Sudan University of Science and Technology

College of Graduate Studies

Effect of Urea Fertilizer on Growth and Oil Content of Lemongrass (*Cymbopogon citratus* DC. Stapf)

تاثير سما د اليوريا على نمو ومحتوى الزيت فى حشيشة الليمون

(*Cymbopogon citratus* DC. Stapf)

By
Enas Ahmed Ali Jbreel
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Supervisor
Prof. Mohamed Osman Warrag
Department of Horticulture
College of Agricultural studies
Sudan University of Science and Technology

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Dedication

To my dear mother, the soul of my

Father, my teachers and my sisters

To all who assisted me during this

Study. With love and respect…

ACKNOWLEDGMENT

I would like to express my deepest sense of gratitude to my supervisor Prof.Mohamed Osman for his helpful, guidance and encouragement throughout this work.

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I would like to take this opportunity to express my profound gratitude to my beloved parents, who gave help, sisters and friends. At the end I would like to thank also indebted to those who cooperated with me but not mentioned.

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ABSTRACT

Lemongrass (*Cymbopogon citratus* DC. Stapf.) a tropical grass that belongs to the family Poaceae (Gramineae) is one of the most important medicinal and aromatic plants. It produces an essential oil which has a high economical value. Lemongrass can be successfully produced in Sudan at a commercial level. This study was carried out to investigate the effect of urea fertilizer on the vegetative growth, herbage yield and oil content of lemongrass (*Cymbopogon citratus*). An experiment was conducted at the Department of Medicinal and Aromatic Plants of Horticulture Sector Administration, Khartoum, Sudan, during the period from August to November of the year 2015. The treatments of this study consisted of two doses five levels of urea (1.0, 2.0, 3.0, 4.0 and 6.0g urea /plant) and the control (no urea). The treatments were arranged in a completely randomized design with four replications. The fertilizer was applied to the plants which were already growing in plastic pots measuring 34×32 cm. The plants were cuts to the level of the soil and the fertilizer was applied immediately. The parameters measured are the plant height, the number of tillers per plant, the number of leaves per plant before and after first cut and the oil contents at the two cut. The first cut was 30 days after the fertilizer application and the second cut was 60 days after the first one and the second dose of the fertilizer. Application of urea had significant effects on vegetative growth and herbage. The fertilizer application resulted in a significant difference in oil content in the first cut, while the second cut was not greatly affected with respect to oil content. The best result was obtained by the application of 3.0 g urea/plant in plant height, 4.0 and 6.0 g/plant regarding the number of tillers and 3.0, 4.0 and 6.0 g/plant for number

of leaves per plant. All fertilizer treatments resulted in significantly more values of these parameters than the control. With respect lemongrass oil content, there was significant difference in the first cut, whereas the second cut showed no significant differences among the treatments. Although the percentage oil content showed no significant difference, the total oil content seems to be affected.

الخلاصة

حشيشة الليمون حشيشه مداريه ، تنتمى للعائله النجيليه ، هي إحدي النباتات الطبية والعطرية المهمة ، منتجه للزيت الاساسي الذى له قيمه إقتصاديه عاليه ويمكن ان تنتج في السودان علي المستوى التجاري .

أجريت التجربة بهدف معرفة تأثير سماد اليوريا علي النمو الخضري وإنتاج العشب وكمية الزيت في حشيشة الليمون . أجريت التجربة في قسم النباتات الطبية والعطرية –إدارة القطاع البستاني –الخرطوم –السودان. في الفترة ما بين أغسطس – نوفمبر / 2015 م .

إشتملت التجربة علي خمس مستويات من اليورياعلي جرعتين (1.0 , 2.0 , 3.0 , 4.0 , 6.0 جم / للنبات) والشاهد(من غير يوريا). نفذت التجربة بإستخدام التصميم الكامل العشوائية بأربع مكررات. وتم إعطاء جرعة السماد إلي النباتات التي كانت تنمو مسبقا في أكياس مقاس 34×32سم ، والتي تم قطعها علي مستوي واحد من سطح الارض وكان معامل القياس في التجربة طول النبات وعدد الخلف وعدد الأوراق وكمية الزيت علي قطعتين ، أول قطعة كانت بعد 30 يوم من إضافة السماد كجرعة أولي والقطعة الثانية كانت بعد 60 يوم من القطعة الأولي والجرعة الثانية من السماد.

إضافة سماد اليوريا كان له أثر معنوي علي النمو الخضري وإنتاج العشب قبل وبعد القطعة الأولي .بينما كمية الزيت إضافة السماد كان لها أثر معنوي في القطعة الأولي بينما في القطعة الثانية كان ليس لها تأثير علي كمية الزيت . أفضل النتائج المتحصل عليها عند إضافة 3.0 جم / للنبات في حالة طول النبات 4.0 و6.0 جم / للنبات في حالة عدد الخلف 6.0,4.0,3.0 جم / للنبات فيما يتعلق بعدد الأوراق . كل مستويات التسميد المضافة أعطت تقديرات معنوية مقارنة بالشاهد ،.بالنسبة لكمية زيت حشيشة الليمون في القطعة الأولي إضافة السماد كان لها أثر معنوي مقارنة بالقطعة الثانية التي لم يكن لها أي أثر معنوي علي كمية الزيت عند إضافة السماد.

CHAPTER ONE

Introduction

Sudan is a large tropical –sub-Saharan country approaching 2.5 million square kilometers in area. It has been considered as a treasure house of valuable aromatic plant species due to its characteristic geographical position .This unique position of Sudan is reflected in its diverse habitats. In addition, there are areas with a high-naturalized value, rich in official botanical species, which grow wild and are potential candidates for crop development and commercialization. Moreover, the use of aromatic plants and/or aromatic material of plant origin is widely practiced in Sudan (ElGhazali *et al*., 2004).

Lemongrass *(Cymbopogon citratus* DC. Stapf) is a perennial grass plant, widely distributed worldwide especially in tropical and subtropical countries (Francisco *et al*., 2011). The global demand of lemongrass oil is about 4,500 million tonnes/annum; however the production is only about 600 tonnes/annum (Barbosa *et al*., 2008). Its aqueous extract is commonly used as a drink while the whole plant is well incorporated into traditional food for its lemon flavour. It also enjoys a wide application in folk medicine (Figueirinha *et al*., 2008). The characteristic smell of lemongrass oil makes its use in scenting of soaps, detergents and insect repellent preparations (Rangari, 2009; Handa and kapoor, 2009; Arumugam and Murugesh, 2010). The major importance of the oil is that it is a source of citral which imparts the lemon- like odor to the oil. The oil contains a high percentage (over 75%) of citral (Gupta and Sharma, 2009). Citral goes in perfumery, cosmetics, beverages, and starting material for manufacture of ionone, which produces vitamin A(Rangari, 2009; Handa and kapoor, 2009; Arumugam and Murugesh,2010).Application of nitrogen to lemongrass induces a significant increase in both vegetative growth and oil content (Singh *et al* .,2008; Elkashif and

Osman,2009). An interaction was reported between climatic conditions and water relations (Ahmed, 1982; Singh *et al*., 2005). Although some studies were conducted in Sudan with respect to lemongrass, more research will enrich the information about this valuable aromatic plant species.

Thus this study was conducted to explore the effects of urea fertilizer on the vegetative growth and oil content of lemongrass under Khartoum, Sudan, conditions.

CHAPTER TWO

LITERATURE REVIEW

Classification

Scientific name: *Cymbopogon citratus* DC. Stapf

English names: lemongrass

Family: Gramineae (Poaceae)

Class: Liliopsida (Monocotyledonae)

Division: Magndiophyta

2.1. History:

Lemongrass has been used in medicine in India for more than 2000 years. However, its first recorded distillation is in Philippines in the 17 th century, and in 1799 it was introduced to Jamaica. In 1917 it was grown for the first time in USA by Hood and during the first World War, grown in Guatemala by Julia Samayoa. Although the oil has been known, since very early times in India, the systematic cultivation and distillation of the grass were started in Kerala (India) only about 100 years ago. Cultivation has assumed the status of a plantation crop after the second World War (Jayasinha, 1999**).**

2.2. Botanical description:

Lemongrass is herbaceous ornamental grass (Gilman, 1999) equally versatile in the garden. This tropical grass grows in dense clumps that can grow up to 6 ft. (1.8 m) in height and about 4 ft. (1.2 m) in width, with short rhizome (Reitz, 1982).

2.2.1. Leaves:

The straps like leaves are 0.5-1.0 in (1.3-2.5 cm) wide, about 3 ft. (0.9 m) long, and have gracefully drooping tips. The evergreen leaves are bright bluish-green and release a citrus aroma when crushed. The leaves mostly emerge from the soil, usually without a stem. The leaf is simple with margin entire, linear shape and parallel venation. Leaf blade length is 18 to 36 in, and leaf color is green.

2.2.2. Flowers:

The lemongrass plants that are likely to encounter don't produce flowers and flowering panicles are rarely formed (Ross, 1999).

2.2.3. Trunk/bark/Branches: typically multi-trunked or clumping stems.

2.2.4. Fruit: no fruits are produced.

2.3. Propagation: by means of division of root stock (stools).

2.4. Mature plant: is about 50-70stools (Gilman, 1999).

2.5. *Cymbopogon spp*:

Cymbopogon is one of the most important essential oil yielding genera of the family Poaceae (Gramineae). The genus comprises about 140 species that are widely distributed in semi-temperate to tropical regions of Asia, Africa and America. Approximately 45 species have been reported to occur in India. The *Cymbopogon species* that produce volatile oils are called aromatic grasses (Rao, 1997). Different types of essential oils, such as palmarosa oil, lemongrass oil, citronella oil and ginger grass or Rosa oil, are very popular in perfumery (Rao, 1997; Sangwan *et al*., 2001). *Cymbopogon* species display wide variation in morphological attributes and essential oil composition at inter- and intraspecific levels (Rao, 1997).

2.5.1. Some *Cymbopogon* species, common name, regions, plant part used and the uses.

Species	**Region**	**Common Name**	**Parts**	**Medicinal Uses**
C. nardus (L.) Rendle	India	Citronella oil	Leaves	Insect repellent and as perfumes (Noor *et al*., 2012).
C. parkeri Stapf	Pakistan	Lemon grass	Aerial	Antiseptic and stomachic treatment (Bagheri *et al*., 2007).
C. excavatus Hoscht	South Africa	Bread-leavened Turpentine grass	Sheaths	Used as insecticides (Govere *et al*., 2000).
*C. olivieri (*Boss*)*	Pakistan	Pputar	Aerial	Pyretic, vomit, diuretic, rheumatism, and as anti-malaria condiment (Mahboubi and Kazempour, 2012; Abbas and Hassan, 2003).
*C.winterianus (*Jowitt*)*	Brazil	Java grass	Fresh leaves	Treatment of epilepsy and anxiety (Leite *et al*., 2011).
*C. validus (*Stapf*)*	Eastern and Southern Africa	African bluegrass	Essential oils	Skin toner, anti-ageing in men, fumigant and for rodent control (Kepe, 2004).
*C.marginatus (*Steud.)	South Africa	Lemon-Scented grass	Root	They are used as moth repellent (Secoy and Smith, 1983).
C. citratus Stapf.	India	Lemongrass	Aerial	Fever, digestive disorders (Jeong *et al*., 2009).
C. citratus Stapf.	Nigeria	Lemongrass	Leaves	Diabetes, inflammation and nerve disorders (Aibinu *et al*., 2007).
C. flexuosus (Nees ex Steud.) Wats	India	Lemongrass	Leaves	Cosmetics, antiseptic and for treatment of fever (Desai and Parikh, 2012).

2.5.2. Species in Sudan:

1-*Cymbopogon citratus*

Distribution: cultivated in many parts.

2- *Cymbopogon commutatus*

Distribution: Darfur (Jeble Marra).

3- *Cymbopogon excavates*

Distribution: Khartoum, Blue Nile State.

4- *Cymbopogon nervayus*

Distribution: Central Sudan. (ElGhazali *et al*., 2004).

2.6. Chemical constituents:

The major constituent of roots, stems and leaves are geraniol (30-5%), citronellol (24-1%), neral (10-3%) and geranial (13.6%).The constituent of lemongrass oil are citral (31-52%), Z.citral (28-82%), linalool (4-82%), geranyl acetate (3-57%) and trans-geraniol (3-66%). It acts as a natural precursor for production of semi synthetic vitamin A (Rao *et al*., 1995and Kulkarni *et al*., 1997).

2.7. Environmental requirements:

The crop can grow practically on all types of soil under a variety of geographic climates (Rangari, 2009). The crop grows well in both tropical and sub-tropical climates at an elevation up to 900m above sea level. However, ideal conditions for growing lemongrass are warm and humid climate with sufficient sunshine and 250-330 cm rainfall per annum evenly distributed over most parts of the year (Qadry, 2008-2009).Temperature ranging from 20-30 ˚ C. and well sunshine throughout the year is conducive to high crop yield. Lemongrass can also be grown in semi –arid regions receiving low to moderate rainfall (Handa and Kapoor, 2009). Lemongrass can grow well over medium fertile soils and moderate irrigation. Well drained sandy loam is most suitable for the growth of the plant. However it can be grown on a variety of soils ranging from loam to poor laterite (Eavan, 2004)). Calcareous and water logged soils should be avoided as they are unsuitable for cultivation (Arumugam and Murugesh, 2010).

2.8. Land preparation and planting:

The plant rarely flowers and propagation is by division of the root stock (stools). The land is well prepared, divided into plots, the plants were planted at a rate of three stools per hole at a distance of 60 cm between the plants and 75 cm between the rows (Gunther, 1950).

2.9. Harvesting and Yield:

The first harvest is generally obtained after 4-6 months of transplanting the seedlings. Harvesting (cutting) is done at the interval of 60 to70 days depending upon the fertility of the soil and other seasonal factors (Eavan, 2004). Under normal condition three harvests are possible during the first year and 3 to 4 in subsequent years, depending on the management practices followed. Harvesting is done with the help of sickles and the plants are cut 10 cm above ground level and allowed to wilt in the field, before transporting to the distillation site (Handa and

Kapoor, 2009). Depending upon soil and climate condition, plantation lasts on an average 3 to 4 years only .The yield of the oil is less during the first year but it increases in the second year and reaches a maximum in the third year, then the yield declines .On an average 25 to 30 tons of fresh vegetative yield is harvested per hectare per annum from 4-6 cuttings which yield about 80 kg of oil (Eavan, 2004).

2.9. Post-harvest Management:

2.9.1. Drying:

The grass is allowed to wilt for 24 h before distillation as it reduces the moisture content by 30% and improves oil yield .The crop is chopped into small pieces before filling in stills(Handa and Kapoor, 2009). Drying in full sunlight for 3 days reduces oil yields but has little effect on oil quality (Oyen and Nguyen, 1999).

2.10. Uses of *Cymbopogon citratus:*

Traditionally the leaf infusion has been used in oral health care as a diuretic and as an abortifacient (Morton, 1981). The aerial parts are also sold as herbal tea, used commercially in baked foods and confectionary and also are used in sachets as an insect repellent. The essential oil shows significance antimalarial activity in the four-day suppressive in-vitro tests in mice (Tchoumbougnang *et al.*, 2005).

Initially, the use of lemongrass extract in folk medicine for the treatment of certain ailment was disputed, but recent findings have confirmed its efficacy (Adejuwon and Esther, 2007).Tea from its leaves is used popularly in Brazil as an antispasmodic, analgesic , anti-inflammatory, antipyretic, diuretic and sedative and its essential oil is widely used for perfumes and cosmetics (Carlini *et al*.,1986).The oil from *C.citratus* were also found to pose anti-microbial , antifungal, insect repellent and antioxidative action (Velluti *et al*.,2004;Singh *et al*.,2010;Sonker *et al*.,2014). Lemongrass oil may be used in the treatment of acne, athlete's foot, excessive perspiration, flatulence, muscle aches, oily skin, scabies and stress (Cruz, 2002). Other studies reported that lemongrass oil is active against keratinophilic fungi, 32 ringworm fungi (Kishore *et al*., 1993; Abe *et al*., 2003) and food storage fungi (Misrha and Dubey, 1994). The fresh leaf essential oil has a larvicidal activity (Cavalcanti *et al*., 2004). The essential oils of *C. citratus* were found to produce 86.6% suppression of *Plsmodium berghei* growth (Tchoumbougnang *et al* .,2005). Several investigations have been carried out on the potentials of lemongrass extract as a source of hypolipidemic and hypoglycemic substances which may lower the risks of hypertension and obesity (Adejuwon and Esther, 2007). Essential oil fraction of lemon tea has been reported to exhibit anti-fungal effects against filamentous fungi of different classes thereby showing its broad spectrum of activity against both disease causing and non-pathogenic fungi (Dharmendra *et al.*, 2001).

2.11. Effect of nitrogen (N) fertilizer on growth and oil content:

Previous reports revealed that lemongrass responds well to nitrogen fertilizer application and optimum use of irrigation water markedly increases the yield of several oil-yielding aromatic crops (Singh *et al*., 2005). The favorable effect of increased N rates on nitrogen uptake of aromatic plants was also observed by Novak *et al*., (2003) and Azizi *et al.*, (2009). The effect of varying nitrogen rates has manifested its effect on herbage production. The use of 90 kg N/ha/year resulted in significantly higher herbage yield of lemongrass in all harvests. The interaction effect of irrigation and N on oil yield of lemongrass was also found to be statistically significant at all the harvests stages except the first and fourth. It was found that the response of lemongrass to increasing rate of N was greater at higher water regimes and lower levels of irrigation could achieve comparable yields with increasing rate of N application (Chakraborty *et al*., 2010). Although the herbage production is higher in the summer harvest in the first year but in the second year, i.e. rainy (kharif) season harvest recorded the highest production because of better dissolution of the applied nutrients due to rainfall received during that period (1 1.85 mm).This might be due to some biochemical and microbiological changes in the soil which in the presence of adequate available moisture aided in decomposition of plant litters and uprooted weeds and enhanced the uptake and utilization of the nutrients (Fatima *et al.*, 2002). Miyazaki (1962) found that in lemongrass, both plant growth and oil content declined due to nitrogen deficiency. In *Cymbopogon martini* the plant height was increased due to balanced application of 80 kg /hectare each of NPK, but it was not reflected on the final yield of oil content (Gupta *et al*., 1978).Working with *Cymbopogon flexuosus* and *Cymbopogon martini,* Ghosh and Chatterjee (1976) found that 25, 20, 15 Kg NPK fertilizer /hectare increased the vegetative growth, hastened flowering and increased essential oil content, of both species, compared with unfertilized plants. Addition of nitrogen fertilizer increase growth rate but did not appear to influence appreciably the oil content. Also, autumn (kharif) planting gave the earliest emergence than summer and winter plantings. It was also observed that the plants give higher growth rate and oil yield in autumn season compared to the other two seasons (Ahmed, 1982). Application of 86kg N/ha resulted in the highest vegetative growth and oil content as compared to the other treatments in both cuts (Elkashif and Osman, 2009). Nitrogen fertilization of lemongrass has been reported to result in more tillers, more number of leaves, large size of leaves, and a higher rate of regrowth after cutting (Singh *et al*., 2008). Application of nitrogen at 400kg/ha to lemongrass resulted in the highest herb and oil content, with no significant effect on the quality of the essential oil (Singh *et al.*, 2008). Omer *et al.* (2008) reported that nitrogen fertilizer was effective in increasing essential oil content of sweet basil, and a positive correlation between nitrogen fertilizer rate and essential oil content, in all cuts. Fresh yield significantly increased as N rate increased and the highest yield was obtained at the rate of 86 kg Nha^{-1} in both cuts(first and second) the yield 12.7- 14.3ton /hectare respectively (Elkashif and Osman, 2009).

2.12. Fertilizer:

Fertilizers are substances applied to soil to increase yield by providing one or more of the elements that are essential plant nutrients. Fertilizers raise soil fertility by increasing the plant nutrients in the cycle of growth and decay .With good farming practices much of the extra plant foods that are applied can be maintained in circulation, so raising cropping potential or fertility of the land. Fertilizers have another function that is not always realized, they lessen costs of production per ton since they raise yields without a corresponding large increase in total costs per hectare.

Organic manures supply nutrients that have already served to grow the crops that produced the wastes or excreta used to make the manure.

Chemical fertilizers have a unique function, they supply extra nutrients and make farmers independent of supplies in their soils if fertilizers can be bought, yields need not be limited by the natural fertility of soils.

2.13.1. Nitrogen fertilizer:

There are three forms of inorganic nitrogen in fertilizers

-Nitrates supply NO^{-}_3 ions.

-Ammonium salts supply NH_4^+ions.

-Simple amides are not ionized salts but contain nitrogen in NH_2 (amide) form or forms derived from this grouping urea. Plants take up both ammonium and nitrate ions. Except in very acid soils, ammonium N is quickly converted to nitrate by microbial action. Where this conversion is slow due to extreme acidity, plants adapted to condition may take up much ammonium.

Simple amides (e.g. urea) are hydrolyzed quite quickly to ammonium compound and are then nitrified. If ammonia gas (NH_3) is used as a fertilizer, the N is also converted to the ammonium from the soil and then behaves as ammonium salt (Cooke ,1975).

2.13.2. Role of Nitrogen in plants:

Established as an essential mineral element for rooted plants in the last century, nitrogen has been recognized to be responsible for lush vegetative growth and a dark green leaf color. Its deficiency is usually recognized first by the pale green or yellowish-green color of the leaves, especially in the grasses, and the premature necrosis of the older leaves beginning from the tip and the portion with green coloration is likely to be associated with the fact that nitrogen is one of two soil –derived constituents of chlorophyll (C_{33} H_{72} O_5N_4 Mg). Abundance of nitrogen for plants promotes aerial vegetative growth, increases the top-to-root ratio, and is essential for fruit and seed formation. In fact, nitrogen atoms may out number all other soil-derived essential atoms combined (C, H, and O, excepted). In mass nitrogen in plant material is often found in larger quantities than any other element as well. Sometimes, the concentration of potassium may be higher in some plant materials, but nitrogen exceeds the combined total of all other soil-derived essential mineral elements in the seeds of the commonly grown agricultural crops (Olson *et al*., 1981).

2.13.3. Amides:

Urea has the chemical formula CO $(NH^2)_{2.}$ It is also called carbamide. Urea is very concentrated and contains about 46%N, all soluble in water. Ordinary crystalline urea is hygroscopic and difficult to handle, but it can be made in granules or prills that store and spread satisfactorily. In soil, urea is rapidly converted the enzyme urease to ammonium carbonate which is unstable and releases free ammonia. If this change occurs on or near the soil surface ammonia may be lost to the air, and the fertilizer is inefficient. If it occurs near germinating seeds or the roots of the young plants the crop may be damaged by the high concentration of ammonia (Cooke, 1975).

2.14. Essential oil:

Essential oil (EO) is a valuable natural plant product that has been used in various fields from medicine to flavours and fragrances since antiquity. The extensive applications of EO are largely attributed by a long list of biological properties that are not only functionally important to the plant itself but also beneficiary to human such as anti-oxidants (Adorjan and Buchbauer, 2010; Amorati *et al*., 2013; Bakkali *et al*., 2008), anti-cancer (Sharma *et al*., 2009), anti-allergic, anti-inflammatory (Passos *et al*., 2007), antiviral (Astani *et al*., 2011), antibacterial (Bourgou *et al*., 2012; Inouye *et al*., 2001), antimicrobial (Gkogka *et al*., 2013), insect repellent (Rajkumar and Jebanesan, 2007).

The EO components consist of diverse complex mixtures of potentially hundreds of chemical constituents with low molecular weights ranging from 50 to 200 (Rowan, 2011). The active organic compounds can be categorized into four groups defined by chemical structures namely terpenes (mono- and sesquiterpene), terpenoids (alcohols, esters, aldehydes, ketones, ethers, phenols and epoxides), phenylpropenes and other aromatic compounds (sulfur- and nitrogen- derivatives) (Hyldgaard *et al*., 2012).

The production of EO depends on the interaction between genetic, ontogenesis and physiological state of the plant with environmental conditions. In fact, the regulation of the volatile compounds within the plant is further complicated by dynamic differential components of the abiotic factors such as physicochemical characteristics of the soil, moisture, temperature and light intensity (Srividya *et al*., 2015).

Variations of EO yield at different developmental stages have been reported in a number of commercially important aromatic plants. The EO yield obtained from the stem bark of *Cinnamomum cassia* of different ages ranged between 0.41 and 2.61%. Twelve years old bark had the highest oil yield (2.61%) compared to five year old bark (0.58%) (Geng *et al*., 2011). In contrast, EO obtained from young leaves of *Myrtus communis* has the highest yield (0.92% on dry weight basis) compared to matured leaves (0.48%) (Rowshan *et al*., 2012). Jaafar *et al*. (2007) found a considerable variation in the EO analyzed from different plant parts namely leaves, stems, flowers and rhizomes of torch ginger (*Etlingera elatior*).There is also a significant correlation between developmental stages and the composition of EO. Li *et al*. (2013) reported that the oil of juvenile leaves oils of *Cinnamomum cassia* contain more volatile compounds (29) than in the older leaves (21).

CHAPTER THREE

Materials and Methods

An experiment was conducted in the Department of medicinal and aromatic plants of Horticulture Sector Administration of Elmogran Area Khartoum Sudan. The area lies between the latitudes $15^{\circ}.9^{I}$ and $16^{\circ}.45^{I}$ North and the longitudes $24^{\circ},45^{I}$ and $21^{\circ},25^{I}$ East (Ali, 2000). The climate can be considered a semi-arid of rainy season from July to September (Oliver, 1965).

3.1. The plant material source:

Lemongrass (*Cymbopogon citratus*), of the family Graminae (Poaceae) plants were raised from tillers obtained from fully mature plants grown in the nursery of Horticulture Sector Administration of the Ministry of Agriculture-Elmogran Area, Khartoum, Sudan. Similar plants were planted at the rate of one tiller per bag which was 34×32 cm, in the loam soil. All the tillers were cut at the same level.

3.2. Experimentation:

The experiment have six treatments consisting of different level of urea ($CO(NH_2)_2$) fertilizer as a source of nitrogen. The six treatments were: control (no fertilizer), 1.0, 2.0, 3.0, 4.0 and 6.0 g urea per plant. Urea fertilizer was chose because it is cheap and can be easily obtained. Fertilization was conducted by adding urea at two doses. The first dose was added after two weeks from planting and then another dose after a month from first one. Water was added immediately after fertilizer application. Then weekly watering was done until the first harvest. After that plants were irrigated every other day till the second harvest.

Harvesting was done twice. The first one after a month from fertilizer application and the second one was after two months from the first harvest, and the second fertilizer application.

3.3. Data collection:

To study the effect of different levels of urea on growth rate the collected data were:

3.3.1. Plant height:

A month from the first fertilizer application and before cut, then 2 months after the second fertilizer application and the first cut, four plants per treatment per replicate were measured, the plant height was measured from the soil surface to the highest tip of the tallest leaf (cm).

3.3.2. Number of tillers:

The number of the tillers of four plants per treatment per replicate was recorded every month for three months, starting a month after the first dose of fertilizer application and before cut and two months after the first cut.

3.3.3. Number of leaves:

The number of the leaves per plant from four plants per treatment per replicate was recorded every month for three months, starting a month after the first dose of fertilizer application and before cut and two months after the first cut.

3.3.4. Fresh weight:

All the leaves from one plant per treatment per replicate were weighed. Weighing was done twice: first one after the first harvest, and the second after the second harvest.

3.3.5. Dry weight:

All the fresh leaves from one plant per treatment per replicate were weighed, then put at room temperature (25 ˚C) for 48 hours. After drying, each plant leaves were weighed, for the two harvests.

3.3.6. Oil content of dry leaves:

Oil was extracted by water distillation method. Twenty grams of each sample was placed in 2000 ml rounded bottom capacity flask. 500 ml of distilled water was added and the clevenger receiver and condenser were attached to the top of the flask. The system was heated at 100℃ for about four hours till the volume of oil above the water layer in the receiver was constant. Oil was pipetted, dried over anhydrous sodium sulphate and stored in dark containers. After distillation yield percentages were calculated as volume of oil /weight of plant sample * 100.

3.4. Statistical design and data analysis:

The experiment was laid out in a completely randomized design, with four replication. The data were subjected to analysis of variance and the means were separated using Duncan's Multiple Range test.

CHAPTER FOUR

RESULTS

4.1. Plant height:

The effect of urea fertilizer on plant height (cm) was shown in Table1and figs. 1and 2. Addition of 3.0g urea per plant resulted in the tallest plants before and after the cuts. The difference was significant between this treatment and the control. The 1.0, 2.0 and 3.0g urea/plant were not significantly different from each other before cut. After cut 1.0, 2.0 and 3.0 g urea/plant were not significantly different from each other. The control treatment was significantly shorter than all other treatments after cut (Table1).

4.2. Number of tillers /plant:

The effects of urea fertilizer on number of tillers are shown in Table2 and figs. 3and 4. Addition of urea fertilizer showed that no significant difference occurred among the different levels of urea fertilizer before cut (Table2), but after cut 4.0 g urea/plant resulted is highly significant difference; whereas both 4.0and 6.0g urea/plant resulted in significantly more tillers. The 1.0, 2.0 and 3.0 g urea /plant were not significantly different from each other, but resulted is significantly more tillers than the control (Table 2).

4.3. Number of leaves /plant:

The highest number of leaves per plant was brought about by the 1.0.3.0 and 4.0 g urea/plant before cuts. After cut 3.0, 4.0 and 6.0 g urea /plant gave highest number of leaves (Table 3, figs. 5and 6). The lowest number of leaves was brought about by the control. The results of the other treatments were not consistent.

Table 1: Effect of different levels of urea (g/plant) on plant height (cm).

g urea/plant	Before cut	After cut	
	1st Month	2nd Month	3rd Month
0	79.00b*	65.75c	70.25d
1.0	80.25ab	73.00ab	76.25ab
2.0	81.50ab	72.25ab	74.75ab
3.0	83.00a	76.25a	77.75a
4.0	73.75c	67.00c	72.25cd
6.0	79.75ab	71.75b	73.25bcd
Lsd	3.195	3.852	3.295

*The means followed by the same letter are not significantly different at the 5% level according to Duncan´s Multiple Range Test.

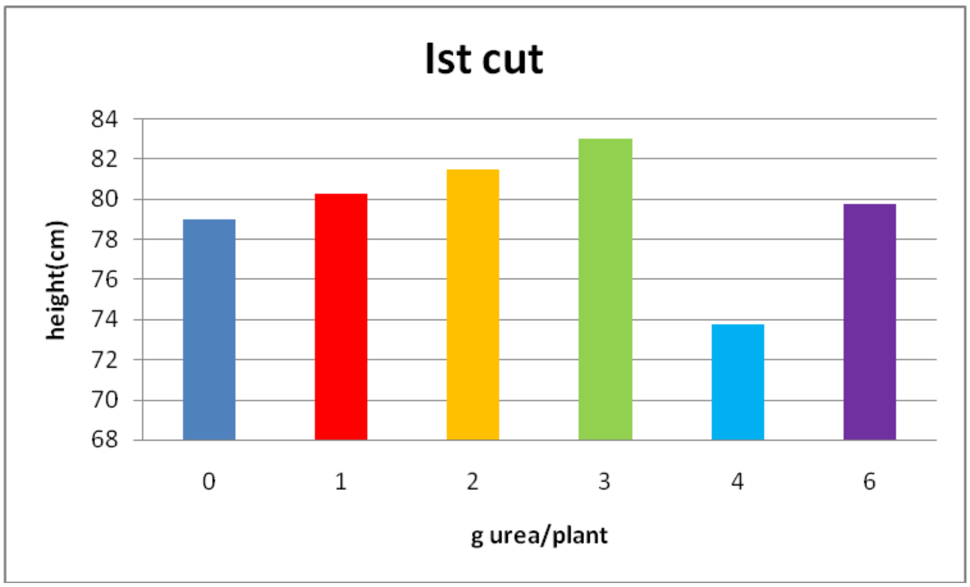

Fig.1: Effect of different levels of urea g/plant on plant height.

Fig.2: Effect of different level of urea g /plant on plant height.

Table 2: Effect of different levels of urea (g/plant) on Number of Tillers/plant.

g urea/plant	Before cut	After cut	
	1st Month	2nd Month	3rd Month
0	5.75a*	9.50c	9.75c
1.0	6.25a	15.50b	16.50b
2.0	7.25a	16.00b	17.25b
3.0	6.25a	15.50b	18.00b
4.0	7.50a	23.25a	25.50a
6.0	5.00a	16.50b	23.00a
Lsd	2.269	1.622	3.985

*The means followed by the same letter are not significantly different at the 5% level according to Duncan´s Multiple Range Test.

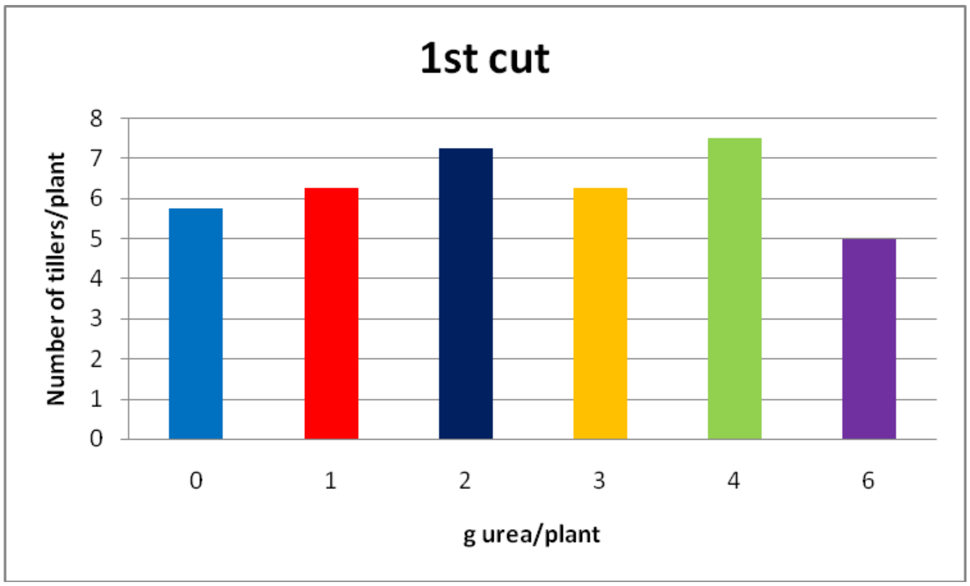

Fig.3: Effect of different levels of urea g/plant on number of tillers per plant.

Fig.4: Effect of different levels of urea g/plant on number of tillers per plant.

Table 3: Effect of different levels of urea (g/plant) on Number of leaves/plant.

g urea/plant	Before cut	After cut	
	1st Month	2nd Month	3rd Month
0	47.0c*	61.0b	88.0d
1.0	64.8a	99.2a	127.5c
2.0	46.2c	108.8a	144.2b
3.0	60.8ab	101.0a	156.0ab
4.0	59.0ab	111.5a	161.2a
6.0	51.8bc	76.2b	149.5ab
Lsd	11.60	16.44	14.94

*The means followed by the same letter are not significantly different at the 5%level according to Duncan´s Multiple Range Test.

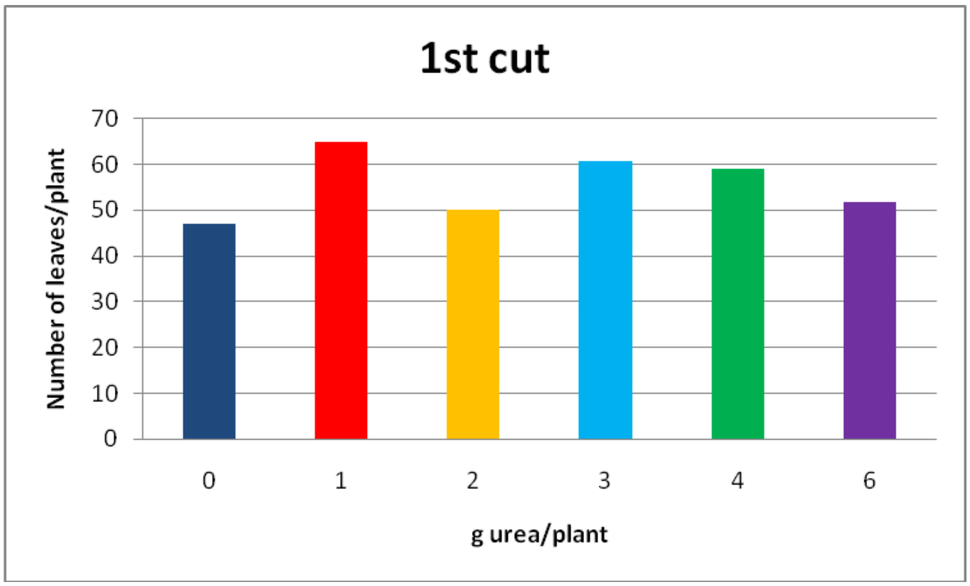

Fig.5: Effect of different levels of urea g/plant on number of leaves per plant.

Fig.6: Effect of different levels of urea g/pant on number of leaves per plant.

4.4. Fresh weight /plant:

The effect of different levels of urea fertilizer on fresh weight is shown in Table 4 and fig.7. The results indicated highly significant differences in fresh weight in both cuts. In the first cut the 1.0 and 3.0 g urea/plant resulted in significantly higher fresh weight than the other levels. In the second cut both these levels together with the 4.0 g urea /plant resulted in significantly higher fresh weight than the other fertilizer levels. The control plants exhibited the lowest fresh weight in the second cut, whereas they did not in the first cut.

4.5. Dry weight /plant:

In the second cut all fertilizer levels resulted in higher dry weight compared with the control, whereas there were no significant differences among the different fertilizer levels. In the first cut the 1.0 and 3.0 g urea per plant resulted in significantly higher dry weight than the rest of the treatments which were not significantly different from each other (Table 6 and fig. 8).

4.6. Oil content:

Effect of different levels of urea on oil content is shown in Table 7. Addition of different levels of fertilizers to lemongrass plants showed significant difference between the fertilizer treatments in the first cut. In the second cut no significant differences were found.

Table 4: Effect of different levels of urea (g/plant) on fresh weight (g)/plant.

g urea/plant	Cut 1	Cut 2
0	34.0b*	44.4c
1.0	28.12bc	76.9bc
2.0	42.25a	96.2a
3.0	42.88a	88.8ab
4.0	25.75c	82.0ab
6.0	33.88b	79.4b
Lsd	6.713	14.01

*The means followed by the same letter are not significantly different at the 5% level according to Duncan´s Multiple Range Test.

Table 5: Effect of different levels of urea (g/plant) on dry weight (g)/plant.

g urea/plant	Cut1	Cut 2
0	8.75b*	13.25b
1.0	7.62b	25.88a
2.0	11.38a	29.50a
3.0	11.25a	27.75a
4.0	7.62b	26.88a
6.0	9.12b	26.62a
Lsd	1.93	4.39

*The means followed by the same letter are not significantly different at the 5% level according to Duncan´s Multiple Range Test.

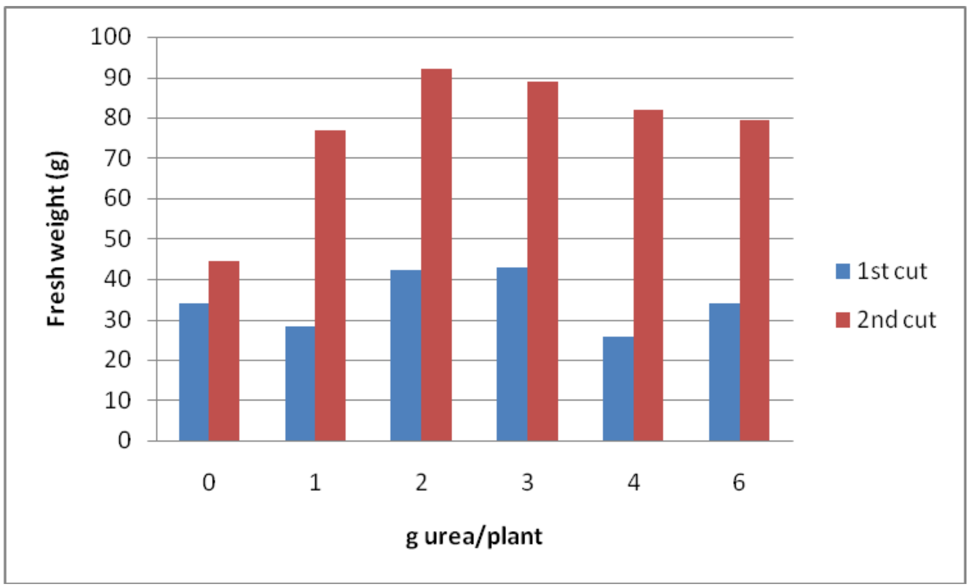

Fig.7: Effect of different levels of urea g/plant on fresh weight per plant.

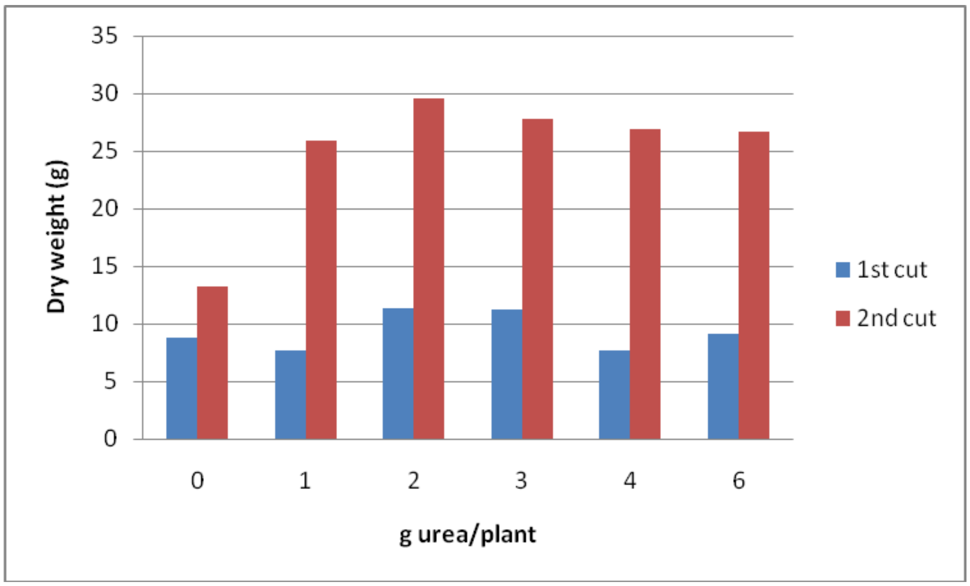

Fig.8: Effect of different levels of urea g/plant on dry weight per plant.

Table 6: Effect of different levels of urea (g/plant) on oil content (ml).

g urea/plant	Cut 1	Cut 2
0	0.4333ab*	0.467a
1.	0.5000a	0.533a
2.0	0.4333ab	0.567a
3.0	0.4000b	0.500a
4.0	0.4333ab	0.533a
6.0	0.5000a	0.533a
Lsd	0.07263	0.0938

*The means followed by the same letter are not significantly different at the 5% level according to Duncan´s Multiple Range Test.

Table 7: Effect of different levels of urea (g/plant) on percentage yield of oil of lemongrass.

g urea/plant	Harvest 1	Harvest 2
0	2.1	2.3
1.0	2.5	2.6
2.0	2.1	2.8
3.0	2.0	2.5
4.0	2.1	2.6
6.0	2.5	2.6

CHAPTER FIVE

DISCUSSION

Lemongrass *(Cymbopogon citratus* DC. Stapf) is a perennial grass, widely distributed worldwide especially in tropical and subtropical countries (Francisco *et al.*, 2011), *Cymbopogon* is one of the most important essential oil yielding genera of the family Poaceae (Graminae) (Rao, 1997; Sangwan *et al*., 2001). The major importance of the oil is that it is a source of citral which imparts the lemon- like odour to the oil. The oil contains a high percentage (over 75%) of citral (Gupta and Sharma, 2009).

Fertilizers are substances applied to soil to increase yield by providing one or more of the elements that are essential plant nutrients (Cooke, 1975). Nitrogen has been recognized to be responsible for lush vegetative growth and a dark green leaf colour. Its deficiency is usually recognized first by the pale green or yellowish-green colour of the leaves, especially in the grasses. Abundance of nitrogen for plants promotes aerial vegetative growth, increasing the top-to-root ratio, and is essential for fruit and seed formation (Olson *et al*., 1981).

The results obtained from this experiment reflect significant variation in plant height (cm), number of tillers, number of leaves and oil content as affected by different levels of nitrogen (control ,1.0 ,2.0 ,3.0 ,4.0 and 6.0 g urea /plant), these showed significant variation before and after cutting, reflectied the importance of nitrogen fertilization.

Application of different urea levels significantly affected plant height. Addition of 1.0, 2.0 and 3.0 g urea/plant resulted the highest plant height before and after cutting. This agrees with Gupta et al. (1978) who found that in *C. maritini* the plant height was increased due to balanced application of 80kg of NPK/hectare.

Addition of urea fertilizer had no effect on number of tillers. There was no significant difference between the different levels of fertilizer before cut. While after cut addition of fertilizer appears to have resulted in significant differences between the levels of fertilizer. Application of 4.0g urea /plant resulted in more number of tillers after a month from cut and fertilizer application. Addition of 4.0 and 6.0g urea/plant recorded the number of tillers after two months from the first cut and fertilizer application.

There were significant differences among the levels of fertilizer. Application of 1.0, 3.0and 4.0 g urea /plant resulted in higher number of leaves after a month of fertilizer application and before cut, also after a month from the cutting and fertilizer this application gave higher number of leaves . However addition of 3.0, 4.0and 6.0 g urea/plant gave higher number of leaves after two month from the cutting and fertilizer. This agrees with Singh *et al.* (2008) who reported that nitrogen fertilization of lemongrass has resulted in more tillers, more number of leaves, large size of leaves, and a higher rate of regrowth after cutting. The favourable effect of increased N rates on nitrogen uptake of aromatic plants was also observed by Novak *et al*., (2003) and Azizi *et al.* (2009).

Addition of fertilizer had significant effects on fresh weight. In the first cutting 1.0 and 3.0 g urea/plant resulted in higher fresh weight while in the second cut 1.0, 3.0 and 4.0 g urea /plant resulted in higher fresh weight.

In the first cut addition of fertilizer resulted in significant difference. Application of 1.0, 2.0, 4.0, and 6.0 g urea /plant resulted in higher oil content. This agrees with that of Ghosh and Chatterjee (1976) who, working with *Cymbopogon flexuosus* and *Cymbopogon martini*, found that 25, 20, 15 Kg NPK fertilizer /hectare increased the vegetative growth, hastened flowering and increased essential oil content, of both species, compared with unfertilized plants. Also Elkashif and Osman (2009) reported that application of 86kg N/ha resulted in the highest vegetative growth and oil content as compared to the other treatments in both cuts. Likewise, Omer *et al.* (2008) reported that nitrogen fertilizer was effective in increasing essential oil content of sweet basil, and they found a positive correlation between nitrogen fertilizer rate and essential oil content, in all cuts. In the second cut, addition of fertilizer had no significant differences between the different levels of fertilizer. This result agrees with that of Ahmed (1982) who observed that addition of nitrogen fertilizer increased growth rate but did not appear to influence appreciably the oil content.

Conclusion

Application of urea as nitrogen source had appositive effect on the vegetative growth of lemongrass (*Cymbopogon citratus*), whereas the oil content was increased at the first cut only. The best result was obtained by 1.0g urea/plant for the plant height. The highest number of tillers was brought about by the application of 4.0 and 6.0 g urea/plant whereas1.0, 4.0 and 6.0 g urea/plant resulted in the highest number of leaves. The effect of urea on oil content was in consistent.

The adoption of this important aromatic plant species seems to be very promising. Probably more research would be helpful to make use of it in boosting the economy of Sudan.

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Appendix:

Analysis of variance

Variate: p_H

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	200.708	40.142	8.68	<.001
Residual	18	83.250	4.625		
Total	23	283.958			

CV%
2.7

Analysis of variance

Variate: P_H_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	309.000	61.800	9.19	<.001
Residual	18	121.000	6.722		
Total	23	430.000			

CV%
3.7

Analysis of variance

Variate: P_H_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	149.333	29.867	6.07	0.002
Residual	18	88.500	4.917		
Total	23	237.833			

CV%
3.0

Analysis of variance

Variate: N_S

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	17.333	3.467	1.49	0.243
Residual	18	42.000	2.333		
Total	23	59.333			

CV%
24.1

Analysis of variance

Variate: N_S_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	382.208	76.442	14.52	<.001
Residual	18	94.750	5.264		
Total	23	476.958			

CV%
14.3

Analysis of variance

Variate: N_S_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	605.833	121.167	16.84	<.001
Residual	18	129.500	7.194		
Total	23	735.333			

CV%
14.6

Analysis of variance

Variate: N_L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	1180.83	236.17	4.50	0.008
Residual	18	945.00	52.50		
Total	23	2125.83			

CV%
13.2

Analysis of variance

Variate: N_L_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	7991.7	1598.3	13.05	<.001
Residual	18	2205.2	122.5		
Total	23	10197.0			

cv%
11.9

Analysis of variance

Variate: N_L_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	14583.0	2916.6	28.82	<.001
Residual	18	1821.5	101.2		
Total	23	16404.5			

cv%
7.3

Analysis of variance

Variate: F_W

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	992.18	198.44	9.72	<.001
Residual	18	367.56	20.42		
Total	23	1359.74			

cv%
13.1

Analysis of variance

Variate: F_W

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	6393.59	1278.72	14.38	<.001
Residual	18	1600.56	88.92		
Total	23	7994.16			

cv%

12.1

Analysis of variance

Variate: D_W

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	56.208	11.242	6.69	0.001
Residual	18	30.250	1.681		
Total	23	86.458			

CV%
14.0

Analysis of variance

Variate: D_W

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	691.177	138.235	15.84	<.001
Residual	18	157.062	8.726		
Total	23	848.240			

CV%
11.8

Analysis of variance

Variate: V_O_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	0.025000	0.005000	3.00	0.055
Residual	12	0.020000	0.001667		
Total	17	0.045000			

CV%
9.1

Analysis of variance

Variate: V_O_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
T	5	0.017778	0.003556	1.28	0.334
Residual	12	0.033333	0.002778		
Total	17	0.051111			

CV%
10.1

Analysis of variance

Variate: To_yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	0.30907	0.10302	1.07	
rep.*Units* stratum					
harvest	1	39.97845	39.97845	417.14	<.001
fertilizer	5	8.06365	1.61273	16.83	<.001
harvest.fertilizer	5	4.96485	0.99297	10.36	<.001
Residual	33	3.16271	0.09584		
Total	47	56.47872			

CV%

13.1

Ministry of Environment, Forestry and Physical Development Meteorological Authority Weather –Climate data.

Station Khartoum
Period 2015

Month	Mean temperature ˚c	
	Max.	Min.
August	38.3	26.4
September	39.6	27
October	38.4	31.1
November	36	21.6