Sudan University of Science and Technology

College of Graduate Studies

Investigations on the bio-stimulating potential of some selected local flora on growth and productivity of Lemongrass (*Cymbopogon citratus*)

إستقصاءات علي قدرة بعض النباتات المحلية المختارة كمحفزات حيوية لنمو وإنتاجية حشيشة الليمون

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Dedication

I would like to dedicate this work to
my parents,
Brothers and sisters,
my husband,
To all who assisted me during this study
With love and respect

Hind

Acknowledgement

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

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Abstract

For increased yield per unit area, growers intensified the use of agricultural chemicals which is hazardous to humans and environment. In conformity with the growing interest in the use of botanicals as alternatives to these chemicals in Sudan ,this study was undertaken to investigate the bio-stimulating potential of shoot powder and water extracts of Henna (*Lawsonia inermis*), Moringa (*Moringa oleifera*), Hargel (*Solenostemma argel* Del. Hayne),and Haza (***Haplophyllum tuberculatum*)** on growth attributes and productivity of Lemongrass (*Cymbopogon citratus*) . Besides synthetic hormones namely; Gibberellic Acid and Benzyladenine were also tested for stimulation of growth and productivity of lemongrass. All treatments were conducted under nursery conditions at Khartoum, Sudan. Henna, Moringa , Hargel and Haza powders were tested as soil dressing for Lemongrass in doses of: 0, 4, 8, 12 and 16 g/ plant, while tap water extracts of their powders were tested as foliar applications on Lemongrass in concentrations 0, 4, 8, 12 and 16 g/l. All tests were arranged in complete randomized design. Each treatment was replicated six times. Data were collected 6 months after treatments for number of leaves, leaf length (cm), leaf width (cm), leaf chlorophyll content, leaf fresh weight (g), leaf dry weight (g), root fresh

weight (g),root dry weight (g),and leaf oil contents (%). Data were subjected to analysis of variance . Means were separated by Duncan's Multiple Range Tests at 95% confidential tests.

According to the test of bio-stimulating potential of Henna powder and water extracts on the growth and oil yield of Lemongrass , considerable gains in growth parameters were obtained upon soil dressing with 16g of Henna powder, whereas these parameters were enhanced substantially by the 8 g/l foliar treatment. On the other hand, the impact of Moringa applications on the growth attributes and yield of Lemongrass plant were obtained upon soil dressing with 4g of Moringa leaves per plant whereas these parameters were enhanced substantially by the 8 g/l foliar treatment. Also the results of this study confirmed that applications of Hargel as a bio-stimulant source had a positive effect and enhanced the growth attributes of lemongrass. The best growth result in adding Hargel as soil dressing was obtained with 16g Hargel / plant ,while the 4g/plant hargel resulted in the best significant increase in shoots and roots fresh and dry weights. The best results of adding Hargel as foliar applications were 8g /plant .

The Impact of Haza (*Haplophyllum tuberculatum*) applications on growth attributes of Lemongrass revealed a general increase in growth parameters in the treated plants comparing to the control ,the highest values of all growth parameters were obtained upon soil dressing with

4g of Haza leaves per plant whereas these parameters were enhanced substantially by the 8 g/l foliar treatment.

The results of hormones experiment indicated that Gibberellic Acid and Benzyladenine altered the growth of Lemongrass. The highest values of leaves number, leaf length , width , Chlorophyll content , shoot fresh and dry weights were obtained with BA treatment, while roots fresh and dry weights were obtained with the combination treatment (BA+GA_3).

These results elucidated the signification of these uses of the tested plants as bio-stimulators for Lemongrass plants .Yet further confirmatory tests are needed coupled with phyto-chemical studies to define the active constituents responsible for these enhancements.

Key words:

Lemongrass (*Cymbopogon citratus*),Henna (*Lawsonia inermis*), Moringa (*Moringa oleifera*), Hargel (*Solenostemma argel* Del. Hayne), Haza (***Haplophyllum tuberculatum***), GA_3 , 6BA.

المستخلص العربي

لزيادة إنتاج وحدة المساحة كثف المنتجون إستخدام الكيماويات الزراعية وهي ذات مخاطر علي الإنسان والبيئة. في إتساع مع الرغبة المتنامية في إستخدام المواد النباتية كبدائل لهذه الكيماويات في السودان أُجريت هذه الدراسة لإستقصاء قدرة التحفيز الحيوي لمسحوق الأوراق والمستخلصات المائية لنباتات الحناء, المورنقا, الحرجل والحزا علي نمو وإنتاجية نبات حشيشة الليمون. إضافة إلي ذلك أختبرت قدرة الهرمونات الصناعية (الجبرلين- بنزايل ادينين) علي تحفيز نمو وإنتاجية نبات حشيشة الليمون.

أجريت كل التجارب تحت ظروف المشتل بمدينة الخرطوم- السودان .مسحوق الحناء , المورنقا ,الحرجل والحزا أستخدمت كإضافة لتربة حشيشة الليمون بجرعات 8,0, 4, 12و16جم/نبات كما انّ المستخلص المائي لمساحيق هذه النباتات قد إختبر كمعاملات رش علي حشيشة الليمون بتركيزات 0 , 8,4, 12و16جم/لتر.

كل الإختبارات قد أُعدت بتصميم كامل العشوائية. كل معاملة كررت ستة مرات. رصدت البيانات بعد 6 أشهر من المعاملات للمقاييس التالية:عدد الأوراق ,طول الأوراق,عرض الأوراق , محتوي الكلوروفيل في الورقة , الوزن الرطب للأوراق , الوزن الجاف للأوراق ,الوزن الرطب للجذور, الوزن الجاف للجذور ومحتوي الزيت بالأوراق. أخضعت البيانات لتحليل التباين وتمّ الفصل بين المتوسطات بإستخدام إختبار دنكن في حدود ثقة 95%.

وفقا لإختبار إمكانية التحفيز الحيوي للمسحوق والمستخلص المائي للحناء علي نمو وإنتاج الزيت بحشيشة الليمون فإنه قد تم التوصل لنتائج معنوية في معايير النمو ومحتوي الزيت عند الإضافة الأرضية للمعاملة 16 جم/نبات تليها المعاملة 8جم/ لتر حناء رش . من ناحية اخري فإنّ إضافة المورنقا لحشيشة الليمون كان له اثرعلي خصائص النمو ومحتوي الزيت حيث تم التوصل لنتائج معنوية عند الإضافة الأرضية للمعاملة 4 جم/ نبات تليها المعاملة 8جم/ لتر مورنقا رش. كما أكدت نتائج هذه الدراسة أنّ إستخدام الحرجل كمحفز حيوي كان له أثر ايجابي في تعزيز خصائص النمو لحشيشة الليمون, حيث تمّ التوصل لأفضل نتائج للنمو عند الإضافة الأرضية للمعاملة 16 جم/نبات حرجل , بينما أدت المعاملة 4جم/ نبات حرجل إلي افضل زيادة معنوية في الوزن الرطب والجاف للمجموع الخضري والجذري. و كانت المعاملة8جم/ لتر حرجل رش هي الأفضل عند إضافة الحرجل بالرش. عكست إضافة

الحزا لحشيشة الليمون زيادة عامة في معايير النمو للنباتات المعاملة مقارنة بالكنترول , حيث تمّ التوصل لأعلي قيم لمعايير النمو عند الإضافة الأرضية للمعاملة 4جم/نبات تليها المعاملة 8جم/ لتر حزا رش .

اظهرت نتائج تجربة الهرمونات ان حمض الجبرليك والبنزايل ادينين قد حفزا النمو في حشيشة الليمون , وقد تم الحصول علي اعلي القيم لعدد الاوراق, طول الورقة ,عرض الورقة, محتوي الكلوروفيل , الوزن الرطب والجاف للمجموع الخضري عند معاملة النباتات بالبنزايل ادينين , بينما كانت اعلي القيم للوزن الرطب والجاف للجذور عند المعاملة بخليط البنزايل ادينين وحمض الجبرليك.

هذه النتائج أوضحت معنوية إستخدام النباتات المختبرة كمحفزات حيوية لنمو نبات حشيشة الليمون وعلي الرغم من ذلك هنالك حاجة لمزيد من الإختبارات التأكيدية المستقبلية.

كلمات مفتاحية:

حشيشة الليمون , الحناء, المورنقا , الحرجل , الحزا , حمض الجبرليك , البنزايل ادينين.

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Chapter One

Introduction

CHAPTER ONE

INTRODUCTION

The relationship between Man and the plants has always been a very close one throughout the development of human culture, and no doubt, the herbalist is probably one of the first professionals in the evolution of human cultures. Today, the plant kingdom still remains a virtually untapped reservoir of new compounds, some provide novel structures from which synthetic chemists may derive even more interesting compounds.

Medicinal and aromatic plants are produced and offered in a wide variety of products, from crude materials to processed and packaged products like pharmaceuticals, herbal remedies, teas, spirits, cosmetics, sweets, dietary supplements, varnishes and insecticides (Ohrmann, 1991; Gorecki, 2002; Lange, 1996). The use of botanical raw material is in many cases much cheaper than using alternative chemical substances. An estimated number of 70,000 plant species are used in folk medicine worldwide (Farnsworth and Soejarto, 1991), a figure that has been confirmed by (Schippmann *et al*., 2002). Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anti-carcinogenic potential (Cai *et al*., 2004). Moreover, approximately 120 drugs in western medicine are obtained from plants, while many other drugs are obtained either by semi synthesis from plant products, or synthesis based on plant molecules (Pezzuto.,1997).The plant's therapeutic value is usually

related to the source of secondary metabolites, Unlike primary metabolites, secondary metabolites are not of vital importance for plants and they usually play a role in the interaction of the plants with the environment such as toxins to defense against harmful microorganisms or various predators, messengers, repellents, or camouflages (Verpoorte.,1998).

Essential oils were the first group of secondary metabolic products in sagebrush foliage to be isolated and studied chemically (Adams and Oakberg,1934, Kinney *et al*.,1941). As a common feature, essential oils possessed the essence of a plant, the identifiable aroma, flavour, or other characteristic that was of some practical use. They were used as perfumes, food flavours, deodorants, pharmaceuticals, and embalming antiseptics (Lawrence and Reynolds, 1984).

Sudan has a large area, with multi- culture, habits rich biodiversity and medicinal plants. And although it was divided into two countries, it is still rich with valuable medicinal and aromatic plants. Generally Sudan is a very flat country, most of its parts range between 400 and 450 m above mean sea level 4, and with the vast variety of climate and flora, thus the flora of the Sudan consists of 3137 species of flowering plants belonging to 170 family and 1280 genera. Of these, 278 species, 210 genera and 72 families have already been identified as medicinal, culinary and aromatic (MCA) plants (El-Amin, 1990; El Ghazali *et al*., 1994,1997).Therefore traditional medicine together with use of medicinal plants became an important part of the cultural heritage of Sudan (Elkhalifa , 2003; El badwi *et al*., 2014).

Cymbopogan citratus (commonly named, lemongrass), is a great interest due to its commercially valuable essential oils and widely used in food technology as well as in traditional medicine. Owing

to the new attraction for natural products like essential oils, despite their wide use and being familiar to us as fragrances, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the environment. Based on literature data, it appears that geranial, neral, geraniol, limonene and β-myrcene have been found as major compounds in many other Cymbopogan species with the main chemicalcomponent of lemongrass oil is citral (Luiz *et al*.,2001; Huynh, 2008). Citral or 3,7-dimethyl-2,6- octadienal is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (transcitral, citral A) and neral (cis-citral, citral B) (Huynh, 2008). Citral is an important raw material used in the pharmaceutical, perfumery and cosmetic industries, especially for the synthesis of Vitamin A and ionone (Efraim *et al*., 1998). Chemically, Citral is a mixture of two aldehydes that have the same molecular formula, C10H16O, but different structures. However, it is noteworthy that the composition of any plant essential oil studies is influenced by several factors, such as local, climatic, seasonal and experimental conditions (Perry *et al*., 1999; Daferera *et al*., 2000).

A plant bio-stimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, a biotic stress tolerance and/or crop quality traits, regardless of its nutrients content (Patrick, 2015). Plant bio-stimulants are organic materials that appear to impact several metabolic procedures such as respiration, photosynthesis, nucleic acid synthesis and ion uptake and when applied in small quantities, improve the plant growth and development (Castro and Vieira, 2001). In recent years, plant bio-stimulants are being extensively used in farming and cultivation . Positive effects of its application have yielded

extraordinary results as confirmed by many studies (Poincelot ,1993 ; Starck ,2005).

1.2 Objectives:

In efforts and desire to enhance the scientific production of *Cymbopogan citrates* under Sudan conditions, this study aimed to fulfill the following objectives:

1. To test the bio-stimulative effects of foure Sudanese indigenous plants, namely Hargel (*Solenostemma argel* (Del.), Henna (Lawsonia inermis) , Haza (*Haplophyllum tuberculatum*) , Moringa (*Moringa oleifera*) on growth attributes of *Cymbopogon citratus* plants.
2. To investigate the influence of plant hormones namely , Gibberellic acid (GA_3) and Benzyladenine (BA) and the combination between them on growth attributes of *Cymbopogon citratus* plants.

Chapter Two

Literature Review

CHAPTER TWO

LITERATURE REVIEW

2.1 The lemongrass plant

Lemongrass (*Cymbopogon citratus*) is an aromatic tall herbaceous plant belonging to genus Cymbopogan of family Poaceae (Akhila, 2010). Lemongrass essential oil possesses biologically active constituents including citral contents comprising more than 75% (w/w) of its essential oil (Huynh *et al.*, 2008; Tajidin *et al.*, 2012). The oil quality is judged by its citral content and its solubility in alcohol . It bears reddish yellow to reddish-brown colour, with strong, lemon odor properties. It is used in the perfume, soap and cosmetics industries and forms the starting material in the manufacture of synthetic Vitamin A. Further the oil serves as an input to pharmaceutical preparations, such as pain balm, disinfectants, and mosquito-repellent creams (Prommegger *et al.*, 2005).

2. 1.1 Classification of *Cymbopogon citratus* DC. Stapf

Scientific name: *Cymbopogon citratus* DC. Stapf

English names: lemongrass

Family: Gramineae (Poaceae)

Class: Liliopsida (Monocotyledonae)

Division: Magnoliophyta

2.1.2 *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).

2.1.3 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).

2.1.4 Ecology and distribution

Lemongrass 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).

Lemongrass is distributed in Africa, Indian subcontinent, South America, Australia, Europe and North America. It is widely distributed throughout the tropics and is grown in West Indies, Guatemala, Brazil, Congo, Tanzania, India, Thailand, Bangladesh, Madagascar and China. Jammu.

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

typically multi-trunked or clumping stems, and there is no fruits are produced.

2.1.6 Propagation

By means of division of root stock (stools).

2.1.7 Cultural Practices

Lemongrass can be grown on a wide range of soils but a well drained and fertile soil is more favorable. The plant rarely flowers and propagation is

by means of division of the root stock (stools). The land well prepared, divided into plots. Seedlings are planting at a distance 40×40cm , 40×30 cm, 40×60cm apart depending upon fertility of land and inter culture implements used. It is better to plant on ridges in areas receiving high rainfall (Qadry and shah prakashan,2008-2009).

(Singh, 1970) concluded that the plant harvested after 4-5 months from planting, when the plant height 60 - 70 cm above 20 cm from the soil, thus the oil yield depend on plant height. The optimum time of harvest for Cymbopogon winterianus was 72 days after growth, although the total weight of leaves was rather small. Harvesting the plant after 4 or 5 month from planting when the plant height 60 - 70 cm thus the oil yield depend on plant height . (Herat *et al*., 1979) found that both the Photosynthetic rate, and oil content, in lemongrass (*Cymbopogon citratus*) and citronella grass (*Cymbopogon nardus*) were decreased with the increase in leaf age. (Singh *et al*., 1979) studied the effect of age on citronella grass (*Cymbopogon winterianus*) oil content of leaves decreased in maturity.

2.1.8 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*., 1995 ; Kulkarni *et al*., 1997).

2.1.9 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 a biotic 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 than in the older leaves .

Lemongrass essential oil possesses biologically active constituents including citral contents comprising more than 75% (w/w) of its essential oil (Huynh *et al.*, 2008; Tajidin *et al.*, 2012). The oil quality is judged by it's citral content and its solubility in alcohol . It bears reddish yellow to reddish-brown colour, with strong, lemon odor properties. It is used in the perfume, soap and cosmetics industries and forms the starting material in the manufacture of synthetic Vitamin A. Further the oil serves as an input to pharmaceutical preparations, such as pain balm, disinfectants, and mosquito-repellent creams (Prommegger *et al.*, 2005).

2.1.10 Factors affecting oil yield and herbage production in *Cymbopogon species*:

A. Effect of seasonal variation

Lemongrass oil depend on growth and temperature thus the amount of oil increase in worm summer than cold winter (Miyazaki, 1969). In Sudan (Mohammed,1982) studied the effect of seasonal variation on the growth and oil yield of *Cymbopogon citratus* and found that autumn season gave the highest plant growth rate and oil content. in the Philippians,(Oliveros and Aureus, 1977) found that leaves of wild lemon grass (*Cymbopogon citratus*) that was collected during hot dry season rendered the highest amount of oil ranging from 0.31 % to 0.48 % which contain 31.05 % to 45.2 % citral a decline was noticed in the rainy season with yield raging from 0.10 % to 0.36 % . The lowest oil yield was during the cool month ranging between 0.1% and 0.2 with Citral content of 25.60 % to 39.14 %

most of these studies based on studies done by (Miyazaki,1969) were carried to investigate the impact of temperature as indicated by seasonal variation, on plant, oil, content and Citral content in open field cultivation. It has been found that the plant is vigorous under high temperature, and the both the oil content, and Citral content of oil are high. On the other hand, the fall in temperature invites the lowering in plant growth, oil and Citral content.

B. Effect of leaf age on the oil content

Study under high temperature conducted by (Miyazaki and Kiyoshi, 1962) found that the oil was higher in young leaves and declines along with advances in age. How-ever, it has been found that the difference in citral content with leaf age, under high temperature was comparatively small. (Herath *et. al*., 1979) found that both the Photosynthetic rate, and oil content, in lemongrass (*Cymbopogon citratus*) and citronella grass (*Cymbopogon nardus*) were decreased with the increase in leaf age.

2.1.11 Lemongrass economical value

Lemon grass (*Cymbopogan citratus*) is an aromatic plant which grows in many parts of tropical and subtropical South East Asia and Africa, origin in Indo-Burma region and is native to India. The crop is cultivated to obtain Citral-rich essential oil and 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).

2.1.12 Uses of lemongrass

It is known that oil of lemongrass (*Cymbopogon citratus*) is one of the most important essential oil-bearing herbaceous species of

the Gramineae because of its high citral content of up to at least 75% of the oil (Jayasinha,1999). The oil and citral are both used in the perfumery and soap industries and in the manufacture of synthetic vitamin A. Lemongrass has powerful pain relieving properties and useful for all types of pain including abdominal pain, headache, joint pains, muscle pain, digestive tract spasms, muscle cramps, stomachache and others. It can work as antifungal and anti-bacterial agent. Due to presence of vitamin A, lemongrass is helpful for skin issues such as acne pimples. Citral, found in lemongrass, can harm cancer cells (http://healthers.org/lemongrass/).Lemongrass is a tall, perennial hedge throwing up dense fascicles of leaves from a short rhizome. Traditionally lemongrass is grown in high rainfall area as a rain fed crop. But under semi-arid tropical conditions, lemongrass thrives well under irrigated conditions. A temperature ranging from 20–30 ºC and good sunshine throughout the year is conducive to high crop yield with better oil content. Research showed that herb yield can be increased with nitrogen application (Rao *et al.*, 1991).

2.2 Plant Bio-stimulants

Recently, the agricultural sector is facing concomitant challenges of rising the productivity to feed the growing global population and increasing the resources use efficiency, while reducing the environmental impact on the ecosystems and human health. In fact, fertilizers and pesticides play a crucial role in agriculture, representing a powerful tool for growers to increase yield and guarantee continuous productivity throughout the seasons under both optimal and suboptimal conditions. In the last three decades, several technological

innovations have been proposed to enhance the sustainability of agricultural production systems, through a significant reduction of synthetic agrochemicals like pesticides and fertilizers. A promising and environmental-friendly innovation would be the use of natural plant bio-stimulants (PBs) that enhance flowering, plant growth, fruit set, crop productivity, and nutrient use efficiency (NUE), and are able also to improve the tolerance against a wide range of a biotic stressors (Colla and Rouphael, 2015). PBs were initially defined by excluding some functionalities like fertilizers or plant protection products. In 1997, in Grounds Maintenance web-journal, Zhang and Schmidt from the Department of Crop and Soil Environmental Sciences of the Virginia Polytechnic Institute and State University, defined PBs as "materials that, in minute quantities, promote plant growth". By using the statement "minute quantities" for describing PBs, the authors implicitly wanted to discriminate bio-stimulants from nutrients and soil amendments, which also promote plant growth, but are clearly applied in larger quantities. The PBs mentioned in this web article were two important categories such as humic acids and seaweed extracts, and their action on plants was proposed to be essentially hormonal. In 2012, the European Commission has assigned an ad hoc study on plant bio-stimulants to evaluate the substances and materials involved, which was published by (du Jardin , 2012) as: "The Science of Plant Bio-stimulants - A bibliographic Analysis".

2.2.2 Bio-stimulants categories and mode of action

Based on the scientific literature (250 scientific articles using the term 'bio-stimulant' in their titles and/or abstracts), the following definition was proposed: "Plant bio-stimulants are substances and materials, with the exception of nutrients and pesticides, which, when applied to plant, seeds or growing substrates in specific formulations, have the capacity

to modify physiological processes of plants in a way that provides potential benefits to growth, development and/or stress responses". (du Jardin,2012) concluded that PBs are very heterogeneous materials, and proposed in his study eight categories of substances that acts as bio-stimulants:

1. humic substances, complex organic materials (obtained from agro-industrial and urban waste products, sewage sludge extracts, composts, and manure),
2. beneficial chemical elements (Al, Co, Na, Se, and Si);
3. inorganic salts including phosphite;
4. seaweed extracts (brown, red, and green macroalgae);
5. chitin and chitosan derivates;
6. anti transparent (kaolin and polyacrylamide);
7. free amino acids ;
8. N-containing substances (peptides, polyamines, and betaines);

but all those categories of substances did not include any microbial bio-stimulants. Three years later in the frame of a special issue on "Bio-stimulants in Horticulture" conducted by (Colla and Rouphael ,2015), a new definition was proposed by (du Jardin , 2015), which was supported by scientific evidence about the mode of action, nature and types of effects of PBs on agricultural and horticultural crops. PBs were defined by (du Jardin ,2015) as follows: "A plant bio-stimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, a biotic stress tolerance and/or crop quality traits, regardless of its nutrient content". This definition could be completed "By extension plant bio-stimulants also designate commercial products containing mixtures of such substances and/or microorganisms"

Although the term “bio-stimulant” has been used for many years, it is still not fully defined. As European Bio-stimulant Industry Council (EBIC) defined that “plant bio-stimulants contain substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit: nutrient uptake, nutrient efficiency, tolerance to a biotic stress, and crop quality” (du Jardin, 2012). According to (du Jardin, 2015), the review of the relevant literature reveals a wide range of compounds act as a bio-stimulant including: humic and fulvic acids; protein hydrolysates and other N-containing compounds; Seaweed extracts and botanicals; chitosan and other biopolymers; beneficial fungi and bacteria potentially.

2.2.3 Importance of Bio-stimulants as organic farming substances

Modern agriculture is searching for new biotechnological solutions that would allow reducing the use of chemical synthetic products without affecting crop yield or the income (Hong *et al*., 2007). Organic farmers have a special interest in biological products that improve the quality and fertility of the soil and that promote the growth of the crops and increase their resistance to pests and diseases, since the use of chemical fertilizers and pesticides is restricted (Verkleij, 1992).

Plant bio-stimulants are natural substances that stimulate some processes within plants and influence several metabolic activities such as respiration, photosynthesis, nucleic acid synthesis and ion uptake resulting in improved plant growth and development (Castro and Vieira, 2001; Saa-Silva et al., 2013).

Growth bio-stimulants include diverse formulations of compounds, plant products and micro organisms that are applied to plants or soils to improve crop vigour, yield and quality (Atherton,1998). The crop life cycle from seed germination to plant maturity can be influenced by bio-stimulants in some ways such as increase in plant metabolism efficiency, improved tolerance to a-biotic stresses, efficient nutrient assimilation, translocation and use, enhanced quality attributes of produce, increased water use efficiency, enhanced physiochemical properties of the soil and development of complementary soil micro-organisms (Davis, 2010). Reports on growth and yield stimulations in horticultural crops upon use of bio-stimulants are frequent (Abdelrahman, 2016; Hamed, 2016; Idris and Modawi , 2016; Idris *et al.,* 2014; Idris *et al.,* 2011; Cerdan *et al*., 2009; Foidle *et al*., 2001).

2.2.4 Bio- stimulants bibliographic review

Several studies documented that bio-stimulants promote plant growth, development and productivity (Brown and Saa, 2015; Bulgari *et al*., 2015), however the mechanism of action is poorly or not understood. It is possible that the beneficial effects of bio-stimulant on growth parameters could be ascribed to auxin and gibberellin-like activity, and enhanced nitrogen uptake, as documented for the bio-stimulant action of plant-derived protein hydrolysate in corn, tomato, and gibberellin-deficient dwarf pea plants (Colla *et al*., 2014). Another suggested function of bio-stimulants was linked to reactive oxygen/nitrogen species and hormonal signaling. For example, chitosan, a natural biopolymer produced from chitin, is the major constituent of arthropods exoskeleton and fungi cell walls and has been extensively studied as an elicitor for inhibiting

postharvest senescence and diseases in many fruit, such as apple, citrus, kiwifruit, peach, pear, strawberry, and sweet cherry (Kerch, 2015).

2.2.5. Bio-stimulants in horticulture

Several commercial and experimental sea weed extracts can also be employed to produce potent bio-stimulants. Foliar application of *Ascophyllum nodosum* extract in kiwi plants after flowering increased the weight and maturity of the harvested fruits (Chouliaras *et al*., 1997). In Clementine and orange trees, foliar spray of seaweed extracts at budding stage positively affected bud sprouting and full bloom, and enhanced gibberellins content and fruit yield (Fornes *et al*., 2002). Apple trees treated with seaweed extract exerted an improved flowering, vegetative growth and yield (Basak, 2008). Treatment of seaweed extracts to olive plants before bloom improved oil quality characteristics (Chouliaras *et al*.,2009) as well as mineral content, leaf dry weight and stem diameter (Zulaikha,2013).Application of seaweed extract to peanut leaves enhanced seed yield and increased the protein content of the harvested seed (Featonby-Smith and van Staden, 1987). In the work of (Colavita *et al*., 2011), foliar application of seaweed extract in pear tree increased fruit diameter, fruit weight and number of cell per area of parenchymatous tissue. Additionally, seaweed extracts have shown promising results as growth-yield- promoting agents in tropical trees (Mohamed and El-Sehrawy, 2013; Karthikeyan and Shanmugam,2014).

Experiments with citrus plants showed enhanced yield and fruit quality (mineral status/acidity) following foliar application of moringa leaf or pollen grain extract/yeast extracts (El-Boray *et al*.,2015;Nasir *et al*., 2016).

In addition to the above, some interesting data revealed that bio-stimulants are able to regulate specific physiological features in fruit trees. For example, their application has been correlated with increased biosynthesis of antioxidant-related compounds. Chicken feather-derived protein hydrolysate applied in banana plants at flower induction period enhanced the accumulation of several bioactive substances, like amino acids, phenolics, and flavonoids (Gurav and Jadhav,2013). Finally, (Viti *et al.*,1990) demonstrated the positive effect of foliar application of a commercial protein hydrolysate upon in vivo and in vitro pollen tube elongation in olive plants.

2.3 Moringa (*Moringa oleifera*)

Since the inception of human civilization man has been searching for food which can keep him healthy and active. In this modern era of research and technology this futile search still continues to discover novel herbal drugs and alternate source of nutritional supplements. One such promising tree which has successfully cleared all the tests of nutritional benefits, medicinal properties, environmental and consumption safety is the perennial, multipurpose, softwood-tree '*Moringa oleifera*' of the monogeneric family Moringaceae. *oleifera* is reputedly known as 'horseradish' tree, 'drumstick' tree, 'ben-oil tree' or 'benzoil tree', 'cabbage tree', 'mother's best friend' a 'miracle tree'.

2.3.1 Origin and geographic distribution

Moringa oleifera is a native of India. Its cultivation extends in the Himalayan foothills of south Asia from north eastern Pakistan to north-western Bengal in India and north-eastern Bangladesh at an elevation of 1400m above sea level. Its cultivation and usage has gained momentum in South East Asia, West Asia, Arabian Peninsula, East and West Africa, West Indies and Southern Florida, Central and South America from Mexico to Peru including Brazil and Paraguay (Ganatra *et al.*, 2012). About 33 species have been reported in the family Moringaceae (Arora *et al.*, 2013). Among those, thirteen species namely, *M. arborea*, *M. borziana*, *M. concanensis*, *M. drouhardi*, *M. hildebrandtii*, *M. longituba*, *M. oleifera*, *M. ovalifolia*, *M. peregrina*, *M. pygmaea*, *M. rivae*, *M. ruspoliana*, *M. stenopetala* are well known and found worldwide.

2.3.2 Botanical description

Morphology and physical characteristics: Moringa is a slender softwood tree that branches freely and can be extremely fast growing. Although it can reach 3 heights in excess of 10 m (33 f) and a diameter of 20-40 cm at chest height, it is generally considered a small- to medium-size tree (Radovich, 2009). The stem is normally straight but occasionally is poorly formed. The tree grows with a short, straight stem that reaches a height of 1.5-2 m before it begins branching but can reach up to 3.0 m (Foidl *et al.*, 2001). The extended branches grow in a disorganized manner and the canopy is umbrella shaped. The trip innate compound leaves are feathery with green to dark green elliptical leaflets 1-2 cm (0.4-0.8 in) long. The tree is often mistaken for a legume because of its leaves. The alternate, twice or thrice pinnate leaves grow mostly at the branch tips. They are 20-70 cm long, grayish-downy when young, long petiole with 8-10 pairs of pinnae each bearing two pairs of opposite, elliptic or

obovate leaflets and one at the apex, 1-2 cm long (Morton,1991).The flowers Conspicuous, lightly fragrant flowers are borne on inflorescences 10-25 cm (4-10 in) long, and are generally white to cream colored, 2.5 cm in diameter, borne in sprays, with 5 at the top of the flower, although they can be tinged with pink in some varieties. The flowers, which are pleasantly fragrant and 2.5 cm wide are produced profusely in axillary, drooping panicles 10-25 cm long (Sachan *et al*., 2010). They are white or cream colored and yellow-dotted at the base. The five-reflexed sepals are linear-lanceolate. The five petals are slender-spatulate. They surround the five stamens and five staminodes and are reflexed except for the lowest (Foidl *et al.*, 2001; Proyecto Biomasa ,1996).The Fruits are trilobed capsules, and are frequently referred to as pods. Immature pods are green and in some varieties have some reddish color. Pods are pendulous, brown, triangular, splitting lengthwise into 3 parts when dry, 30-120 cm long, 1.8 cm wide, containing about 20 sec embedded in the pith, pod tapering at both ends, 9-ribbed. Fruits production in March and April in Sri Lanka (Burkill, 1966). The Seeds The seeds are round with a brownish semi-permeable seed hull, with 3 papery wings. Seed hulls are generally brown to black, but can be white if kernels are of low viability. Viable seeds germinate within 2 weeks. The hull itself has three white wings that run from top to bottom at 120° intervals. Each tree can produce between 15,000 and 25,000 seeds/year. The average weight per seed is 0.3 g and the kernel to hull ratio is 75:25 (Makkar and Becker, 1997).

2.3.3 Ecology

Moringa oleifera is a fast-growing evergreen or deciduous tree and attains a height of 10-12 meters. It bears drooping, fragile branches covered with thick, corky, whitish bark (Rollof *et al*., 2009). Moringa

does not require much water for growth and so can be grown in dry regions where there is scanty or uneven rainfall. Although, it tolerates a wide range of soil conditions, but prefers a neutral to slightly acidic (pH 6.3 to 7.0), well-drained sandy or loamy soil. Moringa is widely adapted to the tropics and subtropics. Optimum leaf and pod production requires high average daily temperatures of 25-30 ° C, well-distributed annual rainfall of 1000-2000 mm (40-80 in), high solar radiation and well-drained soils (Odee,1998). Growth slows significantly under temperatures below 20 ° C. Ideal elevation is less than 600 m (1, 970 f). Moringa is relatively tolerant of drought and poor soils and responds well to irrigation and fertilization.

2.3.4 Propagation and planting

The plant is propagated by planting limb cuttings 1-2 m long, from June to August. The plant starts bearing pods 6-8 months after planting, but regular bearing commences after the second year. The tree bears for several years. It does not tolerate freeze or frost. It can also be propagated by seed. As with all plants, optimum cultivation depends on producing the right environment for the plant to thrive. Moringa is a sun and heat loving plant. Seeds are planted an inch below the surface and can be germinated year-round in well-draining soil (Rajangam *et al*., 2001).

2.3.5 Uses

Moringa Oleifera has been used in the traditional medicine passed down for centuries in many cultures around the world, it is known for long time as an important nutritional supplement with a range of medicinal properties. According to India's ancient tradition of ayurveda, the leaves of the Moringa tree prevent 300 diseases. M. oleifera leaves works as antioxidant (Arabshahi *et al*., 2007 ; Sreelatha, 2011; Padma ,2009 ;

Asma *et al*., 2005; Suphachai, 2014) which adds one more attribute to its known pharmacological importance. The iron content of the leaves is high, and they are reportedly prescribed to overcome anaemia (Anwar *et al*., 2007) It's proved by research work that M. oleifera leaves extract is good to regulate the hyperthyroidism (Pankaj and Anand,2000), antineoplastic agent to treat Sickle cell disease (Saalu *et al*., 2011) and antiulcer agent (Kodia *et al*., 2014). It works as hypocholestrolemic agent in obese patients (Ghasi *et al*., 2000), antiproliferation and induction of apoptosis on human cancer cell (Sreelatha *et al*., 2011) and was supported the study of (Suphachai,2014) which provided evidence that *M. oleifera* leaves possess chemo preventive and cytotoxic properties. Therefore, it might prove beneficial as an alternative to anticancer drugs. *M. oleifera* leaves as ethnomedicine to treat diabetes mellitus (Jaiswal *et al*., 2009), in addition the experimental findings by (Soliman, 2013; Yassa,2014) have indicated the potential benefits of using the aqueous extract of *M. oleifera* leaves as a potent antidiabetic treatment. As per the recommendation by the World Health Organization (WHO) 1-4 gram/day of Moringa leaf-powder is sufficient to meet the nutritional demands of an adult. Moringa has long been considered a panacea for improving the nutrition of poor communities in the tropics and subtropics. Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Protein content of leaves is high (20-35% on a dry weight basis). Most important is that the protein is of high quality having significant quantities of all the essential amino acids. This amino acid balance is very unusual in plant foods. Moringa leaves also contain high quantities of nutrients (per 100 g fresh weight): vitamin A (7564 IU), vitamin C (51.7 mg), calcium (185 mg) and potassium (337 mg) (Foidl and Paull, 2008).

2.3.6 Phytochemical properties

Moringa species contain various phytoconstituents such as alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes. The diversity of these phytochemicals in the genus contributes to its numerous pharmacological uses. About 110 compounds were identified from the genus**.** Some of these compounds showed positive results when tested for various biological activities. In addition to these 110 compounds, the genus contains more compounds as detected by GC-MS. The Moringa genus has high antioxidant activity mainly due to its high content of flavonoids. Most of the flavonoids present in the genus are in the flavanol and glycoside form. The most common flavonoids of the genus are rutin , quercetin , rhamnetin , kaempferol , apigenin , and myricetin . Optimization research has been conducted to discover the best way to extract flavonoids from *M. oleifera* with highest yield. As a result, subcritical ethanol extraction yielded 26.7% more flavonoid than a reflux method (Wang *et al*., 2017).

Moringa species contain abundant glucosinolates. The most abundant glucosinolate present in the species is 4-O-(α-L-rhamnopyranosyloxy)-benzyl glucosinolate, also known as glucomoringin (GMG). Three isomers of 4-O-(α-L-acetylrhamnopyrosyloxy)-benzyl glucosinolate were also detected in M. oleifera leaves, depending on the maturity and physiological properties of the leaves (Leone *et al*., 2015).

Recently, isothiocyanates have become a major research interest of Moringa for their various biological activities such as their anticancer, antidiabetic, antimicrobial, and anti-inflammatory effect (Park *et al*., 2011; Padla *et al*., 2012; Waterman *et al*., 2014, 2015).

M. oleifera leaves contain gallic acid as their major phenolic acid. Ellagic acid , ferulic acid , caffeic acid , o-coumaric acid , and chlorogenic acid , are also detected in the leaves and gentisic acid , syringic acid , ρ-coumaric acid , and sinapic acid were detected in trace amounts (Leone *et al*., 2015; Teixera *et al*., 2014 ; Saini *et al*.,2014) reported that the major carotenoid detected in *M. oleifera* leaves is lutein. (Saini *et al*., 2014) reported that *M. oleifera* did not contain α-carotene which can usually be found in green leafy plants. The author assumed that all of the α-carotene had been fully converted into lutein. Other carotenoids that can be found in the plant are all-E-luteoxanthin, 13-Z-lutein , 15-Z-β-carotene , and all-E-zeaxanthin (Saini *et al*., 2014). Lupeol acetate, β-amyrin , and α-amyrin were isolated from an n-hexane fraction of an ethanol extract of the aerial part of *M. peregrina* (El-Alfy *et al*., 2011). Two new pyrrole alkaloid glycosides were isolated from *M. oleifera* leaves, marumoside A and marumoside B together with pyrrolemarumine-4″-O-α-L-rhamnopyranoside (Sahakitpichan *et al*., 2011).

A sterol glycoside, namely β-sitosterol-3-O-β-D-galactopyranoside , was isolated from a chloroform extract of *M. oleifera* stem bark (Bargah and Das, 2014). The main steroidal components in *M. peregrina* oil were β-sitosterol (56.76%), campesterol (23.24%), and stigmasterol (8.11%) (Abd El Baky and El-Baroty, 2013).

β-sitosterol was isolated from the leaves and seeds of *M. oleifera* (Maiyo *et al*., 2016) and an acetone extract of *M. stenopetala* root wood has been reported to contain cholest-5-en-3-ol (Tesemma *et al*., 2013). The species contain high oleic acid levels from 68 to 79%. However, the fatty acid content is dependent on the location where the oil is obtained. *M. oleifera* seed oil has a nutty flavor and has light

yellow color (Nadeem and Imran, 2016). The oil contains a high concentration of oleic acid which constitutes 75–77% of the fatty acid composition of the seeds.

Lately, various workshops are conducted worldwide for dissemination of information on the nutritional, medicinal and various other promising benefits of Moringa. In a nut-shell, *M. oleifera* is an asset to mankind and therefore rightly called as a "Miracle tree" (Omotesho *et a*l., 2013).

Moringa is among plants with bio-stimulative properties. Several lab-experimentation has shown that Moringa spray has marked beneficial effects on crops plants. The effects of sprays accelerated the growth of young plants that became firmer, more resistant to pests and disease, longer life-span, heavier roots, stems and leaves and large fruits with increased yield (20–35%).

Rajamani *et al.* (2014) reported that Moringa fermented leaf juice was enhancive for growth attributes in *Brassica oleracea.* Likewise, Foidle *et al.*(2001) reported that a spray made from Moringa leaf extract resulted in increased strawberry productivity and claimed the possibility of its use as a foliar spray to accelerate growth of young plants as the leaf extract contains significant quantities of calcium, potassium, Cytokinin, anti-oxidants, proteins, ascorbic acid and phenols. Besides, Nasir *et al.*(2016) reported that Moringa leaf extract added as foliar spray and soil applications to citrus plants increased leaf N, P, K, Ca, Mn and Zn, minimized fruit drop and increased yield, fruit juice, total soluble solids, vitamin C, sugars, total antioxidants and phenolics contents. Priming seeds of the rangeland grass *Echinochloa crusgalli* with Moringa leaf extract resulted in significant increase of shoot vigour coupled with higher number of leaves and fertile tillers (Nouman *et al.*, 2011).

Other researchers claim the benefits of using relatively low doses of Moringa (Wagentrisl, 2003; Kohata *et al.*,2004). The growth promotion obtained from Moringa applications may be attributed to several possibilities. Moringa leaves have been characterized to contain a desirable nutritional balance of minerals, amino acids and fatty acids (Razis *et al* 2014; Teixeira *et al*., 2014). They also contain various antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids. In addition, they contain vitamin B, chromium, copper, magnesium, manganese, phosphorus, zinc, calcium, potassium and cytokinin in the form of zeatin (Alhakmani *et al*., 2013; Kesharwani *et al.*, 2014; Vongsak *et al.*, 2014).

2.4 Henna (*Lawsonia inermis*)

Henna (*Lawsonia inermis* L.) belongs to the family Lythraceae. It is traditionally used to develop a red or black coloring to hands, feet and hair in some occasions such as weddings and religious festivals. It is among flora adapted to growth conditions of Sudan where it had been grown in home gardens as hedges and as ornamental. The cultivation of Henna is practiced in Sudan coupled with processing at a commercial level in the River Nile State.

2.4.1 Origin and geographic distribution

The origin of henna plant (*Lawsonia inermis*) is North Africa and south west Asia. It is grown as an ornamental and dye plant, and it is widely cultivated in tropical regions of the world in Sudan, Egypt, China, and India. Thus, the Major producing countries include Sudan, Egypt and India (Leung,1980).

2.4.2 Botanical description

Henna is a tall shrub or small tree, standing 1.8 to 7.6 m (5 ft 11 in to 24 ft 11 in) tall. It is glabrous and multi-branched, with spine-tipped

branchlets. The leaves grow opposite each other on the stem and are glabrous, sub-sessile, elliptical, and lanceolate (long and wider in the middle; average dimensions are 1.5–5.0 cm x 0.5–2 cm or 0.6–2 in x 0.2–0.8 in), acuminate (tapering to a long point), and have depressed veins on the dorsal surface. Henna flowers have four sepals and a 2 mm (0.079 in) calyx tube, with 3 mm (0.12 in) spread lobes. Its petals are obvate, with white or red stamens found in pairs on the rim of the calyx tube. The ovary is four-celled, 5 mm (0.20 in) long, and erect. Henna fruits are small, brownish capsules, 4–8 mm (0.16–0.31 in) in diameter, with 32–49 seeds per fruit, and open irregularly into four splits (Zumrutdal and Ozaslan,2012).

2.4.3 Ecology

Lawsonia inermis plant is widely distributed across the Sahel and Central Africa. It also exists in the Middle East (Orwa *et al.,* 2009). It grows mainly along waterways and in semi-arid regions and is adapted to a wide range of environmental conditions. It can withstand low air humidity and drought conditions. The seeds of henna plant require high temperatures for germination, growth and maximal development (Orwa *et al.,* 2009).

Henna plant grows on any type of soil, from light loam to clay loam, but does best on heavy soils, which are retentive of moisture. It tolerates a little alkalinity in the soil. (Council of Scientific and Industrial Research, 1962).

2.4.4 Propagation and planting

Propagation is carried out through seeds and cuttings (Council of Scientific and Industrial Research, 1962).

2.4.5 Uses

Lawsonia inermis plant is cultivated in Africa and Asia for both medicinal and industrial (dyeing) purposes. Just as people use it for staining hair, nails and beard, it is pointed that *Lawsonia inermis* is used for various fields in medicine. Hepatoprotective and immunomodulatory effect, antimicrobial, anthelminthic, antifungal, antitrypanosomal, abortifacient, antioxidant and anticancer activity of *Lawsonia inermis* were reported from all over the world by previous studies (Zumrutdal and Ozaslan,2012).

Lawsonia inermis (Lythraceae) is a very useful medicinal plant in all parts of the world. The leaf powder of henna sap is used for staining hair, nails and beard (Chengaiah *et al.*, 2010). The leaves of *Lawsonia inermis* are used to treat poliomyelitis, measles among the Yoruba tribe of South Western Nigeria (Oladunmoye and Kehinde, 2011). The seeds of henna have been reported to possess deodorant action and are used in most cases of gynecological disorders such as menorrhagia, vaginal discharge and leucorrhoea (Nawagish *et al.*, 2007). The leaves of *Lawsonia inermis* with those of Hibiscus rosa-sinensis, Eclipta prostrata and seeds of Abrus precatorius when they are taken in equal quantities and ground into paste which is soaked in sesame oil for 5 days is used as hair oil by the tribes of Andra Pradesh, India (Suneetha *et al.*, 2011). In Turkey, henna which is an extract of Lawsonia sp. is used as hair dye and nail dye in many cultures as decorative dye centuries (Ozaslan *et al.*, 2009). Henna is widely used in the cosmetic industry as dyeing agent also in India (Chengaiah *et al.*, 2010). Reports show that methanolic root extracts of Lawsonia is used in Nigeria for cosmetic purposes, as antimalarial (Idowu *et al.*, 2010) as well as for abortifacient purposes (Aguwa, 1987). The powdered roasted seed is mixed with gingerly oil to make a paste which is used for the treatment of ring worm. Decoction of the leaves is

used for aseptic cleaning of wounds and healing (Kumari *et al*., 2011). *L. inermis* is also used by some individuals as 'blood tonic', thus implying its multifaceted use (Idowu *et al*., 2010).

2.4.6 Henna phytochemicals

Lawsonia inermis has been well investigated phytochemically by various researchers. The occurrence of B- sitosterol glucoside , (Mahmoud *et al*., 1980) flavonoids , (Afzal *et al*., 1980) quinoids , (Nakhala *et al*., 1980) naphthalene derivatives , (Afzal *et al*.,1984) gallic acid (Nakhala *et al*., 1980) coumarins (Dzhuraev *et al*., 1982) , and xanthones (Bhardwaj *et al*., 1978) in Lawsonia leaves has been reported. Earlier work establishes the use of henna as an alternative vegetable retanning agent (Musa *et al*., 2008).

According to phytochemical analysis, the powdered of its leaves contain about 0.5-1.5% lawsone; the chief constituent responsible for the dyeing properties of the plant. Henna plant also contains alkaloids, glycosides, flavonoids, saponin, mannite, tannic and gallic acids, coumarins, mucilage, and naphoquinone (Ahmed *et al*., 2000; Chukwu *et al*., 2011; Khan *et al*., 1991; Kirkland and Marzin, 2003; Nayak *et al*., 2007; Rosenberg, 1999; Vardamides *et al*., 2001).

In a study, the nitrogen content was measured by the Kjeldahl technique. The results showed that the leaves had a level of Ca, Na, P and K contents, ranging from 0.2 to 4%. The Mg content was less than 0.2% while Cu, Zn and Fe contents were above 0.5, 1.1 and 15%, respectively, Mn content was less than 1.5% while the Nitrogen Matter (NM) content was less than 1.5%. In the stems, P and K contents were respectively, above 5.12 and 0.5%, Mg content was less than 0.08%, while Na and Ca contents were less than 0.2%. Cu, Zn, Fe and Mn contents were less than 0.95, 1.7, 4 and 0.5%, respectively and NM contents were less than 0.2% (Boubaya *et al*., 2011).

The extract of henna leaves as environmentally friendly corrosion inhibitors of metals was also investigated by Al-Sehaibani (2000). The water extracts of henna, (*Lawsonia inermis*) leaves powder were investigated for their corrosion inhibitory ability on steel and commercial aluminum in saline, acid and alkaline water. The maximum efficiency was attained by just 20 g L-1 of the extract. The inhibition efficiency of mild steel corrosion in HCl by the extract was 96% and that of aluminum in Na OH was up to 99.8% and no inhibition was observed for steel and aluminum in Na Cl solution.

Rehan (2003) conducted a research on the effect of water extracts from dry leaves of some economic plants, date palm (*Phoenix dectylifera*), henna (*Lawsonia inermis*) and corn (*Zea mays*) on the corrosion inhibition of commercial grade metals; steel, aluminum and copper in acidic chloride and sodium hydroxide solution using the weight loss, solution analysis and potential measurements. The inhibition action was found to critically depend on the metal type and solution composition. Only date palm and henna extracts were found to be highly effective in reducing corrosion rate of steel in acidic chloride solution and aluminum in sodium hydroxide solutions. The inhibition efficiency increased with increasing concentration of the extract. The inhibition was interpreted in terms of chemisorption of some active ingredients in the leaves according to Temkin isotherm (Buchweishaija, 2009). Regarding its bio-stimulating property, Chandrasekaran *et al.* (2000) mentioned that treatment of soyabean seed with *Lawsonia inermis* leaf extract at 10%, increased shoot length significantly. Besides, Pathak and Srivastava (2000) stated that treating of sunflower with *Lowsonia inermis* increased its total phenols content. On the other hand, Singh *et al.* (2006) reported that the extract of Henna gave significant control of the white fly in tomato compared to the untreated control.

2.5 Hargel (*Solenostemma argel* Del. Hayne)

The Hargel plant (*Solenostemma argel*), belongs to the *Asclepiadaceae* family. This family includes many wild growing medicinal Plants such as (*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 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, 1996). Sudan is regarded now as the richest source of this plant (Organgi, 1982; El-Ghazali, 1997 and Ahmed, 2003).

Solenostemma argel is considered to be medicinally important in the Sudan, Libya and Chad (Ahmed, 2003).

2.5.1 Origen and distribution

Solenostemma argel (Del.) Haynes plant is a member of the family *Asclepiadaceae*, it is a desert plant which widely distributed in Egypt, Libya, Chad, Algeria, Saudi Arabia, Palestine, Central and Northern parts of the Sudan (Ahmed, 2004).

In the Sudan , Hargel (*Solenostemma argel*) is considered as a desert plant of traditional medical uses. It grows wild in the area extending from Dongola to Barber, particularly around Abu Hamad, where it is grown under irrigation (Elkamali and Khalid, 1996). However, Sudan is regarded as the richest source of Hargel plant.

2.5.2 Botanical description

The plant is an erect herbaceous perennial plant that grows up to 60-100 cm tall, with several vigorous stems. The leaves are, oval, leathery and

covered with fine hairs. It has numerous flowers with white petals, and a strong smell, flowering period extends from March to June. The fruits are box shape about 5 cm. long and 1.5-2 cm- wide, green with violet lines; they contain pubescent seeds (Elkamali, 2001)**.**

2.5.3 Ecology

Solenostemma argel occurs in dry sandy and rocky localities as well as gravelly wadis, with an annual rainfall as low as 50-100 mm.It is drought and frost tolerant (Khalid *et al*.,1974).

2.5.4 Propagation and planting

Solenostemma argel is propagated by seed. Average 1000 seed weight is 24.7g.Seeds are sown directly in the field or the plants are raised in nursery beds and transplanted to the field .When directly sown about 2.5 kg seed/feddan is required ;irrigation is necessary as the seeds fail to germinate with less than 10mm rainfall. Seeds germinate in a wide range of temperatures, but maximum germination was observed at 35 °C and at alternating higher and lower temperatures. Pre- treatment of seeds with growth stimulators promote germination, The seeds are very sensitive to salinity during germination. The suitable depth for sowing is the upper surface layer. Methods of in vitro propagation have been developed using meristematic tips (Plaza *et al*.,2005).

2.5.5 Harvesting

The best stage for harvesting the leaves of *Solenostemma argel* is in pre-flowering stage. It is a perennial plant, but under cultivation, the crop can be grown as un annual , as the first year the yield is highest. The leaves can be harvested 3 times during the season. Under irrigated conditions, about 1000 kg of dry leaves per ha per season can be obtained. The aerial parts and leaves are dried in the shade, and later stored in jute bags (Elkamali,1991).

2.5.6 Uses

Solenostemma argel is considered to be medicinally important in the Sudan, Libya and Chad (Ahmed, 2003). Argel leaves are used to treat many diseases including kidney, liver, stomach diseases, and some allergies (Tharib *et al*., 1986; Elawad *et al*., 2014; Faten *et al*., 1994). Infusion of leaves is used to treat gastrointestinal cramps, as laxative (Filipescu *et al*.,1985) stomach ache; anti-colic, urinary tract infections, cold and anti-syphilitic for prolonged period time between 40- 80 days (Elkamali and Khalid ,1996; Boulos ,1983; Taj Al- Deen *et al*., 2014) and as an anti-inflammatory (Jobeen *et al*., 1984). Also, argel leaves are used to treat measles as incense, sometimes crushed leaves of argel are used as remedy to treat sciatica, neuralgia, and for supporting wounds and bronchitis (Tharib *et al*., 1986; Boulos, 1983).

(Taj Al- Deen *et al*., 2014) reported that, in Yemen argel leaves are used as herbal medicine for prevention of diabetes and the leaves are consumed as tea, and his result study was carried out biological investigation on argel leaves as a hypoglycemic agent. The whole of argel plant is used as incense to treat many diseases including: diabetes mellitus, hypercholesterolemia, cough, cold, jaundice and measles (Elkamali and Khalid ,1996).

2.5.7 Phytochemical properties

Phytochemical of *S. argel* had been studied by many researchers (Kamel *et al*., 1982; Roos *et al*.,1980; Hamed , 2001; Sulieman *et al*., 2009).It has been studied from different parts of *S. argel* (leaves, stems, and flowers) and provided numerous ingredients and crystalline compounds (Idris *et al*.,2011; Plaza *et al*.,2005) .Previous study conducted by (Elkamali, 2001) reported and classified some chemical constituents of *S. argel* are including acylated phenolic glycosides, namely argelin and argelosid,

choline, flavonoids, monoterpenes, pregane glucoside, sitosterol, and a triterpenoid saponin .

(Hamed , 2001) described two of stemmosides (A, and B) as pregnane ester glycosides, and stemmin C as polyhdroxy pregnane of Argel plant. Also, leaf extracts contain; quercetin, rutin, flavanones, and alkaloids, flavonoids, and kaempferaol (Tigani and Ahmed, 2009; Shafek and Michael, 2012; Murwan *et al*., 2010) reported that leaves of *S. argel* are characterized by having (64.8%) carbohydrates, about (15 %) was protein, and he found that crude oil, moisture content, and ash (1.6%, 4.4% and 7.7%, respectively), they estimated that minerals content, whereat, they found high potassium about 0.54%, calcium, magnesium, and sodium(0.06%, 0.03%, and 0.01% respectively), while manganese, ferrous, lead, and cupper were estimated as low percentages (0.001%, 0.002%, 0.002%, 0.001%, and 0.0001% respectively). And (Murwan *et al*., 2010) fractionated the protein of argel leaves to different compounds including: albumin, non-nitrogenous protein, prolamine, globulins, and glutulin. In addition leaves of argel also contained anti-nutrition factors (phytic acid, and tannin) (Murwan *et al*., 2010).

Phyto-chemicals of medicinal properties from argel shoots had been reported by many workers (Roos *et al.*, 1980; *Hamed,* 2001). Sulieman *et al.*, (2009) reported that the aqueous extracts of argel have antifungal and antibacterial properties. Orange, (1982), Idris *et al*.(2012) reported significant increase in date palm yield and improvements in the physical quality characters of the fruits upon addition of small quantities of dry leaves of Argel to the soil of the palms ,under the conditions of the Northern State, Sudan. They attributed the yield and quality gains to a growth regulator-like effect of Argel.

Also, Idris *et al.* (2014) reported restoration of normal growth in vegetatively malformed mango trees, beside enhancements in

characteristics of it. Argel water was effective in controlling aphids and white flies in summer tomatoes and Egyptian bull worm in okra respectively (Unpublished observation). In a pilot field experiment on *Brassica nigra,* some peripheral plots were severely infested by aphids. The infestation caused stunting of shoots and delayed flowering compared to non-infected plots. However, upon treatment with argel as a soil additive, or a spray of shoot water extract or a combination of soil additive and spray, the vegetative growth was restored in all plots after pest disappearance and the plants flowered within 10-15 days after treatments. The inflorescence was abnormally thick and profusely branched in plants that received the combined treatment suggesting a growth-regulator-like effect and indicating the efficiency of argel as a pesticide (Abdelwahab, 2002).

2.6 Haza (*Haplophyllum tuberculatum*)

The genus *Haplophyllum*, belonging to the Rutaceae family, comprises about 70 species distributed from the Mediterranean area to eastern Siberia (Willis ,1980). This genus distributed throughout temperate and subtropical zones of Eurasia and the northern tropical zone of eastern Africa (Somalia) oils (Kashiwada *et al*., 1996 ; Javidnia *et al*., 2006).

2.6.1 Origen and distribution

Haplophyllum genus is distributed from Morocco and Spain in the west to China in the east. It extends north to Romania and south to Somalia and in the east it extends north to the Lake Baikal region (Townsend,1986). Its range spans five different floristic regions: Mediterranean, Saharo-Arabian, Irano-Turanian, and Sudano-Zambezian regions (Takhtajan ,1986). The main center of diversity is the Irano-Turanian region, Iran, Turkey, and Central Asia which harbours 60% of

the species diversity. *Haplophyllum tuberculatum* is found in central and eastern areas of Asia.

2.6.2 Botanical description

Perennial herb, up to 40 cm tall, glabrous to short-hairy; stem usually much branched from the base, yellowish green to almost white; glands numerous on all parts; and very variable**.** Leaves alternate, strong smelling, variable in shape, from narrowly linear to short in size. Flowers are yellow and variable in size. Petiole short below, absent above; blade very variable, shortly obovate, elliptical, lanceolate or linear, sometimes deeply cut into 3 lobes. The flowers are in loose corymbose terminal panicles, with five free ovate sepals. The stamens 10 are filamentous and hairy. The petals are five and bright yellow in color (Al-Burtamani *et al* ., 2005).

2.6.3 Ecology

This common perennial herb is found wild even growing as a common weed among summer crops. *Haplophyllum tuberculatum* occurs in sandy desert, on a variety of soils, often on silt deposits, and also in dried watercourses, cultivated and ruderal localities, from sea-level up to 1330 m altitude. The psammophytic community inhabits the sand dunes of *Haplophyllum tuberculatum* on the upper positive part of axis 1 are correlated with species concentration of dominance. These combinations are typical of grass communities inhabiting the wadi bed and sand dunes (Alatar *et al* ., 2012).

2.6.4 Uses

Hazza is used in traditional medicine as a remedy for headaches and arthritis, skin discoloration, the juice is applied as a wart removal, and against parasitic diseases and other infections (Al-Burtamani *et al.,* 2005).

It is also used to treat nervous system, infertility and fever (Said *et al.*, 2002). Decoctions of the plant are recommended by herbalists for preparations used as carminatives for children. In the north of Oman, the juice expressed from the leaves is used as a remedy for headaches and arthritis (Mossa *et al.*,1992). In Saudi Arabia, *Haplophyllum tuber-culatum* is used to treat malaria, rheumatoid arthritis and gynecological disorders (Al-Yahya *et al.*, 1992). While, in Sudan the herb is used as an antispasmodic, to treat allergic rhinitis and gynecological disorders, asthma and breathing difficulties (Mohamed *et al.*, 1996) and so on, indicating a large degree of variability in its traditional uses as a function of geographic and ecological location. In Sudan it is called "a plant of all disease". It is used in most of Sudanese homes as emergency medication and is mostly used by old Sudanese in the rural areas.

2.6.5 Haza phytochemicals

The plant's chemical composition has been shown to vary as a function of geographic location and time of collection. It includes alkaloids, lignans, flavonoids and essential oils (Kashiwada *et al.*, 1996; Javidnia *et al.*, 2006). Many studies have evaluated the medicinal properties and phytochemistry of some of these species, analyzing their contents for alkaloids, lignanes, glycosides and flavonoids, etc. Two new alkaloids, haplotubinone and haplotubine, were isolated from the aerial parts of *Haplophyllum tuberculatum* together with the known Lignan Diphyllin (Adnan *et al.*, 2001).

The chemical components of the *Haplophyllum tuberculatum* essential oil was analyzed by gas chromatography– mass spectral (GC–MS) as well as 13C NMR spectroscopy. More than 30 compounds, constituting about 99.7% of the total oil, were identified. The most abundant oil components were β-phellandrene (23.3%), limonene (12.6%), (Z)-β-ocimene (12.3%),

β-caryophyllene (11.6%), myrcene (11.3%), and α-phellandrene (10.9%) (Al-Burtamani *et al*., 2005).

Jepreel (2019) investigated the responses of Periwinkle plants to soil and foliar applications of Haza plant. The foliar treatments were for boiled water extracts of hand crushed Haza shoots in concentrations: 0.0, 5, 10, 15 and 20 g/l, while the soil dressing test was for powder of dry shoots of Haza applied in doses of: 0.0, 5, 10, 15 and 20 g per plant. The results showed substantial increments in vegetative and reproductive growth parameters coupled with high alkaloids content from soil dressing with 10 g/plant Haza treatment or the foliar application of the 10 g/l Haza extract. These findings elucidated the bio-stimulating potential of Haza applications for enhancing vegetative and reproductive growth beside alkaloids content of Periwinkle. She reported that stimulating potential may be of value for trials on organic production of other horticultural crops.

(Sad AL-ah and Mubarak, 2017**)** conducted a Laboratory experiment to evaluate the insecticidal effects of Plant of the mosquito *Haplophyllum tuberculatum*, mesquite *prosopis juliflora*. Against lesser grain borer *R.dominica.* The aqueous extract of each plant were used under three concentrations from each plant (5%, 10%, 15%)-were used in this study. The results showed that the higher concentrations of all tested plants in aqueous formulations gave significantly higher mortality percentage than the control after 24 hours of exposure. The highest concentration 15% of *H. tuberculatum* aqueous extract of all plant parts revealed (3.3 to 6.0) mortality after 72 hours of exposure, whereas, the same concentration of *P.juliflora* extract gave lowest mortality percentage (3.1 to 5.0). Also the experiment showed that all tested plants effects are repellants more than lethal effects, and this effects are disappear with increase of time period.

Eisa (2016) studied the impact of Hazza (*Haplophyllum tuberculatum*) at various rates on the growth attributes and quality of Aloe vera plants under nursery conditions. The treatments were applications of Haza as foliar and soil applications ; the foliar treatments were for cold, hot and boiled water extracts of 15 g dry shoots of Haza per litre and the soil application test were for 0.0, 2.5, 5, 7.5 and 10 g/ plant dry Haza shoot treatments. The results obtained indicated that relatively, there was a general increase in growth parameters in Haza treated plants compared to the control. Except for the root fresh and dry weights, the highest values of all growth parameters were obtained from the boiled Haza water extract and the 7.5 and 10 g soil dressing treatments. He reported that the improvements in growth and gel content are indicators of the agronomic benefit of Haza applications; a step towards organic farming.

2.7 Growth hormones, Gibberellic Acid (GA_3) and Benzyladenine (BA)

2.7.1 Plant hormones

Plant hormones have got an important role in growth and deployment of all plants. It has been stated that a plant hormone is a natural substance , by the plant it self and acts to control plants activities. There are five recognized groups of natural plant hormones which are auxins, gibberellins, cytokines, ethylene and abscisic acid (Leopold and Kriedemann, 1975). Leopold reported that each of the mentioned organic substances is distinctive both in chemical characteristics and in being able to bring about characteristic growth responses and each group of regulators is capable of altering growth, including cell division, cell elongation ,differentiation and differential growth phenomena . Changes in primary metabolic processes due to nutrient or external growth

conditions may play an important role in the regulation of secondary metabolism (Singh *et al.*, 2001).

2.7.2 Plant growth regulators

Plant growth regulators include plant hormones, natural and synthetic ,other non-nutrient chemicals, not naturally found in plants, but when applied to plants influence their growth and development. These are organic substances that are biologically active at very low concentrations. In different aromatic plants, the plant growth and terpenoid biosynthesis is regulated by plant growth regulators which gives significant effect in both properties and content of terpenoids (Shukla *et al.*, 1992) Terpenoid biosynthesis is based on primary metabolism such as photosynthesis and oxidative pathways for carbon and energy supply (Singh *et al.*, 1990) Triacontanol, a natual plant growth regulator plays significant role in enhancing biomass production which results in increased biosynthesis of secondary products. Physiological changes like growth, photosynthesis, flowering and cellexpansion in plant was observed by the application of phytohormone Gibberellic acid (GA_3) (Yuan and Xu , 2001; Taiz and Zeiger,2006) The metabolic activity withinpathways increased by the application of GA_3 which results to stress and anthocyanin biosynthesis (Ohlsson and Bjork,1988).

Plant growth regulator or phytohormone are active at low concentration and have particular effect on plant growth (Nambara and Marion-Poll , 2005) and (Teale *et al.*, 2006). Plant growth regulators are classified as auxin,cytokinin,gibberellins, abscisic acid and ethylene (Teale *et al.*, 2006) whereas jasmonate and brassinosteroids are also recognized as plant growth regulator (Taiz; and Zeiger,2006).

Growth regulators can improve plant growth ,development , yield, and essential oil quality (Singh *et al.*, 2001). Foliar applicatuon of triacontanol and mixtalol have been shown to significantly increase yield

attributes of rose-scented geranium (Bhatacharya and Raw 1996). Plant growth regulators can also confer plant resistence to abiotic stresses such as drought and osmotic stress (Chatterjee , 1995; Zhao and Oserhuis,1997; Varooqi *et al*., 2000; Singh *et al*., 2001).

Plant growth regulators can influence growth and essential oil production. Endogenous levels as well exogenous application could affect essential oil production (Prins *et al*., 2010).

Growth responses , flowering quality and active chemical constituents of gladiolus plants were studied by Bedour *et al.* (2011) using some vitamins such as thiamin, ascorbic acid and their compination during two seasons . Plants which received the compined treatments of both vitamins recorded the highest growth ,flower quality,and cormlet induction.

2.7.2.1 Gibberellins (GA)

Gibberellins (GA) are phytohormones that are responsible for the regulation of plant height. Seed germination, flowering and stem elongation are regulated by gibberellins which are diterpenes. Gibberellins play an essential role in seed germination by activation of embryo vegetative growth and mobilization of energetic reserves from endosperm and they are also linked with juvenile to adult transition processes and promote fructification (Taiz and Zeiger,2006) and (Clouse,2001) Gibberellins (GAs) are a family of plant hormones controlling many aspects of plant growth and development. Gibberellic acid is a member of this family.

2.7.2.2 Benzyladenine (BA)

Benzyladenine is one of the Cytokinins . Cytokinin functions in whole plant ontogeny from fertilized ovule to senescence and death. It play a role in processes like cell division, shoot initiation and growth,

senescence delay and photomorfogenic development, control of chloroplast division and growth, modulation of metabolism and morphogenesis in response to environmental stimulus (Pozo *et al*.,2005; Chernyad , 2000 and Hirose *et al* ., 2008). Cytokinins are involved in the regulation of various processes of plant growth and development while interacting with other phytohormones.

2.7.3 The Role of Plant growth regulators on essential oil in aromatic plants

It is well known that essential oil is derived from mevalonic acid via the isoprene pathway in a manner similar to that for other terpenes. Thus plant growth regulators that exert their effect at the level of gibberellin metabolism might increase the accumilation of essential oil in plants.Growth retardants such as phosphone D and chlormequat chloride (CCC) which influence gibberellin metabolism have been shown to increase terpene formation resulting in increased essential oil content of peppermint and sage (El-keltawi and Croteau,1987).

(Abbas *et al*., 2012) carried out a study on lemongrass (*Cymbopogon citratus*) and they studied the effects on plant growth, hormonal content and essential oil content by the application of plant growth regulators such as indole butyric acid (IBA) and mepiquat chloride(MC).The application of plant growth regulators IAA and MC had no significant effect on essential oil content and growth in lemongrass (Abbas *et al*.,2012). The most important essential oil of species *Cymbopogon citratus* Stapf (lemongrass) belong to family Gramineae has high content of citral up to 75% (Jayasinha *et al*., 1999). Also it was observed that the content,recovery and properties of lemongrass essential oil was changed by the application of plant growth regulator (Misra *et al* .,1991). Similarly it was observed in peppermint, sage, spearmint (El-keltawi *et al*., 1986) and in rose scented geranium (Eid *et al*.,1980). In rose scented geranium,

the content of essential oil was enhanced by the foliar application of tricontanol and mixtalol which is a long chain aliphatic alcohol. Number of branches, height of the plant,composition and yield of essential oil was influenced (Bhattacharya *et al*., 1996).

In aromatic plant Basil (*O. gratissimum*), (Hazzoumi *et al*., 2014) studied the effect of plant growth regulator gibberellic acid (GA), indole 3-acetic acid (IAA) and benzyl-amino-purine (BAP) on the yield and composition of essential oil and also on the main compound (methyl chavicol) and its isomer (the trans-anethole).The content of essential oil was enhanced by the application of IAA and IBA. The effect of GA, IAA and kinetin was studied in O. basilicum on the content and composition of essential oil.They concluded that the content of essential oil was reduced by the application of GA whereas by the application of IAA and kinetin the content of essential oil was enhanced. Due to this the main compound methyl chavicol was reduced from 75.16% in the control to 74.1% kinetin 73.2% IAA and 70.7% GA (Hazzoumi *et al*., 2014).

Abou Zied and Sherbeany (1971) indicated that chloremquat enhanced the volatile oils of chamomile. (Sharafzadeh *et al*., 2012) revealed that naphthaleneacetic acid and spermidine altered oil constituents of German chamomile. Another research showed that α-bisabolol oxide A, increased in chamomile with application of 100 ppm IAA (Reda *et al*., 2010). Growth and essential oil yield of *Mentha piperita* were improved by the application of polyamines (Youssef *et al*., 2002) . Silva *et al*.(2005) reported that auxin and cytokinin increased some components of the lemon balm oil. Povh and Ono (2007) showed that application of gibberellic acid influenced the chemical composition of Salvia oil. A report revealed that sodium salt of NAA and IAA increased the essential oil of *Mentha piperita* (Koseva-kovacheva and Staev, 1978).

Chapter Three

Materials &Methods

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

This study was conducted in the nursery of the Horticultural Sector Administration , Federal Ministry of Agriculture, Khartoum, Sudan . The area lies between the latitudes 15 .9 ; and 16 .45 North; and the longitudes 24 ,45 ; and 21 ,25 East (Ali, 2000). The climate can be considered a semi-arid of rainy season from July to September (Oliver, 1965).

3.2 The plant material source

The Lemongrass experimental materials were obtained from mature field grown plants. Tillers of uniform size and shape were severed to 10 cm length prior to planting in 30×40 cm black polyethylene bags containing River Nile sedimentary soil. Four weeks after planting, they were used as test materials in two separate experiments for every test

3.3. Experiments

3.3.1 Experiment (1): The investigation on the bio-stimulating property of Moringa:

Ground Moringa leaves was tested as soil treatments at rates of: 0, 4, 8, 12, and 16 g / Lemongrass plant. The cold water extract of Moringa, was tested as foliar treatments in concentrations of: 0, 4, 8, 12, and 16 g/l. The two tests were arranged in completely randomized design with 6 replicates to determine the impact of Moringa, soil and foliar applications on the performance of Lemongrass plants.

3.3.2. Experiment (2): The investigation on the bio-stimulating property of Henna:

Ground Henna, leaves was tested as soil treatments at rates of: 0, 4, 8, 12, and 16 g / Lemongrass plant. The cold water extract of Moringa , was tested as foliar treatments in concentrations of: 0, 4, 8, 12, and 16 g/l. The two tests were arranged in completely randomized design with 6 replicates to determine the impact of Henna, soil and foliar applications on the performance of Lemongrass plants.

3.3.3. Experiment (3): The investigation on the bio-stimulating property of Hrgel:

Ground Hargel leaves were tested as soil treatments at rates of: 0, 4, 8, 12, and 16 g on Lemongrass plants. The cold water extract of Hargel , was tested as foliar treatments in concentrations of: 0, 4, 8, 12, and 16 g/l. The two tests for were arranged in completely randomized design with 6 replicates to determine the impact of Hargel soil and foliar applications on the performance of Lemongrass plants.

3.3.4. Experiment (4): The investigation on the bio-stimulating property of Haza:

Ground Haza leaves were tested as soil treatments at rates of: 0, 4, 8, 12, and 16 g on Lemongrass plant. The cold water extract of Haza, was tested as foliar treatments in concentrations of: 0, 4, 8, 12, and 16 g/l. The two tests for were arranged in completely randomized design with 6 replicates to determine the impact of Haza , soil and foliar applications on the performance of Lemongrass plants.

3.3.5 Experiment (5): The investigation on the growth bio-stimulating property of Benzyladenine (BA) and Giberellic acid (GA_3):

Four treatments of different concentrations of BA and GA_3 hormones were added as a foliar application to study the impact of GA_3 and BA hormones applications on growth attributes of Lemongrass. The four treatments were: control (no hormone), BA (100mg/litter) , GA_3 (100mg/litter) and the combination of BA(100mg/litter) +GA_3 (100mg/litter) per plant.

3.4. Replications:

Each of the above-mentioned treatments was replicated 6 times. Each plant was considered a replicate.

3.5. Data collection:

Six months after applications, wherever appropriate, data were collected for the following parameters:

1. Number of leaves.
2. Leaf length (cm).
3. Leaf width (cm).
4. Leaf chlorophyll content: was determined with Spad device.
5. Leaf fresh weight (g).
6. Leaf dry weight (g).
7. Root fresh weight (g).
8. Root dry weight (g).
9. leaf oil contents (%). The oil content was determined according to Guenther (1948).

3.6. Data analysis:

Data were subjected to analysis of variance and means were separated at 95% confidence limits according to Duncan's Multiple Range Tests with the aid of MStat computer program.

Chapter Four

Results

CHAPTER FOUR

RESULTS

4.1.Experiment (1):

The investigation on the bio-stimulating property of Moringa on lemongrass plants.

A.The soil applications:

All Moringa applications resulted in significant increase in leaf length compared to the control .This parameter was best enhanced by the 4 g/plant treatment which was statistically equal to the 8g/plant treatment (Table 1). Regarding leaf width, the best value was recorded for the 4 g/plant Moringa treatment, but the difference was not significant compared to the control, the 8 and 12 g/plant Moringa treatment. However the best value was recorded for the 16 g/plant treatment which was significantly lower than the 4 g/plant treatment (Table1). Leaf chlorophyll content was significantly increased by the 8g /plant treatment compared to other treatments that shared the second rank (Table 1).

According to Figure (1) except the 16g/plant all Moringa soil applications increased the number of leaves significantly over the control. The least dose (4g/plant) ranked top and therefore decreases were recorded with increase of dose (Figure1).

According to Table (2), the 8 g/plant Moringa treatment ranked top for the fresh and dry weights for leaves and roots. However, the 4 g/plant treatment ranked second for leaves fresh and dry weights, while the 12 g/plant treatment ranked second for roots fresh and dry weights. The 16 g/plant was deteriorative for leaves fresh and dry weight, but was enhancive for root fresh and dry weights compared to the control.

The leaves oil content was only enhanced over the control by the 4g/plant treatment (Table 2).

B. The foliar applications:

All Moringa treatments increased the leaf length over the control. The longest leaves resulted from the 16g/l Moringa treatment (Table3). The leaf width was enhanced significantly over the control by the 4g/l Moringa treatment, while the highest leaf chlorophyll content was recorded for the 12 g/l treatment followed by the 16g/l treatment (Table3).

Regarding the number of leaves the 8g/l Moringa treatment was the most enhancive for the number of leaves /plant. The other Moringa treatments decreased this parameter significantly compared to the control that ranked second (Figure 2).

According to Table (4), all Moringa treatments increased leaves fresh and dry weights significantly over the control .The best values were recorded for the 8g/l Moringa treatment followed by the 12 g/l treatment. The 8g/l Moringa treatment also ranked top for both roots fresh and dry weights. The 4 and 12 g/l treatments were also enhancive compared to the control, but the 16 g/l treatment reduced roots fresh weights but increased their dry weights when compared to the control. The highest leaf oil content resulted from the 8g/l treatment with significant differences from the 12 and 16 g/l treatments but without significant difference from the control and the 4 g/l Moringa treatment (Table 4).

Table 1. Impact of Moringa soil applications on the, length, width and chlorophyll content of Lemongrass leaf

Moringa treatments (g/plant)	Leaf length (cm)	Leaf width (cm)	Chlorophyll content
0	58.65[c]	1.650[ab]	30.35[b]
4	62.90[a]	1.725[a]	31.05[b]
8	61.70[ab]	1.650[ab]	34.10[a]
12	60.92[b]	1.650[ab]	31.38[b]
16	54.38[d]	1.525[b]	31.20[b]

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

Figure 1. Impact of Moringa soil applications on the number of leaves of Lemongrass plant

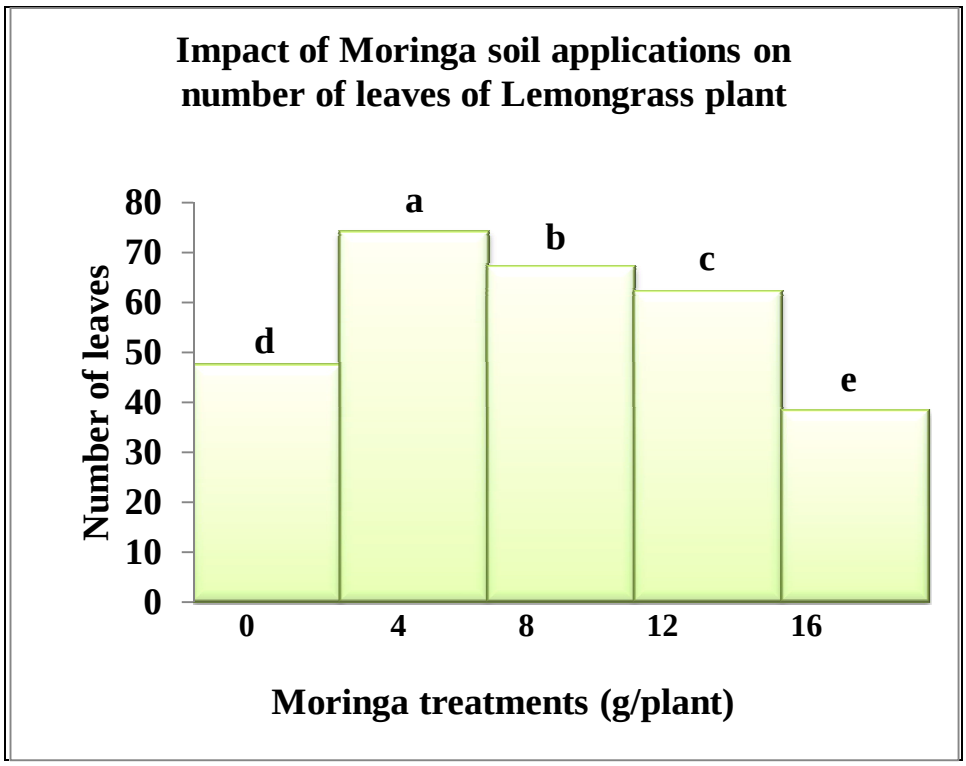

Table 2. Impact of Moringa soil applications on Lemongrass shoot and root fresh and dry weights and leaf oil content

Moringa treatments (g/plant)	Leaf fresh weight (g)	Leaf dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Leaf oil content (%)
0	279.5^{c}	83.63^{c}	40.63^{e}	32.00^{e}	0.2333^{b}
4	304.4^{b}	87.13^{b}	58.00^{c}	43.88^{c}	0.4000^{a}
8	309.3^{a}	90.75^{a}	86.53^{a}	67.25^{a}	0.1667^{bc}
12	222.9^{d}	74.25^{d}	64.25^{b}	57.38^{b}	0.1333^{bc}
16	144.0^{e}	42.00^{e}	46.00^{d}	40.50^{d}	0.1000^{c}

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

Table 3. Impact of Moringa foliar applications on the length, width and Chlorophyll content of Lemongrass leaves

Moringa extract conc. (g/l)	Leaf length (cm)	Leaf width (cm)	Chlorophyll content
0	58.40^{d}	1.700^{bc}	30.38^{c}
4	62.83^{c}	1.900^{a}	30.52^{c}
8	63.90^{bc}	1.875^{ab}	31.13^{bc}
12	64.25^{b}	1.650^{c}	33.03^{a}
16	66.07^{a}	1.875^{ab}	31.6^{b}

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

Figure 2. Impact of Moringa foliar applications on the number of leaves of Lemongrass plant.

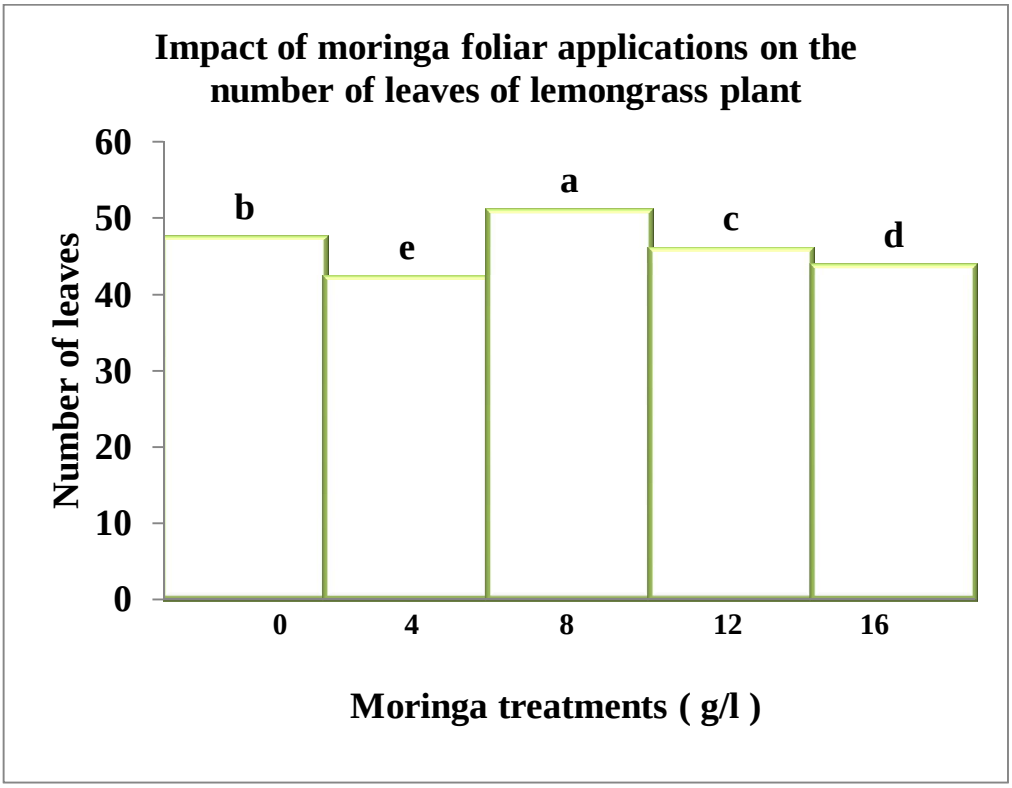

Table 4. The impact of Moringa foliar applications on Lemongrass shoots and roots fresh and dry weights, and leaves oil content

Moringa extract conc. (g/l)	Leaf fresh weight (g)	Leaf dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Leaf oil content (%)
0	112.1^{e}	32.72^{e}	27.5^{d}	14.45^{e}	0.2333^{ab}
4	176.6^{c}	48.25^{d}	31.13^{c}	26.25^{b}	0.2333^{ab}
8	226.8^{a}	62.50^{b}	43.5^{a}	35.00^{a}	0.2667^{a}
12	214.0^{b}	67.13^{a}	27.25^{b}	25.13^{c}	0.1333^{bc}
16	160.1^{d}	51.38^{c}	21.25^{e}	16.00^{d}	0.1000^{c}

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

4.2. Experiment (2):

The investigation on the bio-stimulating property of Henna on lemongrass plants.

A. The soil applications:

All Henna treatments resulted in significant increase in leaf length compared to the control . This parameter was equally enhanced by the 12 and 16 g/plant treatment (Table 5). Regarding leaf width, the best value was recorded for the 4 g/plant Henna treatment, whiles the other treatments induced significant increase over the control at a statistical equal level (Table5). Leaf chlorophyll content was best increased by the 12g /plant treatment compared to other treatments (Table5).

Regarding the number of leaves , all Henna soil applications increased the number of leaves significantly over the control. The highest dose (16g/plant) ranked top (Figure 3).

According to Table (6), the 16 g/plant Henna treatment ranked top for the fresh and dry weights of leaves. The 8 g/plant Henna treatment ranked top for the fresh and dry weights of roots. However, the 8 g/plant treatment ranked second for leaves fresh and dry weights, while the 16 g/plant treatment ranked second for roots fresh and dry weights. The 8 g/plant Henna treatment resulted in significant increase in leaf oil content, while the other Henna treatments were ineffective as promoters of this parameter (Table 6).

B. The foliar applications:

The 8 g/l Henna treatment was the most enhancive for the leaf length. However, all Henna treatments increased the leaf length over the control (Table7). The widest leaves were obtained from the 4g/l treatment, while

the highest leaf chlorophyll content was recorded for the 4 and 8g/l treatments (Table7).

Figure (4) illustrates the impact of Henna foliar applications on leaves number of lemongrass. The 8 g/l Henna treatment was the most enhancive for the number of leaves /plant.

According to (Table8), all Henna treatments increased leaves fresh and dry weights significantly over the control .The best values were recorded for the 12g/l Henna treatment. The 16g/l Henna treatment also ranked top for both roots fresh and dry weights, followed by 8 g/l Henna treatment. The 4 and 12 g/l treatments were also enhancive compared to the control. No significant differences obtained among the treatments in oil content (Table 8).

Table 5. Impact of Henna soil applications on the number, length, width and chlorophyll content of Lemongrass leaves.

Henna treatments (g/plant)	Leaf length (cm)	Leaf width (cm)	Chlorophyll content (%)
0	52.03[d]	1.450[c]	30.38[d]
4	58.27[c]	1.825[a]	33.60[b]
8	60.50[b]	1.550[b]	30.90[d]
12	62.10[ab]	1.625[b]	36.63[a]
16	62.38[a]	1.625[b]	32.40[c]

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

Figure 3. Impact of Henna soil applications on number of leaves of Lemongrass plant

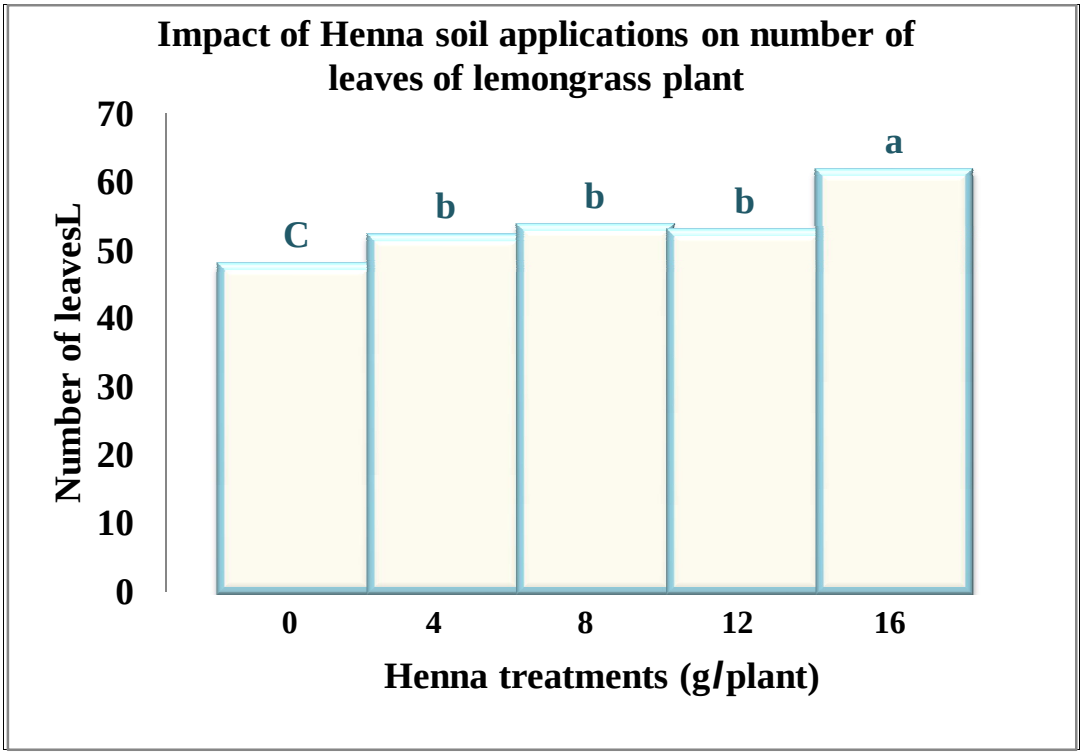

Table 6. Impact of soil applications of Henna on the fresh, dry weight and oil content of shoots, and roots of Lemongrass plant.

Henna treatments (g/plant)	Leaf fresh weight (g)	Leaf dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Leaf oil content (%)
0	087.50^{e}	31.50^{e}	40.13^{a}	33.00^{ab}	0.1667^{a}
4	156.5^{d}	46.13^{d}	31.63^{c}	25.25^{d}	0.1667^{a}
8	203.3^{b}	64.38^{b}	39.75^{a}	33.25^{a}	0.2333^{a}
12	173.1^{c}	59.00^{c}	32.00^{c}	26.88^{c}	0.1667^{a}
16	312.3^{a}	86.88^{a}	36.75^{b}	31.63^{b}	0.1333^{a}

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

Table 7. Impact of foliar applications of Henna on the length, width and chlorophyll content of Lemongrass plant.

Henna extract conc. (g/l)	Leaf length (cm)	Leaf width (cm)	Chlorophyll content
0	51.78[d]	1.425[b]	30.38[c]
4	63.63[b]	1.700[a]	33.33[a]
8	65.28[a]	1.525[a]	33.53[a]
12	52.15[d]	1.425[b]	30.70[bc]
16	58.22[c]	1.575[a]	32.10[ab]

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

Figure 4. Impact of Henna foliar applications on the number of leaves of Lemongrass plant.

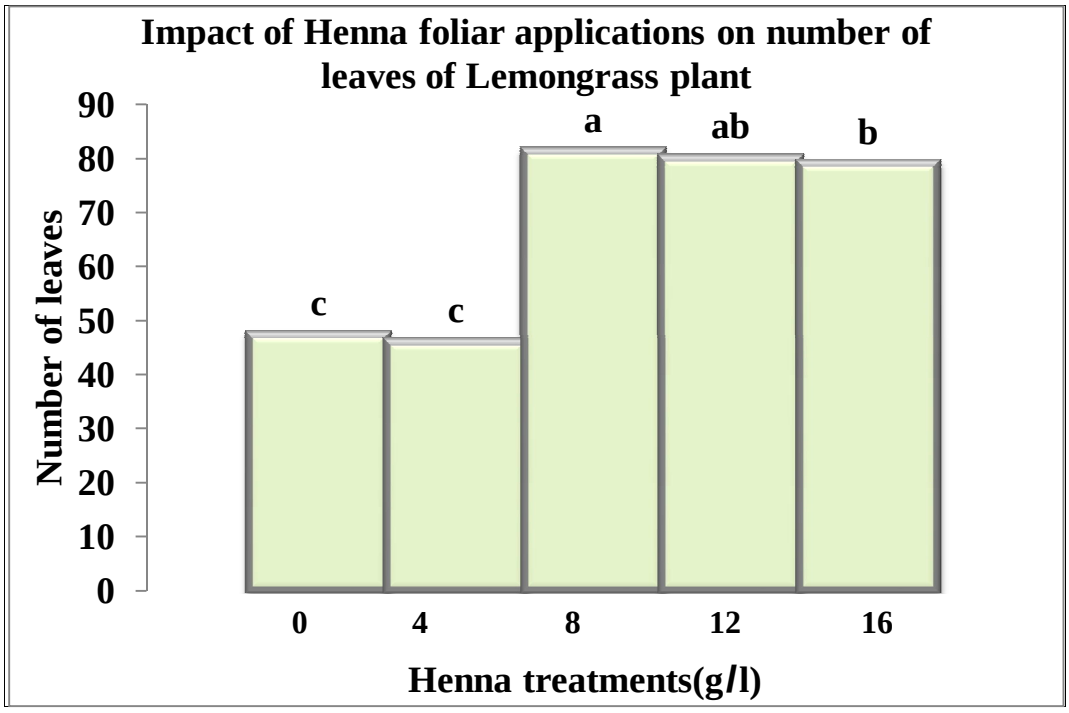

Table 8.The impact of Henna foliar applications on Lemongrass shoots and roots fresh and dry weights, and leaf oil content.

Henna extract conc. (g/l)	Leaf fresh weight (g)	Leaf dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Leaf oil content (%)
0	087.5^{e}	031.5^{e}	40.13^{e}	33.00^{e}	0.1333^{a}
4	189.0^{d}	059.3^{d}	46.00^{d}	38.13^{d}	0.1667^{a}
8	313.1^{b}	079.8 ^{c}	54.63^{b}	47.13^{b}	0.1667^{a}
12	335.3^{a}	108.8^{a}	50.50^{c}	43.63^{c}	0.1667^{a}
16	306.1^{c}	099.9^{b}	143.0^{a}	111.0^{a}	0.1333^{a}

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

4.3. Experiment (3):

The investigation on the bio-stimulating property of Hargel on lemongrass plants.

A. The soil applications:

According to Table (9) The leaf length was significantly different between the control and the other treatments, The dose 8 and 16 g/plant of hargel was the highest value in leaf width with significant difference from the control (Table 9). On the other hand, the results of chlorophyll content showed significant differences between the treatments and the control (Table 9).

Regarding (Fig 5) the highest dose of Hargel resulted in significant increase in leaves number compared to the control.

Table (10) illustrates the impact of soil applications of hargel treatments on fresh and dry weights of shoots and roots. The treatment 4g hargel resulted in a significant increase in shoot fresh and dry weights, followed by the 12g, 16g and then the 8g hargel treatments compared to the control. The 4 g hargel treatment increased roots fresh weight significantly over the 16 , 12 and 8 g hargel compared to the control. (Table 10).

B. The foliar applications:

According to (Table 11) The leaf length was significantly different between the control and the highest value of hargel treatments 12g/l, followed by the 8,16 and then 4 g/l. (Table 11) . The dose 16g /l of hargel was the highest value in leaf width with significant difference from the control (Table 11). On the other hand, the results of chlorophyll content showed significant differences from the control between the treatment 16 and 12 g/l followed by the dose 4 then 8 g/l

and the least chlorophyll content resulted from the control (Table 11).

According to (Fig 6) significant differences were observed among treatments in number of leaves that the highest number recorded in the dose of 8 g/l followed by 16 and 12 g/l, then the least leaves number that resulted from the 4 g/l hargel foliar treatment .

Table (12) illustrates the impact of foliar applications of hargel treatments on fresh and dry weights of shoots and roots. The highest hargel level 8g/l resulted in a significant increase in shoot fresh weights, followed by the 16 , 4 g/l and then the 12 g/l hargel treatments resulted in significant increase in the fresh and dry weights of shoots compared to the control which recorded as the least shoots fresh and dry weights. The 8g/l hargel treatment increased roots fresh and dry weight significantly over the 16 , 4 and 12 g/l hargel treatments compared to the control which recorded the least weights of fresh and dry roots (Table 12).

Table 9. Impact of soil application of Hargel on the length, width and chlorophyll content of Lemongrass plant.

Hargel treatments (g/plant)	Leaf length (cm)	Leaf width (cm)	Chlorophyll content
0	58.92^{c}	1.125^{c}	22.00^{c}
4	64.38^{a}	1.325^{b}	34.30^{b}
8	62.75^{b}	1.500^{a}	36.13^{ab}
12	62.63^{b}	1.400^{b}	37.33^{a}
16	62.50^{b}	1.525^{a}	34.60^{b}

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

Figure 5. Impact of Hargel soil application on the number of leaves of Lemongrass plant.

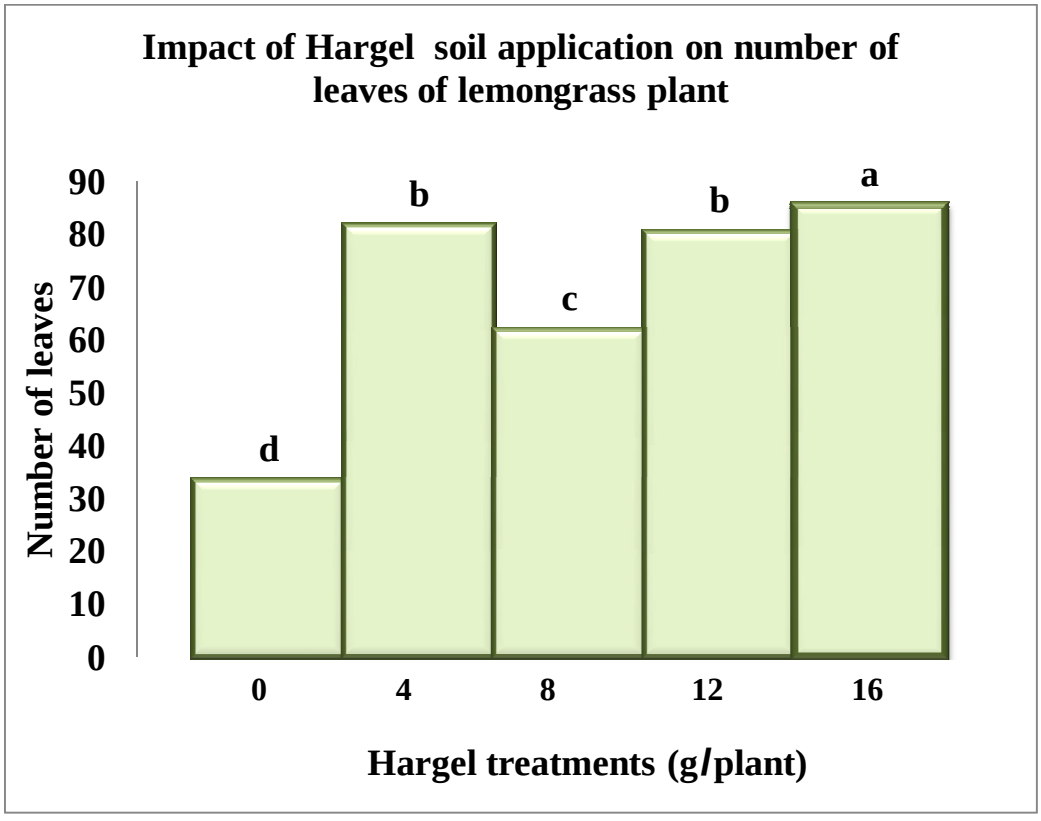

Table 10. Impact of Hargel soil application on lemongrass shoots and roots fresh and dry weights and leaf oil content.

Hargel treatments (g/plant)	Leaf fresh weight (g)	Leaf dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Leaf oil content (%)
0	87.50^{e}	22.95^{e}	27.50^{e}	14.45^{e}	0.1366^{a}
4	351.5^{a}	111.8^{a}	107.1^{a}	67.75^{a}	0.1366^{a}
8	125.9^{d}	32.13^{d}	34.45^{d}	17.33^{d}	0.1553^{a}
12	271.9^{b}	98.80^{b}	64.88^{c}	38.38^{c}	0.1557^{a}
16	201.1^{c}	85.78^{c}	75.88^{b}	43.13^{b}	0.1558^{a}

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

Table 11. Impact of Hargel foliar application on the length, width and Chlorophyll content of Lemongrass plant.

Hargel extract conc. (g/l)	Leaf length (cm)	Leaf width (cm)	Chlorophyll content
0	56.33^{e}	1.125^{c}	22.00^{e}
4	57.88^{d}	1.425^{b}	35.33^{c}
8	60.75^{b}	1.450^{b}	33.38^{d}
12	63.88^{a}	1.375^{b}	38.40^{b}
16	58.88^{c}	1.550^{a}	40.10^{a}

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

Figure 6. Impact of Hargel foliar application on number of leaves of Lemongrass plant.

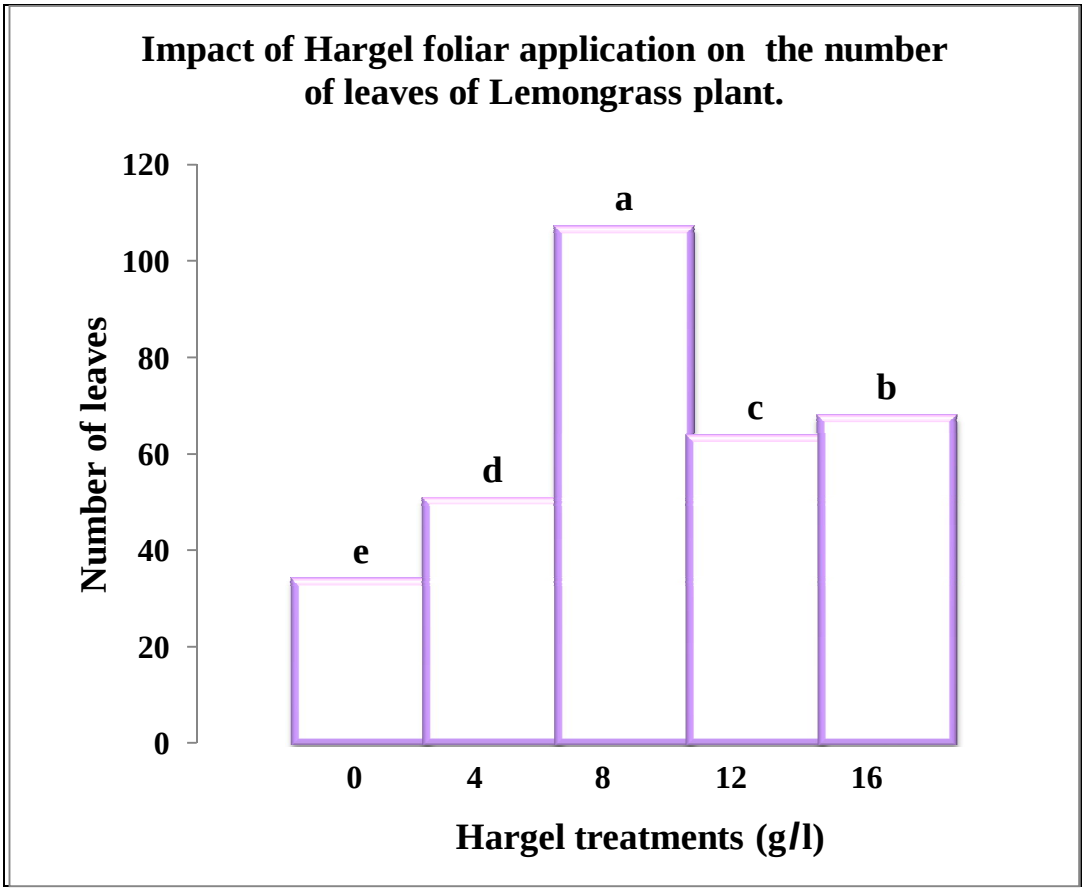

Table 12. The impact of Hargel foliar application on Lemongrass shoots and roots fresh and dry weights , and leaf oil content

Hargel extract conc. (g/l)	Leaf fresh weight (g)	Leaf dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Leaf oil content (%)
0	087.50^{e}	022.95^{e}	018.33^{e}	14.45^{e}	0.1443^{a}
4	194.9^{c}	062.65^{c}	058.88^{c}	30.75^{c}	0.1467^{a}
8	450.8^{a}	137.9^{a}	133.1^{a}	63.38^{a}	0.1557^{a}
12	164.0^{d}	052.42^{d}	033.25^{d}	19.50^{d}	0.1567^{a}
16	196.1^{b}	070.65^{b}	091.38^{b}	49.13^{b}	0.1563^{a}

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

4.4. Experiment (4):

The investigation on the bio-stimulating property of Haza on lemongrass plants.

A. The soil applications:

The dose 4g/plant of Haza was the highest value in leaf length with significant difference from the control (Table 13). The leaf width was significantly different between the control and Haza treatments (Table 13)., On the other hand, the treatments 4g and 8g/plant resulted in the highest values of chlorophyll content followed by 12g and 16g/plant showed significant differences between the treatments and the control (Table 13).

According to Figure (7) significant differences were observed among treatments in number of leaves. the treatments 4g ,8g , 12g, and then 16g/plant resulted in significant increase in leaves number compared to the control (Figure 7) .

Table (14) illustrates the impact of soil application of Haza treatments on fresh and dry weights of shoots and roots. The Haza level 8g resulted in a significant increase in shoot fresh and dry weights, followed by the 12g Haza treatment resulted in significant increase in the fresh and dry weights of shoots compared to the control. (Table 14). The 12 g Haza treatment increased root fresh and dry weights significantly over the the control and ranked top (Table 14).

A. The foliar applications:

The leaf length was significantly different between the control and the highest value of Haza treatments 8g/l, followed by the 4, 12, and then 16 g/l. However, the control resulted in the smallest value in leaf length (Table 15). The dose 4 g/l of Haza was the highest value

in leaf width with significant difference from the control (Table 15). On the other hand, the results of chlorophyll content showed significant differences between the treatments and the control, however, the Haza dose 4 g /l ranked as the top value in chlorophyll content followed by the doses 8, 12, then 16 g/l and the least chlorophyll content resulted from the control (Table 15).

Table (16) illustrates the impact of foliar application of Haza treatments on fresh and dry weights of shoots and roots. The highest Haza level 16 g/l resulted in a significant increase in shoot fresh and dry weights comparing to the control which recorded as the least shoots fresh and dry weights (Table 16). The 16 g/l Haza treatment resulted in increase in the roots fresh and dry weights significantly compared to the control which recorded the least roots fresh and dry weights (Table 16).

According to Figure (8) significant differences were observed among Haza foliar treatments in number of leaves that the highest number recorded in the dose of 8 g/plant followed by 4, 16 and then 12 g/plant , and the least leaves number resulted from the control (Figure 8).

Table 13. Impact of soil application of Haza on the length, width and chlorophyll content of Lemongrass plant.

Haza treatments (g/plant)	Leaf length (cm)	Leaf width (cm)	Chlorophyll content
0	58.92[d]	1.125[b]	30.38[d]
4	73.15[a]	1.375[a]	38.10[a]
8	71.22[b]	1.325[a]	38.17[a]
12	71.40[b]	1.375[a]	36.47[b]
16	70.20[c]	1.350[a]	34.97[c]

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

Figure 7. Impact of Haza soil application on the number of leaves of Lemongrass plant.

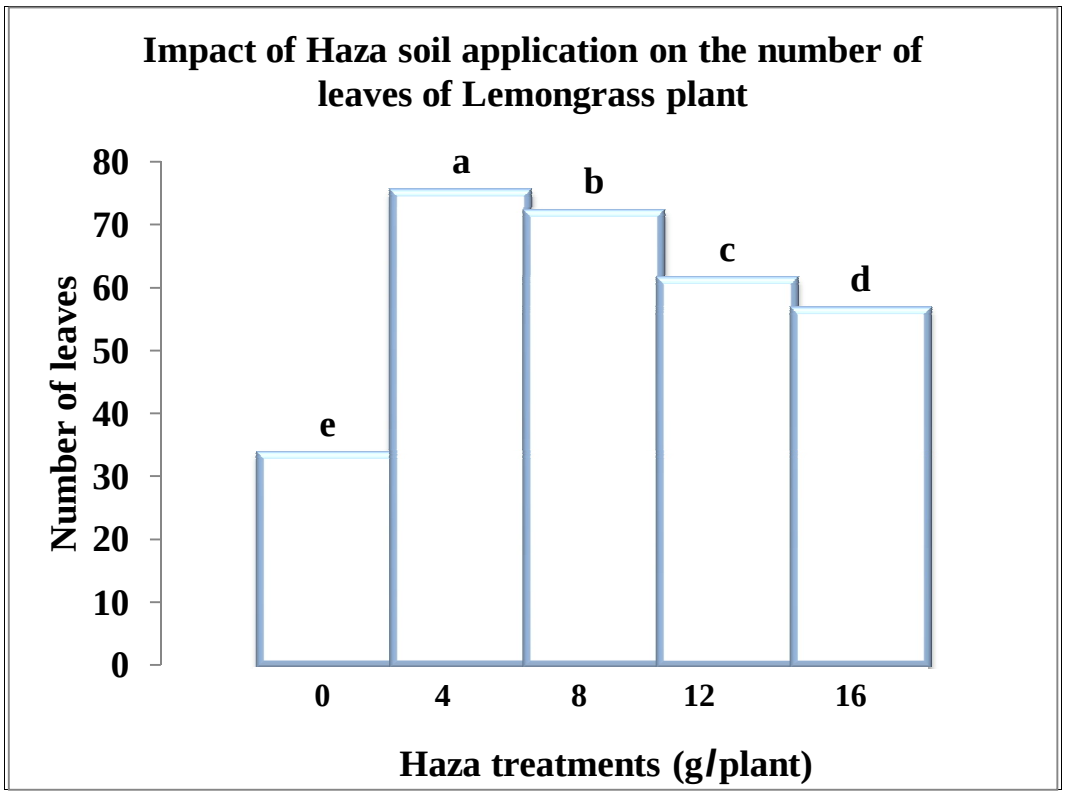

Table 14. Impact of soil application of Haza on the fresh and dry weight of shoots , and roots in Lemongrass plant.

Haza treatments (g/plant)	Leaf fresh weight (g)	Leaf dry weight (g)	Root fresh weight (g)	Root dry weight (g)
0	87.50^{e}	33.20^{c}	18.33^{d}	13.75^{c}
4	140.1^{c}	39.35^{a}	20.38^{c}	15.63^{b}
8	148.3^{a}	38.20^{a}	22.13^{b}	17.55^{a}
12	144.3^{b}	35.88^{b}	26.13^{a}	18.67^{a}
16	123.9^{d}	35.13^{b}	22.75^{b}	15.75^{b}

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

Table 15. Impact of foliar applications of Haza on the length, width and chlorophyll content of Lemongrass plant.

Haza extract conc. (g/l)	Leaf length (cm)	Leaf width (cm)	Chlorophyll content
0	55.25[e]	1.125[d]	30.38[e]
4	74.13[b]	1.475[a]	37.72[a]
8	78.03[a]	1.325[c]	36.33[b]
12	68.03[c]	1.400[b]	34.10[c]
16	62.58[d]	1.325[c]	31.38[d]

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

Figure 8. Impact of Haza foliar application on the number of Leaves of Lemongrass plant.

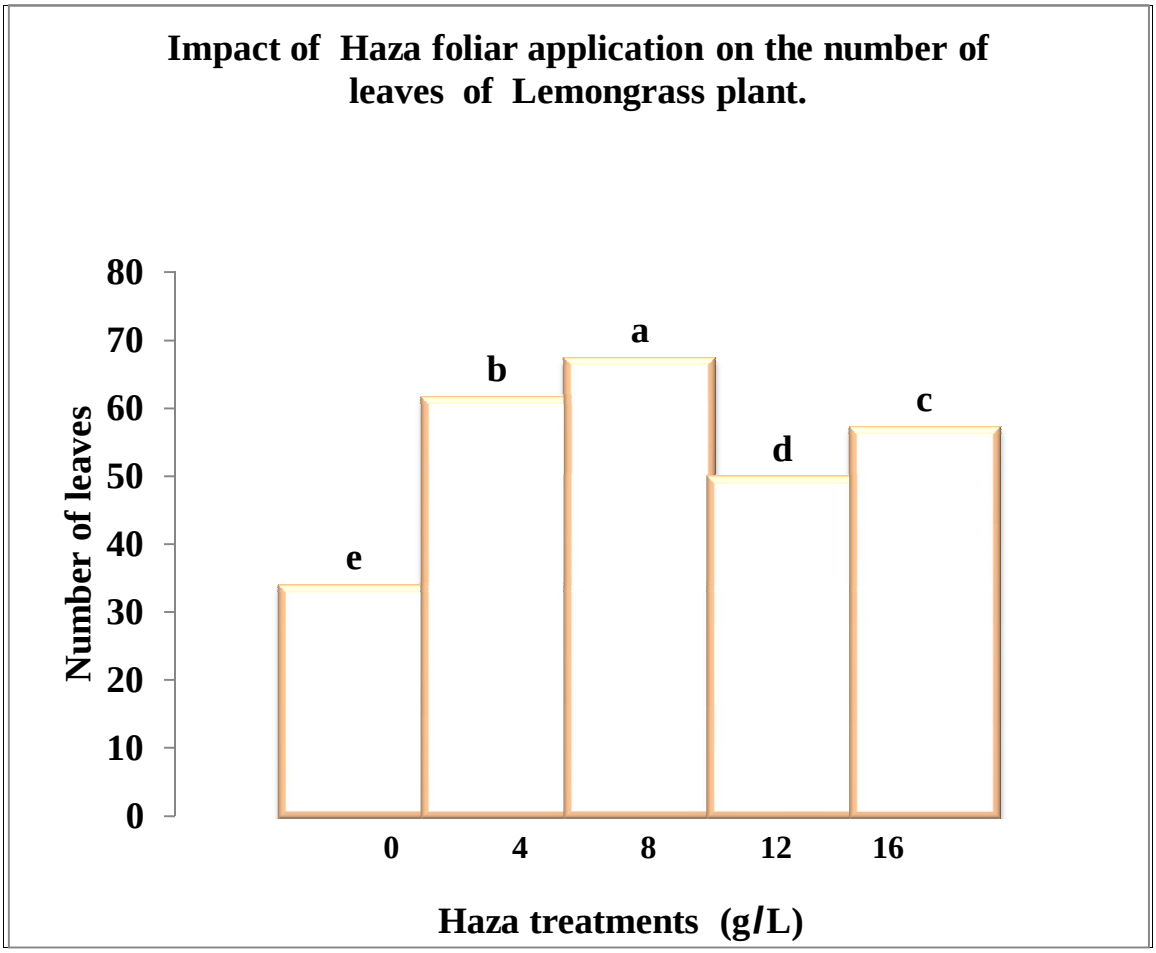

Table 16. Impact of foliar application of Haza on the fresh and dry weight of shoots , and roots of Lemongrass plant.

Haza Fertilizer (g/plant)	Leaves fresh weight (g)	Leaves dry weight (g)	Roots fresh weight (g)	Roots dry weight (g)
0	87.50^{e}	22.95^{d}	18.33^{c}	13.02^{c}
4	126.5^{b}	35.60^{b}	25.38^{b}	14.50^{b}
8	123.5^{c}	36.47^{b}	24.63^{b}	16.52^{a}
12	104.3^{d}	31.20^{c}	25.50^{b}	13.85^{bc}
16	139.8^{a}	38.45^{a}	28.00^{a}	15.82^{a}

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

4.5. Experiment (5):

The investigation on the bio-stimulating property of Benzyladenine (BA) and Giberellic Acid (GA_3) on lemongrass plants.

According to Table (17) The treatments BA , GA_3 ,and BA+GA_3 performed similarly and gave the best results in the leaf length comparing to the control (Table17). The highest leaf width observed from the treatment BA , followed by the treatments GA_3 and BA+GA_3 which performed similarly, and the least leaf width resulted from the control(Table17).

The highest chlorophyll content obtained from the treatment BA and BA+GA_3 , while the least chlorophyll content obtained from the GA_3 treatment. (Table17).

According to Figure (9) significant differences were observed among treatments in number of leaves, the highest number of leaves obtained from the treatment BA followed by BA+GA_3 treatment and then GA_3 treatment , and the least number of leaves resulted from the control. (Figure 9).

The results of the effect of hormones treatments on shoots and roots fresh and dry weights are presented in Table (18). It is noteworthy that the best shoots fresh and dry weight resulted from the treatment BA , followed by GA_3 , and then the treatment BA+GA_3 , comparing to the control (Table18). While analysis of the data showed that the highest roots fresh and dry weights resulted from the treatment BA+GA_3 followed by BA and then the treatment GA_3 comparing to the control (Table18).

Table 17. Impact of foliar application of BA,GA_3 and BA+GA_3 hormones on the length , width of leaves and chlorophyll content in Lemongrass plant.

Hormone (100 mg/litter)	Leaf length (cm)	Leaf width (cm)	Chlorophyll content
Control	67.50[b]	1.200[c]	30.42[b]
BA	71.00[a]	1.650[a]	31.80[a]
GA_3	70.25[a]	1.500[b]	29.17[c]
BA+GA_3	70.50[a]	1.475[b]	31.95[a]

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

Figure 9. Impact of foliar application of BA,GA_3 and BA+GA_3 hormones on number of leaves of Lemongrass plant

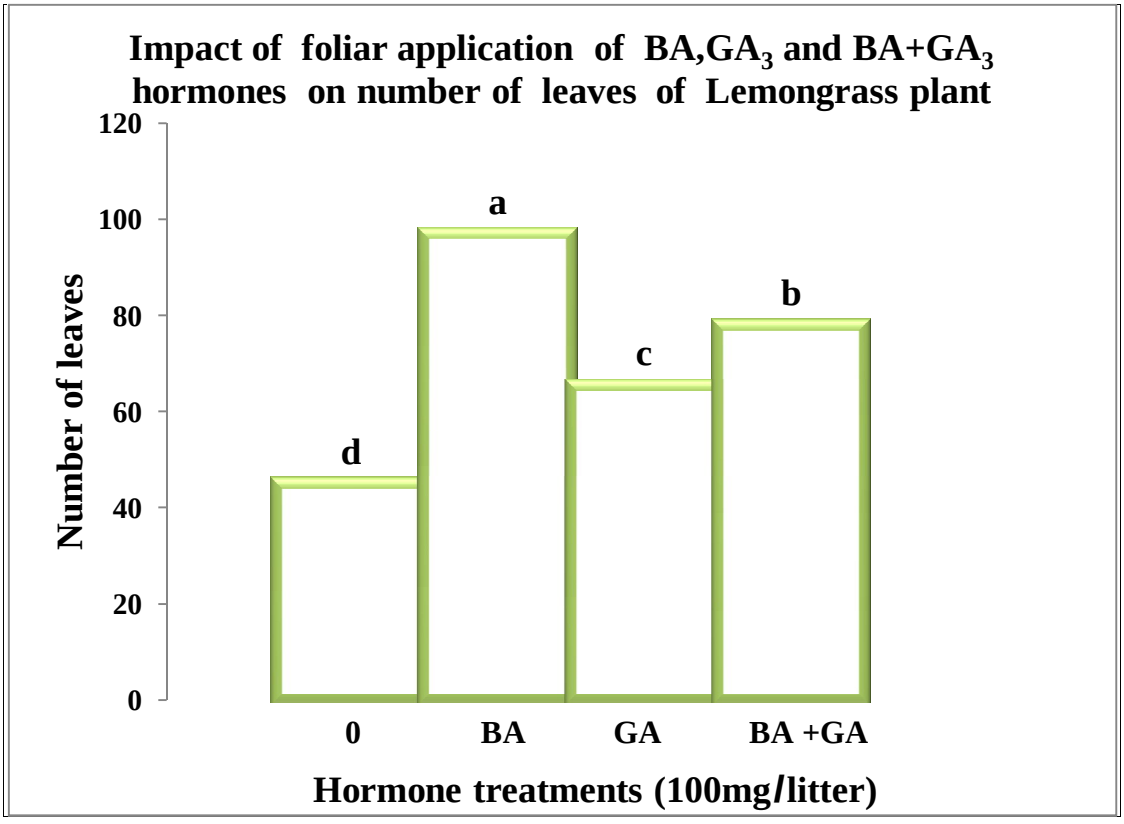

Table 18. Impact of foliar application of BA,GA_3 and BA+GA_3 hormones on the fresh and dry weight of shoots and roots of Lemongrass plant.

Hormone (100 mg/L)	Leaf fresh weight (g)	Leaf dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	31.50^{d}	31.50^{d}	40.13^{d}	33.00^{d}
BA	124.6^{a}	124.6^{a}	61.38^{b}	49.33^{b}
GA_3	117.5^{b}	117.5^{b}	53.63^{c}	36.85^{c}
BA+GA_3	77.53^{c}	77.53^{c}	90.88^{a}	68.07^{a}

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

Chapter Five

Discussion

CHAPTER FIVE

DISCUSSION

The chances of Sudan in the agricultural business are numerous; yet this potential is not fully exploited due to lack of a master plan to exploit the huge resources in accordance with standard modern agribusiness. Hence, there is a need to focus on environment friendly and sustainable means to increase the productivity per area unit. Organic farming renders better returns than chemically produced crops. In organic production, bio-fertilizer and bio-pesticides are used instead of agri-chemicals to grant provision of safe food without harming the environment.

Among modern agriculture priorities is the search for new technological solutions that would allow the reduction of chemical inputs without affecting crop yield or the income (Hong *et al*., 2007). Bio-stimulants are organic materials that, when applied in small quantities, enhance plant growth and development such that the response cannot be attributed to application of traditional plant nutrients (Schmidt *et al*., 2003). According to Ali *et al*. (2009), the scarce scientific literature combined with less validated producer information on natural growth enhancers shows that the growth-enhancing effect of different products can be roughly divided into three categories: compounds enhancing nutrients availability or facilitate their uptake, or decreasing damage by pests and diseases, and /or interfering with the plant hormone system either directly or indirectly through microbes. Applications of different plant bio-stimulants in various concentrations and diverse mechanisms of action has resulted different effects on medicinal plants. Plant bio-stimulants are organic materials that appear to impact several metabolic procedures such as respiration, photosynthesis, nucleic acid synthesis and ion uptake and

when applied in small quantities, improve the plant growth and development (Castro and Vieira, 2001) or in other words, a mixture of two or more PGRs or combination of these with other substances (amino acids, nutrients, vitamins) is known as a plant growth promoter or plant bio-stimulant. Plant bio-stimulants are effective when applied in small doses, thus leads to the plant growth, and production enhancement (Li WJ and Ni YZ,1996). In general, they stimulate metabolic processes for more yields in plants (Saa-Silva.,*et al* 2013). In *Anethum graveolens* L. plants, morphological traits of umbel number per plant, dry weight of plant, seed weight per plant were improved by seed inoculation and foliar application of vermicompost and bio-stimulant mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum* (Darzi and Haj Seyed Hadi., 2012). Some other studies have reported the positive effect of these bio-stimulants on other medicinal plants such as coriander (Kumar *et al*.,2002), celery (Migahed *et al* .,2004), fennel (Mahfouz and Sharaf Eldin,2007), turmeric (Velmurugan *et al*.,2008) and hyssop (Koocheki *et al*., 2009, El-Sharabasy *et al.* (2012) reported about amino acids significant influence on the synthesis of secondary metabolites. They expressed that the steroid biosynthesis have a positive correlation with increasing the glutamine concentration, so that the highest steroid content was recorded in the application of glutamine at 500 mg.

However, according to Moringa experiment the results of the study proved the benefits of Moringa applications on growth and oil content of Lemongrass. These findings are in conformity with several preceding studies. Priming seeds of the rangeland grass *Echinochloa crusgalli* with Moringa leaf extract resulted in significant increase of shoot vigour coupled with higher number of leaves and fertile tillers (Nouman *et al*., 2011). Besides, Foidle *et al*.(2001) reported that a spray made from Moringa leaf extract resulted in increased strawberry production and

claimed the possibility of its use as a foliar spray to accelerate growth of young plants. Moreover, Moringa fermented leaf juice was also tested for its growth promoting attributes in *Brassica oleracea* and the results were promising (Rajamani *et al*., 2014). The benefits of use of relatively low doses of Moringa in this study are also in line with claims of other researchers (Wagentrisl,2003;Kohata *et al.*, 2004). The growth promotion obtained from Moringa applications may be attributed to several possibilities. The Moringa treatments might have improved the nutritional status of Lemongrass plants. Apart from nutrition, the possibility of hormonal role prevails. The enhancements in leaf number, length and width and number of tillers are indicative of cytokinin role. Moringa leaves have been characterized to contain a desirable nutritional balance of minerals, amino acids and fatty acids (Razis *et al*., 2014, Teixeira *et al* .,2014). They also contain various antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids. In addition, they contain vitamin B, chromium, copper, magnesium, manganese, phosphorus, zinc, calcium, potassium and it also contain cytokinin in the form of zeatin (Alhakmani *et al*., 2013, Kesharwani *et al.*, 2014, Vongsak *et al.*, 2014).

The core and objectives of this study lied within this context as the growth stimulating potential of Henna was tested on Lemongrass; a plant well adapted to Sudan's agro-climatic conditions (ElGhazali *et al*., 2004). The data analysis of this test revealed substantial growth gains upon Henna applications, The finding of Henna experiment might be considered as a first report claiming of the growth stimulating property of these plant especially when used as foliar treatment in concentration of 12 g/l; a treatment that increased the leaf fresh weight by 352.3% coupled by oil content slightly increased over the control. Likewise, the soil application of 6 g Henna was also stimulated of leaf weight as it increased

this parameter by 202% beside significant increase in leaf oil content. The employment of these cheap doses of Henna proved to be of significant impact on growth of Lemongrass and this potential can be tested on other horticultural crops for further confirmation. Regarding interpretation, this stimulation might owe growth stimulating hormones or their precursors or a bio-pesticide constituent within Henna tissues. However, further research on the growth bio-stimulation potential of Henna is needed. Further phyto-chemical studies to define the phyto-chemicals responsible for the stimulations of growth might provide solid interpretation of the results. Yet, the encouraging high growth rates of Lemongrass obtained in this study might warrant further research on this plant aiming towards its production at an economical level. Thus, *Lawsonia inermis* L. as a very useful medicinal plant was used in this experiment as a bio-stimulant source . Chandrasekaran *et al*. (2000) mentioned that soyabean seed treatment with *Lawsonia inermis* leaf extract at 10% increased shoot length significantly. (Pathak and Srivastava , 2000) overlaid the total phenols content in sunflower was maximum after treatment with *Lowsonia inermis*. However, contradictory explanations were reported on the compounds within Henna tissues. (Oladunmoye and Kehinde, 2011) overlaid the leaves of *Lawsonia inermis* are used to treat the poliomyelitis, measles among the Yoruba tribe of South Western Nigeria, also, (Dixit *et al*.,1980; Habbal *et al*.,2005; Bhomick and Chooudhay,1982; Natarajan and Lalithakumar , 1987) investigated the antimicrobial properties of Henna. (Boubaya *et al*.,2011) overlaid leaves of Henna had a level of Ca, Na, P and K contents, ranging from 0.2 to 4%. The Mg content was less than 0.2% while Cu, Zn and Fe contents were above 0.5m 1.1 and 15%, respectively, Mn content was less than 1.5% while the Nitrogen Matter (NM) content was less than 1.5%. In the stems ,P and K contents were respectively, above 5.12 and 0.5%,Mg

content was less than 0.08% , while Na and Ca contents were less than 0.2%.Cu , Zn, Fe and Mn contents were less than 0.95, 1.7, 4 and 0.5%, respectively and NM contents were less than 0.2%. According to phytochemical analysis of Henna, powdered leaves contain about 0.5-1.5% lawsone, the chief constituent responsible for the dyeing properties of the plant. Henna plant also contain mannite, tannic acid, mucilage, gallic acid and naphoquinone (Ahmed *et al*., 2000; Rosenberg, 1999; Vardamides *et al*., 2001; Khan *et al*.,1991). The extracts of *L. inermis* gave positive tests for alkaloids, saponin, tannin and glycosides and negative for flavonoids and resin (Chukwu *et al*., 2011). Kirkland and Marzin (2003) reported that natural constituents of *Lawsonia inermis* were essential oils, 1,4-naphtho-quinone, tannins, gallic acid, flavonoids, lipids, sugars, tri-acontyl tri-decanoate, mannitol, xanthones, coumarins (5-alkyloxy-7-hydroxy-coumarin), (2-3%) resins, (5-10%) tannic ingredients and up to 2% lawsone (2-hydroxy-1,4-naphtho-quinone). Nayak *et al*. (2007) stated that *Lawsonia inermis* contain a soluble matter tannin, gallic acid, glucose, mannitol, fat, resin and mucilage. 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.* (2011a and 2014b) that such enhancements might owe to growth regulator- like constituents in Hargel.

Many Studies have been conducted so far on Hargel . (Sabahelkheir and Mohamed,2010) revealed that the mineral composition of (*Solenostemma argel*) is extremely rich in Potassium and moderately high in manganese and Iron . Murwan *et al*. (2010) characterized the leaf of (*Solenostemma argel*) by high carbohydrates (64.8%) and low crude fiber (6.5%). In addition the leaf contained 15 % protein, 1.6% crude oil, 7.7% ash, and 4.4% moisture content. The results revealed that the leaf contained high potassium (0.54%), calcium (0.06%), magnesium (0.03%)

and sodium (0.01%), but it characterized by low cupper (0.0001%), ferrous (0.002%), manganese (0.002%) and lead (0.001%). The protein fractionation of leaf characterized by high Albumins (16.7%), Non-Nitrogenous Protein (15.3%), Prolamine (11.7) and low Globulins (8.7 %), and Glutulin (6.2%). Leaf contained phytic acid (3.2 g/100g and tannin content (0.4%).

More over , (Sabahelkheir and Mohamed,2010) reported the presence of elevated phytic acid concentration in Argel leaves, this constituent might have an influence on the enzymatic processes of the recipient plant. Phyto-chemicals of medicinal properties from argel shoots had been reported by many workers (Roos *et al.*, 1980; Kamel *et al.*, 2000*; Hamed,* 2001). Sulieman *et al.* (2009) reported that the aqueous extracts of argel have antifungal and antibacterial properties. (Idris *et al.* (2011) revealed that Argel treatments enhanced flowering and yield parameters of date palms and improved the physical characteristics of the fruits. Argel water was effective in controlling aphids and white flies in summer tomatoes and Egyptian bull worm in okra respectively (Unpublished observation). In a pilot field experiment on *Brassica nigra,* some peripheral plots were severely infested by aphids. The infestation caused stunting of shoots and delayed flowering compared to non-infected plots. However, upon treatment with Argel as a soil additive, or a spray of shoot water extract or a combination of soil additive and spray, the vegetative growth was restored in all plots after pest disappearance and the plants flowered within 10-15 days after treatments. The inflorescence was abnormally thick and profusely branched in plants that received the combined treatment suggesting a growth-regulator-like effect and indicating the efficiency of Argel as a pesticide (Abdelwahab, 2002).

An extensive literature survey indicated that very few Studies have been conducted so far on *H.tuberculatum* species either grown in Sudan or

elsewhere. Because this plant is reported to contain coumarins, flavonoids, glycosides and alkaloids and it might possess beneficial biological activities including antioxidant activity. The plant's chemical composition has been shown to vary as a function of geographic location and time of collection. It includes alkaloids, lignans, flavonoids and essential oils (Kashiwada *et al*., 1996- Javidnia *et al*., 2006).The results of this study revealed growth benefits in Lemongrass plants treated with Haza as soil dressing , and the foliar treatment 4g resulted in best enhancement of leaves number , leaf length and width , this enhancement is coupled with chlorophyll content. The function of chlorophyll is to absorb light energy and transfer it to photosynthesis reaction centers (Gilpin, 2001). Nevertheless, the increase in the levels of chlorophyll content obtained in this study are indications of healthy and active growth . The gains in growth also might be related to growth regulator like constituents in Haza shoots. Such gains in date palm yield resulting from the soil application of Argel were attributed to a growth regulator like effect of Argel (Idris *et al.*,2010). In conclusion **,** the stimulation of growth in Lemongrass plants treated with Haza in this study require more biochemical studies of Haza to recognize the ingredients responsible of such growth enhancement .

Other non-nutrient chemicals, not naturally found in plants, but when applied to plants influence their growth and development. These are organic substances that are biologically active at very low concentrations. There are five recognized groups of natural plant hormones, which are, auxins, gibberellins, cytokines, ethylene and abscisic acid (Leopold and Kriedemann, 1975). Leopold reported that each of the mentioned organic substances is distinctive both in chemical characteristics and in being able to bring about characteristic growth responses and each group of regulators is capable of altering growth, including cell division, cell

elongation, differentiation and differential growth phenomena. Triacontanol, a natual plant growth regulator plays significant role in enhancing biomass production which results in increased biosynthesis of secondary products. Physiological changes like growth, photosynthesis, flowering and cell expansion in plant was observed by the application of phytohormone Gibberellic acid (GA_3) (Yuan and Xu , 2001; Taiz and Zeiger,2006) .The metabolic activity within pathways increased by the application of GA_3 which results to stress and anthocyanin biosynthesis (Ohlsson and Bjork,1988).

Plant growth regulators can also confer plant resistance to a biotic stresses such as drought and osmotic stress (Chatterjee , 1995; Zhao and Oserhuis,1997;Vardhini and Rao, 2003). The responses of some aromatic grasses to plant growth regulators have been studied (Farooqi *et al.*,2000 ; Singh *et al.*,2001).The use of plant growth regulators is directed towards improving the yield, quality and/or quantity of many crops. Gibberellic acids (GA_3) are the most important natural growth regulators in use. They are used to induce major changes in the growth required to increase the quantity and quality of edible characters, chemical composition and yield criteria of *Hibiscus sabdariffa* L. plants (Rabie,1996). Balbaa *et al.* (2008), Abou Zied and Sherbeany (1971) indicated that chloremquat enhanced the volatile oils of chamomile. Sharafzadeh *et al.* (2012) revealed that naphthaleneacetic acid and spermidine altered oil constituents of German chamomile. Silva *et al.* (2005) reported that auxin and cytokinin increased some components of the lemon balm oil. Povh and Ono (2007) showed that application of Gibberellic acid influenced the chemical composition of *Salvia* oil. Growth and essential oil yield of *Mentha piperita* were improved by the application of polyamines (Youssef *et al.*, 2002). Another research showed that α-bisabolol oxide A, increased in chamomile with

application of 100 ppm IAA (Reda *et al.*, 2010). A report revealed that sodium salt of NAA and IAA increased the essential oil of *Mentha piperita* (Koseva-kovacheva and Staev, 1978). The studies show that plant growth regulators can influence the yield of essential oils such as lemongrass, peppermint and rose scented geranium (Eid and Rofaeel, 1980; El-Keltawi and Croteau, 1986; Mishra and Srivastava, 1991).Growth regulators can influence essential oil production through effects on plant growth, essential oil biosynthesis and the number of oil storage structures (Sharafzadeh and Zare, 2011). As conclusion , the results of this experiment indicated that Gibberellic acid and benzyladenine altered growth of Lemongrass. The highest values of tillers number , leaves number, leafe length , width and chlorophyll content were obtained by BA treatment.The highest values of shoot fresh and dry weights were obtained by BA treatment , while roots fresh and dry weights were obtained by the combination treatment ($BA+GA_3$),thus , the results of this experiment are in agreement with previous studies reported by researchers indicated that plant growth regulators can influence plant growth. Nevertheless, these findings are in line with other preceding studies on the growth stimulation by applications of local flora of Sudan as substitutes for agro-chemicals. Within this context, Idris *et al*. (2011) reported enhanced growth and yield in date palm by soil application of low doses Argel. Similarly, other researchers reported growth stimulation by soil and foliar applications of local flora on *Aloe vera* (Eisa, 2016), *Catharanthus roseus* (Jbreel, 2016), *Mangifera indica* (Idris and Albashir,2018), *Duranta plumier* (Hamid, 2016) and *Euphorbia splendenes* (Osman, 2017). Generally, Several studies were conducted in Sudan with respect to lemongrass such as application of nitrogen to lemongrass which 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).

In conclusion **,** the adoption of this important aromatic plant species seems to be very promising. Yet, further biochemical studies are needed to determine the Moringa ,Henna ,Hargel and Hazza active ingredients contents on levels which are the critical quality determining factors and it would be helpful to make use of it in boosting the economy of Sudan. Besides, the encouraging results of this study elucidated an economical potential for possible large scale production of lemongrass plant under Sudan conditions.

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