EFFECTS OF NITROGEN, PHOSPHORUS AND WATERING REGIMES ON GROWTH, LEAF YIELD AND ESSENTIAL OILS OF SAGE (*Salvia officinalis* L.)

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A THESIS SUBMITTED TO GRADUATE SCHOOL IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN HORTICULTURE

EGERTON UNIVERSITY

SEPTEMBER, 2015

DECLARATION AND RECOMMENDATION

Declaration

This Thesis is my original work and has not been presented for examination in any other university/institution.

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Recommendation

The Thesis has been submitted with our approval as the University supervisors.

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DEDICATION

This work is first dedicated to the Almighty God for his goodness, mercies and favour along this journey. Secondly, I dedicate this work to my late grandmother, Roda Nyosubo Wankyo, my late mother Rebecca Otaigo Marwa and to my beloved father Lameck Rioba Wankyo. I will never forget all the effort you put into my life.

ACKNOWLEDGEMENT

I wish to sincerely thank the Almighty Father for His care, support and grace to have taken me through this long journey. I also thank Egerton University lecturers, technical staff and any other member of staff of the University for their support whenever I called on them. Thank you for your advice and guidance. To my first supervisor, Prof. F.M. Itulya, PhD, you have surely been a strong pillar in my academic endevours since my undergraduate and masters studies, where you were my academic supervisor. You have now successfully taken me through my PhD studies. How much should I thank You? So much. Dr Mwanarusi Saidi, PhD, my second supervisor, deserves great appreciation. You have mentored me from the time I was an undergraduate student and guided me through my academic progression to this level. I wish to deeply appreciate Prof. Hornermeier of Justus Liebig University, Giessen, Germany, for hosting part of this study in his laboratory. To other members of the institute, thank you all for your assistance particularly in language translation. To my Kenyan friend living in Germany, Emilly Koske,I have no words to thank you for your hospitality, and for taking your time to show me around and making my weekends enjoyable. I am indebted to Prof. Matasyo (Egerton University), who facilitated the extraction of essential oils in his laboratory. Many thanks to Prof Nativ Dudai and Prof. Nirit of Newe Yaar research centre in Israel for hosting the essential oil analysis (GC-MS). May God bless you abundantly for the great gesture. My gratitude also goes to the University of Kabianga, German Academic Exchange Programme (DAAD) and National Commission for Science, Technology and Innovation (NACOSTI), I appreciate the financial support which enabled me to complete the enormous research objectives. I must thank the people who did the actual work in the field including assisting in data collection and tending the crop. My sincere gratitude also goes to my colleagues who encouraged me when at times I almost gave up. I also extend my appreciation to my PhD classmates who were sweating it out with me. Thank you for your encouragement and advice.

Last but most important, I wish to thank my husband Dr Babere, my sons Kerata and Joel and my daughter Rebecca for the sacrifices and prayers they have had to offer in this worthy course. Thanks to my parents and my parents in-law and the members of the extended family of both sides, my friends the church leadership and members of my church for the support you gave me.Words will never be enough to thank all of you. May God bless you in abundance.

ABSTRACT

Sage (*Salvia officinalis* L.) belongs to Lamiacea family. It is well known as a common medicinal and aromatic plant widely used in food as well as herbal medicine products. It has wide applications in food flavouring, cosmetics and perfumery by the use of its essential oil. In Kenya, it is increasingly becoming important mostly being grown by export farms. Its leaf productivity is however often limited by nitrogen and phosphorus, which are deficient in many Kenyan soils. The problem is even exacerbated by irregular rainfall in most parts of the country where it is grown, thus necessitating irrigation. The main objective of this study was therefore to determine the effects of nitrogen (N), phosphorus (P) and watering regimes on vegetative and leaf yield and essential oil of sage. The experiment was conducted at the Horticultural Research and Teaching Farm of Egerton University, laid out in a three factor Split Block, arrangement, in a Randomised Complete Block Design (RCBD, with three replications. Treatments consisted of N supplied as urea (46% N) at four rates; 0, 40, 80 and 120kg N/ha while P was supplied as Triple Superphosphate (46% P_2O_5) at four rates; 0, 30, 60 and 90 kg P/ha. Watering regimes included W1= Watering to field capacity once after every week, W2= Watering to field capacity once after every two weeks, and W3= watering to field capacity once after every four weeks. N was assigned to the main plots; watering to the strip plots, and P to the sub-sub plots. The study was conducted in four experiments; experiment 1 (June 2011-October 2011), experiment 2 (October 2011-February 2012) experiment 3 (March 2012-May 2012) and experiment 4 (March 2014-July 2014). Data were collected on plant height, primary and secondary branches/plant, number of internodes/plant, Leaf Area Index (LAI), Specific Leaf Weight (SLW), Leaf Fresh Weight (LFW) and Leaf Dry Weights (LDW), Total Phenolic Compounds (TPC) (Experiment 3 only), essential oil yield (Experiment 3 and 4 only) and essential oil composition (Experiment 3 only). All data were subjected to Analysis of Variance (ANOVA) and where F test was significant; treatment means were separated using the Duncan Multiple Range Test (DMRT) at $P \leq 0.05$. Results indicated that sage responded to N and P application at 80 kg N/ha and 60 kg P/ha.The growth and leaf yield parameters were maximum when these treatments were combined with watering once after every two weeks. Lower and higher N and P application rates as well as too close or far apart watering intervals reduced growth and leaf fresh and dry weights. N, watering and P regimes did not significantly influence the total phenolic compounds. The mean effects of N, P and watering frequency did affect essential oil content of the crop. Furthermore, interactive effects between these variables affected the composition of the oil. Specifically, (i) the percentage of β-Pinene increased with increasing N levels, (ii) β-Pinene decreased with reducing irrigation frequency, (iii) interactive effects of N and P treatments were identified for contents of both α- and β-thujones, and (iv) α-thujone accumulation was also affected by the interaction of watering regime and P application. Camphor was the major ingredient under all treatments and its percentage in the oil was higher than the recommended threshold by ISO standard (ISO, 9909). Based on the results of this study, N and P application at 80 kg N/ha and 60 kg P/ha is sufficient enough to support sage growth and leaf fresh yield, under watering once after two weeks regime whereas production of both α- and β-thujones can be maximized by application of 40 kg N/ha and 60 kg P/ha and watering once a week.There is also need to develop agrotechnical practices aimed at reducing the levels of camphor in sage growing in Kenya to conform to the recommended standards (ISO, 9909). More so, there is need for economic evaluation of these practices before they can be recommended for use in Kenya.

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ANOVA	-	Analysis of Variance
Ca	-	Calcium
CoA	-	Coenzyme A
Cu	-	Copper
DMAPP	-	Dimethylallyl Diphosphate
DMRT	-	Duncan's Multiple Range Test
DNA	-	Deoxyribonucleic Acid
DW	-	Dry Weight
EI	-	Electron Ionization
FDA	-	Food and Drug Administration
GAE	-	Gallic Acid Equivalent
GC-MS	-	Gas Chromatography-Mass Spectrophotometry
GPP	-	Geranyl Diphosphate
GRAS	-	Generally Recognised As Safe
He	-	Helium
IPP	-	Isopentyl Diphosphate
K_2O	-	Potassium Oxide
KARI	-	Kenya Agricultural Research Institute
K	-	Potassium
LAI	-	Leaf Area Index
LA	-	Leaf Area
LDW	-	Leaf Dry Weight
LFW	-	Leaf Fresh Weight
LRI	-	Linear Retention Indices
LSD	-	Least Significant Differences
MDA	-	Malondialdehyde
Mg	-	Magnesium
Mn	-	Manganese
MS	-	Mass Spectrophotometer

N	-	Nitrogen
P_2O_5	-	Phosphorous Pentoxide
PEP	-	Phosphoenol Pyruvate
Phe	-	Phenylalanine
Pi	-	Inorganic phosphate
PLP	-	Pyridoxal-5-Phosphate
P	-	Phosphorus
PPi	-	Inorganic pyrophosphate
RT	-	Retension Time
RWC	-	Relative Water Content
SLW	-	Specific Leaf Weight
SWC	-	Soil Water Capcity
TPC	-	Total Phenolic Acid
Trp	-	Tryptophane
Try	-	Tyrosine
TSP	-	Triple Super Phosphate
USA	-	United States of America
UV	-	Ultra Violet
W	-	Watering
Zn	-	Zinc

CHAPTER ONE

INTRODUCTION

Sage (*Salvia officinalis* L.) (Fig. 1) is an evergreen woody stemmed shrub with strong spicy aroma and slightly bitter and astringent taste. It belongs to the family Lamiaceae and is one of the three genera commonly referred to as sage. Lamiaceae is one of the large plant families used as a framework to evaluate the occurrence of some typical secondary metabolites (Wink, 2003).

The typical secondary metabolites of Lamiaceae include various terpenoids and phenolic compounds (Hegnauer, 1989). Lamiaceae is subdivided into two major groupings: the Lamioideae and Nepetoideae (Bremer et al., 1998), with *S. officinalis* belonging to the latter group. In addition to essential oil, members of Nepetoideae produce special "tannin", mainly represented by the phenolic compound 'rosmarinic acid'.

Fig. 1. Sage (*S. officinalis* L.) branch. In Lima, C.F.M. (PhD Thesis, Universidade do Minho Escola de Ciencias, 2006).

Sage centre of origin and diversity is able in that it is believed to be Central and Western Asia (Kintzios, 2000), but has also indicated to have originated from Southern e (Tutin et al., 1972). Hedge (1992) reported there were three main speciation centres: tal Mediterranean, South- West Asia, and Africa and America.

Sage produces long, angular and erect stems ng heights between 50 to 100 cm, depending ecies and environmental conditions. A number anches (usually 3 to 5) are produced from buds of the main stem (Karamanos, 1995), branching being more intensive after cutting. s are opposite, simple, ovate and petiolate. The inflorescence is a terminal verticillaster consisting of 4 to 10 violet, blue, lilac or pale-blue flowers. All above ground parts are covered by glandular hairs which impart a silver colour to mature plants.

The species *(S. officinalis* L.) also known as Dalmatian sage is the most representative within the genus and has been the object of numerous studies, especially regarding its richness in volatile constituents (Putievski et al., 1986; Dudai et al., 1999; Putievski et al., 1986; Perry et al., 1999). It is an aromatic plant used in the pharmaceutical and food industries for its richness in essential oils (Rau et al., 2006) and flavonoids and phenolic diterpenes which have been shown to have high antioxidant capacity (Frutos and Hernandez-Herrera, 2005). Several other studies have also shown sage to be one of the sources of some potent antioxidants (Cuvelier et al., 1996; Grzegorczyk et al., 2007; Santos-Gomes et al., 2002).

Sage has long been used in folk medicines for the treatment of all kinds of ailments, although to most people, it is known as a culinary herb. The antioxidant properties were found to be related to the presence of rosmarinic acid and carnosic acid (Chang et al., 1977; Cuvelier et al., 1996). More studies have revealed the presence of a large number of diterpenoids (Gonzalez et al., 1992; Tang and Elsenbrand, 1992) and phenolic acids (Tanaka et al., 1998) including a number of novel caffeic acid metabolites, such as, sagerinic acid (Lu and Foo, 1999) and sagecoumarin (Lu et al., 1999), but comparably few flavonoids and phenolic glycosides (Wang et al., 1998; 1999).

Sage is a popular kitchen herb. It has been used in a variety of food preparations since ancient times. It is now extensively cultivated all over the world mainly to obtain dried leaves to be used as raw material in medicine, perfumery and food industry (Bruneton, 1999; Santos-Gomes et al., 2002). It is an aromatic herb and thus was previously considered mainly for its essential oil content (Heath, 1978; Perry et al., 1999; Perry et al., 1996; Santos-Gomes and Fernandes- Ferreire, 2001; Tucker and Maciarello, 1990). The essential oil and flavourants of sage are used as basic material for various foods, cosmetic and pharmaceutical preparations (Heath, 1978; Tucker and Maciarello, 1990). It is reported to have a wide range of biological activities, such as anti-bacterial, fungistatic, virustatic, astringent, eupeptic and anti-hydrotic effects (Farag et al., 1986). The Food and Drug Administration (FDA) includes sage on its list of substances “Generally Recognised As Safe (GRAS)” for use as spices and other natural seasonings and flavourings, as well as on the list of GRAS essential oils, oleoresins (solvent-free), and natural extractives (including distillates).

Sage owes its versatile application to complex composition of essential oils mainly consisting of thujone, cineol, camphor, borneol and pinene (Longaray et al., 2007; Langer et al.,

1996). Apart from these, it also contains triterpenes, flavonoids, carnosol and organic acids, vitamins B_1 and C, Propelargonidin (PP) and carotene (Radulescu et al., 2004; Lu and Foo, 2002). The essential oils are applied in the treatment of a wide range of diseases of the nervous system, heart and blood circulation, respiratory, digestive, metabolic and endrocrine system (Radulescu et al., 2004). The essential oils have been shown to play an important role in preventing diseases of the 21st century like arteriosclerosis, diabetes, cataract, Parkinson's disease and Alzheimer's Disease (Akhondzadeh et al., 2003).

Extracts from common sage protect cells from DNA damage, stimulate its repair and also have an inhibiting effect on development of cancer cells (Ramos et al., 2010; El-Hadri et al., 2010). Additionally, sage is considered a phytoestrogen (Balacs, 1993). Investigations have shown that the herb has strong effects in cases of oligomenorrhea and amenorrhea (Salbei, 2007). As such, it can be used to control hormone imbalances as well as premenstrual tension, irregular or heavy menses, pelvic congestion, fibroids, endometriosis and cysts, and should never be used in pregnancy (Yardley, 2004). Sage has also been used in the food industry due to the presence of phenolic compounds which have antioxidant activity (Heim et al., 2002). The quality of antioxidant activity of sage is highly correlated with phenolic compounds (Thorsen and Hildebrandt, 2003).

All species of the genus *Salvia* can grow from sea level up to altitudes of 1500m. Consequently, their favourable habitats are found in most subdivisions of the Mediterranean climate (Papadakis, 1975), whereas considerable frost damage occurs in more acute climatic conditions (Rey, 1991). Both herbage and oil yields are reduced in cold and shady environments (Bernath et al., 1991) which induce a reduction in plant size and the density of peltate hairs (Li et al., 1996). In general, essential oil concentration tends to be higher in warmer and drier regions (Kargiolaki et al., 1994). Oil chemical composition has also been found to depend on environmental conditions (Bernath et al., 1991). Apart from extremely coarse or fine-textured soils, sage species can be grown over a wide range of soil types, especially in the medium-textured, well drained calcareous soils with a pH around 6.5.

S. officinalis can be propagated sexually and asexually. When seeds are used, they are drilled either directly in the field or hand sown in the nurseries. In the former case, seed rate varies between 3 to 5 kg/ha (Scroumbis, 1988). Basic fertilizers are applied before planting while surface application is done during growth. The recommended rates for the basic application

range from 40 to 100 kg N, 30 to 80 kg P_2O_5, and 30 to 100 kg K_2O per hectare. Harvesting is achieved through cutting the whole plant at a height of 10 to 15 cm from ground surface, using mowers or other motorized cutters just before flowering. Leaves are the main yield components (more than 50 % in total DW) followed by stems 34 % and flowers 14 % (Karamanos, 1995). Essential oil yields also vary between 110 and 200 litres/ha. The essential oil yields are highest in the leaves as compared to roots and stems. Increased leafiness brings about higher oil yields (Karamanos, 1995).

Creating an optimal system for growing volatile oil crops, such as sage, requires assessment of the crop responses to variation in environmental and crop management factors. Due to the pronounced xerophytic characteristics of the commercially cultivated sage, it has been previously assumed that sage would produce high quality oils only under stressful conditions (high temperature, drought and low fertility) (Hay and Waterman, 1993). However, according to Hay and Waterman (1993), yield improvement can be achieved by adjusting elements of the growing systems, such as, fertilizer application and water supply.

1.1 Statement of the Problem

Sage is an aromatic perennial herb that is considered economically important. It is cultivated as a culinary herb and for medicinal purposes. Sage is a 'newly' introduced crop in Kenya mainly being grown by export farmers who have reserved their agronomic package. Kenyan small scale farmers who could greatly benefit from production of sage for health, nutrition and income generation are not able to take up the opportunity of growing sage because of lack of information regarding the agronomic practices occassioned by limited research on sage especially so under Kenyan conditions. Moreover, documented fertilizer application rates for sage are given as a wide range. The problem is made worse by the fact that N and P are considered to be limiting factors for crop production in Kenya, because most of the soils are deficient in these elements. Due to climate change, there is unreliable rainfall necessitating irrigation during certain periods of crop production cycle. There is scanty information on irrigation water management for optimum sage productivity under Kenyan conditions. Being a xerophyte, over irrigation may alter the potential leaf and essential oil yield as well as essential oil composition.

1.2 Research Justification

Sage is one of the important aromatic crops with great potential in Kenya. In addition to its essential oil yield production, it is a good source of natural phenolic antioxidants. Being a 'newly' introduced crop in Kenya, it is important to develop the appropriate production technology for different environments. A better understanding of the effects of nitrogen and phosphorus rates on sage growth and performance under local conditions will help in designing N and P management strategies that will optimize N and P utilization, leading to increased sage yield and essential oil content. Determining the effects of watering regime will be beneficial in establishing the watering levels that will optimize on leaf yields and essential oil content. This will help growers to avoid under or over-watering, and hence, save on production cost and improve on returns. Results of this study will also bridge the knowledge gap that exists on sage culture under local conditions.

1.3 Objectives of the Study

1.3.1 General Objective

The general objective of this research was to contribute towards improved herbage yield and essential oil content of sage through establishment of optimal levels of N, P fertilization and watering regimes for the crop.

1.3.2 Specific Objectives

The specific objectives were to determine:

i. The effects of N, P and watering regimes on sage plant growth and leaf yield.
ii. The effects of N, P and watering regimes on sage essential oil yield and composition.
iii. If interaction exists between N, P and watering regimes on growth, leaf yield and essential oil yield and composition of sage.

1.4 Research Hypotheses

The following hypotheses were tested:

i. Increasing N, Pand watering levels increase sage plant growth and leaf yield.
ii. Increasing N, Pand watering levelsincrease sage essential oil yield and composition.
iii. Interaction effects of N, Pand watering levels influence growth, leaf yield and essential oil yield and composition of sage.

CHAPTER TWO

2.0 LITERATURE REVIEW

Lamiaceae is a family of flowering plants that includes 250 to 258 genera and approximately 6,000 to 6,970 species across the world (Mabberley, 1997). The family has a cosmopolitan distribution and contains many plant species with culinary and medicinal purposes. Examples of the plants with culinary and medicinal use include basil, mint, rosemary, sage, savory/satureja, marjoram, oregano, thyme, lavender (*Lavendula angustifolia* L.), and perilla [*Perilla frutescens* L. (Britt.)] (Naghibi et al., 2005). Plants in the family have been used since ancient times as folk remedies for various health problems such as common cold, throat infections, acaricidal, psoriasis, seborrheic eczema, hemorrhage, menstrual disorders, miscarriage, ulcer, spasm and stomach problems (Takayama et al., 1989; Loizzo et al., 2010; Ribeiro et al., 2010). Their constituents, particularly diterpenoids and triterpenoids, have been found to have antiseptic, antibacterial, anti-inflammatory, cytotoxic, cardio-active and other properties (Ulubelen, 2003). Antioxidant and radical scavenging activities are the main properties of these compounds making them contribute to preventing cardiovascular or inflammatory diseases and cancer, which are caused among others by harmful effects of free radicals.

Typical secondary metabolites of the Lamiaceae include various terpenoids, especially monoterpenes, sesquiterpenes, as well as various phenolic acids (Wink, 2003). The secondary metabolites are generally concentrated in one particular region of a plant such as the leaves, roots, bark, fruits or glandular hairs. In cases where they occur in various organs of the same plant, they frequently have different chemical profiles (Araujo et al., 2003).

2.1 Plant Secondary Metabolites

Plants synthesize a vast range (more than 100,000 different substances) of organic compounds that are traditionally classified as primary and secondary metabolites (Raven et al., 2006). Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration and growth and development. These include phytosterols, chlorophyll, acyl lipids, nucleotides, carbohydrates, amino acids and organic acids. The other categories of phytochemicals, many of which accumulate surprisingly in high concentrations in some species are referred to as secondary metabolites. They are low molecular mass compounds (Wink, 2003; Hadacek, 2002). They are structurally diverse and many are distributed among a

very limited number of species within the plant kingdom and so can be diagnostic in chemotaxonomic studies.

The secondary metabolites are non essential for the basic (primary) metabolic processes of the plant that result in growth and development (Taiz and Zeiger, 1991). They are present in very small quantities with no specific functions in plants in contrast to primary metabolites (Pichersky and Gang, 2002). However, it has been indicated that some secondary molecules may function as co-substrates or coenzymes serving the needs of primary pathways (Hadacek, 2002). Hartmann (1996) gave a distinction between the two. He indicated that primary metabolism was universal, uniform, conservative and indispensable, while secondary metabolism was singular, diverse, adaptive and dispensable for growth and development, but indispensable for survival of the plant.

2.1.2 Role of Secondary Metabolites in Plants

Secondary metabolites are indispensable for plant survival, because they have been shown to have several roles other than being end products of metabolism or metabolic wastes (Taiz and Zeiger, 1991; Bennett and Wallsgrove, 1994; Wink, 2003), detoxification products, results of shunt and over-flow metabolism, degradation products or cell storage compounds (Hadacek, 2002). Extensive investigations have revealed that they have complex biological activities and functions that render them essential for plant fitness and survival and reproduction (Bennett and Wallsgrove, 1994 and Pichersky and Gershenzon, 2002).

Secondary metabolites play a role in the adaptation of plants to their environment by interacting with the ecosystem (Bourgaud et al., 2001). Being sessile in nature and cannot fully rely on their immune system against pathogens and pests, they produce the defence chemicals to inhibit their natural enemies as explained by Wink (2003). Natural products/secondary metabolites have also been attributed to antibiotic, antifungal, antiviral and insecticidal activities that protect plants against pathogens and insects due to the presence of terpene, phenolic or alkaloidal secondary molecules. In addition, many secondary compounds (alkaloids) described as poisonous, serve as a defence against various herbivores (Bennett and Wallsgrove, 1994).

On the other hand, plants need animals and insects for pollination or seed dispersal. In some cases, secondary metabolites like monoterpenes, anthocyanins, carotenoids, phenolics, tannins and saponins function as attractants to these organisms (Wink, 2003; Bennett and Wallsgrove, 1994). In several instances, attractant and defensive activities are exhibited by the

same molecule as in the case of anthocyanins and terpenes (Hadacek, 2002; Wink, 2003). Furthermore, the phenomenon of allelopathy is due to the presence of specific secondary molecules; alkaloids, cyanogenic glycosides, glucosinolates, terpenes and tannins among others (Wink, 2003) that are released in the surrounding environment in order to inhibit germination and or growth of neighbouring plants (Bourgaud, 2001).

There are also a number of secondary metabolites that carry out other physiological functions like N transport and storage in a toxic form (alkaloids) or UV-absorption (flavonoids) that protect plants from UV- radiation hazards (Taiz and Zeiger, 1991;Wink, 2003 and Bourgaud, 2001). Wink (2003), Bourgaud (2001), and Gershenzon (1985) suggested that specific secondary metabolites can function to reduce transpiration under hot environments by inducing stomatal closure (abscisic acid) or by providing a vapour shield on the leaf surface (volatile terpenes).

Scientific knowledge on phytochemicals has grown over the years. There has been knowledge on their functions and potential with regard to the reducing capacity of cancer or coronary heart diseases (Knekt et al., 2002; Sesso et al., 2003).

2.1.3 Classification of Secondary Metabolites

Plant secondary metabolites are present in plants in highly structural diversity and intraspecific variability (Hadacek, 2002 and Wink, 2003).Wink (2003) reported that a single secondary metabolite group dominates within a certain taxon. Plant secondary metabolites are usually classified according to their biosynthetic pathways (Bourgaud et al., 2001). In general, four major groups of secondary metabolites have been identified; terpenoids, phenolics, nitrogen/sulphur- containing compounds and fatty acid/ polyketide derivatives (Dixon, 2001). Figure 2 below presents a general overview of the different secondary metabolites produced by plants with particular emphasis on the five types of phenolic compounds that are of interest in this study.

2.1.4 Chemical Constituents of Sage

Sage has a range of chemical constituents which are categorized into volatile compounds or essential oils or terpenes and non volatile compounds (phenolic compounds and nitrogen-containing compounds).

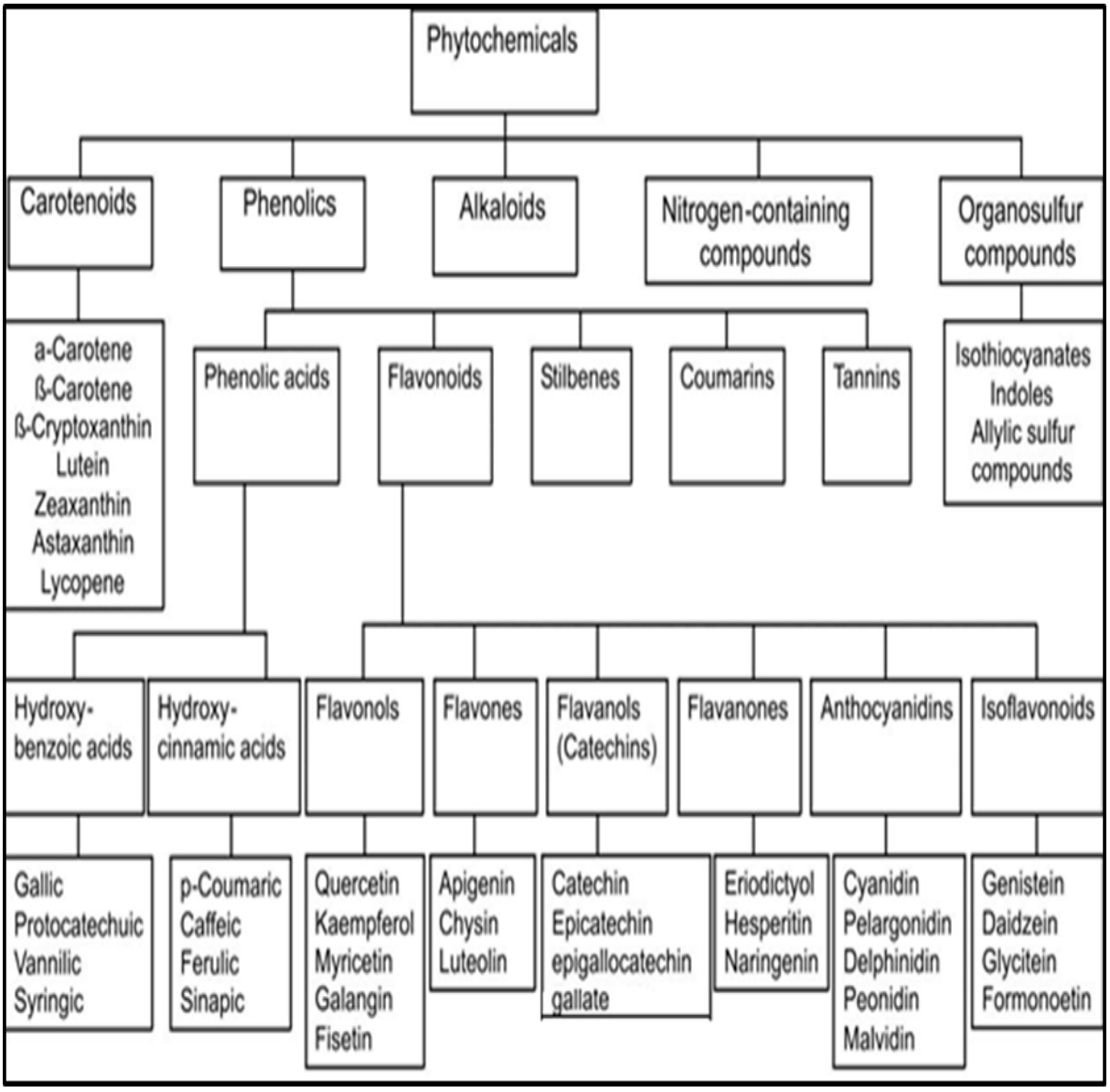


Fig. 2. Classification of dietary phytochemicals (modified by Liu, 2004) in Engert N. (Ph.D.Thesis, Justus Liebig University, Giessen, 2011).

2.1.4.1 Volatile Compounds/Essential Oils/Terpenes of Sage

The essential oils also known as ethereal oils, are defined as, the oils obtained by the steam distillation of plants. From the view point of practical applications, these materials may be defined as odiferous bodies of an oily nature, obtained almost exclusively from vegetable organs, including, flowers, leaves, barks, woods, roots, rhizomes, fruits, and seeds (Celiktas et al., 2006; Skocibusic et al., 2006). An essential oil is generally identified with the name of the source plant. Essential oils are mostly liquid, aromatic and possess pleasant odour and essence. The term "essential oil" is often used in cosmetics and perfume industries as synonymous with perfume oil, base or, "compound".

Chemically, the essential oils are a complex and highly variable mixture of constituents that belong to two groups: terpenoids and aromatic compounds. The name terpene is derived from the English word "Turpentine" (Guenther, 1952; 1985). The terpenes are the unsaturated hydrocarbons which have a distinct architectural and chemical relation to the simple isoprene molecule (CH_2=C (CH_3)-CH=CH_2). Those having molecular formula $C_{10}H_{16}$, are thus constituted by two isoprene units combining by head to tail union (Guenther, 1960; Pinder, 1960). In addition to the terpenes $C_{10}H_{16}$ the essential oils often contain more completed hydrocarbons of the same composition, but of higher molecular weight. Their composition can be expressed by the general formula $(C_5H_8)n$. For monoterpene n=2; for diterpene ($C_{20}H_{32}$) and sesquiterpenes $(C_{15}H_{24})n$ is greater than 2.

Although essential oils are comprised of many types of compounds, the major ones are monoterpenes (Seigler, 1998). The volatile oils of sage are chemically complex mixtures, often containing in excess of 100 individual components (Waterman, 1993 and Hay and Waterman, 1993), although terpenoid molecules predominate. They have low boiling points and can be recovered from the plant tissues by steam distillation.

Commercial sage may be substituted with *S. fruticosa (S. triloba)* whose principal essential oil component of which is 1, 8-cineole, with *a*-thujone only accounting for 1- 5%. Langer et al. (1996) analysed by gas liquid chromatography the essential oils of commercially available samples of leaves of *S. officinalis* and *S. fruticosa* (used as medicinal and culinary herbs) obtained by steam distillation and dichloromethane extraction. Although standardized conditions of sample preparation were employed, differences in the composition of the oils were found: steam distillation yielded a reduced amount of the less volatile compounds, and the

accuracy of determination was significantly lower than in the case of dichloromethane extraction. The commercial samples, which differed considerably in the composition of their essential oils, were of different ages of the leaves. Extraction of individual leaves of *S. officinalis* showed a decrease in the *a*-thujone content, with a corresponding increase in the relative amount of camphor, related to leaf age. At least two chemotypes of *S. officinalis* exist, one with a low α-thujone content (4-8%) and another with a relatively high content (16-32%) (Boelens and Boelens, 1997). Owing to the observed variability of the essential oil composition of *S. officinalis*, the relative contents of *a*-thujone, *ß*-thujone and camphor have to be totalled in order to form a significant parameter for the characterization of *Salvia* species. This parameter varied between 45 and 68% in *S. officinalis* and between 4.8 and 15.9% in *S. fruticosa* with a small standard deviation. Consideration of this parameter, together with the amount of 1, 8-cineole (eucalyptol) *S. officinalis (*2.8-23%), *S. fruticosa (*55-75%), permits the differentiation between these species and respective mixtures.

The terpene alcohols thujol, menthol and thymol were found in *ß*-glucosides in the leaves of Dalmatian sage (*S. officinalis*) (Boelens and Boelens, 1997). Kustrak (1988) identified the following constituents in the essential oil (1.55% yield) of *S. officinalis* ssp. *Minor f. auriculata:* α-thujonene (35.3%), camphor (18.1%), 1,8-cineole (7.3%), camphene (6.4%), α-terpineol (5.9%), *ß*-thujonene (5.6%), α-pinene (5.5%), limonene (2.4%), linalyl acetate (1.7%) and borneol (1.7%). In another subspecies, ssp. *angustifolia,* Pace and Piccaglia (1995) identified 34 components, with the most abundant being α-thujone (39%), *a*-humulene (12.5%), 1, 8-cineole (8%), *ß*-pinene (7%), *ß*-thujone (3%), camphor (2%), and globulol (2%).

2.1.4.2 Physiological Process Underlying Essential Oil Production in Sage

Essential oil production does not depend only on plant genetics or developmental stage. The environment and its changes can influence in a significant way biochemical pathways and physiological processes that alter plant metabolism and therefore, the essential oil biosynthesis (Sangwan et al., 2001). Among the diversity of secondary metabolite classes, the isoprenoids (known as terpenes or terpenoids), whose name is related to its five-carbon structure: isopentenyl disphosphate (IPP) has been shown to be important due to their ecological functions like attraction of pollinators, seed dispersion, protection against herbivores and allelophathy (Pare and Tumlinson, 1999; Wink, 2003). Isoprenoids occur in plants as primary metabolites

(Ubiquinone, plastoquinones, gibberellins, brassinosteroids, carotenoids and others) (Rodriguez-Concepcion and Boronato, 2002).

Culinary sage produces a number of monoterpenes, including (+)- and (-)-α-pinene, (+)- and (-)-β-pinene, (+)- and (-)-camphene, (+)-sabinene, (+)- and (-)-limonene, myrcene, 1,8-cineole, and (+)-bornyl diphosphate (Croteau, 1987). Because sage produces this broad range of acyclic, monocyclic, and bicyclic monoterpenes, including several olefin isomers, a cyclic ether and a diphosphate ester, this plant has provided an ideal system for the study of a variety of synthases, all of which utilize the same substrate but produce different products by variations on a single reaction mechanism (Croteau, 1987; Wise and Croteau, 1998). These include (+)-bornyl diphosphate synthase (the enzyme producing the precursor of (+)-camphor) (Croteau and Karp, 1979a and Croteau and Karp, 1979b), 1,8-cineole synthase (Croteau et al., 1994), (+)-sabinene synthase (the enzyme producing the precursor of (−)-3-isothujone) (Croteau, 1992a and Croteau, 1992b), and several pinene synthases (Gambriel and Croteau,1984; Wagschal et al., 1994 and Pyun et al., 1994). As is typical of monoterpene cyclases (Wise and Croteau, 1998 and Wagschal et al., 1991), many of these enzymes from sage appear to generate multiple products from geranyl diphosphate. Investigations with the partially purified native enzymes have suggested that a single enzyme, termed (+)-pinene synthase (cyclase I), is responsible for the synthesis of both (+)-α-pinene and (+)-camphene, with lesser amounts of (+)-limonene and myrcene, whereas a second enzyme, (-)-pinene synthase (cyclase II), has been shown to produce (-)-α-pinene, (-)-β-pinene, and (-)-camphene, with minor amounts of (-)-limonene, terpinolene, and myrcene (Gambriel and Croteau, 1982 and Croteau, 1984). More recently, a third synthase from sage, termed cyclase III, has been described which produces a mixture of (+)-α-pinene and (+)-β-pinene, along with minor amounts of myrcene (Wagschal et al., 1994 and Pyun et al., 1994).

Terpenes are biosynthesised through two pathways: mevalonate and methylerythritol phosphate. The first is located in the cytosol and endoplasmatic reticulum (Hadacek, 2001), which has acetyl-CoA as its precursor, while the second occurs in the plastids from glyceraldehydes-3-phosphate and pyruvate (Rodriguez-Concepcion and Boronato, 2002). Both generate isopentenyl diphosphate (IPP), which is isomerised (isopentenyl diphosphate isomerise) forming dimethylallyl diphosphate (DMAPP), the isoprene synthase substrate, an enzyme that is present in the chloroplasts responsible for the diphosphate break and isoprene (a five-carbon compound) formation. Adding an IPP molecule to DMAPP through prenyltransferases will generate geranyl

diphosphate (GPP), a monoterpene (C_{10}) precursor. Consecutive condensation of IPP (by special prenyltransferases) produces farnesyl diphosphate (FPP) and geranyl diphosphate (GGDP), which are precursors of sesquiterpenes (C_{15}) and diterpenes (C_{20}), respectively. These terpene groups are converted by terpene synthases giving rise to other compounds. There are also triterpenes, tetraterpenes and polyterpenes, with 30, 40 and more than 45 carbons, respectively, (Bohlmann and Keeling, 2008). Figure 3 gives a scheme through which conversion of geranyl diphosphate to the monoterpenes of sage occurs as illustrated by Wise et al. (1998).

The main essential oil of sage contains camphor as a major component (i.e upto 20% of the oil) (Croteau and Karp, 1976) and the biosynthesis of this monoterpene ketone has been shown to involve, as a first co mmitted step, the cyclization of geranyl diphosphate to bornyl diphosphate (Croteau and Karp, 1979) and borneol dehydrogenase (Croteau et al., 1978), which have been partially purified and characterised as having two distinct types of phosphohydrolases capable of cleaving bornyl diphosphate to alcohol (Croteau and Karp, 1979). Terpene synthases products may suffer many reactions (oxidation, reduction, isomerisation, conjugation, etc) giving rise to stereochemicals and metabolic variants (Kesselmeier and Staudt, 1999; Sangwan et al., 2001), which deliver range of chemical diversity found in this secondary metabolite class.

The production and accumulation of essential oils is associated with specialized structures because being very toxic to cells, they must be contained in such structures. There are numerous sorts of specialized secretory structures like, for instance, glandular trichomes, secretory cavities, idioblasts and others (Gershenzon, 1994). According to Gottieb and Salatino (1987), the essential oil production and the secretory structure formation are closely connected.

Sage is considered to have the highest essential oil yield among *Salvia* species, along with a higher total ketone content and lower total alcohol content (Ivanic and Savin, 1976; Newall et al.,1996). The major components of the essential oil of sage are the terpenes *a*- and *ß*-thujones (35-50%, mainly). Others include 1, 8 cineole, borneol, camphor, caryophyllene and linalyl acetate (Newall et al.,1996).

2.1.4.3 Non-Volatile Compounds of Sage

2.1.4.3.1 Phenolic Compounds

Phenolic molecules characterised by the presence of the phenol group (a hydroxyl function on a benzene ring) in their structure, constitute another major class of secondary metabolites. All phenolic compounds i. e. the phenylpropanoids formed by a 3-carbon side chain attached to an a

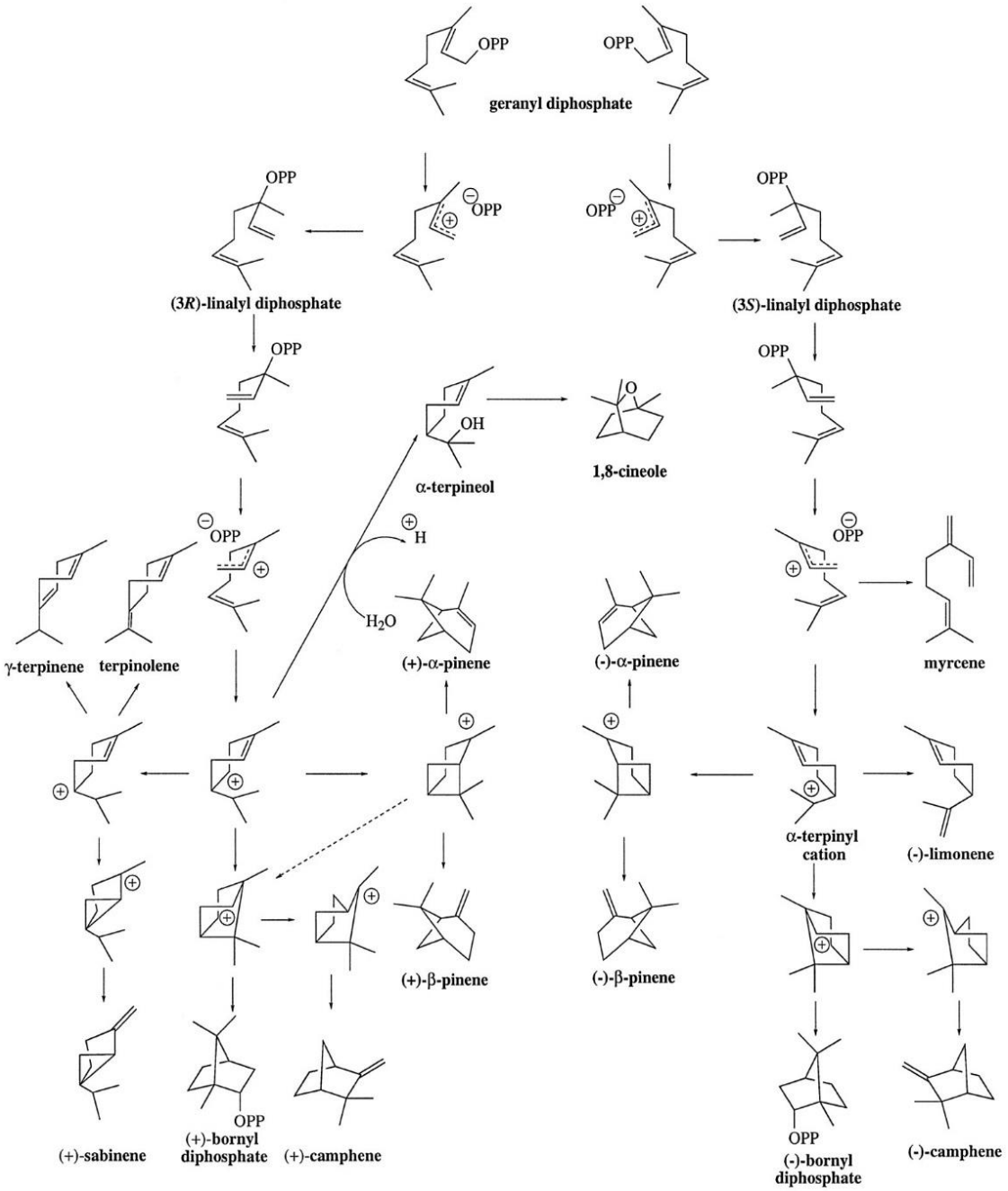


Fig. 3. Schematic representation of conversion of geranyl diphosphate to the monoterpenes of sage (Wise et al., 1998).

aromatic ring (Hanson, 2003) and their polymers lignins; the flavonoids made up of two aromatic rings connected by a 3-carbon bridge, for example, anthocyanins, flavones, flavonols, and soflavonoids and their polymers tannins (Taiz and Zeiger, 1991) are biochemically synthesised via the shikimate pathway. The enzyme-catalysed condensation of a phosphoenol pyruvate (PEP) derivative with the aldehyde of erythrose 4-phosphate yields shikimic acid, a precursor in the biosynthetic routes leading to formation of the aromatic amino acids phenylalanine (phe), tyrosine (tyr) and tryptophane (trp) (Hanson, 2003; Sangwan et al., 2001).

2.1.4.3.2 Nitrogen Containing Compounds

A large variety of plant secondary products also entail N in their structures in contrast to the fore-going molecules. This group of secondary metabolites consists of alkaloids, non-protein amino acids, amines, cyanogenic glycocides, glucosinolates and alkamides (Wink, 2003). Most nitrogenous secondary products are biosynthesized from common amino acids.

Alkaloids which are present in approximately 20% of all plant species are derived through decarboxylation of amino acid precursors i. e. ornithine and lysine, phenylalanine and tyrosine, tryptophan or from nicotinic or anthranilic acid (Hanson, 2003). The aromatic amino acids (phenylalanine, tyrosine and tryptophan) are derived from the shikimate pathway, whereas lysine and ornithine biosynthesis is via transamination of oxaloacetate (a ketoacid produced by Krebs cycle) mediated by the enzyme pyridoxal-5-phosphate (PLP) (Diamandithis, 1994). In alkaloids, the N atom is usually part of a heterocyclic ring on which classification is based. Wink (2003), reported that more than 12,000 alkaloid structures have been elucidated and examples of well-known important alkaloids are nicotine, morphine, caffeine and cocaine (Taiz and Zeiger, 1991).

2.2 Effects of Nitrogen Fertilizer on Plant Growth and Yield

Nitrogen is the most needed essential element for plant growth and development constituting 1-5% of plant dry matter (Marschner, 1995; Souri et al., 2009). This element has important role in structural macromolecules. The response of plants to N (amount and time of application) generally differs depending on species, tissue and physiological age of the plant. Vegetative phase of plants has been shown to respond better than reproductive growth to N levels (Souri et al., 2009).

Nitrogen is an indispensable element for plant growth and development. Its importance for plant function is demonstrated in the vast number of metabolic molecules i.e. amines, amides,

amino acids, peptides, proteins, coenzymes, nucleic acids, chlorophyll pigments and secondary compounds like alkaloids, which entail N in their structure (Marchner, 1995). Up to 75% of leaf organic N is located in the chloroplasts, primarily as enzyme protein or incorporated in chlorophyll molecule, suggesting a positive relationship between assimilated N and photosynthesis and consequently, plants growth (Andrews et al., 1999; Meziane and Shipley, 2001). Chlorophyll content in the leaf has been associated with plant yield (Richardson et al., 2002) and is closely linked to inorganic N supply from the roots. The amount of chlorophyll and pigment content in general (chlorophyll a and b and carotenoids) is altered under adverse environmental factors, including nutrient deficiency (Richardson et al., 2002; Shevchenko et al., 2004).

Numerous works examining the effects of N on growth and yield of medicinal and aromatic plants including plants of the Lamiaceae family have been carried out. Some of these researches include: *S. officinalis* (Bezzi et al., 1992; Geneva et al., 2010), *S.fruticosa* L. (Karioti et al., 2003) and *S. sclarea* Linn. (Sharma and Kumar, 2012), *Mentha* species (Clark and Menary, 1980b and Piccaglia et al., 1993), basil (Sifola and Barbieri, 2006 and Nguyen and Niemeyer, 2008), thyme (Baranauskien et al., 2003; Shams et al., 2013), oregano (Said-Ahl et al., 2009 and Kareem et al., 2005); marjoram (Trivino and Johnson, 2000); anise hyssop (*Agastache foeniculum* Kuntze.) (Kabaudani and Omidbaigi, 2008), satureja (Babalar et al., 2010) and White Horehound (*Marrubium vulgare* L.) (AL-Lyeithy et al., 2013)
in the family Lamiaceae; java citronella (*Cymbopogon winterianus* Jowitt) (Prakasa Rao et al., 1983) and Palmarosa (*Cymbopogon martini* Roxb.) (Rajeswara Rao, 2001) of the Poaceae family; geranium (*Pelargonium graveolens* L. Her) (Araya et al., 2006 and Singh, 1999) in the Geranaceae family and St. John's Wort (*Hypericum perforatum* L.) (Berti et al., 1999) of the Clustaceae family. This is an indication of numerous attempts to improve the quality and content of essential oils through the application of various nutrients including N (Gershenzon, 1983; Hornok, 1986; Economakis et al., 1999).

The supply of inorganic nutrients is known to affect plant growth and development. Experiments with sage plants (*S. fruticosa*) grown in solution culture (Economakis, 1993) have shown that N concentrations of 100 to 150 ppm are high enough to promote vegetative growth at satisfactory levels. On the other hand, higher concentrations (200 ppm) reduced stem, leaf and root fresh and dry weights. In the same experiments (in solution culture) with four levels of N-

fertilization (0, 80,160 and 240 kg N/ha) on Greek or Mediterranean wild sage *(S. fruticosa* L.*)* crop (Karamanos, 1995), it was found that N application increased plant height, branching and herbage yields in comparison with the unfertilised plots. Similarly, essential oil yields increased significantly with N-application mainly because N promoted herbage yield (Karamanos, 1995). Rohrich et al. (1996) emphasised that in sage plant, essential oil yield and top branches yield were augmented by increasing N upto 100 or 150 kg N/ha.

In a more recent study, Sharma and Kumar (2012) reported that N fertilizer encouraged growth parameters of Clary sage (*Salvia sclarea* Linn.) as compared with the control. Application of 1.5 g N/plant recorded significantly higher plant canopy spread (East-West) and number of flowering stalks/plant than the control, but remained at par with other treatments. However, in the North-South orientation, they observed significantly higher plant spread and number of spikelets/plant when the plants were supplied with 3.0 g N/plant than the plants that received no fertilizer (control) but remained at par with the other treatments. All the N treatments were higher than the control. Similarly, Ezz El-Din et al. (2010) reported enhanced growth in caraway as a result of N-fertilization which they attributed to the positive effects of N on activation of photosynthesis and metabolic processes of organic compounds in plants, which in turn, encourage plant vegetative growth. Sharma and Kumar (2012) also noted that different levels of N fertilization significantly influenced the biomass accumulation of plants. Their results were in agreement with those reported by Agamy (2004) for Fennel and Said-Al Ahl (2005) for Dill.

Geneva et al., (2010) studied the effects of foliar fertilization and arbuscular mycorrhizal colonization on *S. officinalis* growth, antioxidant activity and essential oil composition. They used foliar feed (Agroleaf®) which had N: P: K (20:20:20) plus microelements and *Glomus intraradices* (the mycorrhizal fungi). They observed that the application of foliar fertilization and/or mycorrhizal colonization improved dry biomass accumulation and increased the content of antioxidant metabolites (ascorbate peroxidise and superoxide dismutase) while guaiacol peroxidise increased. Combined application of foliar feed and *Glomus intraradices* significantly promoted 1,8-cineole and α- thujone; mycorrhizal colonization enhanced bornyl acetate, 1,8-cineole, α- thujone and β-thujones while foliar fertilization increased bornyl acetate and camphor.

Singh et al. (2011) conducted an experiment on the effect of different levels of nitrogen and phosphorus on the growth and flowering of Scarlet sage (*Salvia splendens* L.). They reported that the interaction of N X P levels significantly affected all the growth and flowering parameters. The best interaction level was 120 kg N/ha and 80 kg P/ha which was found to be superior over all the other treatment combinations. Abbes (1998) studied population, clonal and nitrogen effect on growth of *Salvia fruticosa* Mill. He found that nitrogen supply affected nitrate reductase activity (measured in situ) and growth rates.

He et al. (2013) worked on the effects of different N levels and ratios of ammonium and nitrate on root development and contents of bioactive compounds in red/Chinese sage (*Salvia miltiorrhiza* Bunge.). They observed that the longest root length, the largest root diameter, the number of roots and the amount of dry matter of each plant decreased significantly with increasing N application. The shoot/root ratio tended to increase with increasing N application. They also reported that the contents of all three lipophilic components significantly decreased with increasing application of N.

A high rate of N application increases leaf area development and increases overall crop assimilation, thus contributing to increased seed yield (Bhardwaj and Kaushal, 1989). Patra et al. (1993) reported that straw mulching significantly affected the fertilizer N use efficiency and essential oil yield in Japanese Mint (*M. arvensis* L.). Alkire and Simon (1996) and Piccaglia et al. (1993) concluded that N increases essential oil yield of peppermint by influencing a variety of growth parameters such as tillers per plant, the total plant dry weight and the leaf area index (LAI). In field grown Lemon or Orange Mint (*Mentha citrata* Ehrh.) and *M. arvensis*, N fertilization upto 240 kg N/ha increased biomass and plant height, but markedly decreased the leaf to stem ratio (Ram et al., 1995 and Ram and Kumar, 1997).

Tanjia et al. (2009) reported that plant height of mint was significantly affected by various levels of N-fertilizers. Generally, plant height was increased as the level of N increased. Rahman, (1999) had reported that plant height of mint was increased as the level of N increased from 0 to 200 kg N/ha. He further noted that fresh herb yield increased with increasing N rates. The maximum value (103.54 g) of fresh herb product was obtained with the treatment of 120 kg N/ha. Generally, herb productivity increased as the level of N increased from 0 to 120 kg N/ha.

Field studies in Greek Oregano have shown that N application significantly affect a number of vegetative traits, inducing both biomass and oil yield peaks at a rate of 80 kg N/ha

(Sotiropoulou and Karamanos, 2010). Contrary, Ozguven et al. (2006) reported a significant increase in fresh and dry weight of *O. syriacum* species at the rate of 40 kg N/ha in the field. Barreyro et al. (2005) reached a similar conclusion working with an Oregano hybrid (*Origanum* x *appli*).

Aziz et al. (2009) reported that in comparison with low N level, higher N levels significantly increased the dry matter production of Oregano. In addition to that, the increment was 9% and 26% for the harvest in 2006 and 2007, respectively. Since dry matter of low N level was comparable between two harvests, the results indicate a N dose dependency of dry weight of Oregano.

Arabaci and Bayram, (2004) working on the effects of nitrogen fertilization and different plant densities on agronomic and technological characteristics of Basil reported that all characteristics except for the essential oil yield were significantly affected by fertilizer usage. The green herb yield (4029.4 kg/ha) under N fertilizer condition was higher than non-N fertilizer condition. Similar results were reported by Arabaci, (1995); Ceylan et al., (1994); Koo, (2000).

Biesiada et al. (2008) reported that the most suitable level of N application to achieve best yields of Lavender was 100 kg N/ha. In their study, the lowest yields were achieved from plants fertilized with N at 50 kg N/ha. However, they reported that heavy N fertilization (200 kg N/ha) caused a decrease in the yield of Lavender flowers. Ruminska et al. (1991) and Kordana et al. (1991) stated that intensive N fertilization in total rates of 100-200 kg N/ha decreased the yield of Lavender as well as having had detrimental effect on overwintering of the plants.

The findings by John et al. (1999) indicate that the seed oil and dry matter yields of Lesquerella/Desert mustard [*Lesquerella fendleri* (Gray) S. Wats.] showed linear responses to N rates. Seed oil content was reduced as the N rate was increased resulting in a negative linear response. The increased seed yield related to N tended to offset the decreased oil content so that oil yields were increased by added N at all rates tested. Nitrogen fertilizer has been reported to have a similar effect on oil content of other oil seed crops, such as Sunflower (*Helianthus annuus* L.) and Meadowfoam (*Limnanthes alba* L.) (Zubriski and Zimmerman, 1974; Pearson and Tolliff, 1986).

Kumar et al. (2008) working on Coriander (*Coriandrum sativum* L.) showed that each successive increase in the N level from 0 to 80 kg N/ha enhanced the plant height, while the number of branches/plant increased up to 120 kg N/ha. This could be due to large cells

development at increasing N levels (Black, 1967) with higher meristematic activities, which consequently benefited the growth. Barreyro et al. (1993) and Rahman et al. (1990) reported a similar trend. Regarding yield parameters, Kumar et al. (2008) observed that each successive increase in N level from 0 to 80 kg N/ha led to a marked increase in the umbels/plant, umbellets/umbel, seeds/umbellet and 1000-seed weight, but a further increase in the N level from 80 to 120 kg/ha did not result in a significant improvement in these yield attributes. Vigorous vegetative growth and a better supply of photosynthates to the sink at higher N levels might have resulted in these increased yield attributes. Sharma and Israel (1991) also found higher values of yield attributes with increasing N levels. In their work, Kumar et al. (2008) showed that N application at 40, 80 and 120 kg N/ha produced 29.6, 44.8 and 46.4% higher seed yield and 49.7, 70.7 and 78.2% higher stover yield, respectively, compared to no N application. However, significant increases in seed and stover yield were only found up to 80 kg N/ha levels. Barreyro et al. (1993), Garg et al. (2004) and Oliveira et al. (2003) also reported the positive response of Coriander to N application.

Nitrogen application upto 160 kg N/ha increased yield of fresh biomass of Geranium (*P. graveolens*) (Ram et al., 2003) while Arganosa et al. (1998) found that maximum biological yield, seed yield, oil yield and seed weight were obtained from 80 kg N/ha and highest oil percentage gained by using 40 kg N/ha.

A number of studies have found an increase, no increase or a decrease in the quantity and quality of herbal plant yields following N fertilizer application. For example, Golcz et al. (2006); Dzida and Jarosz, (2006) and Biesiada and Kus, (2010) have shown that N fertilization results in a significant increase in quantity and quality of herbal yields. Contrary to the foregoing observation, a number of other studies suggest that elevated nitrate levels do not induce analogous increase in plant growth (Karamanos, 1995; Andrews et al., 1999; Akanbi and Togun, 2002; Omidbaigi and Arjmandi, 2002; Baranauskiene et al., 2003). Marschner (1995) indicated that elevated amounts of nitrates are of limited use for plant metabolism. Biesiada et al. (2006) did not observe a clear correlation between N fertilization and the chemical composition of Calendular flower heads, although they noted a slight decrease in polyphenol content at higher N rates. Also, Ruminska et al. (1991) reported that Calendula does not require intensive mineral fertilization and that high doses of N result in the decrease of yields of flower heads.

2.3 Effects of Phosphorus Fertilizer on Plant Growth and Yield

Phosphorus nutrition has a major impact on plant growth. The assimilated and highest oxidised form of inorganic phosphate (Pi), PO_{4-3} is indispensable for important cell functions (Ticconi and Abel, 2004). Phosphate esters constitute intermediates in metabolic pathways of biosynthesis and catabolism, where energy transduction depends upon phosphorylated molecules and the energy-rich pyrophosphate bonds (PPi) (Marchener, 1995). Phosphorus regulates key enzyme activity [e.g. phosphorylation of Phosphoenolpyruvate (PEP) carboxylase in C_3 and C_4 plants] (Theodorou and Plaxton, 1995). Moreover, Pi ensures the stability of nucleotides via its presence in the structure of these macromolecules and the stability of biomembranes that contain phospholipids (Abel et al., 2002). Thus availability of Pi has profound consequences for plant metabolism and growth. In plants, P is therefore required in relatively large amounts for the biosynthesis of primary and secondary metabolites (Marschner, 2002), since P has essential functions as a constituent of nucleic acids and phospholipids (biomembranes) and plays a key role in the energy metabolism of cells.

Most of the researches conducted on the effects of P on the productivity of medicinal and aromatic plants have mainly been done alongside innoculation with mycorrhizal fungi in Peppermint; (Arango et al., 2012), garden sage (Nell et al., 2009) Basil, (Copetta et al. 2006; Oregano (Khaosaad et al., 2006), Coriander (Kapoor and Mukerji, 2002) and Fennel (Kappor et al., 2004) to enhance P uptake by plants.

Nell et al. (2009) reported that P application to garden sage increases the leaf biomass, total phenolic compounds and rosemarinic acid concentration and the rosemarinic acid yield in leaves. On the other hand, findings for *M. piperita* indicated that the production of biomass was not significantly influenced by P fertilization (Santos de Souza et al., 2012). They reported a higher main branch length with the highest level of P. Rodrigues et al. (2004) also found lower *M. Pepirita* growth in plants grown in low P concentrations which they attributed to probable commitment of P in protein synthesis instead of being used in biomass production. They also noted that P concentrations did not influence the accumulation of dry matter, shoot and root of plants of *M. piperita* during its development. Contrary to these observations in *M. Piperita, Thymus vulgaris* L. cultivated in 50% P showed a better relative growth rate (Bueno, 2004).

Vegetative growth of selected aromatic plants was promoted by P in solution culture up to a concentration of 34 ppm. Higher concentrations caused suppression in growth and

development. The peak in P demand was observed later than that of N, at the seed formation stage (Economakis, 1993). Alsafar and Al-Hassan et al. (2009) reported an increase in LAI with increased rate of fertilizer application similar to Kumar et al. (1999) and Lacy et al. (1981). These studies were conducted on mint. They reported that LAI increased significantly with the increasing rate of fertilizer application from 75/50 kg N/P_2O_5/ha to 100/75 kg N/P_2O_5/ha, compared to the control and the other lower application rates. The same treatment combination resulted in increased total dry matter and essential oil yield, which increased with the corresponding increase in the total number of leaves per plant and leaf area. Contrary to the foregoing findings, Maia, (1998) observed that deficiencies in N and P drastically reduced the production of fresh matter of mint. Kilic et al. (2012) reported that the herbal yield of thyme was highest when P was 135 kg P_2O_5/ha.

2.4. Effects of Nitrogen Fertilizer on Essential Oils

Essential oils are chemical mixtures of highly functionalised volatile compounds derived from plant secondary metabolism. Terpenes, mainly monoterpenes and sesquiterpenes are the predominant constituents of the oils although the presence of other molecules like phenylpropanoids is possible (Sangwan et al., 2001). However, phenylpropanoids are not present in *Salvia*. *Salvia officinalis* essential oil consists of more than 100 individual components, primarily of terpene nature (Ginnouli and Kintzios, 2000). Their concentration is maximum in leaves, intermediate in flowers and minimal in stems (Bellomaria et al., 1992). Among *Salvia* species, *S. officinalis* is considered to have the highest essential oil yield (Newall et al., 1996). However, Karousou et al. (2000) demonstrated that the essential oil content of three sage species in Greece ranged from 1.0 to 5.5 % in *S. fruticosa* Mill.; 1.3 to 4.2 % in *S.pomifera* and only 0.9 to 2.3 % (ml /g dry weight) in *S. officinalis* L. The last species (*S.officinalis* L) has exhibited variations in oil content under different seasons and geographical origins (Putievsky et al., 1986; Chalchat et al., 1998; Perry et al., 1999; Santos-Gomes and Fernandes-Ferreira, 2001). Karioti et al. (2003) also reported significant differences in oil content and composition of *S. fruticosa* due to plant growth stage.

Major components of *S. officinalis* essential oil are α- and ß- thujones (mainly α), camphor, 1,8 cineole, borneol and α-caryophyllene (Kintzios and Gianouli, 2000). *Salvia officinalis* oil has been described in several studies (Langer et al., 1996; Perry et al., 1999; Piccaglia et al., 1997; Santos-Gomes and Fernandes-Ferreira, 2001; Savelev et al., 2004) and a

great variation in its composition has been revealed (Langer et al., 1996). Oil of *S. officinalis* var *purpurea* has been chemically analysed only once by Savelev et al. (2004). Data from the study by Savelev et al.(2004), indicated an oil synthesis similar to *S. officinalis* with α-caryophyllene being the predominant constituent (24-32% depending upon plant growth stage).

Sharma and Kumar (2012) observed significant differences in the composition of oil constituents when different N doses were used. Nitrogen may enhance essential oil biosynthesis process through its direct or indirect role in plant metabolism which result in more plant metabolites. Other similar results were reported by Omer (1998) and Omer et al. (2008) who reported that N fertilizer was effective in increasing essential oil of *Origanum syriacum* Linn. and *Ocimum americanum* Linn. Application of 3.0 g N/plant enriched the essential oil sample with higher percentage of major constituents as linalool (19.10%), α-terpineol (7.15%) and linalyl acetate (32.11%) as compared to other treatments (Sharma and Kumar, 2012). Such a response might have been due to de novo meristematic cell metabolism in building dry matter with essential oil production (Said-Al Ahl et al., 2004).

Karioti et al. (2003) conducted a study to investigate the effects of N on the content and composition of the essential oils of *Salvia fruticosa* Mill. They used the nutrient film technique-hydroponics. Their results showed qualitative and quantitative differences due to the sampling dates as well as different concentrations of N in the nutrient solution. Leaf essential oil content increased with time for all the treatments, the highest values being recorded at the end of seed formation stage. However, the effect of N was minor with a remarkable decrease for 200 mg of N/L at the end of seed formation stage. This is in agreement with results reported by Singh et al. (1989) with *M. arvensis* in sand culture. They observed that the essential oil content increased with an increase in nitrate-N upto 16 meq/L beyond which it decreased. Bhardwaj and Kaushal (1990) working with peppermint in soil culture also reported an increase in essential oil content with increased nitrogen levels upto 150 kg N/ha, beyond which they reported no effect with higher nitrogen doses. In the contrary on an experiment by Singh et al. (1989) reported that for mint species grown in soil, the essential oil content decreased with an increased N concentration from 0-50 to 100 kg N/ha, with the highest essential oil content being obtained with no nitrogen application under their experimental conditions.

Alkire and Simon (1995) conducted a research on Peppermint (*Mentha* x *piperita* L. cv. 'Black Mitcham') and native Spearmint (*M. spicata* L.) to evaluate their response to N rates and

form. Their treatments were N forms (ammonium sulphate, calcium nitrate, urea and anhydrous ammonia), anhydrous NH_3 treatments of 0, 45, 90, 135, 179, 224 kg N/ha, and N rates rates of 0, 56, 112 and 168 kg N/ha. They reported increased essential oil yields for the 168 kg N/ha rate over the 112 and 56 kg N/ha N rates. In addition, they noted that calcium nitrate gave the highest essential oil yields with 93.0 kg N/ha, compared to urea (87.1 kg/ha) and ammonium sulphate (85.0 kg/ha). Piccaglia et al. (1993) also showed that N increases essential oil yield of Peppermint.

Clark and Menary (1980) in a study on Peppermint observed that irrigation and N fertilizer influenced essential oil yield with the highest oil yield achieved with application of 300 kg N/ha coupled with 50 mm of irrigation weekly. In another study by Alsafar and Al-Hassan (2009), application of 75 kg N/ha significantly increased the total dry matter and essential oil yield of wild mint (*Mentha longifolia* L.).

Sotiropoulou and Karamanos (2010) reported that, fertilizer treatments significantly affected the levels of some compounds, in one season of their study. In their study, application of 80 kg N/ha significantly increased linalool percentage in inflorescences (0.48%). In leaves, significantly higher percentages of α-pinene (1.88%), camphene (0.39%), π-cymene (21.4%), thymol (0.59%) and caryophyllene (1.48%) were detected in leaves of plants from unfertilized plots whereas carvacrol levels were significantly higher at 40, 80 and 120 kg N/ha (61.5-63.78%) in comparison to the control (56.46%).There was however, no N effect on oil concentration as is the case in other studies of the Lamiaceae family (Amr et al., 2003; Baranauskiene et al., 2003; 1999; Ram et al., 2006;Ram and Kumar, 1997). Contrary to these, an increase in oil concentration by N application was observed in Basil (Sifola and Barbieri, 2006), Egyptian Oregano (Ozguven et al., 2006) and sage (Karioti et al., 2003). However, in another study, a reduction in oil concentration by N fertilization in *Origanum vulgare* var. *samonthrake* and *Origanum vulgare* var. *creticum* has been reported by Azizi et al. (2009).

A study by Omer (1999) showed that N fertilization with more than 2 g N/pot significantly decreased essential oil content of wild Egyptian Oregano. A similar effect was also reported for plants in other genera of the family Lamiaceae, *Rosemarinus officinalis* (Boyles et al., 1991) and *Thymus vulgaris* (Baranauskiene et al., 2003).

Nitrogen fertilization affected the composition of the essential oils of Egyptian Oregano by increasing the percentage of thymol and carvacrol with a simultaneous decrease of γ -

terpinene and ρ-cymene (Omer, 1999). Furthermore, Alvarez-Castellanos and Pascual-Villalobos, **(**2003) conducted a study on nine Chrysanthemum (*Chrysanthemum coronarium*L.) (Asteraceae) populations. Looking at the effect of N, P_2O_5 and K_2O fertilizer application against no fertilizer application on yield of flower heads and essential oil composition, they observed a decrease in essential oil content with increase in fertilizer application. On the nutrient fertilized plots, the main compound (13.9-26.9%) obtained and in higher concentrations (2.32-5.47% more) was camphor. The accumulation of germacrene-D in the oil, on the other hand, was negatively affected by the fertilization treatment. In a similar way, fertilization significantly changed geranial and citronelal in essential oil of Lemon-scented tea-tree (*Leptospermum petersonii* Bailey.) (Diatloff, 1990).

Application of N upto 160 kg N/ha increased yield of essential oil of Geranium beyond which a further increase in N dosage did not significantly affect the essential oil yield (Ram et al., 2003). Ram et al. (1998b) observed a reduction in oil content of menthol in mint leaves with increased N application. Another study showed that the percentage of compounds of Garden thyme was not significantly affected by applying N fertilizer in the range of 0-135 kg N/ha (Baranauskiene et al., 2003).

In a study on Chamomile, (*Matricaria recutita* L.), Meawad et al. (1984) and Letchimo, (1993) reported that increasing N fertilizer increased flower yield. Despite this, they found that further nitrogen application did not affect flower yield. They also observed that nitrogen consumption had a negligible impact on extract content. Nitrogen plays a vital role in development and division of essence-bearing new cells, biosynthesis of essential oils and active ingredients in medicinal plants. Abou-Zeid and EI-Sherbeeny (1974) also indicated that the increase in essential oil yield by application of N fertilizer could be due to the role of N in development and division of essence-bearing new cells, essence channels, secretory canals and glandular trichomes. However, high N application led to significant reduction in essence. This finding complies with the results of the study conducted by Omidbaigi et al. (2003) on Sweet Basil.

Venskutonis et al. (1999) stated that N fertilizer had little effect on Caraway essential oil content whereas it did not affect the phenols amount. In Dill (*Anethum graveolens* L.), Bist et al. (2000) indicated that increase in N increased Dill's carvone essence from 39 to 60%. However, it

decreased dill's apiol essence from 7.9-3.39% but there was no significant difference between the control and N application at the rate of 25 kg N/ha regarding the studied compounds.

Increasing N level from 0-30 kg N/ha had a positive effect on the major component (ρ-cymene) of Black Cumin seed (*Nigella sativa* L.) essential oil whereas no change in the levels of α-pinene or β-pinene was observed (Ashraf et al., 2006). Shah (2008) reported that various levels of N fertilization (0, 176, 264, 352 or 442 mg N/pot) decreased essential oil content of Black Cumin seeds. In another study, essential oil yield of Cumin (*Cuminum cyminum* L.) was significantly increased by adding N fertilizer (Azizi and Khahrizi, 2008).

2.5. Effects of Phosphorus Fertilizer on Essential Oils

In plants, P has multi-functional roles as a constituent of nucleic acids or biomembranes. Furthermore, it is highly involved in the energy metabolism of cells and it is therefore required for the biosynthesis of primary and secondary metabolites in plants (Marschener, 2002). Phosphorus application was revealed to make a positive effect with increasing doses on essential oil content and yield of cumin plant (Tuncturk and Tuncturk, 2006).

Praszna and Bernath (1993) observed that plants grown under nutrient deficient conditions show less essential oil production. However, P concentration in the nutrient solution did not cause any significant variation in the yield or relative composition of *S. officinalis* var *purpurea* essential oil (Nell et al., 2009). This study also indicated that neither the quality nor quantity of essential oil was significantly affected by the different treatments while essential oil yield increased by 1.2-fold as a result of P fertilization, compared with the other treatments. Contrasting results from numerous other experiments have highlighted P impacts on oil content and quality from various species (Kapoor et al., 2004; Economakis et al., 2002). However, Economakis (1995) reported no effect of P levels on the oil content of *S. fruticosa* cultivated under hydroponics. Mairapetyan and Tadevosyan (1999) studied optimization of N:P:K ratios in hydroponics, and concluded that mint requires higher P supply for maximum accumulation of essential oil. This is because increased biosynthesis of essential oils is correlated with the optimization of plant nutrition (Mairapetyan, 1999).

Munsi (1992) found that application of N and P improved productivity of Japanese mint, increasing the dry matter production and essential oil yield. Piccaglia et al., (1993) evaluated for two consecutive years, levels of P (0.75 and 150 kg P_2O_5/ha) and nitrogen (0, 100 and 120 kg

N/ha) and found that the two seasons of planting and levels of N and P supplied did not influence the composition of essential oil of mint.

Zheljazkov and Margina (1996) observed that essential oil yield increased with increasing levels of fertilization with N, P and K. They also observed that there was no significant effect on the chemical compounds of the essential oil in the first harvest, while in the second harvest, there was increased content of menthol. Freitas et al. (2006) reported that in *M. arvensis*, when P was not added to the substratum, the essential oil content increased by upto 89% in mycorrhizal plants compared to non inoculated ones. No increment in essential oil content occurred when the P levels increased. On the other hand, Fatima et al. (2006) reported that plants of *M. piperita* subjected to 7.75/15.5 mg/L of P showed a higher increase in essential oil content than those grown at 233.0/46.5 mg/L of P.

High P rates (more than 7.47 kg P/ha) decreased Chamomile essential oil yield (Emongor, 1990). Within Sweet Basil leaves essential oil, eugenol, linalool, 1,8-cineol, acetate-D-amyl and germacrene-D concentrations were affected (increased or decreased) by the amount of applied P. On the other hand, α-bergamotene and β-elemene in Basil concentration was not affected by amount of P applied (Chimura et al., 1993). Application of 100 kg P/ha significantly increased the fresh and dry weights in feverfew (*Tanacetum parthenium* L.) (Schultz B.P.) plant, while all P levels (50, 100 and 150 kg P/ha) significantly enhanced the essential oil concentration as compared to control (Saharkhiz and Omidbaigi, 2008).

2.6 Effects of Water Stress on Plant Growth and Yield

Water deficit has been shown to affect growth and essential oil content and composition of aromatic crops. It has been suggested that under water stress, a high density of oil glands due to the reduction of leaf area results in an elevated amount of oil accumulation (Simon et al., 1992). In addition, the stimulation of essential oil production under water stress could be due to the fact that plants produce high terpene concentrations under environmental stress conditions, because of a low allocation of carbon to growth and defence (Turtola et al., 2003).

Bettaieb et al. (2009) working on water deficit effects on *S. officinalis* observed that the depressive effects of drought on plant morphology occurred from the second week of treatment and was more pronounced with the intensity of water constraint. They also noted that plants subjected to severe water deficit presented thinner stems with fewer, dry and smaller leaves than the control ones. They also noted that water treatments reduced significantly plant height, and

this effect was more pronounced with the severity of drought. They showed that under severe and moderate water deficit, the aerial plant height was significantly reduced by 46.2 and 23%, respectively, compared with the control. This decrease was attributed to a preferential allocation of biomass production to the roots (Albouchi et al., 2003) or a reduction in chlorophyll content and, consequently, photosynthesis efficiency, as reported by Viera et al. (1999).

Changes in essential oils extracted from aromatic plants and their composition were observed with water stress in Palmarosa (Sabih etal.,1999). Water stress resulted in significant reduction of fresh and dry matter, nutrient content, and essential oil yield of Japanese mint plants (Mirsa and Strivastava, 2000). Fresh and dry weights of *O. basilicum* L. were decreased as plant water deficit increased (Simon et al.,1992). Arnon and Gupta, (1995) also reported a reduction in plant height due to water stress.

Drought stress during the vegetative period (before flowering stage) of medicinal plants has been shown to result in shorter plants and smaller leaf areas of Mint (Abbaszadeh et al., 2008), Yarrow (Sharifi et al., 2005) and Chicory (Taheri et al., 2008); reduced water use due to the reduction in plant size of calendula (Rahmani et al., 2008) and decreased vegetative dry matter of balm (Aliabadi et al., 2009). Khalid (2006) noted that total fresh and dry weights of plants were decreased due to exposure to injurious levels of drought (50%) or excessive water (125%).

Khalil et al. (2010) studied the effect of different levels of water stress (30, 50 and 70% depletion of available soil moisture), on some morphological and biochemical characteristics of greenhouse container grown Basil plant. Results showed that water stress has significant effect on morphological and biochemical characteristics of Basil. Plant height, number of branches, number of leaves, leaf area, fresh and dry weights of the first cut showed significant increase under 50% soil moisture level, while further increase in water stress level showed significant decrease in studied parameters. A similar tendency was observed for relative water content percentage as well as photosynthetic pigments concentrations (Chl a, Chl b, total Chl a+b and Carotenoids), while in the second cut, the studied characters showed progressive decrease with increasing water stress level (except for photosynthetic pigments which revealed the same trend as in the first cut). Reverse trend was observed for oil percentage and proline content.

Mohamad et al. (2012) reported that drought stress treatments had a significant effect on dry yield of Basil. They showed that increasing drought stress level from 100% (control) of field

capacity to 60% of field capacity, reduced dry yield per plant by 22.84 kg/ha. They further indicated that plant dry weight reduced by 0.298 g/ pot, height reduced by 6.38 cm, number of sub-shrub per plant reduced by 1.64 and the rate of reduction for number of internodes was 5.50. Similar results were obtained on Chamomile (Pirzad et al., 2006); Basil, (Hasani et al., 2004); *Dracocephalum moldarica* (Safikhani et al., 2008) and on Japanese Mint (Mirsa and Srivastava 2000). They suggested that the reduction could be because of more allocation of photosynthetic material to root as indicated by Sreevalli et al. (2001).

In an Iranian study, the effects of different levels of water stress on some morphological and biochemical characteristics of Purple Basil was investigated. Different levels of water stress were 100 (control), 90, 80, 70, 60 and 50% of field capacity. The results showed that as the soil water content decreased, the plant height, stem diameter, number and area of leaves, leaf area index, herb yield and leaf chlorophyll contents decreased but the amount of anthocyanin and proline increased (Moeini et al., 2006).

Azizi et al. (2009) used three watering treatments while studying three oregano species; the control where soil water content was maintained at 60% of maximal water holding capacity during the seedling development stage, water treatment two was maintaining soil water content at 70% of maximal water-holding capacity during stem elongation and flowering (at the beginning of flowering) stage and the third watering treatment (water deficiency treatment) where the soil water content was maintained at 50% of the maximal water holding capacity throughout the cultivation period, and the late water deficiency treatment where soil water content was maintained at 60% and 70% of the maximal water holding capacity during the seedling and stem elongation development stages, respectively. During the flowering stage, the water content was then reduced to 50% of maximal water-holding capacity. Based on these treatments, they reported that water deficiency significantly decreased dry matter production for two harvests in 2006 and 2007 in comparison with control; a dry matter decrease of 17% and 11% was recorded for consistent and later water deficiency, respectively, during the experimental period of 2006. In the following year, an additional differentiation of the effect of water deficient treatments on dry matter was observed.

In an experiment conducted in Egypt by Aziz et al. (2008), the effects of four irrigation intervals (3, 5, 7 and 10 days) were investigated on plant growth, essential oil yield and its main constituents of *Thymus vulgaris* plants. The plants that received irrigation every three days

recorded significant increase in plant height, fresh and dry weight of herb per plant as compared with plants irrigated every 5, 7 and 10 days.

An experiment undertaken by Khazaie et al (2008) to determine the herbage biomass and oil production of thyme (*T. vulgaris* L.) in 2003 and 2004 in the semi-arid region of Khorasan in Iran using three irrigation intervals (7, 14, and 21 days) revealed that irrigation intervals did not change total harvested herbage biomass and oil production. The results showed that there is a high potential for saving water through longer irrigation intervals (e.g. 14 days) using locally adapted plants in the semi-arid conditions of Khorasan (Khazaie et al., 2008).

Bahreini-Nejad et al. (2013) reported that plant height, leaf area and root length and dry weight reduced under water stress in both years of their study on *T. daenensis* subsp *daenensis* Celak. (Iranian sub-species). These results were generally in line with Letchamo and Gosselen (1995) in *T.vulgaris*, Bettaieb et al. (2009) in *S. officinalis*, Davatgar et al. (2009) in *Oryza sativa* and Laribi et al. (2009) in *C. carvi*. The reductions in plant height, leaf area, root length and dry weight under water stress were perhaps due to a decline in the cell enlargement and more leaf senescence resulting from reduced turgor pressure (Shao et al., 2008). They also observed that fresh and dry weight of aerial parts decreased as water stress level increased in both years. In agreement with this findings are observations by Letchamo and Gosselin (1995) in *T. vulgaris* , Said Al Ahl et al. (2009) in Oregano and Houshmand et al. (2011) in Chamomile also reported that drought stress reduced herbage yield of tested species. Decrease in fresh and dry weight was as a result of reduction in plant height and leaf areas. Reduction in fresh and dry weight of the plant may also be due to a decrease in plant growth, photosynthesis and canopy structure during water stress as reported by Shao et al. (2008). Moreover, the pronounced effect of decreased irrigation on overall growth of *T. daenensis* may be attributed to the lower availability of sufficient moisture around the root and thus a lesser proliferation of root biomass resulting in the lower absorption of nutrients and water leading to production of lower biomass (Singh et al., 1997).

The plant height and number of branches/plant of coriander differed significantly under various moisture regimes (Kumar et al., 2008). The tallest plants with the maximum number of branches/plant were obtained with the application of three irrigations at the branching, flowering and seed formation stages. They showed that omitting irrigation at the branching stage markedly reduced both plant height and number of branches/plant, which may be due to the lower

availability of water and poor nutrient uptake at that stage. Tomar et al. (1994) also reported similar findings in coriander. Omitting irrigation at the seed formation stage, however, did not reduce the plant height or the number of branches/plant.

2.7 Effects of Water Stress on Essential Oil

Drought stress increases the essential oil percentage of most medicinal and aromatic plants, since in case of stress, more metabolites and other biological substances that prevent cells from oxidization are produced. However, essential oil content decreases under drought stress, because of the interaction between the amount of the essential oil percentage and shoot yield both of which are important components of the essential oil content. This is as a result of drought stress increasing the essential oil percentage but lowering shoot yield which results into a reduction in essential oil content (Aliabadi et al., 2009). Appropriate irrigation strategies showed a great potential for improvement of the yield of monoterpenes in field-grown spear mint and Rosmary (Define et al., 2005).

The effects of water supply are undoubtedly positive on herbage production of most plants. Define et al. (2005) are less clear, however, on the effect of water supply on concentration of active substances in aromatic and medicinal plants. There are indications that the effects of water may vary substantially among Lamiaceae species of contrasting origin. Thus, one could expect a different response of hydrophytes (e.g *Mentha* species) from xerophytes (e.g *Lavandula* species) (Bernath, 1991). On this account, sage species, as xerophytes might exhibit a reduction in their essential oil concentration with increased water supply as a result of the reduction in the concentration of glandular hairs per unit leaf area due to a more intensive cell enlargement.

Both moderate water deficit and severe water deficit improved sage essential oil yields (Bettaieb et al., 2009). Similar results were reported earlier on other plant spices cultivated in the same conditions; Parsley (Petropoulos et al., 2008), Mexican oregano (Dunford and Vasquez, 2005) and Satureja(Baher et al., 2002). Conversely, water deficit decreased oil yield of Rosemary and Anise (*Pimpinella anisum* L.) (Singh and Ramesh, 2000; Zehtab-Salmasi et al., 2001). It has been suggested that under water stress, a high density of oil glands due to the reduction of leaf area results in an elevated amount of oil accumulation (Simon et al., 1992). In addition, the stimulation of essential oil production under water stress could be due to the fact that plants produce high terpene concentrations under environmental stress conditions, because of a low allocation of carbon to growth and defense (Turtola et al., 2003).

Baher et al. (2002) noted that the main constituents of *Satureja hortensis* L, such as carvacrol, increased under moderate water stress, and λ-terpinene content decreased under moderate and severe water stress. They also noted that essential oil, total carbohydrate, and proline contents were pronouncedly increased with increasing stress levels of *S. officinalis* plants (Hendawy and Khalid, 2005).

Manukyan (2011) studied the effects of drought stress on the productivity and quality of lemon catmint (*Nepeta cataria* L. *f. citriodora*), lemon balm and sage under soiless greenhouse production. He reported that the essential oil content of lemon catmint and lemon balm was significantly influenced by substrate moisture. High drought stress (250 hPa) induced a higher amount of essential oil in lemon catmint and lemon balm (0.151% and 0.068%), respectively, while in the case of sage, there was no significant difference between the treatments. However, the essential oil yield was significantly affected by the treatments with 50 hPa giving the best oil yield (lemon catmint = 0.363 ml/plant; lemon balm = 0.087 ml/plant and sage = 0.198 ml/plant).

Manukyan (2011) also reported that essential oil composition of lemon catmint was affected by the substrate moisture. High drought stress of 250 hPa provided high content of trans-ocimen, geranyl acetate and phytol in lemon catmint while the contents of 6-methyl-5-heptan-2-on and cis-3-hexen-1-ol decreased. On the other hand, drought stress did not have any influence on the main components of lemon catmint essential oil. In lemon balm, geranial and neral peaked up with increasing drought stress. In sage, drought stress had considerable influence on most identified compounds.

Changes in essential oils extracted from aromatic plants and their composition were observed with water stress (Sabih etal., 1999). Water stress resulted in significant reduction of fresh and dry matter, nutrient content, and essential oil yield of Japanese mint plants (Mirsa and Strivastava, 2000). Drought stress during the vegetative period of medicinal plants (before flowering stage) has been shown to result in shorter plants and smaller leaf areas of mint (Abbaszadeh et al., 2008).

Bahreini-Nejad et al. (2011) conducted two experiments on peppermint (*Mentha piperita* L.) to determine the effect of drought stress on growth parameters, essential oil constituents and yield. Five levels of water deficit stress including D1 (100% field capacity-control), D2 (85% field capacity), D3 (70% field capacity), D4 (60% field capacity) and D5 (45% field capacity) were investigated during 4 months. Results indicated that drought stress motivated a significant

reduction in all of the growth parameters and essential oil yield and percent. The highest values of growth parameters and essential oil percent and yield were observed under 100% field capacity (control). Also, the highest values of menthone and menthofuran were obtained under 100% field capacity (control) while the highest values of menthol were obtained under 70% field capacity.

Khalid (2006) evaluated the influence of water stress on essential oil of two species of basil: Sweet Basil (*Ocimum basilicum* L.) and American Basil (*Ocimum americanum* L.) and noted that for both species under water stress, essential oil percentage and the main constituents of essential oil increased. Also, three parsley cultivars (plain-leafed, curly-leafed and turnip-rooted) were grown under conditions of 35-40% and 45-60% water deficit in order to evaluate the effect of this form of stress on essential oil yield and composition. It was shown that water stress increased the yield of essential oil (on a fresh weight basis) from leaves of plain-leafed and curly-leafed, but not turnip-rooted parsley. However, on a m^2 basis, foliage oil yield increased significantly only in curly-leafed parsley. Water stress also caused changes in the relative contribution of certain aroma constituents of the essential oils (principally 1, 3, 8-*p*-menthatriene, myristicin, terpinolene + *p*-cymenene), but these changes varied between cultivars. Rahmani et al. (2008) also showed that drought stress had significant effect on oil yield and oil percentage of Calendula. Their results showed that highest oil yield was achieved under non-drought condition while highest oil percentage was achieved under drought condition.

Radacsi et al. (2010) showed that different levels of soil water capacity (SWC 30-50-70%) resulted in significant changes in physiological parameters of *O. basilicum* L. 'Genovese' in a pot experiment. Compared to the control plants (water supply to 70% SWC) the driest condition (30% SWC) caused a decrease of the relative water content (RWC) in the plants by 20%. Additionally, their water potential was reduced to 45% of the control plants. The same treatment increased the concentration of malondialdehyde (MDA) indicating the level of oxidative stress by 52% compared to the leaves of the control plants. However, under a moderate water deficit (50% SWC), the plants exhibited a less severe decrease in RWC and water potential, as well as less oxidative stress. Although the reduction of the SWC to 50% did not significantly affect the dry shoot mass, a more severe water stress (30% SWC) reduced it by 34%, compared to the control plants. The essential oil concentration per plant dry mass showed only a slight increase in consequence of drought stress (significant at 90% confidence level). The

essential oil production calculated to a single individual is determined basically by the changes in the biomass, thus, the lowest results were found in the driest soil. Water supply modified the quantitative composition of the oil: the proportion of linalool decreased from 59.68 to 44.39 %. The samples of the stressed plants contained some minor components, (ß-myrcene and 2-octanone) not present in the control samples.

Azizi et al. (2009) had three watering treatments: the control where soil water content was maintained at 60% of maximum water holding capacity during the seedling development stage. Water treatment two was maintaining soil water content at 70% of maximum water-holding capacity during the stem elongation and flowering (at the beginning of flowering) stage. The third watering treatment (water deficiency treatment) where the soil water content was maintained at 50% of the maximum water holding capacity throughout the cultivation period and the late water deficiency treatment where soil water content was maintained at 60% and 70% of the maximal water holding capacity during the seedling and stem elongation development stages, respectively and during the flowering stage, the water content was then reduced to 50% of maximal water-holding capacity. Based on these treatments, they reported that later water deficiency significantly increased essential oil content of oregano. Conversely consistent water deficiency showed no effect on essential oil content. Compared to the experimental period of 2006, the essential oil content of oregano in 2007, was higher for control and for later water deficiency, but not for later water deficiency. In 2007, both control and later water deficiency showed a comparable essential oil yield, which was significantly higher than that of consistent water deficiency.

In Egypt, an experiment by Aziz et al. (2008) four irrigations intervals (3, 5, 7 and 10 days) were investigated on plant growth, essential oil yield and its main constituents of *T. vulgaris* plants. Plants irrigated every 10 days gave the highest relative percentage of thymol. They also indicated that the rate of transformation of p-cymene to thymol to be higher under stress conditions.

Bahreini-Nejad et al. (2013) studying the effects of water deficit on essential oil content and yield of *T.daenensis* observed that essential oil content increased while essential oil yield decreased under water stress. It was reported that water stress increased essential oil accumulation via a higher density of oil glands due to the reduction in leaf area (Simon et al., 1992). In addition, the increase in essential oil concentration under water stress could be due to

the fact that plants produce high terpene concentrations under water stress conditions due to a low allocation of carbon to growth, suggesting a trade-off between growth, and defense mechanism (Turtola et al., 2003). Water deficit decreased oil yield of *T. daenensis* under moderate and severe water deficit. Reduction in essential oil yield was due to reduction in herbage yield, thus indicating that essential oil yield is positively related to soil water content and herbage yield (Singh et al. 1997).

Bahreini-Nejad et al. (2013) reported that the effect of water deficit on essential oil compositions of thymol, carvacrol, ρ-cymene, γ-terpinene, β-caryophyllene and borneol were 66.41-70.48%, 6.72-11.69%, 5.05-6.17%, 3.67-4.22%, 3.72-3.92% and 1.68-3.07%, respectively, for two years, and they were the main compositions of essential oil of *T. daenensis*. Thymol content was 63.29 in 2010 and 66.83% in 2011, under controlled conditions. On the other hand, Bahreini-Nejad et al. (2010) reported that thymol concentration of *T. daenensis* population was from 1.9-7.2%.

Thymol contents increased under moderate and severe water stress and these results were in agreement with those of Aziz et al. (2008) in *T. vulgaris* and Said- Al Ahl and Hussein (2010) in Oregano. The ratio of thymol to other constituents, particularly, carvacrol, plays an important role for cosmetic, culinary and pharmaceutical purpose (Letchamo and Gosselin, 1995). Thus, the results indicated that quality of essential oil of *T. daenensis* may increase under drought stress. Carvacrol content reduced under both moderate and severe drought stresses. Other researchers; Letchamo and Gosselin (1995) and Aziz et al. (2008) in *T. vulgaris* and Jordan et al. (2003) working with *T. heymalis* reported that carvacrol content of such plants reduced under water stress.

Singh and Ramesh (2000) reported that water deficit stress reduced the oil yield of rosemary on a hectare basis, but oil yield on a plant fresh weight basis was not affected. Fatima et al. (2006) studied the effect of water stress on essential oil of excised leaves of palmarosa (*Cymbopogon martinii* var. *motia*) and citronella java (*C. winterianus*). They noted that essential oil percentage was increased under water stress while essential oil content was decreased under such condition. In coriander, it has been observed that water stress has significant effect on flowering shoot yield, essential oil yield and essential oil percentages with the highest oil percentage achieved under non-stress condition (Aliabadi et al., 2008). According to Zepak (1998) the higher the dry matter yield of plants the higher their essential oil yield.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

Four experiments were conducted at the Egerton University Horticulture Field. Experiment 1 was conducted from June, 2011 – October, 2011, experiment 2 (October, 2011 – February, 2012), experiment 3 (March, 2012 – May, 2012), and experiment 4 (March, 2014-July, 2014). Egerton University lies at Latitude 0° 23' South, Longitude of 35° 35' East, and Altitude of 2200 m above the sea level. Soils at the site are vintric mollic andosols (Kinyanjui, 1979). The monthly temperature variations during the experimental period are shown in Figure 4. Overall mean temperatures of the experimental site were 19.00 ^{0}C, 20.10 ^{0}C, 20.73 ^{0}C, and 20.49 ^{0}C for experiments 1, 2, 3 and 4, respectively.

3.2 Treatments and Experimental Design/Layout

The study was conducted in a plastic tunnel to shield the plants from rainfall that would otherwise reverse the watering treatment effect. The plant material used in this study was sage (*Salvia officinalis*) obtained from East African Dehydrates Nursery- Njoro, Kenya. The three factors studied were; (i) nitrogen (N), (ii) phosphorus (P), and (iii) watering regime (W). Nitrogen was applied at 4 levels [0 (N1), 40 (N2), 80 (N3), and 120 (N4) kg N/ha]. Phosphorus was applied at 4 levels [0 (P1), 30 (P2), 60 (P3), and 90 (P4) kg P/ha], and Watering was watering to field capacity by drip irrigation, once every week (W1), once every two weeks (W2), and once every four weeks (W3). In total, there were 48 treatment combinations per replication as given in Figure 5.

The experimental design was a three factor Split- Block, arranged in a Randomized Complete Block Design (RCBD) with three replications as shown in the Fig 5. The experimental design was chosen on the basis of convenience since both water and N factors are mobile making it necessary to strip water as a strip plot factor and apply N as the main plot factor. Nitrogen levels were the main plot factor, watering regimes being the strip-plot factor, while phosphorus was the sub-sub plot factor. Main plots measured 8 m x 2.75 m each, strip-plots were 2 m x 14 m each, and sub-sub plot 2 m x 0.5 m each. The main plots within a block were separated by 1 m paths. The strip- plots within a block were separated by 1 m paths, while sub-plots within a main plot were separated by 0.25 m paths. Individual blocks measured 8 m x 14 m, separated by a 2 m

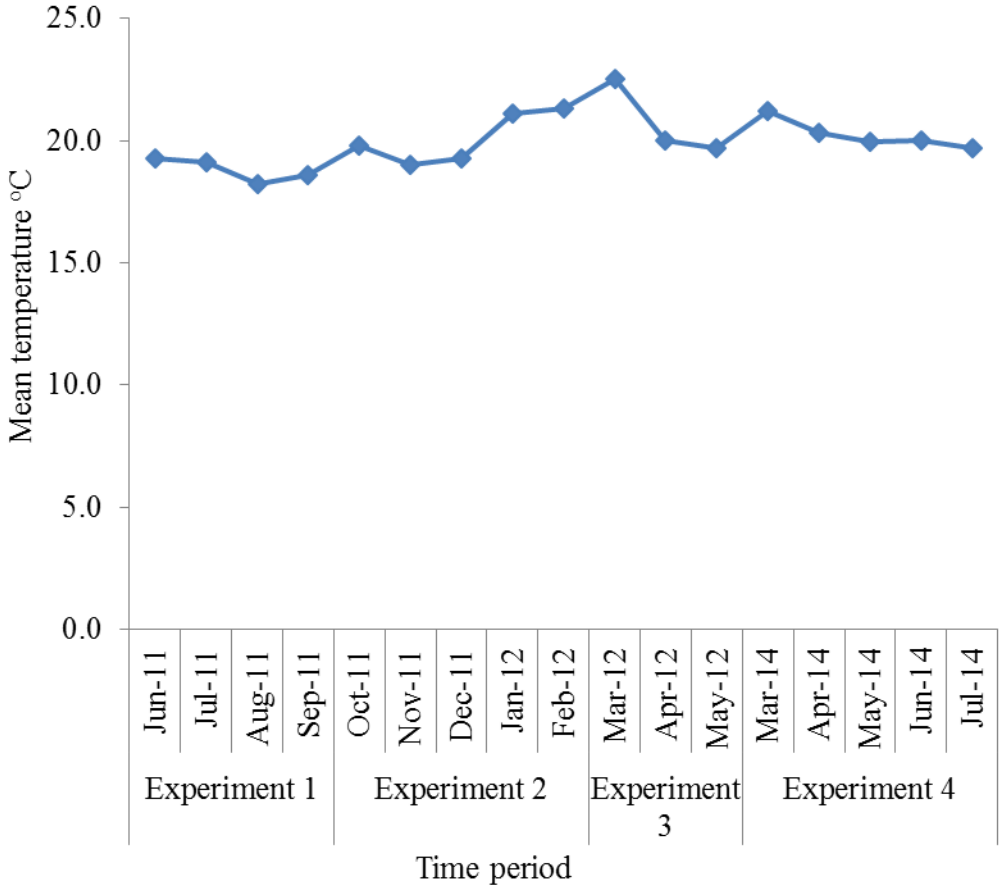


Fig. 4. Temperature Data for Egerton University Tatton Farm during the period of study. (Source: Egerton University Weather Station).

BLOCK 1

	N1					N4					N3					N2			
W1	**P4**	**P2**	**P1**	**P3**		**P3**	**P4**	**P1**	**P2**		**P3**	**P1**	**P4**	**P2**		**P2**	**P3**	**P1**	**P4**
W2	**P4**	**P2**	**P1**	**P3**		**P3**	**P4**	**P1**	**P2**		**P3**	**P1**	**P4**	**P2**		**P2**	**P3**	**P1**	**P4**
W3	**P4**	**P2**	**P1**	**P3**		**P3**	**P4**	**P1**	**P2**		**P3**	**P1**	**P4**	**P2**		**P2**	**P3**	**P1**	**P4**

BLOCK 2

	N3					N2					N1					N4			
W2	**P3**	**P1**	**P2**	**P4**		**P3**	**P2**	**P4**	**P1**		**P3**	**P4**	**P1**	**P2**		**P2**	**P3**	**P1**	**P4**
W1	**P3**	**P1**	**P2**	**P4**		**P3**	**P2**	**P4**	**P1**		**P3**	**P4**	**P1**	**P2**		**P2**	**P3**	**P1**	**P4**
W3	**P3**	**P1**	**P2**	**P4**		**P3**	**P2**	**P4**	**P1**		**P3**	**P4**	**P1**	**P2**		**P2**	**P3**	**P1**	**P4**

BLOCK 3

	N4					N1					N3					N2			
W3	**P3**	**P1**	**P4**	**P2**		**P2**	**P4**	**P3**	**P1**		**P4**	**P1**	**P3**	**P2**		**P4**	**P1**	**P3**	**P2**
W1	**P3**	**P1**	**P4**	**P2**		**P2**	**P4**	**P3**	**P1**		**P4**	**P1**	**P3**	**P2**		**P4**	**P1**	**P3**	**P2**
W2	**P3**	**P1**	**P4**	**P2**		**P2**	**P4**	**P3**	**P1**		**P4**	**P1**	**P3**	**P2**		**P4**	**P1**	**P3**	**P2**

KEY

Nitrogen levels	Phosphorus levels	Watering regimes
N1= 0 kg N/ha	P1= 0 kg P/ha	W1= once a week for four hours
N2= 40 kg N/ha	P2= 30 kg P/ha	W2=once every two weeks for four hours
N3= 80 kg N/ha	P3= 60 kg P/ha	W3=once every four weeks for four hours
N4= 120 kg Nha	P4=80 kg P/ha	

Fig. 5. Experimental Design and Field Layout

Nitrogen was applied in the form of urea [CO ($NH_{2)2}$ (46% N)] in two equal splits; the first one two weeks after planting and the second one four weeks later (Experiment 1). During the second and third experiment, the first split was applied one week after the first and second cuts, respectively, and the second split, six weeks after the first and second cuts, respectively. Application on the fourth experiment involved first split, two weeks after planting and the second one four weeks later.

Phosphorus was applied in the form of Triple Super Phosphate (TSP) (48% P_2O_5) and incorporated in the soil at planting (Experiment 1 and 4) while the same was applied one week after first and second cuts (Experiment 2 and 3) following weeding of the plots.

Watering at each regime was done until field capacity was achieved. The duration of irrigation water application was determined by using a Waterscout (Model SM 100 Sensor) connected to 2475 Plant Station (Watch Dog Model, Spectrum Technologies, Plainfirld, IL 60585, USA) based on the time taken to achieve field capacity.

3.3 Crop Establishment, Management and Harvesting

Before the crop was planted out, soil samples were taken at 30 cm and 45 cm depth. Soil samples were taken for laboratory nutrient analysis, at the then Kenya Agricultural Research Institute (KARI)- Njoro. Selected soil nutrients/characteristic determined are shown in Table 1.

Soil of the greenhouse was dug to a depth of approximately 20 cm. The layout of the experiment was done and then trenches 60 cm deep dug in between the beds and on the sides of the greenhouse in which a plastic sheet lining was mounted to prevent water from flowing from one treatment to another. Drip lines to be used for water application were also laid down, four lines per bed to serve five rows of plants per bed.

During planting, holes were made using a hoe. Fertilizer (TSP) (48% P_2O_5) was applied as per the layout. Plant splits of same height which is the common method of propagating sage were then planted at a spacing of 50 x 25 cm giving 15 plants per sub-plot. Thereafter, weeding was done manually as needed. Pests and diseases were also judiciously controlled whenever they were noticed using appropriate pesticides.

Crop management practices were conducted as is documented for the crop (Kintzios, 2000). Plant shoot cuttings were obtained from East African Dehydrates Company- Njoro, Kenya, and a rooting hormone applied to the cut end and planted into a propagation chamber where they were adequately watered and allowed to root. Since they were few, they were

Table 1. Selected soil chemical properties for the experimental site

Depth of collection of sample	pH	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
0-30 cm	6.1	1.14	3	334	655	405	0.68	25	1119	49
0-45cm	6.1	0.77	5	362	824	432	0.66	18	1040	49

transferred to a greenhouse where they were planted and allowed to multiply for four months. Splits were then obtained from these mother stock plants and used for planting.

Harvesting of the first crop was done at the end of the fifth month and subsequent second and third cuts were made at intervals of 14 weeks. Harvesting was achieved through cutting the plants at the base (15 cm above the ground). Only three inner plants per plot were cut for data collection. The rest of the plants were cut to the same level after data collection was completed to allow for re-growth.

3.4 Data Collection

3.4.1. Growth Parameters

Data collection on growth parameters (plant height, number of primary branches, number of secondary branches and number of internodes) was done on a weekly basis, starting two months after planting for the first crop, and one month after the cut for the subsequent cuts. Height was determined using a ruler on the terminal/main branch from the ground level to the tip of the branch, while the rest of the parameters were determined through counting with reference to the terminal main stem.

3.4.2. Yield Parameters

3.4.2.1 Leaf Fresh Weight (LFW) and Leaf Dry Weight (LDW)

After harvesting (cutting back), the candidate plants (inner row 3 plants) were stripped off all the leaves and weighed to determine the LFW. The fresh leaves were put in paper bags and placed in an oven at 65^{o}C and dried to a constant weight. They were then weighed using an electric balance once the leaf disks for the respective samples had been returned and readings recorded to give the LDW.

3.5.2.2 Leaf Area (LA) and Leaf Area Index (LAI)

A random sample of one hundred leaves per plot was taken for determination of the LA. This involved boring disks whose number was counted and recorded. The LA was determined by first determining the leaf area of sampled leaves by using a cork borer to maximally get leaf discs from each of the leaves, then using the radius of the cork borer (0.6 cm), the area of each disc was computed as 0.6 x 0.6 x 22/7=1.13cm^2 . The number of disks obtained from the sampled leaves was used to calculate the leaf area of all sampled leaves. The discs were then put in paper bags and oven dried at 65^{o}C to a constant weight. The dried discs were then weighed

and the relationship between area and dry weight of the discs was used to estimate leaf area as given by Remison (1997);

$$\text{LA} = \frac{\text{Area of discs} \times \text{Total leaf dry weight + dry weight of dried discs}}{\text{Dry weight of discs}}$$

Leaf Area Index (LAI) was then calculated by dividing leaf area of the plants in each sample by the ground area covered by the plants for which leaf area had been determined.

3.4.2.3 Specific Leaf Weight (SLW)

The SLW was determined as the ratio of total LDW to leaf area.

3.4.3 Analysis of Essential Oil Content and Total Phenolic Compounds (TPC)

3.4.3.1 Extraction of Essential Oil

Essential oil extraction was done for experiments 3 and 4 because it was going to be very expensive to do it for the four experiments. It was done from leaves that had been dried at 65^{o}C to a constant weight and stored in a dry and well ventilated room. The whole dried leaves were then weighed before (to determine the weight that would be used in calculating the percent essential oil yield on g/g basis) extraction of the essential oils, which was done by hydrodistillation using a Clevenger type apparatus for four (4) hours at 100^{o}C as described by Kapoor et al.(2004) and Sephidkon (2002). The essential oil was collected in vials and the essential oil yield determined by weighing the essential oil (grams) and subtracting the weight of the bottle. The oil in glass vials was then sealed and stored away from light in a refrigerator, awaiting GC-MS analysis which was conducted in Israel [Agricultural Research Organization (ARO), Volcani Centre, Newe Ya'ar].

3.5.3.2 GC-MS Analysis of Essentail Oil Components

Analysis of essential oil components was done for experiment 3 only because with a large sample size, it was too expensive to run it for all the experiments. Thirty (30)µl of each essential oil sample were diluted in 1 ml of Petroleum ether (40-60^{o}C Pesti-S Bio-lab) and analyzed with a GC/MSD apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto sampler Combi PAL (CTC Analytic, CH-Zwingen) and a Restek Rtx-5SIL MS cap. column (95% dimethyl/ 5% diphenyl polysiloxane, 30 m 0.25 mm i.d., film thickness 0.25 mm).

The oven temperature was programmed isothermal at 50^{o}C for 1 minute, rising from 50 to 200 ^{o}C at 5 ^{o}C /min and then from 200 to 280 ^{o}C at 20 ^{o}C /min, and finally held isothermal at 280 ^{o}C for 10 minutes; injector temperature, 250 ^{o}C; transfer line temperature, 280^{o}C. Helium

(He) was used as the carrier gas at constant pressure with retention-time locking. The pressure range was 8-14 psi (linear velocity of 32 cm/s at 8 psi) to achieve a constant retention time (RT) for the internal standard of 7.5 min on a non-polar column. The oil samples were injected in split (1:50) mode. The Mass Spectrophotometer (MS) was operated in Electron Ionization (EI) mode at 70 eV over an m/z range of 42-350 amu. The identification of the compounds was based on the comparison of the mass spectra, performed with MSDChem software (Agilent), with the commercial libraries Wiley 7, and HPCH2205. The Linear Retention Indices (LRI) determination was carried out by injecting a homologous series of n-alkanes under the same chromatographic conditions.

3.4.4 Total Phenolic Compounds (TPC) Assay

3.4.4.1 Extract Preparation

The total phenolic compounds were assayed for experiment 3. A sample of leaves dried at 65^{o}C to a constant weight was ground into powder using a mill. Approximately 50mg of finely powdered (ground) and thoroughly mixed plant material (sample) was put into a 50ml volumetric flask, and then 12.5ml Methanol/water (80/20, v/v) was added to the sample. The mixture was placed in the ultra-sound water bath for 30 minutes.The volume was made 1:1 (v/v) with water (double distilled) by adding 12.5ml of double distilled water and shaken by hand. The samples were then filtered into 50ml plastic sample containers and stored in the refrigerator at -5^{o}C until used for assay (with slight modification on that used in Sajid (PhD Thesis, Justus Liebig University, Giessen, 2011).

3.4.4.2 Total Phenolic Assay by Folic- Ciocalteu Method

The total phenolic compounds assay was done using the Folin-Ciocalteu micro method (Waterhouse, 2001) using gallic acid as a standard. The assay is electron transfer reaction based which measures the samples reducing capacity (Huang et al., 2005). Folin-Ciocalteu reagent consists of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids. Fourty (40)µL of the sample extract was mixed with 3.16ml double distilled water in a test-tube and 200 µL of Folin Ciocalteu reagent added. It was mixed at 2000rpm and left to stand for 5 minutes after which, 600 µL of saturated sodium carbonate solution was added. The reaction blend was again mixed at 2000 rpm and kept at 40^{o}C for 30 minutes in a water bath. Folin-Ciocalteu solution was reduced to blue oxides of tungsten and molybdenum and the absorbance was

measured spectrophotometrically at 765nM. The total phenolic content was expressed as Gallic Acid Equivalent (GAE).

3.5 Data Analysis

Yield and yield data obtained were analysed by Analysis of Variance (ANOVA) using MSTAT C (1991). For treatments found to be significantly different at F-test, means were separated using the Duncan's Multiple Range Test at $P \leq 0.05$. Essential oil yield was expressed as percentages based on sample dry weight, and percent composition of major essential oil components/compounds of sage were analysed by use of a GC-MS.

The model that was used to analyse the data was:

$$Y_{ijkl} = \mu + (\rho)_i + (\alpha)_j + (\rho\alpha)_{ij} + (\beta)_k + (\rho\beta)_{ik} + (\alpha\beta)_{jk} + (\rho\alpha\beta)_{ijk} + (\delta)_l + (\alpha\delta)_{jl} + (\beta\delta)_{kl} + (\alpha\beta\delta)_{jkl} + (\varepsilon)_{ijkl}$$

Where;

Y_{ijkl} = Observations corresponding to ith replication, k^{th} level of nitrogen, j^{th} level of watering and l^{th} level of phosphorus

μ = General mean

$(\rho)_i$ = i^{th} block effect

$(\alpha)_j$ = effect of j^{th} level of nitrogen

$(\rho\alpha)_{ij}$ = Interaction between i^{th}block and the j^{th} level of nitrogen

$(\beta)_k$ = effect of k^{th} level of watering

$(\rho\beta)_{ik}$ = Interactionbetween i^{th} block and the k^{th} level of watering

$(\alpha\beta)_{jk}$ = Interaction between the j^{th} level of nitrogen and the k^{th} level of watering

$(\rho\alpha\beta)_{ijk}$ = Interaction between i^{th} block, the j^{th} level of nitrogen and the k^{th} level of watering

$(\delta)_l$ = Effect of the l^{th} level of phosphorus

$(\alpha\delta)_{jl}$ = Interaction between the j^{th} level of nitrogen and the l^{th} level of phosphorus

$(\beta\delta)_{kl}$ = Interaction between the k^{th} level of watering and the l^{th} level of phosphorus

$(\alpha\beta\delta)_{jkl}$ = Interaction between j^{th} level of nitrogen, the k^{th} level of watering and the l^{th} level of phosphorus

$(\varepsilon)_{ijkl}$ = Interaction between i^{th} block, j^{th} level of nitrogen, the k^{th} level of watering and the l^{th} level of phosphorus.

The error components $(\rho\alpha)_{ij}$, $(\rho\beta)_{ik}$, $(\rho\alpha\beta)_{ijk}$ and $(\varepsilon)_{ijkl}$ are independently and normally distributed with means zero and respective $\delta^2 a, \delta^2 b, \delta^2 c$ and $\delta^2 \varepsilon$.

CHAPTER FOUR

4.0 RESULTS

This chapter presents results on the effects of N, watering and P regimes on (i) sage growth measured as plant height, number of primary branches/plant, number of secondary branches/plant, number of internodes/plant, LAI and SLW, (ii) yields as LFW and LDW and (iii) TPC, essential oil yield and composition.

4.1.1 Effects of N, Watering and P Regimes on the Growth of Sage

Results on the effects of N, watering and P regimes on plant height, number of primary branches/plant, number of secondary branches/plant, number of internodes/plant, leaf area index and specific leaf area are presented in this section.

4.1.1.1 Effects of N, Watering and P Regimes on Plant height

Sage height was significantly ($P \leq 0.5$) affected by N levels, at particular stages of plant growth and only in experiments 1 and 4 (Fig. 6a and b). During experiment 1, N significantly influenced plant height from week 5 through to the first cut (week 14), with the greatest response noted on plots that received 40 kg N/ha and the least on plots that received 120 kg N/ha (Fig 6a). However, during experiment 4, plant height was significantly affected by nitrogen application at week 7 with the tallest plants on 120 kg N/ha and shortest on plots that received no N (Fig. 6b). No significant ($P \leq 0.5$) effects of N were noted for plant height during the second and third experiments (Fig. 6a and b).

Watering regimes significantly ($P \leq 0.05$) affected plant height in experiments 1 and 4 at weeks 13 and 14 in experiment 1, and week 2 through to harvesting (week 8) in experiment 4 (Fig. 7a and b). It was observed that watering once after every two weeks resulted in the tallest plants than the rest of the watering treatment while plants were shortest on watering once after four weeks for both experiments 1 and 4 (Fig. 7a and b).

Plant height was significantly influenced by P in experiment 1 at week 2 upto week 5 and then week 12, whereas in experiment 2, it significantly affected plant height between week 7 and 8 (Fig. 8a). During experiment 3, the significant effect was noted during weeks 6, 7 and 8, and in experiment 4, the effects were noted throughout the growth period (Fig. 8b). During the first experiment, the highest height response was noted with 30 kg P/ha and lowest on plots that

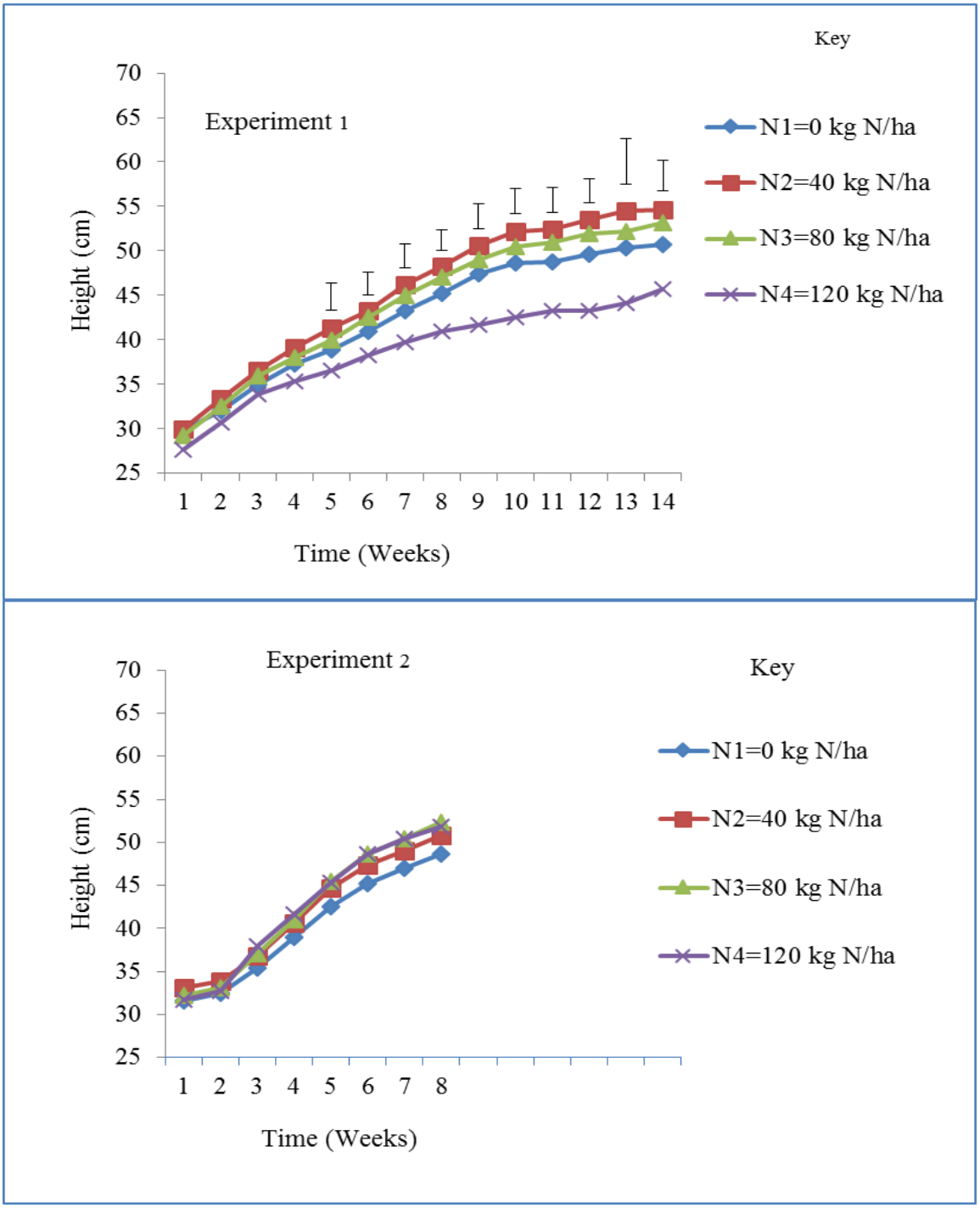


Fig. 6a. Effects of nitrogen on plant height (I=LSD at $P \leq 0.05$)

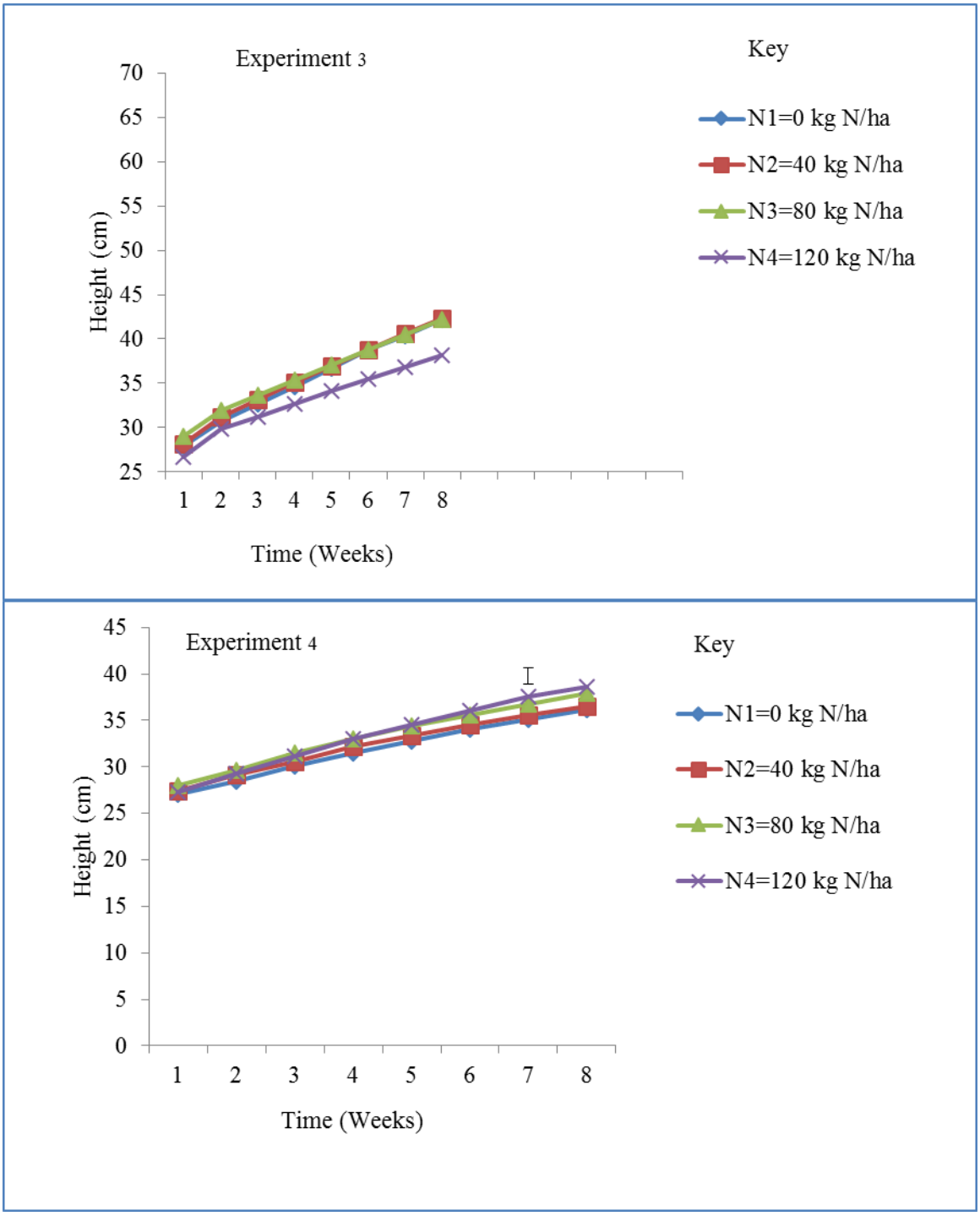


Fig. 7b. Effects of nitrogen on plant height (I=LSD at $P \leq 0.05$)

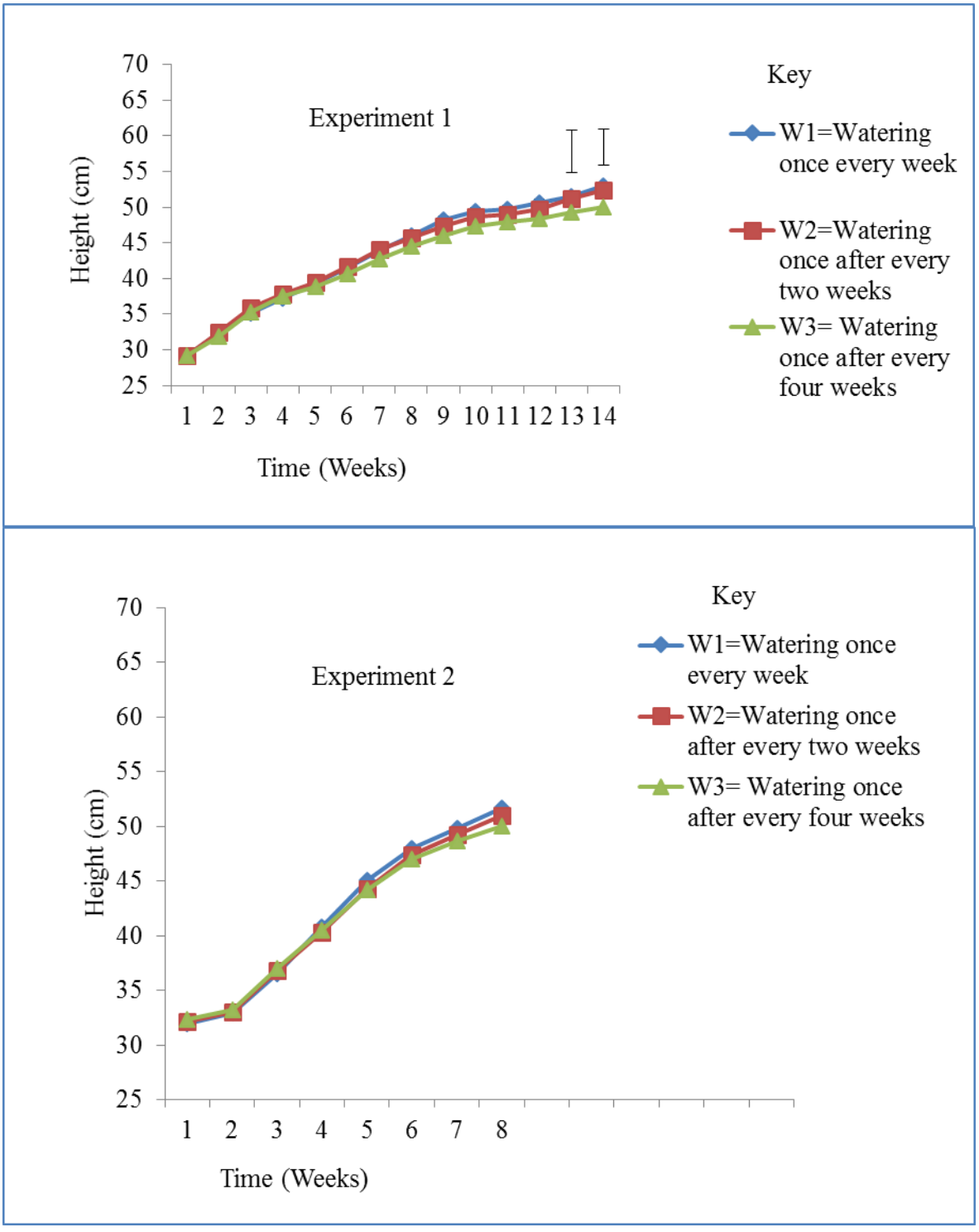


Fig. 7a. Effects of watering on plant height (I=LSD at $P \leq 0.05$)

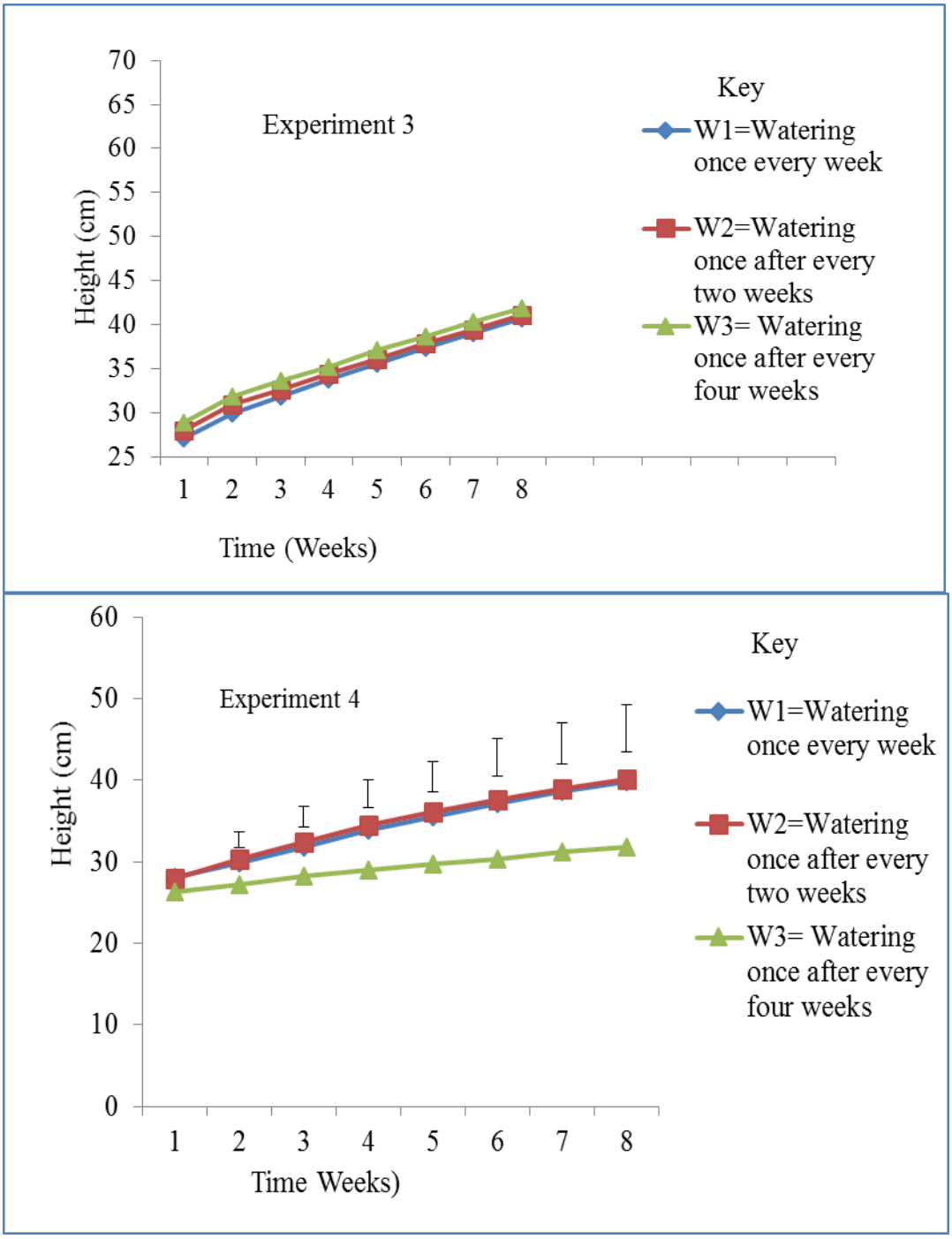


Fig. 7b.Effects of watering on plant height (I=LSD at $P \leq 0.05$)

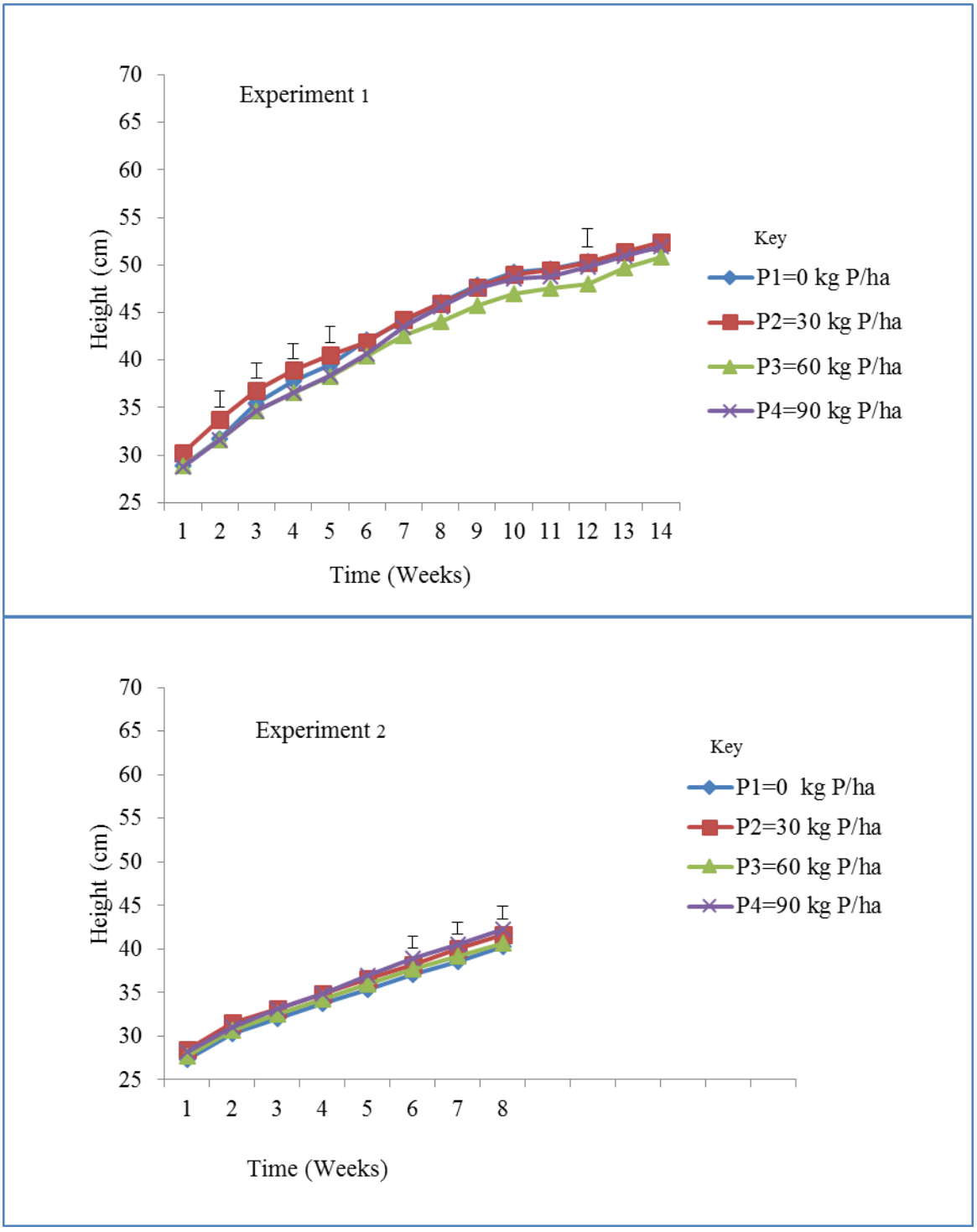


Fig. 8a. Effects of phosphorus on plant height (I=LSD at $P \leq 0.05$)

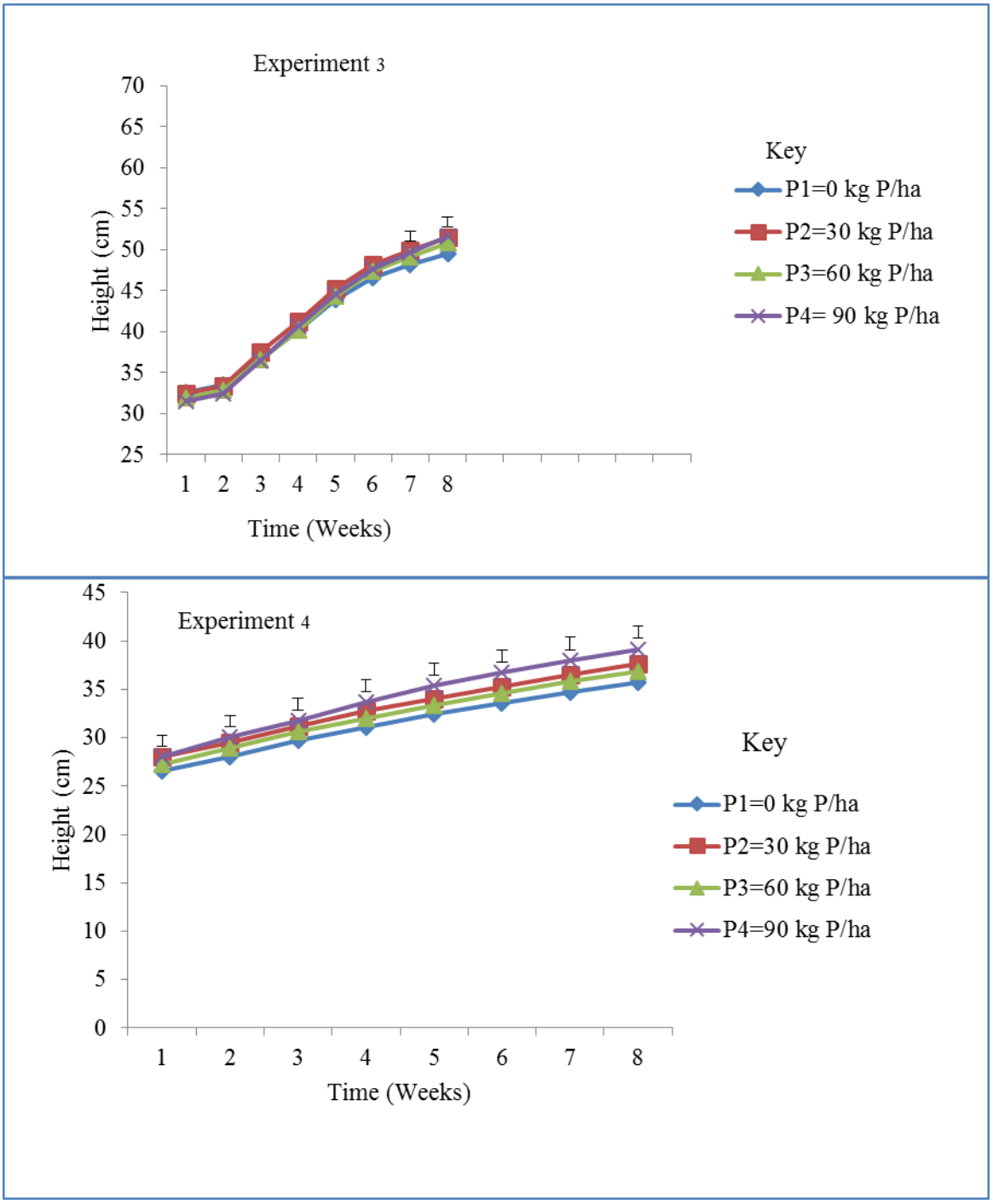


Fig. 8b. Effects of phosphorus on plant height (I=LSD at $P \leq 0.05$)

This trend however changed during the second and third experiments. Except in weeks 1, 2 and 8, the highest plant height was attained with 30 kg P/ha in experiment 2, and during experiments 3 and 4, 90 kg P/ha resulted in taller plants from week 3 to 8and 2 to 8, respectively. On the other hand, the shortest plants were recorded on plots that received 60 kg P/ha in experiment 1, while higher levels and no P resulted into shorter plants during early and later stages of growth, respectively, in the second experiment. During the third and fourth experiment, plants receiving no P were the shortest in height (Fig. 8b). There were significant three-way interaction effects between N, watering, and P during the first and second experiments (Tables 2 and 3), while the interaction effects were not significant for experiments 3 and 4 (Appendix 1 and 2).

Shortest plants (39.7 cm, 41.67 cm and 35.67 cm) were found on plots that received 80 kg N/ha, were watered once after every four weeks with 60 kg P/ha and in experiments 1, 2 and 3 (Tables 2 and 3; Appendix 1), respectively. During the fourth experiment, however, shortest plants (28.7 cm) were recorded on plots that received no N x no P or 40 kg N/ha x 30 kg P/ha when watered after every four weeks (Appendix 2). For experiments 1, 2, 3 and 4 the height increase as affected by all the treatments was 20 cm (33.5%), 23.03 cm (35.6%), 14 (28.2%), and 18.3 cm (38.9%) (Tables 2 and 3; Appendices 4 and 5).

4.1.1.2. Effects of N, Watering and P Regimes on Number of Primary Branches/Plant

Nitrogen had significant effect ($P \leq 0.05$) on the number of primary branches inexperiments 2 and 4 (starting in week 3) (Fig. 9a and b). The number of primary branches per plant positively responded to increasing levels of N application with the highest numbers observed at 120 kg N/ha and lowest on plants that received no N fertilizer in both experiments (Fig. 9a and b). However, no significant effects were observed for this parameter in experiments 1 and 3 (Fig. 9a and b).

Watering significantly influenced the number of primary branches per plant in experiment 1 at week 1, and later in weeks 10 to 14 (Fig. 10a). Significant watering effects were also observed during experiment 4 at week 3 through to week 8 (Fig. 10b). Watering once every week and once every two weeks favoured development of more primary branches as compared to watering once after every four weeks which resulted into least primary branches in experiment 1 (Fig. 10a). In experiment 4, watering once a week had significantly higher number of primary branches than once every two or four weeks (Fig. 10b). On the other hand, watering had no significant effects on number of primary branches in experiments 2 and 3 (Fig.10a and b).

Table 2. Effects of nitrogen, watering and phosphorus on plant height (Experiment 1)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	51.0c-k**	49.3e-l	49.3e-l	49.0f-l	
	W2	53.3a-h	56.0a-g	52.0a-k	53.0a-i	
	W3	41.7lm	47.3h-m	44.0k-m	46.0h-m	**49.3***
40	W1	53.0a-i	55.7a-g	50.3d-k	49.3e-l	
	W2	52.7a-j	56.3a-f	58.0a-d	53.3a-h	
	W3	51.3b-k	45.7h-m	44.0k-m	47.0h-m	**51.4**
80	W1	50.3d-k	57.0a-c	51.7a-k	52.3a-j	
	W2	57.3a-b	55.7a-g	59.7a	56.0a-g	
	W3	48.0g-l	45.0i-m	39.7m	51.3b-k	**52.0**
120	W1	48.3f-l	53.3a-h	50.3d-k	51.3b-k	
	W2	58.7a-c	51.3b-k	51.3b-k	57.0a-e	
	W3	46.0h-m	42.0l-m	45.3j-m	44.7j-m	**50.0**
P Means		**51.0**	**51.2**	**51.2**	**50.9**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

**Values followed by the same letter within a column are not significantly different according to the Duncan's Multiple Range Test at $P \leq 0.05$

Table 3. Effects of nitrogen, watering and phosphorus on plant height (Experiment 2).

Nitrogen	**Watering**	**Phosphorus (kg P/ha)**				
(kg N/ha)		None	30	60	90	**N Means**
None	W1	47.3r-x**	43.0w-y	43.7u-y	46.0t-y	
	W2	47.0s-x	46.3t-y	48.0q-w	46.7t-y	
	W3	52.3n-r	56.0k-n	53.7m-p	54.0m-p	**48.67***
40	W1	58.3j-m	59.3j-l	58.3j-m	62.7i-j	
	W2	44.3u-y	46.3t-y	45.7t-y	44.3u-y	
	W3	45.7u-y	47.7q-x	48.7q-u	48.3q-v	**50.81**
80	W1	48.0q-w	55.3l-o	56.0k-n	54.0m-p	
	W2	58.3j-m	60.7i-k	64.7i	58.7j-m	
	W3	42.7x-y	44.0u-y	41.7y	43.3v-y	**52.28**
120	W1	48.0q-w	50.7o-t	47.0s-x	48.3q-v	
	W2	49.7p-t	52.3n-r	52.0n-s	50.7o-t	
	W3	52.7n-q	56.0k-n	57.0k-n	57.0k-n	**51.78**
P Means		**49.5**	**51.5**	**51.4**	**51.2**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

**Values followed by the same letter within acolumn are not significantly different according to the Duncan's Multiple Range Test at $P \leq 0.05$

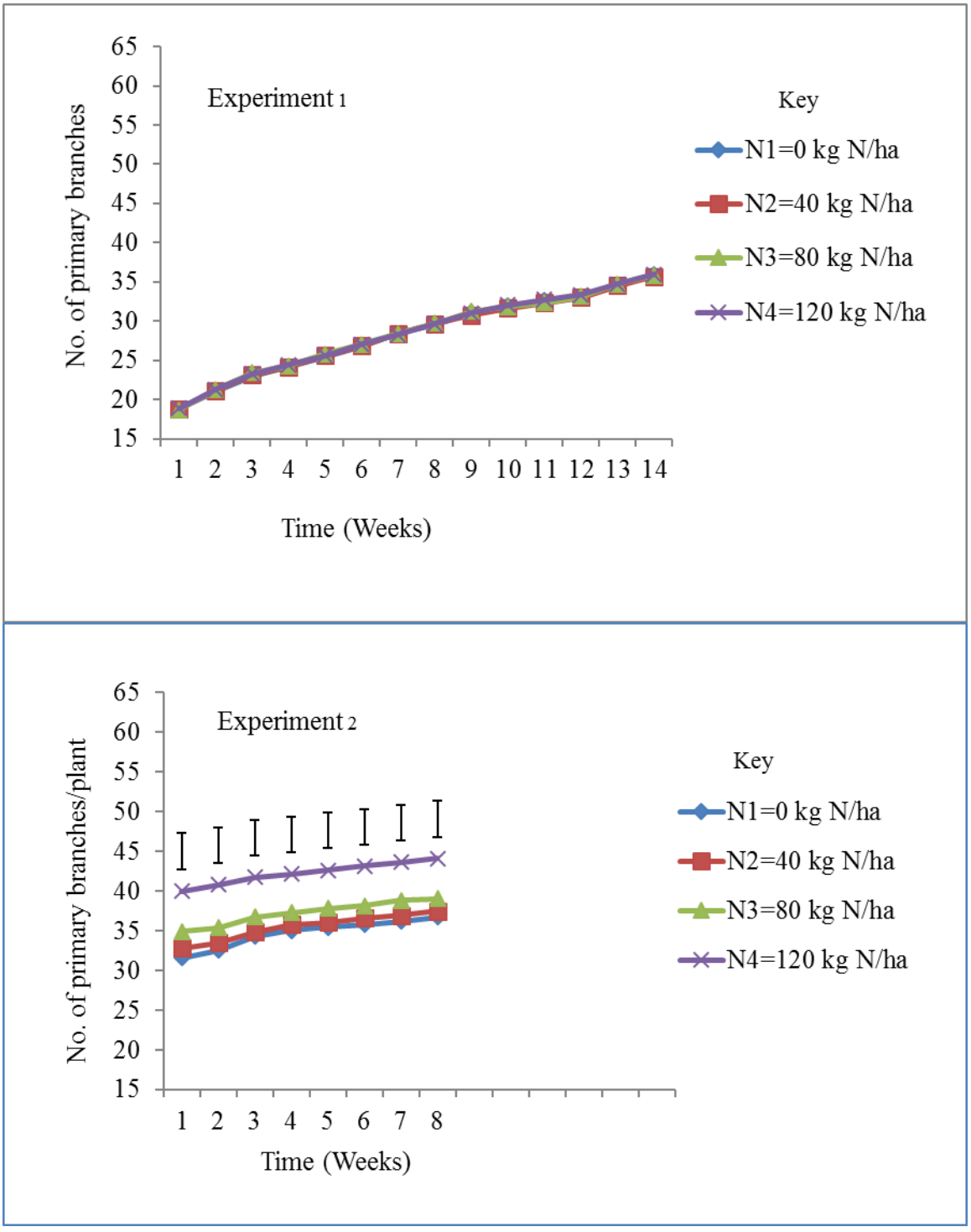


Fig. 9a. Effects of nitrogen on the number of primary branches/plant (I=LSD at P ≤ 0.05)

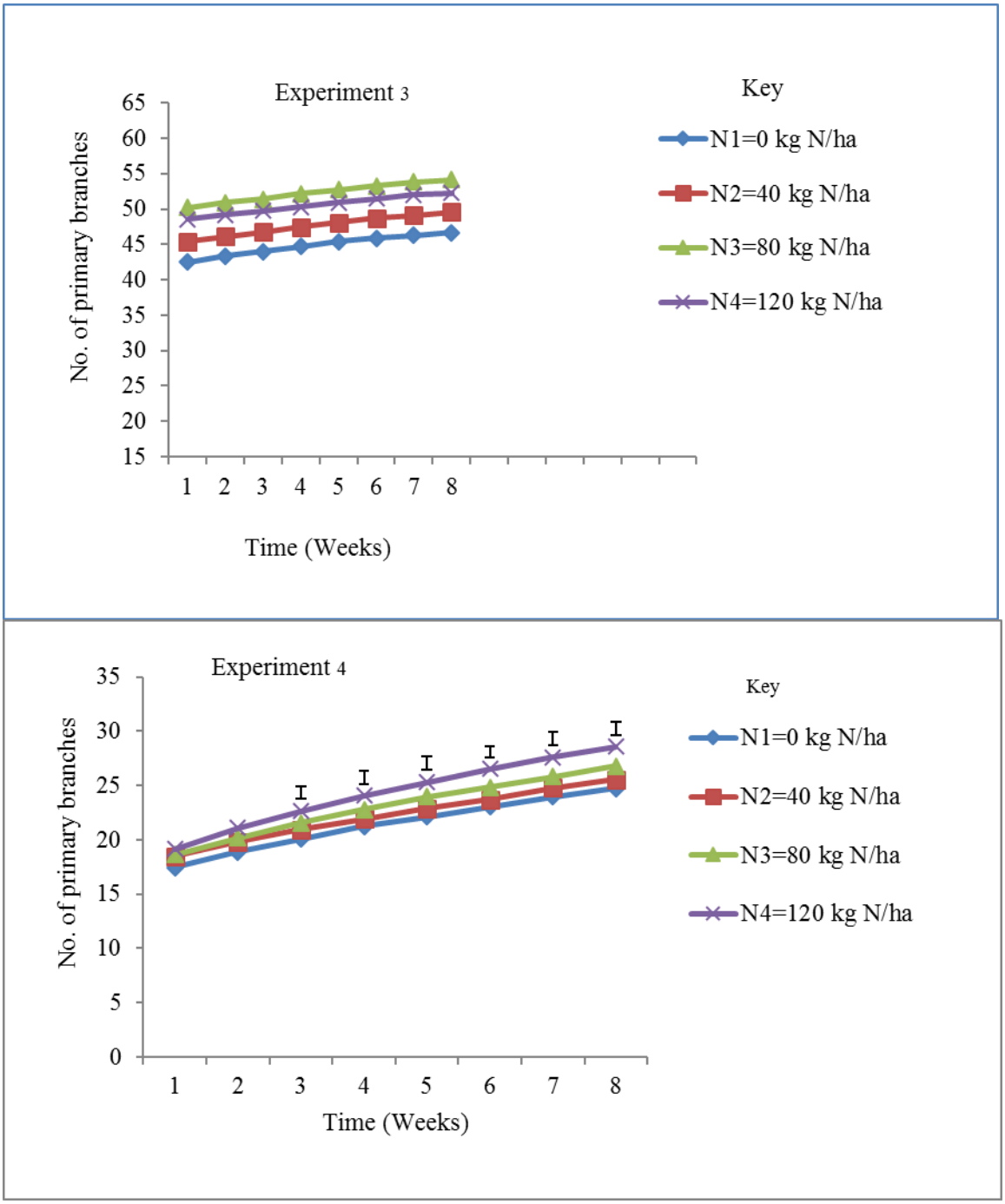


Fig. 9b. Effects of nitrogen on the number of primary branches/plant (I=LSD at $P \leq 0.05$)

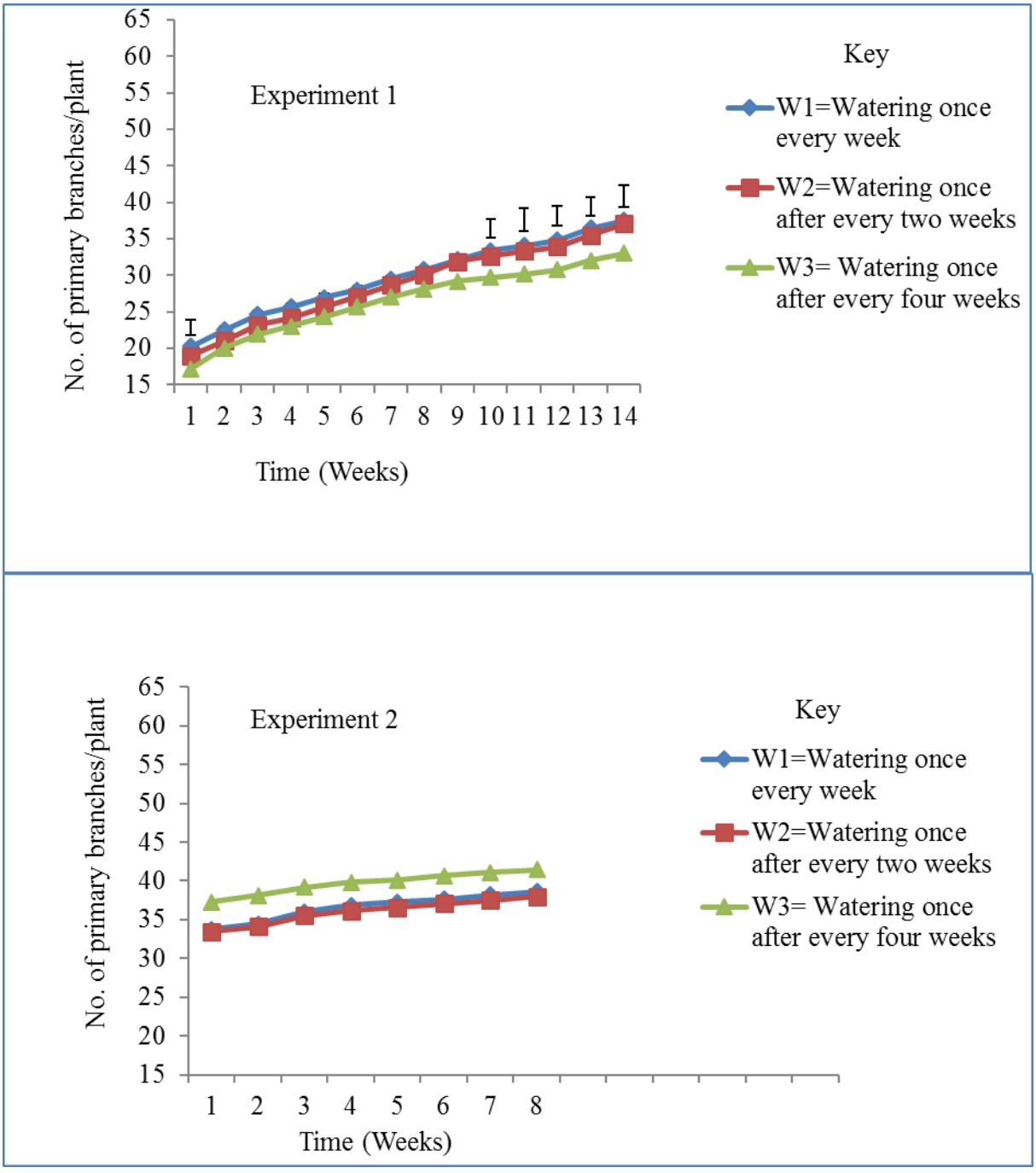


Fig. 10a. Effects of watering on the number of primary branches/plant (I=LSD at $P \leq 0.05$)

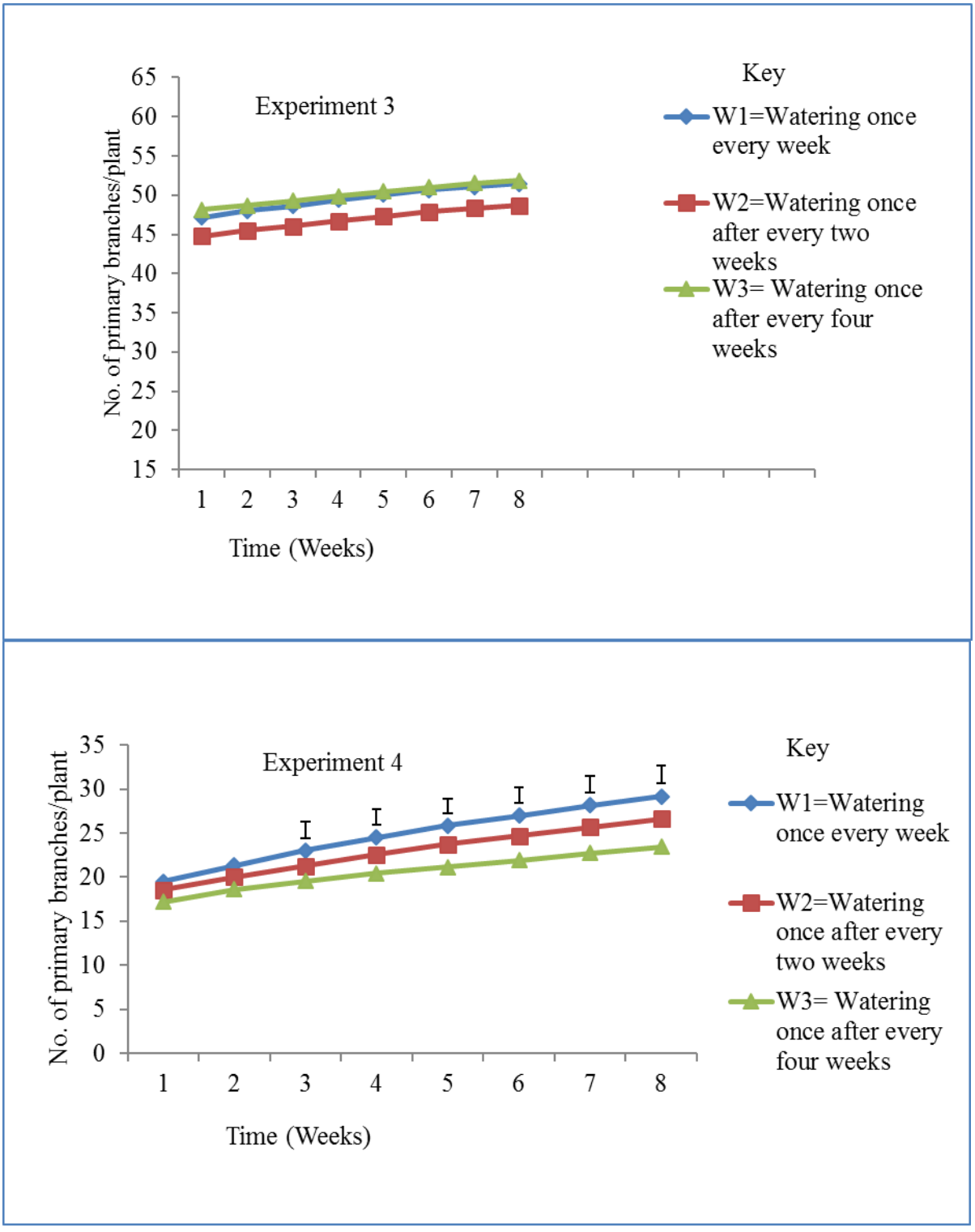


Fig. 10b. Effects of watering on the number of primary branches/plant (I=LSD at $P \leq 0.05$)

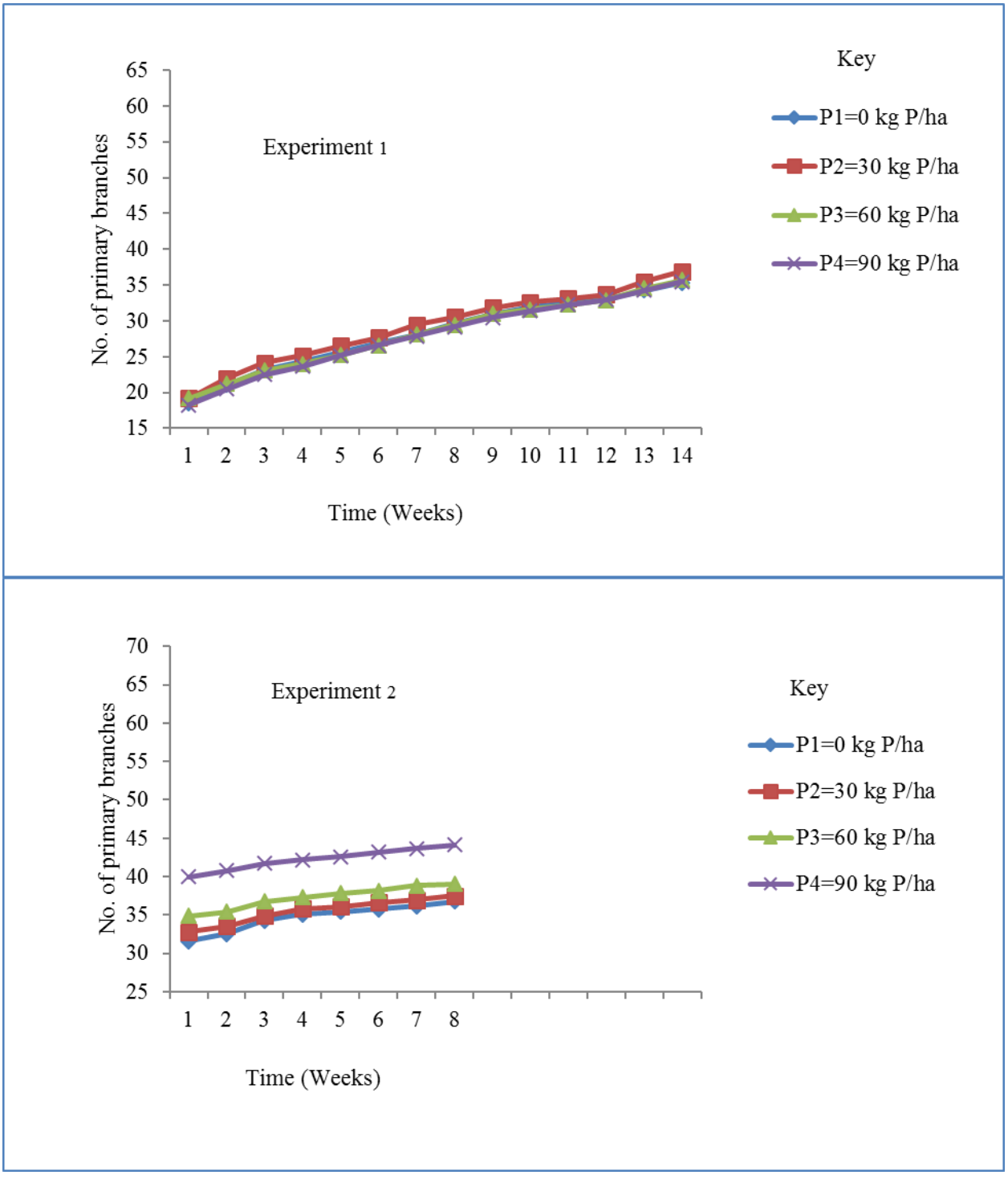


Fig.11a. effects of phosphorous on the number of primary branches/plant (I=LSD at P ≤ 0.05)

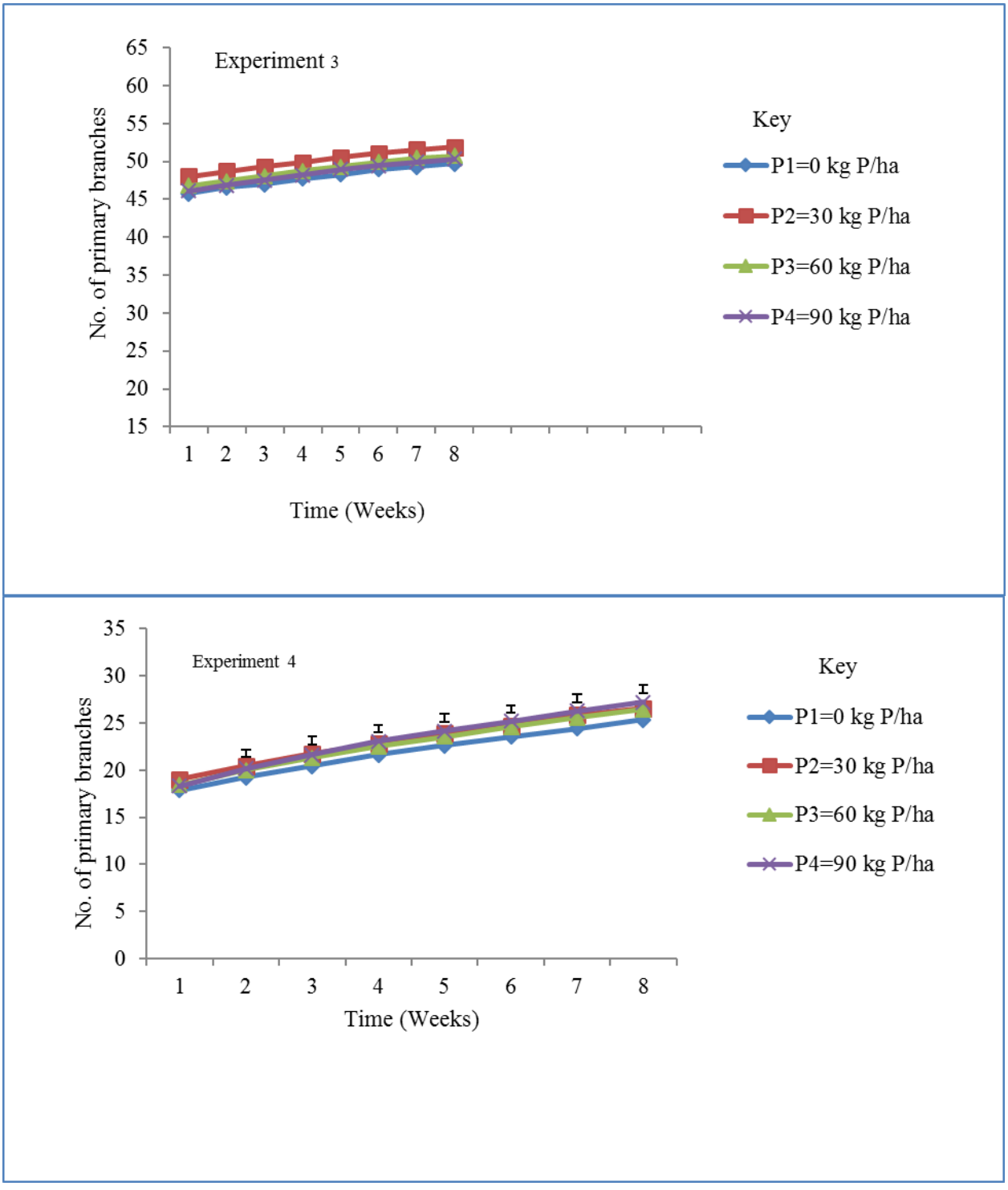


Fig. 11b. Effects of phosphorus on the number of primary branches/plant,(I=LSD at $P \leq 0.05$)

Phosphorus significantly ($P \leq 0.05$) influenced the number of primary branches/ plant in experiment 4 (Fig. 11b). Except at weeks 2 and 3, the greatest numbers of primary branches/plant were recorded with 90 kg P/ha and the lowest on no P fertilizer. No significant effects ($P \leq 0.05$) were observed in experiments 1, 2 and 3 (Fig. 11a and b). There were no significant interaction effects ($P \leq 0.05$) on the number of primary branches/plant among the. different experiments as shown in Appendices 3,4,5 and 6. No trend was noted for this parameter in all the experiments.

4.1.1.3 Effects of N, Watering and P Regimes on the Number of Secondary Branches/ Sage Plant

There were significant effects ($P \leq 0.05$) of N on the number of secondary branches/plant in experiment 2, 3 and 4, in weeks 3 and 4; 4 and 5 and 2 through to 8, respectively (Fig. 12a and b). Nitrogen had no significant effects on number of secondary branches/plant in experiment 1 (Fig, 12a). The general trend was that no N resulted in higher number of secondary branches/plant while 120 kg N/ha gave the lowest (Fig.12a and b).

Watering had significant ($P \leq 0.05$) influence on the development of secondary branches/plant in experiment 4 and not in experiments 1, 2 and 3. (Fig.13a and b). In experiment 4, watering once every week resulted in higher number of secondary branches/plant compared to watering once after every four weeks while watering once after every two weeks gave intermediate results (Fig. 13b).

Phosphorus treatment had significant ($P \leq 0.05$) effect on the number of secondary branches/plant in experiments 1 (at weeks 1, 4, 5, 6, 7 and 13) and 2 (at week 4 through to 8) (Fig. 14a). No significant effect was observed in experiments 3 and 4 (Fig.14b). In experiment 1, the general trend was a higher number of secondary branches/plant where 30 kg P/ha was applied and lowest with 90 kg P/ha. In Experiment 2, plants growing without P generally gave lower numbers of secondary branches/plant though closely overlapping with the 90 kg P/ha, with higher values recorded for 30 and 60 kg P/ha (Fig. 14a).N x watering x P interaction effects was not significant ($P \leq 0.05$) in all experiments (Appendices 7,8,9 aand 10).

4.1.1.4 Effects of N, Watering and P Regimes on Number of Internodes per Sage Plant

Nitrogen significantly ($P \leq 0.05$) influenced the production of internodes per plant later in experiment 1 (week 11); experiment 2 (at week 7 and 8) and experiment 4 (week 1 to 8). There were no significant ($P \leq 0.05$) effects of N on the number of internodes/plant in experiment 3

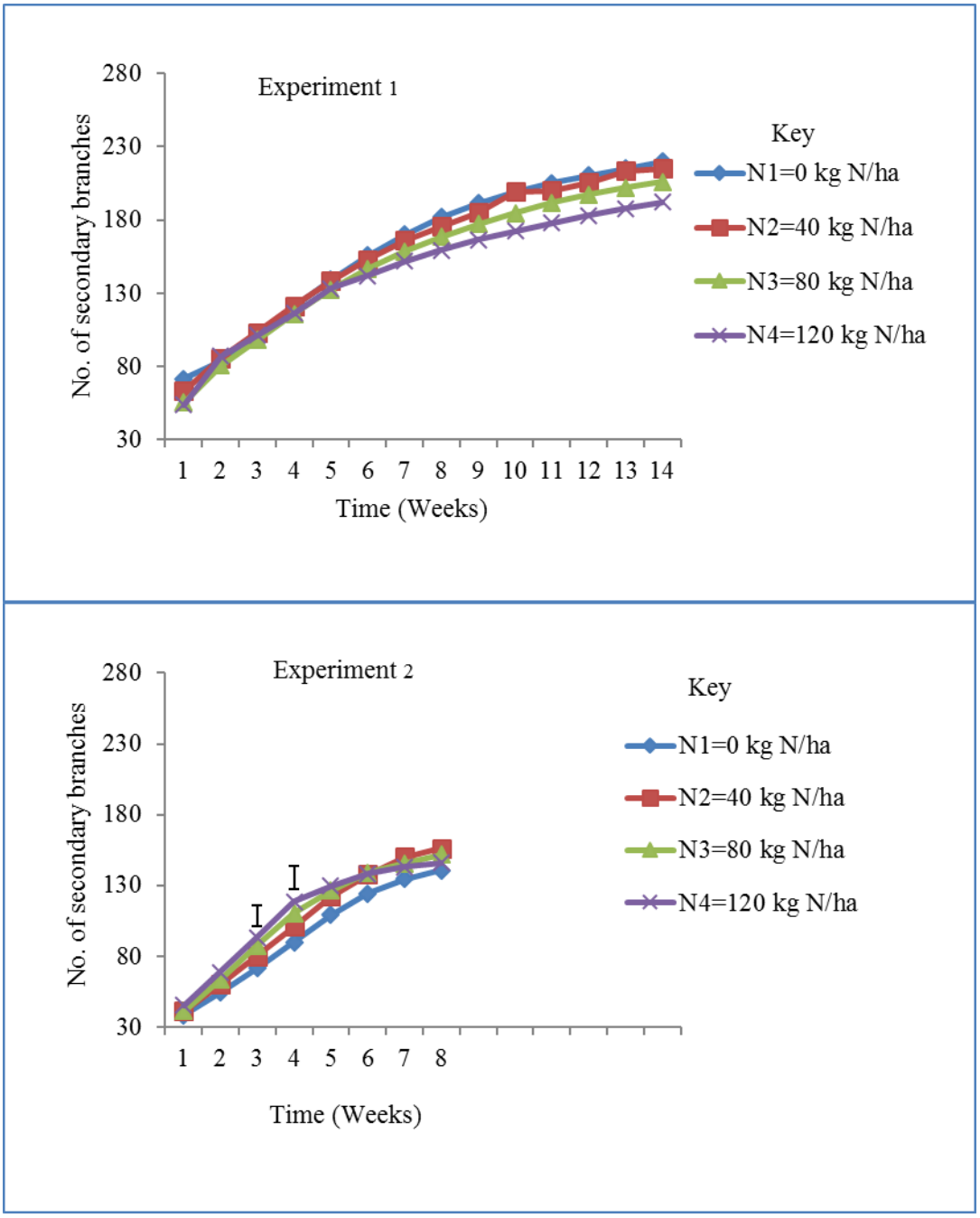


Fig. 12a. Effects of nitrogen on the number of secondary branches/plant (I=LSD at $P \leq 0.05$)

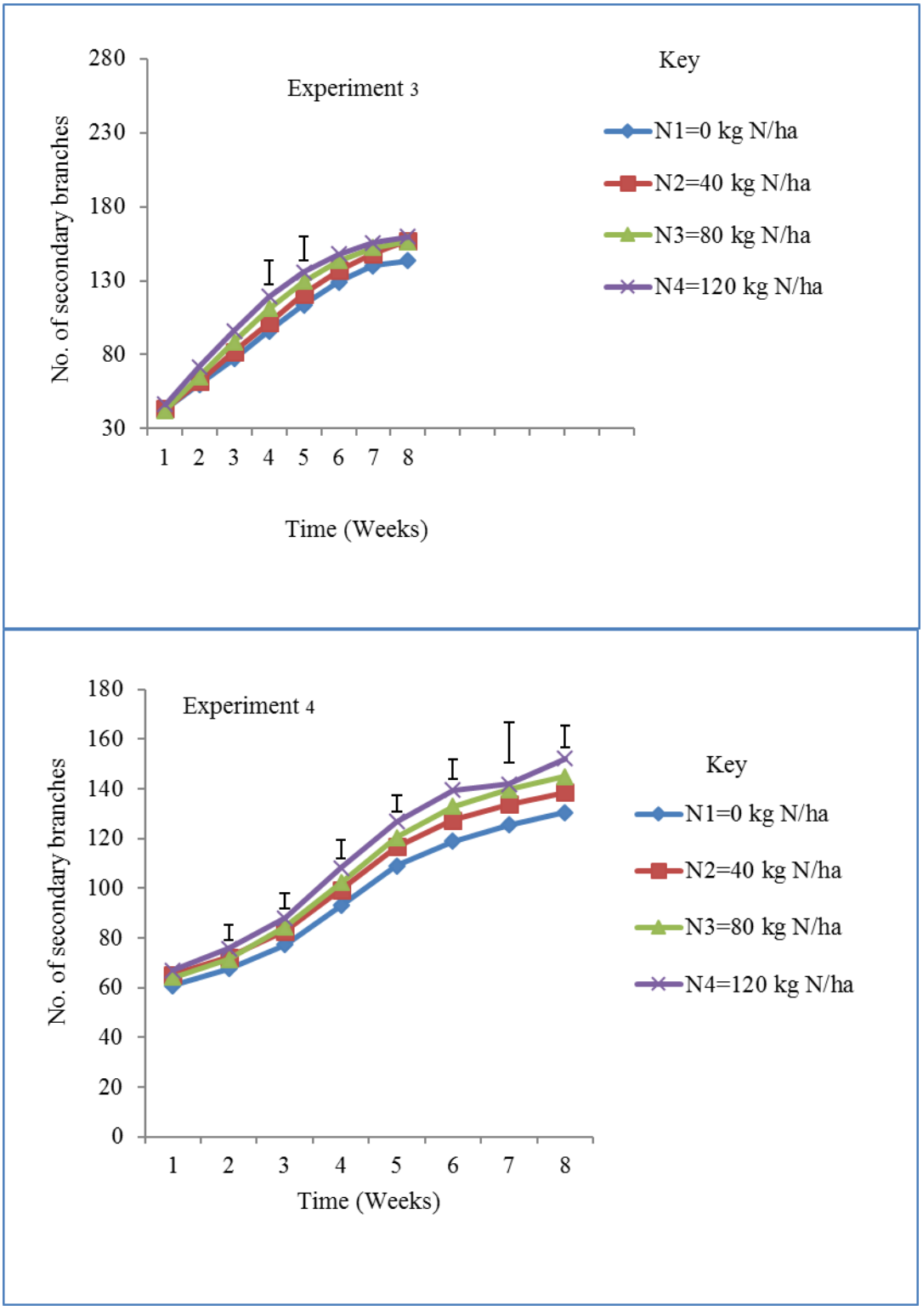


Fig. 12b. Effects of nitrogen on the number of secondary branches/plant (I=LSD at $P \leq 0.05$)

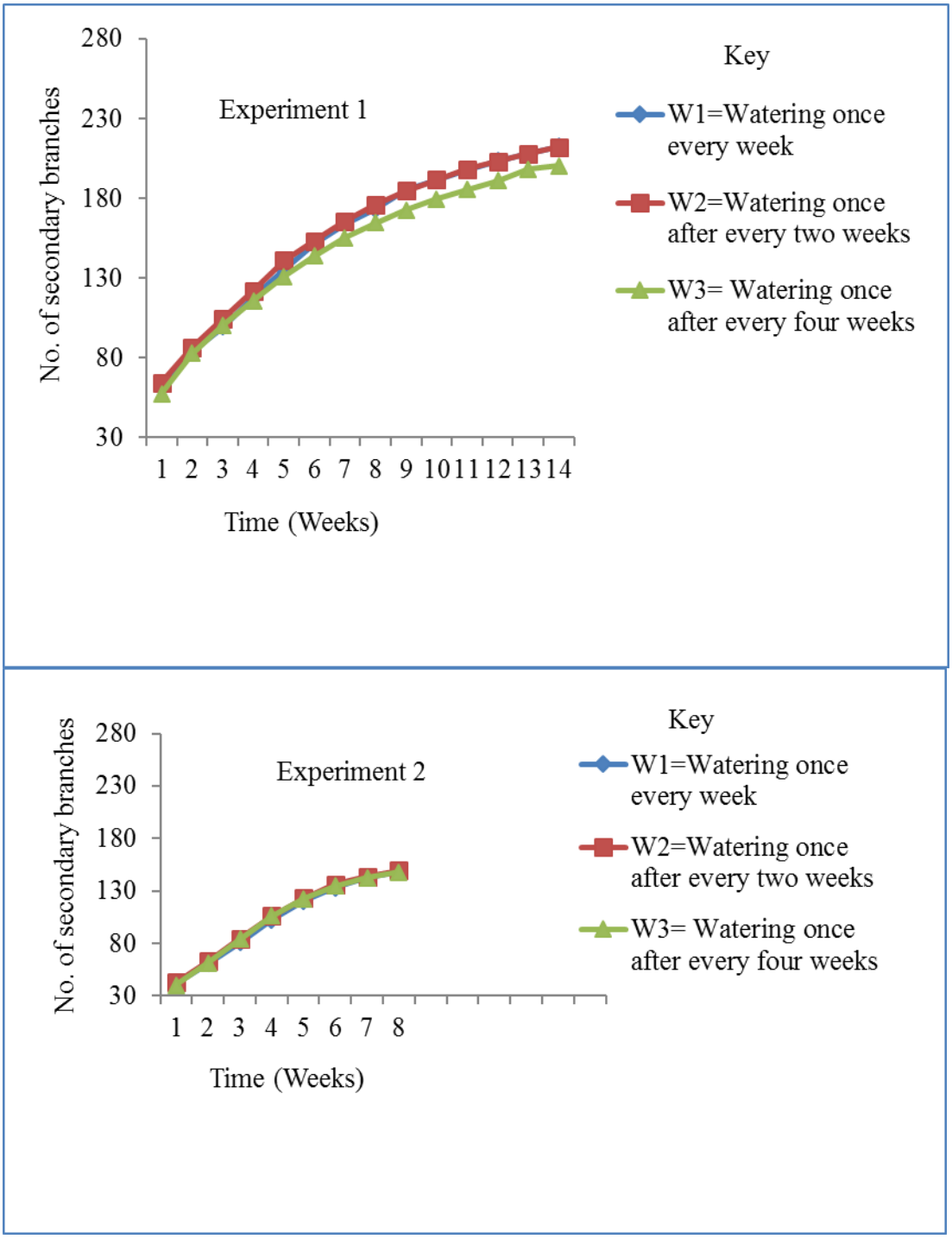


Fig. 13a. Effects of watering on the number of secondary branches/plant (I=LSD at $P \leq 0.05$)

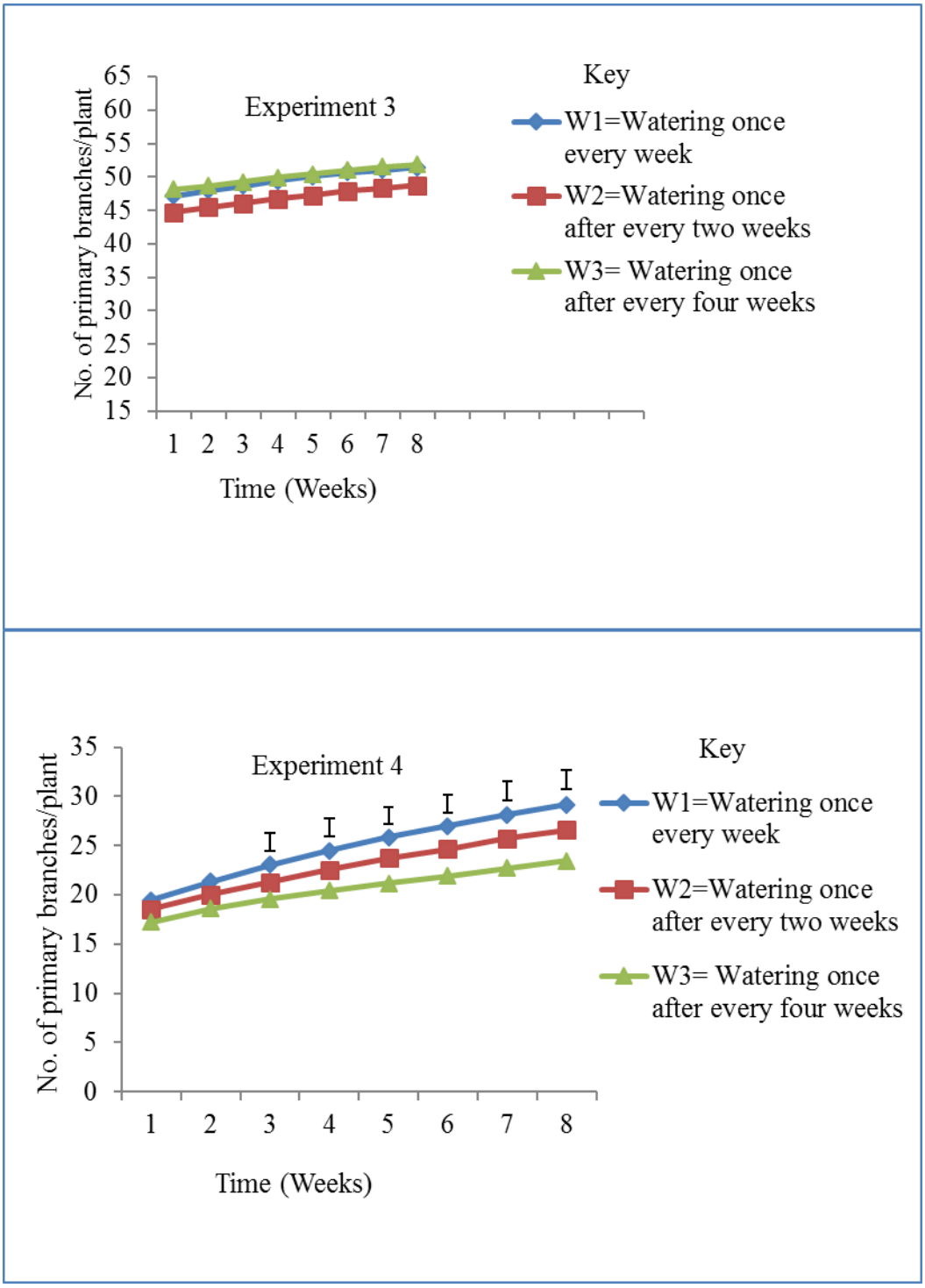


Fig. 13b. Effects of watering on the number of secondary branches/plant (I=LSD at $P \leq 0.05$)

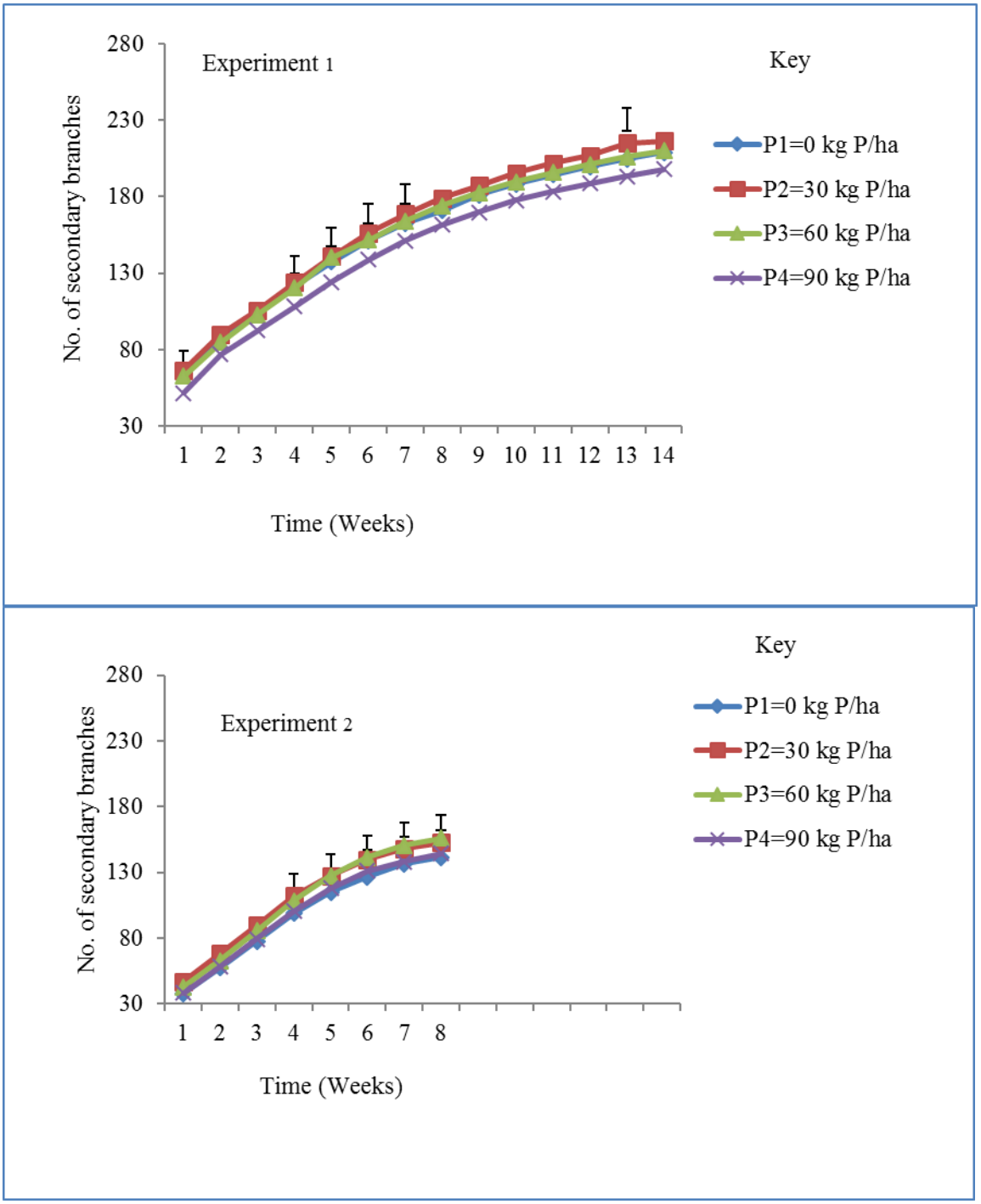


Fig. 14a. Effects of phosphorus on the number of secondary branches/plant (I=LSD at $P \leq 0.05$)

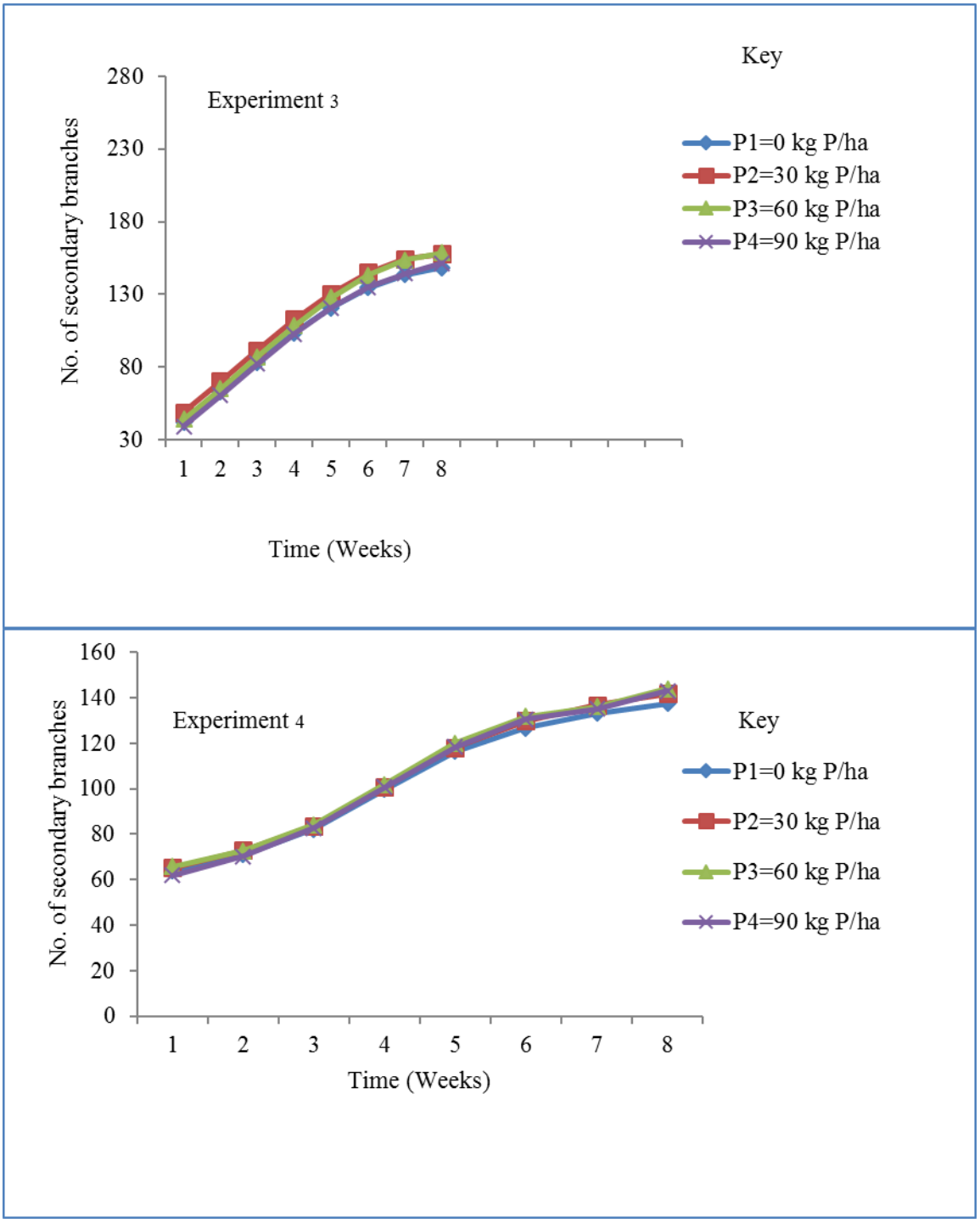


Fig. 14b. Effects of phosphorus on the number of secondary branches/plant (I=LSD at $P \leq 0.05$)

(Fig. 15a and b). There were significant ($P \leq 0.05$) effects of watering on the number of internodes/plant in experiment 1 (week 7) while no significant effects were observed for experiments 2, 3 and 4 (Fig. 16a and b). Watering once after every four weeks resulted into more number of internodes per plant while watering once after every two weeks resulted into the least number of internodes per plant (Fig. 16a and b).

Except for experiment 3, P significantly ($P \leq 0.05$) influenced the number of internodes/plant in experiments 1 (week 6 and 7), 2 (week 3) and 4 (weeks 1 to 8) (Fig. 17a and b). The number of internodes/plant tended to be high with 120 kgN/ha. There were no significant interaction effects between

N, watering and P regimes on the number of internodes/plant and no trends were also observed (Appendices 11, 12, 13 and 14).

4.1.1.5 Effects of N, Watering and P Regimeson LAI

The highest LAI values though not significant ($P \leq 0.05$), were reported on 80 kg N/ha combined with 60 kg P/ha and watering once after every two weeks across the experiments 1 (6.90), 2 (5.98), 3 (6.70) and 4 (11.06), while the lowest was 2.90, 1.99, 2.30 and 2.20 in experiments 1, 2, 3 and 4, respectively, recorded on plots that received no nitrogen, watered once after every four weeks and received the highest level of phosphorus (90 kg P/ha) (Tables 4, 5, 6 and 7).

4.1.1.6 Effects of N, Watering and P Regimes on SLW

No significant ($P \leq 0.05$) effects of both main factor and interactions were noted for the SLW, and no trends in response were observed for all the experiments (Appendices 15, 16, 17 and 18).

4.1.2 Effects of N, Watering and P Regimes on Yield

4.1.2.1 Effects of N, Watering and P Regimes on LFW

There were no significant effects of single factors on LFW (Table 8). However, nitrogen x phosphorus interaction was significant only in experiment 4, where the highest leaf fresh weight was recorded on plots that received no nitrogen x 30 kg P/ha, and lowest on no nitrogen x 60 kg P/ha (Table 8). No significant interactions (N x W x P) were noted during the experiments 1, 2, 3, and 4 (Tables 9, 10, 11 and 12).

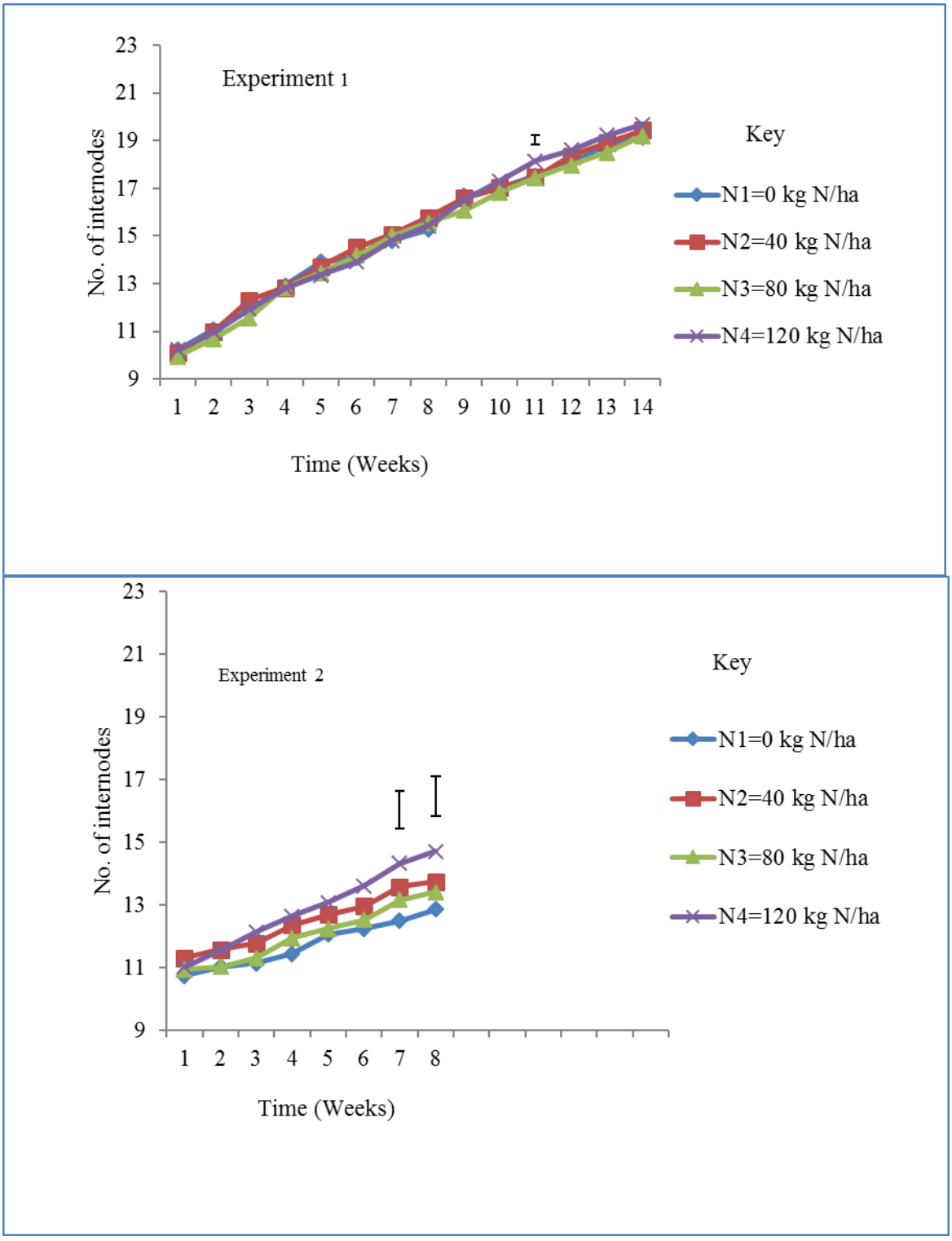


Fig. 15a. Effects of nitrogen on the number of internodes/plant (I=LSD at P ≤ 0.05)

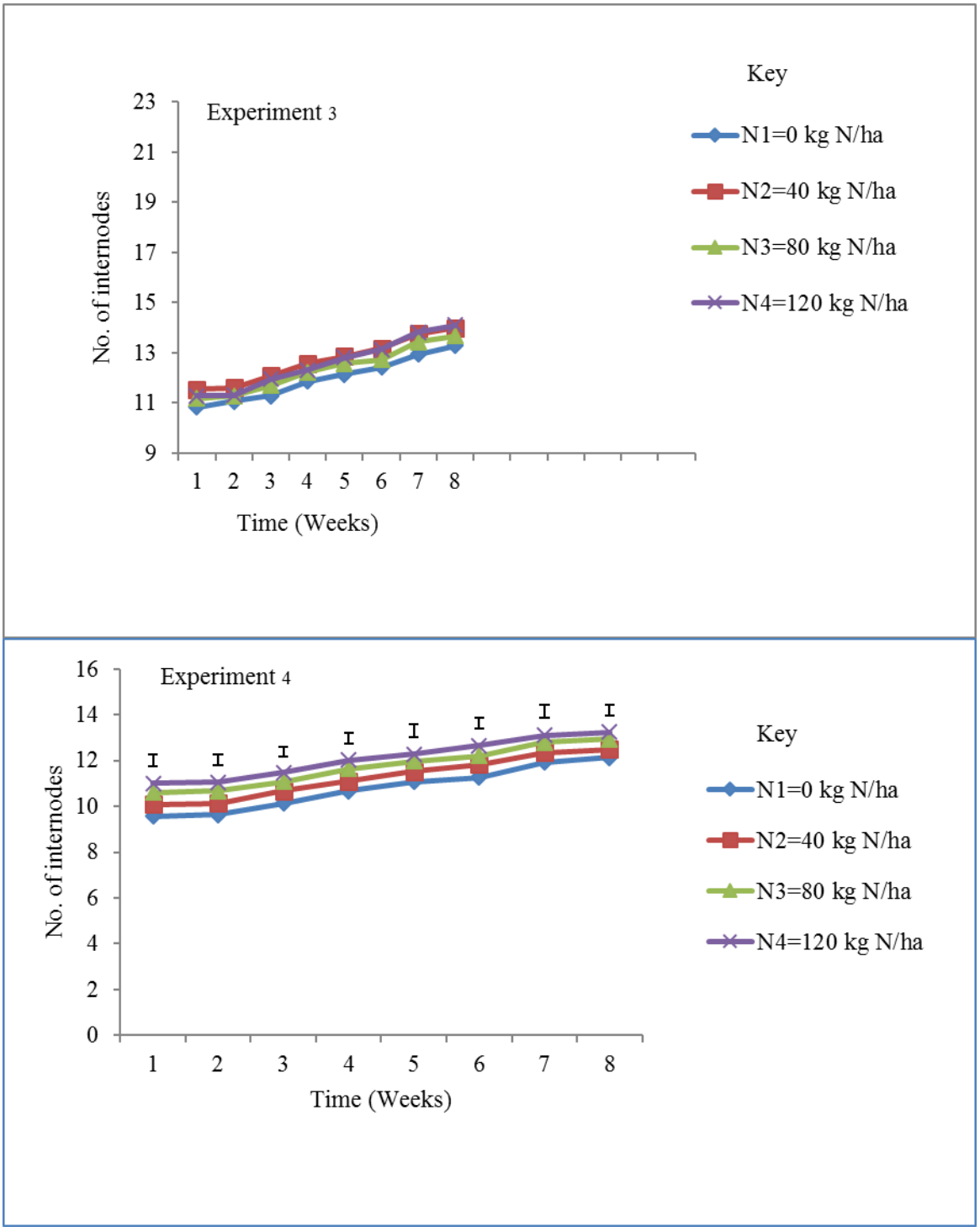


Fig. 15b. Effects of nitrogen on the number of internodes/plant (I=LSD at P ≤ 0.05)

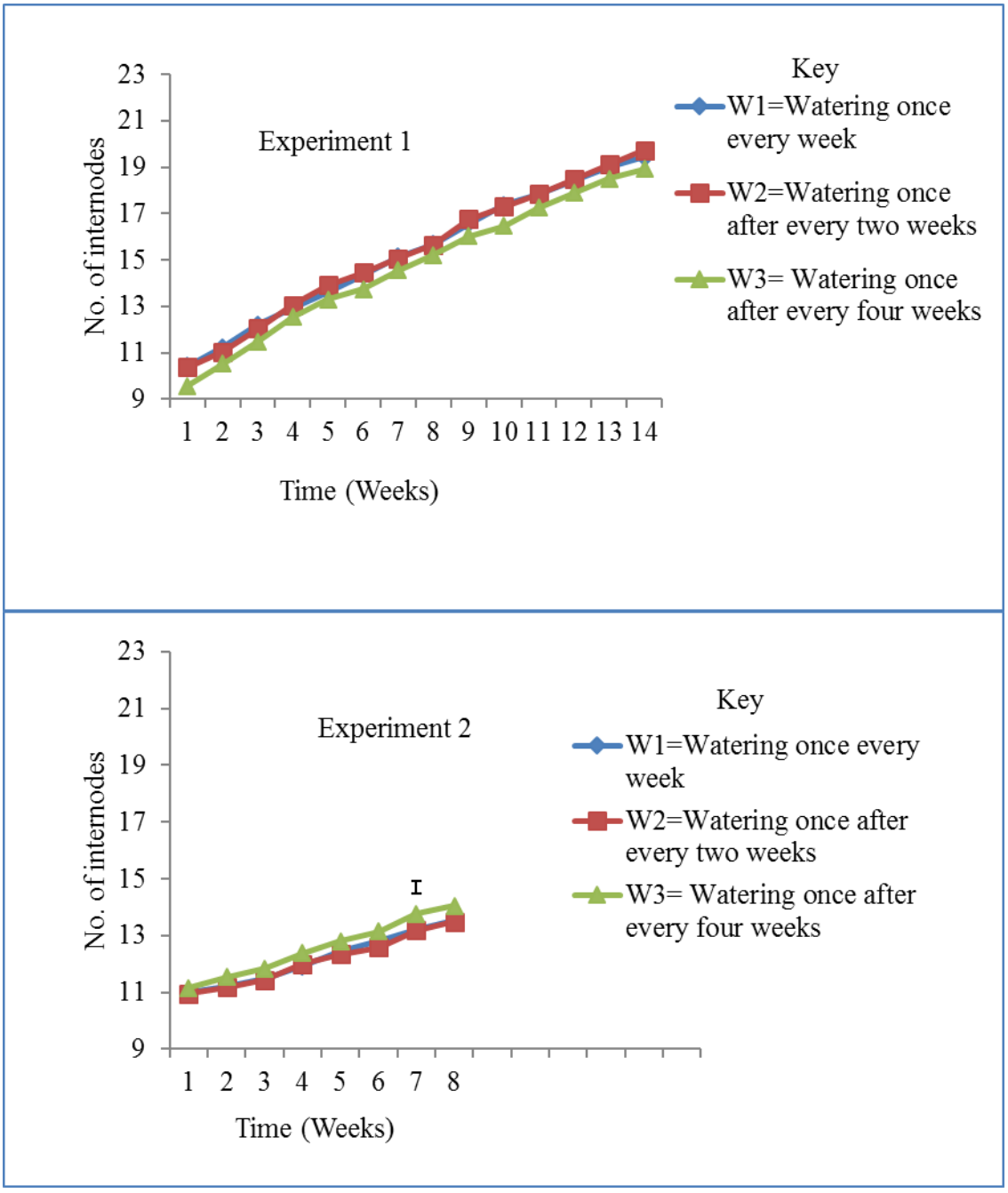


Fig. 16a. Effects of watering on the number of internodes/plant,(I=LSD at $P \leq 0.05$)

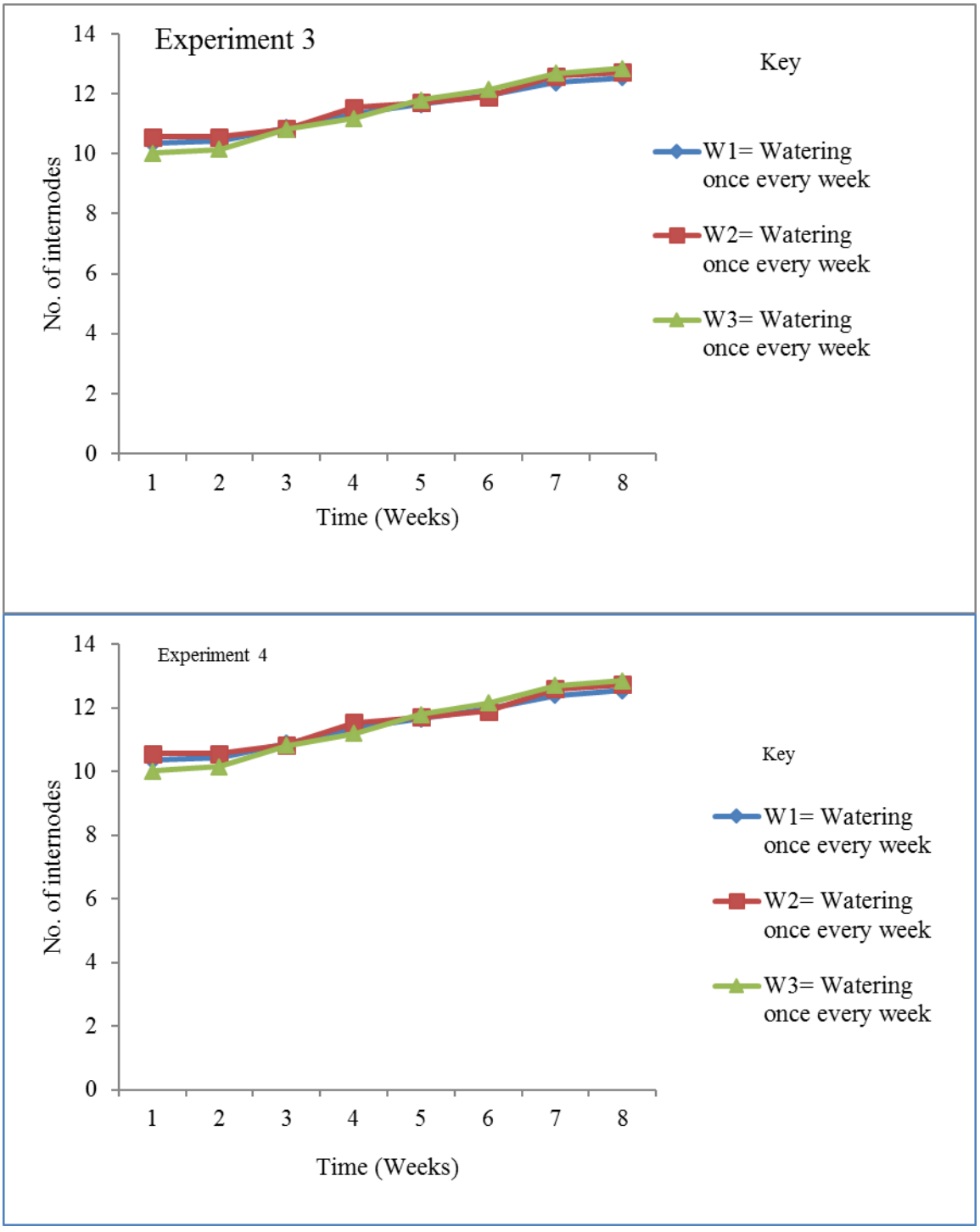


Fig. 16b. Effects of watering on the number of internodes/plant (I=LSD at $P \leq 0.05$)

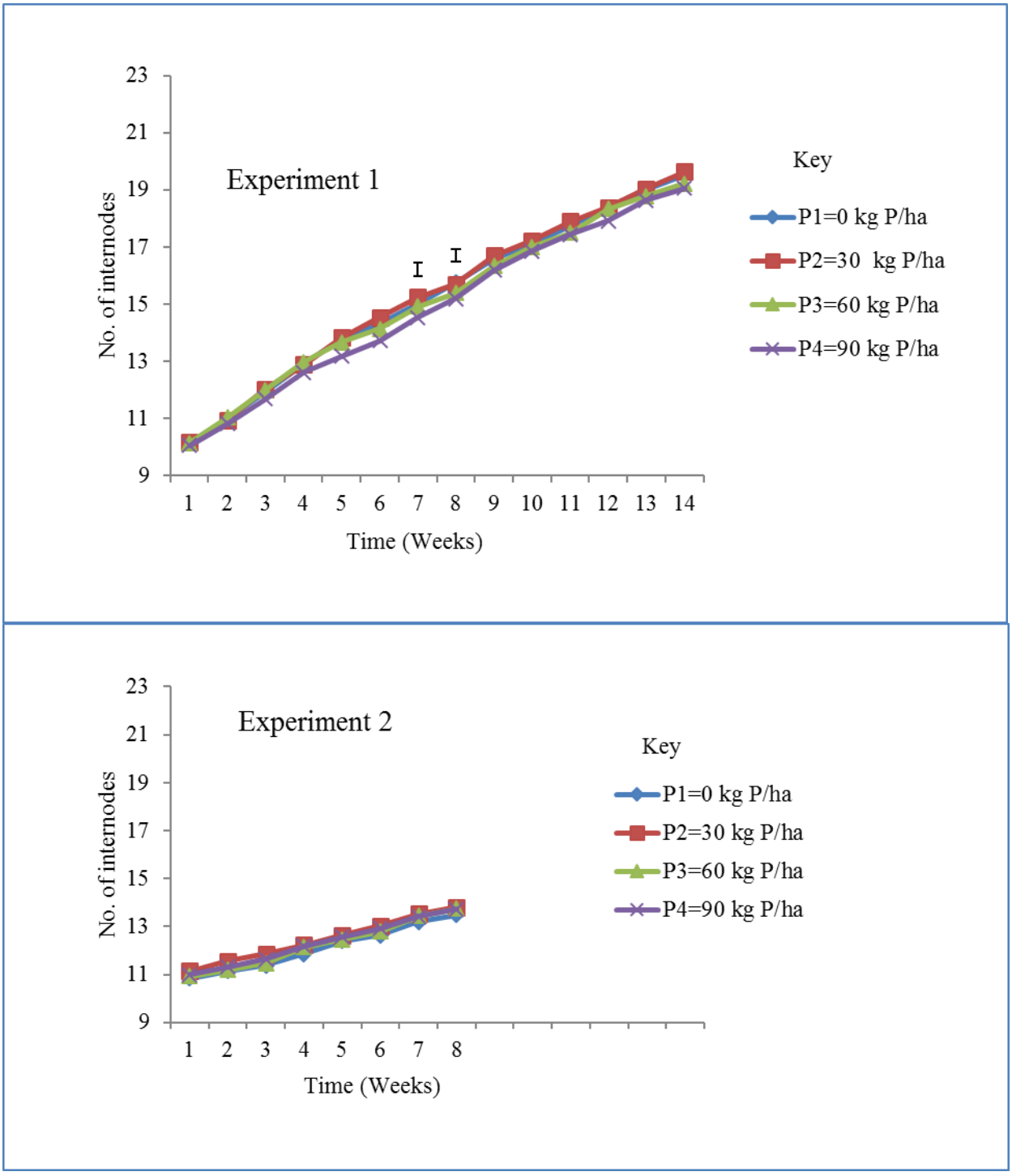


Fig. 17a. Effects of phosphorus on the number of internodes/plant (I=LSD at $P \leq 0.05$)

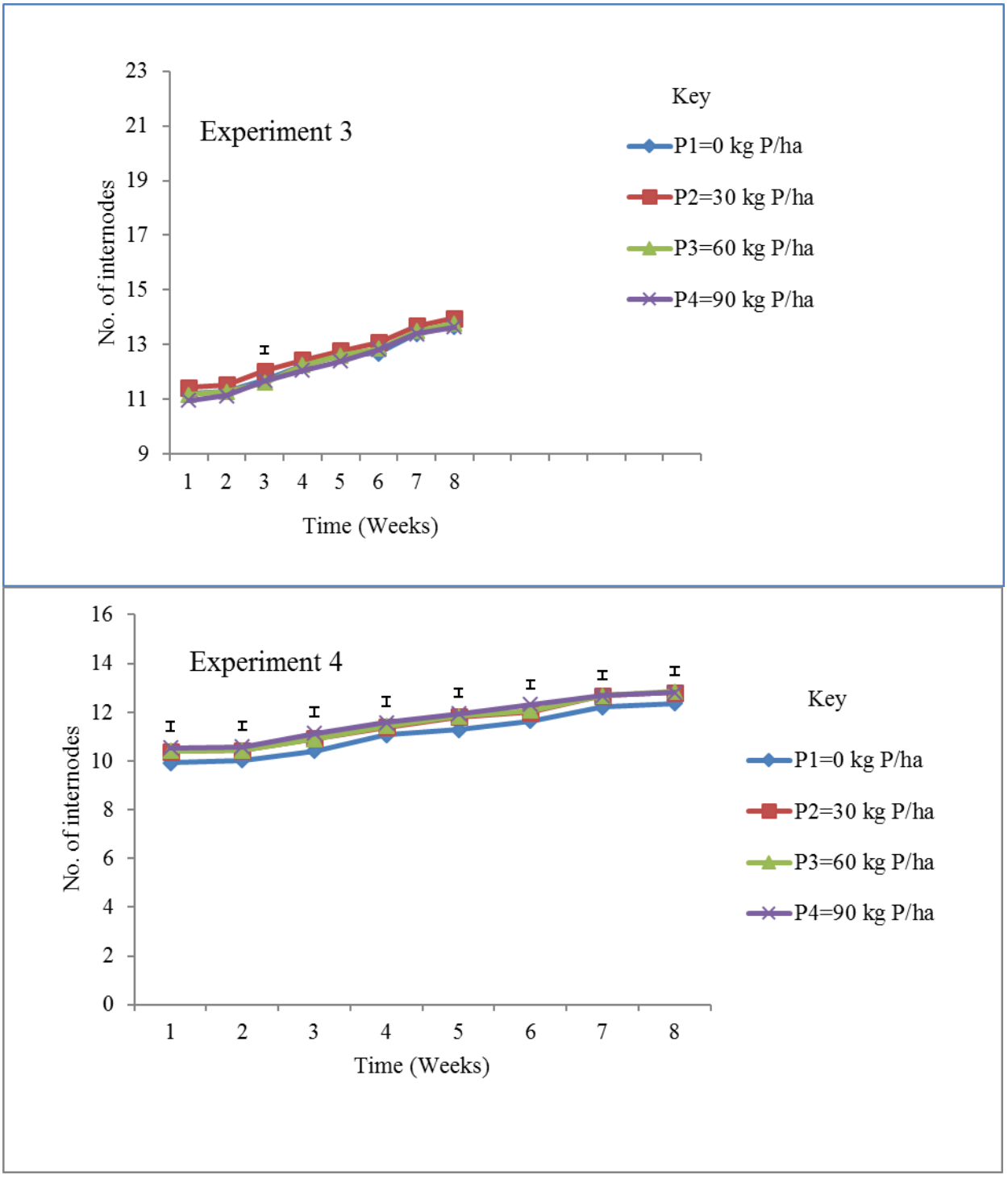


Fig. 17b. Effects of phosphorus on the number of internodes/plant (I=LSD at $P \leq 0.05$)

Table 4. Effects of nitrogen, watering and phophorus on the Leaf Area Index (Experiment 1)

N	**Watering**		**P (kg P/ha)**			
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	3.85*	5.70	3.87	4.87	
	W2	6.83	6.27	5.23	4.60	
	W3	3.70	3.50	3.63	2.90	**4.59**
40	W1	5.07	5.33	4.47	4.90	
	W2	5.83	5.63	4.97	4.77	
	W3	3.73	3.23	4.53	4.37	**4.74**
80	W1	3.53	4.52	4.34	5.33	
	W2	6.43	5.63	6.90	5.43	
	W3	4.90	3.40	4.13	4.57	**4.93**
120	W1	4.10	5.30	5.20	3.67	
	W2	5.47	6.20	5.67	5.53	
	W3	3.40	4.43	4.53	4.76	**4.86**
P Means		**4.74**	**4.93**	**4.79**	**4.65**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Table 5. Effects of nitrogen, watering and phosphorus on Leaf Area Index (Experiment 2)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	4.87*	3.79	4.70	3.03	
	W2	3.53	4.91	4.94	4.96	
	W3	3.05	3.57	3.64	1.99	**3.92**
40	W1	2.99	2.54	2.28	3.36	
	W2	2.60	3.53	2.79	2.61	
	W3	2.90	3.64	3.31	3.45	**3.00**
80	W1	4.09	3.18	2.46	3.65	
	W2	2.20	3.87	5.98	3.08	
	W3	4.57	3.74	3.35	3.59	**3.65**
120	W1	3.07	4.88	3.38	5.63	
	W2	4.64	5.22	4.96	4.93	
	W3	4.45	4.38	4.23	5.62	**4.62**
P Means		**3.58**	**3.94**	**3.84**	**3.83**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Table 6. Effects of nitrogen, watering and phosphorus on Leaf Area Index (Experiment 3)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	4.27*	3.20	2.40	2.77	
	W2	4.00	2.83	3.53	3.67	
	W3	4.03	4.60	4.53	2.30	**3.51**
40	W1	3.70	6.57	3.60	5.33	
	W2	4.93	5.47	3.13	3.37	
	W3	4.83	4.90	3.97	5.30	**4.59**
80	W1	3.20	4.03	4.23	4.13	
	W2	4.33	4.80	6.70	4.47	
	W3	4.63	5.37	4.67	3.90	**4.54**
120	W1	4.07	6.03	4.47	5.20	
	W2	5.90	5.50	5.13	5.07	
	W3	4.63	5.80	5.33	5.23	**5.20**
P Means		**4.38**	**4.93**	**4.31**	**4.23**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Table 7. Effects of nitrogen, watering and phosphorus on Leaf Area Index (Experiment 4)

	Watering		**P (kg P/ha)**			
N						
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	4.55*	6.64	3.93	5.12	
	W2	6.47	7.25	6.81	5.99	
	W3	4.77	4.95	3.63	2.20	**5.20**
40	W1	3.49	5.75	3.01	3.72	
	W2	5.41	5.98	3.97	4.58	
	W3	4.23	4.24	4.15	3.79	**4.36**
80	W1	4.06	4.97	5.56	4.88	
	W2	5.79	5.62	11.06	5.24	
	W3	5.56	5.31	4.56	4.87	**5.62**
120	W1	3.84	4.83	4.77	2.82	
	W2	6.90	4.53	6.65	5.52	
	W3	4.44	5.59	3.47	4.82	**4.85**
P Means		**4.96**	**5.47**	**5.01**	**4.58**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 8. Effects of nitrogen and phosphorus on leaf fresh weight (tons/ha)

N (kg N/ha)	P (kg P/ha)				
	0	**30**	**60**	**90**	**N Means**
			Experiment 1		
0	11.79*	17.11	11.53	13.98	**13.60**
40	15.74	19.98	19.27	18.01	**18.25**
80	20.69	19.91	19.19	14.33	**18.53**
120	15.74	14.61	12.34	15.56	**14.56**
P means	**15.99**	**17.90**	**15.58**	**15.47**	
			Experiment 2		
0	12.71	12.31	12.18	9.48	**11.67**
40	8.99	9.73	9.93	9.54	**9.55**
80	9.07	11.39	11.18	8.78	**10.10**
120	8.74	9.52	9.60	9.26	**9.28**
P means	**9.88**	**10.74**	**10.73**	**9.26**	
			Experiment 3		
0	10.82	8.88	11.71	8.73	**10.04**
40	10.23	9.93	12.16	9.63	**10.49**
80	11.12	11.19	11.06	10.73	**11.03**
120	9.13	12.00	11.62	12.57	**11.33**
P means	**10.33**	**10.50**	**11.64**	**10.42**	
			Experiment 4		
0	24.27ab**	25.13a	14.28c	16.59c	**20.07**
40	14.76c	16.59c	17.84c	19.88abc	**17.27**
80	14.70c	17.16c	18.46bc	16.47c	**16.70**
120	16.24c	18.93bc	18.70bc	15.97c	**17.46**
P means	**17.49**	**19.45**	**17.32**	**17.23**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

**Values followed by the same letter within a letter series and within an experiment are not significantly different according to the Duncan's Multiple Range Test at $P \leq 0.05$

Table 9. Effects of nitrogen, watering and phosphorus on leaf fresh weight (tons/ha) (Experiment 1)

N (kg N/ha)	Watering	P (kg P/ha) None	30	60	90	N Means
None	W1	15.20*	20.60	9.93	10.70	
	W2	8.80	16.43	13.80	17.70	
	W3	11.37	14.30	10.87	13.53	**13.60**
40	W1	13.43	19.40	14.67	10.37	
	W2	16.90	20.63	23.40	15.57	
	W3	16.90	19.90	12.40	28.10	**17.64**
80	W1	21.13	23.40	23.20	16.43	
	W2	20.00	23.47	27.10	17.93	
	W3	13.73	12.87	14.37	8.63	**18.52**
120	W1	17.23	12.97	11.90	15.60	
	W2	16.33	16.27	12.70	14.93	
	W3	13.67	14.60	12.43	15.80	**14.54**
P Means		**15.39**	**17.90**	**15.56**	**15.44**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 10. Effects of nitrogen, watering and phosphorus on leaf fresh weight (tons/ha) (Experiment 2)

N (kg N/ha)	Watering	P (kg P/ha) None	30	60	90	N Means
None	W1	10.80*	15.13	13.35	8.00	
	W2	14.23	11.90	9.77	13.23	
	W3	7.87	9.90	13.43	8.87	**11.37**
40	W1	10.10	7.00	10.67	10.77	
	W2	8.10	9.87	9.23	7.10	
	W3	10.77	12.33	12.57	10.77	**9.94**
80	W1	10.33	11.73	13.28	9.23	
	W2	7.67	11.67	16.03	9.43	
	W3	10.77	10.77	9.47	6.10	**10.54**
120	W1	6.90	10.43	10.37	9.80	
	W2	9.90	8.23	12.10	9.87	
	W3	9.43	9.90	6.33	8.10	**9.28**
P Means		**9.74**	**10.74**	**11.38**	**9.27**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 11. Effects of nitrogen, watering and phosphorus on leaf fresh weight (tons/ha) (Experiment 3)

	Watering	**P (kg P/ha)**				
N (kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	11.57*	7.10	9.33	12.90	
	W2	8.00	8.43	14.23	7.10	
	W3	9.80	11.10	11.57	6.20	**9.78**
40	W1	12.43	12.00	14.23	11.13	
	W2	8.03	8.90	11.57	9.77	
	W3	10.23	8.90	10.67	8.00	**10.49**
80	W1	8.90	12.43	9.33	9.77	
	W2	11.13	9.33	14.67	10.53	
	W3	13.33	11.80	12.27	5.77	**10.77**
120	W1	11.90	14.20	8.90	11.53	
	W2	10.93	10.23	12.43	12.87	
	W3	10.93	11.57	13.53	13.30	**11.86**
P Means		**10.60**	**10.50**	**11.89**	**9.91**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 12. Effects of nitrogen, watering and phosphorus on leaf fresh weight (tons/ha) (Experiment 4)

N (kg N/ha)	Watering	P (kg P/ha) None	30	60	90	N Means
None	W1	15.91*	23.82	14.76	17.16	
	W2	20.62	16.44	12.53	16.80	
	W3	18.85	24.09	15.56	15.82	**17.70**
40	W1	15.20	16.09	20.98	21.42	
	W2	16.09	18.22	15.91	19.38	
	W3	12.98	15.47	16.62	18.84	**17.27**
80	W1	11.29	17.78	13.69	17.25	
	W2	16.36	33.33	34.84	17.15	
	W3	16.44	17.78	17.87	15.02	**19.07**
120	W1	17.15	20.80	23.56	18.66	
	W2	16.62	18.49	16.89	15.47	
	W3	14.93	17.51	15.64	13.78	**17.46**
P Means		**16.04**	**19.99**	**18.24**	**17.23**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

4.1.2.2 Effects of N, Watering and P Regimes on LDW

There were neither significant main factors nor interaction effects on the leaf dry weight in all the experiments (Tables 13, 14, 15 and 16). The highest leaf dry weights were recorded on plots receiving 80 kg N/ha x watering once after every two weeks x 60 kg P/ha.

4.1.3 Effects of N, Watering and P Regimes on TPC, Essential Oil Yield and Composition.

This section presents data on TPC, essential oil yield and composition of sage.

4.1.3.1 Effects of N, Watering and P Regimes on TPC

The data analysis did not show any significant differences both for the main factors and the interactions. However, N showed an inverse relationship, where higher TPC (60.94 mg GAE/g) were accumulated on plants that received no N fertilizer and the lowest accumulation (56.89 mg GAE/g) on plants that received 120 kg N/ha (Table 17).

Application of 90 kg P/ha resulted in the highest level of TPC (61.40 mg GAE/g) while 60 kg P/ha gave the lowest (58.83 mg GAE/g) (Table 17).

Furthermore, the three- way response was greatest (70.52 mg GAE/g) on no N x watering once after four weeks x 90 kg P/ha, and the lowest (46.66 mg GAE/g) on 120 kg N/ha x watering once after four weeks x 90 kg P/ha (Table 17).

4.1.3.2 Effects of N, Watering and P Regimes on Essential Oil Yield

There were no significant effects of N, watering and P or their interactions on the essential oil content in experiments 3 and 4 (Table 18). However, the effect of N was highest at the rateof 40 kg N/ha (in experiment 3), while in experiment 4, 80 kg/ N/ha gave the highest essential oil yield.

Watering once every week gave the highest percentage of essential oil content and lowest percentage was obtained when watering was done once after two weeks in both experiment 3 and 4. Applying 90 kg P/ha resulted into relatively higher amount of essential oil in both experiments and the lowest was recorded on 30 kg P/ha (experiment 3) and no P (experiment 4) (Table 18).

The interaction effects though not significant showed varying effects in terms of the treatment combinations. Nitrogen x watering combination of 120 kg N/ha x watering once after four weeks resulted in the highest percentage of essential oil yield (Experiment 3), while 120 kg N/ha x watering once a week gave the highest essential oil yield in experiment 4 (Fig. 18). On the other hand, the lowest percentage of essential oil yields were obtained at 80 kg N/ha x watering once after four weeks and 120 kg N/ha x watering once after two weeks in experiments

Table 13. Effects of nitrogen, watering and phosphorus on leaf dry weight (tons/ha) (Experiment 1)

	Watering	**P (kg P/ha)**				
N (kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	3.25*	4.10	2.64	2.51	
	W2	2.69	4.23	3.08	3.67	
	W3	2.92	3.10	3.34	3.66	**3.27**
40	W1	2.89	3.69	3.86	4.07	
	W2	3.84	4.26	3.83	3.14	
	W3	4.21	4.37	4.37	3.56	**3.84**
80	W1	4.14	4.16	4.47	4.02	
	W2	4.13	4.50	5.26	4.31	
	W3	3.51	3.35	3.63	2.60	**4.01**
120	W1	3.67	3.10	3.48	3.74	
	W2	3.71	3.18	3.66	4.30	
	W3	3.06	4.10	3.38	3.62	**3.58**
P Means		**3.50**	**3.85**	**3.75**	**3.60**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 14. Effects of nitrogen, watering and phosphorus on leaf dry weight (tons/ha) (Experiment 2)

	Watering	**P (kg P/ha)**				
N (kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	3.73*	3.10	2.47	4.08	
	W2	2.40	3.87	3.60	2.30	
	W3	2.37	2.97	2.90	1.77	**2.96**
40	W1	3.80	3.48	4.20	3.82	
	W2	2.33	2.80	2.97	2.30	
	W3	2.93	2.43	3.27	2.23	**3.05**
80	W1	2.37	3.37	3.10	3.87	
	W2	2.77	2.53	5.03	3.00	
	W3	4.00	2.77	3.57	3.13	**3.29**
120	W1	2.10	3.80	3.05	3.13	
	W2	3.90	2.67	3.83	2.77	
	W3	3.97	4.97	4.07	2.93	**3.43**
P Means		**3.06**	**3.23**	**3.51**	**2.94**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 15. Effects of nitrogen, watering and phosphorus on leaf dry weight (tons/ha) (Experiment 3)

N (kg N/ha)	Watering	P (kg P/ha) None	30	60	90	N Means
None	W1	2.50*	1.70	1.50	1.63	
	W2	2.37	1.6	2.67	2.17	
	W3	1.87	2.87	2.70	1.67	**2.10**
40	W1	1.93	3.40	2.03	2.27	
	W2	3.10	3.00	2.93	2.30	
	W3	2.97	3.00	2.47	3.17	**2.71**
80	W1	1.97	2.87	2.70	2.63	
	W2	2.33	2.67	3.70	2.77	
	W3	2.87	3.23	3.00	2.47	**2.77**
120	W1	2.67	3.57	2.60	3.33	
	W2	3.43	2.47	3.23	3.30	
	W3	2.80	3.40	2.97	3.07	**3.07**
P Means		**2.57**	**2.82**	**2.71**	**2.57**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 16. Effects of nitrogen, watering and phosphorus on leaf dry weight (tons/ha) (Experiment 4)

	Watering	**P (kg P/ha)**				
N (kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	4.66*	5.52	4.74	4.34	
	W2	4.94	4.93	4.55	4.11	
	W3	4.78	3.35	4.32	3.78	**4.50**
40	W1	4.22	5.03	3.14	3.97	
	W2	4.33	4.82	4.43	3.96	
	W3	3.81	4.04	4.00	4.05	**4.15**
80	W1	4.76	4.95	4.50	4.39	
	W2	3.50	4.48	6.61	3.96	
	W3	3.94	4.88	4.44	4.57	**4.58**
120	W1	4.77	5.24	5.31	3.53	
	W2	3.85	4.20	4.23	4.51	
	W3	3.70	4.08	4.37	3.58	**4.28**
P Means		**4.27ab**	**4.63a**	**4.55ab**	**4.06b**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 17. Effects of nitrogen, watering and phosphorus on total phenolic compounds (mg GAE/g) (Experiment 3)

	Watering		**P (kg P/ha)**			
N (kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	58.83*	57.70	61.66	56.93	
	W2	62.79	58.68	59.24	62.74	
	W3	57.60	59.44	65.16	70.52	**60.94**
40	W1	64.76	53.91	64.33	62.67	
	W2	62.08	58.93	58.07	64.80	
	W3	60.03	62.52	58.24	58.66	**60.75**
80	W1	69.60	62.40	66.78	60.38	
	W2	56.43	57.24	61.03	64.64	
	W3	57.35	55.11	52.47	65.85	**60.77**
120	W1	49.30	63.32	52.56	63.78	
	W2	60.42	64.36	57.48	59.13	
	W3	56.21	60.54	48.91	46.66	**56.89**
P Means		**59.62**	**59.51**	**58.83**	**61.40**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 18. Effects of nitrogen, watering and phosphorus on the essential oil yield (%) of sage.

N (kg N/ha)	**Experiment 3**	**Experiment 4**
0	0.43*	0.45
40	0.49	0.44
80	0.44	0.51
120	0.44	0.47
Watering		
Watering once a week	0.53	0.53
Watering once after two weeks	0.37	0.40
Watering once after four weeks	0.46	0.46
P (kg P/ha)		
0	0.40	0.43
30	0.40	0.44
60	0.50	0.49
90	0.51	0.50

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

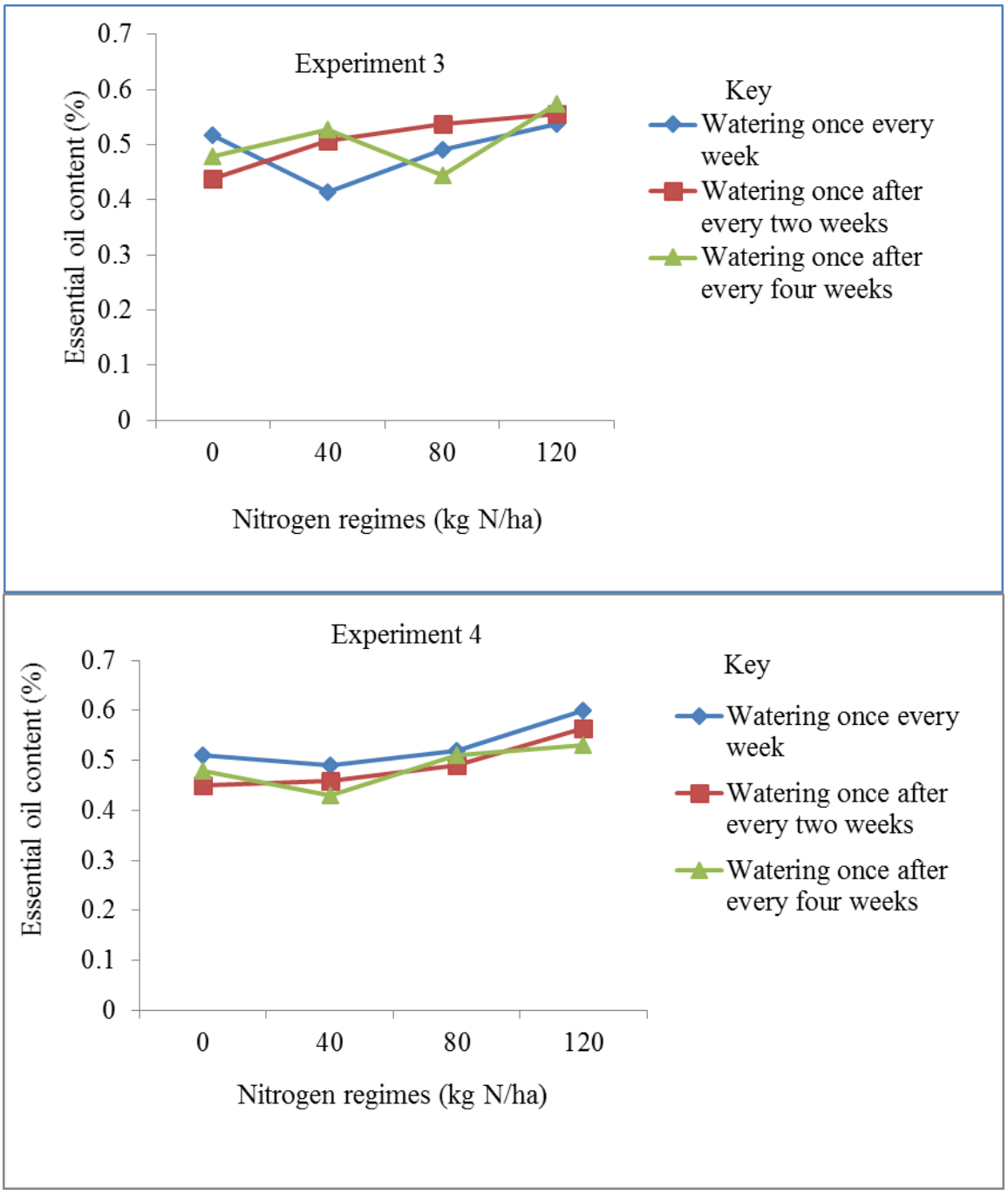


Fig. 18. Effects of nitrogen and watering on the essential oil content

Nitrogen x P on the other hand indicated that 80 kg N/ha x 30 kg P/ha gave a better response in essential oil yield in experiment 3 while the highest rate of N application (120 kg N/ha) combined with 60 kg P/ha resulted into highest amounts of essential oil yield in experiment 4. During experiment 3, 40 kg N/ha x 0 kg P/ha resulted in the lowest content of essential oils while in experiment 4, the lowest essential oil content was recorded on 40 kg N/ha x 60 kg P/ha (Fig. 19).

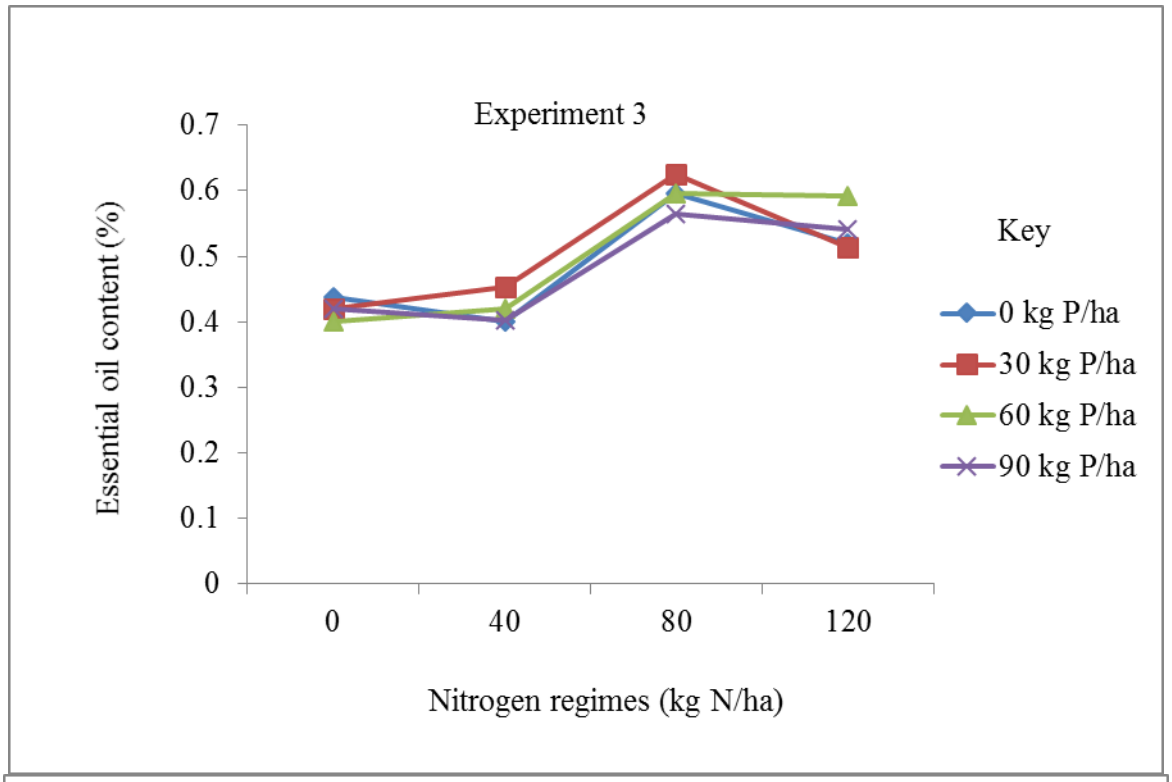
Watering once every week x 90 kg P/ha resulted in the highest essential oil yield in experiment 3, and watering once every week x 30 kg P/ha gave highest oil yield in experiment 4. Lowest essential oil content was observed on plots that were watered once after two weeks x 30 kg P/ha in both experiment 3 and 4 (Fig. 20).

There were no significant three-way interaction effects (N x W x P) on essential oil yield in experiments 3 and 4 (Tables 19 and 20). The treatment combination of 40 kg N/ha x watering once after two weeks x 60 kg P/ha resulted into the highest essential oil yield (0.85%) in experiment 3 (Table 19), while 80 kg N/ha x watering once after two weeks x 90 kg P/ha gave the highest essential oil yield in experiment 4 (Table 20). Lower essential oil yields were observed on treatment combinations of 120 kg N/ha x watering once after two weeks x 30 kg P/ha in experiments 3 and 4 (Tables 19 and 20).

4.1.3.3. Effects of N, Watering and P Regimes on Essential Oil Composition

Fifty four compounds were identified in the essential oil of sage. The 12 major components were α- and β-Pinene, α- and β-thujone, 1,8-cineol, camphor, camphene, Borneol, β-Caryophyllene, α-Humulene, Viridoflorol and Manool (Table 21A and B).The remaining 42 components were microcomponents, with their concentrations in the oil <1% (Table 22).
β-Caryophyllene, α-Humulene, Viridoflorol and Manool (Table 21A and B). The remaining 42 components were microcomponents, with their concentrations in the oil <1% (Table 22). Of the 12 major components, both α- and β-pinene had the least percentage composition in the essential oil of sage, while camphor recorded the highest percentage (≥ 29%) of the essential oil of sage, regardless of the nitrogen fertilization level (Table 21 A).

Nitrogen and watering regimes significantly influenced the concentration of β-Pinene in the essential oil of sage. Significant N x P interaction effects were also noted for α- and β-thujone while W x P interaction effects only influenced α-thujone (Table 21 A). Among the main components recorded in this study, both α- and β-pinene were lowest while 1-8 cineol, α- and β-



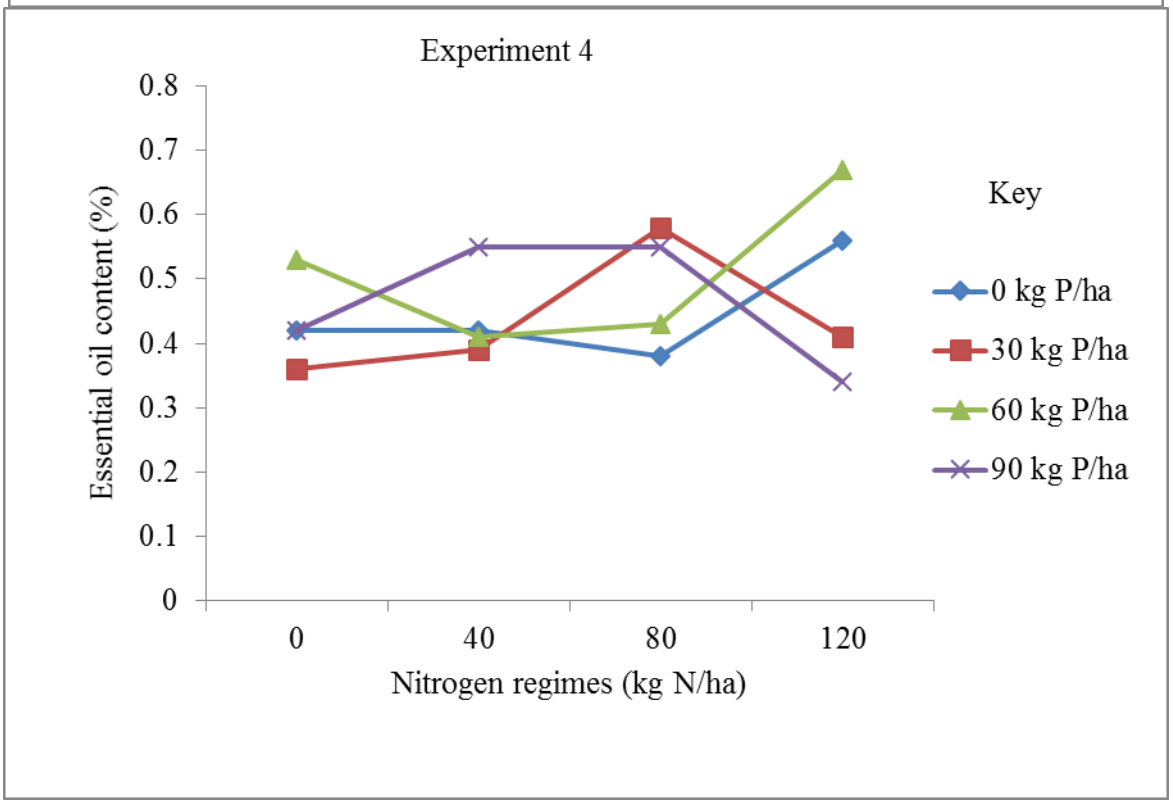


Fig.19. Effects of nitrogen and phosphorus on the essential oil content

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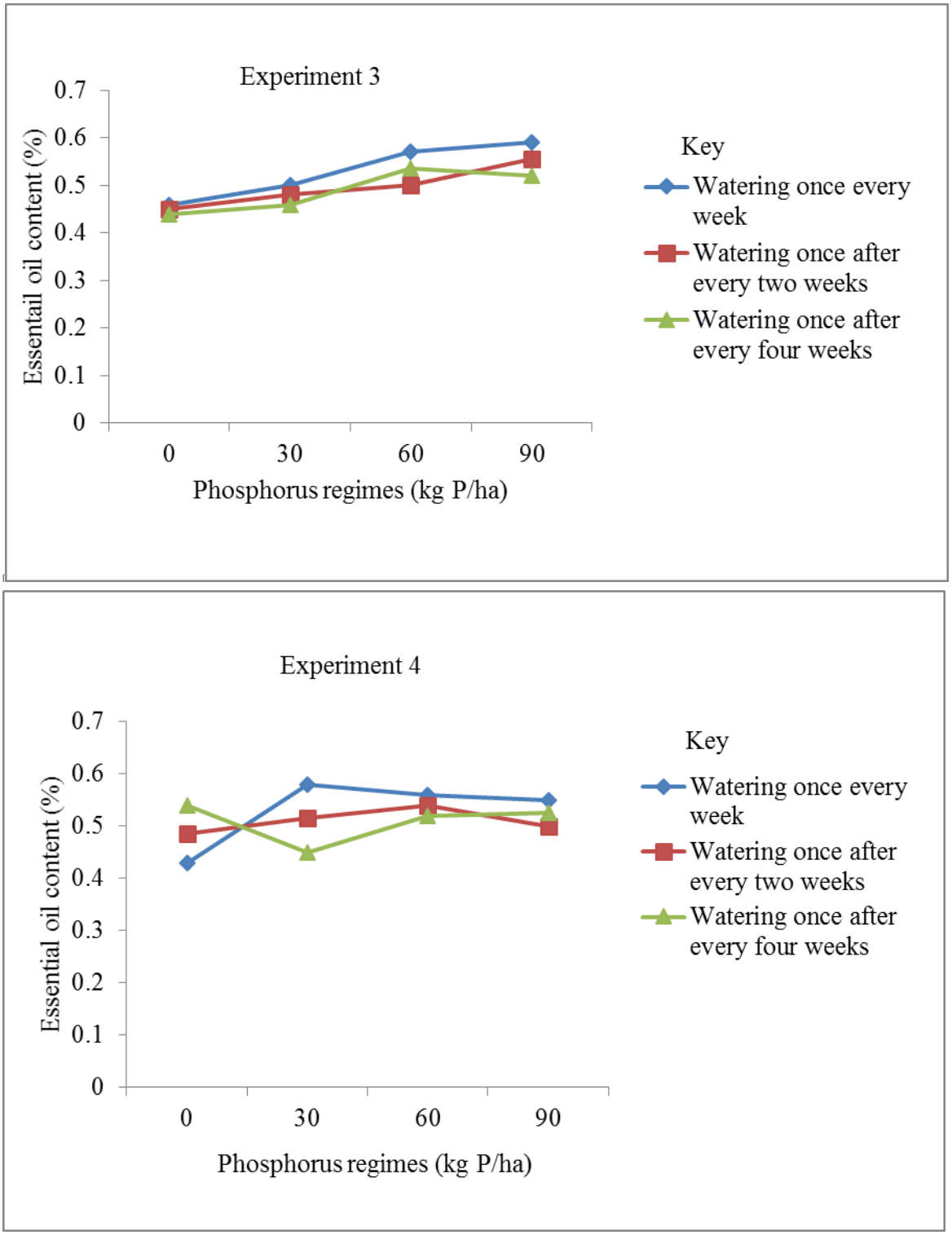

Fig. 20. Effects of watering and phosphorus on the essential oil content

Table 19. Effects of nitrogen, watering and phosphorus on essential oil yield [% (g/g)] (Experiment 3)

N (kg N/ha)	Watering	P (kg P/ha) None	30	60	90	N Means
None	W1	0.45*	0.51	0.52	0.56	
	W2	0.45	0.41	0.47	0.50	
	W3	0.41	0.46	0.46	0.50	**0.48**
40	W1	0.46	0.51	0.54	0.50	
	W2	0.23	0.30	0.30	0.37	
	W3	0.46	0.55	0.59	0.53	**0.45**
80	W1	0.49	0.54	0.55	0.55	
	W2	0.34	0.30	0.38	0.34	
	W3	0.38	0.35	0.42	0.38	**0.42**
120	W1	0.46	0.39	0.42	0.42	
	W2	0.19	0.25	0.31	0.26	
	W3	0.49	0.53	0.54	0.56	**0.46**
P Means		**0.40**	**0.43**	**0.46**	**0.46**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 20. Effects of nitrogen, watering and phosphorus on essential oil yield [% (g/g)] (Experiment 4)

N (kg N/ha)	Watering	P (kg P/ha) None	30	60	90	N Means
None	W1	0.48*	0.53	0.55	0.55	
	W2	0.37	0.34	0.40	0.37	
	W3	0.40	0.41	0.37	0.46	**0.44**
40	W1	0.43	0.44	0.42	0.43	
	W2	0.24	0.32	0.39	0.32	
	W3	0.42	0.41	0.48	0.40	**0.39**
80	W1	0.35	0.56	0.63	0.55	
	W2	0.37	0.32	0.38	0.36	
	W3	0.39	0.34	0.39	0.37	**0.42**
120	W1	0.48	0.46	0.46	0.44	
	W2	0.33	0.30	0.30	0.27	
	W3	0.45	0.45	0.41	0.32	**0.39**
P Means		**0.39**	**0.41**	**0.43**	**0.42**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Table 21. Content of the 12 major constituents in the essential oil of sage. (A) α-Pinene, β-Pinene, 1,8-cineol, α-thujone, β-thujone and camphor. (B)Camphene, Borneol, E- Caryophyllene, a-Humulene, Viridiflorol, Manool.a,b,c

A

Treatment	**α-Pinene/ %**	**β-Pinene/ %**	**1,8-cineol / %**	**α-thujone/ %**	**β-thujone / %**	**Camphor / %**
N(0)	1.39	0.95b	7.25	7.031	7.09	31.37
N(40)	1.74	1.21ab	8.70	8.35	9.04	31.55
N(80)	1.33	0.89b	7.75	6.99	7.22	29.89
N(120)	1.60	1.41a	8.23	7.62	8.33	32.57
P(0)	1.57	1.15	7.91	7.59	7.79	31.81
P(30)	1.44	1.11	8.47	7.17	8.34	32.01
P(60)	1.57	1.21	8.23	7.93	8.35	31.23
P(90)	1.35	0.99	7.31	7.30	7.20	30.33
W(1)	1.64	1.43a	8.63	8.67	8.57	31.51
W(2)	1.48	0.86 b	7.44	7.05	7.38	31.42
W(3)	1.31	0.95 b	7.87	6.78	7.81	31.10
Source of variation			**Significance**			
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.
W	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.
N x W	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N x P	n.s.	n.s.	n.s.	<0.05	<0.05	n.s.
W x P	n.s.	n.s.	n.s.	<0.05	n.s.	n.s.
W x N x P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

B

Treatment	Camphane/ %	Borneol/ %	β – Caryoph-yllene/ %	α - Humulene/ %	Viridoflorol / %	Manool/ %
N(0)	1.66	1.76	7.73	5.36	7.33	3.30
N(40)	1.89	1.45	8.56	5.59	6.42	2.65
N(80)	1.56	1.38	8.84	5.87	8.19	4.67
N(120)	1.91	1.50	9.22	5.86	6.17	2.54
P(0)	1.75	1.50	8.00	5.38	7.42	4.01
P(30)	1.64	1.54	7.98	5.59	7.40	3.26
P(60)	1.84	1.53	8.68	5.77	6.69	3.85
P(90)	1.67	1.47	8.51	5.79	8.48	4.39
W(1)	1.81	1.49	8.84	5.99	7.17	3.35
W(2)	1.92	1.45	8.62	5.85	6.40	2.51
W(3)	1.78	1.43	7.85	5.35	7.76	4.69

[a]Levels of significance are from ANOVA.

[b] n.s., not significant; N, nitrogen; P, phosphorous; W, Watering.

[c]Numbers with identical superscript are not significantly different (Duncan Multiple Range Test; DMRT, α= 5%).

Table 22. Micro-components (< 1%) in the essential oil of sage leaves.

Compounds	
Tricyclene	Eugenol
Myrcene	Isoledene
α-Phellandrene	α-Copaene
α-Terpinene	Aromadendrene
ρ-Cymene	α-Guaiene
Limonene	9-epi-E-Caryophyllene
γ-Terpinene	γ-Muurolene
Terpinolene	E-β-Lonone
ρ-Cymenene	γ-Gurjunene
Linalool	Viridiflorene
Iso-3-Thujanol	α-Selinene
Trans-Pinocamphone	α- Muurolene
Iso-Borneol	γ-Cadinene
3-Thujanol	δ-Cadinene
Terpinene-4-ol	Trans-Calamenene
ρ-Cymen-8-ol	β-Calacorene
α-Terpineol	Spathulenol
Bornyl acetate	Caryophyllene oxide
Trans-Sabinyl acetate	Globulol
Thymol	Humulene epoxide II
Carvacrol	Selina-3-11-diene-6-a-ol

thujones were almost at the same concentration. Camphor remained at the highest concentration (> 30%) regardless of the P fertilizer application level, although the effects of P application rates were not statistically significant ($P \leq 0.05$) for all the main components of sage essential oil (Table 21 A and B).

Watering once every week tended to favour the accumulation of all the main essential oil components despite there being no significant ($P \leq 0.05$) effects of watering on the main components of sage essential oil (Table 21 A and B). The levels of both α- and β-pinene were lowest while that of camphor was > 30% regardless of the watering regimes (Table 21 A).

4.1.3.4. Interrelationship between Essential Oil Content (%) and Growth Parameters of Sage.

The data presented in Table 23 indicates the coefficients of correlation between the essential oil yield and the plant height, number of primary and secondary branches/plant, number of internodes/plant, LA, LAI and SLW.The results indicate positive correlation between essential oil yield and plant height, number of primary and secondary branches/plant, number of internodes/plant (Experiment 3) and plant height, number of primary branches/plant and number of internodes/plant (experiment 4) (Table 23).

On the other hand, there were negative correlations between the essential oil yield and LA, LAI and SLW (Experiment 3), and with number of secondary branches/plant,LA, LAI and SLW (Experiment 4) (Table 23).

Table 23. Coefficients of Correlation (R) between essential oil yield and growth parameters

Growth	**Experiment 3**		**Experiment 4**	
parameters	**P values**	**R**	**P values**	**R**
Plant height	0.982	0.002	0.0812	0.020
Primary branches	0.945	0.006	0.899	0.011
Secondary branches	0.191	0.110	0.806	-0.021
Internodes	0.466	0.061	0.940	0.006
Leaf Area	0.013	-0.093	0.269	-0.207
Leaf Area Index	0.349	-0.079	0.695	-0.033
Specific Leaf Weight	0.585	-0.046	0.714	-0.031

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effects of N, Watering and P Regimes on the Growth of Sage

5.1.1 Plant Height

The findings of taller plants on plots that received 120 kg N/ha (Fig 6a and b) are supported by other research findings (Karamanos, 1995; Rohricht et al., 1996; Rahman 1999; El-Din et al., 2010; Ram et al., 1995; Ram and Kumar, 1997; Sharma and Kumar, 2012; Kumar et al., 2008 and Tanjia et al., 2009). According to Black (1967), this could be due to large cells development at increasing N levels with higher meristematic activities which consequently benefited growth. However, other researchers (Economakis, 1993b; Karamanos, 1995; Schenk, 1996; Andrews et al., 1999; Akanbi and Togun, 2001; Omidbaigi and Arjamandi, 2002; Baranauskiene et al., 2003; de Groot et al., 2003 and Martin et al., 2006) reported that elevated N levels do not induce analogous increase in plant height growth, which could be explained by Marschner's (1995) report that elevated amounts of nitrates are of limited use for plant metabolism.

Watering regimes only significantly affected plant height in experiment 1 and 4 (Fig 7) with taller plants being recorded for the watering once every two weeks treatment, and shortest on watering once after every four weeks. Several authors have reported a decrease in plant height with increasing severity of water stress (Bettaieb et al., 2009; Arnon and Gupta, 1995; Abbaszadeh et al., 2008; Sharifi et al., 2005; Taheri et al., 2008; Khalil et al., 2010; Mohamad et al., 2012 and Pirzad et al., 2006; Hasani et al., 2004, Safi Khani et al., 2008; Misra and Srivastava, 2000, Moeni et al., 2006; Bahreini-Nejad et al 2013; Gosselen, 1995; Bettieb et al., 2009; Davatgar et al., 2009 and Laribi, 2009). The decrease could be the result of preferential allocation of biomass production to the roots (Albouchi et al., 2003 and Sreevalli et al., 2001) or a reduction in chlorophyll content, and consequently, photosynthesis efficiency as reported by Viera et al. (1999). It could also be due to a decline in cell enlargement, resulting from reduced turgor pressure (Shao et al., 2008). Hassan et al. (2013), also attributed this morphological change to an adaptation of the plant to water and environmental stress to reduce transpiration, and to induce a lower consumption of water (Banon et al., 2003; Stanhill and Albers, 1974).

Generally, growth reduction as a result of water deficit has been widely reported in many plant species such as in *Azadirachta indica* (Burman et al., 1991); *Schinus Molle, Schinus*

terembithifolius and *Myoporum acuminatum* (Farahat, 1990); potato (Heuer and Nadler, 1995); dry lands crops (Arnon and Gupta, 1995); *Khaya senegalensis* (Sayed, 2001), soybean (Specht et al., 2001; Zhang et al., 2004), basil (Yassen et al., 2003; Telci, 2005; Telci et al., 2005); *Simmodsia chinensis* (Uday et al., 2001; Soad, 2005); *Melia azedarach* (Azza and Sahar 2006), *Bauhinia variegata* (Azza et al., 2007); citrus seedlings (Wu et al., 2008), rosemary (Sanchez-Blanco et al., 2004; Leithy et al., 2006; Nicolas et al., 2008), *Abelmoschus esculentus* (Sankar et al., 2007; 2008), *Vigna unguiculata* (Manivannan et al., 2007a),parsley (Petropoulos et al., 2008), sage (Bettaieb et al., 2009), mint (Shormin et al., 2009), purple basil (Ekren et al., 2012) and Coriander (Jamali and Martivosyan, 2013).

Phosphorus significantly affected plant height in all the experiments, although at different growth stages for the respective experiments (Fig 8a and b) with 90 kg P/ha yielding the tallest plants,and the shortest plants on plots that received no P. Similar observations were made by Jeliazkov et al. (1999), who found that plant height of three mint cultivars increased with increasing N, P and K and Santos de Souza et al. (2012) who reported a higher main branch length with the highest level of phosphorus for *Mentha piperita* L. This increase in plant height could be attributed to the fact that P plays an effective role in root growth (Mahmudi, 2005). Adequate root growth further enhances nutrient uptake thereby enhancing growth. Moreso, being a constituent of ATP, phosphorus has significant role in energy transformation in plants and various physiological processes (Shivasankeb et al.,1982) thus enhancing growth.

5.1.2 Number of Primary Branches/plant

Nitrogen effects on the number of primary branches were significant in experiments 2 and 4 (Fig. 9a and b) with the highest numbers observed at 120 kg N/ha, and the lowest on plants that received no N fertilizer in both experiments (Fig. 9a and b). These results are in agreement with reports by Karamanos et al. (1995) and Frabboni et al. (2011) that N fertilization improves the number of branching/ plant.

Watering had significant effects on this parameter (Experiments 1 and 4), with the watering more frequently (once every week) giving the highest number of primary branches/plant, and the less frequent watering giving the least (Fig. 10a and b). Similar results were obtained with *Dracocephalum moldavica* by Hasani (2006) who reported a decrease in the number and length of auxiliary branches with decrease in moisture stress. Furthermore, Halvorson et al. (2001) found that the number of branches per plant was closely correlated with

soil moisture regime during the growing season. Hassan et al. (2013) also reported significant effects of water deficit on the number of branches/plant in rosemary, while Clarke and Simpson (1978) showed that *Brassica napus* cv. Tower was scarcely affected by irrigation. Seghatoleslami et al. (2013) also observed that the number of auxiliary branches of roselle (*Hibiscus sabdariffa*) was not significantly affected due to irrigation treatment.

Kumar et al. (2010) reported that nitrogen and phosphorus had significant effect on number of branches when considered individually, but their interaction showed no significant variation on the number of branches of clary sage. This observation was similar to those observed in the current study. The observed nitrogen and phosphorus interaction effects could be linked to the fact that both nitrogen and phosphorus are structural components of biomolecules like proteins, phospholipids and nucleic acids and more still, phosphorus is an integral part of ATP, playing a role in energy metabolism in the plant. This in turn translates into an increase in foliage development by the plant by providing the energy and stimulating cell division and elongation (Davlin and Witham, 1986). Similar findings were reported by Prasad and Shukla (1986); Bhat et al. (2006) and Mishra and Negi (2009).

5.1.3 Number of Secondary Branches/plant

Nitrogen had significant effects on the number of secondary branches/plant in experiment 2, 3 and 4. Phosphorus had significant effects on secondary branches, whereas nitrogen and watering interaction influenced the same parameter. More secondary branches/plant were obtained with 40 kg N/ha and watering once after every two weeks as compared with 80 kg N/ha and watering once after every four weeks that gave the lowest number. This is a contrast to findings by Girase (1976) on safflower.

5.1.4 Leaf Area Index

There were no significant nitrogen effects on LAI in all the experiments. Similar observations were reported by Frabboni et al. (2011) on basil. They however, noted that greater values were observed for plants fertilized than the plants that received no fertilization.

The LAI was lowest on unfertilized plots, while high LAI was recorded on plots that received 80 kg N/ha. Similar findings were noted by Rajeswara Rao (2001) who reported that addition of 80 kg N/ha/year enhanced the total biomass yield of *Cymbopogon martinii* (Roxb.) Wats. Var *Motia Burk*by 57.6% relative to untreated plots. Elsewhere, Ozguven et al. (2008); Ram et al. (2006); Singh and Sharma (2001) had similar observations for wormwood, mint and

palmarosa, respectively. This is supported by the report by Ezz El- Din et al. (2010) that there is a positive response of nitrogen on activation of photosynthesis and metabolic processes of organic compounds in plants which in turn encourage plant vegetative growth. Empirical data have shown that nitrogen is an important factor affecting crop leaf area index either for early growth (Zhong, 1999) or for the whole growth season (Booij et al., 1996). Hodges and Kanemasu (1977) found that photosynthesis, respiration and dry matter accumulation could be expressed as a function of LAI. Shih and Gascho (1980) reported a positive correlation between LAI and sugarcane biomass yield, which could be attributed to higher radiation interception that has been linked to high LAI (Singels and Donaldson, 2000).

A high rate of N application increases leaf area development (Bhardwaj and Kaushal, 1989). Alsafar and Al-Hassan et al. (2009) reported an increase in LAI with the increased rate of fertilizer application similar to Kumar et al. (1999) and Lacy et al. (1981). They reported that LAI increased significantly with the increasing rate of fertilizer application from 75 kg N/ha and 50 kg P/ha to 100 kg N/ha and 75 kg P/ha, compared to the control and the other lower application rates.

It was also observed that except for 120 kg N/ha, all the nitrogen levels indicated a trend of decreasing LAI from the first cut to the third cut. The highest LAI was recorded in the first cut and the lowest in the third cut (Fig. 7). Similar results were reported for sugarcane by Sandhu et al. (2012) who reported that mean LAI increased with time and decreased with crop cycle (plant cane, first and second ratoon). However, such was not the trend for watering and phosphorus effects on LAI (Fig.7).

5.2.0 Effects of N, Watering and P on Yield

5.2.1 Leaf Fresh Weight

There were no significant N, watering and P effects on LFW in all experiments. However, in experiment 4, nitrogen x phosphorus interaction effects were significant (Table 26). Nitrogen x watering x phosphorus interactioneffects were not significant in all the experiments. The highest response was noted on 80 kg N/ha x watering once every two weeks x 60 kg P/ha. Gardener et al. (1985) stated that fertilization may play a direct or indirect role in plant anabolism through activating the photosynthetic processes as well as the accumulation of their products in plant organs, resulting in more plant material expressed as fresh weight. These effects could be due to the effects of N on the root establishment and nutrient uptake (Wange et.,

1995). El-Mekawy (2009), reported that fresh weight of *Thymus capitatus* L considerably increased with decreasing irrigation intervals and raising the nitrogen levels. In the same year (2009), he also observed that *Achillea fragrantissima* fresh weight increased at the treatment of short irrigation intervals with the mixture of bio and NPK fertilization. This was not the case with the current study since moderate watering resulted into increased fresh weight than the most frequent watering. Watering once after every two weeks, was better than watering once after four weeks probably because of the effect of more frequent watering on increasing growth of the root system, consequently increasing the nutrient uptake needed for plant growth as was also reported for thyme (El-Mekawy, 2009). Moreso, as a result of water availability increasing the biosynthates accumulation, consequently the fresh matter of the herb (Bouton et al., 1985). Similar results were reportedon basil (Ekren et al., 2012; Telci et al., 2005); anise (Eid et al., 1996); *Calotropis procera*, *Peganum harmala* and *Marrubium vulgare* plants (El- Mekawy, 1999); *Salvia splendens (*Khattab et al., 2002) and sweet marjoram (*Majorana hortensis* L.*)* (Kandeel and Sharaf (2003). Ramos et al. (1999) established that water deficit inhibits accumulation of fresh plant mass in greater extent than dry biomass as was observed for cowpeas and common bean plants (Auge et al., 2002). Halil et al. (2006) reported 27-43% total plant weight reduction under severe water stress. These differences in the rate of yield reduction due to water deficit depended on the genotypes, crop growth stage, severity and duration of water deficit as outlined by Webbe et al. (2001).

5.2.2 Leaf Dry Weight

There were no significant main factor effects on dry weight. The lowest dry weight was recorded in the plots that received no nitrogen fertilization, while the highest dry weight was on 80 kg N/ha across the watering regimes and phosphorus levels. From the literature searches, it is clear that there are varying responses of dry weight to nitrogen rates of application for many of the medicinal and aromatic plants. For example, for *Salvia* species, Economakis (1993b), documented a range of 100-150 ppm and higher than 200 ppm reduced dry weight; Rohricht et al. (1996) reported that 100-150 kg N/ha increased top branch yield, while Sharma and Kumar (2012) proposed 1.5-3.0 g N/plant. In the case of basil, as low as 50 kg N/ha has been reported to provide good yields (Arabaci and Bayram, 2004), while other studies with the same crop show that increasing N application rates of upto 80 kg N/ha (Singh et al., 2004a), 80-120 kg N/ha (Yassen et al., 2003); 100 kg N/ha (Dadvand Sarab et al., 2008); 30-50 kg N/ha (Dedio et al.,

1986; Biesiada et al., 2006); and 200 kg N/ha or even upto 300 kg N/ha (Sifola and Barbieri, 2006) would give the best dry weight yields. Similarly in mint, there has been shown to be a linear increment in yields with increase in fertilizer application from 60 kg N/ha - 225 kg N/ha (Gulati and Duhan, 1975; Sarma et al., 1975, Shelke and Morey, 1978; Chandra et al., 1983; Singh, 1983). They indicated that application of nitrogen at rates lower than 60 kg N/ha to 225 kg N/ha resulted in a decrease in dry weight yield, contrary to the studies on Greek oregano, where Tanjia et al. (2009) reported herb yield increase from 0 to 120 kg N/ha. A range where findings of the current study fall. Sotiropoulou and Karamanos, (2010) indicated that oregano peak biomass yield was achieved at 80 kg N/ha, while Ozguven et al. (2006) and Barreyo et al. (2005) reported a significant increase in dry weight at 40 kg N/ha for both *Origanum syriacum* and *Origanum x appli* hybrid. Varying rates were also associated with marigold dry weight yield; 100 kg N/ha (Mili and Sable, 2003); 100-200 kg N/ha (Gontait and Chattopadhyay (2004) and 80 kg N/ha (Krol, 2011), who observed that 120-160 kg N/ha practically had no effect on the yields of dry flower heads.

There were no significant differences amongst the phosphorus treatments in all the experiments as was reported by Fatima et al. (2006) that the total dry mass was not significantly affected by P concentrations in mint, as well as Santos de Souza et al. (2012), who also noted that P concentrations did not significantly influence the accumulation of dry matter, although the highest was attained at 120 kg P/ha. Contrary, Munsi (1992) found that the application of N and P improved the productivity of Japanese mint, increasing the dry matter production. It was also reported by Alsafar and Al-Hassan (2009), that nitrogen and phosphorus fertilizer play a key role in enhancing mint crop yields and application of 75 kg N/ha and 50 P_2O_5/ha significantly increased total dry weight of coriander.

The intermediate watering treatment in combination with 80 kg N/ha and 60 kg P/ha resulted into higher dry weights across the seasons. This finding on watering conforms to that reported by Corell et al. (2009) that *Salvia* dry weight production increased with the watering rate. Decreasing dry weight under water deficit could be as a result of a reduction in the chlorophyll content as indicated by Hassan (2013), who showed that lower chlorophyll contents were associated with low moisture levels. The consequence of low chlorophyll is a reduction in photosynthetic efficiency (Khalid, 2006). Fresh and dry weights of *O. basilicum* L. were decreased as plant water deficit increased (Simon et al.,1992).

5.3.1 Effects of N, Watering and P on Total Phenolic Compounds

No nitrogen treatment resulted into higher levels of total phenolic compounds as was also observed by He et al. (2013) in *Salvia miltiorrhiza.* Nguyen and Niemeyer (2008) illustrated that nutrient availability affects the production of polyphenolic compounds in three cultivars of basil. Other studies have shown that high concentrations of nutrients do not consistently lead to lower phenolic concentrations (Chapin et al., 1986; Iason and Hester, 1993). Elsewhere, it has been shown that plant nutrient status can significantly influence or have little or no effect on production.

In the current study, watering once after every four weeks under highest rates of N and P resulted in increased levels of total phenolic compounds as was also reported by Petridis et al. (2012) who reported that water stress induced the accumulation of phenolic compounds especially oleuropein, suggesting their role as antioxidation. Similar findings were noted by Hazzoumi et al (2015) in basil where the contents of total phenolic compounds increased in non-mycorrrhizal plants under water stress. This could be explained by a positive correlation between phenolic compounds and soil water content as was reported by Chunlong et al. (2008). This could be attributed to the fact that phenolic compounds have strong hydrophobicity with phenolic hydroxyl and other hydrophilic grouped in molecules inspite of hydrophobic parts such as aromatic rings (song et al 2000), which means that phenolic compounds could hold internal water of plants and reduce water loss in drought environment. So leaves with higher phenolic compounds would lessen transpiration and therefore, sage synthesized and accumulated phenolic compounds as an adaptation to variation in soil water.

Higher levels of P resulted into higher levels of TPC. Similar results were reported by Jing et al. (2011) in American Ginseng. They noted that TPC significantly decreased under P-deficient conditions. Contridicting results were observed by Sarker and Karmoker (2011) in lentil (*Lens culinaris*) and Dinkelaker et al. (1995) in root exudates. Dinkelaker et al. (1995) further indicated that in many plants, P-deficiency enhanced production and root exudation of phenolic compounds. Therefore the observations in the current study could have been due to the compounded effect of the interaction effects of both N and watering treatments.

5.3.2 Effects of N, Watering and P on Essential Oil Yield

The results indicate no significant ($P \leq 0.05$) effects of N on the essential oil yield. There are several studies showing that N fertilizer application affects yield and composition of essential

oils of some medicinal plants (Ashraf et al., 2006; Sekeroglu and Ozguen, 2006). Some authors have indicated no significant effects of N application on several herbs, medicinal and aromatic plants (Arabaci and Bayram, 2004; Barreyro et al., 2005) while others showed that N fertilization enhanced essential oil yield (Abbaszadeh et al., 2009).

Application of either 60 or 90 kg P/ha (Experiment 3) and 60 kg P/ha (in Experiment 4) resulted in the highest levels of essential oils in sage although there were no significant P treatment effects (Tables 16 and 17). Several authors have also reported no significant effects of P on essential oil content; Nell et al. (2009) in *Salvia officinalis var purpurea*; and Economakis, (1995) in *Salvia fruticosa*. This is contrary to reports by other researcherswho indicated that P application significantly increased the essential oil content. For instance, Ramezani et al. (2009) in basil; Kapoor et al. (2004) in fennel (*Foeniculum vulgare* Mill) and Tuncturk and Tuncturk (2006) in cumin.

Watering once every week resulted into higher accumulation of essential oil, although watering regimes did not have any significant effect ($P \leq 0.05$) on sage essential oil (Table 16 and 17). Baghalian et al. (2011) indicated that drought stress had no significant effect on essential oil content of German chamomile. Contrary to this, another research showed that both moderate water deficit and severe water deficit improved sage essential oil yields (Bettaieb et al., 2009). Similar results have been reported on other plant spices; parsley (Petropoulos et al., 2008) and Mexican oregano (Dunford and Vasquez, 2005).

5.3.3 Effects of N, Watering and P Regimes on Essential Oil Composition

Fifty four compounds were identified in the essential oil of sage (Tables 21 A and B). Most of these components have been identified by other studies on the essential oil of sage (Raal et al., 2007; Mohamed et al., 2009; Baranauskiene et al., 2011). The main medicinally valuable components of the essential oil in sage are the monoterpenes α and β thujone, 1,8-cineole, and camphor. The composition ofsage essential oil in this study did not match the profile defined by the standard ISO 9909 (ISO, 9909), which requires camphor concentration in the essential oil to be within the range of 4.5-24.5%.

S. officinalis produces monoterpenes with a broad range of carbon skeletons, including acyclic, monocyclic, and bicyclic compounds. Three distinct monoterpene synthases are responsible for the first steps in the formation of the most characteristic monoterpenes of *S. officinalis* essential oil. The (+)-sabinene synthase catalyzes the production of sabinene, which

undergoes further rearrangements leading to the two major monoterpenes, α- and β-thujone. The 1,8-cineole synthase produces in one-step 1,8-cineole. Finally, (+)-bornyl diphosphate synthase produces bornyl diphosphate, which is subsequently hydrolyzed to borneol and then oxidized to camphor (Croteau et al., 1994). Environmental conditions such as temperature, day length, light, fertilizers and water status influence quantitative compositions of essential oils (Dudai, 2005) since they take part in the biosynthesis process. In Salvia, the major monoterpenes 1,8-cineole, camphor, and the two thujones show pronounced dynamics during a vegetative cycle (Grausgruber-Groger et al. 2012). In the present study, fertilization treatments had but a small effect on composition of the essential oil in *S. officinalis*.

Effects of N on the main components of sage essential oil were not significantly different. However, both α- and β-pinene had the least percentage composition in the essential oil of sage, while camphor recorded the highest percentage (> 29%) of the essential oil of sage, regardless of the nitrogen fertilization level. Effects of N application rates on concentrations of essential oil components vary between plants and components. For example, in *Ocimum basilicum*, foliar spray of N increased the concentration of linalool and epi-α-cadinol, and decreased 1,8-cineol, geraniol and euginol (Nurzynska- Wierdak, 2012); in clary sage, 3.0 g N pot-1 induced the highest concentration of linalool, trans-geraniol and linaly- acetate (Sharma and Kumar, 2012); in oregano, N application increased carvacrol concentration and decreased α-pinene, camphene, π-cymene, thymol and caryophylllene concentrations in leaves, and increased linalool in inflorescences (Sotiropoulou and Karamanos, 2010). Effects of N on essential oil composition might result from its involvement in synthesis of primary, as well as secondary metabolites (Koeduka et al., 2006). In the present study, levels of N application affected the accumulation of the monoterpene β-Pinene in the essential oil of sage (Table 39 A). The percentage of β-Pinene in the oil increased with increasing N levels.

Effects of P application on the composition of essential oils varies between plant species, components of the essential oil and environmental conditions. In the present study, the level of P application did not affect the composition of the essential oil of sage (Table 39 A and B). Similarly, Kumar et al. (2010) noted that phosphorus levels did not affect essential oil composition in *Desmostachya bipinnata*; in Sweet Basil, eugenol, linalool, 1,8-cineol, acetate-D-amyl and germacrene-D concentration were not affected by P application (Chimura et al., 1993),

while in *Chamomilla recutita* a-bisabolol content was increased by elevated P applications (Heneka, 1993).

There was a trend for lower accumulation of all the main essential oil components by reduction of watering frequency, but the changes were not significant ($P \leq 0.05$) except for β-Pinene which was significantly decreased with reducing irrigation frequency (Table 39 A and B). Irrigation frequencies were demonstrated before to affect essential oil composition of several herbs, medicinal and aromatic plants (Corell et al., 2009; Bettaieb et al., 2009; Petropoulous et al., 2008; and Taha et al., 2013). Manukyan (2011) reported an increase of α-thujone and β-thujone with increased drought stress levels. Aziz et al. (2008b) showed a water-stress induced decrease in ρ-cymene content in *Thymus vulgaris*. However, in Chamomile drought stress decreased epigenin content, but did not affect essential oil composition (Baghalian et al., 2011). The lack of response to reducing the frequency of irrigation in the present study, other than on β-Pinene content suggests that the plants were not severely water stressed, probably due to the high water holding capacity of the vitric mollic andosol soil at the experimental site (Shoji et al., 1993).

In addition to the individual effects of N fertilization, P fertilization and watering regime interactive effects between these factors were demonstrated before to affect oil production in various aromatic plants. In the present study with sage, significant interactive effects ($P \leq 0.05$) between N and P treatments were identified for contents of both α- and β-thujones (Table 39 A and B). Under low N concentrations (no N supply), increased P supply significantly ($P \leq 0.05$) reduced production of α-thujone. However, with increasing supply of N, P had a stimulation effect on α-thujone production.For example, high levels of N were required to allow P-induced accumulation of α-thujone. The highest levels of α-thujone accumulation occurred in plants that received 120 kg N/ha and 30-60 kg P/ha. The interactive effects on β-thujone were different from that observed for α-thujone (Table 39 A and B). Duncan Multiple Range Test at $\alpha = 5\%$ demonstrated that under low N concentrations (no N supply) increased P supply increased production of β-thujone. However, with increasing supply of N, P had an inhibiting effect on β-thujone production.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

Based on the findings of this study, it can be concluded that:

1. There were considerable variations in all growth parameters. Sage growth responded to N and P application at 80 kg N/ha and 60 kg P/ha. The growth parameters were maximum when combined with watering once after every two weeks. Lower and higher N and P application rates as well as too close or far watering intervals resulted into reduced growth at different combinations.
2. There were varied responses in both fresh and dry leaf yield of sage with the highest responses at 80 kg N/ha and 60 kg P/ha when combined with watering once after every two weeks.
3. Biochemical compounds (total phenolic compounds and essential oil yields) were not significantly influenced by different N, watering and P regimes. Furthermore, interactive effects between these variables on the content of the essential oil were also not significant. However, these variables affected the composition of the oil. Specifically, (i) the percentage of β-Pinene increased with increasing N levels, (ii) β-Pinene decreased with reducing irrigation frequency, (iii) interactive effects between N and P treatments were identified for contents of both α- and β-thujones, and (iv) α-thujone accumulation was affected also by the interaction between watering regime and P application. Camphor was the major ingredient under all treatments and its percentage in the oil was higher than the recommended threshold by ISO standard (ISO, 9909).

From the foregoing conclusions, the following recommendations can be made:

1. Nitrogen and phosphorus application at 80 kg N/ha and 60 kg P/ha is sufficient enough to support sage growth and leaf fresh yield, under watering once after two weeks regime for the study area,
2. Production of both α- and β-thujones can be maximized by application of 40 kg N/ha and 60 kg P/ha and watering once a week.
3. There is need to develop agrotechnical practices aimed at reducing the levels of camphor in locally grown sage to conform to the recommended standards (ISO 9909).

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APPENDICES

Appendix 1. Effects of nitrogen, watering and phosphorus on plant height (Experiment 3)

Nitrogen	**Watering**	**Phosphorus (kg P/ha)**				
(kg N/ha)		None	30	60	90	**N Means**
None	W1	37.3*	39.7	39.7	42.7	
	W2	40.0	40.3	44.7	41.3	
	W3	42.7	46.0	45.0	47.0	**42.2**
40	W1	46.0	48.0	40.3	47.7	
	W2	39.0	42.3	39.0	37.7	
	W3	43.0	41.0	41.7	43.0	**42.4**
80	W1	38.3	40.7	41.0	41.0	
	W2	44.7	46.3	49.7	45.3	
	W3	38.7	41.7	35.7	40.3	**42.0**
120	W1	37.7	38.0	36.0	37.7	
	W2	36.7	36.0	38.0	38.0	
	W3	39.7	40.0	41.7	41.3	**39.0**
P Means		**40.3**	**41.7**	**41.0**	**41.9**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 2. Effects of nitrogen, watering and phosphorus on plant height (Experiment 4).

N	**Watering**		**P (kg P/ha)**			
(kg N/ha)		None	30	60	90	**N Means**
None	W1	36.0*	39.0	39.0	40.3	
	W2	37.7	39.0	36.0	40.3	
	W3	28.7	34.7	31.3	32.3	**36.2**
40	W1	34.0	43.3	39.7	40.7	
	W2	39.0	37.0	39.3	43.7	
	W3	31.0	28.7	29.7	32.0	**36.5**
80	W1	37.7	41.0	39.7	43.0	
	W2	40.3	41.3	36.7	41.7	
	W3	32.0	32.7	34.3	34.7	**37.92**
120	W1	39.7	42.7	41.0	41.7	
	W2	41.0	41.3	41.7	47.0	
	W3	31.0	31.0	33.7	31.7	**38.61**
P Means		**35.7**	**37.6**	**36.8**	**39.1**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 3. Effects of nitrogen, watering and phosphorus on the number of primary branches (Experiment 1)

N (kg N/ha)	Watering	P (kg P/ha) None	30	60	90	N Means
None	W1	39*	39	37	36	
	W2	37	40	39	34	
	W3	33	33	33	32	**36**
40	W1	33	40	36	39	
	W2	38	39	33	38	
	W3	34	33	32	34	**36**
80	W1	36	41	36	39	
	W2	36	38	37	36	
	W3	33	33	33	34	**36**
120	W1	37	37	40	35	
	W2	38	38	38	36	
	W3	30	33	35	33	**36**
P Means		**35**	**37**	**36**	**36**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 4. Effects of nitrogen, watering and phosphorus on the number of primary branches (Experiment 2)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	33*	30	39	36	
	W2	31	34	47	35	
	W3	45	37	38	36	**37**
40	W1	42	45	38	39	
	W2	34	36	34	32	
	W3	41	33	37	39	**38**
80	W1	36	39	35	38	
	W2	40	32	36	42	
	W3	46	39	47	38	**39**
120	W1	37	43	41	47	
	W2	46	40	47	42	
	W3	43	49	46	46	**46**
P Means		**40**	**38**	**42**	**39**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 5. Effects of nitrogen, watering and phosphorus on the number of primary branches (Experiment 3)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	49*	57	41	41	
	W2	46	46	44	42	
	W3	48	44	48	53	**47**
40	W1	47	60	46	57	
	W2	48	45	44	47	
	W3	48	50	54	47	**50**
80	W1	51	55	56	52	
	W2	57	52	55	59	
	W3	49	54	62	47	**54**
120	W1	51	57	48	52	
	W2	47	47	53	47	
	W3	54	56	55	58	**52**
P Means		**50**	**52**	**51**	**50**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 6. Effects of nitrogen, watering and phosphorus on the number of primary branches (Experiment 4)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	24*	29	27	30	
	W2	25	26	25	26	
	W3	19	22	23	22	**25**
40	W1	26	30	28	28	
	W2	25	25	25	26	
	W3	24	22	22	23	**25**
80	W1	28	29	31	31	
	W2	27	26	27	27	
	W3	23	23	32	25	**27**
120	W1	31	31	31	30	
	W2	28	29	28	29	
	W3	24	25	26	29	**29**
P Means		**25**	**26**	**27**	**27**	

*Values not followed by a letter are not significantly differently different according to the F-Test at $P \leq 0.05$

Appendix 7. Effects of nitrogen, watering and phosphorus on the number of secondary branches (Experiment 1)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	199*	210	181	176	
	W2	225	247	220	231	
	W3	247	234	225	243	**220**
40	W1	231	263	275	194	
	W2	177	220	206	146	
	W3	225	219	212	215	**215**
80	W1	235	226	253	208	
	W2	223	250	241	211	
	W3	137	157	158	173	**206**
120	W1	210	169	180	191	
	W2	211	214	178	193	
	W3	186	188	193	193	**262**
P Means		**209**	**216**	**210**	**198**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 8. Effects of nitrogen, watering and phosphorus on the number of secondary branches/plant (Experiment 2)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	131*	137	129	135	
	W2	106	123	147	108	
	W3	147	147	151	156	**135**
40	W1	163	170	161	137	
	W2	147	150	160	128	
	W3	149	152	132	152	**150**
80	W1	131	164	153	132	
	W2	157	158	177	157	
	W3	111	133	149	125	**146**
120	W1	120	141	142	148	
	W2	150	137	156	143	
	W3	127	165	153	136	**143**
P Means		**137**	**148**	**151**	**138**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 9. Effects of nitrogen, watering and phosphorus on the number of secondary branches/plant (Experiment 3)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	122*	140	119	128	
	W2	103	117	140	108	
	W3	151	135	140	147	**129**
40	W1	136	168	129	101	
	W2	138	146	151	121	
	W3	146	142	118	147	**137**
80	W1	128	153	149	129	
	W2	151	144	160	168	
	W3	124	137	157	123	**144**
120	W1	133	147	147	163	
	W2	157	145	157	153	**148**
	W3	125	165	149	132	
P Means		**135**	**145**	**143**	**135**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 10. Effects of nitrogen, watering and phosphorus on the number of secondary branches/plant (Experiment 4)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	150*	159	139	133	
	W2	133	131	143	130	
	W3	108	111	116	112	**131**
40	W1	143	167	156	161	
	W2	137	132	142	141	
	W3	125	116	120	124	**139**
80	W1	162	153	155	167	
	W2	153	141	151	138	
	W3	124	134	128	130	**145**
120	W1	147	162	176	169	
	W2	141	155	162	168	
	W3	126	138	139	143	**152**
P Means		**137**	**142**	**144**	**143**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 11. Effects of nitrogen, watering and phosphorus on the number of internodes (Experiment 1)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	19*	20	19	18	
	W2	19	21	20	18	
	W3	19	19	18	19	**19**
40	W1	19	20	19	20	
	W2	20	20	19	20	
	W3	20	19	18	19	**19**
80	W1	20	20	19	20	
	W2	20	19	20	19	
	W3	18	19	18	18	**19**
120	W1	19	19	20	19	
	W2	20	20	20	20	
	W3	19	20	19	19	**20**
P Means		**20**	**20**	**19**	**19**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 12. Effects of nitrogen, watering and phosphorus on the number of internodes (Experiment 2)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	13*	13	12	12	
	W2	12	13	12	13	
	W3	13	13	13	14	**13**
40	W1	14	14	13	14	
	W2	14	13	14	13	
	W3	13	15	15	14	**14**
80	W1	12	13	14	13	
	W2	14	13	13	14	
	W3	13	14	14	14	**13**
120	W1	13	15	15	15	
	W2	14	15	15	15	
	W3	15	14	15	15	**15**
P Means		**13**	**14**	**14**	**14**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 13. Effects of nitrogen, watering and phosphorus on the number of internodes (Experiment 3)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	14*	13	13	13	
	W2	12	13	12	13	
	W3	14	14	14	14	**13**
40	W1	14	15	14	14	
	W2	14	14	14	13	
	W3	13	15	15	14	**14**
80	W1	14	13	13	13	
	W2	14	14	14	14	
	W3	13	13	14	14	**14**
120	W1	13	15	14	14	
	W2	14	14	14	14	
	W3	14	14	14	14	**14**
P Means		**14**	**14**	**14**	**14**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Appendix 14. Effects of nitrogen, watering and phosphorus on the number of internodes (Experiment 4)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	11*	13	12	12	
	W2	12	13	12	12	
	W3	12	12	13	12	**12**
40	W1	12	12	12	12	
	W2	12	12	13	13	
	W3	13	13	13	13	**13**
80	W1	13	13	13	13	
	W2	13	13	14	13	
	W3	12	13	13	13	**13**
120	W1	13	13	14	13	
	W2	13	13	13	13	
	W3	13	13	13	14	**13**
P Means		**12**	**13**	**13**	**13**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 15. Effects of nitrogen, watering and phosphorus on the Specific Leaf Weight (Experiment 1)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	0.009*	0.008	0.008	0.006	**0.008**
	W2	0.007	0.008	0.008	0.008	**0.008**
	W3	0.009	0.009	0.008	0.010	**0.009**
40	W1	0.007	0.010	0.007	0.008	**0.008**
	W2	0.007	0.007	0.010	0.009	**0.008**
	W3	0.008	0.009	0.008	0.009	**0.009**
80	W1	0.008	0.007	0.006	0.007	**0.007**
	W2	0.008	0.007	0.007	0.007	**0.007**
	W3	0.008	0.009	0.009	0.009	**0.009**
120	W1	0.007	0.007	0.008	0.008	**0.008**
	W2	0.008	0.008	0.009	0.008	**0.008**
	W3	0.009	0.009	0.009	0.008	**0.009**
P Means		**0.008**	**0.008**	**0.008**	**0.008**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 16. Effects of nitrogen, watering and phosphorus on Specific Leaf Weight (Experiment 2)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	0.008*	0.008	0.007	0.010	**0.008**
	W2	0.009	0.009	0.011	0.008	**0.009**
	W3	0.010	0.008	0.007	0.007	**0.008**
40	W1	0.008	0.010	0.009	0.008	**0.009**
	W2	0.008	0.008	0.010	0.009	**0.009**
	W3	0.008	0.008	0.007	0.009	**0.008**
80	W1	0.008	0.008	0.008	0.008	**0.008**
	W2	0.008	0.008	0.007	0.010	**0.008**
	W3	0.008	0.007	0.008	0.008	**0.008**
120	W1	0.009	0.007	0.008	0.007	**0.008**
	W2	0.008	0.009	0.011	0.011	**0.010**
	W3	0.010	0.012	0.008	0.009	**0.010**
P Means		**0.009**	**0.009**	**0.008**	**0.009**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

Appendix 17. Effects of nitrogen, watering and phosphorus on Specific Leaf Weight (Experiment 3)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	0.006*	0.005	0.007	0.006	**0.006**
	W2	0.006	0.005	0.011	0.007	**0.007**
	W3	0.006	0.006	0.006	0.007	**0.006**
40	W1	0.007	0.006	0.009	0.006	**0.007**
	W2	0.006	0.006	0.006	0.006	**0.006**
	W3	0.007	0.006	0.006	0.007	**0.007**
80	W1	0.005	0.006	0.006	0.007	**0.006**
	W2	0.007	0.007	0.007	0.007	**0.007**
	W3	0.006	0.006	0.006	0.007	**0.006**
120	W1	0.006	0.005	0.005	0.005	**0.005**
	W2	0.005	0.006	0.006	0.006	**0.006**
	W3	0.006	0.006	0.006	0.006	**0.006**
P Means		**0.006**	**0.006**	**0.007**	**0.006**	

*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$.

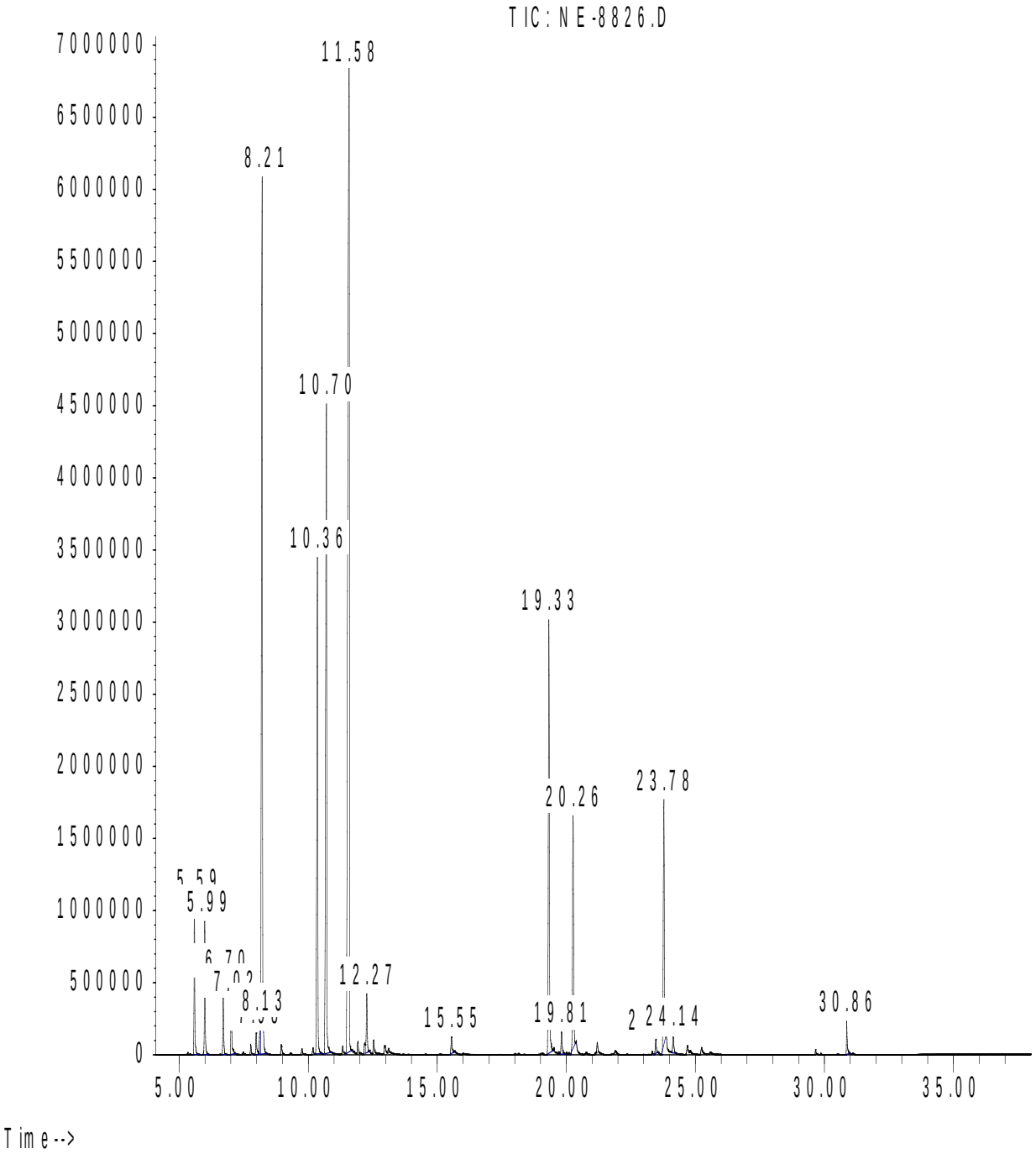
Appendix 18. Effects of nitrogen, watering and phosphorus on Specific Leaf Weight (Experiment 4)

N	**Watering**	**P (kg P/ha)**				
(kg N/ha)		**None**	**30**	**60**	**90**	**N Means**
None	W1	0.008*	0.008	0.007	0.006	**0.007**
	W2	0.009	0.008	0.008	0.008	**0.008**
	W3	0.010	0.007	0.011	0.010	**0.010**
40	W1	0.010	0.009	0.011	0.011	**0.010**
	W2	0.005	0.009	0.011	0.008	**0.008**
	W3	0.009	0.009	0.008	0.008	**0.009**
80	W1	0.008	0.007	0.008	0.008	**0.008**
	W2	0.008	0.010	0.008	0.007	**0.008**
	W3	0.007	0.009	0.009	0.007	**0.008**
120	W1	0.009	0.007	0.010	0.010	**0.009**
	W2	0.008	0.017	0.009	0.009	**0.011**
	W3	0.009	0.008	0.009	0.008	**0.009**
P Means		**0.008**	**0.009**	**0.009**	**0.008**	

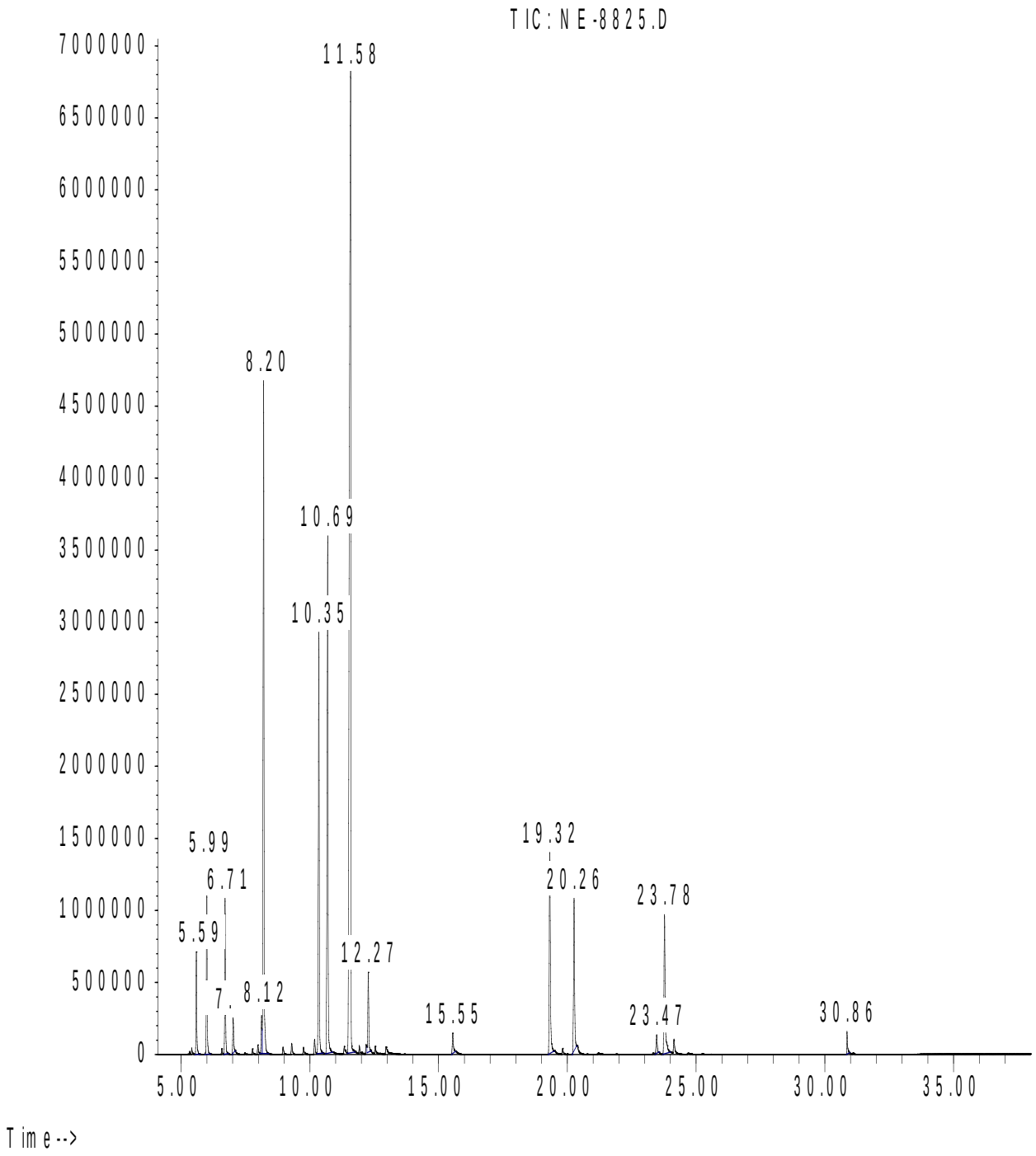
*Values not followed by a letter are not significantly different according to the F-Test at $P \leq 0.05$

Appendix 19. Selected chromatographs for sage essential oil

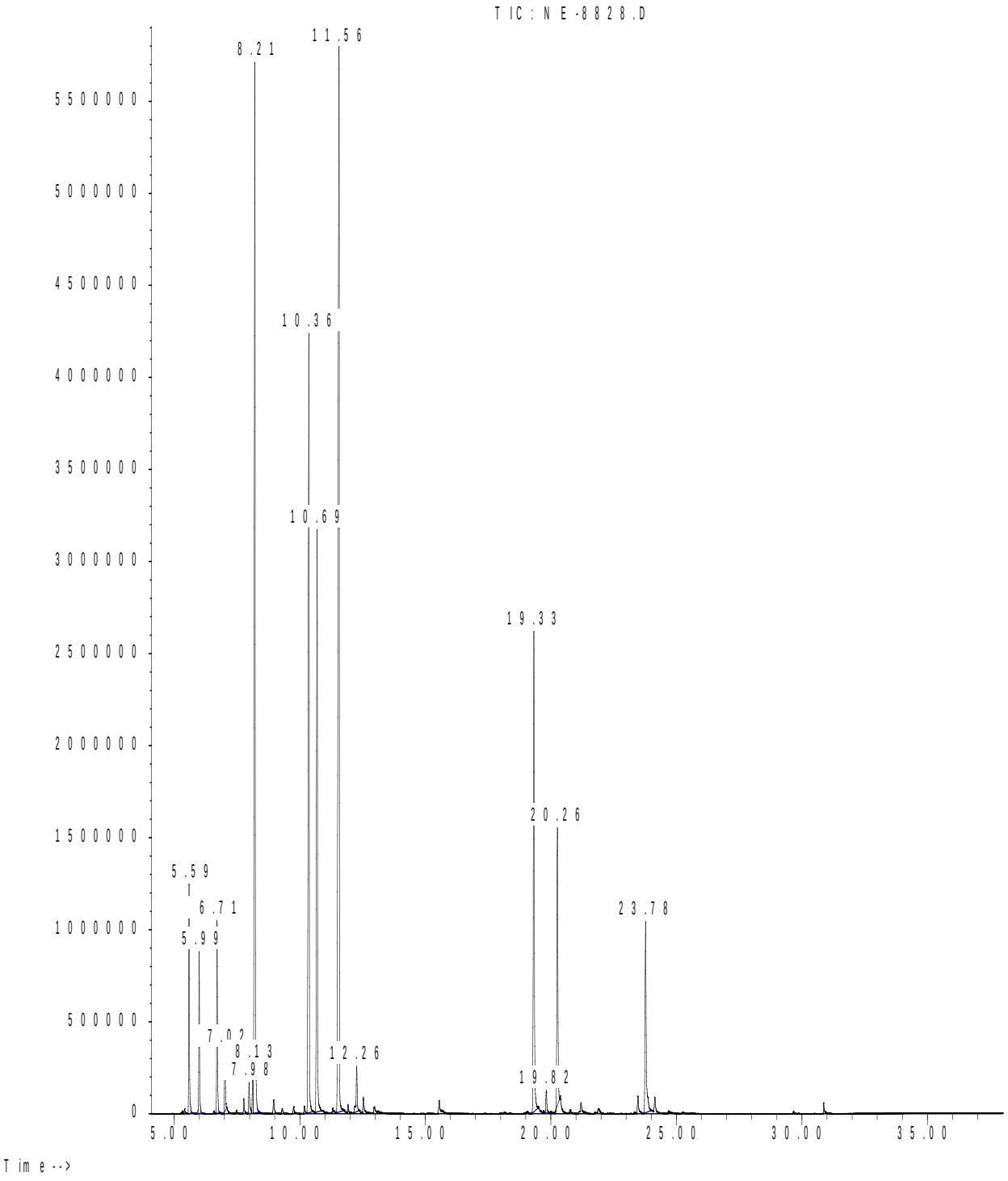
A. 40 kg N/ha X watering once after every two weeks X 0 kg P/ha



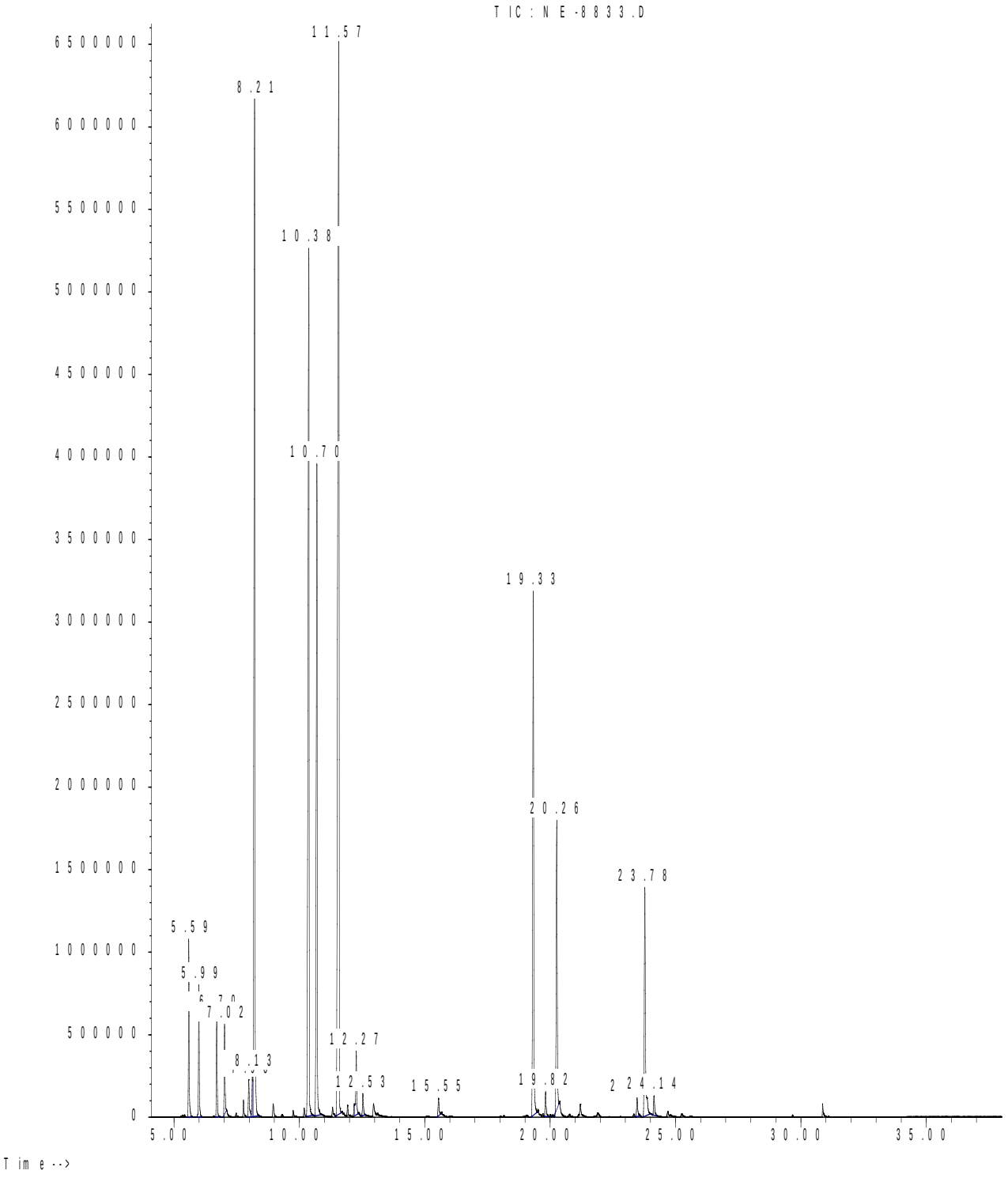
B. 120 kg N/ha X watering once after every week X 0 kg P/ha



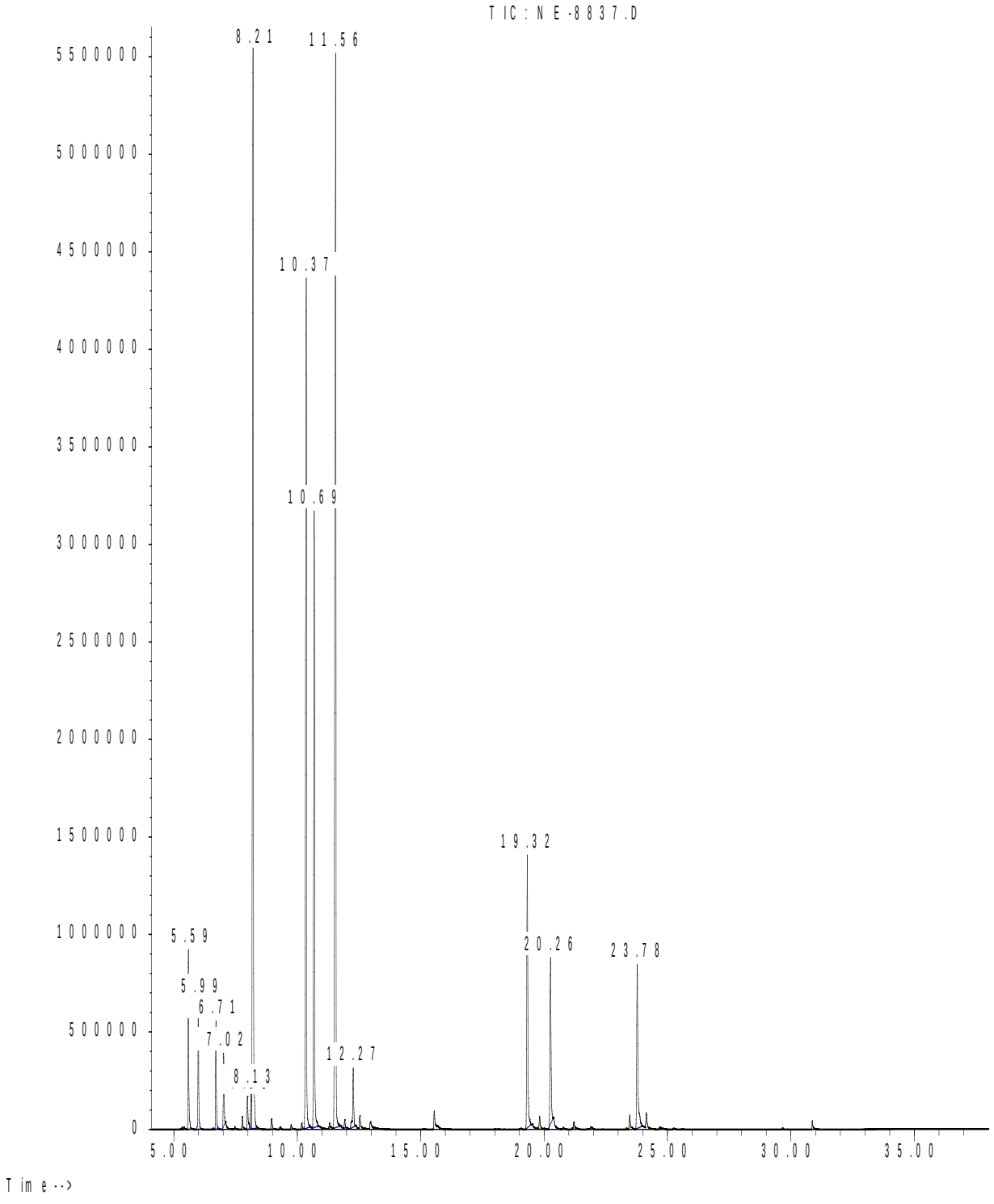
C. 120 kg N/ha X watering once after every week X 0 kg P/ha



D. 0 kg N/ha X watering once after every two weeks X 90 kg P/ha



E. 120 kg N/ha X watering once after every two weeks X 30 kg P/ha



Appendix 20. Publications Based on this Research

1. Rioba, N.B., F.M. Itulya, M. Saidi, B. Nirit, and N. Dudai. 2015. Effects of nitrogen, phosphorus and irrigation frequency on essential oil content and composition of sage (*Salvia officinalis* L). Journal of Applied Research in Medicinal and Aromatic Plants. 2(1):21-29.
2. Rioba, N.B., M. Saidi and F.M. Itulya. 2014. Effects of nitrogen, phosphorus and irrigation regimes on growth and leaf productivity of sage (*Salvia officinalis* L) in Kenya. Annals of Biological Research. 5(2):84-91.