmental factors. *J. Food Agr. Environ., 10:*680*–688.*

Rouphael, Y., Cardarelli, M., Lucini, L., Rea, E., Colla, G. (2012b). Nutrient solution concentration affects growth, mineral composition, phenolic acids and flavonoids in leaves of artichoke and cardoon. *Hort. Science, 47:*1424*–1429.*

Salih, S.E.M. (2013). Response of micro-propagated banana to fertilizers. *M.Sc. Thesis, Sudan University of Science and Technology.*

Smriti, S., Kumar, R., Singh, S.K. (2002). Effect of sulphur and boron nutrition on growth, yield and quality of onion (*Allium cepa* L.). *Journal of Applied Biology*, 12(1/2): 40-46.

Tawaraya, K., Turjaman, M. and Ekamawanti, H.A. **(2007).** Effect of arbuscular mycorrhizal colonization on nitrogen, sulfur and phosphorus uptake and growth of *Aloe vera* L. *HortScience*, 42 (7):1737–1739.

Zapata, P.J., Navarro, D., Guillen, F., Castillo, S., Martinez-Romero, D., Valero, D., Serrano, M. (*2013*). Characterization of gels from different *Aloe* spp. as antifungal treatment: Potential crops for industrial applications. *Ind. Crops Production, 42:*223*–230.*

Zhanhai, Y., Jin, C., Xin, M., Jian, M.H. (2009). Effect of *Aloe vera* polysaccharides on immunity and antioxidant activities in oral ulcer animal models. *Carb. Pol., 75:*307–*311*.

Zheng, Q., Liu, L., Liu, Z., Chen, J., Zhao, G. (2009). Comparison of the response of ion distribution in the tissues and cells of the succulent plants *Aloe vera* and *Salicornia europaea* to saline stress. *J. Plant Nutr. Soil Sci., 172:*875–*883.*

Table 1: Impact of sulfur applications on leaf number, length, width and thickness of *Aloe vera*

Sulfur levels (g/plant)	**No. of leaves**	**Leaf length** (cm)	**Leaf width** (cm)	**Leaf thickness** (cm)
0.0	15.4a	37.20ab	4.02a	0.88a
0.5	17.0a	38.90ab	4.25a	1.08a
1.0	16.0a	39.54ab	4.26a	1.02a
2.0	15.8a	36.02b	4.40a	1.04a
4.0	16.8a	41.00a	3.30a	1.10a
C.V.	7.87	8.65	25.11	18.70

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 2: Impact of sulfur applications on number of tillers and roots and root length of *Aloe vera* plants.

Sulfur levels (g/plant)	**No. of tillers**	**No. of roots**	**Root length** (cm)
0.0	19.8b	26.2c	19.0c
0.5	19.8b	29.4b	22.5ab
1.0	22.0ab	29.4b	22.4ab
2.0	22.0ab	35.4a	21.4ab
4.0	23.6a	36.4a	25.2a
C.V.	12.27	10.87	11.70

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 3: Impact of sulfur applications on shoot and root fresh and dry weights of *Aloe vera* plants.

Sulfur levels (g/plant)	**Shoot fwt** (g)	**Shoot dwt** (g)	**Root fwt** (g)	**Root dwt** (g)
0.0	654.2d	065.12d	15.77d	1.562c
0.5	753.0c	075.10c	23.89bc	2.310b
1.0	807.4b	080.74bc	21.83c	2.290b
2.0	657.2d	084.06b	26.90a	2.670a
4.0	913.2a	132.00a	24.46b	2.660 a
C.V.	3.58	5.41	8.19	8.83

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 4: Impact of sulfur applications on leaf, gel and peel weights and chlorophyll content of *Aloe vera* plants.

Sulfur levels (g/plant)	**Leaf Wt** (g)	**Gel Wt** (g)	**Peel Wt** (g)	**Chlorophyll (mg/g)** a	b
0.0	212.8d	103.0c	109.8a	14.10d	10.08c
0.5	245.4c	141.2b	104.2b	25.06a	13.06a
1.0	258.8b	155.4ab	103.8b	21.58b	11.48b
2.0	258.9b	156.0ab	097.8c	17.69c	10.60c
4.0	267.0a	166.0a	094.4c	19.93b	08.06d
C.V.	3.46	7.34	5.21	6.98	6.81

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Chapter Eight

Response of *Aloe vera* plants to foliar applications of 6-benzyladenine (BA)

Abstract:

Aloe vera is progressively gaining importance in global markets due to its numerous uses as a base in preparations of medicines, cosmetics and food supplements and therefore research effort is needed in Sudan to explore possible factors that may qualify for its production at commercial level. This study aimed to investigate the possibilities of growth and yield enhancements of *Aloe vera* plants upon their treatment with foliar applications of 6-Benzyladenine (BA) under nursery conditions, at Shambat, Khartoum North, Sudan. BA concentrations 0.0, 75,150, 300 and 600 ppm were tested on 12-15 cm Aloe tillers planted in 25X30 cm plastic bags containing (Gureira). The complete randomized design was used with eight replicates. The applications were repeated every 3 months and data were collected 12 months after commencement of the test. The analysis revealed a general increase in growth and yield parameters in BA-treated plants compared to the control. Except for the root fresh weight, the highest values of all growth parameters were obtained from the 600 ppm BA treatment. The enhanced growth attributes and gel yield obtained from BA applications in this study, might put this growth regulator among success supporting factors for large scale production of the plant under Sudan conditions.

Key words: *Aloe vera*; 6-Benzyladenine; Foliar; Growth; Chlorophyll; Gel content.

Introduction:

6-Benzylaminopurine, 'benzyl adenine' or '"BA"' is a first-generation of synthetic cytokinins that elicits plant growth and development responses, setting blossoms and stimulating fruit richness by stimulating cell division. It is an enzyme inhibitor (inhibitor of respiratory kinase) in plants, and increases post-harvest life of green vegetables (Siddiqui, *et al.,* 2011).

Aloe vera (Liliaceae) is a succulent plant indigenous to Northern Africa and Mediterranean countries and has become naturalized almost in all parts of India (Klein, *et al.* 1988). The plant has stiff gray-green lance-shaped leaves containing clear gel in a central mucilaginous pulp. *Aloe vera* has been used for several thousands of years in folk

medicine in many cultures from ancient Egypt, Greece, and Rome to China and India (Kemper and Chiou 1999). Some of the most important pharmacological activities of *Aloe vera* are antiseptic (Capasso, *et al.* 1998), anti- tumor (Winter, *et al.* 1981), anti-inflammatory (Yagi, *et al.* 1998), wound and burn healing effect (Heggers, *et al.* 1993), anti diabetic (Rajasekaran, *et al.* 2006) and as an adjunct to current AIDS therapy (Mc Daniel, *et al.* 1990).

Aloe vera propagates vegetatively in its natural state. However, propagation rate in some varieties is very slow because a single plant can produce only seven to nine lateral shoots in a year. Moreover, the production of *Aloe* leaves is insufficient to meet the industry demand in India (Aggarwal and Barna 2004) and the production of cosmetics, foods and pharmaceuticals containing *Aloe vera* has experienced a slow increase due to limited availability of raw material with high quality (Campestrin, *et al.* 2006).

Aloe vera L. is an economically important herbaceous plant from Liliaceae (Reynolds, 2004). The geographic origin is believed to be in Sudan and was subsequently dispersed in the tropical, subtropical and Mediterranean regions (Grindlay and Reynolds, 1986). It is a succulent perennial that can thrive well under xerophytic conditions (Heber, 2007). It was officially listed as a purgative and skin protectant by the American Pharmacopoeia in 1930 (Park and Lee, 2006). The major products of *Aloe vera* are the latex and the gel, which are obtained from the storage tissues of the leaf (Ni, *et al*., 2004). The main constituents of the latex are anthraquinones (Bradley, 2002). Aloin and emodin are the most important anthraquinones having anti- (bacterial, viral, fungal, diabetic) and analgesic properties (Hamman, 2008; Steenkamp *et. al.*, 2007; Eshun and He, 2005 and Reynolds, 2004). The uses of *Aloe vera* are diverse in Sudan. In traditional medicine it is used for skin moisturing, treatment of burns and a purgative especially for injuries in diabetic patients (Hasanuzzaman, *et al.*, 2008). Moreover, it is among the drought tolerant ornamentals used in rocky gardens. The exploitation of the potential of the plant is increasing with growth of cosmetic and neutraceutical markets (Danhof, 1987). According to Ndhlala *et al.*, (2016), the global market of aloe products is currently estimated to be USD 13 billion and it has appeared in more than 50 different product categories globally since 2012. In response to the international awareness about the significance of *Aloe vera* in human health and its applications in development of

cosmetic products, it is important to study the means of increasing its production and quality. Nevertheless, research aiming to the economic exploitation of this plant under Sudan conditions is almost lacking.

Cytokinins are important plant hormones that regulate numerous processes of plant growth and development. They influence cell division, differentiation and enhance leaf extension, beside mobilization of nutrients (Shudo, 1994). Zeatin and 2ip are the naturally occurring cytokinins, while 6-benzyladenine (BA) and 6-furfurylaminopurine (kinetin) are the two most pronounced synthetic forms of this group of hormones. However, plant species respond differently to cytokinins. Regarding *Aloe vera* plant, the preceding research reports highlighted the benefits of cytokinins applications on *Aloe vera* plant. According to Khandelwal, *et al*., (2009), the foliar application of BA on this plant was enhancive for growth attributes and gel yield. More-over, Babatunde and Yongabi (2008) observed that, the growth parameters such as number of leaf, leaf length, leaf width, leaf weight and the whole plant weight of Aloe plants increased significantly, by increase in BA rates. In addition, Hazrati, *et al*., (2011) reported that, the gel and peel weights were significantly increased by BA application and the maximum gel and peel weights were observed in plants that received a dose of 1000 ppm. In as much as, Ji-Dong, *et al*. (2006) reported that, the BA application increased leaf weight and total biomass. Hence, the objective of this study was to determine the effects of different levels of BA on the growth attributes and gel concentration of *Aloe vera* plants under nursery conditions in Sudan.

Materials and methods:

This test was conducted in the nursery of Sudan University of Science and Technology, at Shambat, Khartoum North, Sudan, in a complete randomized design for determination of the impact of 6-benzyladenine applications on growth attributes of *Aloe vera*. Tillers, 12-15 cm long were planted in 25X30 cm plastic bags containing river Nile sedimentary silt soil (Gureira). A month after establishment, they were used as test plant material. 6-Benzyladenine was tested in rates of: 0.0, 75, 150, 300 and 600 ppm as foliar application. Each treatment was replicated 8 times and each plant in a bag was considered a replicate. All tested plants received a dose of 2 g urea once at the beginning of the study. The BA applications were repeated every 3 months and irrigation was applied according to need.

Final data were collected after the 12th month for number of leaves, leaf length, width and thickness, number of tillers, number of roots, root length, shoot fresh and dry weights, root fresh and dry weights, leaf weight, leaf gel weight, leaf peel weight, and chlorophyll 'a' and 'b' contents. For dry weights, the harvested shoots and roots were subjected to sun drying for 30 days, prior to oven drying for a week at 70° C until weights were constant. For determination of leaf gel content, the leaves were cut into several portions with scalpel blades to ease gel extraction after weighing the leaves. The remaining peels were weighed separately with a portable digital balance. Determination of chlorophyll content was performed according to the method of Arnon (1949) by using the chlorophyll flourometer (Li-Cor, Lincoln, NE, USA). Two hundred milligrams of fresh leaf samples were ground with 10 ml of 80% acetone at 4ºC and centrifuged at 2500 rpm for 10 minutes at 4ºC. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 665 and 649 nm with spectrophotometer after which the chlorophyll (a) and (b) were determined by Vernon's models. The collected data were subjected to analysis of variance and means were separated by Duncan's Multiple Range Tests with the aid of MstatC computer program.

Results:

The impact of BA foliar applications on number of leaves, leaf length, width and thickness of *Aloe vera* plants is illustrated in Table (1). All BA rates resulted in significant increase in these parameters compared to the control. The number of leaves was equally increased by various rates of BA with significant difference from the control. The highest value of leaf length was obtained from the 600 ppm BA treatment with significant difference from the other BA treatments. The leaf width was also increased by the 600 ppm BA treatment with significant difference from all treatments except the 300 ppm BA treatment. The leaf thickness was also increased significantly by all BA treatments at an equal statistical level except the 150 ppm level that ranked second.

Results of the impact of BA foliar applications on number of tillers, number of roots and root length of *Aloe vera* plants are compiled in Table (2). Except the 75 ppm, all BA treatments increased the number of tillers per plant significantly. The highest value was obtained from the 600 ppm treatment that ranked top without significant difference from

the 300 ppm treatment. The number of roots was not affected by all BA treatments which were statistically equal to the control. The root length was significantly increased by the 600 ppm BA treatment, while the other concentrations were similar to the control.

The impact of BA treatments on shoot fresh and dry weights, root fresh and dry weights of *Aloe vera* plants is demonstrated in Table (3). BA concentrations 150-600 ppm increased shoot fresh weight at an equal statistical level with significant difference from the 75 and 0.0 ppm BA levels. The shoot dry weight was also increased significantly by all BA treatments except the 75 ppm treatment which was not different from the control. On the other hand, the least root fresh weight was obtained from the 600 ppm BA treatment, while the highest values for this parameter were recorded for the control, the 75 and 300 ppm BA treatments. However, the highest root dry weight was recorded for the 300 ppm BA treatment with significant difference from the 150 and 600 ppm BA treatments, but without statistical difference from the control and the 75 ppm BA treatment.

The result of foliar applications of BA on leaf, gel and peel weights and chlorophyll content of *Aloe vera* plants is shown in Table (4). Except for the peel weight and chlorophyll 'b' content, all parameters were significantly enhanced by BA applications compared to the control. The gel weight and chlorophyll 'a'content increased in the same pattern with the increase of BA concentration. The leaf weight also increased with increase of BA concentration with the 600 ppm ranking top, the 300 ppm ranking second while the third position was shared by the 150 and 75 ppm BA treatments. However, the highest peel weight resulted from the 150 ppm treatment without significant difference from the 600 and 75 ppm BA treatments. Leaf chlorophyll 'a' content of the control was almost doubled by the 300 and 600 ppm BA treatments. As for chlorophyll 'b', its content increased progressively with increase of BA concentration and the increase was statistically significant except for the 75 ppm treatment that shared the fourth position with the control.

Discussion:

Cytokinins are important plant hormones that regulate various processes of plant growth and development with cell division and differentiation, enhancement of leaf extension

and nutrient mobilization (Shudo, 1994). Taking in consideration that, Plant species have different responses to Cytokinins, there are usually dramatic improvements in both quantity and quality of plant growth when appropriate fertilizers are added (Sakakibara, *et al*., 2006). It had been reported that, the foliar application of BA enhanced the growth and yields of *Aloe vera* (Khandelwal, *et al*., 2009; Babatunde and Yongabi, 2008); they observed that, the growth parameters of Aloe plants increased significantly, by increase in BA rates. Application of Benzyladenine in *Codiaeum variegatum* plants significantly increased plant height, number of leaves and fresh weight of leaves in comparison to the control plants (Van Schaik, *et al*., 1997). In addition, Hazrati, *et al*., (2011) reported that, the gel and peel weights were significantly enhanced by BA application as the maximum gel and peel weights were observed in the dose of 1000 ppm BA treatment. A leaf of *Aloe vera* is an important factor in yield determining in *A.vera* plant (Eshun and He, 2005), thus the application of foliar BA had significantly increased the yield; similar results were obtained by Khandelwal, *et al*. (2009). On the other hand, Cytokinin can increase division, cell enlargement and distribution of assimilates in the succulent plants and thus cause to better development of the leaves and increase in gel weight (Carey, *et al.*, 2008). Hernández-Cruz, *et al*. (2002) showed that the yield of aloe gel was better with a low frequency of watering and high rates of BA. It was observed that, the BA increased the number of offsets in *Aloe vera* plants that might be due to the apical dominance and stimulation of offsets (Duck, *et al.*, 2004). Davies, (2004) reported that, exogenous application of cytokinin increased the chlorophyll content in the chloroplast and it was observed that the application of N +BA increased the chlorophyll content in leaves of the *Aloe vera* plants. Generally, the highest levels of chlorophyll 'a' and 'b' were obtained in the highest level of BA (Davies, 2004). Therefore, he concluded that, the foliar application of BA increased growth parameters, chlorophyll content and gel concentration in *Aloe vera* plants. Similar results were also obtained by Khandelwal, *et al*. (2009). Ji-Dong, *et al*. (2006) reported that, the BA application in rates of (1000-1500 ppm) increased leaf weight and total biomass.

The results of this study revealed improvements in growth attributes upon BA application and the best results were obtained with the highest dose of BA. The result is in agreement with the findings of (Sakakibara, *et al.*, 2006), who reported that, Aloe plants treated with

various levels of BA, showed significant increase in number of leaves and the growth of all BA treated plants was better than that of the control. Besides, Babatunde and Yongabi (2008), reported enhancements in the number of leaves, leaf weight, leaf and root length and leaf diameter of Aloe vera plants with the higher BA application rates. Similar results were also reported by (Carey, *et al*., 2008) who reported that, the application of BA enhanced the growth attributes of *Aloe vera* plants. The results are also matching those of (Duck, *et al.,* 2004), Babatunde and Yongabi (2008) who observed an increase in growth parameters of *Aloe vera* plants with increase in BA levels. However, the results of this study revealed the benefits of foliar application of BA on the potting plants as it enhanced the growth attributes of *Aloe vera* plants. The enhanced formation of tillers and gel weight due to BA treatments is an advantage to aloe propagators as tillers are the major propagation means of the plant. The increment in gel formation by the high rates of BA treatments is a privilege as the gel is the economically valued harvest of this promising plant, and therefore the 600 ppm BA treatment might seem more attractive on cost reduction bases. In conclusion, regarding commercial production of *Aloe vera* under Sudan conditions, the overall results of this study may seem encouraging enough for adoption of a pilot project to find the basis for future expansions. The BA application should be accompanied as a cultural practice due to its significant impact on growth and gel yield of the plant. Besides, the positive result obtained here from BA application, fortifies the limited preceding reports on the positive impact of BA application and may justify further research on cytokinins and other plant growth promoters on other plant genera and species under Sudan conditions.

REFERENCES:

Aggarwal, D. and Barna, K.S. **(2004).** Tissue culture propagation of elite plant of *Aloe vera* Linn. *J. Plant Biochemistry & Biotechnology*, 13: 77-79.

Arnon, D.I. **(1949).** Enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24: 1-15.

Babatunde, F.E. and Yongabi, K.A. **(2008).** Effect of BA on productivity of *Aloe barbadensis* L. and its inhibitory effect on *Trichophyton rubrum*. *Advances in Horticultural Science*, 22(3): 187-190.

Bradley, P.R. **(1992).** British Herbal Compendium, *British Herbal Medicine Association:* Vol. 1.l. Bournemouth, UK.

Bradley, A., Andresen, L.L., Gundersen, K.E. Malterudb and Risea, F. (2002). Antioxidant Activity of Synthetic Cytokinin analogues: 6-Alkynyl- and 6-Alkenylpurines as novel 15-lipoxygenase inhibitors. *Bio-organic and Med. Chem.*, 10:1581-1586.

Campestrini, L.H., Kuhnen, S., Lemos, P.M.M. Bach, D.B., Dias, P.F. and Maraschin, M. (2006). Cloning protocol of *Aloe vera* as a study-case for "Tailor-Made" biotechnology to small farmers. *Journal of Technology, Management and Innovation*. 1(5):76-79.

Carey, D., Whipker, B., McCall, I. and Buhler, W. (2008). Benzyl adenine foliar sprays increase offsets in *Sempervivum* and *Echeveria*. *J. of Horticultural Science,* 53: 19-21.

Danhof, I..E. (**1987).** Remarkable Aloe: Aloe through the Ages. Grand Prairie, TX: *Omnimedicus Press*.

Davies, P.J. (2004). The plant hormones: their nature, occurrence and function. In Davies, P.J. (Eds). Plant hormones biosynthesis, signal transduction, action, *Kluwer Academic Publishers*, Dordrecht, Boston, London, pp. 471-492.

Duck, M.W., Gregg, B.M., Fernandez, R.T., Royal , D.H. and Cardoso, F.F. (2004). Height control of *Picea spp* and *Chamaecyparis lawsoniana* with uniconazole and 6-benzyladenine. *J. Environ. Hortic*., 22 (3): 165-169.

Eshun, K. and He, Q. (2004). *Aloe vera*: A valuable ingredient for the food, pharmaceutical and cosmetic industries—*A review*. Crit *Rev Food Science and Nutrition*, 44:91–6.

Grindlay, D., Reynolds, T. (1986). The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. *Journal of Ethnopharmacology*, 16:117–51.

Hamman, J.H. (2008). Composition and Applications of *Aloe vera* Leaf Gel. *Molecules*, 13: 1599-1616.

Hasanuzzaman, M., Ahamed, K.U., Khalequzzaman, K.M., Shamsuzzaman and Nahar, K. **(2008).** Plant characteristics, growth and leaf yield of *(Aloe vera* L.) as affected by organic manure in pot culture. *Australian Journal of Crop Science*, 2(3): 158-163.

Hazrati, S. **(2011).** Effect of Various Extracts of Organic Materials and PGR against orchid plantlets Growth Crosses Results on Culture Media. UNS Faculty of agriculture, Semarang. (*Indonesian*).

Heber, D. (2007). Physicians' Desk Reference for Herbal Medicines. 4th. Montvale, NJ: Thomson; 2007.

Heggers, J., Pelley, R. and Robson, M. (1993). Beneficial effects of *Aloe* in wound healing. *Phytotherapy Research.* 7: S48-S52.

Hernández-Cruz, L.R., Rodríguez-García, R., Rodríguez, D.J., Angulo-Sánchez, J.L. (2002). *Aloe vera* Response to Plastic Mulch and N. In En Janik J, Whipkey A (Eds). *Trends in new crops and new uses*, ASHS Press. Alexandria: VA, EEUU, pp. 570-574.

Ji-Dong, W., Zaho-pu, L., Qing-song, Z., Ling, L. and Feng- zhi, P. (2006). Effects of different BA levels on seedling growth, nitrat and its secondary metabolites in Aloe vera seeding. *Plant Nutrition and Fertilizer Science*, 12(6): 864-868.

Kemper, K.J. and Chiou, V. **(1999).** *Aloe vera*: The Longwood Herbal Task Force and The Center for Holistic Pediatric Education and Research. 1-29 pp.

Khandelwal, S.K., Meenakshi, J., Choudhary, M.R. and Gupta, K.N. **(2009).** Effect of BA and spacing on growth and yield of Indian aloe (*Aloe barbadensis* L.). J. *Medicinal and Aromatic Plant Science*, 31(3): 203-205.

Klein, A.D., Penneys, N.S. (1988). *Aloe vera*. *J. Am. Acad. Dermatol*. 18:714-720.

Mc Daniel, H., Carpenter, R., Kemp, M., Kahlon, J. and Mc Analley, B. (1990). Extended survival and prognostic criteria for Acemannan (ACE-M) treated HIV Patients. *Antiviral Res.* Suppl. 1: 117.

McDaniel., J.E. Lewis., H.R. M.E. Agronin., D.A. Loewenstein., J. Riveros., R. Mestre., M. Martinez., N. Colina., D. Abreu., J. Konefal., J.M. Woolger and K.H. Ali. (2012). The effect of an Aloe polymannose multinutrient complex on cognitive and immune functioning in Alzheimer's disease. *Journal of Alzheimer's Disease*, 33(2) 393-406.

Ndhlala A. R., du Plooy, C. P. and Abdelgadir, H. A. (2016). The potential of *Aloes* as medicine and cosmetic ingredients in South Africa. Abstracts of workshop: (*Linking Sudanese Medicinal and Aromatic Plants Producers to the Global Markets*), Coral Hotel, Khartoum, Sudan, 22-24 March, 2016. Pp. 25.

Ni, Y., Turner, D., Yates, K.M. and Tizard, I. (2004). Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. *International immunopharmacology*, 4:1745–55.

Park, Y. and Lee, S. (2006). New Perspectives on Aloe. New York: Springer Verlag.

Rajasekaran, S., Sivagnanam, K. and Subramanian, S. (2006). Modulator effects of *Aloe vera* leaf gel extract on oxidative stress in rats treated with streptozotocin. *J. pharm. and pharmacol*ogy, 57 (2): 241-246.

Reynolds, T. **(2004).** Aloe Chemistry. In Reynolds T. (Ed). *Aloes: the genus Aloe*, *CRC Press*, Boca Raton. Florida, USA, pp. 39-74.

Sakakibara, H., Takei, K. and Hirose, N. (2006). Interactions between N and cytokinin in the regulation of metabolism and development. Trends Plant Science*;* 11: 440-448.

Shudo, K. (1994). Chemistry of Phenylurea cytokinins. In Mokk DV, Mok Mc (Ed) Cytokinins: Chemistry, activity and function, *CRC Press*, Boca Raton, pp. 35-42.

Siddigi, R., Taiz, L. and Zeiger, E. **(2011).** Plant physiology. 4th Edition. *Sinauer Associates*. Sunderland, pp: 690 - 691.

Steenkamp, V., Stewart, M.J. **(2007).** Medicinal applications and toxicological activities of Aloe products. Pharm Biol., 45:411–20.

Van Schaik,S., Struik, P.C. and Damian, G. (1997). Effects of irrigation and BA on the vegetative growth of *Aloe barbadensis* Mill. Trop. Agric., 74(2): 104-109.

Winter, G., Thomas, J., Mandal, A.K.A., Raj Kumar, R. and Chordia, A. (1981). Role of biologically active amino acid formulations on quality and crop productivity of tea (*Camellia* sp.). *International Journal of Agricultural Research,* 4: 228 – 236.

Yagi, A., Tsunoda, M., Egusa, T., Akasaki, K. and Tsuji, H. (1998). Immunochemical distinction of *Aloe vera, A. arborescens,* and *A. chinensis* gels. Planta Med 64: 277-278.

Table 1: Impact of BA foliar applications on number of leaves, leaf length, width and thickness of *Aloe vera* plants

BA conc. (ppm)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)
0.00	13.20b	36.88c	4.20d	0.80c
075	17.40a	40.40b	5.44c	1.24a
150	17.80a	41.40b	5.72bc	0.98b
300	19.20a	40.60b	6.28ab	1.16a
600	19.20a	44.60a	6.66a	1.24a
C.V.	8.67	5.24	9.12	12.08

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 2: Impact of BA foliar applications on number of tillers, number of roots and root length of *Aloe vera* plants

BA conc. (ppm)	No. of Tillers	No. of roots	Root length (cm)
0.00	18.4c	30.2a	18.0b
075	18.8c	29.8a	20.4b
150	21.4b	33.4a	20.6b
300	22.4ab	33.8a	18.8b
600	24.6a	34.8a	24.6a
C.V.	9.27	13.75	10.65

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 3: Impact of BA foliar applications on *Aloe vera* shoot and root fresh and dry weights

BA conc. (ppm)	**Shoot fwt** (g)	**Shoot dwt** (g)	**Root fwt** (g)	**Root dwt** (g)
0.00	436.5b	39.64c	41.44a	4.28ab
075	416.1b	40.20c	41.60a	4.18ab
150	582.9a	55.26b	37.26b	3.72bc
300	625.1a	62.54a	43.34a	4.32a
600	640.5a	64.20 a	33.43c	3.24c
C.V.	8.23	9.38	5.64	11.04

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 4: Impact of BA foliar applications on the leaf, the gel and peel weights and chlorophyll 'a' and 'b' contents of *Aloe vera* plants.

BA conc.	**Leaf wt**	**Gel wt**	**Peel wt**	**Chlorophyll (mg/g)**	
(ppm)	**(g)**	**(g)**	**(g)**	**a**	**b**
0.00	205.0d	101.4e	103.6c	10.76e	07.66d
075	230.4c	113.8d	114.6ab	13.44d	08.14d
150	240.4c	125.0c	121.4a	15.86c	11.34c
300	256.0b	148.6b	111.4bc	21.62b	12.08b
600	276.0a	157.8a	118.8ab	24.12a	14.28a
C.V.	4.04	4.72	6.31	8.96	8.07

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Chapter Nine

Response of *Aloe vera* plants to foliar and soil applications of Argel (*Solenostemma argel* Del. Hayne)

Abstract:

This study aimed to investigate the response of *Aloe vera* plants to foliar and soil application treatments with Argel in 2 separate tests under the conditions of the nursery at Shambat, Khartoum North, Sudan. The foliar treatments were cold, hot and boiled water extracts of 15 g dry leaves of Argel per litre. The soil dressing test was for 0, 2.5, 5, 7.5 and 10 g dry Argel leaves per plant. The two tests were arranged in complete randomized design with 7 replicates per treatment. Treatments were repeated every 3 months. At the termination of the experiments growth attributes were evaluated in the terms of number, length, width, thickness and fresh weights of leaves, number of tillers and roots, root length, fresh and dry weights of shoots and roots, weights of peel and gel beside chlorophyll a and b contents. Data were analyzed separately and combined. The analysis revealed a general increase in growth parameters in the treated plants compared to the control. Except for the root fresh and dry weight, the highest values of all growth parameters were obtained from the boiled Argel extract and the 7.5 and 10 g/plant soil dressing treatments. The significant improvements in growth and gel content are indicatives to the possibility of commercial organic production of this plant under Sudan conditions.

Keywords: *Aloe vera*; Argel; Extracts; Soil dressing; Growth; Gel content

Introduction:

This plant is a member of Liliaceae family and is a native of Sudan (Heck, *et al.*, 1981). It can thrive well under xerophytic conditions. *Aloe vera* is an important constituent of cosmetics and pharmaceuticals and had been used in folk medicine for over 2000 years, and still remains as an important component in the traditional medicine of many contemporary cultures, such as China, India, West Indies, and Japan (WHO, 1999). Some of its most important pharmacological activities are the antiseptic (Capasso, *et al.*,1998), antitumor (Winter *et al.*,1981), antiinflammatory (Yagi *et al.*,1998), wound and burn

healing (Heggers *et al.,* 1993), anti diabetic (Rajasekaran, *et al.,* 2006) and the adjunct to current AIDS therapy effects (Daniel *et al.,* 1990). *Aloe vera* is an anti-oxidant rich plant that contains vitamins such as A, C, and E plus zinc and selenium minerals which boost the immune system and combat free radicals in the body (AOAC, 1990). The gel extracted from leaves contains more than 75 potentially active medicinal ingredients including vitamins, minerals, saccharides, amino acids, anthraquinones, enzymes, lignin, saponins, salicylic acids, and the anthraquinones, particularly barbaloin, are responsible for its bitter taste and cathartic effect (Cook and Samman, 1996, Dagne *et al.* 2000; Boudreau and Beland, 2006). *Aloe vera* also contains products of the isoprenoid pathway, including carotenoids, steroids, terpenes, and phytosterols (Samman, 1998).The demand for aloe products in the world markets is increasing due to its multiple uses in industries (Ndhlala *et al.,* 2016). However, this plant did not receive scientific or even cultural attention in Sudan for regular economical production except the limited production for local ornamental and ethno-medicine uses.

Recently there is a growing interest in Sudan in the use of some plant products for agriculture production as these plants were assumed to contain bioactive compounds that can be used as growth promoters or biostimulants. Such tendency is in conformity with international efforts. Bio-stimulants are organic materials that had been shown to influence several metabolic processes such as respiration, photosynthesis, nucleic acid synthesis and ion uptake and when applied in small quantities, enhance plant growth and development (Arpiwi, 2007). According to Sandra (2011), mung bean sprouts (*Vigna radiata*), bananas (*Musa paradisiaca* L.) and onions (*Allium cepa* L.) contain auxin; snap beans (*Phaseolus vulgaris* L) contain cytokinins; coconut water (*Cocos nucifera* L.) contains auxin, cytokinins and gibberellins. Besides, Wijaya (2002), reported cytokinin content in corn grain (*Zea mays* L.). In as much as, Zamroni and Darini (2009) studied effects of coconut water on growth of chili (*Capsicum annuum* L.) and reported significant enhancements in growth parameters. Likewise, Idris *et al.,* (2012) reported significant increase in date palm yield and improvements in the physical quality characters of the fruits upon addition of small quantities of dry leaves of Argel (*Solenostemma argel* Del., Hayne) to the soil of the palms, under the conditions of the Northern State, Sudan. They attributed the yield and quality gains to a growth regulator-

like effect of Argel. It is noteworthy to recognize that, the argel plant is a native of the northern parts of Sudan, where it grows wild. Besides, Eldoash *et al*., (2012), reported efficient control of the green pit-scale insect on date palm coupled with yield enhancement when the palms were treated with argel. In addition, Idris *et al*., (2014), reported restoration of normal growth in vegetatively malformed mango trees, beside enhancements in characteristics of inflorescence, fruit set and fruit retention upon treatment of the malformed mango trees with different forms of Argel. Such efforts are of value when considering means for organic production of horticultural crops. Nevertheless, organic production is also highly valued for the production of medicinal plants (Merestala, 1996). Therefore, this study aimed to investigate the impact of Argel applications on growth and gel yield of *Aloe vera* plants.

Materials and methods:

This test was conducted in complete randomized design in the nursery of Sudan University of Science and Technology, at Shambat, Khartoum North, Sudan, for determination of the impact of the foliar application of water extracts and soil dressing with dried leaves of Argel on growth attributes and gel content of *Aloe vera* plant. Tillers, 12-15 cm long were planted in 25X30 cm plastic bags containing river Nile silt soil. A month after establishment, they were used as test plant materials. Two separate tests were conducted as follows:

1. Test of Argel water extracts: The impact of foliar treatment with either cold, hot or boiled extracts of 15 g of dry Argel leaves per liter were compared against an untreated control.
2. Test of soil dressing with dry Argel leaves powder: The Argel concentrations tested were: 0.0, 2.5, 5.0, 7.5 and 10 g/ plant.

The cold extraction was prepared by direct submersion of dry leaves in tap water. For preparation of hot water extract, tap water was heated to around 90° C prior to submersion of the dry leaves, while leaves were submersed in tap water and heated to boiling for 10 minutes for the preparation of the boiled water extract. In the three cases, leaves were left for 60 minutes in water and then filtered with sach cloth for the final preparation of the extracts. The treated plants were sprayed to run-off. Each treatment

was replicated 7 times and each plant in a plastic bag was considered a replicate. Each plant received a dose of 2 g urea at the beginning of the study. The Argel applications were repeated every 3 months and irrigation was applied according to need. Data were collected after 12 months for number of leaves, leaf length, leaf width, leaf thickness, number of tillers, number of roots, root length, shoot fresh and dry weights, root fresh and dry weights, leaf weight, leaf gel weight, leaf peel weight, and chlorophyll 'a' and 'b' contents. For dry weights, the harvested shoots and roots were subjected to sun drying for 30 days, prior to oven drying for a week at 70° C until weights were constant. For determination of leaf gel content, the leaves were cut into several portions with scalpel blades to ease gel extraction after weighing the leaves. The remaining peels were weighed separately with a portable digital balance. Determination of chlorophyll content was performed according to the method of Arnon (1949) by using the chlorophyll flourometer (Li-Cor, Lincoln, NE, USA). Two hundred milligrams of fresh leaf samples were ground with 10 ml of 80% acetone at 4ºC and centrifuged at 2500 rpm for 10 minutes at 4ºC. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 665 and 649 nm with spectrophotometer after which the chlorophyll (a) and (b) were determined by Vernon's models. Data were analyzed separately and combined by analysis of variance and means were separated by Duncan's Multiple Range Tests with the aid of MstatC computer program.

Results:

1. Foliar application of different water extracts:

Data on the impact of the type of extract on the number of leaves, leaf length, width, thickness and number of tillers of *Aloe vera* plants are illustrated in Table (1). All Argel extracts resulted in significant increments in the above mentioned parameters compared to the control. The boiled extract ranked top for these parameters with significant differences from other treatments except the leaf thickness. The performance of plants treated with the hot extract was better than that of plants treated with the cold extract.

Table (2) demonstrates the impact of applied extracts on the number and length of roots, shoot and root fresh and dry weights of aloe plants. The highest number of roots and root length resulted from plants treated with the boiled extract with significant difference from

other treatments. Again, better gains in these parameters were obtained from the hot extract compared to the cold one. All Argel treatments resulted in statically equal increment in shoot fresh weight with significant difference from the control. However, the highest shoot dry weight was recorded for plants treated with the cold water extract, followed in rank by the hot, then the boiled extract and finally the control. All extracts enhanced root fresh weight significantly compared to the control and the best gain in weight was achieved in plants treated with the hot water extract. The highest root dry weight was equally enhanced in plants treated with hot and boiled extracts with significant difference from plants sprayed with the cold extract that equaled the control statistically.

The effects of Argel water extracts on weights of leaf, gel and peel beside chlorophyll 'a' and 'b' contents are compiled in Table (3). Except for chlorophyll 'b' content, the different types of extracts enhanced these parameters significantly compared to the control. The values for leaf weight and chlorophyll 'a' content were equally increased by the boiled and hot extracts. The hot extract resulted in best gel yield with significant difference from other treatments, while the weight of peel was increased significantly in plants treated with boiled extract. However, the highest values for chlorophyll 'b' content were equally obtained from the control and the hot extract.

2. Soil applications analysis:

Results of the impact of Argel soil application on the number of leaves, leaf length, width, thickness and number of tillers of *Aloe vera* plants are presented in Table (4). Significant enhancements in these parameters were obtained in all Argel treated plants compared to the control. Regarding the number of leaves, the 7.5 and 10 g treatments resulted in significant statistically equal enhancement of this parameter. The 7.5 g treatment was found best as it induced significant increments in leaf length, width, thickness and number of tillers.

The impact of Argel soil applications on number of roots, root length, shoot and root fresh and dry weights of *Aloe vera* plants are shown in Table (5). The 7.5 g Argel treatment resulted in significant increase in the values of these parameters compared to other treatments. The number of roots was equally enhanced by the 2.5, 5 and 10 g treatments that shared a second rank. Otherwise, the 10 g treatment ranked second for

root length, shoot and root fresh and dry weights with significant difference from the 2.5 and 5 g Argel treatments.

The results of impact of Argel soil dressings on leaf gel and peel weights, beside chlorophyll content of *Aloe vera* plants are presented in Table (6). Except for chlorophyll "b" content, all Argel-treated plants showed significant enhancement of these parameters compared to the control. The 7.5 and 10 g equally enhanced the leaf and gel weights with significant difference from other treatments. The highest peel weight resulted from the 7.5 g treatment with significant difference from the other Argel treatments that shared a second position. The 10 g treatment ranked top for chlorophyll 'a' content, while the 5 and 7.5 g treatments ranked second. However, the lowest chlorophyll 'b' content was recorded for the 2.5 and 5 g treatments and the highest was recorded for the 10 g treatment that did not differ from the 7.5 g and the control treatments.

3. Combined analysis:

The combined analysis of Argel water extracts and soil application revealed similarity in outstanding performance of the boiled extract and the 7.5 g dressing treatments for their effects on number of leaves, leaf length, width and thickness, but the number of tillers was significantly enhanced by the boiled extract compared to the 7.5 g soil dressing treatment (Table 7).

According to Table (8), the number of roots and root length were also increased significantly by the boiled extract and the 7.5 g soil dressing treatments that ranked top compared to other treatments. Regarding shoot and root fresh and dry weights, the 7.5 g soil dressing treatment was the most enhancive for these parameters compared to other treatments (Table 8).

The hot and boiled extracts and the soil dressing with 5, 7.5 and 10 g treatments increased leaf weight at the same statistical level and shared the top rank (Table 9). The highest gel weight was achieved from the boiled extract beside the 7.5 and 10 g soil dressing treatments (Table 9). The peel weight was highest in plants that received the 7.5 g soil dressing treatment, but the difference was not significant from those that received 2.5 and 5 g dressing or those sprayed with the hot extract (Table 9). The highest chlorophyll 'a' content was recorded for the 10 g soil dressing treatment which was statistically equal in plants sprayed by the boiled extract, whereas chlorophyll 'b' content was also highest in

plants that received the 10 g soil dressing treatment without significant difference from the control, the hot extract or the 7.5 g soil dressing treatments (Table 9).

Discussion:

Among the 422,000 plant species documented worldwide, 12.5% were reported to have medicinal value (Rao *et al.*, 2004). Medicinal plants have a global recognition in health care apart from monetary benefits to local communities (Rashid, 2009) and are gaining momentum as means of livelihood due to their marketing potential, provision of labor opportunities and contribution to efficient land use (Caniago and Siebert, 1998). Sudan is a flora-rich country owing to diversity of its ecological zones. This flora is mostly exploited as pasture or limited uses in traditional medicine based on wild collection. Argel is among these plants that did not receive sufficient attention for economical production, despite the scientific confirmation of its pharmacological potency Capasso *et al.*, (1998), beside scientific reports on its pesticide properties for agricultural and medical insect pests (Eldoash *et al*., 2012; Elkamali 1991) and recent reports on its growth, flowering and yield promotion of some horticultural crops Idris *et al*., (2014). However, the particular feature of this study is the test of Argel which is an indigenous under-exploited medicinal to explore its potential as an enhancer of growth and gel yielder of *Aloe vera* which is also a medicinal plant of Sudanese origin. The results clearly exhibited the advantages of Argel applications particularly the foliar spray of boiled water extract of 15 g/l, or the soil dressing of 7.5 g of dry leaves powder per plant, for enhanced growth and gel yield. This result is in line with preceding research findings as Idris *et al.,* (2011) reported significant increments in yield and yield components coupled with improved fruit size upon dressing date palm trees soil with dry leaves of Argel. Likewise, Idris *et al.,* (2014), reported enhanced flowering, fruit set and fruit retention in malformed trees of 'Tommy Atkins' mango cultivar, upon foliar spray with shoot water extract or soil dressing of the trees with ground Argel shoots. In both cases, the authors owed the enhancements to growth regulator-like effects of Argel. Basically, the natural plant hormones are classified into juvenility and senescence hormones. Auxins, cytokinins and gibberellins are the most outstanding juvenility hormones that regulate and promote growth beside morphogenesis (George and Deklerk, 2008). The growth and yield stimulated by Argel applications on *Aloe vera* may also be attributed to

its mineral composition as Argel is extremely rich in potassium and moderately high in manganese and iron (Sabahelkheir and Mohamed, 2010). Potassium influences the water potential of plant cells beside its role in translocation and accumulation of carbohydrates in plant tissues. More-over, Sabahelkheir and Mohamed (2010) reported the presence of elevated phytic acid concentration in Argel leaves. This constituent might have an influence on the enzymatic processes of the recipient plant. The increase in chlorophyll content is an indication of adequacy of active chloroplasts and therefore efficient photosynthesis (Avenson *et al.*, 2005). It is noteworthy that iron and manganese are necessary for photosynthesis (Yaronskay *et al* 2007). The beneficial responses of some plant species to the applications of plant extracts had been frequently reported and attributed to their elevated endogenous hormones levels. Auxins were isolated from extracts of mung bean sprouts (*Vigna radiata*), bananas (*Musa paradisiaca* L.) and onions (*Allium cepa* L.), while cytokinins were isolated from snap beans (*Phaseolus vulgaris* L) and a mix of auxin, cytokinins and gibberellins was obtained from coconut water (*Cocos nucifera* L.) (Sandra, 2011). The enhanced tillers formation obtained in this study through Argel applications is a celebrity to propagators and is needed for large scale production of the *Aloe vera* which is conventionally propagated by tillers. Nevertheless, the possibility of economical production of *Aloe vera* under Sudan conditions seems valid as no pests or diseases were encountered throughout the study and the growth and gel yield were within limits reported in preceding studies (Calvo *et al.*, 2014; Khan *et al.*, 2009; Thomas *et al.*, 2009 and Mandal *et al.*, 2007). In conclusion, the study demonstrated significant enhancements in growth and yield of *Aloe vera* plant treated with Argel which are indicative to the bio-stimulating effect of Argel, but further biochemical studies are needed to define the constituent(s) behind the enhancements especially its content of juvenility hormones. The encouraging agronomic responses of *Aloe vera* to Argel applications are a step towards its organic production that may add to its commercial value.

Determination of chlorophyll content: Determination of chlorophyll content was performed according to the method of Arnon (1949). Two hundred milligrams of fresh leaf samples was ground with 10 ml of 80% acetone at 4oC and centrifuged at 2500 rpm

for 10 minutes at 4oC. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm with spectrophotometer.

References:

AOAC. (1990). Official methods of analysis of the Association of Official Analytical Chemists. 15th Ed. Association of Official Analytical Chemists Inc. Suite 400 2200 Wilson Boulevard, Arlington, Virginia 22201, USA. Pp. 69-81.

Arnon, D.I. (1949). Enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris*. *Plant. Physiol*., 24: 1-15.

Arpiwi, N.l., (2007). Concentration effect of gibberellins on seed production of potato (*Solanum tuberosum* L. cv. Granola) size m (31-60 grams). *thesis*. department of biology, faculty of Udayana University, Bali. *Indonesian*.

Avenson, T., Cruz, J., Kanazawa, A. and Kramer, D. (2005). Regulating the proton budget of higher plant photosynthesis. *P Natl Acad Sci. USA*, 102: 9709– 9713.

Boudreau M.D and Beland F.A. (2014). An evaluation of the biological and toxicological properties of Aloe barbadensis (Miller), Aloe vera. Journal of Environmental Science and Health, 24:103–54.

Calvo, P., Nelson, L. and Kloepper, J.W. (2014). Agricultural uses of plant biostimulants. *Plant Soil*, 383: 3 - 41.

Caniago, F. and Siebert, R. T. (1998). Some new compounds from the Aloe of India grown in green houses. *Indian Journal of Chemistry*. **27B** : 856-857.

Cook, N.C. and Samman, S. (1996). Flavonoids: Chemistry, metabolism, cardioprotective effects and dietary sources. Journal of Nutritional Biochemistry,7: 66–76.

Dagne, E., Bisrat, D., Viljoen, A. and Van Wyk, B.E. (2000). Chemistry of Aloe species. Curr Org Chem., 4: 65–78.

Daniel, H., Carpenter, R., Kemp, M., Kahlon, J. and Mc Analley, B. (1990). Extended survival and prognostic criteria for Acemannan (ACE-M) treated HIV Patients. *Antiviral Res.* Suppl. 1: 117.

Eldoash, k.O., Taha, A.W., Idris, T.I., Sidahmed, O. A., Musa, F.A. and Mardi, H.G. (2011) .Application of plant based extracts for control of the green pit scale insect on date palm. *Emirate Journal of Agriculture*, 23(5): 387-403.

Elkamali., H.H. (1991). Botanical and Chemicall studies on *Solenostemma argel* (Del) Hayne growing in Khartoum. M. Sc., University of Khartoum.

Heck, E., Head, M., Nowak, D., Helm, P. and Baxter, C. (1981). *Aloe vera* (gel) cream as a topical treatment for outpatient burns. Burns, 7: 291–294.

George, E.F., Hall, M.A. and Decklerk, G. J. (2008). Plant propagation by Tissue Culture. 3rd Edition. Springer, Netherland; Volume (1) pp 504.

Heggers, J., Pelley, R. and Robson, M. (1993). Beneficial effects of *Aloe* in wound healing. *Phytotherapy Research.* 7: 48-52.

Idris, T. I.M., Mohamed, A.A. Ibrahim, Mahdi, E. M. and Taha, A.K. (2011). Influence of argel (*Solenostemma argel* Del. Hayne*)* soil applications on flowering and yield of date palm (*Phoenix dactylifera* L.*).Agriculture and biology journal of North America*, 2(3): 538.542.

Idris, T. I.M., Elnour H.S. and Mahdi E.M. (2014).effect of different forms of Argel (*Solennostemma argel* Del Hayne) applications on flowering, fruit set and fruit retention in vegetatively malformed' Tommy Atkins' mango cultivar. *SUST J. Agric. And Veterinary Sc*., 15: 79- 86.

Khan, W., Rayirath, U.P., Subramanian, S., Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J., Norrie, J. and Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regulation*, 28: 386 - 399.

Mandal, A.K.A., Kumar, R. and Thomas, J. (2007). An overview of PGR trials in UPASI TRF. *Planters Chron.,* 103: 12 - 16.

Merestala, T. (1996). Abstract Bibliography of researches of Bio and Organic Fertilizers on growth of *Aloe vera* plants. *Agriculture and biology journal of North America, 67*: 217-224.

Ndhlala, A. R., du Plooy, C. P. and Abdelgadir, H. A. (2016). The potential of *Aloes* as medicine and cosmetic ingredients in South Africa. Abstracts of workshop: (*Linking Sudanese Medicinal and Aromatic Plants Producers to the Global Markets*), Coral Hotel, Khartoum, Sudan, 22-24 March, 2016. Pp. 25.

Rajasekaran, S., Ravi, K., Sivagnanam, K. and Subramanian, S. (2006). Beneficial effects of Aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clin Exp Pharmacol Physiology, 33: 232–237.

Rao, M. Parmar, B. S. and Ketkar, C. M. (2004). Commercialisation. In Aloe Research and Development (Randhava, N. S. and Parmar B. S.), *SBS publication No.3, Society of Horticultural Science*, India. 270- 283.

Rashid, B. (2009). Medicinal and aromatic plants based cropping systems in South Asia, FAO. . *International Journal of Agricultural Research,* 2(11): 210-214.

Sabahelkheir and Mohamed, M.A. (2010). Chemical Composition, Minerals, Protein Fractionation, and Anti-nutrition Factors in Leaf of Argel Plant (Solenostemma Argel). *European Journal of Scientific Research,* 43(3):430-434.

Samman, S. (1998). Lipid metabolism. In: Kuchel P.W, Ralston G.B, editors. In Schaum's Outlines of Theory and Problems of Biochemistry. New York: McGraw Hill Book Company, 362–401.

Sandra, E. (2011). plant hormones and growth, accessed october 25, bogor (*indonesian).*

Thomas, J., Mandal, A.K.A., Raj Kumar, R. and Chordia, A. (2009). Role of biologically active amino acid formulations on quality and crop productivity of tea (*Camellia* sp.). *International Journal of Agricultural Research,* 4: 228 - 236.

WHO (1999). World Health Organization. Monographs on Selected Medicinal Plants. Vol. 1. Geneva.

Wijaya, H. (2002). shoots response interfascicular pinus jung et de vries against cuts and giving hormone treatment 6 -bap. faculty of forestry, hasanuddin university, makassar. (*Indonesian*).

Winter, W.D., Benavides, R. and Clouse, W.J. (1981). Effects of *Aloe* extracts on human normal and tumor cells *in vitro*. Econ. Bot. 35: 89-95.

Yagi, A., Tsunoda, M., Egusa, T., Akasaki, K. and Tsuji, H. **(1998).** Immunochemical distinction of *Aloe vera, A. arborescens,* and *A. chinensis* gels. *Planta Med* 64: 277-278.

Yaronskay, E.B., Gritskevich, E.R., Trukhanovets, N.L. and Averina, N.G. (2007). Effect of macro-nutrient elements on early stages of chlorophyll biosynthesis in streptomycin-treated barley seedlings, *Russian Journal of Plant Physiology*, 54:388-395.

Zamroni, and darini (2009). growing an organizing influence defolasi natural and herbal leaves on growth cuttings. faculty of agriculture. Tamansiswa sarjawinaya university of Yogyakarta. *(Indonesian*).

Table 1: Impact of foliar applications with different types of Argel water extracts on the number, length, width and thickness of leaves and number of tillers of *Aloe vera* plants.

Type of argel extract	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)	No. of tillers
Control	13.0d	32.52d	3.88d	0.54d	19.40d
Boiled extract	19.6a	48.66a	5.18a	1.12a	33.00a
Hot extract	17.6b	42.90b	4.58b	1.10ab	27.80b
Cold extract	15.4c	38.86c	4.06c	0.76c	21.80c
C.V.	10.11	7.23	6.88	13.56	9.42

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 2: Impact of foliar applications with different types of Argel water extracts on shoot and root fresh and dry weights, number of roots and root length of *Aloe vera* plants.

Type of argel extract	No. of roots	Root length (cm)	Shoot fwt (g)	Shoot dwt (g)	Root fwt (g)	Root dwt (g)
Control	26.80c	14.86d	0757.1b	27.44d	070.78d	2.86b
Boiled extract	39.80a	27.10a	1232.0a	32.97b	113.10c	3.30a
Hot extract	31.80b	22.70b	1226.0a	35.54a	118.00b	3.56a
Cold extract	27.20c	19.52c	1259.0a	29.28c	130.50a	3.02b
C.V.	6.21	11.94	5.02	8.17	2.23	8,61

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 3: Impact of foliar applications with different types of Argel water extracts on leaf, gel and peel weights and chlorophyll 'a' and 'b' contents of *Aloe vera* plants.

Type of extract	Leaf wt (g)	Gel Wt (g)	Peel Wt (g)	Chlorophyll (mg/g) a	b
Control	199.0c	110.0d	089.0d	14.14c	12.74a
Boiled extract	264.0a	156.4a	103.6b	23.14a	05.96c
Hot extract	258.2a	145.8b	111.2a	21.74a	12.72a
Cold extract	242.0b	136.8c	098.8c	18.10b	10.68ab
C.V.	7.86	6.57	8,14	8.16	18.31

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

2. Soil applications:

Table 4: Impact of Argel soil applications on the number of leaves, length, width and thickness and number of tillers of *Aloe vera* plants.

Argel levels (g)	No. of leaves	Leaf length (cm)	Leaf width cm)	Leaf thickness (cm)	No. of tillers
Control	13.0c	32.52d	3.88d	0.54d	19.40d
02.5	14.8b	40.90c	3.74d	0.70c	20.60d
05.0	15.6b	45.40b	4.40c	1.04b	26.60b
07.5	18.2a	51.06a	5.04a	1.22a	30.60a
10.0	18.0a	46.60b	4.00b	1.12ab	23.20c
C.V.	7.82	6.17	8.21	11.48	8.22

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 5: Impact of Argel soil applications on number of roots, root length, shoot and root fresh and dry weights of *Aloe vera* plants.

Argel levels (g)	No. of roots	Root length (cm)	Shoot fwt (g)	Shoot dwt (g)	Root fwt (g)	Root dwt (g)
Control	26.80d	14.86e	0757.10d	27.44d	070.78d	2.86d
02.5	21.60e	18.14d	0773.40d	24.14e	089.08c	2.42d
05.0	23.60c	20.56c	0897.40c	33.40c	090.00 c	3.38c
07.5	39.00a	27.24a	1537.00b	36.54b	148.00 b	3.84 b
10.0	32.20b	24.42b	1666.00a	40.40a	163.90a	4.20 a
C.V.	11.20	9.04	5.02	8.65	2.24	8,69

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 6: Impact of Argel soil applications on leaf, gel, peel weights and chlorophyll 'a' and 'b' contents of *Aloe vera* plants.

Argel levels (g)	Leaf wt (g)	Gel wt (g)	Peel wt (g)	Chlorophyll (mg/g) a	b
Control	199.0d	110.0d	089.0c	14.14d	12.74ab
02.5	245.2c	142.2c	109.0ab	17.86c	10.80b
05.0	262.2a	154.4a	107.8ab	19.82b	10.48b
07.5	254.8b	146.2b	114.6a	20.56b	11.48ab
10.0	266.6a	157.0a	104.2b	23,90a	14.72a
C.V.	4.46	5.65	8,14	7.16	22.40

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

3. Combined analysis:

Table 7: Impact of foliar and soil applications of Argel on the number of leaves, length, width and thickness and number of tillers of *Aloe vera* plants.

Type of Argel extract	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)	No. of tillers
Control	13.0d	32.52d	3.88d	0.54d	19.40d
Boiled extract	19.6a	48.66a	5.18a	1.12a	33.00a
Hot extract	17.6b	42.9b	4.58b	1.10ab	27.80b
Cold extract	15.4c	38.86c	4.06c	0.76c	21.80c
02.5g	14.8c	40.90c	3.74d	0.70c	20.60d
05.0g	15.6c	45.40bc	4.40b	1.04b	26.60b
07.5g	18.2a	51.06a	5.04a	1.22a	30.60a
10.0g	18.0a	46.60b	4.00bc	1.12a	23.20c
C.V.	8.09	6.24	7.88	15.56	8.22

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 8: Impact of foliar and soil applications of Argel on number and root length, shoot and root fresh and dry weights of *Aloe vera* plants.

Type of Argel extract	No. of roots	Root length (cm)	Shoot fwt (g)	Shoot dwt (g)	Root fwt (g)	Root dwt (g)
control	26.80cd	14.86d	0757.10e	070.78f	27.44d	2.86d
Boiled extract	39.80a	27.10a	1232.00a	113.10d	31.97c	3.30bc
Hot extract	31.80b	22.00bc	1226.00c	118.00d	35.54b	3.56a
Cold extract	27.20c	19.52c	1259.00b	130.50c	29.28d	3 .02c
02.5g	21.60e	18.14c	0773.40e	089.08e	24.14e	2.42e
05.0g	23.60c	20.56c	0897.40d	090.00 e	33.40c	3.38b
07.5g	39.00a	27.24a	1537.00b	148.00 b	36.54b	3.84a
10.0g	32.20b	24.42b	1666.00a	163.90a	40.40a	4.20 a
C.V.	7.25	6.72	5.02	2.24	8.65	8,66

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 9: Impact of foliar and soil applications of Argel on leaf, gel, peel weights and chlorophyll 'a' and 'b' contents of *Aloe vera* plants.

Type of extract	Leaf wt (g)	Gel wt (g)	Peel wt (g)	Chlorophyll (mg/g) a	b
Control	199.0d	110.0e	089.0d	14.14d	12.74a
Boiled extract	264.0a	156.4a	103.6b	23.14a	05.96c
Hot extract	258.2a	145.0b	111.2 a	21.74ab	12.72a
Cold extract	242.0c	137.8d	098.8 c	18.10c	10.68ab
02.5g	245.2c	142.2c	109.0ab	17.86c	10.80ab
05.0g	262.2a	154.4a	107.8ab	19.82b	10.48b
07.5g	254.8b	146.2b	114.6a	20.56b	11.48ab
10.0g	266.6a	157.0a	104.2 b	23,90a	14.72a
C.V.	4.86	5.55	8,14	8.16	25.29

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Chapter Ten

Responses of *Aloe vera* to applications of Haza (*Haplophyllum tuberculatum* L.)

Abstract:

Various plant extracts are being marketed based on claims of their potencies as yield and quality enhancers and/or for the environmentally friendly pesticidal properties. In two separate tests, this study aimed to investigate the response of *Aloe vera* plants to Haza ground shoots applied as soil dressing and foliar sprays. The soil application test was for 0, 2.5, 5, 7.5 and 10 g/ plant and the foliar treatments were for cold, hot and boiled water extracts of 15 g dry shoots of haza per litre. Tillers, 12-15 cm long, of *Aloe vera* plants established in 25X30 cm polyethylene plastic bags were employed for the tests under nursery conditions. All the tested plants received a dose of 2 g urea prior to treatments. The two tests were arranged in complete randomized design with 7 replicates. Treatments were repeated every 3 months. Data were collected 12 months after first application for number, length, width, thickness and fresh weights of leaves, number of tillers and roots, root length, peel and gel weights, shoots and roots fresh and dry weights beside chlorophyll 'a' and ' b' contents. Data were analyzed separately and combined. The analysis revealed a general increase in growth parameters in treated plants compared to the control. Except for the root fresh and dry weight, the highest values of all growth parameters were obtained from the boiled water extract and the 7.5 and 10 g soil dressing treatments. The improvements in growth and gel content are indicators of the agronomic benefit of Haza applications; a step towards organic farming.

Keywords: *Aloe vera*; Haza; Extracts; Soil application; Growth stimulation; Gel content

Introduction:

Growth is a multifactor dependent rhythmic phenomenon. Besides the availability of nutrients, plants require hormones for their growth and development. Under favorable conditions that accelerate plant growth and development, the levels of endogenous growth promoting substances are higher than that of inhibiting substances (Marshall,

1999; Davis, 2010). Several organic products called “biostimulants”, assumed to make agriculture more sustainable, were available in markets (Tyler, 1993). These biostimulants include diverse formulations of compounds, plant products and micro organisms that are applied to plants or soils to improve crop vigour, yields and quality (Atherton, 1998). The crop life cycle from seed germination to plant maturity can be influenced by biostimulants in some ways such as increase plant metabolism efficiency, improved tolerance to a biotic stresses, efficient nutrient assimilation, translocation and use, enhanced quality attributes of produce, increased water use efficiency, enhanced physicochemical properties of the soil and development of complementary soil microorganisms (Davis, 2010). According to Ro, (2000) and Hutter, (1996) foliar application of biostimulants were able to enhance plant productivity. Cerdan *et al*., (2009) and Ertani *et al*., (2009) reported increased nutrient uptake, in particular nitrogen and iron, when corn and tomato plants were treated with protein hydrolysates. The increase in nitrogen and iron uptake, leads to enhanced activities of nitrate reductase and glutamine synthetase, and Fe (III)-chelate reductase activity, respectively (Chithra *et al.* 1998); Heggers *et al.* 1996). Nutrients spray on plants can accelerate absorption and consumption of elements ending in fast growth (Byeon, *et al*. 1988). Priming seeds of the rangeland grass *Echinochloa crusgalli* with Moringa leaf extract resulted in significant increase of shoot vigour coupled with higher number of leaves and fertile tillers (Nouman *et al*., 2011). According to Makkar *et al.* (2007), Moringa leaf extract contains significant quantities of calcium, potassium, and cytokinin in the form of zeatin, antioxidants proteins, ascorbates and phenols. Mølgaard *et al.*, (2000) investigated the response of *Vinca rosea* to foliar spray with saponins rich extracts of *Daldomera triangulata* L. and reported higher values for shoot and root characters in treated plants compared to the untreated control. Furthermore, DeKroon and Hutchings (1995) studied the response of mustard plants to extracts from corn seed, shoot of *colythanthus spp.* and bark from *Quillaja tree. Their findings revealed* improvements in shoot growth and plant yield upon treatment with corn seed extract without influence on the root system, while the *colythanthus spp.* extract resulted in maximum shoot weight, plant height, leaf number and the total chlorophyll content. However, the extract from the *Quillaja* tree

significantly increased the root length and number but reduced the total chlorophyll content.

The *Aloe vera* plant has been known and used for centuries for its health, beauty, medicinal and skin care properties (Ea *et al.*, 2008). *Aloe vera* is perennial, succulent evergreen plant of the family Liliaceae (Omidbeigi *et al.*, 2009). The geographic origin of *Aloe vera* was believed to be in Sudan, with the plant subsequently being introduced into the Mediterranean regions and most other warm areas of the world (Grindlay and Reynolds, 1986). This perennial succulent plant is drought tolerant and can thrive well under xerophytic conditions (Heber, 2007). The useful parts of the plant are leaves which contain the gel. The chemical composition of the leaf gel is very complex, composed mainly of polysaccharides and soluble sugars followed by proteins, many of which are enzymes, amino acids, vitamins, and anthraquinones (Liu *et al*., 2007). It had been shown that polysaccharides derived from *Aloe vera* enhance immunity activity and exert antioxidant effects (Zhanhai *et al.,* 2009) and most of the activity was attributed to β-polysaccharides (Ramachandra and Srinivasa, 2008). Anthraquinones are the second class of bioactive metabolites, including C-glucosyl derivatives such as barbaloin (10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9–10H-anthracenone), a mixture of the two diastereoisomers aloin A and B, as well as glucose-free compounds such as aloe-emodin (Fanali *et al*., 2010). Anthraquinones were reported to have cathartic effects, anti-inflammatory effects *in vivo* as well as anti- (bacterial, viral and cancer) effects (Park *et al.*, 2009; Pellizzoni *et al*., 2012). The demand for the aloe products is increasing with growth of cosmetic and neutraceutical markets (Danhof, 1987). According to Ndhlala *et al.* (2016), the global market of aloe products is currently estimated to be USD 13 billion and it has appeared in more than 50 categories of products since 2012. Research aiming to the economic exploitation of this plant under Sudan conditions is almost lacking, but badly needed.

The genus *Haplophyllum* belongs to Rutaceae family which contains about 70 species distributed in tropical, subtropical and Mediterranean regions of the world (IJAS, 2011; *Townsend, 1996*). Various members of the genus are used in traditional medicine for the treatment of herpes, warts, erysipelas, toothache, stomach and skin diseases (Bessonova, 1989) beside testicular cancer (Ea *et al.*, 2008). *Haplophyllum tuberculatum* is an

endogenous plant of Sudan where it is locally named, Haza. It grows wild along the banks of the Nile and its tributaries as well as banks of seasonal streams. Growth starts after water recession from banks at the end of the rainy season. It is of wide use in Sudanese ethno-medicine for treatment of rhinitis and skin allergies, spasmo-colon, gynecological disorders, asthma and breathing difficulties (Mohamed *et al.,* 1996). Khalid and Waterman (1981) reported isolation of alkaloid, lignan and flavonoids constituents from *Haplophyllum tuberculatum* collected from Sudan. Under conditions of Egypt, essential oils were isolated from the aerial parts of *Haplophyllum tuberculatum*, collected from different locations, showed significant differences in their yield and chemical composition of estragole, myrtenal, spathulenol and homologous series of medicinally important monoterpene and sesquiterpenes (El Naggar *et al.,* 2014). Nevertheless, Haza shoot is rich in triterpenoid saponins, and around 20 types of saponins had been identified in samples from the shoots of *Haplophyllum tuberculatum* plant (Chaicharoenpong and Petsom, 2009; Morikawa *et al*., 2007). Reports on its agronomy or its probable benefits when applied exogenously on other plants are lacking according to our intensive literature search. Therefore, this study aimed to investigate the growth and yield responses of *Aloe vera plant to Haza soil and foliar applications.*

Materials and methods:

The study was accomplished in complete randomized design in the nursery of Sudan University of Science and Technology, at Shambat, Khartoum North, Sudan, to determine the impact of Haza applications on growth and yield of *Aloe vera*. Tillers, 12-15 cm long, were planted in 25X30 cm plastic bags containing river Nile silt soil. Four weeks after planting, they were used as test plant materials. Two separate tests were conducted as follows:

1. Test of Haza water extracts: *Aloe vera* plants received foliar spray with cold, hot or boiled water extracts of 15 g of dry Haza shoots per liter and were compared against an untreated control.
2. Test of soil dressing with concentrations of ground Haza shoots powder: The impact of soil dressing with 2.5, 5.0, 7.5 and 10 g/ plant was compared against an untreated control.

The extraction was made 60 minutes after dry shoots submersion in water. Foliarly treated plants were sprayed to run-off. Each treatment was replicated 7 times and each plant in a plastic bag was considered a replicate. Each plant received a dose of 2 g urea at the beginning of the study. The Haza applications were repeated every 3 months and irrigation was applied according to need. Data were collected after 12 months for number of leaves, leaf length, leaf width, leaf thickness, number of tillers, number of roots, root length, shoot fresh and dry weights, root fresh and dry weights, leaf, gel and peel weights and chlorophyll 'a' and 'b' contents. For dry weights, the harvested shoots and roots were subjected to sun drying for 30 days, prior to oven drying for a week at 70° C until weights were constant. For determination of leaf gel content, the leaves were cut into several portions with scalpel blades to ease gel extraction after weighing the leaves. The remaining peels were weighed separately with a portable digital balance. Determination of chlorophyll content was performed according to the method of Arnon (1949) by using the chlorophyll flourometer (Li-Cor, Lincoln, NE, USA). Two hundred milligrams of fresh leaf samples were ground with 10 ml of 80% acetone at 4^{o}C and centrifuged at 2500 rpm for 10 minutes at 4^{o}C. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 665 and 649 nm with spectrophotometer after which the chlorophyll (a) and (b) were determined by Vernon's models. Data were analyzed separately and combined by analysis of variance and means were separated by Duncan's Multiple Range Tests with the aid of MstatC computer program.

Results:

1. *Foliar applications of water extracts:*

Data on the impact of the extracts on the number of leaves, leaf length, width, thickness and number of tillers of *Aloe vera* plants are shown in Table (1). The boiled water extract ranked top for all parameters with significant difference from all treatments except for leaf thickness where it shared the rank with other treatments. The hot extract ranked second differing significantly from the cold water extract and the control except for leaf thickness. The cold water extract performed similarly to the control except for leaf length where it induced significant increase.

The impact of extracts on number and length of roots, shoot and root fresh and dry weights, is presented in Table (2). The results revealed the advantages of the boiled extract compared to other treatments. However, the hot extract shared the top rank with the boiled extract for root dry weight and ranked second for other parameters.

Table (3) illustrates the effects of Haza water extracts on leaf, gel and peel weights, beside chlorophyll 'a' and 'b' contents. Best values for leaf weight and chlorophyll 'a' content were equally achieved from the boiled and hot extracts. The boiled extract resulted in highest gel content. It also ranked top for chlorophyll 'b' content but without significant difference from the hot extract. However, the highest peel weight was recorded for the treatment of hot water extract.

2. *Soil applications*:

Results of the impact of Haza soil applications on leaf number, length, width and thickness beside number of tillers of *Aloe vera* plants are presented in Table (4). The leaf length was equally enhanced by the 7.5 and 10 g treatments significantly compared to other treatments. Best leaf length was obtained from the 7.5 g treatment. Maximum leaf width resulted from the 10 g treatment with significant difference from all treatments except the 7.5 g treatment. Regarding leaf thickness, no significant difference was obtained among treatments. The highest number of tillers resulted from the 7.5 g treatment with significant difference from other treatments.

Table (5) demonstrates the influence of Haza soil applications on number and length of roots, in addition to shoot and root fresh and dry weights of *Aloe vera* plants. The 7.5 g treatment resulted in highest number of roots and longest roots. However, the highest values for shoot and roots fresh and dry weights were recorded for the 10 g treatment with significant difference from other treatments.

Data regarding the impact of Haza soil dressings on leaf, gel and peel weights as well as chlorophyll 'a' and 'b' contents of *Aloe vera* plants are compiled in Table (6). These parameters were significantly enhanced by Haza treatments compared to the control. The 7.5 and 10 g treatments resulted in equal increase in leaf weight and shared top rank for this parameter. The 10 g treatment also recorded the highest values for gel weight and chlorophyll 'a' and 'b' contents, whereas the highest value for peel weight was recorded for the 2.5 g treatment.

3. *Combined analysis*:

The main features of the combined analysis are presented in Tables 7, 8 and 9. According to Table (7), the boiled extract resulted in highest number of leaves/ plant, while the highest leaf length was recorded for the 7.5 g soil dressing treatment. Leaf width was equally enhanced by the boiled extract and the 10 g treatment, whereas the leaf thickness was not affected by all Haza treatments that equaled the control. Tillers formation was equally increased by the boiled and the 7.5 g treatments.

According to Table (8), the boiled extract and the 7.5 g treatments equally resulted in best enhancements of number and length of roots, whereas the boiled extract was solely ranked top for shoot fresh and dry weights. However, the fresh and dry weights of roots were increased significantly by the 10 g soil dressing treatment.

All Haza treatments resulted in significant increments in the values of leaf, gel and peel weights as well as chlorophyll content of *Aloe vera* plants compared to the control (Table 9). The boiled and hot extracts and the 5, 7.5 and10 g treatments equally shared top rank for leaf weight. The highest gel weight was achieved from the 10 g treatment. The hot extract enhanced peel weight significantly. The 10 g, the hot and boiled extracts increased chlorophyll 'a' and 'b' contents to a statistically equal level.

Discussion:

The results of this study revealed growth and yield benefits in *Aloe vera* plants treated with Haza particularly the boiled extract and the soil dressing with 7.5 and 10 g dry Haza shoots. These enhancements were coupled with increase in chlorophyll content. Chlorophyll molecules are arranged in and around the membranes of chloroplasts. The function of most chlorophyll is to absorb light energy and transfer it to photosynthesis reaction centres (Gilpin, 2001). Chlorophyll 'a' and 'b' are named after the wavelength (in nanometers) of their red-peak absorption maximum Jeffrey *et al.* (1969). Nevertheless, the high levels of chlorophyll content obtained in this study are indications of healthy and active growth and might be among factors related to the gain in gel content. The gains in growth and gel yield might also be related to growth-regulator-like

constituent(s) in Haza shoots. Such gains in date palm yield resulting from the soil application of Argel (*Solenostemma argel*) were attributed to a growth-regulator-like effect of Argel (Idris *et al.,* 2010). Commercially registered growth regulators are all phytohormones and fall into the following categories: auxins, gibberellins, cytokinins, abscisic acid, and ethylene. Recently, several other hormonal compounds such as oligosaccharins, brassinosteroids, jasmonates, salicylates, and polyamines had been shown to have growth-regulating properties (Basra, 2000). According to Ali *et al*., (2009), the scarce scientific literature combined with less validated producer information on natural growth enhancers shows that the growth-enhancing effect of different products can be roughly divided into three categories: compounds enhancing nutrients availability or facilitate their uptake, or decreasing damage by pests, and/or interfere with the plant hormone system either directly or indirectly through microbes. Sato (2004) and Wagentrisl (2003) reported that commercial products based on extracts from saponin-containing plant parts, had been shown to enhance the yield, quality and growth characters of tomatoes, cucumbers, and strawberries and owed the effect to improved uptake of nutrients from soil and air, effectiveness against fungal infections, nematodes, and pathogens and growth stimulating properties. Foidle *et al.* (2001) reported that a spray made from moringa leaves extract resulted in increased strawberry production and claim the possibility of its use as a foliar spray to accelerate growth of young plants. According to Sims *et al*. (2009), the tea seed extract applied on *Euphorbia obtusifolia* grown under non-nutrient-limiting conditions, resulted in increase in number of leaves, plant height, number of branches and roots compared to the untreated plants, and claimed that tea seed powder contains substances with hormone-like properties or substances that interfere with endogenous hormones that can stimulate or affect biomass allocation in plants. Other studies had also shown that treating soil or spraying plants with saponins-containing plant products based on the bark from the soap bark tree (*Quillaja saponaria*) had a positive effect on the yield and quality of strawberries, tomato, and grapes (Wagentrisl, 2003). Apart from the growth-stimulating effects, plant extracts also displayed growth-reducing effects at increased doses in seeded plants such as corn, mustard, barley, and the soil-treated strawberries (Wagentrisl, 2003). Growth-reducing effects have also been found for early water grass (*Panicum crusgalli* L.), green foxtail

(*Setaria viridis* Beauv.L.), and white clover (*Trifolium repens* L.) at saponins different concentrations (Kohata *et al.*, 2004).

Conclusion: The stimulations of growth and gel yield of *Aloe vera* plants treated with Haza in this study necessitate further biochemical studies of Haza to define the constituent(s) responsible for these enhancements; the priority should be for its content of nutrient elements and juvenility hormones namely auxins, cytokinins and gibberellins which might be tools for better interpretation of the stimulative effects. Regardless of the solid interpretation, the results revealed the possibility of organic commercial of *Aloe vera* plants based on Haza applications.

References:

Ali, B., Sabri, A.N., Ljung, K., Hasnain, S. ***(*2009*).*** Auxin production by plant associated bacteria: Impact on endogenous IAA content and growth of Triticum aestivum *L. Lett. Appl. Microbiol.,* 48*:*542–547.

Arnon, D.I. (1949). Enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris. Plant. Physiol*., 24: 1-15.

Atherton, P. (1998). *Aloe vera* the Plants of potential medicinal value, revisited. *British Journal of Phytotherapy*, 4:76–83.

Basra, A.S. ***(*2000*).*** *Plant growth regulators in agriculture and horticulture (Food Products Press,* New York, NY*).*

Batanouny, K. H. **(1999).** Plants of potential medicinal value, In: Wild medicinal plants in Egypt. Edits. K. H. Batanouny, S. Abou Tabl, M. Shabana and F Soliman, *Swiss Development Co-operation* (*SDC*), Switzerland.

Bessonova, E. (1989). Adverse effects of herbal drugs in dermatology. *British Journal of* Dermatol., 143 (9):23–29.

Byeon, S., Pelley, R., Ullrich, S.E., Waller, T.A., Bucana, C.D. and Strickland, F.M. (1988). Aloe barbadensis extracts reduce the production of interleukin-10 after exposure to ultraviolet radiation. *J Invest Dermtol*.;110:811–7.

Cerdan M, Sanchez-Sanchez A, Oliver M, Juarez M and Sanchez-Andreu JJ. **(2009).** Effect of foliar and root applications of amino acids on iron uptake by tomato plants. *Acta Hortic*., 830: 481 - 488.

Chaicharoenpong, C., Petsom, A. *(*2009*).* Quantitative thin layer chromatographic analysis of the saponins in *Haplophyllum tuberculatum. Phytochem. Anal. 20:*253*–255.*

Chithra, P., Sajithlal, G. and Chandrakasan, G. (1998). Influence of aloe vera on the glycosaminoglycans in the matrix of healing dermal wounds in rats. *Journal of Ethnopharmacology*, 59:179–86.

Chithra, R., Sajithlal, G.B. and Chandrakasan, G. **(1998)**. Influence of aloe vera on collagen characteristics in healing dermal wounds in rats. *Mol Cell Biochemistry*, (181):71–76.

Conard, H., Saltman, P. and Eppley, R. **(1959).** Effect of auxin and gibberellic acid on the growth of *Ulothrix* . *Nature*., 184: 556-557.

Danhof, I.E. (1987). Remarkable Aloe: Aloe through the Ages. Grand Prairie, TX: *Omnimedicus Press*.

Davies, P.J. (2010). The plant hormones: their nature, occurrence and function. In Davies PJ (Eds). Plant hormones biosynthesis, signal transduction, action, *Kluwer Academic Publishers,* Dordrecht, Boston, London, pp. 471-492.

de Kroon, H. and Hutchings, M.J. *(*1995*).* Morphological plasticity in clonal plants: The foraging concept reconsidered. *Journal of Ecology,* 83*:*143–152.

Ea, D.Z., Bashir, A. K., Veres, A., Dobos, N., Nagy, I., Mathe, G. and Blunden J.R. (2005). Essential oil of *Haplophyllum tuberculatum* (Forssk.) A. Juss. from the United Arab Emirates. *J. Essent. Oil Res*., 17: 519-521.

El-Naggar , E.B.;. El-Darier S.M.; El-Mekanen A.S.; Švajdlenka E. and Emlièka M. (2014). Chemical Composition of Essential Oil of *Haplophyllum tuberculatum* (Rutaceae) Grow Wild in Different Habitats of Egypt. *Global Journal of Pharmacology,* 8 (3): 385-393.

Ertani A, Cavani L, Pizzeghello D, Brandellero E, Altissimo A, Ciavatta C and Nardi S. **(2009).** Biostimulant activity of two protein hydrolyzates in the growth and nitrogen metabolism of maize seedlings. *J. Plant Nutr.Soil Sci.,* 172: 237 - 244.

Fanali, J., Mandal, A. A., Raj Kumar, R. and Chordia, A. **(2010).** Role of biologically active aminoacid formulations on quality and crop productivity of tea (*Camellia* sp.). *International Journal of Agricultural Research*; 4: 208 - 216.

Foidle, R.H., Murphy, L.S. and Donalua, R.L. (2001). The effects of Moringa leaf extract on growth and yield of strawberry. relationship. *Journal of Plant Nutrition and Bio-technology,* 16: 478 - 481.

Gilpin, L. (2001). Methods for analysis of benthic photosynthetic pigment. School of Life Sciences, Napier University. Retrieved 2010-07-17.

Grindlay, D. and Reynolds, T. (1986). The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. J. Ethnopharmacology, 16:117–121.

Heggers, J., Kucukcelebi, A., Listengarten, D., Stabenau, J., Ko, F. and Broemeling, L.D. (1996). Beneficial effect of aloe on wound healing in an excisional wound model. *J Altern Complement Med*., 2: 271–277.

Hutter, J.A., Salmon, M., Stavinoha, W.B., Satsangi, N., Williams, R.F. and Streeper, R.T. (1996). Anti-inflammatory C-glucosyl chromone from Aloe barbadensis. *J Nat Prod*., 59: 541–543.

Idris, T.I.M., Ibrahim, A. M.A., Mahdi, E. M., and Taha A. K. (2011). Influence of argel (*Solenostemma argel* Del. Hayne*)* soil applications on flowering and yield of date palm (*Phoenix dactylifera* L.*). .Agriculture and Biology Journal of North America,* 2(3):538-542.

IJAS (2011). *International Journal of Agriculture Systems*. Propagation of Orchids. Irvine: Department of Developmental and Cell Biology, University of California.

Jeffrey, S.W.; Shibata, A., Kazuo, E. (1969). Some spectral characteristics of Chlorophyll a and c from *Tridacna crocea* Zooxanthellae *Biological Bulletin* (Marine Biological Laboratory) **136** (1): 54–62.

Khalid, S.A. and P.G. Waterman, F. (1981). Alkaloid, lignan and flavonoid constituents of *Haplophyllum tuberculatum* from Sudan. *Planta Med*., 43: 148-152

Kohata, K., Yamauchi, Y., Ujihara, T. and Horie, H. *(*2004*).* Inhibitory activity of tea-seed saponins and glyphosate to weed seedlings. *Jarq-Japan Agri Res Quarterly* 38*:*267–270.

Lui, Y., Tanizawa, H. and Takino, Y. (2007). Studies of *aloe vera*: Mechanism of cathartic effect. *Biol Pharm Bull*., 17: 651–653.

Marker, A.F.H. **(1972).** The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. *Freshwater Biology* **2**: 361.

Marshall, J.M. (1990). Aloe vera gel: What is the evidence? *Pharmacological Journal*, 24:360–2.

Mohamed, A.H., M.B. Ali, A.K. Bashir and A.M. Salih, **(1996).** Influence of *Haplophyllum tuberculatum* on the Cardiovascular System. *Pharm. Biol*., 34: 213-217.

Mølgaard, P., Chihaka, A., Lemmich, E., Furu, P., Windberg, C., Ingerslev, F. and Halling-Sorensen, B. *(*2000*).* Biodegradability of the molluscicidal saponins of Haplophyllum tuberculatum. *Regul. Toxicol. Pharmacol. 32:*248*–255.*

Morikawa, T., Matsuda, H., Li, N., Li, X. and Yoshikawa, M. *(*2007*).* Bioactive Saponins and glycosides—Part 29—Acylated oleanane-type triterpene saponins*:* Theasaponins A(6), A(7), and B-5, from the seeds of *Camellia sinensis. Helv. Chim. Acta* 90*:*2342–2348.

Ndhlala, A. R., du Plooy, C. P. and Abdelgadir, H. A. (2016). The potential of *Aloes* as medicine and cosmetic ingredients in South Africa. Abstracts of workshop: (*Linking Sudanese Medicinal and Aromatic Plants Producers to the Global Markets*), Coral Hotel, Khartoum, Sudan, 22-24 March, 2016. Pp. 25.

Nouman, W., Siddiqui, M.T. and Basra, S.M.A. (2012). *Moringa oleifera* leaf extract: An innovative priming tool for rangeland grasses. *Turkish Journal of Agriculture and Forestry*, 36: 65–75.

Omidbeigi, R. Deepti, P., Singh, R. and Subashkumar, G. (2009). Production and Processing of Medicinal Plants, vol. 4, *Astan-e-Ghods Publications*, Iran.

Park, J.Y., Mitrou, P.N., Luben, R., Khaw, K.T. and Bingham, S.A. (2009). Is bowel habit linked to colorectal cancer? Results from the EPIC-Norfolk study. Eur J. Cancer, 45:139–145.

Ramachandra, S. and Srinivasa, K. (2008). Priming seeds for improved viability and storability in *Raphanus sativus* cv. Chinese pink. *Indian Journal of Plant Physiology,* 6: 271–274.

Ro, J.Y., Lee, B., Kim, J.Y., Chung, Y., Chung, M.H. and Lee, S.K. (2000). Inhibitory mechanism of aloe single component (Alprogen) on mediator release in guinea pig lung mast cells activated with specific antigen-antibody reactions. *Journal of Pharmacol. Exp Ther*., 292:114–121.

Sato, Y., Ohta, S. and Shinoda, M. (1990). Studies on chemical protectors against radiation XXXI: Protective effects of *Aloe arborescens* on skin injury induced by x-irradiation. *Yakugaku Zasshi*.,110 (8):76–84.

Sims, I., Whitehouse, P. and Lacey, R. *(*2009*).* The Euphorbia growth inhibition test *(*Environmental Agency R&D Dissemination Centre, *Technical Report EMA* 003, Bristol, USA*).*

Townsend, **K.H. (1996).** Plants of potential medicinal value, In: Wild medicinal plants in Egypt. Edits. K. H. Batanouny, S. Abou Tabl, M. Shabana and F. Soliman, *Swiss Development Co-operation (SDC),* Switzerland.

Tyler, V. **(1993).** The honest herbal: A sensible guide to the use of herbs and related remedies. *Pharmaceutical Products Press,* 12(3): 75-84.. Binghamton, New York:
.

Tyler, V. (1993). The honest herbal: A sensible guide to the use of herbs and related remedies. 3rd ed. Binghamton, New York: *Pharmaceutical Products Press.*

Wagentrisl, H. *(*2003*)*. Use of the plant growth enhancer *Quiponin* in vegetable growing. *Nor-Natur APS and Eco-Trade. 1-10 (*Nor-Natur APS, Copenhagen, Denmark*).*

Zhanhi, X., Ervin, E. H. and Schmidt, R. E. **(2009).** Recovery effects of *Aloe arborescens* on skin injury induced by x-irradiation. . *J. Med Crop Sci*., 43: 952- 956.

Table 1: Impact of Haza water extracts on number, length, width and thickness of leaves and number of tillers of *Aloe vera* L. plants.

Haza trt. (g/plant)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)	No. of tillers
Control	14.0c	31.74d	4.14c	1.66a	18.4c
Boiled extract	19.8a	48.34a	5.32a	1.14a	30.8a
Hot extract	17.8b	41.86b	4.64b	1.04a	26.6b
Cold extract	14.8c	39.58c	4.12c	0.92a	20.6c
LSD.	1.461	2.391	0.33	0.709	2.512
C.V.	8.66	5.45	7.58	14.04	10.05

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 2: Impact of Haza water extracts on number of roots and root length, shoot and root fresh and dry weights of *Aloe vera* plants.

Haza trt. (g/plant)	No. of roots	Root length (cm)	Shoot fwt (g)	Root fwt (g)	Shoot dwt (g)	Root dwt (g)
control	26.2c	13.78d	0787.8c	14.36d	078.0c	2.02b
Boiled extract	40.2a	27.7a	1746.0a	26.16a	173.4a	2.82a
Hot extract	30.8b	23.82b	0899.7b	23.08b	090.5b	2.72a
Cold extract	27.2c	19.96c	0782.3c	19.22c	078.5c	1.88c
LSD.	3.347	1.894	38.03	2.172	5.081	0.1991
C.V.	9.97	7.45	3.34	8.82	4.41	7.19

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 3: Impact of foliar applications with Haza water extracts on leaf, gel and peel weights and chlorophyll 'a' and 'b' contents of *Aloe vera* plants.

Haza trt. (g/plant)	Leaf fwt (g)	Gel wt (g)	Peel wt (g)	Chlorophyll (mg/g) a	b
Control	196.2c	112.0d	090.0d	14.06c	06.76c
Boiled extract	261.0a	152.4a	108.6b	22.54a	15.28a
Hot extract	256.2a	144.6b	115.0a	22.30a	14.84ab
Cold extract	239.0b	137.6c	101.4c	18.60b	13.60b
LSD.	11.13	4.73	4.37	1.68	1.34
C.V.	4.87	3.23	4,19	8.27	10.41

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 4: Impact of Haza soil applications on number, length, width and thickness of leaves and number of tillers of *Aloe vera* L. plants.

Haza trt. (g/plant)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)	No. of tillers
control	14.0c	31.74d	4.14d	1.10a	18.4d
02.5	14.4c	41.26c	3.90d	1.22a	19.2d
05.0	16.4b	45.56b	4.46c	1.12a	26.2b
07.5	18.0a	51.66a	4.96ab	1.22a	30.4a
10.0	18.2a	47.20b	5.20a	1.66a	23.6c
LSD.	1.461	2.391	0.35	0.799	2.612
C.V.	7.63	5.46	7.58	12.04	10.55

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 5: Impact of Haza soil applications on roots number and length, fresh and dry weights of shoot and root of *Aloe vera* plants.

Haza trt (g/plant)	No. of roots	Root length (cm)	Shoot fwt (g)	Root fwt (g)	Shoot dwt (g)	Root dwt (g)
control	26.2c	13.78e	0787.8e	14.36d	078.00e	2.02 d
02.5	21.6d	18.94d	0981.9d	14.56d	098.28d	1.76 e
05.0	25.0c	20.96c	1007.0c	24.50c	108.30c	2.82 c
07.5	38.6a	27.78a	1180.0b	32.98b	120.80b	3.60 b
10.0	31.8b	24.72b	1624.0a	39.96a	162.60a	4.26 a
LSD.	3.347	1.894	38.03	2.172	5.081	0.1997
C.V.	10.93	8.41	3.34	8.82	4.41	7.19

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 6: Impact of Haza soil applications on leaf, gel and peel weights and chlorophyll 'a' and 'b' contents of *Aloe vera* plants.

Haza trt. (g/plant)	Leaf wt (g)	Gel wt (g)	Peel wt (g)	Chlorophyll (mg/g) a	b
Control	196.2d	112.0d	090.0c	14.06d	06.76d
02.5	242.2c	146.0c	104.2a	18.44c	10.66c
05.0	251.2ab	148.6c	102.0b	20.26b	12.78b
07.5	259.8a	153.8b	102.4b	20.96b	12.48b
10.0	263.2a	161.6a	101.8b	23.38a	15.46 a
LSD.	12.13	4.725	1.372	1.679	1.341
C.V.	4.87	3.23	4,19	8.27	10.41

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 7: Impact of foliar and soil applications of Haza on the number, length, width and thickness of leaves and number of tillers of *Aloe vera* plants.

Haza trt.	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)	No. of tillers
Control	14.0d	31.74e	4.14ef	1.66a	18.4d
Boiled extract	19.8a	48.34b	5.32a	1.14a	30.8a
Hot extract	17.8bc	41.86d	4.64cd	1.04a	26.6b
Cold extract	14.8df	39.58d	4.12ef	0.92a	21.6d
02.5 g	14.4d	41.26d	3.90f	1.10a	19.2d
05.0 g	16.4c	45.56c	4.46ce	1.22a	26.2bc
07.5 g	18.0b	51.66a	4.96bc	1.12a	30.4a
10.0 g	18.2b	47.20bc	5.20ab	1.22a	24.6c

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 8: Impact of foliar and soil applications of Haza on number of roots, root length, shoot and root fresh and dry weights of *Aloe vera* plants.

Haza trt.	No. of roots	Root length (cm)	Shoot fwt (g)	Shoot dwt (g)	Root fwt (g)	Root dwt (g)
Control	26.2c	13.78e	0787.8e	078.00g	14.36f	2.02d
Boiled extract	40.2a	27.70a	1646.0a	173.40a	23.08d	2.72c
Hot extract	30.8b	23.82b	0899.7d	090.50f	26.16c	2.82c
Cold extract	27.2c	19.96cd	0782.3e	078.50g	19.22e	1.88de
02.5 g	21.6d	18.94d	0981.9c	098.28e	14.56f	1.76 e
05.0 g	25.0c	20.96c	1007.0c	108.30d	24.50cd	2.82 c
07.5 g	38.6a	27.78a	1180.0b	120.80c	32.98b	3.60 b
10.0 g	31.8b	24.72b	1624.0a	162.60b	39.96a	4.26 a

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.

Table 9: Impact of foliar and soil applications of Haza on leaf, gel and peel weights and chlorophyll 'a' and 'b' contents of *Aloe vera* plants.

Haza trt.	Leaf wt (g)	Gel wt. (g)	Peel wt. (g)	Chlorophyll (mg/g)	
				a	b
Control	196.2d	112.0f	090.0d	14.06d	06.76e
Boiled extract	261.0a	152.4bc	108.6b	22.54ab	15.28a
Hot extract	256.2a	144.6cd	115.0a	22.30ab	14.84ab
Cold extract	239.0c	141.6d	101.4c	18.60c	13.60bc
02.5 g	242.2bc	146.0c	104.2c	18.44c	10.66d
05.0 g	251.8ab	148.6c	102.0c	20.26bc	12.78c
07.5 g	259.2a	153.8b	102.4c	20.96bc	12.48c
10.0 g	263.2a	161.6a	101.8c	23.38a	15.46 a

* Means with the same letter (s) in the same column are not significantly different at 95% confidence limits.