بسم الله الرحمن الرحيم

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Stimulation of Lime's (*Citrus aurantifolia*) Seedlings Growth by Macro and Micro-nutrients, Hormones and Botanical Treatments

تحفيز نمو بادرات الليمون بالمغذيات الكبرى والصغرى والهرمونات
والمعاملات النباتية

A thesis submitted in partial fulfillment of the requirements of the Master of Science degree in Horticulture

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Dedication

I dedicate this work to my father, mother, brothers and sisters...

with love

Acknowledgement

Praise is to Allah the almighty who gave me the health, strength and patience to conduct this study.

I would like to express my gratitude to my supervisor Dr. Tagelsir Ibrahim Mohammed for his guidance, encouragement, help and patience throughout the term of this work.

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Abstract

NPK, micro elements, growth regulators namely BA and GA_3, and Argelwere tested in various concentrations to enhance the growth of lime's seedlings. Each seedling was planted in 40X20 cm plastic bag. In each test, the complete randomized block design was used with 5 replicates where each treatment was composed of 2 seedlings. Treatments were repeated every 3 months after data recording.

The 2 g NPK proved to be the most the satisfactory fertilizer treatment. It resulted in seedlings that can satisfy the desired seedling quality traits for transplanting in the field at the second reading.

Regarding micro elements, B and Fe resulted in tallest seedlings, while circumference and leaf length were equally increased by Zn, Fe and Mn. Boron was the best promoter of the number of branches and leaves.

The test of BA and GA_3 concentrations revealed the advantage of BA over GA_3 in promoting growth attributes; and in particular the 20 mg/l BA was the most effective treatment as it resulted in considerable increments in most measured parameters of seedling growth.

According to the final reading, the 15 and 20 g Argel treatments enhanced seedling height substantially and the reverse was true for the number of branches per seedling. The 10 g treatment resulted in widest circumference, while the 20 g resulted in the highest number of leaves. The 15 g treatment was the best enhancer for leaf length and width. The overall growth of seedlings was significantly stimulated by Argel applications.

المستخلص العربي

تم اختباركل من NPKوالعناصر الصغري ومنظمات النمو(GA_3 ، BA) ونبات الحرجل بتراكيز مختلفه لتحفيز نمو شتلات الليمون. تمت زراعة الشتلات في اكياس بلاستيكية مقاس20X40 سم.

تم استخدام تصميم القطاعات كاملة العشوائية وكررت المعاملات 5 مرات وكل معاملة يحتوي علي 2 شتله، وكررت المعاملات كل 3 اشهر بعد اخذ القياسات.

الجرعة 2جرام من سماد NPK كانت الأفضل بين المعاملات لتحسين صفات الجوده النوعيه لشتلات الليمون منذ القراءة الثانية.

بالرجوع للعناصر الصغري فان البورون والحديد اعطياأعلي زيادة في طول الشتلات بينما زاد محيط ساق الشتلات وطول الاوراق زيادة متساوية باستخدام كل من الزنك والحديد والمنجنيز.وادي البورون الي تحفيز وزيادة فى عدد الافرع والاوراق.

في اختبار تراكيز BAو GA_3 وجد ان BA قد تميز علي GA_3 في تشجيع صفات النمو خصوصا التركيز 20 ملجم/لتر BA اذ كان افضل المعاملات اثرا في معظم قياسات نمو الشتلات.

بالرجوع لمعاملات الحرجل فان الجرعات 15 جرام و20 جرام سجلت أعلي طول للشتلات واقل عدد فروع في الشتلة. واعطت معاملة 10 جرام حرجلاًكبرزيادة في محيط ساق الشتله .20جرام حرجل اعطت أكبر عدد اوراق ومعاملة 15 جرام حرجل كانت افضل محفز لطول وعرض الورقه.عموما اضافة الحرجل اثرت معنويا في كل مقاييس نمو الشتلات.

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CHAPTER ONE

Introduction

Lime (*Citrus aurantifolia* Swingle) belongs to the family Rutaceae. It is native of the East Indies and has spread all over the world in tropical and sub tropical regions (Elsheikh, 2005). Citruses come next to grapes as for world production. They are of high economic value as due to the diversity of their uses (Ali, 1997). Major commercial citrus growing areas include southern China, the Mediterranean region, South Africa, Australia, the southernmost United States, and parts of South America. In the U.S., Florida, Texas, and California are the major producers (Douglas, 2005). The major Arab producing countries are Egypt, Morocco, Syria, Algeria, Sudan, Lebanon, Jordan, Tunisia, Saudi Arabia and Yemen (AOAD, 2007). The five commercially important citrus fruit crops (sweet orange, tangerine, grapefruit, lemon, and lime) are recognized as single species within Citrus. Limes rank second to lemons in terms of importance as flavoring agent for foods, drinks and other non-edible products for home and industrial use (Sauls, 1998).

Nurseries are the critical step for the provision of the transplants of the desired cultivars in the required amounts and time. The awareness of nurserymen about the standard practices would enhance the growth of transplants and in consequence would shorten the marketing cycles. little attention is given for growth enhancing factors like fertilizers, hygiene and plant growth regulators in Sudan's fruit nurseries. Previous studies on mature fruit trees revealed the need of these crops for diversity of fertilizers in adequate amounts for enhanced growth, yield and quality (Idris *et al*., 2011). Besides, the few studies on growth regulators proved the advantages of such practices as tools for improving propagation and growth at the nursery stage (Abdorabo *et al*., 2016; Omer *et al.*, 2016). In the last decade, Sudan's government perceived the importance of the horticultural crops as sources of foreign currency upon export, and it therefore encouraged expansions in this agricultural sector. This situation necessitates genuine efforts to improve propagation methods, cultural practices and observation of hygiene at the nursery stages for the provision of the required types of fruit transplants in sufficient quantities and sound vigor.

Sudan grows a diversity of citrus species and cultivar for local consumption and limited exports, but the agro-climatic conditions are largely in favor of lime (*Citrus aurantifolia* Swingle) and grapefruit (*Citrus paradisi*) (Dawoud and Ahmed, 2017). The yield of lime in Sudan is low (6-7 tons / hectare) compared to the actual potential (Ahmed, 2003). This might be attributed to low nutritional inputs, inadequate cultivation, the use of infected planting materials, use of non-budded lime planting material, planting of unimproved cultivars of scions and rootstocks (Elamin, 2004) and inadequate use of chemicals for pest and disease control (Dafalla, 2004). However, within the last 5 years, epidemic spread of the citrus bacterial canker in different states of Sudan had been observed on lime. Deterioration in yield had been reflected on the price of this commodity. Renewal of lime grooves might be a need (Prof. Dafalla, G.; Plant Pathologist; University of Gezira, Sudan; personal communication, 2019). Such move requires hygienic planting material with desired vigor and excellent agronomic traits.

Nutrition is an essential component for citrus growing. There are 13 mineral nutrients that are known to be required for plant growth and development. If one or more of the 13 mineral elements are present in the soil in excessive amount, then toxicity can occur and plant growth may be reduced. Conversely, if one or more of the essential elements are in short supply, then a deficiency can result and plant growth may be reduced (Street and Gammon, 1978). Fertilization rates are commonly based upon seedling nutritional status usually determined by using a combination of diagnostic methods. The three main methods of diagnosis include visual observation, leaf tissue and soil analyses (Plucknett and Sprague, 1989). Best fertilization strategies to increase yields and quality of citrus crops are of great significance for economical growth. Citruses are perennial and evergreen plant with many flowers. They require a large amount of nutrients. Applying proper types and amounts of fertilizers has been shown to enhance the growth, increase yield and improve quality of citrus (Yu, 2000; Wen and Cai, 2001; Chen, 1990). A balanced nutrition of macro and micro-nutrient elements is among factors influencing growth and development of plants. Satisfaction of such needs would enhance growth and reduce the time required for marketing of lime and other fruits transplants.

Besides, the juvenility hormones should be active and in adequate levels to accelerate the growth of fruit crops transplants. If deficient or inactive, the growth would be impaired. Beneficial growth responses were frequently reported upon exogenous applications of growth regulators. Cytokinins enhance cell division, while auxins result in expansion of cell wall. Gibberellins are recognized for their role to elongate cells and internodes (George *et al.*, 2008). Some chemicals occurring naturally within plant tissues (i.e. endogenously), have a regulatory, rather than a nutritional role in growth and development. These compounds, which are generally active at very low concentrations, are known as plant hormones (or plant growth substances). Synthetic chemicals with similar physiological activities to natural plant growth substances, like GA_3 and BA are compounds having the ability to modify plant growth by some other means.

In conformity with global trend to avoid or minimize the use of synthetic agricultural chemicals, the use of botanicals as bio-pesticides or growth stimulants had been frequently reported under the conditions of Sudan (Sidahmed *et al.*, 2009; Idris *et al.*, 2011 and 2014). Argel (*Solennostemma argel* Del Hayne) had been proposed as a potent bio-pesticide (Eldoash *et al.*, 2011; Taha *et al.*, 2012) and a growth enhancer for numerous horticultural genotypes (Idris *et al.*, 2011 Hamid, 2016; Eisa, 2016).
As the existence of lime had been threatened by an epidemic spread of the citrus bacterial canker which may end in eradication of lime grooves in Sudan, intensive effort to replace the infected trees is badly needed. Shortening the nursery stage would be a benefit. Therefore, the objectives of this research were to investigate the influence of NPK, micro-elements, growth regulators and soil application of Argel plant for growth stimulation of lime's (*Citrus aurantifolia*) seedlings at the nursery stage.

CHAPTER TWO
LITERATURE REVIEW

2.1. Genus Citrus:

Citrus fruits plants belong to the family *Rutaceae* which is a large family that includes 130 genera (Samson, 1986). All commercial citrus fruits belong to the subfamily *Aurantioideae* and the tribe *Citreae*. There are three important genera of commercial value in the subfamily Aurantioideae, *Poncirus, Fortunella* and *Citrus* **(**Freitas, *et al.*, 2003). A high degree of cross-fertility exists between the species of Citrus as well as between the genera of *Citrus*, *Fortunella*, and *Poncirus.* This has permitted breeders to develop various simple and multiple hybrids, some of which have become of considerable economic importance (Cameron and Soost, 1969). The genus *Citrus* is divided into two very distinct subgenera, *Citrus* and *Papeda*, easily distinguished by leaf, flower, and fruit characteristics. The subgenus *Citrus* includes all the commonly cultivated species fruits like oranges, lime, mandarins and grapefruit, all of which have pulp-vesicles filled with pleasantly acid, subacid, or sweet juice, free or almost free, from droplets of oil. On the contrary, none of the species of citrus belonging to the subgenus *Papeda* have edible fruits, as the pulp-vesicles have dense aggregations of droplets of acrid oil that form an axile column and usually give the juice a very disagreeable, acrid, bitterest flavor (Webber and Batchelor, 1943and (Hussien, 2007).

2.2. Lime (*Citrus aurantifolia*):

The limes include both acid or sour limes and the acidless or sweet limes, but in addition the sour limes consist of two kinds, the small-fruited Indian, West Indian, Key lime or Mexican lime and the large-fruited Tahiti or Persian lime, both of which have very few varieties. The West Indian or Mexican lime (*Citrus aurantifolia* Swingle) has numerous local names. In Egypt and the Sudan it is called Limûn Baladi **(**Morton, 1987).

2.2.1 Origin and distribution of citruses:

The genus Citrus is one of 33 genera in the sub – family *Aurantioideae* of the family *Rutaceae*. The taxonomy and geographic origin of the *Aurantioideae* has been reviewed by Swingle and

Reece (1967). Citrus and related genera are natives to South East Asia (north-eastern India, southern China, Indo-Chinese peninsula) which is the center of diversity for these species. Tanaka (1954) proposed theoretical dividing line (the Tanaka line) which runs south east worldly from the north west border of India, above Burma, through the Yunnan Province of China, to the south of Hainan Island. Citron, lemon, lime, sweet and sour oranges and pummelo originated south of this line, while mandarin, Kumquat, and trifoliate orange originated north of the line. The mandarins apparently developed along a line north east of the Tanaka line, along east China, through Formosa and Japan, while the trifoliate orange and Kumquat developed in a line crossing south – central China in an east west direction. Gmitter and Hu (1990) have proposed that Yunnan in China through which the Tanaka line runs, is itself a major center of origin for citruses. Some related Aurantioideae genera are natives to Asia, Africa and Australia. Moreover, Gmitter and Hu (1990) reported that, cultivation of citruses dates back to 2200 B.C.Citrus seeds were taken to the Mediterranean area, and according to Samson (1986), the citron was the first introduction, followed by sour oranges, then lemon and lime. Limes are almost exclusively tropical, while lemons thrive in cooler climates. Mexico and the West Indies are the major producers of lime and distilled lime oil. Limes are also grown in other parts of Central America, south Florida, south Texas and southern California (Lenug and Foster, 1996). Citrus species were introduced to northern Sudan from Egypt in 1886 (Bedri, 1984). Citrus individuals have spread to other parts of the country following succeeding introductions. The lemon or lime juice could be used to individuals with diets low in vitamin (Grieve, 1971).

2.2.2 Botanical Description of limes:

Lime is an ever green tree small, spiny and irregularly branched. Leaves are small, elliptic to oblong with pale green color. The flowers are white, small and produced in auxiliary clusters. The fruit is small, roundish and thin-skinned. The pulp is greenish and has about ten sections. The juice is acidic with distinctive flavor (Elsheikh, 2005). The lime tree is of exceptional vigorous growth and may be considered a shrub as its height ranges from 2 to 4 m, with many slender spreading branches and it usually has numerous, very sharp auxiliary spines about 1 cm long (Morton 1987). When the seed germinates, the primary root is the first organ to appear in deep soil, the primary roots whish has not been severed in transplanting, grows straight downwards and becomes the tap root. Secondary roots are of two types: large pioneer roots and bunches of fine fibrous roots. The pioneer root are large, they form with the primary roots the root system framework. The fibrous roots occur in small bunches 20 to 30 cm long on the tap root of the young seedling and on pioneer roots of the older trees (Walter *et al*.,1968).

The evergreen, alternate leaves are pleasantly aromatic with winged petioles. The auxiliary flowers have 4 to 6 oblong spreading petals, white but purple-tinged when fresh, and 20 to 25 bundled white stamens, with yellow anthers.

The fruit is borne singly or in 2 or 3 cluster (some times large cluster). The twig tips are round, obviate, or slightly elliptical, some time with slight nipple at the apex, the base is round or faintly necked, 2.5 to 5 cm, in diameter. The peel is green and glossy when immature, pale yellow when ripe. The fruit is thick, about 1.5 to 3 mm; the pulp is green-yellow in 6 to 15 segments which do not readily separate. The seeds are few and small and the juice is very acid (Morton, 1987).

2.2.3 Environmental Requirements:

2.2.3.1 climate:

The suitable climate for citruses is tropical and subtropical humid region of the world. The fruit achieves its highest flesh quality in subtropical humid climates or drier regions with irrigation (Riger, 2002). Lime seems to be the citrus best spacies adapted to all environments (Thasman and Whistler, 1996). Mean maximum temperature of hottest month is 31- 32˚ C (88- 90˚ F) in Florida and optimum day time is 25-35˚C (77-86˚F), but temperature can reach 43˚C (110˚ F) in southern California and other citrus growing regions. The fruit is killed by 30 minutes of temperature at -3- -2˚ C (26-28˚ F). Stems and leaves can be killed after few minutes at -7 to -3˚ C (20-26˚F). This is dependent on pervious climatic conditions and age of fruit, leaves and branches (Riger, 2002). The Mexican lime is more sensitive to cold and can thrive well in warm, moist climates with relative humidity from 10-80% and with annual rainfall between2o3 -381 mm (Morton, 1987; Ibrahim 2013)

2.2.3.2 Soil

Lime trees are well adapted to soils having good internal and surface drainage. Growth on heavy clays or poorly–drained soils will be reduced and problematic as limes do not tolerate flooding conditions. Nutritional deficiencies can occur on soils high in calcium (Sauls, 1998). Extremely light sandy soil or very heavy clay soils are unsuitable. The best pH range is 5.5 -6.0 for sandy soil. Lime is more tolerant to alkalinity than most other citrus species. Mexican limes are more sensitive to cold than lemon and is drought tolerant than other citrus species (James, 1993).

2.2.4. World production:

Citruses are extremely important fruit crops. According to FAO (2014), total world production of citrus fruits was estimated as 121 million metric tons, and the major citrus producing countries were USA, China, Mexico and Spain.

The world production of lemon and lime was estimated at 13,032,388 tons in 2007. The largest lime and lemon producing countries were Afghanistan, Albania, Algeria, American Samoa, Andora, Angola, Anguila, Antigua and Barbuda, and Argentina (FAO, 2009).

2.2.5. Importance:

The juice of citrus fruits contains sugars and acids. The relative proportions of sugar and acid vary between the species. Lime and lemons contain higher proportion of acid than sugar. Citric acid occurs in all species. The juice also contains soluble pectin which gives the juice a cloudy and colloidal appearance. It also contains mineral salts, glycosides and small amount of protein. The citruses are all excellent sources of vitamin C, ranging between 35 to 70 mg per 100 gm fresh weight (Watt and Merrill, 1975).

The lime juice is used for food flavoring, preparation of fruit drinks, appetite and added to alcoholic beverages. Lime oil from the peel is used in perfumery, cosmetics and confectionaries. Lime juice has traditional medical uses as a purgative, anti-poisonous against snake and scorpion bites and for curing mouth and gum inflammations. The peel has pleasant aroma and is of cosmetic value. Lime extracts have been studied for their ability to repel mosquitoes (Das *et al.*, 2003) and insecticidal effects were reported against mosquitoes, cockroaches and house flies (Ezeonu *et al.*, 2001).

2.2.6 Varieties and types:

Among the renowned types of lime are the following:

1. Mexican lime: It is also known as Key lime and West Indian lime.
2. Tahiti lime: It is also called Bears lime and Persian lime. Bears is believed to have originated from seeds of citrus transported from Tahiti to Sanfrancisco. The tree is larger than Mexican lime, reaching (6 m) under optimum conditions. The fruit is normally seedless, although one or two seeds may occur.
3. Giant Key lime: It was released by ARS -USDA in 1994. The major difference in this lime is that its fruits are more than twice the size of common Mexican lime.

4. Rangpur lime: Has a large fruit that resembles mandarins rather than lime. The fruits are highly acidic, very seedy with loose thin rind it is primarily used as root stock for grafting other citrus species.

5. Palestine sweet lime: It is not a true lime. Its fruits are pale yellow, juicy and sub acid in flavor. It is primarily used as root stock, although there is some production in the Mediterranean's, India and Latin America.

6. Limequats: They are hybrids between Mexican Lime and Kumquat (Sauls, 1998).

2.2.7. Varieties of Mexican lime:

There are few varieties of the Mexican Lime, except for several spineless selections. The main varieties are:

1. Everglade: a seedling of Mexican lime, pollinated by flowers of a grapefruit or pumelo. It is Lime-like, elliptical with fairly nipple at apex, peel light - yellow when ripe, very juicy pulp, of excellent quality and texture.

2. Kagzi: Commonly cultivated throughout India. It is represented by numerous subtypes differing slightly in size, shape and color.

3. Palmetto: A selected seedling from Mexican lime pollinated from the 'Sicily ' lemon; small sized aromatic fruit, elliptical or nearly round with small nipple at apex, pulp light greenish – yellow, tender , very juicy , of fine quality, with sparightly acid flavor, usually having 3 to 6 seeds.

4. Young (spineless Mexican): Of unknown origin, was introduced to California from Mexico by George Young around 1882 (Morton, 1987).

2.2.8.Lime propagation

Propagation of well-grown, compatible, disease-free, true to type citrus tree is very important in citrus industry. Citrus can be propagated sexually by seed or by vegetative means (Ali, 1997).

Basic types of propagation

There are two type of lime propagation

Sexual propagation

Sexual propagation involves the union of male and female sex cells, the formation of seed, and the creation of individual new genotypes.

Asexual propagation

Asexual propagation involves reproduction from vegetative parts of plant and is possible because the vegetative organs of many plants have the capacity for regeneration (Hartman and kester, 1983).

Lime tree can be propagated by vegetative propagation , like grafting, budding, layering, micro propagation and cutting.

2.2.9. Pests and Diseases:

The major pests affecting the acid lime and lemon plants in the Sudan include**:** Lemon butterfly, leaf eating caterpillars, whitefly, leaf miner and mealy bugs (Elamin, 2004). Among the diseases Gummosis and canker are very devastating. For control of Gummosis, use ring and basin system of irrigation to avoid contact of water with main stem so that the disease causing soil borne fungus will not attack the stem. If disease is seen, remove the affected portion of bark and 1 cm of surrounding healthy bark with knife and apply Bordeaux paste thoroughly on the wound. Canker is caused due to bacterial (*Xanthomonas* sp.) infection, which develops considerably during rainy season. It appears as a minute yellowish-brown spots on stem, leaves and fruits which turn into corky brown specks. Affected leaves turn yellow and drop down. Remove all the affected parts and fallen leaves and burn them (Dafalla, 2004)

2.2.10. Chemical constituents:

The main constituents in the distilled lime oil are a-pinene, b-pinene, myrcene, limonene, terpinolene, ceneole, linalool, borneol, citral and traces of neral acetate and geranyl acetate (Lawless, 1992). Limonene was regarded as the major volatile component (Giga and Munetsi, 1990). Further aroma compounds are terpineol, bisabolene and other terpenoids (Lawless, 1992).

2.2.11. Edible uses:

The juice fruit can be used fresh and the fruit can be frozen or canned. Lime juice and leaves play a role in oriental cookery as an ingredient in beverages, ice cream and baked food (Rehm and Espig, 1991). Lime oil is utilized by the food industry to give flavor to carbonated and alcoholic beverages, hard candy, gelatins, puddings, frozen dairy desserts, baked foods, meat, and meat products (Grieve, 1971; Leung, 1996).

2.2.12. Medicinal uses:

Fruit and leaves are used in traditional medicine. The young leaves are used for **cough and** headache relief. The boiled leaves extract has beneficial effects on blood vessels, muscles, and other bodily organs (Perry, 1980). Lime juice is regarded as an antiseptic, tonic, an astringent, and as a diuretic in liver ailments, a digestive stimulant, a remedy for intestinal hemorrhage and hemorrhoids, heart palpitations, headache, convulsive cough, rheumatism, arthritis, falling hair, bad breath, and as a disinfectant for all kinds of ulcers when applied in a poultice (Morton, 1987). D-Limonene has been determined to inhibit mammary tumors in rats and increase the activity of detoxifying enzyme when given orally to mice (Lam and Zheng, 1991).

2.2.13. Other uses:

Lime oil is used externally in cosmetics as a fragrance and as a fixative in detergents, creams, lotions, perfumes, and soaps (Leung, 1996). Oil extracts have also been studied for their ability to repel mosquitoes (Das, *et al*., 2003), and their insecticidal effect against mosquitoes, cockroaches, and houseflies (Ezeonu, 2001). In tropical Africa, lime twigs are popular chew sticks (Morton(1987; Abdelmuttalib, 2009)

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2.3. Factors affecting citrus growth:

2.3.1.Climatic factors:

Climate is the most important factor determining growth. Citrus have been grown successfully in humid tropics, arid and semi-arid tropics and subtropical climates. The most important climatic factor limiting citrus culture is the minimum temperature. The optimum temperature for growth is between 30-32°C. Growth stops completely at a maximum temperature of 48°C and at a minimum temperature of 13°c (Samson, 1986). In tropics, citrus trees grow rapidly, bloom often and set fruit all the year around. A prolific bloom usually comes with heavy rain after a dry spell. In subtropical climates with cool winters, there is a definite season of production because the rhythm of flowering and growth is controlled by low temperature in winter and citrus trees tend to bloom in the spring only (Reuther, 1977). Southwick and Daveport (1986) reported that chilling temperature (15°c) during winter caused dormancy, which induced flowering when followed by warm temperatures. Under the hot climate in the tropics and adequate moisture supply, most of citrus species flower throughout the year (Sidahmed and Geneif, 1984).

The prevailing relative humidity is another climatic factor that affects citrus fruit quality. It is generally recognized that fruits of all citrus varieties tend to be smoother, thinner skinned and contained more juice when grown in an atmosphere of fairly high humidity. Low humidity causes the fruit to be smaller, the axis longer, and the rind rougher, thicker and more easily peeled. The pulp is also low in juice content but richer in flavor because of greater acidity (Reuther, 1977).

Photoperiod has no effect on citrus flowering (Lenz, 1964 and Moss, 1969). However, light intensity is a very important climatic factor, which affects citrus growth, yield and fruit quality (Goren and Monselise, 1969).

2.3.2. Soil types:

The ideal soil for citrus growth is a medium- textured soil, uniform, reasonably deep and fertile, having good internal drainage and free from injurious salts (Platt, 1973). However, citrus trees grow on a wide range of soils from coarse sands to well drained heavy clay. The best soil pH for citrus ranges from 5-7. However, citrus trees have been grown successfully at a pH of 8.3-8.4 (Samson, 1986).

2.3.3. Nutritional factors:

Crop nutrition is an essential component to tropical fruit crop production systems. There are 13 mineral nutrients that are known to be required for plant growth and development .If one or more of the 13 mineral elements are present in the soil in excessive amount, then toxicity can occur and plant growth may be reduced. Conversely, if one or more of the essential elements are in short supply, then a deficiency can result and plant growth may be reduced (Street and Gammon, 1978). Fertilization rates are commonly based upon tree nutritional status which is usually determined by using a combination of diagnostic methods. The three main methods of diagnosis include visual observation, leaf tissue and soil analyses (Plucknett and Sprague, 1989). Best fertilization strategies to increase yield and quality of citrus crops are of great significance to economical growth. Citrus tree is perennial and evergreen plant with many flowers. They require a large amount of nutrients. Applying proper types and amounts of fertilizers has been shown to enhance the growth, increase yield and improve quality of citrus fruit (Yu, 2000; Wen and Cai, 2001; Chen, 1990).

2.3.3.1.Effect of Nitrogen on Citrus Growth:

Nitrogen is most commonly applied as solid mineral or foliar spray fertilizers. Organic fertilizers such as blood and bone, manures and composts are also potential sources of nitrogen

(Mooney *et al*. 1991; Mooney and Richardson 1992). The response of any particular site to nitrogen saturation is affected by numerous factors including species, composition, nitrogen availability, soil characteristics, and land-use history (Aber *et al*, 1998). Environmental and soil factors may control nitrogen uptake efficiency in citrus. Soil pH affects the rates of several reactions involving N and can influence the efficiency of N use by plants. Nitrification, or the conversion of ammonium (NH_4^+) to nitrate (NO_3^-) by soil bacteria, is most rapid in soils with pH values between 7 and 8. Nitrification approaches zero below pH 5 (Norvell, 1991). At high irrigation rates, appreciable amounts of N can be supplied to the crop. However, crop utilization of this dilute N source appeared to be low, and additional fertilization was required to sustain high growth (Maurer *et al.*, 1995; Wheaton *et al.*, 1997). High application rates of water increases canopy growth but, in some cases, reduces leaf N concentrations due to increased N leaching (Wheaton *et al*., 1997).

Nitrogen is a constituent of amino acids, proteins, chlorophyll, alkaloids, amides, and other compounds in the plant. It occurs chiefly in organic combinations, although traces of unassimilated ammonia and nitrate nitrogen may be found sometimes in certain tissues.
Perennial citrus store large quantities of N within various plant components, which can be utilized for tree growth in subsequent seasons (Legaz *et al*, 1995). The annual vegetative growth of citrus trees contains a variable proportion of the fertilizer N applied during the growth period. A great amount of N in the new growth may be drawn from the tree biomass. Therefore, the N reserve in the leaves and structural components play an important role in the development of new flushes of growth in the spring (Kato, 1986). Nitrogen is a constituent of proteins, chlorophyll, and other essential components of plants, therefore acute lack or shortage of this element brings vegetative growth to a halt and results in a general bronzing and yellowing of foliage, followed by dieback of twigs and decreased growth. In lemons particularly, green leaves may develop yellowish, irregular, blotchy areas before becoming entirely yellow. In addition, older leaves may develop vein chlorosis or "yellow vein". This condition can also be caused by root rotting, branch injury, calcium deficiency, and other disorders. Sometimes a yellow-vein condition of old leaves will develop when a particularly vigorous new shoot has emerged or much fruit has been borne on the shoot. This symptom is somewhat less conspicuous in oranges and other species than lemons, but it does occur. If the

leaves are not too old, restoration of nitrogen sometimes will cause such leaves to regreen fully or partially. Completely yellow leaves, if not too old, will regreen when nitrogen becomes available; if temperature conditions are favorable, abundant new growth will commence. New leaves of trees moderately or acutely deficient in nitrogen are apt to be thin and fragile when young, and the angle between stem and leaf may be narrower than when nitrogen is ample (Dasberg, 1987)

2.3.3.2 Phosphorus (P)

Phosphorus has many important functions in citrus seedling, 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 *et al*., 2006).

Nematian *et al.,* (2011) reported that, Aloe plants treated with Phosphorus fertilizer in doses of 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). Similar effect of P application on *Aloe vera* were reported by Luis Rodolfo *et al.,* (2002), Boroomand *et al*. (2011) and Nejatzadeh *et al.,* (2011). The Shoot development requires the production of leaves by shoot apical meristems and their subsequent expansion. In a study on *Phaseolus trilobus* , (Kumar *et al.*, 2002) reported decreased number of leaves upon phosphorus deficiency which implies changes in leaf initiation and activity of shoot apical meristem, while plant height was increased due to increased phosphorus fertilization at 80 kg P_2O_5 /ha^{-1}. Besides, Anilkumar *et al*. (2001) reported significant increase in height of mustard plants fertilized with. In addition, 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. 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.

2.3.3.3 Potassium (K)

Potassium (K) is required as a cofactor for 40 or more enzymes. It is required for many physiological functions, such as formation of sugars and starch, synthesis of proteins, normal cell division and growth, neutralization of organic acids, regulating carbon dioxide supply by controlling stomatal opening and improving efficiency of sugar use, overcoming environmental stress such as frost by decreasing the osmotic potential of cell sap.

Potassium is a useful nutrient in citrus fertilization, it affects fruits production, size and quality. There are no much positive requests on production, but it is recognized as the most important element for citrus fruits quality (Alva and Tucker, 1999; Aso, 1974; Cohen, 1983; Quin *et al*., 1996). Its deficiency is nowadays associated with the citrus variegated chlorosis (Malavolta, 1994).

2.3.3.4. Iron (Fe)

Iron is a micronutrient and is a constituent of cytochromes, non-haeme iron proteins, involved in photosynthesis, and N2 fixation and respiratory linked dehydrogenises. It is also involved in the reduction of nitrates and sulfates, the reduction in peroxidase and adolase. The increase in carbonic anhydrase activity is considered effective marker of Fe -deficiency.

2.3.3.5.Manganese (Mn)

Manganese is one of the redox micronutrients. It is absorbed by the plant roots in the form of Mn^{+2} and is required for the activity of some dehydrogenases, decarboxylases, kinases, oxidases, peroxidases, and non-specifically by other divalent, cation-activated enzymes, and is required for photosynthetic evolution of O2, besides involvement in production of amino acid and proteins. Manganese has a role in photosynthesis, chlorophyll formation and nitrate reduction, and is indispensable for the synthesis of ascorbic acid, emerging from secondary effects of the fertilizer. A metalloenzyme peroxidase concentration is considered the marker of Mn deficiency. Accumulation of xylose and increased activity of peroxidase are considered useful biochemical markers of Mn deficiency in citrus in addition to reduced activity of phenylanine lyase, tyrosine ammonia lyase, and polyphenol oxidase.

2.3.3.6 Zinc (Zn)

Zinc is an essential constituent of many enzymes such as alcohol dehydrogenase, glutamic dehydrogenase, lactic dehydrogenase, carbonic anhydrase, regulating equilibrium between carbon dioxide; alkaline phosphatase, carboxypeptidase, and other enzymes such as dehydropeptidase and glycylglycine dipeptidase for protein metabolism. It also regulates water relations, improves cell membrane integrity, and stabilizes sulflahydryl groups in membrane proteins involved in ion transport. Zinc deficiency is a common problem world over. Zinc deficiency is probably the most diffused nutritional alteration in all citric production areas. It is especially prevalent in sandy soils but also frequently in alkaline soils, and can be aggravated by high level of phosphate or nitrogen fertilization (Aso, 1974; Langthasa and Bhattacharyya, 1995). In general, soil or foliar zinc applications improve trees conditions and make deficiency symptoms decrease, although yield does not always increase (Aso, 1974; Rodríguez *et al*., 1994). Fruits quality improvements (more saccharose contents, better rind texture) are reported (Langthasa and Bhattacharyya, 1991; Quin *et al*.,1986).

2.3.4 Hormones and growth regulators :

Hormones are chemicals occurring naturally within plant tissue (i.e. endogenously), having regulatory rather than nutritional role in growth and development. These compounds are active at very low concentrations and are known as plant hormones (George *et al*, 2008). There are synthetic chemicals with similar physiological activities as hormones and are called growth regulators. There are several recognized classes of plant growth regulators, and the most recognized are only five groups namely: auxins, cytokinins, gibberellins, ethylene and abscissic acid. Auxins and cytokinins are by far the most important for regulating growth and morphogenesis in plant tissue culture.

2.3.4.2 Cytokinins:

Cytokinins application to a single site in the plant (e.g. to one leaf) causes the treated organ to become an active sink for amino acids. The effect of cytokinins in plant where they are used, often with auxins, stimulates cell division and controls morphogenesis (George *et al*., 2008). Cytokinins overcome apical dominance and release lateral buds from dormancy (George *et al*., 2008). Cytokinins activate RNA synthesis, protein synthesis and activities of some enzymes (Kulaeva, 1980).

Cytokinins occur as free molecules in plants, but are also found in cytoplasm and chloroplast. In whole plants, roots appear to be the major sites of natural cytokinin biosynthesis (Chen *et al.*, 1985). Some experimental evidence that the root tip is the primary site of cytokinin synthesis was obtained by Ochatt and Power (1988) in roots developed from callus of *Prunus ceraus*. Cytokinins produced in roots of plants are normally transported in the xylem to other regions. The bleeding sap of the plant is rich in cytokinins and has been shown to promote growth (Skene,1972; Zimmer and pieper,1975). In a wide range of citrus types, 6- benzylaminopurine (BAP) is the cytokinin used to initiate shoot organogenesis, used either singularly or in combination with NAA.

2.3.4.3.Gibberellins:

Various responses can generally be obtained in plant devoid of gibberellins, although their presence may be essential ingredient (Stuart and Street, 1971). The responses of GA_3 are almost of similar nature to those of auxins. High concentrations of GA_3 induce the growth (Gautam *et al*., 1983). It often diminishes or prevents the formation of adventitious shoots or somatic embryos (Sankhla *et al*., 1993). Gibberellins are heat-labile and are rarely used for proliferation, but are used to stimulate normal development of plantlets (Bhojwani and Razadan, 1983).

2.3.4.4 Argel:

Solenostemma argel, belongs to the *Asclepiadaceae* family. This family includes many wild growing medicinal plants (e.g. *Calotropis procera, S. argel, Leptadinea* spp). These plants are known to contain secondary metabolites such as alkaloids, cardinolides flavonoids etc., which are needed in manufacturing important pharmaceuticals. *Solenostemma* is a monotypic genus with *S. argel* (Del.) Hayne, *S. oleifolium* Bullock et Bruce, *S. triste* (Nees) K. Muelli. *S. argel* is known in the Sudan as Hargel. It is widely spread in the Sudan (El-Amin, 1990) and commonly found in the northern region between Bar Bar and Abuhamed in Northern State *(El-kamali, and Khalid, 1996)*. Sudan is regarded now as the richest soruce of this plant *(Ahmd, 2003). S. argel* is considered to be medicinally important in the Sudan, Libya and Chad *(Ahmd, 2003).* Argel leaves are used in herbal medicine for the treatment of some liver and kidney diseases and some allergies. It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica (*Tharib et al.*, 1986). Also, it is used as incense in the treatment of measles and sometimes crushed and used as remedy for supporting wounds. The leaves are infused to treat gastrointestinal cramps, stomach ache, colic, cold and urinary tract infections and are effective as anti-syphilitic if used for prolonged period of 40-80 days (Boulos, 1983). In the experiment conducted by (Idris *et al.*, 2011) on the effect of Argel soil application on date palms under Sudan's conditions, significant increments in yield, fruit length and width were gained as a result of Argel treatments. They owe the overall benefits of Argel applications to either a growth regulator-like effect or an insecticidal effect as proposed by Sid*ahmed et al.* (2011) or anti-microbial property of Argel as claimed by Elhady *et al.,* (1994). 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). However, this plant did not receive agronomic research attention, and such a move might be needed in the near future.

CHAPTER THREE

Materials and methods

3.1 Experimental site:

The experimental area was located within the nursery of the Plant Tissue Culture Laboratory of the College of Agricultural studies, Sudan University of Science and Technology at Shambat, Khartoum North (latitudes 15° 40′ N, longitude 32° 32′ E, and altitude 375 meters above sea level). Two months after germination, lime seedlings were planted in 25x40 cm plastic bags containing alluvial River Nile silt with a pH of 7.8. They were used a month later as plant materials for the test.

3.2 Experiments

3.2.1 Experiment (1):

The effect of NPK on growth of lime seedlings:

NPK fertilizer was applied as soil dressing in doses of: 0, 2, 4, 6 and 8 g/seedling.

3.2.2. Experiment (2):

The effect of micro-elements on growth of lime seedlings:

The following salts of microelements were tested as foliar treatments in concentration of 50 mg/l: Zn SO_4,. $7H_2O$; Mn SO_4. H_2O, Boric acid and Fe SO_4. $5H_2O$.

3.2.3 Experiment (3):

The effect of BA and GA_3 on growth of lime seedlings:

Benzyladenine (BA) and GA_3 were tested as foliar spray to run-off each in concentration of: 0.0, 20, 40, 80 and 160 mg/l.

3.2.4. Experiment (4)

The effect of Argel treatments on growth of lime seedlings:

The crushed dry leaves of Argel **(*Solenostemma argel* L.)** were tested as soil dressing in doses of: 0.0, 5.0, 10.0, 15.0 and 20.0 g/ seedling.

3.3 Experimental design:

The complete randomized block design with one factor (Gomez and Gomez, 1984) was used.

3.4 Replications:

Each treatment was replicated 5 times and each treatment was composed of two seedlings.

3.5 Repetition of treatments:

The seedlings of Balady lime were transplanted in bags in May 2015. The experiment was carried out in June 2015 and final data was recorded in March 2016. The treatments were repeated at 3 month interval after each reading.

3.6 Data collection:

Data were collected every three months after the first application of treatments for the following parameters:

***1*. Plant height (cm):**

Plant height was measured with a tape meter from soil level to shoot tip.

***2*. Circumference (cm):**

Seedling circumference was measured 10 cm above soil level with a vernier caliper,

***3*. Number of leaves per seedling:**

Number of leaves in each of the ten plants was counted.

***4*. Number of branches per seedling:**

Number of branches in each plant was counted.

***5*. Leaf length:**

Leaf length was measured from the tip of mid-rib to the base of leaf lamina above the degenerating leaflets) with a small ruler.

***6*. Leaf width:**

The width was measured from the widest central point of the leaf with a ruler.

3.7. Data analysis:

Collected data were analyze using Mstat-C computer program. The means were separated by Duncan's multiple range tests.

CHAPTER FOUR

Results

4.1 Experiment (1):

The effect of NPK on growth of lime seedlings:

The progress in seedling height as affected by NPK treatments is illustrated in (Table 1 and Figure 1). The lowest dose (2 g) ranked top in the first and second readings, but in the 3rd reading the best height was recorded for the 4 g treatment. Regarding the number of branches per seedling, all NPK treatments increased this parameter significantly over the control in the first and the second readings. In the first reading the 2 g treatment was superior compared to other treatments, while the highest number of branches was recorded for the 6 g treatment in the second reading and thereafter the fertilizer treatments either equaled the control or resulted in decline in this parameter. (Table 1).

Table (1). Impact of NPK treatments on seedling height and number of branches in lime's seedlings

Treatments g/seedling	seedling height (cm)			Number of branch per seedling		
	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
Control	30.28b	38.21b	50.12d	1.50c	4.40d	9.20a
NPK 2	34.27a	49.53a	70.80b	4.33a	6.10bc	9.50a
NPK 4	31.33b	41.75b	79.20a	2.33b	5.60c	6.50c
NPK 6	31.38b	38.49b	26.54c	2.50b	7.20a	8.60b
NPK8	30.67b	41.23b	51.46d	2.83b	6.50b	6.80c
CV%	3.85	9.99	1.67	21.6	11.68	5.98

*Means within a column with the same letter are not significantly different at 95% confidence limits.

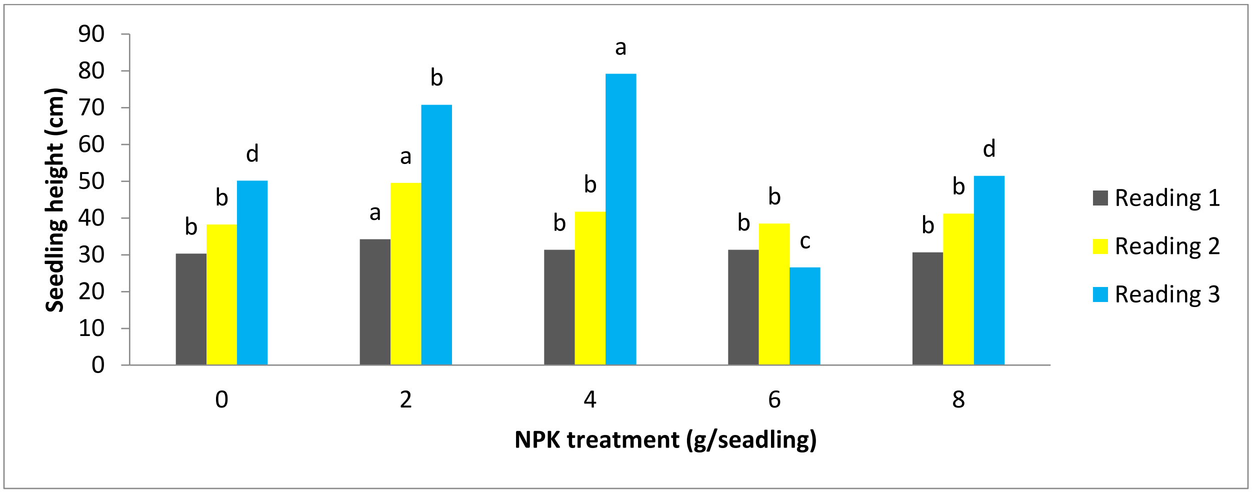


Figure (1). Impact of NPK treatments on the height of lime's seedlings

The progress in circumference of seedlings' stems and the number of leaves per seedling is presented in Table (2). All NPK treatments resulted in significant increase in circumference in the 3rd reading compared to the control. The highest value was recorded for the 4 g NPK treatment, while the 2 and 6 g treatments ranked second. Regarding the number of leaves per seedling, the 4 and 2 g treatments were the most enhancive for this parameter in the 3rd reading. However, the 8 g treatment reduced this parameter compared to the control (Table 2 and Figure 2).

Table (2). Impact of NPK treatments on the circumference and number of leaves in lime's seedlings

Treatments g/seedling	Transplant circumference			Number of leaves		
	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
Control	0.35b	0.49b	0.62d	23.50b	48.00d	127.0c
NPK 2	0.45a	0.57a	0.79b	34.17a	82.00a	181.0a
NPK 4	0.35b	0.48b	0.83a	24.33b	69.80b	178.5a
NPK 6	0.40ab	0.49b	0.79b	23.00b	70.30b	148.0b
NPK 8	0.38ab	0.48b	0.69c	23.00b	53.90c	118.6d
CV%	17.41	16.56	5.07	9.62	8.30	4.9

*Means within a column with the same letter are not significantly different at 95% confidence limits.

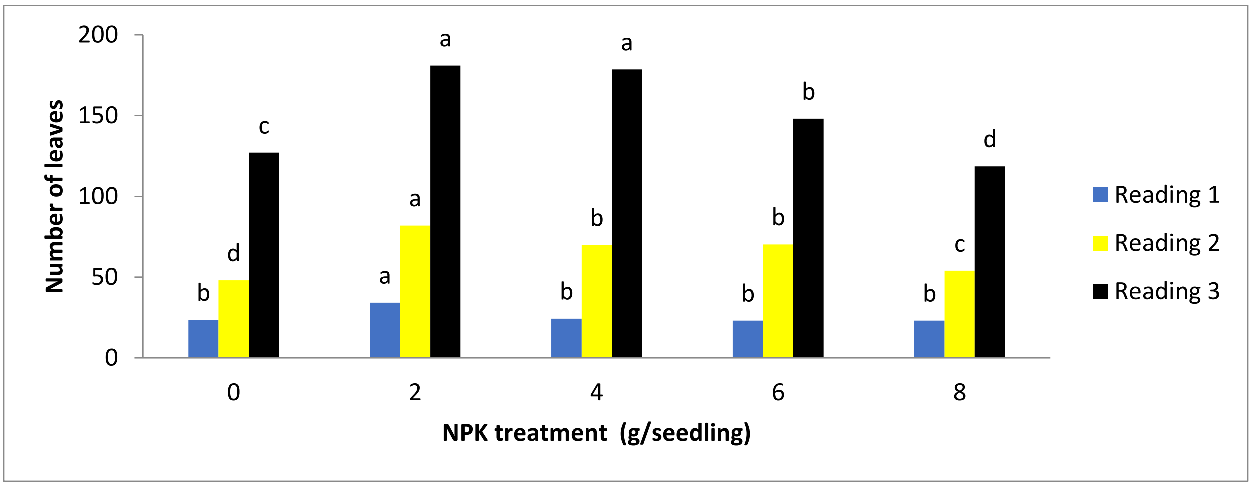


Figure (2). Impact of NPK treatments on number of leaves of lime's seedlings

Table (3) demonstrates the impact of NPK applications on the length and width of leaves. All fertilizer treatments resulted in significant increase in this parameter in the 3^{rd} reading at an equal statistical level compared to the control. However, the NPK treatments did not enhance the leaf width in the 3^{rd} reading compared to the control.

Table (3). Impact of NPK treatments on length and width of leaves in lime's seedlings

Treatments	Leaf length (cm)			Leaf width (cm)		
g/seedling	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
Control	7.15a	7.21b	7.31b	3.28ab	3.47ab	3.47a
NPK 2	7.33a	7.98a	8.07a	3.43a	3.74a	3.48a
NPK 4	6.63ab	6.73bc	8.30a	3.25ab	3.29bc	3.73a
NPK 6	6.90ab	6.37c	8.19a	3.32ab	3.45ab	3.60a
NPK 8	6.17b	6.62bc	8.31a	3.02b	3.08c	3.54a
CV%	9.14	9.73	6.58	8.92	10.18	7.22

*Means within a column with the same letter are not significantly different at 95% confidence limits.

4.2 Experiment (2):

The effect of micro-elements on growth of lime seedlings:

According to Table 4, all micro-elements did not affect the seedling height in the first two readings. In the 3rd reading, the microelements increased the height significantly over the control except Zn treatment. The micro-elements increased the circumference over the control significantly in the first and second readings. In the third reading, Fe equaled the control while the other treatments enhanced the circumference significantly compared to Fe and the control (Table 4).

Table (4). Impact of micro- elements treatments on seedling height and circumference in lime's seedlings

Treatments 50mg/l	seedling height (cm)			Transplant circumference (cm)		
	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
Control	30.15a	38.21a	50.62c	0.35b	0.48e	0.62 b
Zn SO_4, $7H_2O$	30.38a	40.28a	53.23bc	0.42a	0.73a	0.73a
Mn SO. H_2O	29.81a	38.34a	55.65ab	0.41a	0.65c	0.70a
B	30.06a	40.80a	58.77a	0.42a	0.71b	0.72a
Fe SO. $5H_2O$	3.45a	39.19a	59.49a	0.42a	0.51d	0.64b
CV%	11.48	11.24	7.40	15.74	13.14	6.81

*Means within a column with the same letter are not significantly different at 95% confidence limits.

The effect of micro-elements on the number of branches per seedling is shown in (Figure 3). Boron and zinc ranked top in the first reading. In the second reading boron, zinc and iron shared the top rank, while the highest number of branches was recorded for boron only in the 3rd reading.

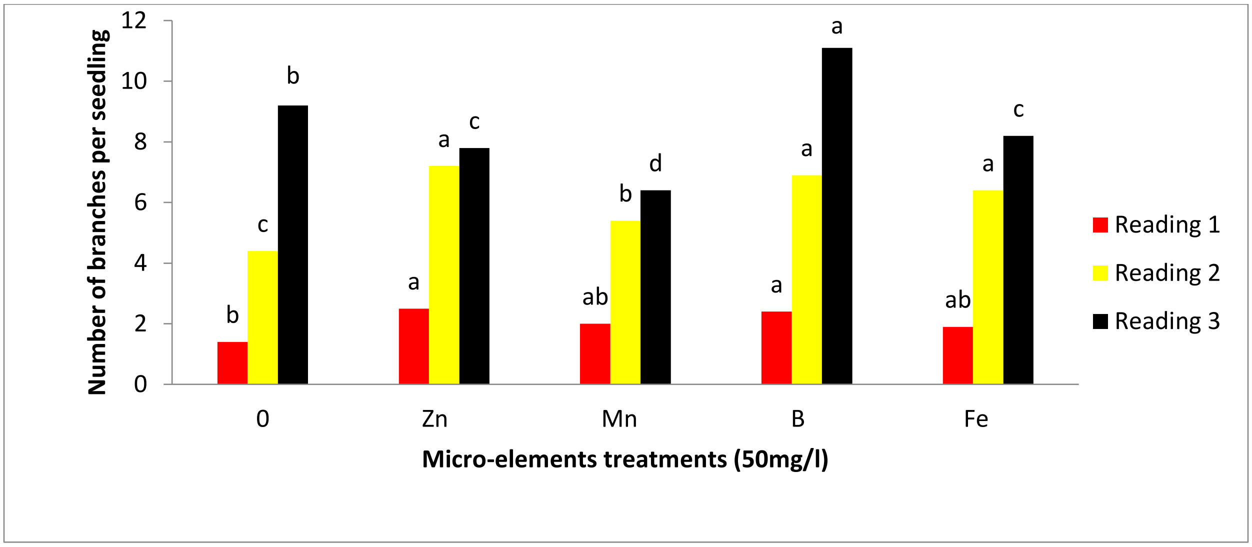
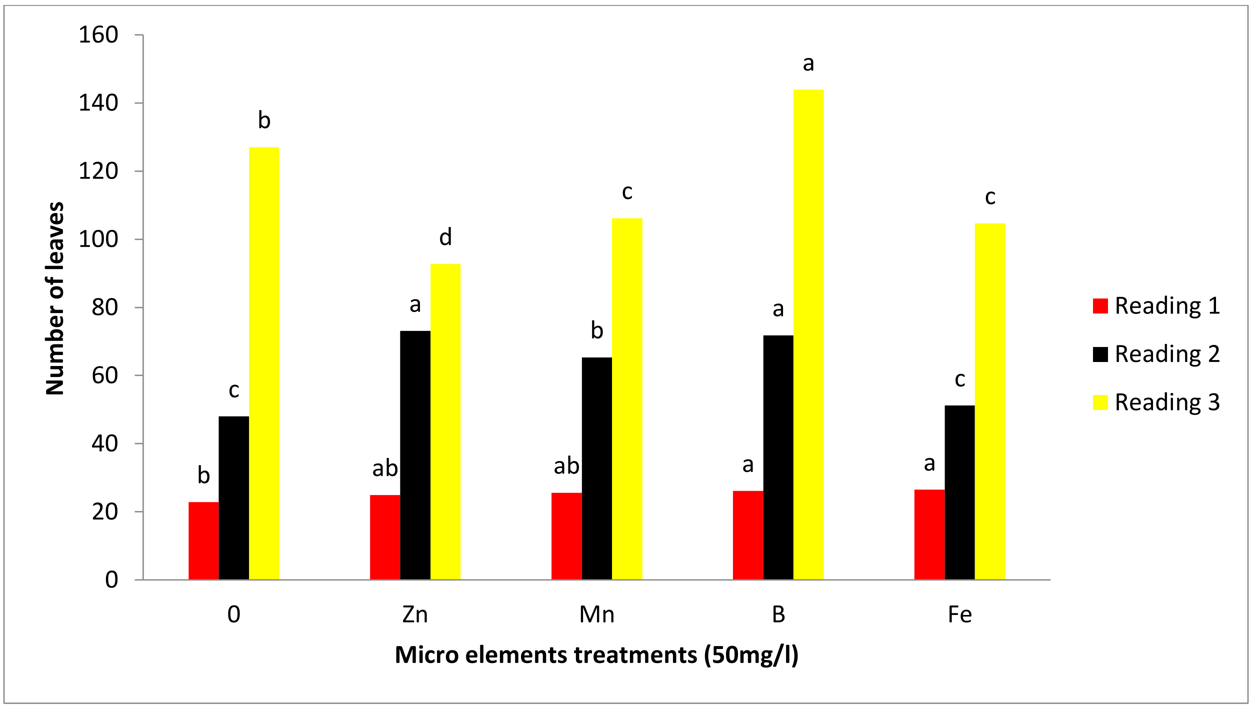


Figure (3). The Impact of micro-elements on the number of branches in lime's seedlings

As for the number of leaves per seedling, this parameter was best enhanced by boron and iron in the first reading. In the second reading, zinc and boron resulted in the highest number of leaves. In the final reading, the highest number of leaves was recorded for boron only (Figure 4).



Figure(4). The Impact of micro-elements on the number of leaves in lime’s seedlings

The leaf length was increased mildly by zinc in the first reading, and no difference was observed in the second reading. However, Mn, B and Fe increased this parameter significantly over the control in the third reading (Table 5). Zinc and boron resulted in slight but insignificant increase in width of leaves over the control in the first reading. In the second and third readings, the impact of micro elements on the leaf width did not differ from the control (Table 5).

Table (5). Impact of micro- elements treatments on length and width of leaves in lime's seedlings

Treatments	Leaf length (cm)			Leaf width (cm)		
50mg/l	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
Control	7.04abc	7.21a	7.31b	3.30ab	3.47a	3.47a
Zn SO_4, $7H_2O$	7.58a	7.18a	7.59b	3.54a	3.54a	3.44a
Mn SO. H_2O	6.50c	6.71a	8.28a	3.21b	3.46a	3.71a
B	7.13ab	7.18a	8.01a	3.46ab	3.52a	3.57a
Fe SO. $5H_2O$	6.68bc	7.01a	8.13a	3.20b	3.41a	3.55a
CV%	8.84	9.28	5.79	8.78	7.19	7.77

*Means within a column with the same letter are not significantly different at 95% confidence limits

4.3 Experiment (3):

The effect of BA and GA_3 on growth of lime seedlings:

The effect of BA and GA_3 on the seedling height is shown in (Figure 5). BA in concentration of 40 and 80 mg/l ranked top for this character in the first reading. In the second reading, the best height was recorded for BA in concentration of 20 and 80 mg/l, while the highest value for seedling height was obtained from 80 mg/l BA treatment, but without significant difference from the 20 and 40 mg/l BA and the 40 mg/l GA_3 treatments in the third reading .

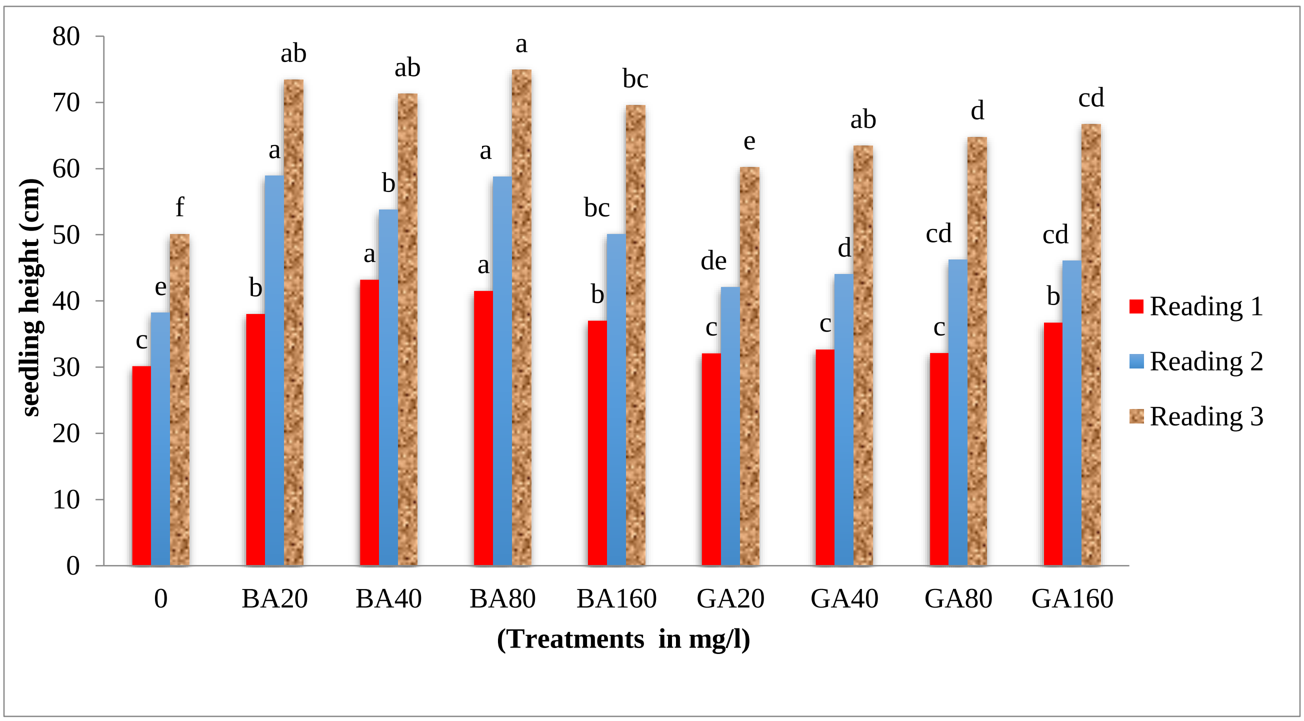


Figure (5). Impact of BA and GA_3 treatments on the height of lime's seedlings

Table (6) illustrates the effect of BA and GA_3 treatments on the number of branches and the circumference in lime's seedlings. The highest number of branches per seedling was recorded for BA at 20 mg/l and GA_3 in concentrations of 40 and 160 mg/l in the first reading. In the second reading, BA concentrations 20 and 80 mg/l were the best enhancers for the number of branches per seedling. However, this character was boosted significantly by the 40 mg/l BA treatment in the third reading.

Regarding seedling circumference, 20, 40 and 80 mg/l BA and 40, 80 and 160 mg/l GA_3 treatments increased this character significantly over the control in the first reading. In the second reading, only BA in concentration of 20 mg/l was the best enhancer for circumference. All growth regulator treatments increased the circumference over the control significantly in the third reading and the 20 mg/l BA treatment resulted in the thickest stems (Table 6).

Table (6). Impact of BA and GA_3 treatments on the number of branches and circumference in lime's seedlings

Treatments (mg/l)	Number of branches per seedling			Seedling circumference (cm)		
	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
Control	1.4c	4.4e	09.2b	0.35b	0.49e	0.62f
BA 20	3.6a	8.5a	09.1b	0.46a	0.67a	0.93a
BA 40	2.0b	9.0a	12.9a	0.46a	0.61ab	0.88b
BA 80	2.2b	6.6b	07.2cd	0.46a	0.61ab	0.83c
BA 160	2.5b	6.0bc	06.8de	0.41ab	0.56bcd	0.74e
GA_3 20	2.5b	6.2b	07.6c	0.41ab	0.50de	0.75de
GA_3 40	3.4a	6.3b	07.2cd	0.44a	0.54cde	0.76de
GA_3 80	2.0b	5.1d	05.9f	0.44a	0.55cd	0.79cd
GA_3 160	3.8a	5.4cd	06.6e	0.45a	0.58bc	0.78d
CV%	23.17	11.31	7.02	16.78	13.10	7.01

*Means within a column with the same letter are not significantly different at 95% confidence limits.

Regarding the number of leaves per seedling, this parameter was best enhanced by the 20 mg/l BA treatment in all readings, but it shared the top rank in the third reading with the 40 mg/l BA and the 160 mg/l GA_3 treatments (Figure 6).

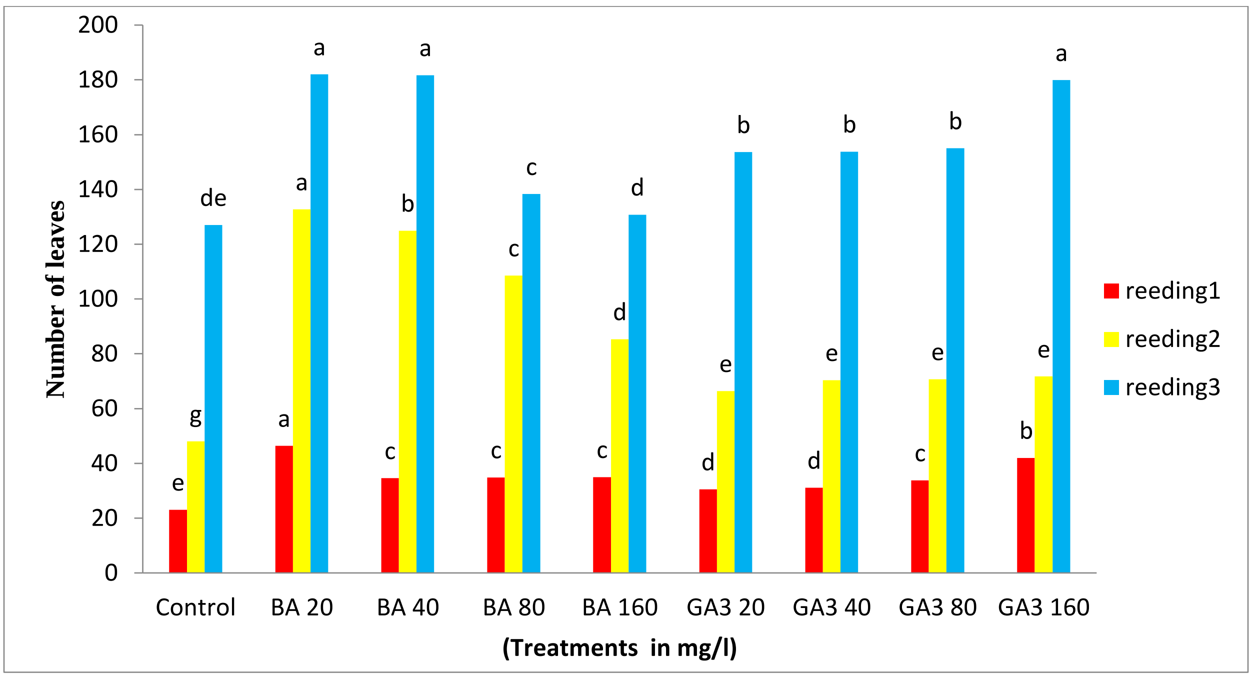


Figure (6). Impact of growth regulators treatments on the number of leaves of lime's seedlings

The leaf length was best increased by the 40 mg/l BA treatment in the three readings. The 20 mg/l BA treatment also performed alike. Both treatments excelled all treatments of GA_3 and the control (Table 7).

BA was found more effective in increasing the width of leaves, and in particular the 40 mg/l BA treatment was the scorer of the highest value for this parameter in all readings. The 20 mg/l BA treatment was not significantly different from the 40 mg/l BA treatment (Table 7).

Table (7). Impact of BA and GA_3 treatments on length and width of leaves in lime's seedlings

Treatments (mg/l)	Leaf length (cm)			Leaf width (cm)		
	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
Control	7.04c	7.12b	7.31cd	3.43c	3.47bc	3.50b
BA 20	7.73ab	7.89a	8.09a	3.65ab	3.84a	3.86a
BA 40	7.91a	8.07a	8.16a	3.75a	3.92a	4.01a
BA 80	7.09b	7.27b	8.05a	3.51b	3.75ab	3.82a
BA 160	6.90c	7.56ab	7.95a	3.33bc	3.60ab	3.68ab
GA_3 20	6.19d	7.31b	7.44 bc	3.05cd	3.38c	3.42b
GA_3 40	6.87c	7.12b	7.74ab	3.23bc	3.54bc	3.70ab
GA_3 80	6.80c	7.05b	7.73ab	3.30bc	3.45bc	3.48b
GA_3 160	6.75c	6.87b	7.780ab	3.39c	3.42bc	3.44b
CV%	8.28	9.51	5.97	9.15	8.37	9.25

*Means within a column with the same letter are not significantly different at 95% confidence limits.

4.4 Experiment (4):

The effect of Argel (*Solenostemma argel* L.) soil applications on growth of lime's seedlings:

The progress in seedling height is illustrated in (Figure 7), The impact of Argel application was most evident in the final reading as all Argel treatments resulted in significant enhancement of height compared to control. The highest doses (15 and 20 g) shared the top rank inducing 50 and 45% increments in this parameter respectively. The highest value was recorded for the 20m g BA treatment

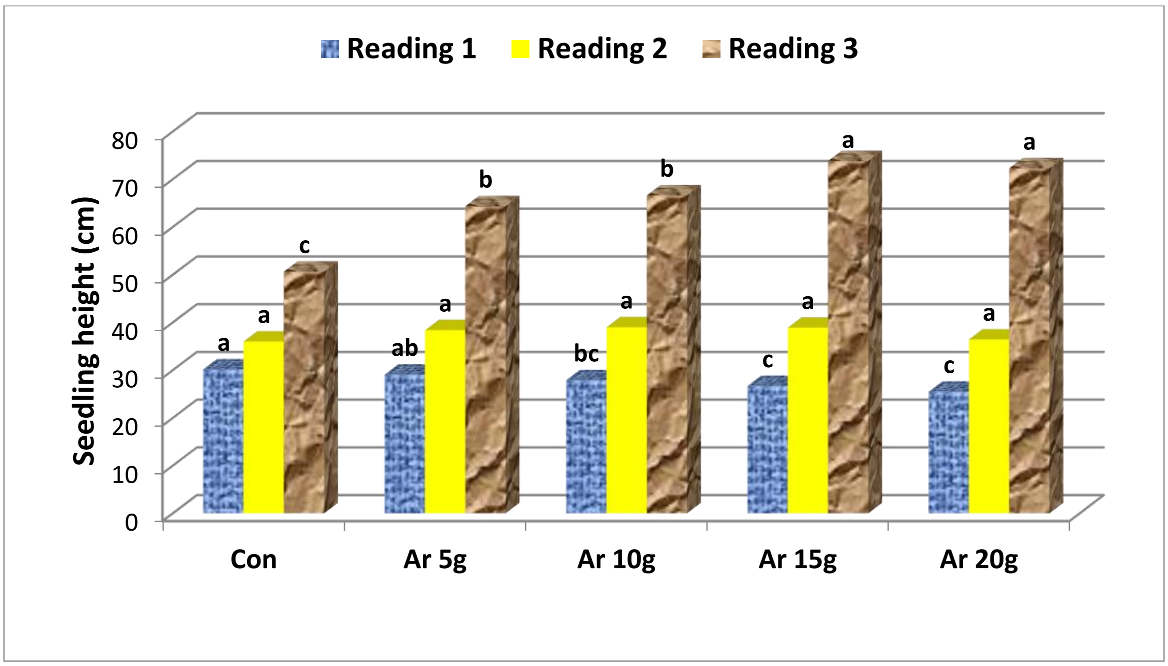


Figure (7). Impact of Argel treatments on the height of lime's seedlings

Regarding the number of branches per seedling, all Argel treatments increased this parameter significantly over the control in the first reading. In the second reading only the 10 g treatment was superior compared to the control that excelled all Argel treatments in the final reading. The deterioration was progressive with increase of Argel dose (Figure 8).

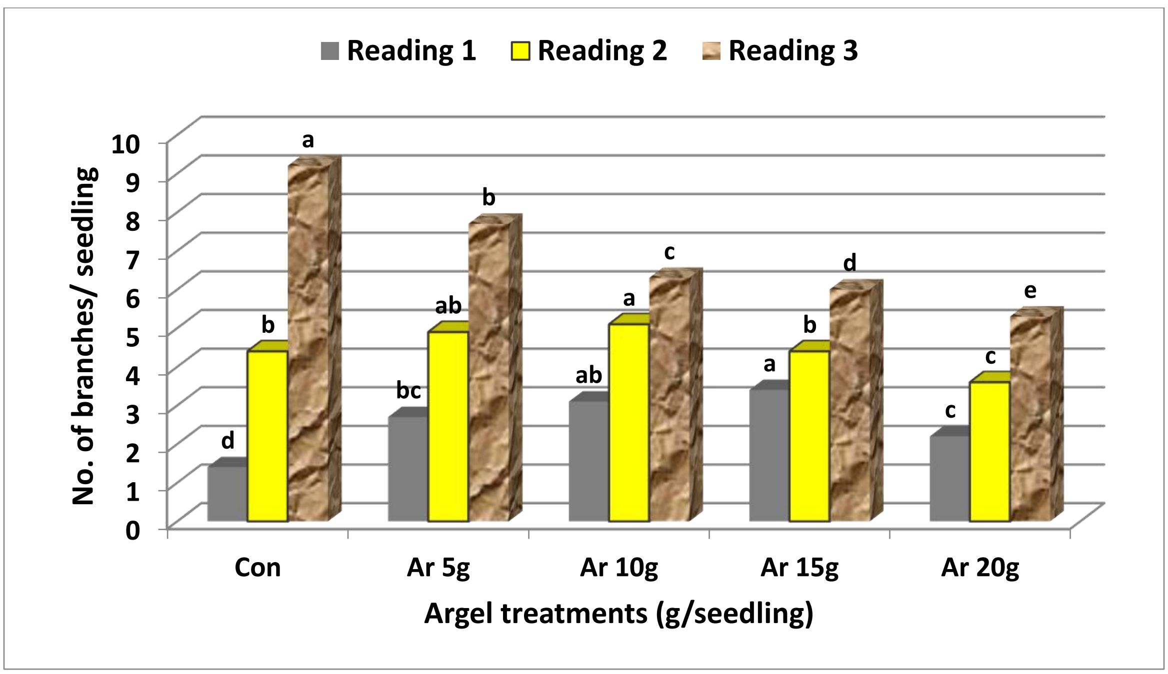


Figure (8). Impact of Argel treatments on branching of lime's seedlings

The increase in circumference of seedlings' stems and the number of leaves per seedling is presented in Table (8). Although the 15 g treatment resulted in about 33% increase of circumference in the first reading compared to control, no significant difference was observed among treatments in the second reading. However, the final reading revealed significant enhancements of this parameter by all Argel treatments in comparison with the control, and the 10 g treatment was the most enhancive. The values of the number of leaves per seedling were higher in Argel treated seedlings in all readings compared to control. The 10 g treatment ranked top in the first reading and also shared the top rank with the 15 g treatment in the second reading, but the highest value in the final reading was recorded for the 20 g Argel dose (Table 8).

Table (8). Impact of Argel treatments on the circumference and number of leaves in lime's seedlings

Argel Treatments (g//seedling)	Transplant circumference (cm)			Number of leaves		
	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
Control	0.36d	0.49a	0.62d	22.5c	48.0c	127.0c
5	0.42bc	0.46a	0.72c	27.1b	53.4b	100.2d
10	0.45ab	0.51a	0.85a	30.3a	59.9a	126.9c
15	0.48a	0.47a	0.80b	25.7bc	60.2a	138.1ab
20	0.39cd	0.49a	0.83ab	24.7bc	57.1ab	149.7a
CV%	12.8	16.06	5.45	12.99	7.65	5.39

*Means within a column with the same letter are not significantly different at 95% confidence limits.

Table (9) demonstrates the impact of Argel applications on the length and width of leaves. The differences in these parameters were quite clear in the final reading where the 15 and 20 g treatments resulted in best enhancements

Table (9). Impact of Argel treatments on length and width of leaves in lime's seedlings

Argel Treatments (g//plant)	Leaf length (cm)			Leaf width (cm)		
	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
Control	7.04a	7.32ab	7.31c	3.5ab	3.4a	3.5b
5	6.36b	6.75b	7.72b	3.1c	3.3a	3.6ab
10	6.89a	7.08ab	7.92ab	3.8a	3.5a	3.9a
15	6.92a	6.86b	8.21a	3.5b	3.3a	3.6ab
20	6.24b	7.48a	8.09ab	3.4ab	3.4a	3.92a
CV%	7.72	8.49	5.70	10.3	10.45	10.65

*Means within a column with the same letter are not significantly different at 95% confidence limits.

CHAPTER FIVE

DISCUSSION

Upon initial establishment of new lime grooves, Sudanese growers prefer seedlings of around 40 - 50 cm in height to avoid drowning of transplants, as flood irrigation is the most common practice in fruit orchards. Besides, a good stem girth and branching, coupled with numerous well expanded leaves are among seedling quality traits. These characters are normally obtained one year or more, after seed germination. In each test conducted in this study, substantial gains in quality characters of lime's seedling were obtained.

Nutrition management is among factors influencing growth and yield of fruit crops. By choosing appropriate fertilizer rates, the grower can drive a crop towards earlier and heavier fruit setting (Alva *et al*., 2006). The NPK is a compound fertilizer composed of three major essential growth elements; namely nitrogen, phosphorous and potassium. Adequate supply of these elements is important for citrus growth (Obreza *et al*.,2008). The seedling is the stage of juvenility; a phase of active growth in term of cell division. Nitrogen is the basic unit of amino acids that form protein, and protein is the basic building unit of new cells. Therefore, the biomass of plants is influenced by the adequacy of nitrogen (Thompson *et al.*, 2002; Menino *et al.*, 2003;Obraza and Zekri, 2008). Phosphorous plays vital roles in photosynthesis, respiration, formation of nucleic acids (Shapley *et al*., 1994). It is also recognized for its influence on the architecture and growth of the root system (Williamson*et al*., 2001; Brady and Weil, 2002). Therefore, an efficient root system will enhance absorption of soil solution which is composed of water plus salts of macro and micro nutrient elements. Potassium is among the macro nutrient elements, required as a cofactor for most plants enzymes, It is necessary for many physiological functions, such as formation and translocation of sugars and starch, synthesis of proteins, normal cell division and growth, neutralization of organic acids, regulation of carbon dioxide supply by controlling the opening and closure of stomata, and overcoming environmental stress such as frost by decreasing the osmotic potential of cell sap. In this study, the lowest NPK dose (2 g/ seedling) proved to be very satisfactory, as it boosted most measured characters to the desired levels. Economically, this treatment is the cheapest. This dose might have satisfied the nutritional requirement of the small seedlings for these elements. Positive impacts on growth upon fertilizing other plant genotypes with NPK had been frequently reported. According to(Mahmoud and Hichem 2011), enhanced growth was obtained in young citrus trees under greenhouse and field conditions when NPK was used for fertilization. Besides, growth stimulations in response to NPK applications had been reported by Boughalleb *et al*., (2011) on Eureka lemon, and Eisa (2016) on *Aloe vera* plants.

Plants vary in their demand for micronutrients, as these are involved in almost all physiological functions. Micronutrients are very important for optimal plant growth, physiological and biochemical pathways in citrus cultivation. They have enzyme-activating functions and play structural role in stabilizing proteins (Hänsch and Mendel, 2009). In this study, boron Stimulated seedling height, the number of branches and the number of leaves substantially and these are the most critical criteria for a qualitative lime seedling. Yet, Fe, Mn and Zn equally boosted the stem girth and leaf length. Therefore, the beneficial response to boron foliar application compared to the application of other micro-elements might owe to deficiency of this element in the soil or a high need of lime seedlings to boron at the juvenility growth phase. According to Rajaie *et al*., (2009), the soil pH is one of the most important factors that affects B uptake by plants. Gupta (1984) found that increasing soil pH by liming to above 6.5 reduces B concentration in many plants. The pH of the this test soil was 7.8, and the high pH might offer an interpretation to the good response of the seedlings to the foliar application of B. As high soil pH generally impairs the uptake of micro-elements from soils, the beneficial responses to the foliar application of other micro-nutrients might also be interpreted as compensation to the existing deficiency resulting from the elevated soil pH.

BA belongs to the cytokinin group of plant hormones which is renounced for its ability to promote cell division and increases the photo-synthetic efficiency (Pierik, 1987), while GA_3 and other forms of gibberellins induce elongation of cells, internodes and are involved in physiological processes such as flowering, set and development of fruits and seeds. The results of this study revealed better growth responses of lime's seedlings to BA compared with GA_3 applications. Both BA and GA_3 are considered juvenility hormones that influence the vegetative growth phase. The increments in measured growth parameters are therefore a function of cell division rather than cell elongation. Besides, the endogenous gibberellins content in BA treated plants might had been stimulated by the exogenous BA application, as synergism in juvenility hormones is a natural phenomenon (George *et al*., 2008). However, the use of 20 mg/l BA dose seemed most satisfactory as it resulted in saleable seedlings with optimum lime seedling quality characters at the time of the second reading.

Such practice can be adopted to reduce the time required to obtain a marketable material. Besides, this low dose is of economical advantage. while the reverse was true in the final reading. This may owe to supra-optimal accumulation of Argel with lime's tissues. However, contradictory explanations were reported on the type of hormone group within Argel tissues. Idris *et al*, (2010) overlaid ginger tissue cultures with high concentration of Argel extract and observed decline in tillering capacity of cultures coupled with elongation of shoots. They owed that to auxin constituent as auxins fortify the apical dominance and improve the expansion of cells walls. In contrast, Ahmed (2018) overlaid the *in vitro* cultures of sugarcane (*Saccharum officinarum*) with low concentration extract of Argel, and obtained striking increase in cultures prolific capacity. He claimed a cytokinin-like effect upon such application of Argel. Nevertheless, the targeted stimulations in this study were achieved but without solid interpretation of the active ingredients behind them other than the claims of Idris *et al.,* 2011 and 2014) that such enhancements might owe to growth regulator-like constituents in Argel. Yet, these results are confirmatory to the findings of preceding research reports claiming growth stimulations on different horticultural genotypes when treated with Argel (Eisa, 2016; Hamid, 2016; Idris and Modawi, 2016, Ahmed, 2018). A marginal remark can be added, as no symptoms of the bacterial canker were observed on the seedlings throughout the study period and thereafter, but the remark cannot be proposed as a mean of control unless considered in future studies by pathologists.

Conclusions and recommendations:

From the above stated results, growth and subsequently marketing of lime's seedlings can be enhanced by either of the following treatments:

1.NPK as a soil treatment in dose of 2 g per seedling.

2. Boric acid application as a foliar treatment in concentration of 50 mg per liter.

3. A foliar treatment of BA in concentration of 20 mg/l.

4. Argel application as a soil treatment in a dose of 15 g per seedling.

For future improvements, combinations and sequential studies on the above stated factors can be tried for further growth enhancements.

CHAPTER SIX

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