A DISSERTATION ON

"Effect of Chlormequat chloride on the Growth, Biochemical attributes and Essential Oil yield in Lemongrass (*Cymbopogon flexuosus*)"

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IN BIOTECHNOLOGY

BY

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M. Tech Biotechnology (4th Semester)

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DECLARATION FORM

I, **Nargis Aftab**, a student of **M. Tech Biotechnology** (**2nd** Year / **4th** Semester), Integral University have completed my six months dissertation work entitled **"Effect of Chlormequat chloride on the Growth, Biochemical attributes and Essential Oil yield in Lemongrass (*Cymbopogon flexuosus*)"** successfully from **Integral University** under the able guidance of **Dr. Ahamad Faiz Khan (Supervisor).**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Name and Signature of Student with Date

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Certificate that Ms **Nargis Aftab** (Enrolment Number: 2000102767) has carried out the research work presented in this thesis entitled **"Effect of Chlormequat chloride on the Growth, Biochemical attributes and Essential Oil yield in Lemongrass (*Cymbopogon flexuosus*)"** for the award of **M. Tech (Biotechnology)** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student himself/herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her **M. Tech (Biotechnology)**.

I wish her good luck and bright future.

Dr. Ahamad Faiz Khan

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I wish her good luck and bright future.

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TO WHOM IT MAY CONCERN

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I wish her good luck and bright future.

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Date: **NARGIS AFTAB**

LIST OF ABBREVIATIONS

CCC – Chlormequat chloride

BSA – Bovine serum album

CEPA – 2 chloro ethyl phosphoric acid

DOXP – 1-deoxy-D-xylulose phosphate

$CuSO_4$ – Copper sulphate

DMAPP – *Dimethyl* allyl pyrophosphate

FID – Flame ionization detector

GA – Gibberellic acid

GPP – Geranyl pyrophosphate

GLC – Gas liquid chromatography

IAA – Indole acetic acid

IBA – Indole -3- butyric acid

IPP – Isopentyl pyrophosphate

IU – International unit

$KHCO_3$ – Potassium hydrogen carbonate

KNO3 – Potassium nitrate

MVA – Mevalonate pyrophosphate

MVAPP – Mevalonate pyrophosphate

mM – millimolar

$Mgcl_2$ – Magnesium chloride

NAA – Naphthalene acetic acid

NaOH – Sodium hydroxide

NADH – Nicotinamide adenine dinucleotide

NDD – N-(1 Naphthyl) ethylene diamine dihydro chloride

NR –Nitrate reductase

OD – Optical density

PGRs – Plant growth regulators

PGRs – Plant growth retardants

PVPP – Polyvinyl poly-pyrrolidone

PPM – Parts per million

PEP – Phospho-enol pyruvate

rpm – Rotation per minute

TCA – Trichloro acetic acid

V – Volume

W – Weight

W/V – Weight/volume

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1. INTRODUCTION

Aromatic plants, also known as herbs and spices, have been used in the Middle East since approximately 5000 BC for their preservative and medicinal properties, in addition to enhancing the aroma and flavour of foods (Chang, 2000; Li, 2006; Piccaglia *et al*., 1993). Their use continues undiminished today and according to the World Health Organization (WHO) nearly 80% of the planet's population, especially in developing countries still depends on plant produced medicines for their healthcare (Collin, 2006; Gurib-Fakim, 2006). Additionally, feed additives derived from plants, also called phytogenics or phytobiotics or botanicals, can be included in animals' diets to improve their productivity and the properties of the resulting feed and animal products (Windisch *et al*., 2009). Among these natural additives, aromatic plants, their extracts and their essential oils have been examined due to their advantages over the antibiotics as growth promoters. They are residue free and generally recognized as safe (GRAS) (Windisch *et al*., 2009; Brenes and Roura, 2010; Varel, 2002). Currently, there is an increasing interest in using herbs and spices in animal nutrition, in order to replace the use of antibiotics and ionophore anticoccidials, especially after the ban of antibiotic feed additives within the European Union countries in 2006 and discussions to restrict their use outside Europe (Greathead, 2003; Barton, 1999). Many herbs and spices can be found worldwide, with many originating from the Mediterranean area, either in the wild or cultivated, such as rosemary, oregano, sage, thymus, peppermint and garlic (Bampidis *et al*., 2005; Botsoglou *et al*., 2009; Ocak *et al*., 2008; Kadri *et al*., 2011). They contain chemical substances such as polyphenols, quinines, flavonols/flavonoids, alkaloids, polypeptides or their oxygen-substituted derivatives (Perumalla and Hettiarachchy, 2011; Negi, 2012; Cowan, 1999). Some of these substances can act synergistically, so their bioactivity is enhanced (Tiwari, 2008). Some bioactive compounds show therapeutic value, such as antioxidant and antiseptic activities (Li, 2006; Madsen and Bertelsen, 1995). Thus, they may reduce the risk of cancer or cardiovascular diseases (Duthie and Brown, 1994; Milner, 2012) and may find application as treatments in curing or managing a wide range of ailments such as respiratory diseases and stomach or inflammatory disorders (Kadri *et al*., 2011). Generally, the bioactive components in the aromatic plants possess the ability to protect the body from damage caused by free radicals induced oxidative stress by quenching singlet oxygen and inducing cytochrome or other enzymes (Li, 2006; Couladis *et al*., 2003). Moreover, herbs and spices can inhibit oxidative rancidity and delay the development of off-flavour in some products (Duke, 2002; Sherman *et al*., 2001). They also contain antimicrobial compounds which contribute to the

retardation of microbial growth on foods and especially snack foods and meat products (Giannenas, 2008; Li, 2006; Elgayyar *et al*., 2001).

Lemongrass, is the most important essential oil (EO) yielding members in the genus Cymbopogon. Their EOs has immense commercial values in flavours, fragrances, cosmetics, perfumery, soaps, detergents, toiletry, tobacco products and pharmaceuticals (Ganjewala *et al*., 2008). It is also used for the synthesis of vitamin A and ionones (b-ionones, methyl ionone, etc.); synthetic citral, derived from conifer turpentine is normally used for these purposes (Dawson, 1995). Lemongrass EO is mainly composed of cyclic and acyclic monoterpenes such as, citral (3,7-dimethyl-2,6-octadienal; a mixture of two isomer geranial and neral), geraniol, citronellol, citronellal, linalool, elemol, 1,8-cineole, limonene, a-carophyllene, methylheptenone, geranyl acetate and geranyl formate.

In recent years medicinal and pharmacological significance of lemongrass EO and its major constituent citral has been rapidly increased. A number of studies have revealed many useful bioactivities such as such as, antimicrobial, allelopathic, anthelmintic, antiinflammatory, anticancer, antioxidant, insect and mosquito repellent of lemongrass extract, oil, citral and citral derived compounds In India, lemongrass (C. flexusosus and *C. Citratus*) are cultivated in Kerala, Assam, Maharashtra and Uttar Pradesh. Apart from India, they are also cultivated in large scale in Brazil, Maxico, Dominica, Haiti, Medagascar, Indonesia and China.

In India, lemongrass (C. flexusosus and *C. Citratus*) is cultivated in Kerala, Assam, Maharashtra and Uttar Pradesh. Apart from India, they are also cultivated in large scale in Brazil, Maxico, Dominica, Haiti, Medagascar, Indonesia and China. India produces around 1000 tons of lemongrass oil per year and is exported to America, England, Germany, Australia and Japan.

Fig 1.1 Healthy slips of lemongrass after transplanting in the pots

Fig 1.2 Lemongrass (*Cymbopogon flexuosus*) grown in pots at Herbal Garden of Integral University

Cymbopogon flexuosus commonly known as Lemongrass, Indian Lemongrass or Lemongrass from Madagascar is an aromatic herb belonging to the Poaceae family. This family is indeed widely distributed counting more than 635 genus and 9000 species. The Cymbopogon genus has for itself more than 140 cultivated species, 52 of them are located in Africa, 45 in India, 6 in Australia, 6 in South America, 4 in Europe, 2 in North America and the rest in South Asia (Suman *et al*.,2004). The rest of the species is distributed between Central America, South America, Africa and other tropical regions (Gagan *et al*.,2011).

Originated from southern India and Sri Lanka, *Cymbopogon citratus* (DC.) grows nowadays spontaneously all over the world, especially in the tropical subtropical and Savannah regions (Negrelle and Gomes, 2007). In West Africa, it has long been cultivated for its medicinal properties. In Tunisia, *Cymbopogon flexuosus* is an introduced species cultivated for decoration, therapeutics and insecticides uses. Indeed, it is grown in gardens especially for its repelling effect of insects like mosquitoes. The essential oil extracted from the lemongrass is used in local industrial products against cockroaches, flies and mosquitoes (Dhaou *et al*.,2010).

Cymbopogon flexuosus is an herb belonging to the Kingdom of Plantae, the Phylum of Spermatophyta (seed plants), the super-branching of Magnoliophyta (flowering plants), the class of Liliopsida (Monocotyledons), the order of Poales, the family of Poaceae (herbaceous), the genus is *Cymbopogon* and the species is *flexuosus* (Karunamoorthi *et al*., 2010).

Cymbopogon flexuosus is a perennial aromatic herb that grows as dense clumps with no ramification. The total length of this plant can reach 2 meters while its width goes up to 1.2 m. It has short rhizomes which also are its way of multiplication. The leaves are green, erect, flat, linear in shape and closed at the base. The majority emerges directly from the ground without stem. Their length exceeds 1m while the width varies from 5 to 15 mm. The leaves give off a characteristic lemon flavor, once pressed by hand or crushed. The upper side is glabrous and whitish with ligaments 4 to 5 mm long.

The glumes are equal to subequal. The lower glume is lance-shaped with an acute apex, while the upper glume is lanceolate 4.3 to 4.5 cm long with a rib. *C. flexuosus* rarely gives flowers. The species identified until now do not show flowers. The inflorescence: *C. flexuosus* has erect inflorescences with a length of 30 to 60 cm. *C. flexuosus* is generally propagated by seedling or by tuft division (Negrelle and Gomes, 2007; Gagan *et al*.,2011).

Cymbopogon flexuosus has different names depending on the country in which it grows. In France and Tunisia, it's called "Citronnelle" or "Herbe Citron". In USA and England, English, they call it "Lemongrass" or "Lemon Grass" which is also the common name in India and Egypt. In Brazil it's called "Capim-cidrao" or "Capim-santo", in Ethiopia "Tej-sar". For the rest of the countries, the most used names are the following: Sereh (Indonesia), Cimbopogone (Italy), Sakumau (Malaysia), Zacate limon (Mexico City), Citrongrass (Sweden), Ta-khrai (Thailand), Limon out (Turkey) (Gagan *et al*.,2011).

1.1 AIMS AND OBJECTIVES OF THE STUDY

- To obtain authentic plant material of lemongrass and its cultivation.
- To study the effect of various concentrations of CCC on growth characteristics.
- To study the effect of various concentrations of CCC on biochemical attributes.
- To study the effect of various concentrations of CCC on quality and yield attributes of essential oil.

2. REVIEW OF LITERATURE:

Mukarram *et al.* (2022) studied that the acute threat of salinity stress is ever-present. Depending on the severity, it poses a reduction in crop growth, development, and productivity, or plant death. Furthermore, different crops respond to salinity differently as a product of their susceptibility. Given the commercial importance of lemongrass, the present experiment was conducted to explore the susceptibility level of lemongrass plants to different salt severities. The result of the study suggests that the lemongrass is a "moderately salt-sensitive" crop. Metabolomic approaches revealed that it can maintain growth and essential oil synthesis under moderate salinity stress (NaCl 80 mM) via un-regulation in ROS and antioxidants metabolism. Nevertheless, higher salinity stress (NaCl 160 mM) inhibits photosynthesis due to PSII retardation and lowers chlorophyll regeneration in addition to restricting stomatal conductance. Furthermore, the innate defence system of the plant comprising CAT, POX, and SOD antioxidants and osmolyte PRO also struggles to render an efficient antioxidative defence and osmo-protection amidst ROS overaccumulation. This leads to significantly lower growth and essential oil accumulation. Further, the authors suggest the lemongrass for reclamation of saline lands considering that the other members of the *Poaceae* family and more sensitive to salinity and cannot grow well in such areas. Therefore, further studies could be relevant in extending the cultivation of lemongrass crops in reclaiming the salt-affected lands (≤ 80mM).

Xiaodeng Shi *et al.* (2021) studied the optimal combination for dwarfing found in the PGR experiments was spraying 1500 ppm uniconazole five times. This treatment combination successfully dwarfed the grafted seedlings of M. wufengensis and enhanced its stress resistance. Uniconazole supplementation results showed that the optimal uniconazole concentration was 1500 ppm. When the uniconazole concentration was 2000 ppm or greater, the new leaves were deformed, and shrinkage or adhesion deformation occurred. Therefore, considering the morphological indicators and given that dwarfing is mainly performed for the ornamental value of M. wufengensis, the best measure for dwarfing M. wufengensis is to spray 1500 ppm uniconazole five times.

Mukarram *et al.* (2021) investigated nanoparticle technology has become a new biotechnological tool. Its application to molecules such as silicon seems to have a promising relevant development in agriculture. Lemongrass has great industrial application including pharmaceutical and cosmetic sectors. The present study evaluated how the exogenous application of silicon nanoparticles (SiNPs) triggers a general trend of growth and yield

enhancement in lemongrass plants. SiNPs up-regulate photosynthesis, gas exchange, antioxidants and nitrogen metabolism. The 150 mg L-1 of SiNPs was found to be the optimum concentration for lemongrass to obtain these eliciting effects. Our study also provides strong evidence and further strengthens the claims that SiNPs can impart beneficial effects in the plant system even in the absence of stress. Lemongrass is an alternative crop in many depressed areas either by social circumstances or adverse environmental conditions, consequently, the biotechnological application of SiNPs to lemongrass crops should be considered an alternative to increase its yield. This will be of special interest when lemongrass crops are under environmental stress conditions such as drought, salinity or heavy metals which have associated oxidative stress since our data indicate that SiNPs significantly ($p \leq 0.05$) increase the antioxidants defence in lemongrass. On the other hand, the present study also provides clear evidence that the application of SiNPs to lemongrass increases the essential oil (EO) production, particularly citral and geranial, which has high commercial values in the different field, especially pharmaceutical and medical areas. Therefore, SiNPs are a promising biotechnological tool for lemongrass crops. Moreover, further studies could be useful to extend the application of SiNPs to other crops of agronomic interest to improve their productivity.

M. Machraoui *et al*. (2018) studied scientific research conducted on *Cymbopogon citratus*helped understanding the beneficial effects on human, animals and plants. Its pharmacological properties explained the different uses of this plant in medicine as a digestive and cardiovascular regulator but also against infectious diseases and psychiatric disorders. The chemical composition of the EO, phenolic compounds and aqueous extracts showed the major compounds which are responsible of the antifungal, antimicrobial, antioxidant and anticancer activities. The different information detailed in this review on the benefits and uses of *C. Citratus* make it a good candidate for further scientific experiments in other fields such as agronomy and pharmaceuticals.

2.1 MAJOR ESSENTIAL OIL YIELDING *CYMBOPOGON* SPECIES

Cymbopogon spp. display wide variation in morphological characters and essential oil composition at inter and intra species level and over the year's different chemo cultivars varying in their aroma have been selected or breed by crossing with other cultivars or closely related species. The most common economically sound species of the genus *Cymbopogon* viz., *C. flexuosus, C. Citratus, C. martinii var. motia and sofia, C. nardus var. nardus, C. pendulus, C. winterianus, C. jwarancusa* and *C. khasianus* yields essential oils of vivid composition

referred as lemongrass oil, palmarosa oil, citronella oil, ginger grass or rusa oil (Rao, 1997; Gupta and Jain, 1978; Kumar *et al*., 2000). The unique characteristics of these aromatic grasses are that they have wide adaptability to grow in different types of soils in different agri-climatic conditions and cropping sequences. In India, total area under cultivation of these aromatic grasses is more than 40 thousand hectares, distributed mainly in Assam, Kerala, Madhya Pradesh, South Gujarat, Karnataka, Maharashtra, Andhra Pradesh and Uttar Pradesh (Husain, 1994). In the following section we have elaborately discussed major elite species of the genus *Cymbopogon*.

2.1.1 Lemongrass (*C. flexuosus*)

Cymbopogon flexuosus is commonly known as lemongrass and locally it is called Cochin or Malabar grass. It is tufted perennial grass, with numerous stiff stems arising from a short, rhizomatous rootstock (Weiss, 1997). The leaf-blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm (Sugumaran *et al*., 2005). The leaf-sheath is tubular in shape and acts as a pseudostem. This plant produces flowers at matured stages of growth (Jaganath *et al*., 2000). Conversely, flowering has never been observed under cultivation due to rapid harvesting time. The rhizome produces new suckers that extend vertically as tillers to form dense clumps. Lemongrass can tolerate a wide range of soils and climatic conditions. However, vigorous growth is obtained on well-drained sandy loam soil with high fertility and exposed to sunlight (Sugumaran *et al*., 2005). In lemongrass, tiller growth usually begins at the apical meristem where cell division occurs, followed by production of axillary buds and the emergence of new tillers. Lemongrass is commonly cultivated as a ratoon crop and first harvested at 4 to 6 months after planting followed by subsequent harvests at 2-3 months interval (Joy *et al*., 2006). Harvesting is done by cutting at 20 cm above the ground level (Sugumaran *et al*., 2005).

Lemongrass is indigenous to India and grown in Kerala, Assam, Maharashtra and Uttar Pradesh. Apart from India, lemongrass is also cultivated in large scale in Brazil, Mexico, Dominica, Haiti, Madagascar, Indonesia and China. The oil from lemongrass is referred as East Indian lemongrass oil. The first variety of lemongrass selected was OD-19 from Kerala in India followed by OD-408 and OD-440 (Kurikose *et al*., 1987). Thereafter, a number of important cultivars have been developed during the course of study of genetic diversity and chemogenetical improvement in a citral producing lemongrass (cultivar OD-19). Some of the important lemongrass cultivars are GRL-1 (geraniol rich lemongrass), Krishna, Cauveri,

Pragati, Chirharit, CKP-25 and SD-68. Among these Krishna is most popular throughout India and was developed at Central Institute of Medicinal and aromatic Plants (CIMAP), Bangalore centre. Krishna yields high bio mass (25-28 Mt/hectare) with high oil yield (230-250 kg /hact.) due to high % of oil in bio mass.CKP-25 is another successful variety which gives good result even in less rainfall area. CKP-25 was developed by Regional Research laboratory (RRL), Jammu. Chirharit is very popular in Tarai region of Uttarakhand as same remains green throughout year producing high quantity of bio mass although % of oil recovery is less due to cold climate in such region. Nima variety is known for its unique citrus clean odor as same contains less grassy component like methyl heptenone. Also, this variety can be grown in west land containing very high salt. All of the above cultivars of lemongrass yield essential oil highly rich in citral except GRL-1 which yields geraniol rich essential oil (Patra *et al*., 1997). Thus, could be easily distinguished from other cultivars by the presence of high amount of geraniol (89.39%). Lemongrass has been used in medicine in India for more than 2000 years. However, its use for distillation is about 100 years old and the first distillation in India was started in about 1890 during the British period from wild grass in Kerala. The total annual world production of East Indian lemongrass oil used to be 1500 tonnes.

2.1.2 Wild Lemongrass (*C. Citratus*)

It is also an important of species in the genus *Cymbopogon* which is a tropical perennial aromatic grass having dense fasicles of leaves from a short/oblique annulate, sparingly branched rhizome. The oil of *C. Citratus* is referred as West Indian lemongrass oil which is characterized by the presence of very high amount of citral. West Indian name however is misnomer as the grass is not indigenous to West Indies but was cultivated to a limited extent. There is hardly any production of lemongrass oil in West Indies now. The tea made from its leaves is popularly used as antispasmodic (Devi *et al*., 2011), analgesic, anti-inflammatory, antipyretic, diuretic and sedative (Liete *et al*., 1986; Sadiq and Khayat, 2010). It is also used as antiseptic, antifever, antidyspeptic, carminative, tranquilizer, stomachic and antihypertensive agent (Borreli and Izzo, 2000). A number of previous studies have also reported its anti-inflammatory, antiseptic, diuretic, neurobehavioral, antimicrobial, and fungistatic activities (Carlini *et al*., 1986; Carbajal *et al*., 1989; Francisco *et al*., 2011). In Mexico, *C. Citratus* is used as a sedative (Tortoriello and Romero, 1992) while in Brazil, an infusion or the cold juice of the leaves has been employed as a sedative and analgesic (Simon *et al*., 1980; Hiruma-Lima *et al*., 2002). An antinociceptive effect of *C. Citratus* has been detected in the rodent hot plate test, an experimental procedure related to central activity (Viana *et al*., 2000). It has been also

recommended against generalized anxiety disorder and epilepsy in experimental procedures in mice (Costa *et al*., 2006; Blanco *et al*., 2009). The leaves decoction has been shown to have antioxidant property (Cheel *et al*., 2005). The stalk or stem of *C. Citratus* is reported to have a small relaxation effect on perfused mesenteric arteries (Runnie *et al*., 2004).

Both lemongrass and wild lemongrass are closely related on the basis of other features like morphology, chemical constituents, geographical distribution and RAPD analysis (Sharma *et al*. 2000; Sangwan *et al*., 2001; Khanuja *et al*., 2005). The strong lemon-like odour of the oil is because of the presence of citral, the main constituent of the essential oil and hence the name lemongrass. Essential oils of both the lemongrasses contain 75-80% of citral but West Indian oil is considered inferior as it is less soluble in 70% alcohol as compared to East Indian lemongrass oil. The lower solubility in 70% alcohol is due to presence of myrcene, an olefinic terpene, which polymerises on exposure to air and light. The major producer of West Indian lemongrass oil is Guatemala producing approximately 250 tons of oil. The total world production of lemongrass oil is approximately 1000-1500 tones. India and Guatemala are the major producers. Smaller amounts are produced in China, Brazil, Indonesis and Haiti (Virmani *et al*., 1988).

2.1.3 Other Cymbopogon Species

Cymbopogon martinii also known as Rosha grass is a tall perennial sweet-scented grass 5-8 feet high used for the extraction of geranium oil which is extensively used as perfumery raw material in soaps, floral rose like perfumes, cosmetics preparation and in the manufacture of mosquito repellent products (Rajeswara Rao, 2001). It is used traditionally in treatment of diabetes. The plant has also been documented in Ayurveda for treatment of urinary tract infection, as anti-inflammatory and as diuretic (Mishra, 2002). *Cymbopogon pendulus* has been recently distilled in India to a limited extent. Essential oil of this grass also contains 70-80% citral. However, it is much harder than the two previous species and can be cultivated in adverse soil and climatic conditions. *Cymbopogon nardus* is a perennial grass cultivated in Southeast Asia. Its oil is known as citronella oil, and has been traditionally used as mosquito repellent, household fumigant, or fragrance agent in food commodities, soaps and cosmetics (Chomchalow, 1993; Jantan *et al*., 1999; Nakahara *et al*., 2003). *Cymbopogon olivieri* (Boiss.) is a plant growing in South East of Iran. The main constituents of this species are alkaloids, saponins and essential oil (Carpenter, 2001). The essential oil of *C. olivieri* which grows in India contains 3-pinene, myrcene, pulgone and piperitone as the major constituents

(Rajendrudu, 1983) and displayed interesting anti-fungi activity. Cymbopogon ambiguus A.Camus is a native Australian lemongrass species found on rocky hillsides throughout the Northern Territory of Australia (Latz, 1995). The leaves have been used traditionally to treat chest infections, sores, muscle cramps as well as headache and associated complaints (infusions and decoctions) (Grice *et al*., 2011; Latz, 1995; Lassak and McCarthy, 1983; Barr *et al*., 1988). Little is known about the chemical constituents present in C. ambiguus apart from a GC-MS study by Barr *et al*. (1988), which identified camphene, borneol, limonene, a-pinene, a-terpineol, camphor, isoborneol, 4-terpineol, myrcene, b-ocimene as being present in the essential oil.

2.2 EXTRACTION OF ESSENTIAL OIL

Lemongrass oil is extracted by various ways such as, the solvent, accelerated solvent, Soxhlet (Sargenti and Lancas., 1997), dense carbon dioxide (Carlson *et al*., 2001), solid-phase matrix (Pham-Tuan *et al*., 2001), and supercritical fluid (Schaneberg and Khan, 2002) extraction methods. However, the common procedure of extracting essential oil is by the hydro-distillation method (Kulkarni *et al*., 2003). Essential oils are extracted from fresh plant material (whole plant or leaves) and air-dried aerial parts using hydro-distillation technique in Clevenger-type apparatus for 3 hours. The essential oil collected after distillation is dried over anhydrous Na2 SO4 to remove moisture and stored in sealed vials under refrigeration. In some cases, hydro-distillation is carried at 100°C for 6 h in an all-glass Dean and Stark apparatus modified to allow lowest phase return (Sukari *et al*., 2008). In such cases, usually 10 ml of the n-hexane are added, to trap the condensed oil, through the top of the condenser. Later and hexane is collected every hour. Then, new portion of hexane is added through the condenser. Essential oil is also extracted with diethylether which is later evaporated. The mixtures are combined and dried over anhydrous Na2 SO4 for 24 h and then filtered. Finally, the hexane solution is evaporated or removed by using a rotary evaporator (Eyela N-1001, Tokyo Rikakikai, Japan) at 40°C to give a yellowish essential oil which is then stored at 4°C for further analysis. The oil yields are calculated on the basis of fresh weight and dry weight of the material (v/w) (Padalia *et al*., 2011; Sukari *et al*., 2008).

2.3 ESSENTIAL OIL YIELD AND COMPOSITION

An EO is defined as the product obtained by hydro-distillation, steam distillation or dry distillation or by a suitable mechanical process without heating (for Citrus fruits) of a plant or some parts of it (Rubiolo *et al*., 2010). They are aromatic oily liquids, volatile, characterized

by a strong odour, rarely coloured, and generally with a lower density than that of water. Essential oils only represent a small fraction of plant's composition; nevertheless, they confer the characteristics by which aromatic plants are used in the food, cosmetic and pharmaceutical industries (Pourmortazavi *et al*., 2004). EOs have a complex composition, containing from a dozen to several hundred components. The great majority of components identified in essential oils includes terpenes (oxygenated or non-oxygenated), with monoterpenes and sesquiterpenes prevailing. Nevertheless, allyl- and propenylphenols (phenylpropanoids) are also important components of some essential oils (Cavaleiro, 2001).

Gas liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS) has been the most applied analytical techniques for essential oil analysis (Masada, 1976) followed by the supercritical fluid extraction-gas chromatography (Liu *et al*., 1993). Due to the complexity of essential oil compositions, sophisticated instruments such as, high performance liquid chromatography in combination with gas chromatography (HPLC-GC) (Mondello *et al*., 1996) is the preferred analysis. HPLC is effective for a broad class separation of a sample, which can be introduced into a GC for further high-resolution separation.

The EO of lemongrass and other members of Cymbopogon have been exhaustively investigated for chemical compositions (Nath *et al*., 1994; Mathew *et al*., 1996; Sahi *et al*., 1997; Sharma *et al*., 1999; Sidbi *et al*., 2001; Nath *et al*., 2002; Khanuja *et al*., 2005; Ganjewala *et al*., 2008; Ganjewala, 2009). Lemongrass yields 1 to 2% EO on a dry weight basis which contains mainly citral (Schaneberg and Khan, 2002; Carlson *et al*., 2001; Pengelly, 2004). Other unusual active components are limonene, citronellal, b-myrcene and geraniol (Schaneberg and Khan, 2002; Ganjewala *et al*., 2008). EOs of most of the Cymbopogon spp. are mainly characterized by citral, geraniol, citronellol, citronellal, linalool, elemol, 1,8-cineole, limonene, b-carophyllene, methyl heptenone, geranyl acetate and geranyl formate (Lewinsohn *et al*.,2008; Sidibe *et al*., 2001; Khanuja *et al*., 2005; Ganjewala *et al*., 2008). The citral imparts characteristic lemon like aroma to EOs of the Cymbopogon spp. (Husain, 1994). The EO of palmarosa (C. martinii), however has high content of geraniol (90%) which gives it a characteristic rose like odor. Besides, geranyl acetate present in the palmarosa EO is reported to influence the quality of EOs (Ganjewala and Luthra, 2009). EOs of the lemongrass cultivars OD-19 and GRL-1 are comprises of several monoterpenes viz., citral, geraniol, borneol, isopulegol and 6-methyl hept-5-en-2-one, geranyl acetate, g-terpinene, a-thujene, a-pinene, sabinene, n-decanol, aterpenyl acetate, b-caryophyllene, a-humulene, germacrene D, bbisabolene and g-cadinene (Nath *et al*., 1994; Mathew *et al*., 1996; Sahi *et al*., 1997; Sharma

et al., 1999; Sidbi *et al*., 2001; Nath *et al*., 2002; Khanuja *et al*., 2005; Ganjewala *et al*., 2008; Ganjewala, 2009). The EO of C. parkeri is consisted of bicyclic monoterpenes, piperitone and sesquiterpenes, isointermedeol (Baqheri *et al*., 2007; Kumar *et al*., 2008). Isointermedeol is a major component in the EO of lemongrass and possess anticancer properties (Kumar *et al*., 2007).

Previously, the author has studied EO compositions of eight lemongrass cultivars. The study revealed that seven of the eight EOs had citral (75-85%) as major constituent, while only one cultivar GRL1 had geraniol (90%) as major component (Ganjewala *et al*., 2008). Khanuja *et al*., (2005) have also reported similar variation in the EO content and composition in 19 Cymbopogon taxa and discerned phylogenetic relationship among these taxa. The EO of C. confortiflorus and C. nardus var. confortiflorus were very rich in geraniol (68% and 46%) whereas C. nardus var. nardus and C. winterianus had very little amount of geraniol. The EO of C. pendulus, *C. flexuosus* and *C. Citratus* were mainly consisted of citral with 80-84% of the total monoterpene content (Khanuja *et al*., 2005).

C. Citratus root oil consisted of ten components with longifolene (577%) and selina-6-en-4-ol (20.03%) as major constituents (Li *et al*., 2005). Shoot EO however has shown an entirely different chemical composition consisting of 12 components with citral (88%) as the major constituent (Li *et al*., 2005). The EO of C. giganteus showed a distinguished composition due to the presence of cis- and trans-p-1(7),8- menthadien-2-ol (19.9% and 22.3%), cis- and trans-p-2,8-menthadien1-ol (10.1% and 14.3%) (Alitonoua *et al*., 2006). Similarly, C. schoenanthus L. Spreng from Tunisia also has a distinguished EO composition due to the presence of limonene (10.5–27.3%), bphellandrene (8.2–16.3%), d-terpinene (4.3–21.2%) and a-terpineol (6.8– 11.0%) (Khadria *et al*., 2008). The GC-FTIR study of the EO of palmarosa has revealed the presence of geraniol (65%) and geranyl acetate (20%) as major constituents (Prashar *et al*., 2003). Palmarosa leaf and flower essential oil are also dominated by geraniol 53.41% and 69.63% respectively in leaf and flower oil (Nirmal *et al*., 2007). In addition, piperitone (6.0%) in flower and nerol (24.76%) and á-pinene (4.32%) in leaf essential oils are also identified (Nirmal *et al*., 2007). The EO Java citronella is mainly comprises of geraniol (40.06%), citronellal (27.44%) and citronellol (10.45%) (Quintans-Junior *et al*., 2008). Another study has revealed the presence of 23 compounds in the EO of citronella with citronellal (27%), trans-geraniol (23%), citronellol (10%), limonene and linalool as major constituents (Simic *et al*., 2008; Lorenzo *et al*., 2000). The EO composition of C. nardus has been found identical to citronella oil with dominance of the geraniol, citronellal, and citronellol. However, EO of C.

nardus harvested from India also has other constituents such as, a-terpineol, cis-sabinene and carvone (Delespaul *et al*., 2000). The EO of C. parkeri from Iran has unique composition with presence of piperitone (81%) as major component and other minor constituents such as, germacrene-D (5%), santolinyl acetate (2.1%) and a-eudesmol (2.1%) (Baqheri *et al*., 2007).

2.4 ESSENTIAL OIL BIOSYNTHESIS IN *CYMBOPOGON* SPECIES

Lemongrass biosynthesize and accumulate EO predominantly in the young and rapidly expanding leaves and floral tops (Singh *et al*., 1990; Dubey *et al*., 2003a; Ganjewala and Luthra, 2007a). Our studies in lemongrass cultivar OD-19 revealed that it accumulates EO in the parenchymal cells which are referred as oil cells (Luthra *et al*., 2007). As discussed previously, EO oils are composed of complex mixtures of cyclic and acyclic monoterpenes. Monoterpenes are the C10 compounds which are mainly derived from geranyl diphosphate (GPP) through various secondary transformations such as, isomerization, acetylation, deacetyaltion, cyclization and dehydrogenation etc. (Banthorpe and Charlwood, 1980; Croteau, 1987). GPP which is believed to be a universal precursor of monoterpenes is synthesized by the fusion in head to tail fashion of the two C5 units called isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). In plants, IPP is biosynthesized by two major pathways the cytosolic acetate-MVA and newly discovered plastidic Methyl-D-Erythritol-4-Phosphate (MEP) or Deoxy-xylulose-5-phosphate (DOXP) or Rohmer pathway (Fig. 3). The acetate-MVA pathway is responsible for the biosynthesis of sterols, sesqui- and tri-terpenes while the MEP pathway for the plastidic isoprenoids such as, carotenoids, phytol side chain of chlorophyll, plastoquinone-9, hemi-, mono- and di-terpenes (Rhomer *et al*., 1993; Lichtenthaler *et al*., 1997; Eisenrich *et al*., 1997; Lichtenthaler, 1999; Luthra *et al*., 1999; Rohmer, 2003).

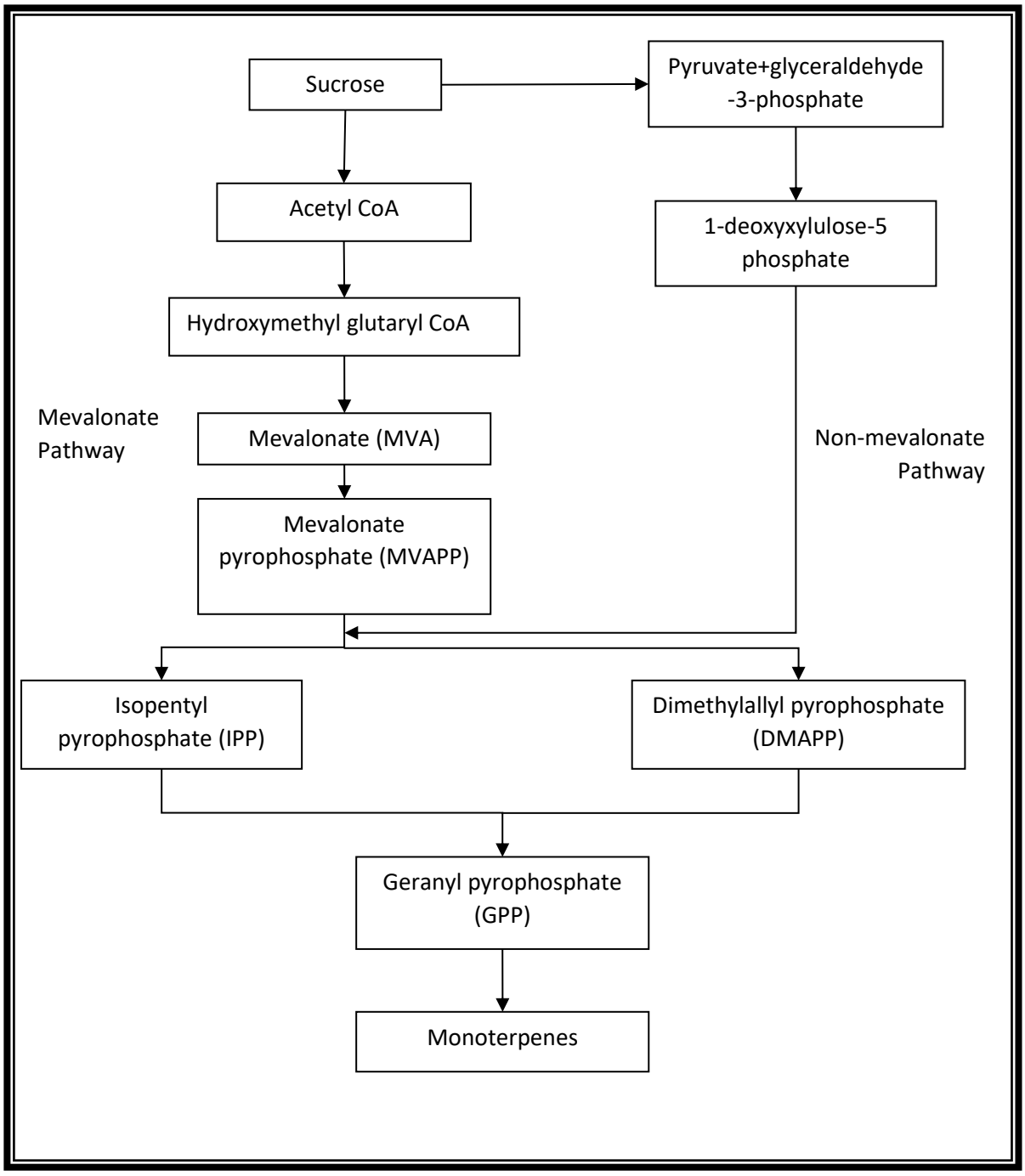


Fig 2.1 General Scheme of Monoterpene Biosynthesis

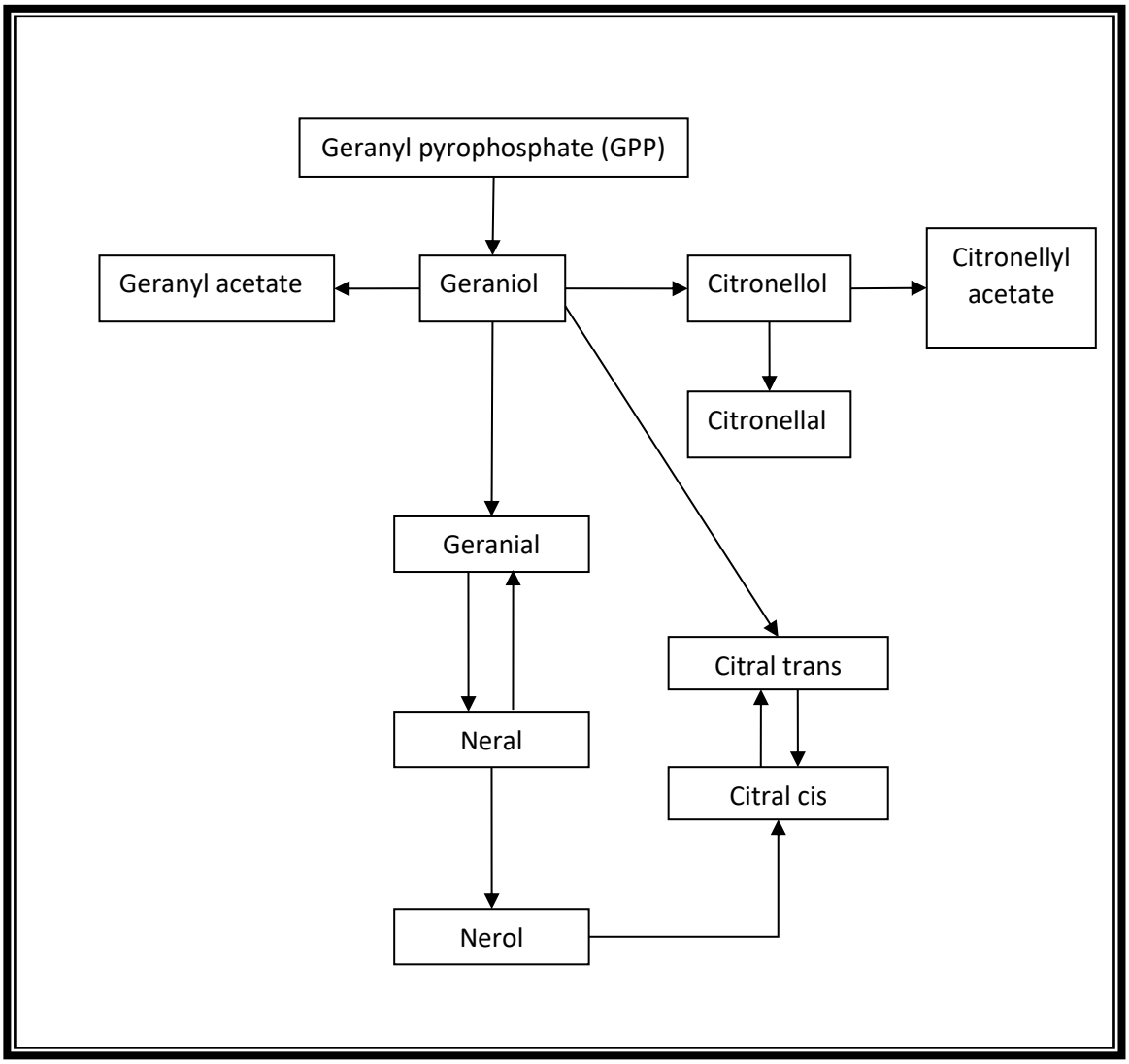


Fig 2.2 Metabolic Interconversion of Monoterpenes in *Cymbopogon* sp.

2.5 ESSENTIAL OIL ACCUMULATING SITE

In members of the genus Cymbopogon essential oils are stored in glandular micro-hairs. In lemongrass EO accumulating sites were detected using Schiff's reagent which gives purple colour after specifically interacting. Based on this method it was found that lemongrass accumulated EO in the leaf mesophyll cells commonly adjacent to non-photosynthetic tissue and in between vascular bundles which are referred as oil cells (Fig. 5) (Lewinsohn *et al*., 1998; Luthra *et al*., 2007). The compartmentalization of EO accumulating sites in lemongrass has important regulatory roles as it prevents other metabolically active cells from these often-toxic components. In citronella EO accumulates in five types of glandular micro hairs found on adaxial surface of the epidermis (Iruthayathas and Herath, 1982).

2.6 FACTORS INFLUENCING EO CONTENT AND COMPOSITION

Several factors such as, temperature, light intensity, soil moisture, fertilizers, and developmental stages of the plant/parts greatly influence EO content and composition of lemongrass (Ganjewala *et al*., 2008). In lemongrass and palmarosa net EO production is dependent on the early growth stages of leaf and inflorescence, respectively (Singh *et al*., 1989). In general, EO production is directly proportional to the yield of biomass of the plant/plant parts. In lemongrass, younger leaves produce EO of higher quality with very high citral content (75%) whereas from older leaves has low level of citral when harvested at a given point.

2.6.1 Developmental Regulation of EO Biosynthesis

The most important characteristic character of the EO accumulation is its dependence on the developmental stages of the concerned plant parts/ organs. The EO accumulation is greatly influenced by the ontogeny of the leaves (in lemongrass) and/or inflorescence (in palmarosa), their origin, their expansion to full development and finally their loss through senescence. The EO yield and proportion of the citral in lemongrass is closely related to leaf growth stages (Singh *et al*., 1989). In lemongrass 80% of the EO is accumulated in the earlier (10-25 day old) leaf developmental phases (Singh *et al*., 1989; Ganjewala *et al*., 2008). Any alteration in the EO content is reflected by the changes in the citral content. Similarly changes in EO content and composition with increase in leaf age have been observed in citronella (Luthra *et al*., 1991). In citronella relative percentage of geraniol and citronellol in the oil increased with corresponding decrease in geranyl acetate and citronellyl acetate as leaves grow older (Luthra *et al*., 1991). Also, leaf ontogeny has mimicked similar changes with steady increase in amount of citronellol, geraniol and citronellal and corresponding decline in amount of geranyl acetate and citronellyl acetate as leaf expands. Towards senescence/maturity, amount of EO, citronellal and geraniol decreased significantly (Luthra *et al*., 1991). Developmental and ontogenic variation in EO content and composition were also studied in lemongrass cultivars (Ganjewala *et al*., 2008). In palmarosa, the geraniol content in the oil increased from 64.8% at vegetative stage to 81.4% at flowering stage with corresponding decrease in geranyl acetate content (Dubey *et al*., 2001). Best quality palmarosa EO is obtained from harvest at early seed formation stage.

2.6.2 Seasonal Variation

Climate/seasons and diurnal factors also affect EO content and composition of lemongrass (Singh *et al*., 1989). It is reported that maximum amount of citral in the EO is accumulated during days when temperature is highest (Singh *et al*., 1979). In lemongrass only EO yield is affected by seasonal fluctuation but the citral content remained unaffected. The EO content in ten diverse but highly selected clones of lemongrass varied during the year with maximal EO yield in the May whilst lowest in the September (Singh *et al*., 1989). Similarly, in *C. Citratus* EO and citral content recorded maximum during the dry hot season which declined during rainy season (Oliveros-Belardo and Aureus, 1977). The EO obtained from lemongrass grown in Tarai climate of Uttar Pradesh had maximum citral content in October and June whilst lowest during the rainy season (Duhan *et al*., 1976). In citronella java EO, citronellal and geraniol contents were recorded maximal during months of October and November, while September harvest gives more citronellal (Malwatkar *et al*., 1984).

In case of palmarosa, summer harvest yields EO with lesser geraniol and higher geranyl acetate contents. The growth stage and harvesting time in particular adapto-climatic conditions have a profound influence on quality of palmarosa oil (Gulati *et al*., 1970; Gupta *et al*., 1978). The EO composition of C. nardus indigenous to Sri Lanka is influenced by temperature, when the temperature was low amount of citronellal in the oil was highest and when the temperature was high amounts of minor constituents such as, borneol and monoterpene hydrocarbons were high.

2.7 PLANT GROWTH RETARDANTS: THEIR MODE OF ACTION AND BENEFITS FOR PHYSIOLOGICAL RESEARCH

The growth behaviour and yield formation of crop plants are governed by their genetic potential, climatic conditions, and the supply of nutrients. Specific control of these processes by the application of plant bioregulators of natural or synthetic origin is increasingly emerging in recent years. Because of their specific properties in regulating shoot growth, the plant growth retardants have become the most widely used group of bioregulators in agricultural and horticultural practice. For detailed summaries of commercially realized and possible future applications of growth retardants the reader is referred to reviews by Davies *et al*. (1988), Rademacher (1991), and Hoffmann (this volume). When applied in appropriate concentrations growth retardants modify plant architecture in a typical fashion (Davies *et al*., 1988; Fletcher and Hofstra, 1988). Internode elongation and thus plant height are reduced without affecting the number of internodes and leaves. Concomitantly, the green colour of the foliage is

intensified and leaf thickness and epicuticular wax may increase. In contrast to the shoot, the growth retardants maintain or slightly enhance root formation. Therefore, the root-shoot ratio is clearly changed in favour of the root. The morphological effects of growth retardants are accompanied by alterations in the developmental and physiological behaviour of treated plants (Davies *et al*., 1988; Grossmann, 1990). The most striking changes include reduction of water consumption, retardation of senescence, and improved resistance to environmental stresses. As a result of this bio-regulation, the economically most important use of growth retardants is in improving lodging resistance and yield formation, particularly in cereals (Bruinsma, 1982; Jung, 1984).

2.7.1 Chemical nature of growth retardants and interference with gibberellin (GA) biosynthesis

Among the growth retardants of the first generation, which have found largescale application in agriculture for decades, the ethylene-releasing ethephon and, particularly, compounds of the onium-type such as cWormequat cWoride, mepiquat cWoride, and AMO 1618 are the most prominent representatives (Jung, 1984).

In addition to these conventional growth retardants, extremely effective compounds have been discovered and developed in recent years (see Rademacher, 1991; for structures). These include substances with a nitrogencontaining heterocycle, such as pyrimidine (e.g, in ancyrnidol), 4-pyridine (in inabenfide), triazole (e.g, in uniconazole, paclobutrazol, triapenthenol, BAS 111 ... W), and norbomanodiazetine (in tetcyclacis), and compounds with a cyclohexanetrione (acylcyclohexanedione) structure as the chemical feature. The latter group with prohexadione calcium, cimectacarb, and LAB 198 999, represents the most recent and thus the third generation of growth retardants known. In model experiments the conventional growth retardants are clearly surpassed by the new types of compounds in activity of reducing shoot growth (Rademacher, 1991). Each derivative of the three chemical classes (except the ethylene-releasing ethephon) share the common action of directly inhibiting gibberellin biosynthesis, but at distinct enzymatic steps (Graebe 1987; Hedden 1990; Rademacher 1991).

The onium-type compounds, with a positively charged moiety, appear to interact with the cyclization of geranylgeranyl pyrophosphate to ent-kaurene, catalyzed by ent-kaurene synthase. However, in the case of cWormequat and mepiquat cWoride unequivocal proof for this mechanism is still pending. The reactions of the next stage in biosynthesis, leading form Ent-kaurene to Ent kaurenoic acid, are the targets of the N-heterocyclics. Finally, the

cyclohexanetriones are known to interfere with certain steps beyond GA12- aldehyde which directly lead to the biologically active gibberellins, particularly to GAl' These are all hydroxylation reactions catalysed by soluble 2- oxoglutarate-dependent dioxygenases. They appear to be inhibited by the compounds competitively with respect to their co-factor (Adams *et al*., Rademacher *et al*., this volume).

The result of this chemical manipulation is a reduction in the endogenous content of gibberellins which is thought to be the main reason for their growth regulating properties. This hypothesis is supported by findings that the dwarfism induced by growth retardants can at least partially be compensated for by applied gibberellins (Rademacher *et al*., 1987). However, not all changes in the growth and physiological behaviour of treated plants can be explained by reduced gibberellin content. In this presentation these other aspects will be discussed in more detail.

2.7.2 Growth retardants with nitrogen-containing heterocycle:

2.7.2.1 Inhibitors of plant cytochrome P450 dependent monooxygenases

At present, most knowledge of the mode of action underlying the morphological and physiological effects of growth retardants has been compiled for the class of chemicals with a nitrogen-bearing heterocycle. All these compounds have a structural element in common: the lone pair of electrons on the Sp2 hybridized nitrogen atom in the heterocycle. This pair of electrons is found on the periphery of the molecules, enabling it to interact with plant cytochrome P450 dependent monooxygenases. It bonds to the protoheme iron of cytochrome P450 as the 6th ligand, preventing the oxygen required for the catalytic reaction from binding. Thus, the enzyme is inactivated (recent survey by Hedden, 1990). Many oxidative reactions in different metabolic pathways are catalysed by such microsomal enzymes. However, the action of this type of growth retardant seems to be Confed to methyl hydroxylases (Hedden, 1990), particularly in the terpenoid pathway. This includes the biosynthesis of gibberellins, abscisic acid (ABA), cytokinin’s and sterols. In a later section this topic will be discussed in more detail. Emphasis should first be laid on the effect of the retardants on growth.

2.7.2.2 Regulation of longitudinal shoot growth

According to Sachs *et al*. (1960), the sites of action of growth retardants are the subapical and intercalary meristems located e.g., at the base of internodes and leaf sheaths. In these areas cells are produced in a zone of meristematic activity with cell division and only slight cell

elongation. Newly formed cells entering the growth zone outside the meristem undergo considerable cell elongation without cell division (Grossmann, 1990). When applied to stems of chrysanthemum (Sachs *et al*., 1960) or basal explants of wheat leaves cultured in vitro (Grossmann *et al*., 1990), growth retardants are able to reduce the meristematic zone with its cell division activity. Histological studies on various shoot sections of sunflower, soybean and maize seedlings treated with tetcyclacis indicated that the type of effect on longitudinal growth depends on the concentration applied (Grossmann, 1988). Thus, the shortening occurring at low retardant concentration (e.g., 10-7 M tetcyclacis in hydroponics) is primarily caused by an inhibition of cell elongation. However, at higher concentrations (10-6-10-4 M) the stunting of the respective shoot sections is increasingly due to a reduced rate of cell division.

In conclusion, cell elongation principally occurring in the growth zones outside the meristems is the more sensitive process to growth retardants as compared to cell division.

2.7.3 Cell elongation and division:

2.7.3.1 Possible backgrounds of their metabolic regulation

As particularly emphasized by studies with genetically defined mutants, gibberellins and auxins are the groups of phytohormones that promote longitudinal shoot and leaf growth, mainly by affecting cell elongation (Graebe, 1987). As mentioned above, growth retardants with N-bearing heterocycles interfere with gibberellin biosynthesis by selectively inhibiting the oxidative steps from ent-kaurene to ent-kaurenoic acid (Graebe, 1987; Rademacher *et al*., 1987; Hedden, 1990). These reactions are catalyzed by the cytochrome P450 dependent kaurene oxygenase, which is affected by the retardant in the same concentration range as the elongation process itself. For example, a 50% inhibition of enzyme activity in vitro was obtained at 10-7 M of tetcyclacis (Rademacher *et al*., 1987). Thus, the reduction in cell elongation caused by Nheterocyclics appears to be closely linked to their influence on gibberellin biosynthesis.

In contrast, the mechanism underlying the effect of growth retardants on cell division in the meristems remains unclear because there is little evidence for direct participation of gibberellins in this process (Rappaport, 1980). In this context, heterotrophically cultivated cell suspensions offer an appropriate model system for meristematic tissue since growth is governed in both cases mainly by cell division activity (Grossmann, 1988; 1990). This was supported by an experiment comparing the effect of various growth retardants on cell division

activity in suspension cultures with their influence on shoot growth of intact plants. At the high concentration of 1Q-4 M, the same relative efficiency of the retardants was obtained for rice, maize, soybean, and sunflower in both systems (Grossmann, 1988). However, as illustrated by the effects of ancymidol, tetcyclacis, and paclobutrazol in cell suspensions, sterols playa more important role in cell division than gibberellins (Goad *et al*., 1988; Grossmann, 1988). It was shown with tetcyclacis that before cell division activity of rice cells ceases their terpenoid synthesis, membrane permeability, and protein, RNA, and DNA synthesis are reduced in a chronological sequence. Concomitantly, the phytosterol content in the cells, particularly stirmasterol, decreases. The addition of cholesterol or stigmasterol - but not applied gibberellins - fully restores normal cell division activity. Sterol production in intact plants is also influenced by Heterocyclic retardants (Grossmann, 1990; Hedden, 1990). However, their relative potency often depends on the plant species and material analysed, the type and - particularly in the case of chiral triazoles - the stereoisomer used, and on the retardant concentration which usually must be higher than that necessary for blocking gibberellin biosynthesis. However, in contrast to the phytohormonal gibberellins, sterols mainly function as membrane components. A strong reduction in sterol production certainly leads to cell damage, whereas a slight change in sterol metabolism creating sterols with membrane-altering properties might rather result in the observed cytostatic effects especially in meristematic cells.

As enzyme targets of growth retardants in sterol biosynthesis cytochrome P 450 dependent obtusifoliol-14a-methyl demethylase and sterol L122-desaturase, which also appears to be a cytochrome P 450, have been proposed (Goad *et al*., 1988; Grossmann, 1990). The latter enzyme catalyses the formation of the L122_ double bond in stigmasterol. Evidence for inhibition of this enzyme is given by a decline in the stigmasterol/sitosterol ratio after treatment with paclobutrazol or tetcyclacis. Hence, there are clear indications that the inhibition of cell division caused by higher retardant concentrations both in heterotrophic cell suspensions and in subapical meristems is mediated by a change in sterol synthesis and, thus, modified membrane properties. With the new types of growth retardants more insights have been obtained into the relative importance of gibberellin and sterol production for cell elongation and cell division.

2.7.3.2 Regulations of developmental and physiological responses

There is no doubt that the retardant-caused morpho-regulation with its alterations in shoot growth and root-shoot ratio by itself can influence the physiological behaviour of a plant

(Davies *et al*., 1988; Fletcher and Hofstra, 1988; Rademacher, 1991). Nevertheless, intensive studies in recent years have revealed further effects of N-heterocyclic retardants on the plant's hormone status, including influences on abscisic acid, cytokinins, and ethylene. Possible examples of the resulting interferences with the regulation of hormone-controlled physiological processes hold the spotlight in the following sections.

2.7.3.3 Influence on abscisic acid (ABA) metabolism and consequences for transpiration and resistance to environmental stresses.

N-heterocyclic retardants appear to cause a biphasic response of endogenous ABA levels. Shortly after treatment, ABA levels are transiently increased as observed in cell suspensions (Hauser *et al*., 1990), detached leaves (Hauser *et al*., 1990; Zeevaart *et al*., 1990) and young plants (Mackay *et al*., 1990).

Experiments with detached leaves of Xanthium strumarium (Zeevaart *et al*., 1990) and cell suspensions of oilseed rape (Hauser *et al*., 1990) suggested an inhibition of the conversion of ABA to inactive phaseic acid (PA) by tetcyclacis and BAS 111 ... W. This presumably cytochrome P450 dependent enzymatic step is involved in the major pathway of ABA catabolism which is initiated by methyl hydroxylation of ABA at C-8' (Zeevaart *et al*., 1990). The induced accumulation of endogenous ABA in suspensions of oilseed rape cells was closely correlated with an enhanced potassium and water content of the cells (Hauser *et al*., this volume). Moreover, in leaves it was accompanied by an increase in stomatal resistance and a reduced transpiration rate (Hauser *et al*., 1990; Mackay *et al*., 1990). Together with reported rises in proline and other amino acids (Mackay *et al*., 1990) these water-conserving effects of Nheterocyclic retardants can explain improved adaptation of treated plants to drought, low temperature, and other environmental stresses (Fletcher and Hofstra, 1988; Dorffling *et al*., 1990).

When analysed later or at higher retardant concentrations ABA levels fall below those of controls as shown in plants of apple (Wang *et al*., 1987), oilseed rape (Hauser *et al*., 1990), and soybean (Grossmann, 1990). The effect might be explained by a newly stimulated ABA catabolism and/or by inhibition of ABA biosynthesis.

However, in each case the influence on ABA metabolism appears to depend on the type of retardant, its endogenous concentration and on the developmental stage and species of the plants used (Hauser *et al*., 1990; Mackay *et al*., 1990).

2.7.3.4 Retardation of senescence

An extended longevity of plants after treatment with retardants has been regularly observed. Since no direct influence of retardants on senescence of leaves or chlorophyll breakdown in chloroplasts appears to exist (Grossmann, K. and MatHe, Ph., unpublished data), a shift in the hormonal constellation of the plant favouring the senescence-delaying cytokinins as opposed to the 'senescence hormones" ABA and ethylene, has been suggested (Grossmann, 1990). The endogenous contents of cytokinins, particularly trans-zeatin and dihydrozeatin and its ribosides, were appreciably increased by N-heterocyclic retardants in rice (Izumi *et al*., 1988) and soybean seedlings (Grossmann, 1990). A close correlation between physiological phenomena and hormone status was demonstrated in detail in senescing cotyledons of pumpkin treated with BAS 111 ... W (Grossmann, K. and Kwiatkowski, J., unpublished data). With increasing retardant concentrations, the reduced loss of total chlorophyll is paralleled with a gradual increase of immunoreactive cytokinins in the order of dihydrozeatin riboside (DZR) > trans-zeatin riboside (ZR) > isopentenyladenosine (IPA). In contrast, the contents of 3-indolylacetic acid (IAA) and gibberellins remain nearly unchanged whereas ABA levels are considerably lowered. lowered. A similar situation has been found in senescing cotyledons of soybean after seed treatment with BAS 111 ... W

The senescence-retarding potency of growth retardants can be further explained by their inhibition of ethylene biosynthesis, which is a common effect of the new types of compounds (Table 1). As shown in sunflower cell suspensions and in leaf discs as well as in intact plants of several species, blocking is most likely at the level of the conversion of 1-arninocyclopropane-1-carboxylic acid (ACC) to ethylene (Grossmann, 1990). The effect cannot be overcome by adding GA3 (Grossmann *et al*., 1989) and, as evident from sunflower cell suspensions, is not functionally related to growth. Hence, this might be seen as a direct effect on the ethylene-forming enzyme or, perhaps more likely, as an indirect influence via a retardant-caused change in the membrane properties (Grossmann *et al*., 1989). In this context, some evidence exists for interrelations between a decreased ethylene production and enhanced levels of (a) cytokinins and (b) polyarnines. Concerning cytokinins, both the application of BAS 111 ... W and aminoethoxyviny1glycine (AVG), a known inhibitor of ACC synthase, depressed ethylene formation in sunflower cell suspensions while cellular cytokinin levels increased (Grossmann K. and Hauser C. unpublished data). A possible explanation of this effect may be the capacity of ethylene to accelerate cytokinin degradation (Bollmark and Eliasson, 1990). In the case of polyarnines, the inhibition of ethylene production by retardants

could lead to an enhanced flux of S-adenosylmethionine (SAM), a common precursor of both pathways, particularly into spermidine and spermine (Grossmann, 1990). Besides cytokinins, spermine and spermidine have also been found to exhibit anti-senescence properties (Nooden and Leopold, 1988)

In conclusion, it is reasonable to assume that the observed changes in the endogenous balance of bioregulators contribute to retardation of plant senescence. As a consequence, yield formation processes are favoured, e.g, in oilseed rape. In this case, treatment of plants grown under field conditions with BAS 111 ... W caused a delay of pod senescence resulting in lowered seed loss by pod shatter and with a reduced susceptibility of pod walls towards fungal infection (Luib *et al*., 1987). Moreover, direct fungicidal properties have been demonstrated for uniconazole and paclobutrazol, possibly due to an inhibition of fungal ergosterol biosynthesis (Aetcher and Hofstra, 1988).

3. MATERIALS AND METHODS:

3.1 Study of Growth retardants in leaves of Lemongrass.

3.1.1 Chlormequat chloride feeding

The post mid expanded (65%-70% expanded) leaves was kept in different concentration of plant growth retardant Chlormequat chloride. One set of leaves was treated as a control while another set was treated with Chlormequat chloride. Thus, treated and untreated leaves were analysed for oil content, protein content, enzyme activity like nitrate reductase, peroxidase, Proline content and chlorophyll content.

- The oil content was determined by hydro-distillation with Clevenger apparatus and is expressed on fresh weight basis (Guenther, 1955).
- Protein content was estimated according to Lowry *et al* (1951).
- Chlorophyll content was calculated by the method of Arnon (1949).
- Proline was estimated calorimetrically by the method of Bates *et al* (1973).
- Peroxidase activity was estimated by Pulter (1974).
- Nitrate reductase activity was measured by Hangeman and Hucklesby (1971).

3.2 CHLOROPHYLL ESTIMATION

0.2 g of the tissue was extracted with 25 ml of 80% acetone and absorbance was recorded at 663nm and 645nm in spectrophotometer. From the absorbance values, amount of chlorophyll was calculated by the method of Arnon (1949).

$$Chl.a = 12.7 \times (A_{663} - 2.69) \times A_{645} \times \left[\frac{V}{1000\ W}\right]$$

$$Chl.b = 22.9 \times (A_{645} - 4.6) \times A_{663} \times \left[\frac{V}{1000\ W}\right]$$

$$Chl.\ a+b=\ 20.2\ \times A_{645}+8.02\times A_{663})\times\left[\frac{V}{1000\ W}\right]$$

Where [V/ 1000 W] is constant which is equivalent to 0.125

Where V= volume of Acetone,

W=Weight of the tissue

3.3 ESTIMATION OF NITRATE REDUCTASE (NR) ACTIVITY

NR (EC 1.6.6.1) activity was measured by *in-vivo* method according to Hageman and Hucklesly (1971). The leaves were washed and pressed in filter paper to remove sticking moisture and cut into small pieces. 0.1g of chopped leaves was taken in 50ml filtering flask containing reaction mixture (2.5ml KNO_3 and 2.5ml phosphate buffer). Each sample was analysed in duplicate. The flasks were kept in 150ml beaker with ice so that the enzyme may not decompose. The reaction mixture was infiltrated into the tissue by using vacuum pump and infiltration was done for one minute. The infiltrated material was kept at 33°C for 1hr. in dark (Klepper *et al.,* 1974).

The reaction was stopped by boiling the reaction mixture. The flasks were removed from the hot plate immediately after the reaction mixture started boiling. The heating also facilitated movement of nitrite out of the tissue. 0.5ml of reaction mixture was taken in a test tube. 1.0ml of sulfanilamide (1%) and 1ml of N-(1 Napthyl) ethylene diamine dihydrochloride (0.02%) were mixed in it. The final volume was made to 10ml. Optical density reading were taken at 540nm against distilled water on spectrophotometer. The nitrate reductase activity is stated as the amount of nitrite formed per gram fresh weight per hour.

3.3.1 Standard Curve of Nitrate Reductase

Sodium nitrite (AR grade) was used for the preparation of standard curve. 69mg sodium nitrite was dissolved in 100ml distilled water in order to obtain solution of 100µmoles/0.1ml strength. By successive dilution, solution of 100 µmoles/0.1ml strength was prepared. From this solution, 0.05ml, 0.1ml, 0.15ml, 0.2ml, and 0.25ml solutions were taken in five different test tubes. To these test tubes 0.95, 0.9, 0.85, 0.8 and 0.75mldistilled water was added to obtain solutions of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 µmoles/ml. Then, 1 ml of 1% sulfanilamide and 1ml of 0.02% N-(1 Napthyl) ethylene diamine dihydrochloride was

added in each test tube. The colour was allowed to develop for 20 minutes. The optical density was read at 540nm on spectrophotometer. The standard curve is presented in fig. 3.3

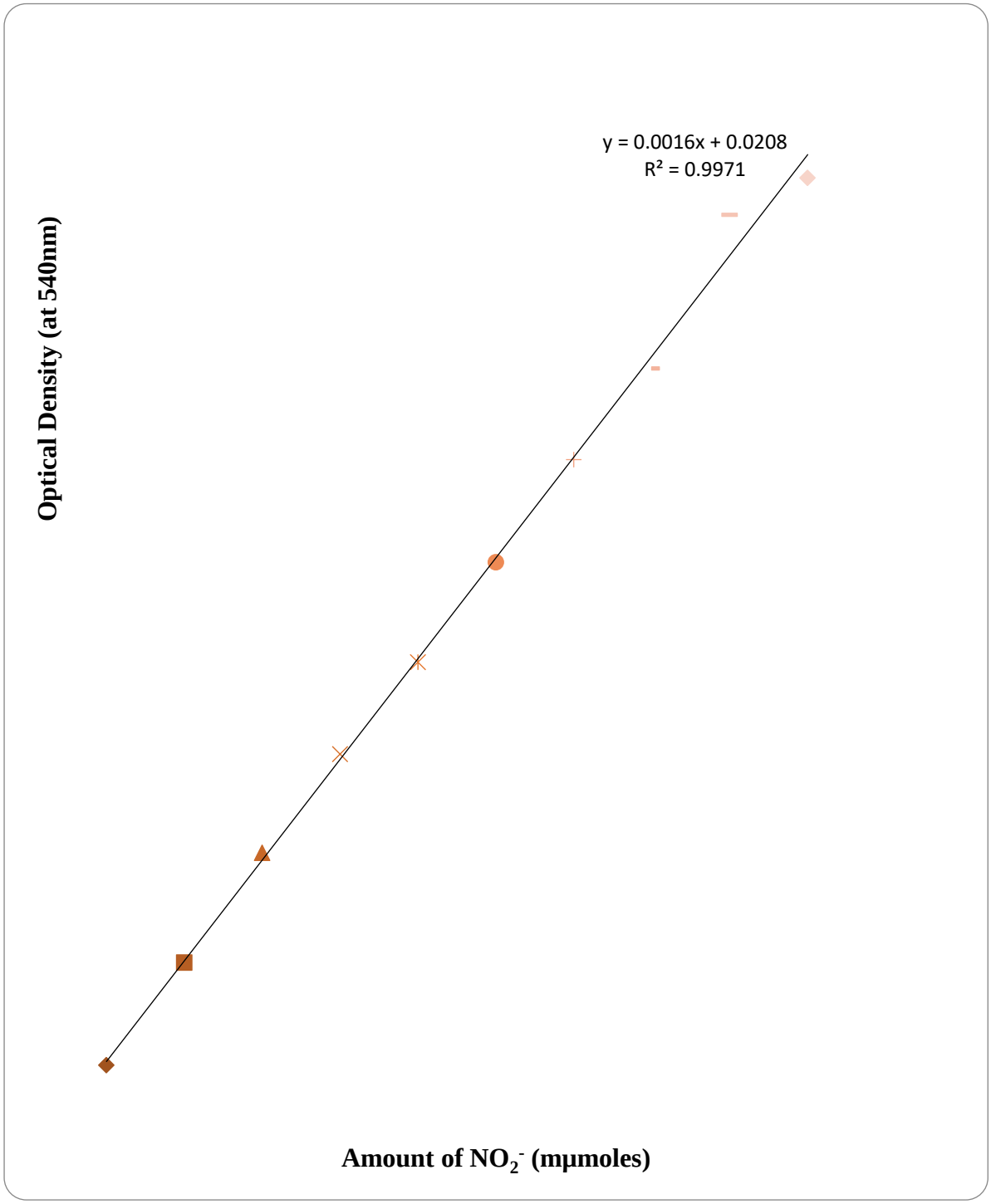


Fig 3.3 Standard Curve for Nitrate Reductase

3.4 ESTIMATION OF PROTEIN

Protein was estimated according to Lowry *et al.* (1951) in crude enzyme extract. 100µl of supernatant and 100µl of 10% trichloroacetic acid (TCA) were mixed and kept for protein precipitation. After 24 hrs., it was centrifuged for 10 minutes at 10,000 rpm to remove TCA. The pellet was dissolved in 1ml of 1N NaOH and shaken well on cyclomixer. 0.5 ml from this solution was taken in the test tube in duplicate and 5ml of alkaline $CuSO_4$ solution was added to this and left for 30 minutes, then 0.5 ml of 1/2 strength Follin's reagent was added to it. Optical density was recorded at 660nm on spectrophotometer. Concentration of the protein was calculated against the standard curve.

3.4.1 Standard Curve of Protein

For the preparation of standard curve of protein, 5mg of Bovine serum albumin (BSA) was dissolved in 25ml of 1N NaOH. From this stock solution, different volumes (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0ml) corresponding to concentrations of 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200µg respectively were pipetted in test tubes in duplicate. Different volumes of 0.1 N NaOH were added to corresponding test tubes to make the final volume 0.5ml. Then 5.0ml of alkaline copper solution was added in each test tube and then 0.5 ml of Folin's reagent was added in each test tube and left for 30 minutes. Optical density was measured on spectrophotometer and standard curve was plotted, which is presented in fig 3.4.

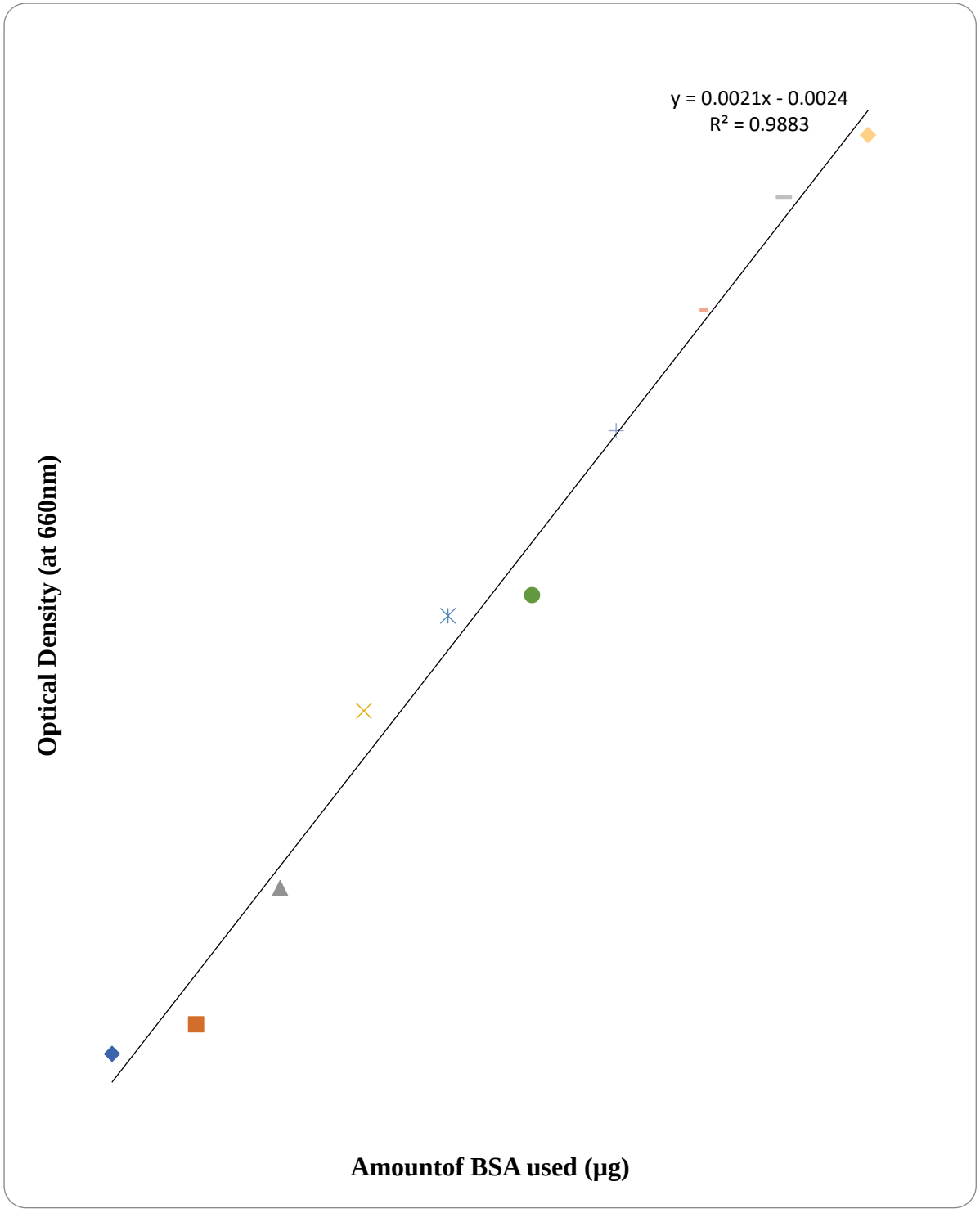


Fig 3.4 Standard Curve for Protein

3.5 ESTIMATION OF PROLINE

Proline was estimated calorimetrically by the method of Bates *et al.*, (1973). 1.5 g fresh tissue was ground in 5ml of 3% sulphosalicylic acid. The homogenate was centrifuged at 10,000 rpm for 10 minutes. 0.5 ml of supernatant was taken in duplicate. To this 0.5 ml glacial acetic acid

and 0.5 ml of ninhydrin solution was added. Ninhydrin solution was prepared by dissolving 1.25 g ninhydrin in 30ml of glacial acetic acid and 20 ml orthophosphoric acid (6M). The test tubes were placed in boiling water for 1hour and then 4ml of toluene was added after cooling the tubes. Optical density was measured at 520nm on spectrophotometer after mixing the contents in cyclomixer.

3.5.1 Standard Curve of Proline

For 5.0mg proline was dissolved in 50ml of distilled water. From this stock solution, different concentrations of proline (20, 40, 60, 80 and 100 ml) were pipetted in test tubes in duplicate. To this 0.5, 0.4, 0.3, 0.2, 0.1 and 0ml sulphosalicylic acid was added. 0.5ml of acetic acid and 0.5 ml of ninhydrin solution was added in each test tube. All the test tubes were placed in boiling water bath for 1hour and then 4ml of toluene was added after cooling the test tubes. Contents were mixed thoroughly on cyclomixer. Optical density was measured at 520nm on spectrophotometer and standard curve was plotted, which is presented in fig 3.5

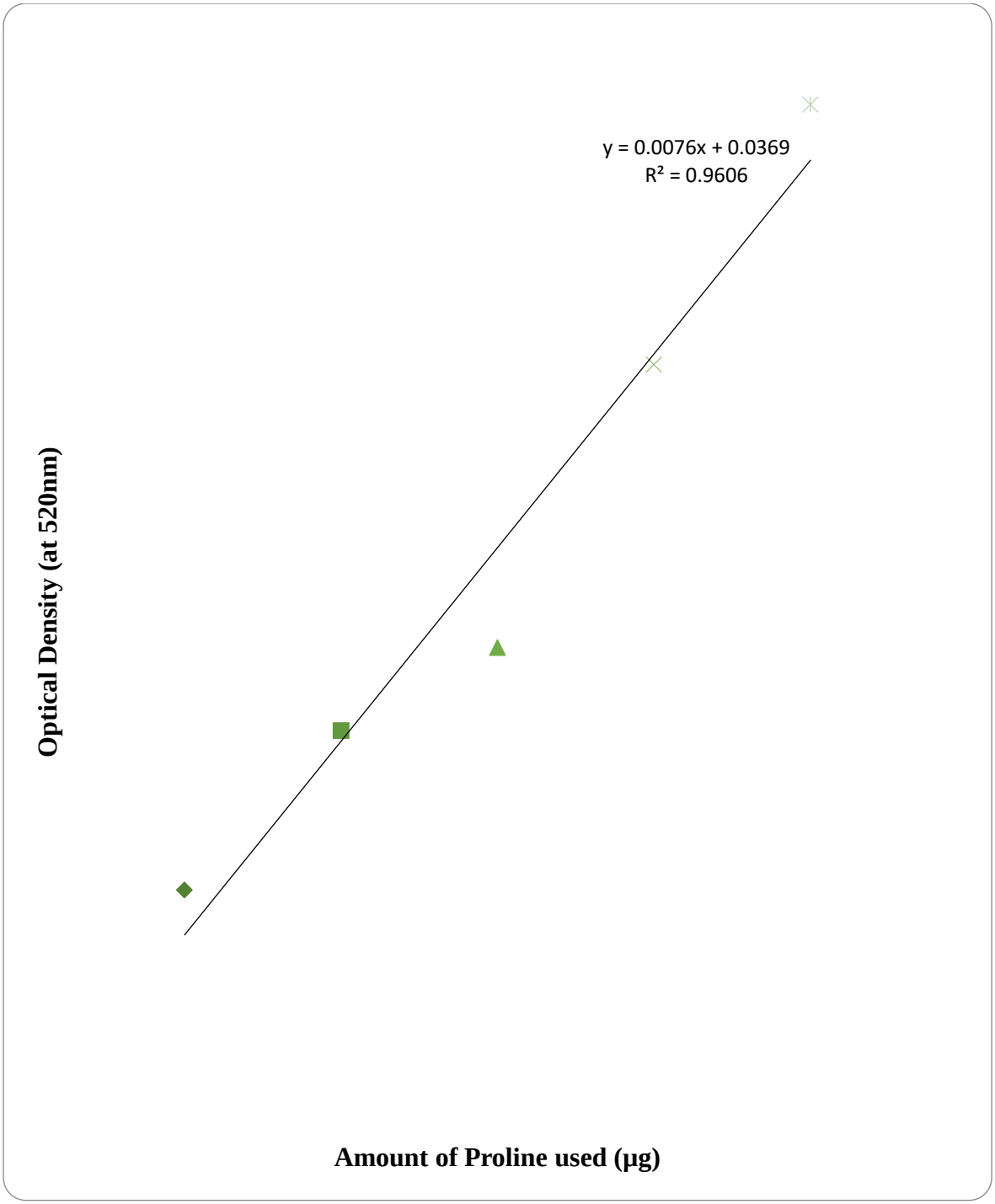


Fig 3.5 Standard Curve of Proline

4. RESULTS AND DISCUSSION

4.1 STATISTICAL ANALYSIS

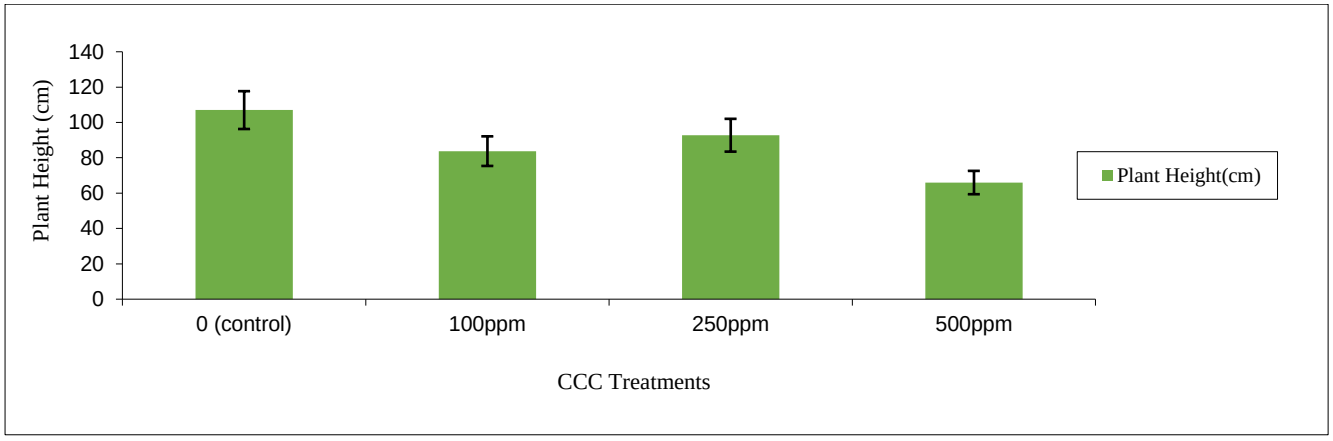
The data were statistically analyzed using one way analysis of variance (ANOVA) by GRAPH PAD Prism 5 software. Mean values were statistically compared by Tukey-Kramer multiple comparison test at (P<0.05. Data were presented as Mean ±SD (n=3).

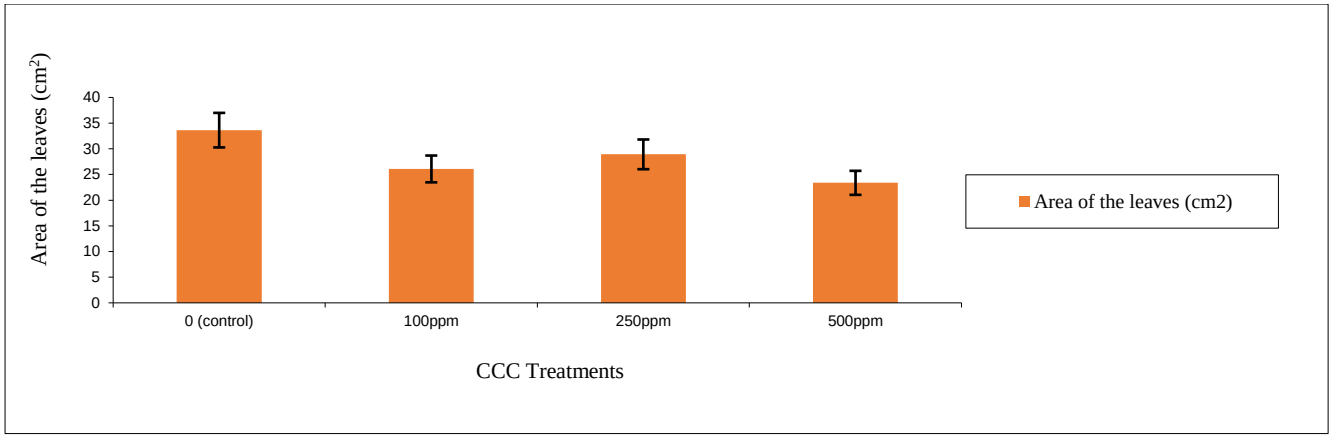
Table 4.1: Analysis of growth and development (plant height, area of the leaves, tiller no and herb yield) of Lemongrass in pot by foliar spraying method of Chlormequat chloride (100ppm, 250ppm and 500ppm).

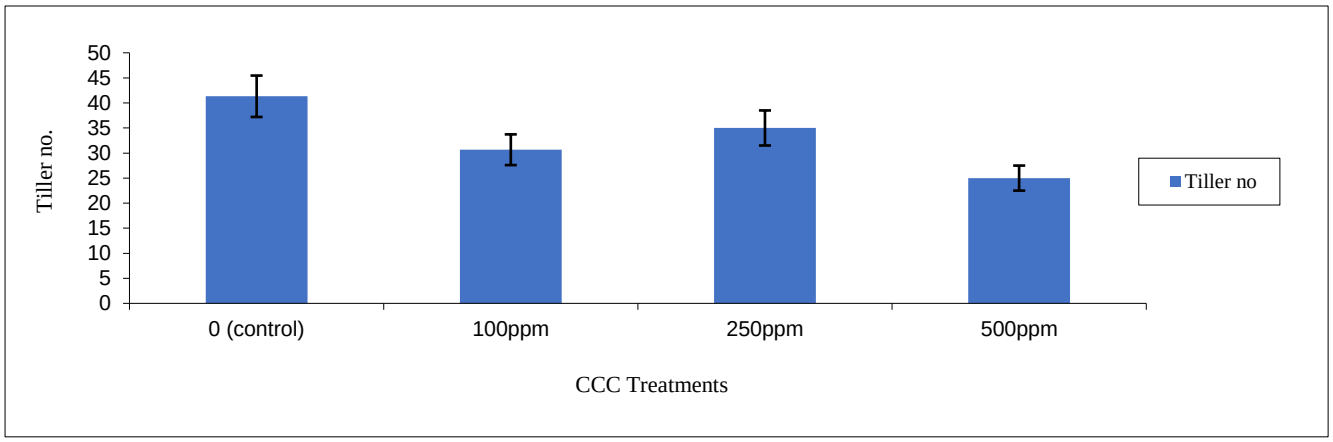
Treatment	Plant Height(cm)	Area of the leaves (cm^2)	Tiller no	Herbage Yield(g)/pod
Untreated	107.89±2.50	28.42±0.57	25±1.00	311.66±2.08
CCC (100 ppm)	83.62±1.60d	23.34±0.90d	19±0.57d	286.95±2.74d
CCC (250 ppm)	92.01±3.04d	26.68±0.37a	22±1.00b	297.28±2.65d
CCC (500 ppm)	62.88±1.05d	20.25±0.90d	15±1.00d	238.88±1.05d

The data represented are Mean ±SD (n=3).

P>0.05-NS=a, P<0.05-b*, P<.001-c**, P<0.001-d***







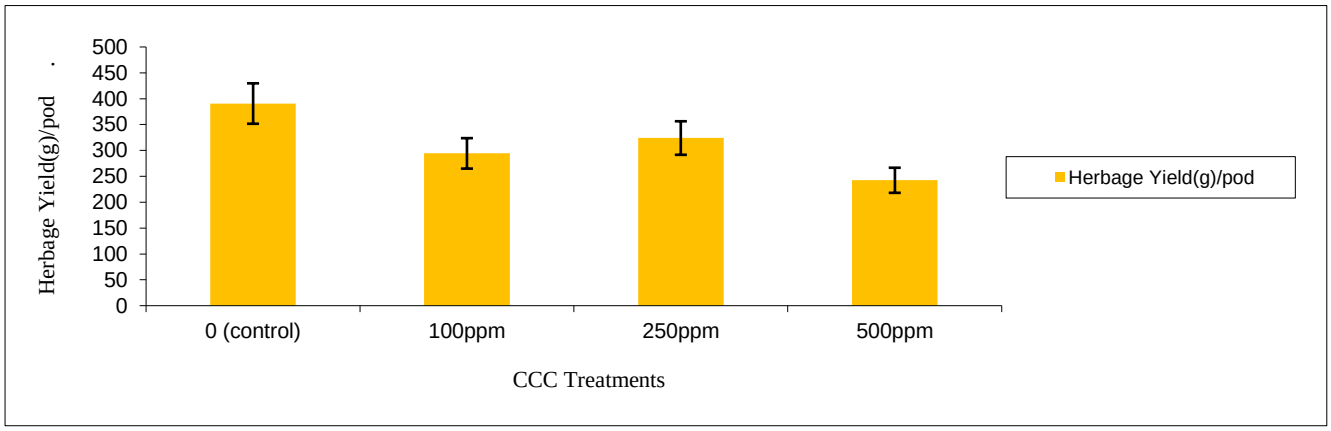
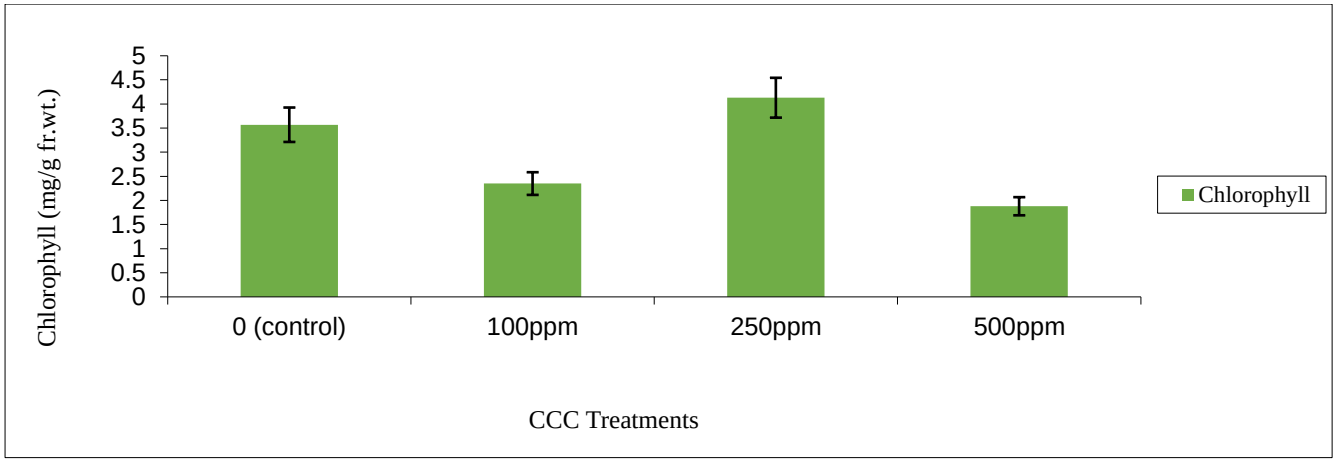
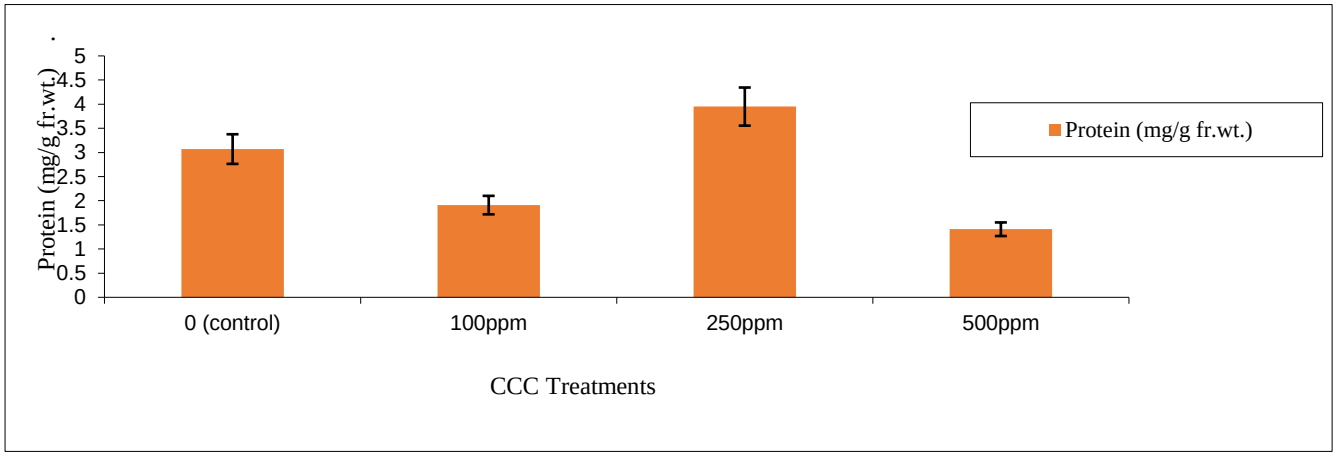
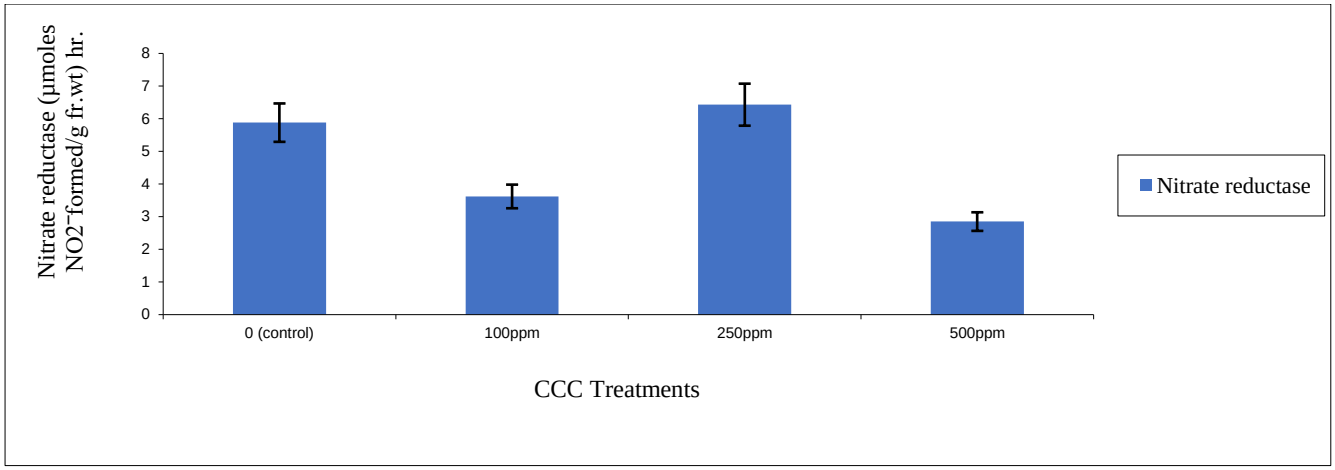


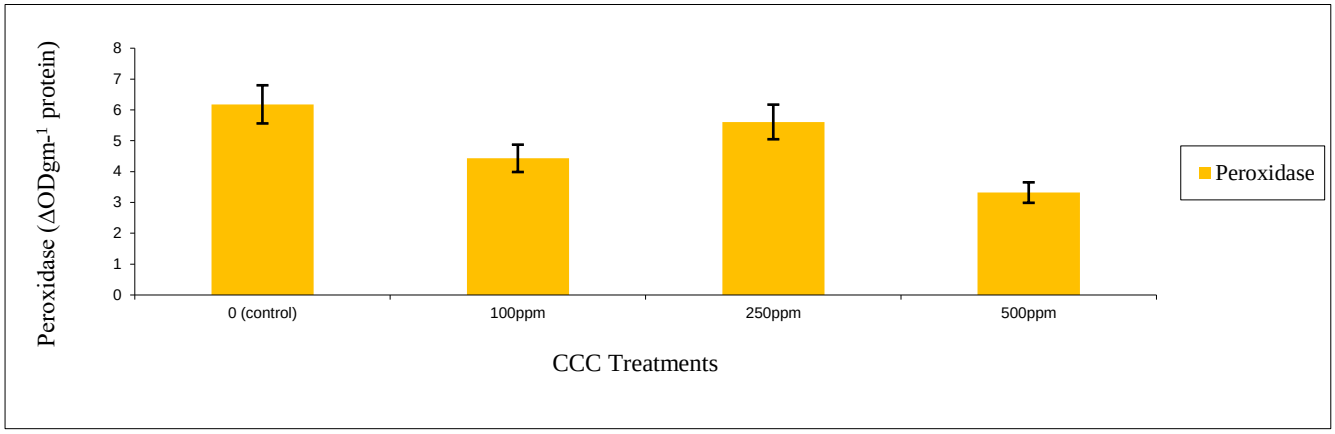
Table 4.2: Effect of Chlormequat chloride (100, 250, 500 ppm) on chlorophyll content, protein content, enzyme activity like nitrate reductase, proline content and oil content and in excised leaves of Lemongrass.

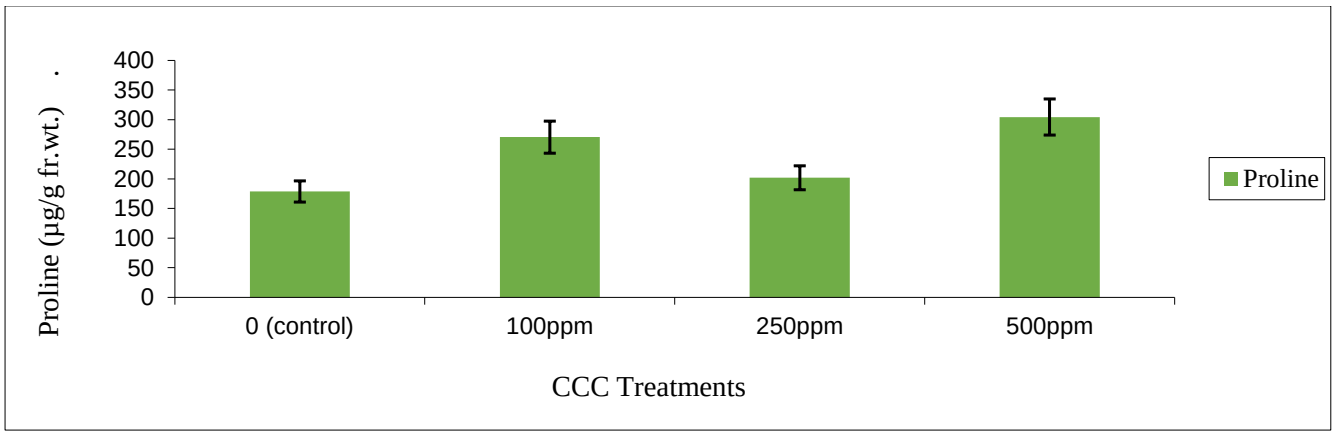
Treatment	Chlorophyll (mg/gfr.wt.)	Protein (mg/g fr.wt.)	Nitrate reductase (µmoles NO_2^-formed /g fr.wt)hr.	Peroxidase (ΔODgm^{-1} protein)	Proline (µg/g fr.wt)	Oil yield (ml/100 g.fr wt.)
Untreated	2.76±0.06	2.12±0.01	5.58±0.05	4.29±0.05	99.08±2.31	0.87±0.01
CCC (100 ppm)	2.45±0.05d	1.47±0.08d	3.32±0.05d	3.32±0.05 d	135.31±1.10d	0.62±0.01 d
CCC (250 ppm)	3.63±0.12d	2.79±0.06d	8.29±0.40d	6.38±0.05 d	86.42±0.57d	1.41±0.01 d
CCC (500 ppm)	1.63±0.02d	1.23±0.02d	2.31±0.10d	2.45±0.15 d	158.38±2.00d	0.46±0.05 d






Nitrate reductase (µmoles NO2¯formed/g fr.wt) hr.
8
7
6
5
4
3
2
1
0
0 (control)
100ppm
250ppm
500ppm
CCC Treatments
Nitrate reductase


Peroxidase (ΔODgm-1 protein)
8
7
6
5
4
3
2
1
0
0 (control)
100ppm
250ppm
500ppm
CCC Treatments
Peroxidase


Proline (µg/g fr.wt.)
400
350
300
250
200
150
100
50
0
0 (control)
100ppm
250ppm
500ppm
CCC Treatments
Proline

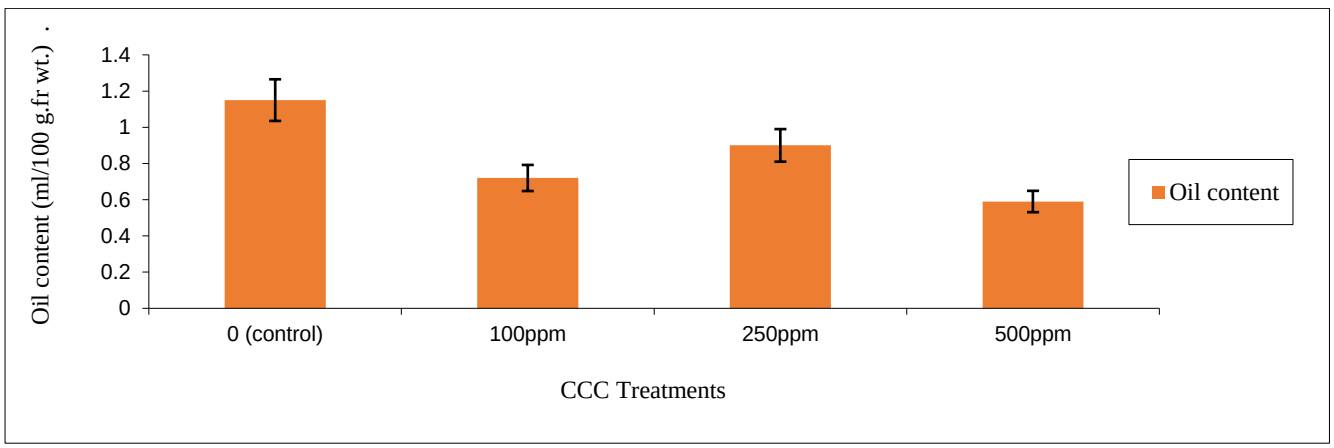
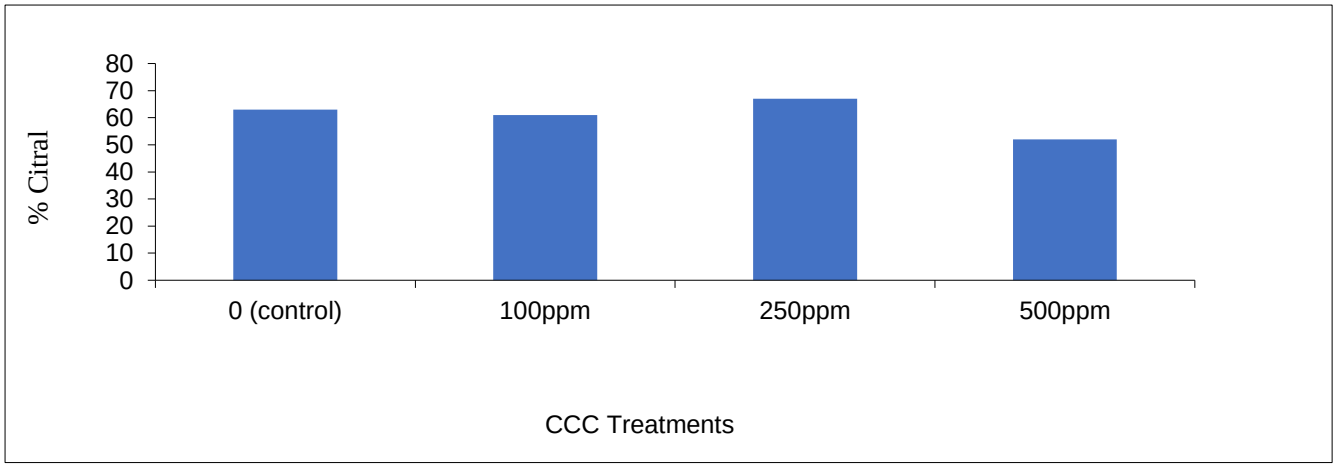

Oil content (ml/100 g.fr wt.)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 (control)
100ppm
250ppm
500ppm
CCC Treatments
Oil content

Table 4.3: Effect of Chlormequat chloride (100, 250 and 500 ppm) on oil constituents in Lemongrass by foliar spaying method in pot.

CCC	% Citral	% Geranyl acetate
Untreated	63	18
CCC (100ppm)	60	15
CCC (250ppm)	76	12
CCC (500ppm)	56	31



5. CONCLUSION

Plant height, leaf area, tiller number and herbage yield decreased significantly due to CCC treatment at 100 ppm, 250ppm and 500ppm concentration. As compared with all the concentrations plant height decreased more at 500ppm. Area of leaves shows good result at 250 ppm. Tiller no. also showed good result at 250 ppm. So, overall herbage yield increased at 250ppm concentrations among all other concentrations. When CCC (250ppm) was applied, chlorophyll content increased significantly over untreated plants. Protein content increased significantly by the application of CCC at 250ppm. NR activity also increased by the application of CCC at 250ppm over untreated plants while peroxidase activity decreased at 250ppm concentration of CCC. Proline content decreased significantly at 250ppm and increased at 500ppm over untreated plants. Oil content increased significantly by CCC at 250ppm over untreated plants. Oil content was not affected significantly due to application of CCC at 250ppm. Geraniol content increased by CCC application at 250ppm over control. Geranyl acetate content decreased at 100ppm, 250ppm and 500ppm significantly.

The treatment of *Cymbopogon flexuosus* plants with Chlormequat chloride resulted in the decrease in the vegetative growth of the plants like plant height, leaf area, tiller number and herbage yield recorded in this study (Table no 1). The Chlormequat chloride enhanced essential oil yield. Among the three concentrations 250ppm proved to be highly affecting in improving the yield of quantity and quality of essential oil. Chlormequat chloride enhanced the biochemical parameters (Table no 2). Enhanced levels of chlorophyll were found coupled with increase in photosynthesis might have contributed increase levels of carbohydrate fractions and carbon dioxide fixation. Chlormequat chloride also influenced the levels of soluble proteins (Table no 2). The enzyme nitrate reductase was significantly increased by gradual increase in the applied levels of Chlormequat chloride with 250ppm proving the best foliar application. The increase in the uptake of various nutrients, including NO_3 and availability of NADH or activation of NR due to Chlormequat chloride treatments. Alternatively, the increased activity of NR can be attributed to the facts that BL stabilizes the plasma membrane, hence preventing damage. This membrane stabilization could have facilitated the increased uptake of nutrients including nitrate (NR activity inducer) increasing the NR activity.

In present study it was observed that Chlormequat chloride employed plants at 250ppm concentration enhanced the essential oil yield and content when compared to untreated plants (Table no 1 and 3). There was significant increase in the content of geraniol and geranyl acetate (Table no 3) The effect of Chlormequat chloride on essential oil yield might have mediated

through the impact on gene transcription in biosynthetic pathways thereby increasing yield and content of oil. PGRs has been documented that 3-hydroxyl-3methylglutaryl coenzyme-A reductase (HMGR) gene catalyses the first step in the biosynthesis of isoprenoids which ultimately culminates in the biosynthesis of sesquiterpenoids. (Chlormequat chloride might have triggered the intrinsic genetic potentiality of the plants to produce more essential oil) Higher levels of carbohydrate and their possible diversion to secondary metabolism might have contributed to elevated levels of essential oils in *Cymbopogon flexuosus*.

6. REFERENCES

Bampidis, V.A.; Christodoulou, V.; Christaki, E.; Florou-Paneri, P.; Spais, A.B. Effect of dietary garlic bulb and garlic husk supplementation on performance and carcass characteristics of growing lambs. Anim. Feed Sci. Technol. 2005, 121, 273–283.

Barton, M.D. The down-side of antibiotic use in pig production: The effect of antibiotic resistance of enteric bacteria. In Manipulating Pig Production; Carnwell, P.D., Ed.; Australasian Pig Science Association: Victoria, Australia, 1999; Volume 7.

Bollmark M. and Eliasson L. (1990) Ethylene accelerates the breakdown of cytokinins and thereby stimulates rooting in Norway spruce hypocotyl cuttings. Physiol. Plant. 80: 534-540.

Botsoglou, N.A.; Taitzoglou, I.A.; Botsoglou, E.; Zervos, I.; Kokoli, A.; Christaki, E.; Nikolaidis, E. Effect of long-term dietary administration of oregano and rosemary on the antioxidant status of rat serum, liver, kidney and heart after carbon tetrachloride-induced oxidative stress. J. Sci. Food Agric. 2009, 89, 1397–1406.

Brenes, A.; Roura, E. Essential oils in poultry nutrition: Main effects and modes of action. Anim. Feed Sci. Technol. 2010, 158, 1–14.

Bruinsma J. (1982) Plant growth regulators in field crops. In: McLaren 1.S. ed., Chemical manipulation of crop growth and development, pp 3-11. Butterworth, London, UK.

Chang, J. Medicinal herbs: Drugs or dietary supplements? Biochem. Pharmacol. 2000, 59, 211–219.

Collin, H. Herbs, spices and cardiovascular disease. In Handbook of Herbs and Spices; Peter, K.V., Ed.; Woodhead Publishing Limited: Cambridge, UK, 2006; Volume 3, pp. 126–137.

Couladis, M.; Tzakou, O.; Verykokidou, E.; Harvala, C. Screening of some greek aromatic plants for antioxidant activity. Phytother. Res. 2003, 17, 194–195.

Cowan, M.M. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 1999, 12, 564–582.

Davies ToO., Steffens G.L. and Sankhla N. (1988) Triazole plant growth regulators. Horticultural Rev. 10: 63-105.

Dawson, F.A. (1995). The amazing terpenes. Naval Stores Rev. 6–12.

Dhaou, S. O., Jeddi, K., & Chaieb, M. (2010). Les Poaceae en Tunisie: systématique et utilité thérapeutique. Phytothérapie, 8(2), 145-152.

Dorffling K., Rademacher W. and Flores-Nimedez A.A. (1990) Use of cryoprotectants in rice. In: Focus on irrigated rice, pp 1-13. The international rice research conference, Seoul, Korea.

Duke, J. Handbook of Medicinal Spices; CRC Press: Boca Raton, FL, USA, 2002.

Duthie, G.G.; Brown, K.M. Functional foods. In Reducing the Risk of Cardiovascular Disease; Goldberg, I., Ed.; Chapman and Hall: London, UK, 1994; pp. 19–38.

Elgayyar, M.; Draughon, F.A.; Golden, D.A.; Mount, J.R. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Protect. 2001, 64, 1019–1024.

Fletcher R.A. and Hofstra G. (1988) Triazoles as potential plant protectants. In: Berg D. and Plempel M. eds., Sterol biosynthesis inhibitors. Pharmaceutical and agrochemical aspects, pp. 321-331. VCH Publishers, Weinheim, Germany.

Gagan Shah, Richa Shri, Vivek Panchal,Narender Sharma,Bharpur Singh and A. S. Mann (2011) Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (Lemon grass). J Adv Pharm Technol Res. 2011 Jan-Mar; 2(1): 3–8.

Ganjewala, D., Kumari A. and Khan, K.H. (2008). Ontogenic and developmental changes in essential oil content and compositions in *Cymbopogon flexuosus* cultivars. In: Prasad BN, Lazer Mathew, editor. Recent Advance in Biotechnology. New Delhi, India: Excel India Publishers. pp. 82–92.

Giannenas, I. How to use plant extracts and phytogenics in animal diets. In World Nutrition Forum, the Future of Animal Nutrition; Binder, E.M., Schatzmayr, G., Eds.; Nottingham University Press: Nottingham, UK, 2008; pp. 111–129.

Goad L.J., Haughan P.A. and Lenton J.R. (1988) Regulation of sterol production and the effects on plant cell growth. In: Pinfield N.J. and Stobart AX eds., Plant lipids: targets for manipulation, pp. 91-105. BPGRG monograph No. 17.

Graebe J. (1987) Gibberellin biosynthesis and control. Annu. Rev. Plant Physiol. 38: 419-465.

Greathead, H. Plants and plant extracts for improving animal productivity. Proc. Nutr. Soc. 2003, 62, 279–290.

Grossmann K. (1988) Plant cell suspensions for screening and studying the mode of action of plant growth retardants. In: Maramorosch K. and Sato G. eds., Advances in cell culture, pp. 89-136. Academic Press, San Diego, USA.

Grossmann K. (1990) Plant growth retardants as tools in physiological research. Physiol. Plant. 78: 640--648.

Grossmann K., Hauser C., Sauerbrey E., Fritsch H., Schmidt O. and Jung J. (1989) Plant growth retardants as inhibitors of ethylene production. I. Plant Physiol. 134: 538-543.

Grossmann K., Kwiatkowski 1, Siebecker H. and Jung I. (1987) Regulation of plant morphology by growth retardants. Effects on phytohormone levels in soybean seedlings determined by immunoassay. Plant Physiol. 84: 1018-1021.

Grossmann K., Sauerbrey E. and Jung J. (1990) Influence of growth retardants and ethylenegenerating compounds on culture response of leaf explants from wheat (Triticum aestivum L.). J. Plant Physiol. 135: 725-731.

Gurib-Fakim, A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol. Aspects Med. 2006, 27, 1–93.

Hauser C., Kwiatkowski J., Rademacher W. and Grossmann K. (1990) Regulation of endogenous abscisic acid levels and transpiration in oilseed rape by plant growth retardants. J. Plant Physiol. 137: 201-207.

Hedden P. (1990) The action of plant growth retardants at the biochemical level. In: Pharis R. P. and Rood S. B. eds., Plant growth substances 1988, pp. 322-332. Springer-Verlag, Heidelberg, Germany.

Izumi K., Nakagawa S., Kobayashi M., Oshio H., Sakurai A. and Takahashi N. (1988) Levels of IAA, cytokinins, ABA and ethylene in rice plants as affected by a gibberellin biosynthesis inhibitor, uniconazole-P. Plant Cell Physiol. 29: 97-104.

Jung J. (1984) Plant bioregulators in cereal crops. Action and use. In: Ory R.L. and Rittig P.R. eds., Bioregulators, chemistry and uses, pp. 29-43. ACS symposium series 257, Washington D.C., USA.

Kadri, A.; Zarai, Z.; Chobba, I.B.; Bekir, A.; Gharsallah, N.; Damak, M.; Gdoura, R. Chemical constituents and antioxidant properties of Rosmarinus officinalis L. essential oil cultivated from South-Western Tunisia. J. Med. Plants Res. 2011, 5, 5999–6004.

Karunamoorthi K, Ilango K, Murugan K (2010) Laboratory evaluation of traditionally used plant-based insect repellent against the malaria vector Anopheles arabiensis Patton (Diptera: Culicidae). Parasitol Res 106:1217–23.

Li, T.S.C. The range of medicinal herbs and spices. In Handbook of Herbs and Spices; Peter, K.V., Ed.; Woodhead Publishing Limited: Cambridge, UK, 2006; Volume 3, pp. 113–125.

Luib M., Koehle H., Hoeppner P. and Rademacher W. (1987) Further results with BAS 111 04 W, a new growth regulator for use in oilseed rape. In: Hawkins A.F., Stead A.D. and 285.Pinfield N.J. eds., Plant growth regulators for agricultural and amenity use, pp. 37-43. BCPC Publications Monograph No. 36, Thornton Heath, UK.

M. MACHRAOUI1*, Z. KTHIRI 1, M. BEN JABEUR 1, W. HAMADA1; Ethnobotanical and phytopharmacological notes on *Cymbopogon citratus*(DC.) Stapf, Volume 55 (5), 2018.

Mackay C.E., Hall I.C., Hofstra G. and Fletcher R.A. (1990) Uniconazole-induced changes in abscisic acid, total amino acids, a proline in Phaseolus vulgaris. Pestic. Biochem. Physiol. 37: 74-82.

Madsen, H.L.; Bertelsen, G. Spices as antioxidants. Trends Food Sci. Technol. 1995, 6, 271–277.

Milner, J.A. Functional foods. In Reducing the Risk of Cancer; Goldberg, I., Ed.; Chapman and Hall: London, UK, 1994; pp. 39–70. Agriculture 2012, 2 238.

Mohammad Mukarram [1,2] *, M. Masroor A. Khan[1], Andleeb Zehra[1], Peter Petrik[3], and Daniel Kurjak[2]; Suffer or Survive: Decoding Salt-Sensitivity of Lemongrass and Its Implication on Essential Oil Productivity, 2022.

Mohammad Mukarram a, *, M. Masroor A. Khan a, Francisco J. Corpas b; Silicon nanoparticles elicit an increase in lemongrass (*Cymbopogon flexuosus* (Steud.) Wats) agronomic parameters with a higher essential oil yield, Journal of Hazardous Materials 412 (2021) 125254.

Negi, P.S. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. Int. J. Food Microbiol. 2012, 156, 7–17.

Negrelle R.R.B.; Gomes EC (2007) *Cymbopogon citratus*(DC.) Stapf: Chemical Composition and Biological Activities. Rev. Bras. Pl. Med., Botucatu, v.9, n.1, p.80-92.

Nooden L.D. and Leopold A.C. (1988) Senescence and aging in plants. Academic Press, San Diego, USA.

Ocak, N.; Erener, G.; Burak, A.F.; Sungu, M.; Altop, A.; Ozmen, A. Performance of broilers fed diets supplemented with dry peppermint (Mentha piperita L.) or thyme (Thymus vulgaris L.) leaves as growth promoter source. Czech J. Anim. Sci. 2008, 53, 169–175.

Perumalla, A.V.S.; Hettiarachchy, N.S. Green tea and grape seed extracts—Potential applications in food safety and quality. Food Res. Int. 2011, 44, 827–839.

Rademacher W. (1991) Inhibitors of gibberellin biosynthesis: applications in agriculture and horticulture. In: Yamana H. ed., Gibberellin symposium, Tokyo, Springer-Verlag, Tokyo, Japan: in press.

Rademacher W., Fritsch H., Graebe I.E., Sauter H. and Jung J. (1987) Tetcyclacis and triazole-type plant growth retardants: their influence on the biosynthesis of gibberellins and other metabolic processes. Pestic. Sci. 21: 241-252.

Rappaport L. (1980) Plant growth hormones: internal control points. Bot. Gaz. 141: 125-130.

Sachs R.M., Lang A., Bretz C.F. and Roach 1. (1960) Shoot histogenesis: subapical meristematic activity in a caulescent plant and the action of gibberellic acid and AMO1618. Amer. J. Bot. 47: 260--266.

Sauerbrey E., Grossmann K. and Jung J. (1988) Ethylene production by sunflower cell suspensions. Effects of plant growth retardants. Plant Physiol. 87: 510--513.

Sherman, P.W.; Flaxman, S.M. Protecting ourselves from food. Am. Sci. 2001, 89, 142–151.

Tiwari, S. Plants: A rich source of herbal medicine. J. Nat. Prod. 2008, 1, 27–35.

Varel, V.H. Livestock manure odor abatement with plant-derived oils and nitrogen conservation with urease inhibitors: A review. J. Anim. Sci. 2002, 80, E1–E7.

Wang S.Y., Sun T., Whitaker B.D. and Faust M. (1988) Effect of paclobutrazol on membrane lipids in apple seedlings. Physiol. Plant. 73: 560--564.

Zeevaart I.A.D., Gage D.A. and Creelman R.A. (1990) Recent studies of the metabolism of abscisic acid. In: Pharis R.P. and Rood S.B. eds., Plant growth substances 1988, pp. 233-240.