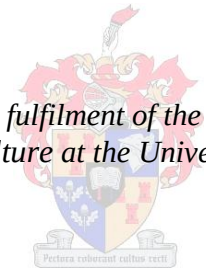
Influence of Soil Texture, Water Management and Fertilizer N on the Biomass Production and Antimicrobial Properties of *Mentha longifolia* L.

by
KOETLISI ANDREAS KOETLISI



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Supervisor: Dr Josias Eduard Hoffman
Co-supervisor: Prof. Dr. Abel J. Pienaar
Faculty of Agrisciences
Department of Soil Science

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Abstract:

Soil texture, plant available water and fertilizer N would influence growth, biomass production and antimicrobial properties of locally used medicinal plants.This research was aimed at investigating how various soil textures (loamy sand, sandy loam and loam) with varying amounts of plant available water (PAW) and nitrogen fertilizer rates would influence the biomass production and antimicrobial properties of *Mentha longifolia* L. In this research, a two-way factorial experiment was used. It was produced by 3 X 3 factors, viz. three different soil textures (loam, sandy loam and loamy sand) and three levels of PAW in the first trial (0 %, 50 % and 90 % depletion of PAW) and three levels of N fertilizer rates in the second trial. The elemental fertilizers KNO_3, K_2SO_4, KH_2PO_4, KCl Ca $(NO_3)_2{\cdot}2H_2O_z$, $CaSO_4{\cdot}2H_2O$ and Mg $SO_4{\cdot}7H_2O$ were used to prepare a nutrient solution for fertigation to meet 0Kg ha^{-1}, 150Kg ha^{-1} and 250Kg ha^{-1} fertilizer N. This was replicated four times. The experiment was conducted in a tunnel. From the first trial the highest biomass production was obtained from 0% depletion of PAW treatments whereas 50% and 90% depletion of PAW matched each other at lower biomass productions. In terms of soil texture a higher biomass production was gained from loamy sand followed by loam and sandy loam. In the second trial similar influences of soil texture were evident and the significant biomass productions were highest, intermediate and low from 250Kg ha^{-1}, 150Kg ha^{-1} and 0Kg ha^{-1} of fertilizer N, respectively. Accordingly, *Mentha longifolia* L revealed a minimal bacterial inhibition activity at 20g $100ml^{-1}$ against Staphylococcus aureus (gram positive bacteria) under Minimum Inhibitory Concentration assay–susceptibility test. It was therefore concluded that soil texture does influence biomass production. In a like manner, the PAW had a significant impact on the total biomass production. An increase in N fertilizer increased vegetative biomass production. Plant material obtained from *Mentha longifolia* L has antimicrobial properties. Medically the plant can be used to combat Staphylococcus aureus – a major and ubiquitous pathogen for humans. The significance of this study is thus that it will benefit and help the medical community and future research as the guide to sustainable production and utilization of *Mentha longifolia* L.

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Opsomming:

Grondtekstuur, plant beskikbare water en kunsmis N sal plantegroei, biomassaproduksie en antimikrobiese-eienskappe van plaaslike medisinale plante affekteer. Die doel van die navorsing was om die effek van grondteksture, plant beskikbare water (PAW) en stikstof op die biomassaproduksie en antimikrobieseeienskappe van *Mentha longifolia* L. te bestudeer. 'n Tweerigting-faktoriaal-eksperiment is gebruik deur drie verskillende grondteksture (leem, sanderige-leemgrond en leemsand) en drie vlakke van PAW in die eerste geval (0%, 50% en 90% uitputting van PAW) en drie vlakke van N-kunsmistoedienings in die tweede geval. Die basiese kunsmis KNO_3, K_2SO_4, KH_2PO_4, $KClCa(NO_3)_2.2H_2O_z$, $CaSO_4.2H_2O$ en $MgSO_4.7H_2O$ is gebruik in so ‘n mate dat 0Kg ha^{-1}, 150kg ha^{-1} en 250 kg ha^{-1} Nas sproeibemesting toegedien is. Dit is vier keer herhaal. Die eksperiment is uitgevoer in 'n tonnel. Die hoogstebiomassaproduksie is van die eerste geval verkry van 0% uitputting van PAW behandelings, terwyl 50% en 90% uitputting van PAW ooreenstem met mekaar op laer biomassaproduksies. In terme van grondtekstuur is 'n hoër biomassaproduksie verkry in leemsand gevolg deur leem en sanderigeleem. In die tweede geval is soortgelyke invloede van grondtekstuur duidelik en die beduidende biomassaproduksies was die hoogste, intermediêre en laagste van 250 kg ha^{-1}, 150kg ha^{-1} en 0Kg ha^{-1} van kunsmis N, onderskeidelik. Gevolglik, *Mentha longifolia* L onthul 'n minimale bakteriese inhibisie aktiwiteit op 20g $100ml^{-1}$ teen Staphylococcus aureus (gram positiewebakterieë) onder Minimum inhiberende konsentrasie assay-vatbaarheidtoets. Die gevolgtrekking is dus dat grondtekstuu biomassaproduksie beïnvloed. In 'n soortgelykewyse, het PAW 'n beduidende impak op die totale biomassaproduksie. 'n Toename in N-kunsmis verhoog vegetatiewe biomassaproduksie. Plantmateriaalverkry van *Mentha longifolia* L het antimikrobiale-eienskappe en kan as Die medisinale plante gebruik word om Staphylococcus aureus te bestry - 'n groot en alomteenwoordige patogeen in die mens. Die belangrikste bydrae van die navorsing is die bydra wat dit tot die mediesegemeenskap gemaak het. Die studie het ook riglyne gestel vir toekomstige navorsing vir volhoubare produksie van *Mentha longifolia* L.

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Dedication:

I dedicate this work to my omnipotent Creator,

Jehovah God Almighty,

without whom I would not have been competent to complete this study.

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Chapter 1: Introduction

"Let us not forget that cultivation of the earth is the most important labour of man. When tillage begins, other arts follow. The farmers are therefore the founders of civilization". **Daniel Webster**

1.1 Introduction

Down the ages, people have used plant and animal resources for their basic needs (Schippmann, Leaman, and Cunningham, 2002). These resources included edible nuts, mushrooms, herbs, fruits, game and other produce for medicinal and cultural uses apart from food and clothing (Schippmann *et al*., 2002). Dahlberg and Trygger (2009) indicated that, according to relevant research in South Africa, villagers have frequently used medicinal plants which helped them with health problems. Knowledge of plants and household remedies was extensive and varied from household to household. Villagers normally relied on common species, and were generally aware of alternative species used to treat different ailments (Dahlberg and Trygger, 2009).

Amongst the medicinal plants, *Mentha longifolia* L. (mint), in the Lamiaceae family, is one of the common plants known for its medicinal properties, which is why it has been adopted as a medicinal and kitchen herb (Van der Walt, 2004). Ecologically, this is a hydrophilic (water-loving) plant that is usually found growing in wet or damp places. *Mentha longifolia* L is a fast growing perennial herb that has a creeping habit and has underground rootstock. Its distribution as an indigenous herb in South Africa is associated mainly with marshes and stream banks. Its uses as a popular traditional medicine are mainly for respiratory ailments, such as coughs, colds and asthma. It is also taken for stomach cramps and indigestion (Maoshing, 2009; Van der Walt, 2004).

Since the 1990s, the medical community has increasingly recognised the importance of indigenous herbs. Up to 80% of African patients in South Africa consult African indigenous healers before attending Primary Health Care facilities

(Setswe, 1999; Shackleton, 2003). The World Health Organization (WHO) (2000) supports Setswe's findings, stating that indigenous herbs make an important contribution to people's lives. The interest in medicinal herbs has increased among westerners in recent decades and it seems likely to continue increasing. As the demand for indigenous herbs has increased, the interest and involvement of scientific community also increase (WHO, 2000). With reference to the latter, WHO (2002 – 2005) states that the popularity of indigenous herbs among developing and developed stakeholders comes, at least in part, from a reaction against the effects of chemical drugs and allopathic medicine.

Msuya and Kideghesho (2009) note that, even where modern medical services are available in many parts of Africa, indigenous herbs have remained a more feasible option due to their affordable prices. Because the supply of indigenous herbs is declining with over-harvesting, cultivation might become economically feasible (Cunningham, 2001 in: Schippmann *et al*., 2002). Small-scale cultivation of indigenous herbs requires low financial investment, as it mainly depends on readily available organic inputs (Agelet *et al.*, 2000 in: Schippmann *et al.*, 2002).

In this chapter, the following topics shall be discussed; literature review and affirmation, present knowledge about the cultivation of medicinal plants, medicinbal properties of *Mentha longifolia* L, harvesting and handling of medicinal plants and finally the research aim.

1.2 Literature Review and Affirmation

1.2.1 Present Knowledge about Cultivation of Medicinal Plants

Despite the mentioned evidence that indigenous plants are broadly used for medicinal and nutritional purposes, only limited research results are available on the conservation of these useful plants. According to WHO (2003), agriculture has recently been identified as pivotal for the sustainable production of medicinal plants. However, the cultivation of medicinal plants requires intensive care, management and monitoring. Optimal conditions should apply for the plantation to be sustainable.

The quality of a plant's medicinal properties is influenced by soil, climate and other factors such as extrinsic environmental conditions (WHO, 2003).

The European Agency for Evaluation of Medicinal Products (EMEA) (2002) recommends that medicinal plants should not be grown in sludge and heavy metal contaminated soils, and that the use of chemicals such as herbicides and insecticides should be avoided or kept to a minimum. EMEA (2002) adds that controlled irrigation is a prerequisite and should be carried out according to the needs or levels for normal growth of the medicinal plant (EMEA, 2002).

Mentha longifolia L is found within the taxonomy of mints, genus *Mentha* of the Lamiaceae family. It grows in such a way that soil physical properties remain a complex problem (Tucker *et al.*, 1980 in; Saric-Kundalic *et al.*, 2009). The systematics of the section *Mentha* is especially difficult because of the frequent hybridisation, occurring both in the wild population and in cultivation (Harley and Brighton, 1977 in; Saric-Kundalic *et al.*, 2009). Therefore, the soil requirements are always considered in a general perspective for the section of *Mentha* in general. Mint will grow well in most soil types, including heavy textured moist soils if drainage is sufficient. On lands that are waterlogged during winter, it will not perform well and plants may even die off. Deep, well-drained soils, and rich in humus, with good moisture retention are the most suitable. Further requirements, according to the literature, are that soil samples should be taken for analysis to determine base fertility levels before mint is planted, and the soil pH should be kept between 5.5 and 7.0 (Directorate Plant Production, 2009).

Water requirement is another important factor in the cultivation of medicinal plants, especially where the spicie is a hydrophilic (Van der Walt, 2004). However, the effect of drought stress on the growth and development of medicinal and aromatic plants' has hardly been studied. The results indicate that a water deficit during the vegetative period (before the flowering stage) may result in shorter plants and smaller leaf areas of mint (Abbaszadeh *et al*., 2008 in; Farahani *et al.*, 2009). Reduced water use results in the reduction in plant size and decreased vegetative dry matter. Drought stress reduces yield of medicinal and aromatic plants by three

main mechanisms: firstly, whole canopy absorption of incident photo synthetically active radiation may be reduced, either by drought-induced limitation of leaf area expansion, by temporary leaf wilting or rolling during periods of severe stress, or by early leaf senescence (Farahani *et al.*, 2009). Secondly, drought stress decreases the efficiency with which absorbed photosynthetically active radiation is used by the crop to produce new dry matter (the radiation use efficiency). Lastly, drought stress may limit grain yield of medicinal and aromatic plants by reducing the harvest index (HI). Consequently, according to Farahani *et al*. (2009), drought stress reduces the vegetative growth period and the plants develop to the flowering stage sooner. Therefore, quantity characteristics of medicinal and aromatic plants decrease under drought conditions (Farahani *et al*., 2009).

Subtle variations in soil water levels are known to produce significant effects on plant physiological response and soil nutrient availability, thereby influencing production as a whole (Paul *et al*., 2003 in; Araya, Gowing and Dise, 2010; Davies and Gowing, 1999; Foth, 1990; Hamdy, [s.a.]). Shormin, Khan and Alamgir (2009) indicate that water stress significantly decreased plant height, leaf area index, dry matter accumulation and oil content of *Mentha arvensis* L. (Shormin, Khan and Alamgir, 2009). According to the available literature, soil and water factors have a great influence on the biomass production of the medicinal plants.

According to Havlin *et al*. (2005), production potential of a crop depends on the growing season, environment and the skills of the producer to identify and minimise factors that reduce yield potentials. Elimination of fertilizer would decrease yield (Havlin *et al*., 2005). Sufficient nutrient availability is required to realise maximum yield potential (Havlin *et al*., 2005). *Mentha* has to be fertilized properly to attain a good crop. Nitrogen fertilization is essential for foliage stimulation and improving the flavour and quality of oil. Fertilizer rates should be kept generally high in order to allow good vegetal growth and development of the maximum number of leaves. Frequent nitrogen applications through fertigation are required throughout the growing season to maintain soil fertility and to compensate for in-season nutrient

deficiencies. Application of nitrogen at 200 kg ha^{-1} together with organic mulch enhances essential oil yield (Directorate Plant Production, 2009).

1.2.2 Medicinal Properties of *Mentha longifolia* L

1.2.2.1 Relevence of Mentha in health maintanance

Since time immemorial, people have dealt with disease and accidents that threatened their lives and health. This resulted in herbal medicines that co-evolved with humans within their societies and were used to ward off the diseases suffered by early people (Babajide, *et al*., 2010; Ahmad, 1999 in; Jan *et al*., 2008; Abbaszadeh *et al*., 2009). Large proportions of rural and urban populations (approximately 80%) throughout the world still depend on herbal medicine for symbolic and medicinal value (Ahmad, 1999 in; Jan *et al*., 2008; Abbaszadeh *et al*., 2009).

According to Jan *et al*. (2008) the majority (1.5 billion) of the population of developing countries use indigenous medicine, either because the people cannot afford synthetic medicine or because indigenous medicine is more acceptable. Doughari *et al*. (2009) indicate that the interest in plants with antimicrobial properties has been rejuvenated due to current problems associated with the use of antibiotics with the increased prevalence of multidrug resistance (MDR) (Shahverdi *et al*., 2004). Strains of a number of pathogenic bacteria are mentioned as methicillin resistant: *Staphylococcus aureus, Helicobacter pylori,* and MDR *Klebsiela pneumonia* (Doughari *et al*., 2009). These well-known and publicised problems associated with pharmaceutical drug use encourage people to look for alternative remedies. As is the case with the allopathic medicine system, the traditional herbal system also uses special combinations of plants to treat diseases (Jan *et al*., 2008; Van Andel & Havinga, 2008).

Since ancient times, the uses, knowledge and understanding of wild mint as a medicinal herb, have varied from location to location depending on the beliefs and needs of the community. Wild mint is seen as an aromatic and melliferous plant. It is

used in the pharmaceutical, tobacco, food manufacturing industries (in the development of various liqueurs and sweets), and especially in cosmetology (Stanisavljevic *et al*., 2010). Related to *Mentha longifolia* is an English horse mint. Essential oil of English horse mint has a pleasant and refreshing odour. According to the Physicians' Desk Reference (PDR), it exhibits carminative and stimulant properties of the gastrointestinal tract (Ghoulami *et al*., 2001). It relieves colds, respiratory inflammation, headaches, and pain in muscles and joints. Internally, it is used in the form of infusion, and externally, as a bath additive (PDR, 2004 in; Stanisavljevic *et al*., 2010).

In some contexts, *Mentha* spp. had been used as a folk therapy for treatment of "bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints due to their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue and anticatharral activities" (Dzamic *et al*., 2010; Naseri *et al*., 2008; Gulluce *et al*., 2007). According to Petkar (2008), the AmaXhoza use milk or water decoctions of wild mint for coughs, colds, asthma and other bronchial ailments. It has also been used to treat headaches, fevers, indigestion, flatulence, hysteria, painful menstruation, delayed pregnancy and urinary tract infections (Petkar, 2008; Scott *et al*., 2004). It has been reported to be a diaphoretic and has mild spasmolytic action on the smooth muscle of the digestive tract, hence is useful for cramp-like complaints of the gastro-intestinal tract, gall bladder and the biliary tract (Petkar, 2008). Externally, wild mint is used to treat wounds and swollen glands (Asekun *et al*., 2007), and it is further used as an anti-parasitic and repellent (Guarrera, 1999). As with all folk medicine, the effectiveness of *Mentha* as a remedy comes from its traditional use and reputation, not as per scientific testing (Guarrera, 1999).

Scientists are paying attention to traditional remedies in order to build up a register of effective ones to be accepted within African Health Indigenous Knowledge Systems (WHO, 2003). *Mentha longifolia* L. is a candidate.

In phytotherapy research, phytochemicals have gained strength and recognition and the current research seems to be making strides in bringing to light the importance

of herbs in human life (Liu, 2004 in; Doughari *et al*., 2009). Phytochemicals are defined as bioactive, non-nutrient plant compounds in grains, vegetables, fruits, and other plant foods that have been correlated to reducing the risk of major chronic diseases. The word 'phyto-' is traced from the Greek word *phyto* which means - plant (Liu, 2004 in; Doughari *et al*., 2009). The existence of these bioactive components is believed to make them resistant against bacterial, fungal and pesticidal pathogens. These bioactive components (also called "essential oils") are therefore considered to be responsible for the antimicrobial effects of plant extracts (Abo *et al*., 1991; Nweze *et al*., 2004 in; Doughari *et al*., 2009). Essential oils are volatile, natural, complex compounds characterised by a strong odour and are formed by aromatic plants as secondary metabolites (Odeyemi, 2009) that play a significant role in the protection of the plants as antibacterial, antiviral, antifungal, insecticide agents, and also against herbivores by reducing their appetite for such plants (Hajlaoui *et al*., 2009).

In addition, plant essential oils are considered to be an optional source of natural compounds against pathogenic bacteria because they constitute a loaded source of bioactive chemicals (Bauer *et al*., 2001; Mimica-Duki *et al*., 2003; Hafedh *et al*., 2009 in; Hafedh *et al*., 2010). According to Hafedh *et al*. (2010), the effectiveness of the activity of essential oils with respect to gram-negative and gram-positive bacteria is acknowledged in literature. These properties are due to the presence of active monoterpene constituents (Hafedh *et al*., 2010).

According to Singh *et al*. (2008), the compounds shown in Table 1.1 are found in the essential oil of *Mentha longifolia* L.

These compounds vary in quality, quantity and in composition from place to place, depending on the variations in climate, soil composition, plant organ, age and stage in the vegetative cycle (Masotti *et al*., 2003; Angioni *et al*., 2006 in; Hajlaoui, *et al.,* 2009; Hajlaoui *et al*., 2008; Fahlen *et al.,* 1997). Thefore when plants come from uncontrolled situations, the composition and therefore, effectiveness of the essential oils is unpredictable (Hajlaoui *et al.,* 2009).

Table 1.1 Constituents of *Mentha longifolia* L Essential oil (Singh *et al*.' 2008)

Compounds		
Myrcene, Limonene, α-Terpinene, Limonene, Eucalyptol, Ocimene, Terpinene, Ocimene, α-Terpinolene, 3-Octanol, *trans*-Sabinene hydrate, Caryophyllene, α-Humulene, Linalyl propanoate, α-Terpineol, *cis*-Piperitone oxide	*trans*-Piperitone oxide, *dl*-Carvone, *p*-Menthane *cis*-Pinocarvyl acetate, 2 Hydroxypiperitone, Isopiperitenone, *p*-Cymen-8-ol, Hydroxypiperitone, Piperitenone, Jasmone, Piperitenone oxide, Isocaryophyllene oxide, Caryophyllene oxide,	Isocaryophyllene oxide, Caryophyllene oxide, *trans*-Nerolidol, *m*-Thymol α-pinene, β-pinene, 1,8-cineole, linalool, ocimeno,l menthol, *cis*-isopulegone, pulegone, dihydroedulan I, β-bourbonene, bicyclogermacrene and camphor

The latter discussion gave an account on what constitutes *Mentha*. This is the prerequisite to realise the relevance of *Mentha* in health maintenance. The following section discusses the antimicrobial concept captured inasmuch as medicinal plants are concerned.

1.2.2.2 The antimicrobial concept

Antimicrobial activity may underpin traditional claims about the use of many plant species including *Mentha longifolia* L. South African indigenous medicines including *Mentha* species are frequently prepared as teas for oral administration or topical application. Aqueous infusions are made of all species to create the traditional preparation (Scott *et al.,* 2004) to provide affordable and accessible remedies. An antimicrobial is a drug used to treat a microbial infection. "Antimicrobial" is a general term that refers to a group of drugs that includes antibiotics, antiprotozoals, antifungals, and antivirals (Webster's New World Medical Dictionary, 2008). This explains the wide variety of ailments that *Mentha* infusions are used for.

The literature shows that essential oils obtained from *Mentha longifolia* L. have been used to kill or inhibit the growth of an array of microbes (Gulluce *et al*., 2007) that commonly cause infections in humans. Gulluce at al. (2007) indicated that the essential oil showed strong antimicrobial activity against 30 microorganisms, inter

alia, *Bacillus macerans*-M58, *Pseudomonas syringaepv. tomato* A35, *and Staphylococcus aureus*-A215, *Aspergillus flavus*, *Fusarium oxysporum*, and *Penicillum species,* whereas the methanol extract remained almost inactive. On the other hand, they found that the methanol extract from Mentha longifolia gave much better activity than the essential oil in the antioxidant activity assays employed (Gulluce *et al*., 2007). They then concluded that, *Mentha longifolia* possesses compounds with antimicrobial and antioxidant properties. It was suggested that parallel studies are necessary to confirm the antimicrobial and antioxidant properties of *Mentha longifolia* species (Gulluce *et al*., 2007).

Dzamic *et al*. (2010) investigated the composition and the efficacy of *Mentha longifolia* L essential oil. Their findings were similar to those of Gulluce *et al*. (2007). Dzamic *et al*. (2010) found that a concentration of 10µl/ml showed fungicidal activity against *Aspergillus* and *Fusarium* species, and *Alternaria alternata*, *Penicillium funiculosum* and *T*. *viride*. A concentration of 5µl ml^{-1} was effective against *Trichophyton mentha grophytes* and yeast *Candida albicans*. The most sensitive micromycetes were *Cladosporium fulvum*, *C*. *cladosporium cladosporioides* and *Penicillum ochrochloron*, against which the concentration of 2.5µl ml^{-1} was lethal (Dzamic *et al*., 2010). The results for antioxidant activity supported that of Gulluce *et al*. (2007).

Since 2000, research in the field of medicinal herbs has focused on the antimicrobial properties of the most commonly used species by different communities. Pirbalouti *et al*. (2010), tested one gram-positive (*Staphylococcus aureus*) and three gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacterial strains against plant extracts and essential oils of different medicinal plants, including *Mentha longifolia* Hudson.Their results showed that gram-negative bacteria were more sensitive than gram-positive bacteria. Antibacterial activity of extracts and essential oils of plants varied in relation to the organisms tested. The most dynamic concentration was a 10 mg/ml concentration which absolutely inhibited the growth of all the gram-negative bacteria (Pirbalouti *et al*., 2010).

Shah *et al*. (2010) investigated evidence for *Mentha longifolia's* properties in its usage as a remedy for diarrhea and stomach spasm. In their experiment, they made different doses of crude extract of *Mentha longifolia,* that were then administered orally to selected rats and rabbits (Shah *et al*., 2010). Their main goal was to investigate the antidiarrhoeal and antispasmodic activities of the plant with the intention of providing a pharmacological base to its medicinal use in hyperactive gastric disorders. They used Castor oil-induced diarrhea on experimental animals to test aqueous menthol crude extract for possible antidiarrhoeal effect (Shah *et al*., 2010). According to the results, the crude extract of the leaves of *Mentha longifolia*, like loperamide, a standard antidiarrhoeal agent (Reynolds *et al*., 1984 in; Shah *et al*., 2010), significantly inhibited the frequency and wateriness of stools (Shah *et al*., 2010), indicating that *Mentha longifolia* has antidiarrhoeal and antispasmodic properties (Shah *et al*., 2010).

Another investigation by Hafedh *et al*. (2010) about the antimicrobial activity and the effect of *Mentha longifolia* essential oil on pathogenic bacteria morphology observed by atomic force microscopy (Hafedh *et al*., 2010), is relevant to this research. They tested two gram-positive bacteria: *Micrococcusluteus* NCIMB 8166 and *Staphylococcus aureus* ATCC 25923 and two gram-negative bacteria: *Salmonella typhimurium* LT2 DT104 and *Escherichia coli* ATCC 35218, against essential oil of *Mentha longifolia* (Hafedh *et al*., 2010). They reported that the essential oil presented an antibacterial activity that varied in its antimicrobial effectiveness. The Minimum Inhibitory Concentration (MIC) values indicated that the *Mentha longifolia* essential oil had a broad activity especially for *S. aureus* and *M. luteus* (Hafedh *et al*., 2010). Hafedh *et al.* (2010) found that antimicrobial activity can be attributed to the presence of high concentrations of menthol (32.51%), menthone (20.71%) and pulegone (17.76%) that were found from a particular *Mentha longifolia* plant. They concluded that the essential oil had a stronger and broader spectrum of antimicrobial activity than the methanol extract (Hafedh *et al*., 2010).

Furthermore Nickavar *et al,* (2008) determined and compared the antioxidant activity and total phenolic content (TPC) of five *Mentha* species including *Mentha*

longifolia. They deduced that all the tested plants were rich in phenolic compounds and they had high antioxidant properties (Nickavar *et al*., 2008). these results were supported by Ozgen *et al*. (2006).

Knowing that pathogenic bacteria had become resistant to antimicrobial agents, which posed a major health problem, Shahverdi *et al*. (2004) conducted a study to evaluate the degree to which essential oil of *Mentha longifolia* enhanced the antimicrobial activity of nitrofurantoin, which is used for the treatment of urinary tract infections (Shahverdi *et al*., 2004). Essential oil of *Mentha longifolia* and its main component, piperitone, enhanced the bactericidal activity of nitrofurantoin against Enterobacteriaceae. However, the enhancement of antimicrobial activity of nitrofurantoin by piperitone was concentration dependent for all the species tested (Shahverdi *et al*., 2004).

Then the question arises as to whether soil texture, plant available water and nitrogen fertilizer have an impact on the growth, biomass production and antimicrobial properties of *Mentha longifolia* L.

1.2.3 Harvesting and handling of medicinal plants

At harvest the desired plants or plant parts are harvested and cleared to extract the bioactive components (Kellogg, 2010). The post-harvest handling of the medicinal plants and the methods used for extracting the essential oils affect their quality. Therefore, the chemical composition of the essential oil of *Mentha longifolia* L may depend on the environment where the species grow, on post-harvest handling, on processing and on finishing. Changes in any of these factors could have an impact on the final expected quality (Stanisavljevic *et al*., 2010). Considering the fact that there is a lack of literature on the post-harvest physiology of medicinal plant material, much can be learned and adapted from research on agricultural crops of value to the food industry (Fennell *et al*., 2004). In this field, there are several processes through which the quality of plant material can be degraded. These include chemical breakdown and decomposition, microbial contamination and insect attack (Fennell *et al*., 2004). Immediately after harvesting green leaves, the drying

process is normally the option to preserve the herbs before using or even storing them in the herbarium. It is at this point where degradation processes can occur (Stanisavljevic *et al*., 2010; Fennell *et al*., 2004). Factors such as temperature, light, pH and enzymes bring about a serious change and therefore, the recognition of methods of drying is important. Drying is the easiest way to preserve raw plant material. Drying procedures are not all the same and have an impact on the content of active substances in drugs. In the process of drying the plant material, moisture content is reduced, and the amount and composition of volatile compounds have been proved to change (Moyler, 1994 in; Stanisavljevic *et al*., 2010).

The method of drying has a significant impact on qualitative and quantitative composition of essential oils of aromatic plants. Stanisavljevic *et al*. (2010), reported that three drying procedures were used: firstly, drying of plant material (*Mentha longifolia*) was carried out naturally in the shade of a draughty place. Secondly, it was done in the laboratory oven at the temperature 45°C and lastly, in an absorptive low temperature condensation drying oven at 35°C (low temperature drying). After drying, hydro-distillation was used to isolate the essential oil from the dried samples in three different ways, followed by chemical analysis using Gas Chromatography (GC) and Gas Chromatography – Mass Spectrometry (GC-MS) methods (Stanisavljevic *et al*., 2010; Asekun *et al*., 2007). The uppermost yield of the essential oil was obtained from the herbs that had been dried at low temperature and the lowest from those dried in the laboratory oven. The prime content of the dominant component of essential oils, piperitone, was determined and recorded in the oil from the low temperature dried herb, while the essential oil isolated from naturally dried plants and from the ones dried in the laboratory oven contained piperitone in lower concentrations (average and lower, respectively) (Stanisavljevic *et al*., 2010). According to Indigenous African Knowledge System, drying in a natural way is the most efficient and affordable way for African communities, but, if sophisticated techniques were available, absorptive low temperature condensation drying at low temperature would remain the best (Stanisavljevic *et al*., 2010). Asekun *et al*. (2007) reported three drying methods in another manner. Plant material was obtained and divided into three portions. The

first portion was dried to constant weight in the sun, another portion was left to dry in the laboratory under normal air and at room temperature, while the third part was dried in an oven kept at 40°C (Asekun *et al*., 2007). According to their findings, the most prominent components in both the air-dried and sun-dried leaf oils were close to average and slightly lower, respectively, while the major compounds were not detected in the oven-dried leaf oil (Asekun *et al*., 2007). These experiments show that oven-drying produces the lowest quality essential oil.

Hajlaoui *et al*. (2009) report their isolation of the essential oil by using 100 g of the air-dried aerial parts of the plant and placing such parts in hydro-distillation for three hours with 500 ml distilled water using a Clevenger-type apparatus. In order to obtain methanol extract from the herbal plants, 1 g of dry aerial part powder with 10 ml of pure methanol was stirred for 30 min. The extracts were then kept for 24 hours at 4˚C, filtered through a Whatman No. 4 filter paper, and stored at 4˚C (Hajlaoui *et al*., 2009). In other cases, 20g of dry leaf powder was steeped in 100ml distilled water with occasional shaking for two days and then filtered and the filtrate was used as an aqueous infusion (Javale and Sabnis, 2010). In the same study, an aqueous decoction was prepared by using 20g of leaf powder boiled in 100ml distilled water for 15 minutes, allowed to cool and then filtered (Javale and Sabnis, 2010). Ahmed *et al,* (1998) prepared their extract as follows: they took a 20g portion of powdered plant material and soaked it in 100g of water for 72 hours. The mixture was then stirred every 24 hours by using a sterile glass rod. At the end of the extraction schedule, the extract was passed through Whatman filter paper no.1 (Ahmed *et al*., 1998).

In summary, research literature shows that both the cultivation and the post-harvesting treatment could play a major role in the conservation of the *Mentha longifolia*. That is, proper cultivation would ensure sustainable production of medicinal plants whereas post-harvest conditions and procedures, on the other hand, would ensure that a good quality of medicinal plant is maintained.

1.3 Research Aim

Guided by the literature, this research focuses on the conservation of the indigenous herb, *Mentha longifolia* L. for African indigenous healers. Verification of biomass production possibilities of *Mentha longifolia* L. could lead to sustainable production of this herb. This research aimed to verify the biomass production of this herb by using three different soil textures, varying plant available water and varying nitrogen fertilizer rates.

Specific Objectives were to investigate the following:

I. The impact of soil texture on the growth and biomass production of *Mentha longifolia* L.

II. The influence of plant available water on biomass production of *Mentha longifolia* L.

III. The influence of nitrogen fertilizer on biomass production and the antimicrobial properties of *Mentha longifolia* L.

IV. The interaction of soil texture and plant available water on the growth, biomass production, and the antimicrobial properties of *Mentha longifolia* L.

V. The aim also included that recommendations for sustainable cultivation of the indigenous herb, *Mentha longifolia* L. for growth, biomass production and antimicrobial properties be proposed.

Chapter 2: Materials and Methods

"Thou shalt inherit the holy earth as a faithful steward conserving its resources and productivity from generation to generation ..." **Lowdermilk C Walter**

2.1 Introduction

The purpose of this chapter was to explain the materials and methods used for achieving the aim of this study. The following topics are discussed herein; soils used in this research (texture analysis, bulk density, plant available water (PAW) and pH adjustment), research design (PAW depletion experiment and nitrogen fertilizer rates experiment), growth rate and leaf area development, antimicrobial properties – susceptibility test, data analysis and ethical implications.

2.2 Soils used in this research

Three differently textured soils were collected from three sites near Stellenbosch. Long term weather data indicate that this area ought to be classified as a relatively moderate to high rainfall area (600 – 800 mm pa). Rainfall is received mainly during the cool wet winter months (Bekker, 2011). About 100 kg of each soil texture class was obtained.

Approximately 100 kg of soil was collected from the Nietvoorbij Farm (Loamy sand soil), Luckhoff High School field(Loam soil) and Welgevallen Farm (Sandy loam soil). All these soils (top soil samples – A horizon) were collected from a non-cultivated area. Big (> 5 mm) organic material fragments were removed from the soil samples. These soils were then air-dried and sieved. Five-litre experimental pots were used. The pots were filled up to a depth of approximately 160mm at the weight of 7 kg to provide good rooting depth.

2.2.1 Texture - particle size analysis

Particle size analysis was done with the standard pipette method described by Gee and Bauder (1986). Soil texture analyses are shown in Table 2.1.

Table 2.1 Soil texture analysis of soils used in the research

Sample		Fraction of total mass (%)		
Texture	**Size of particles (mm)**	**Loamy sand**	**Loam**	**Sandy loam**
Coarse sand	(0.5 -2.0)	14.3	10.2	09.2
Medium sand	(0.25 – 0.5)	21.8	11.1	25.3
Fine sand	(0.106 – 0.25)	34.4	16.0	26.6
Very fine sand	(0.05 0.106)	13.5	10.7	07.4
Silt	(0.002 mm – 0.05)	11.3	31.4	23.8
Clay	(< 0.002)	04.6	20.6	07.6

2.2.2 Bulk Density

Soil bulk density is the ratio of the mass of dry solids to the bulk volume of the soil. Bulk density is a widely used physical measurement and is required for the conversion of water percentage by weight to water content by volume (Foth, 1990; Blake and Hartge, 1986). In this experiment, after the collection of different soil samples (field samples), soil bulk densities were determined natural at sampling by making use of an adjusted core method modified from Blake and Hartge (1986). According to this core method, a volume of soil is removed by making use of a steel cylinder of a known volume and then oven dried. Three replicates of small cans/ cylinders of 95.6 cm^3 were pressed into the soil and carefully removed. The soil samples were removed from the cylinders and were weighed, oven dried and re-weighed. Then determination of bulk density was performed. In this investigation, bulk densities of the three soils that were used are shown in Table 2.2.

Table 2.2 Average bulk densities of three soils used in the investigation

Field Sample	Average Bulk density (g/cm^3)
Loamy sand	1.25
Sandy loam	1.33
Loam	1.33

2.2.3 Plant Available Water (PAW)

A standard method of determining PAW described by Cassel and Nielsen (1986) was used in this investigation. The total amount of plant available water was determined as the amount of water held between field capacity (FC) and permanent wilting percentage (PWP) (Table 2.3).

In this investigation, Field Capacity was determined two days after the soil had been saturated and free drainage from the pot had stopped. This procedure is supported by available literature (Troeh and Thompson, 2005; Brady and Weil, 2008; Kirkham, 2005; Water Conservation Factsheet (WCF), 2002). The pots of 0.00723 m^3 volume were initially filled with seven kilogram dry soil. The volume of soil in the pots was 0.0056 m^3 for loamy sand and 0.0053 m^3 for sandy loam and loam soil pots. They were then saturated until the water started to drain. The pots were allowed to drain for two days. Field Capacities were then determined as the weight of the pots after 48 hours. After determining FC, the cuttings of the test plant (*Mentha longifolia* L) were planted. *Mentha spp* are mainly propagated vegetatively. Its seeds do not reproduce true to type offspring (Abbas, 2005).

The best thirty-six plants that looked similar in terms of height and number of leaves were selected and planted. These plants were 5cm tall and had four small leaves each. They were chosen from seventy propagated cuttings. After a period of one week, while these plants were still growing, twelve plants were selected and used to determine the permanent wilting point (PWP) of the test plant. Four plants were selected from each soil texture. This was achieved by recharging soil moisture to FC. The pots were allowed to drain until PWP had been reached. That is, the plants

stayed undisturbed till the day *Mentha longifolia* L. showed the first signs of wilting. Plants that did not receive enough water showed curtailing turgor. This is a symptom of wilting. The weight of the pots at this moisture content was considered to be the PWP. At this stage, the values of field capacity and permanent wilting percentage were then converted into volume water content by multiplying by the ratio of bulk density of soil to the density of water. The volume ratio was multiplied by thickness of the horizon (depth of the pot = 16 mm) (Brady and Weil, 2002; Cassel and Nielsen, 1986) to determine the water content per pot in millimetres (mm). Plant Available Water (PAW) was determined as the moisture content between FC and PWP. PAW is that part of soil water which is held by the soil to an extent that it is available for plant root absorption (Kirkham, 2005; Van der Watt and Van Rooyen, 1995).

Table 2.3 FC, PWP and PAW of three soil textures that were used in the investigation

Moisture Content	Loam (mm)	Loamy sand (mm)	Sandy loam (mm)
FC	45	42	40
PWP	12	11	10
PAW	33	31	30

Depth of rooting zone 160mm

2.2.4 pH Adjustment

After conducting physical properties analyses, soil pH was determined. In this research, pH was determined following the standard method described byThomas (1996). Table 2.4 indicates the average initial soil pH values of the three soils used in the investigation. Three replicates were used in every analysis. In this research H_2SO_4 was used to adjust soil pH of the selected soils. The same standard method was used in determining soil pH after the adjustment had been done.

Table 2.4: Initial soil pH $_{KCl}$ of three soils that were used

Sample	Average pH $_{KCl}$
Loamy sand	6.42
Sandy loam	7.11
Loam	6.38

The aim of this experiment was to ensure one common pH value among the three soils that were used in the investigation. The pH had to be between 5.5 to 7.0 – pH ranges for the test plant. To achieve this, 50g sample of soil was drawn from each texture that was to be used in the main investigation. The sample was then wetted, allowed to reach equilibrium while simultaneously drying. Soil pH in KCl was then analysed by following the standard method described by Thomas (1996). Because distinctive soils react differently, a series of dilutions and molarities were put under investigation.

Table 2.5 Dilutions and molarities used in the acidification procedure

H_2SO_4 volumes in 1000 ml distilled H_2O	Molarities	0.2 ml H_2SO_4 in distilled H_2O
0.2 ml H_2SO_4	0.25 M	2 l distilled H_2O
0.4 ml H_2SO_4	0.125 M	5 l distilled H_2O
1.0 ml H_2SO_4	0.0625 M	7 l Distilled H_2O
2.0 ml H_2SO_4		
3.0 ml H_2SO_4		

At the end of these trials, 0.2 ml H_2SO_4 diluted in 5000 ml distilled H_2O and 3.0 ml H_2SO_4 diluted in 1000 ml distilled H_2O gave desired results (Table 2.6). Thereafter, the entire soils used in this investigation were treated accordingly following the same acidification procedure. Planting of the test plants then followed.

Table 2.6 Adjusted soil pH values of three soils used

Sample	Average pH_{KCl}	
	0.2 ml H_2SO_4 diluted in 5000 ml Distilled H_2O	3.0 ml H_2SO_4 diluted in 1000 ml Distilled H_2O
Loamy sand	6.36	–
Sandy loam	–	6.38
Loam	6.38	–

2.3 Research Design

2.3.1 Plant Available Water (PAW) – depletion experiment

In this experiment, a two-way factorial experiment layout was produced by 3 X 3 factors, viz. three different soil textures (loam soil, sandy loam soil and loamy sand soil) and three levels of PAW (0 % depletion of PAW, 50 % depletion of PAW and 90 % depletion of PAW), replicated four times (Figure 2.1). The aim of this experiment was to investigate the influence of soil texture on the depletion of PAW and also on the biomass production of *Mentha longifolia* L. The PAW experiment was done by weighing. A weighing scale was used to weigh the pots on daily basis every morning between 08h00 and 09h00. All the pots were weighed. Mass was determined between FC and pre-determined soil water content. Water was replenished accordingly based on 0%, 50% and 90% depletion of PAW. Figure 2.2 shows the outlay used in this experiment to determine the water content of each pot, based on weight. The weight of the pot on the day of weighing was subtracted from the FC weight of the pot. Differences in weights were multiplied by 1000 to obtain the equivalent amount of water that was lost due to evapotranspiration (ET). These weight differences were also used to calculate the gravimetric water content of each pot. Bulk densities were used to convert the gravimetric values into volumetric water content.

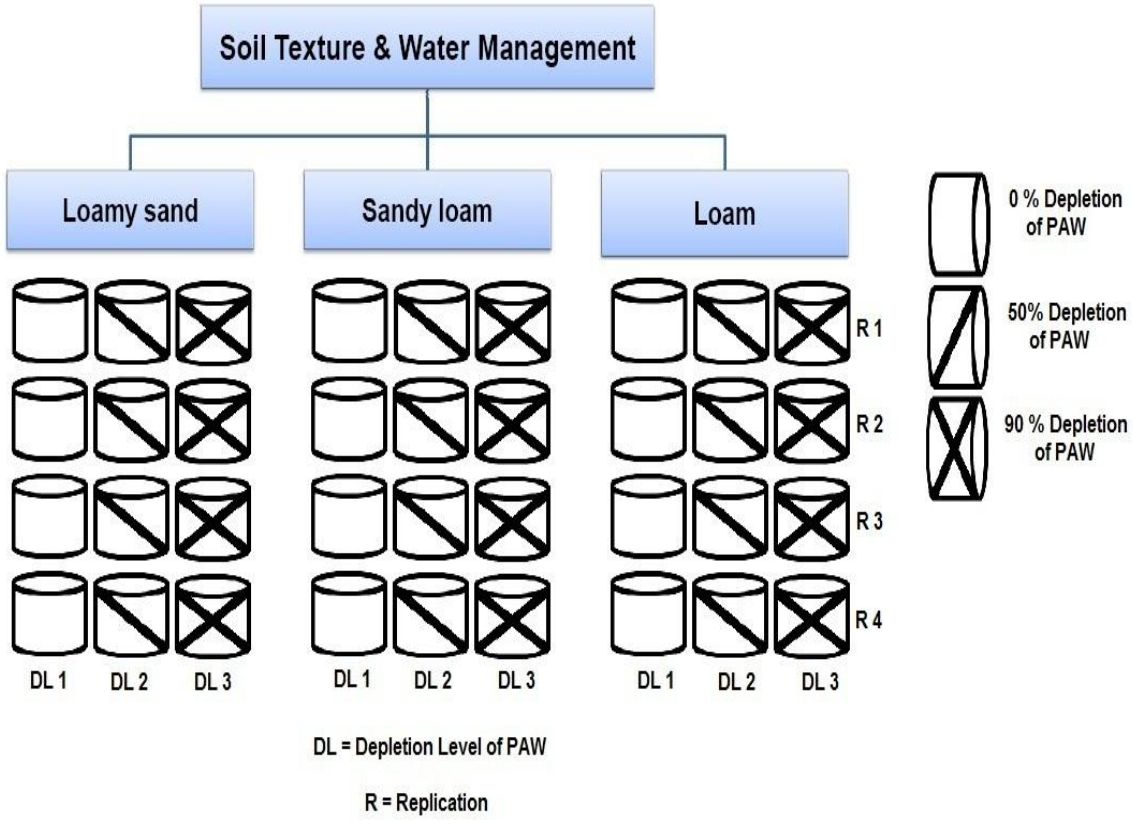

Figure 2.1 Factorial experiment designs for soil texture and PAW depletion experiment

This experiment was done during summer (October to December) in 2010. As previously indicated, the pots were weighed daily between 08h00 and 09h00 until the plants were harvested at three months of age. The average height of the plants was 50cm. This is a normal and desired average height of these plants in the field. As a daily activity, all the pots were treated independently to meet experiment requirements. This was a completely randomised experiment inside the tunnel. Every moment weighing was done, randomisation was done. This was done to avoid preferential conditions towards certain pots. Each pot was treated independently and the averages were used for final calculations of PAW depletion and for plotting the water use that had occurred in each soil. Cumulative water use of each pot was determined by cumulatively adding daily water use.

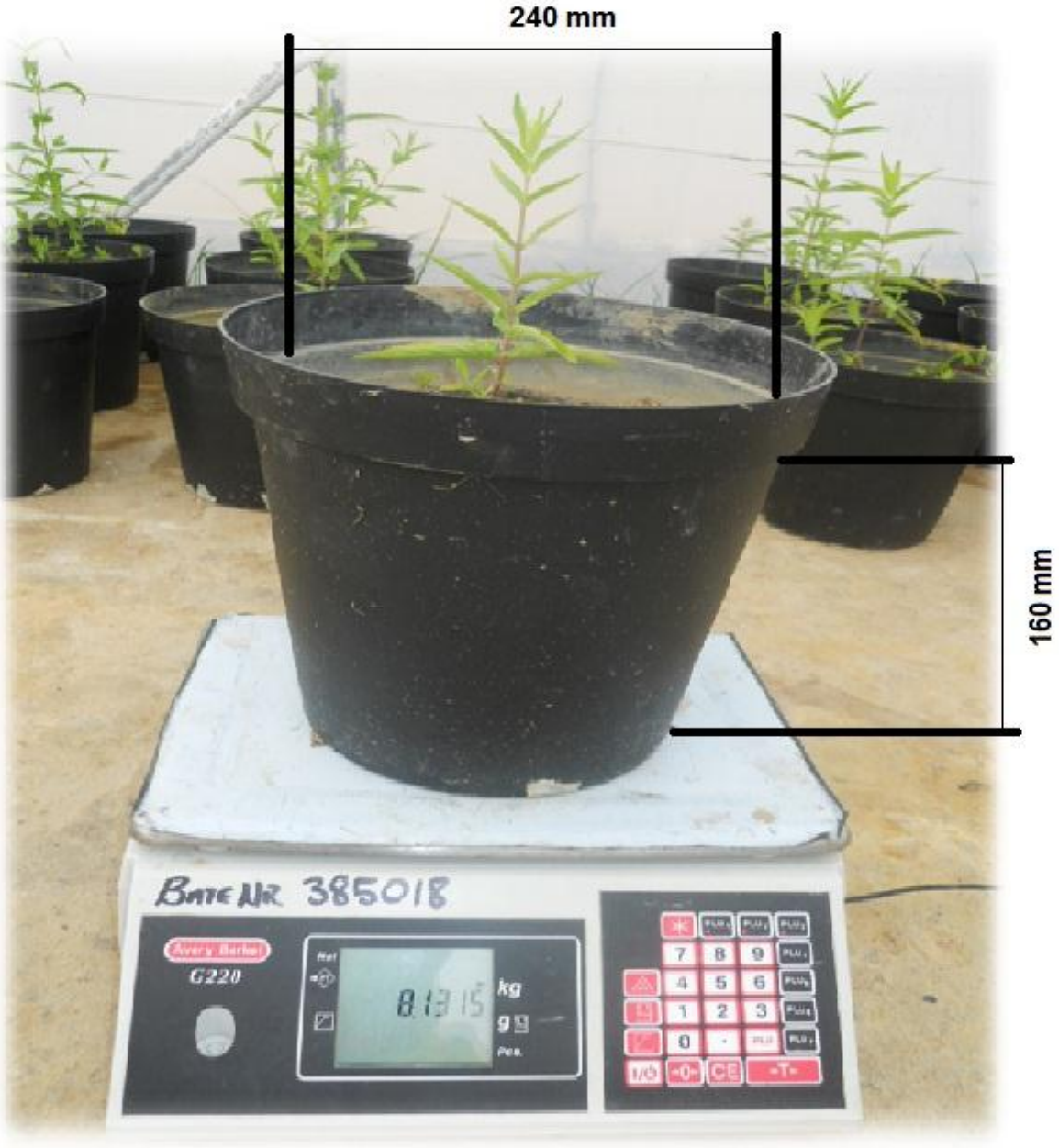

Figure 2.2 Pot on a weighing scale

2.3.2 Nitrogen rates experiment

In this particular experiment, three differently textured soils as had been used in the previous experiment were treated with three different nitrogen fertilizer rates, viz.

0kg ha^{-1}, 150kg ha^{-1} and 250kg ha^{-1} nitrogen fertilizer. They were replicated four times to produce a two-way factorial experiment similar to that of the water management – PAW depletion experiment. The treatment combination is shown in Figure 2.3. Water was applied daily by hand at 0% depletion of PAW as found in the PAW experiment. An average 150 ml solution of 0kg ha^{-1}, 150kg ha^{-1} and 250kg ha^{-1} nitrogen fertilizer was applied daily per pot according to the experimental design indicated in Figure 2.3. The nitrogen fertilizer solutions are described in the nitrogen nutrition section.

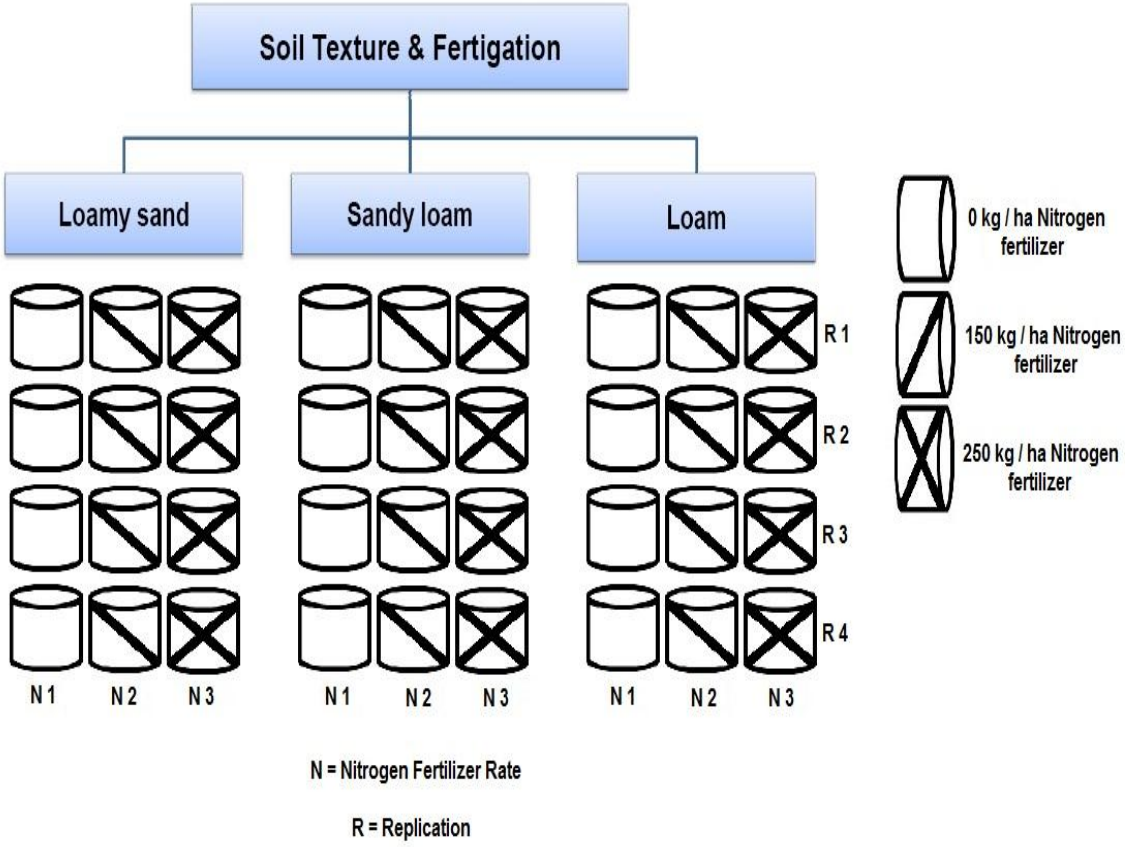

Figure 2.3 Factorial experiment design for nitrogen fertilizer rate and soil texture experiment

2.3.2.1 Nitrogen Nutrition

It is usually undesirable to apply high nitrogen rates near seeds and seedlings at planting or early emergence because of possible injury to the crops, especially in

sandy soils (Havlin *et al*., 2005). Methods discussed by Havlin *et al.* (2005) in soil fertility and fertilizers were considered and adjusted accordingly for this investigation. Standard and basic nitrogen fertilizer application was achieved by fertigation (application of fertilizer with water). A balanced nutrients solution, including nitrogen in different rates, was applied throughout the growing season. To investigate the influence of nitrogen fertilizer on the biomass production and antimicrobial properties of *Mentha longifolia* L, three nitrogen fertilizer solutions were used in this investigation.

These were; 0kg ha^{-1}, 150kg ha^{-1} and 250kg ha^{-1}nitrogen fertilizer solutions. This was prepared through the combination of the following elemental fertilizers; potassium nitrate (KNO_3), potassium sulfate (K_2SO_4), potassium mono-phosphate (KH_2PO_4), potassium chloride (KCl), calcium nitrate (Ca $(NO_3)_2 \cdot 2H_2O_z$), calcium sulfate ($CaSO_4 \cdot 2H_2O$) (gypsum) and magnesium sulfate (Mg $SO_4 \cdot 7H_2O$) (epsomite).

Several elemental fertilizers were used in order to prepare a balanced nutrition for the test plant while varying the nitrogen fertilizer. The solution of 0kg ha^{-1} nitrogen fertilizer was made up of the combination of K_2SO_4, KH_2PO_4, KCl, $CaSO_4 \cdot 2H_2O$ and Mg $SO_4 \cdot 7H_2O$ as shown in Table 2.7. The 150kg ha^{-1} nitrogen fertilizer solution was made up of K_2SO_4, KH_2PO_4, Ca $(NO_3)_2 \cdot 2H_2O_z$, $CaSO_4 \cdot 2H_2O$ and Mg $SO_4 \cdot 7H_2O$ and the 250kg ha^{-1} was a combination of KNO_3, K_2SO_4, KH_2PO_4, Ca $(NO_3)_2 \cdot 2H_2O_z$ and Mg $SO_4 \cdot 7H_2O$ as shown in Table 2.7.

The quantity of each elemental fertilizer is shown in Table 2.7. The final nutrient solution was prepared at an Electrical Conductivity (EC) of 1.0 mS cm^{-1}. Uniform fertigation of balanced plant nutrients plus nitrogen could be accomplished with daily irrigation applied by hand. About 150 ml of nitrogen fertilizer solution was used during this application.

Table 2.7 Composition of plant nutrients solution balanced at an EC of 1.0 $mS.cm^{-1}$

Elemental fertilizers	Nitrogen Level		
	0 kg/ha N	150 kg/ha N	250 kg/ha N
	(Application in g / 1000L)		
KNO_3	-	-	202.0
$K_2 SO_4$	26.1	261.0	87.0
$K H_2PO_4$	176.8	176.8	176.8
KCl	201.2	-	-
$Ca (NO_3)_2 \cdot 2H_2O_z$	-	360.0	400.0
$CaSO_4 \cdot 2H_2O$	280.0	28.0	-
$Mg SO_4 \cdot 7H_2O$	209.1	209.1	209.1

2.4 Growth Rate and Leaf Area Development

Growth rate was measured as total leaf area. All the leaves of the selected plants were measured. Two plants out of four replicates from each nitrogen fertilizer rate and soil texture class were selected. Table 2.8 shows the number of leaves per selected plants. The measurements were taken on the same plants every second day of the week. This activity was conducted for a period of six weeks. This time frame was considered suitable because the test plant is normaly harvested at this age. Another reason was that the plants were well developed to harvest for further experimentation. The task included taking measurement of the length and the width of an individual leaf. The formula of an area of an ellipse (*Mentha longifolia* L leaf shape is similar to an ellipse) was used to calculate leaf area of a given leaf. At the end of the experiment, this method of determining leaf area together with an automated leaf area meter. The LI-COR model 3100 area meter was used. These two methods were then correlated.

Table 2.8 Number of leaves per plant of the three different soil textures at different nitrogen fertilizer rates (P = plant)

Soil Texture Class	Week One		Week Two		Week Three		Week Four	
	P 1	P 2	P 1	P 2	P 1	P 2	P 1	P 2
Loam 0 kg.ha^{-1}	20	22	25	38	34	46	41	61
Loam 150 kg.ha^{-1}	38	32	84	70	104	84	165	123
Loam 250 kg.ha^{-1}	24	42	52	78	82	100	118	161
Loamy sand 0 kg.ha^{-1}	18	30	30	44	44	52	55	82
Loamy sand 150 kg.ha^{-1}	34	40	58	64	90	110	155	177
Loamy sand 250 kg.ha^{-1}	46	43	76	73	106	108	211	155
Sandy loam 0 kg.ha^{-1}	12	26	12	48	12	66	14	84
Sandy loam 150 kg.ha^{-1}	36	26	53	58	76	84	102	112
Sandy loam 250 kg.ha^{-1}	44	40	88	66	108	90	193	159

2.5 Antimicrobial Properties – Susceptibility Test (*in vitro* testing)

In order to test the efficacy of the essential oil of *Mentha longifolia* L*,* a laboratory procedure was followed to introduce decoctions from the plants into Petri dishes containing a selected bacterium colony, so as to measure the effect. The susceptibility tests are normally reported qualitatively as sensitive, intermediate or resistant or quantitatively in terms of the concentration of the agent that inhibits the growth of the organism – the Minimum Inhibitory Concentration (MIC). In the MIC, the susceptibility of organisms is determined against the series of dilutions of the agent (Collins *et al.*, 1995). These series of dilutions/ concentrations of the agent (*Mentha longifolia* L.) were prepared by soaking 20g and 30g of fresh and naturally dried leaves of *Mentha longifolia* L. in 100 ml warm water. A drop of each concentration was placed in a Petri dish inoculated with *Staphylococcus aureus*. The petri dishes were allowed to stand overnight and inhibition zones were determined the next day. *Staphylococcus aureus*, of the *genus Staphylococcus,* is a major and ubiquitous pathogen for humans (Novick *et al*., 2001). Almost every person will have some type of *S. aureus* infection during a lifetime, ranging in severity from minor skin infections, food poisoning to severe life threatening infections (Kowalski *et al.,* 2005). The common occurrence of *Staphylococcus*

aureus made it a good example to be the main pathogen in this research in the investigation of *Mentha longifolia* L.

This experiment was implemented following the guidelines provided by Collins et al, (1995). The test pathogen, *i.e. Staphylococcus aureus*, was obtained from the Department of Food Science, Faculty of Agri Science, at the University of Stellenbosch. Two concentrations were used, namely 20g $100ml^{-1}$ and 30g $100ml^{-1}$. From the *Mentha longifolia* L. plants, fresh leaves and naturally dried leaves in powdered form were then used for the preparation of the aqueous decoction. For obtaining a *Mentha* decoction, these plant samples were soaked in warm water for five and two days for fresh and naturally dried samples respectively. The mixture was shaken every 24 hours and then filtered through a Whatman No.40 filter paper. The filtrate was then used as the antimicrobial agent. Sterilised discs were dipped into this agent and then placed on the Petri dishes containing the *Staphylococcus aureus*. Sample leaves were obtained from 150kg ha^{-1} N fertilizer and 250kg ha^{-1} N fertilizer from the three soil textures that were involved in the investigation. In this regard, one Petri dish accommodated four discs from 150kg ha^{-1} per se. For example, a sample from loam soil 150kg ha^{-1} gave two 20g $100ml^{-1}$ agents prepared from fresh and dried leaves and likewise, two 30g $100ml^{-1}$ agents, one from each of fresh and naturally dried leaves. These agents in the Petri dishes are indicated by the letters A, B, C and D as shown in Figure 2.4. The letters A and B signify 20g $100ml^{-1}$ and 30g $100ml^{-1}$ of the agent prepared from fresh samples while C and D stand for 20g $100ml^{-1}$ and 30g $100ml^{-1}$ of the agent obtained from naturally dried samples. Two growth mediums (rich and poor) were used. Poor medium was used as a control against rich medium as is also shown in Figure 2.4. Inhibition zones were then read after 24 hours and were recorded accordingly. Activity was determined by measuring the diameter of zones showing complete inhibition (mm).

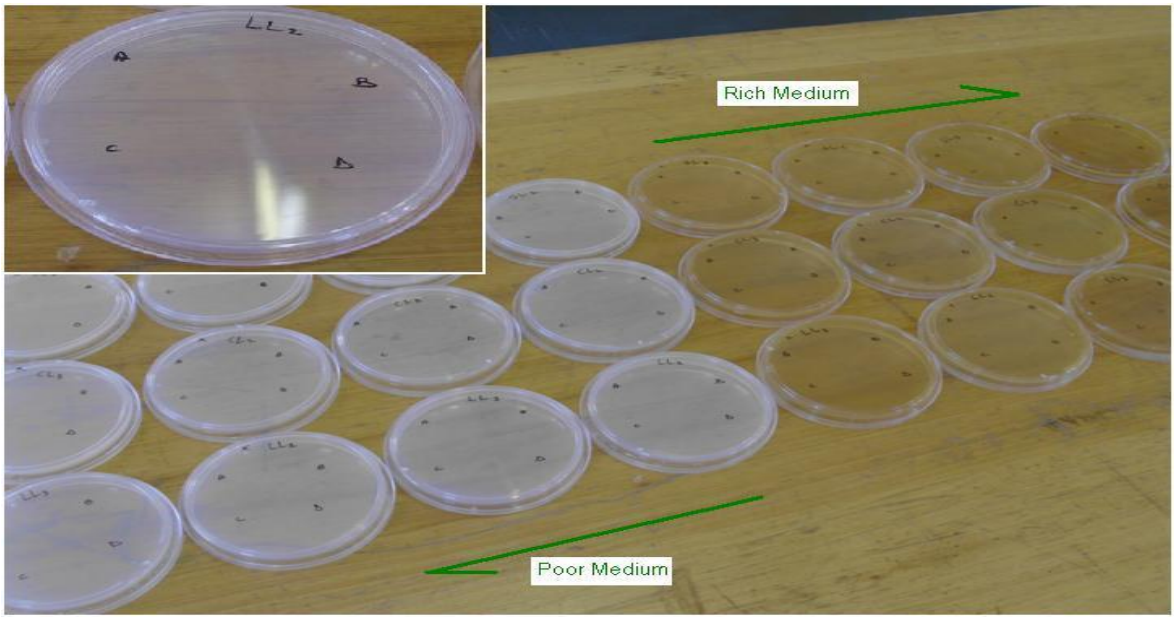

Figure 2.4 Experimental layout; two mediums, four placements of the agent

2.6 Data Analysis

A parametric two-way analysis of variance together with a general linear model was used to describe the separate effects of the treatment factor and the random variable on the sample mean. A multiple comparison was also applied in order to determine the location of significant differences. This was achieved by the use of Least Significant Difference and its calculation based on the standard error of the difference between the means (Ireland, 2010).The basic statistical procedures were performed rapidly by using modern functions within Microsoft Excel® spreadsheet software whereas for more extensive statistical analysis, an advanced statistical software package (Statistica 10) was used.

In this research, adequate replication was implemented. The aim was to address reliability of the measure under study, by involving four replicates. Validity was addressed in two dimensions, *i.e.* firstly by accommodating data source triangulation owing to time, space, and person (Denzin, 1970 in; Thurmond, 2001) and secondly by tracing it from the data collection part instrument introduced in APPENDIX VI in the form a checklist.

2.7 Ethical Implications

Ecological environment and social impact

Cultivation of medicinal plants affects the ecological balance, and in particular, the genetic diversity of the flora and fauna in the surrounding habitat (WHO, 2003). WHO (2003) further states that the social impact of cultivation in local communities needs to be examined to ensure that negative impacts on local livelihoods are avoided (WHO, 2003). This relatively relates to the aromaticity of aromatic plants. This property of plants can either have a positive or a negative impact on the environment. One example is that the aroma of the plant might be a repellent or might cause allergy to other organisms. In this research, this principle was maintained by default due to the nature of the problem under investigation. The experiment was conducted in a confined environment with the result that local communities remained undisturbed. Intellectual Property Rights from Publicly Financed Research and Development Act (51 of 2008) ruled to say that an outcome (intellectual property) arising from publicly financed research and development shall be identifiable, protected, available to utilise and commercialise for the benefit of the people of the Republic. The Act further advocates that the recipient of the funding from the funding agency shall record and report on the benefit for the society. This research therefore observed all these objects of the Act. The structure of this thesis include, *inter alia*, a summary of findings and conclusions and research recommendations for future research to adhere to the application of the Act.

Chapter 3: Soil Texture, Water Management, Water Use Efficiency and Biomass Production

"Both soil and water belong to the biosphere, to the order of nature, and – as one species among many, as one generation among many yet to come – we have no right to destroy them". **Daniel Hillel**

3.1 Introduction

The influence of soil texture, plant available water (PAW) and water use efficiency on the biomass production of *Mentha longifolia* L is central to this study. According to available literature, there is little to no research that has been done specifically on the production requirements for *Mentha longifolia* L (WHO, 2003). The soil texture and PAW that produced the greatest biomass production would indicate the requirements for cultivating *Mentha longifolia* L on a larger scale.

3.2 Results and Discussion

3.2.1 Influence of Soil Texture on PAW and Water Use

Three soils that were used in this experiment were different in terms of texture. The clay content is the most important soil physical property that affects the water holding capacity of the soil. It also influences the rate at which the plants will be able to absorb the soil water. Clay content percentages of these soils are 4.6%, 7.6% and 20.6% for loamy sand, sandy loam and loam respectively (Table 2.1). Other equally important fractions, such as sand and silt percentages, are shown in Table 32.1. The silt content of the selected three soils differs considerably. These are 11.3% (loamy sand), 23.8% (sandy loam) and 31.4% (loam). The combination of clay and silt content determines the water holding capacity, PAW, soil moisture release, as well as other chemical properties of the soil. For example, fine textured soils (those with high clay content) will have more plant available water than coarsely textured soil. Availability of the PAW is limited for uptake by plant roots in fine textured soils.

The second important physical property of the soil is the bulk density (ρb). Brady and Weil (2002) define bulk density as the mass of a unit volume of dry soil. This value is used mainly in quantitative soil studies such as in calculating soil moisture movement within a profile and rates of clay formation and carbonate accumulation. Bulk density affects the total porosity of the soil. Bulk density of the soil is inversely related to porosity of the same soil. Bulk densities of the three soils that were used in the investigation are shown in Table 2.2.

Soil texture and bulk density then influence the water holding capacity of the soil and plant available water (PAW). According to Foth (1990) and Blake and Hartge (1986), bulk density is further used for the conversion of water percentage by weight to water content by volume. Therefore, differences in bulk density will influence the volumetric water content of these soils. FC and PWP are influenced by clay content, total porosity and the bulk density of the soil.

FC and PWP are used to determine PAW. The PAW of these differently textured soils is shown in Table 2.3. This table indicates that PAW increased with increase in clay content, except for the sandy loam. Change in water use over time for these soils is indicated in Figure 2.3. In other words, these are the PAW depletion patterns of the three soils that were used in the investigation.

Change in water use over time was monitored by daily weighing of pots and addition of water to replenish the water uptake by plants. This experiment was implemented according to the three levels of plant available depletion. That is, addition of water on a given pot was based on an assigned level of water depletion for that pot. Literally soil moisture recharge was based on the difference between FC and the water content at which water application was needed based on the 0%, 50% and 90% depletion of PAW schedules. Data were collected by using the daily data collection checklist shown in APPENDIX VI. The observations and data analyses indicated that these three soil textures exhibited similar patterns of plant water use (Figure 3.1). The differences in daily plant water use (expressed in mm) among the three soil textures were significantly different from each other (compare APPENDIX VII).

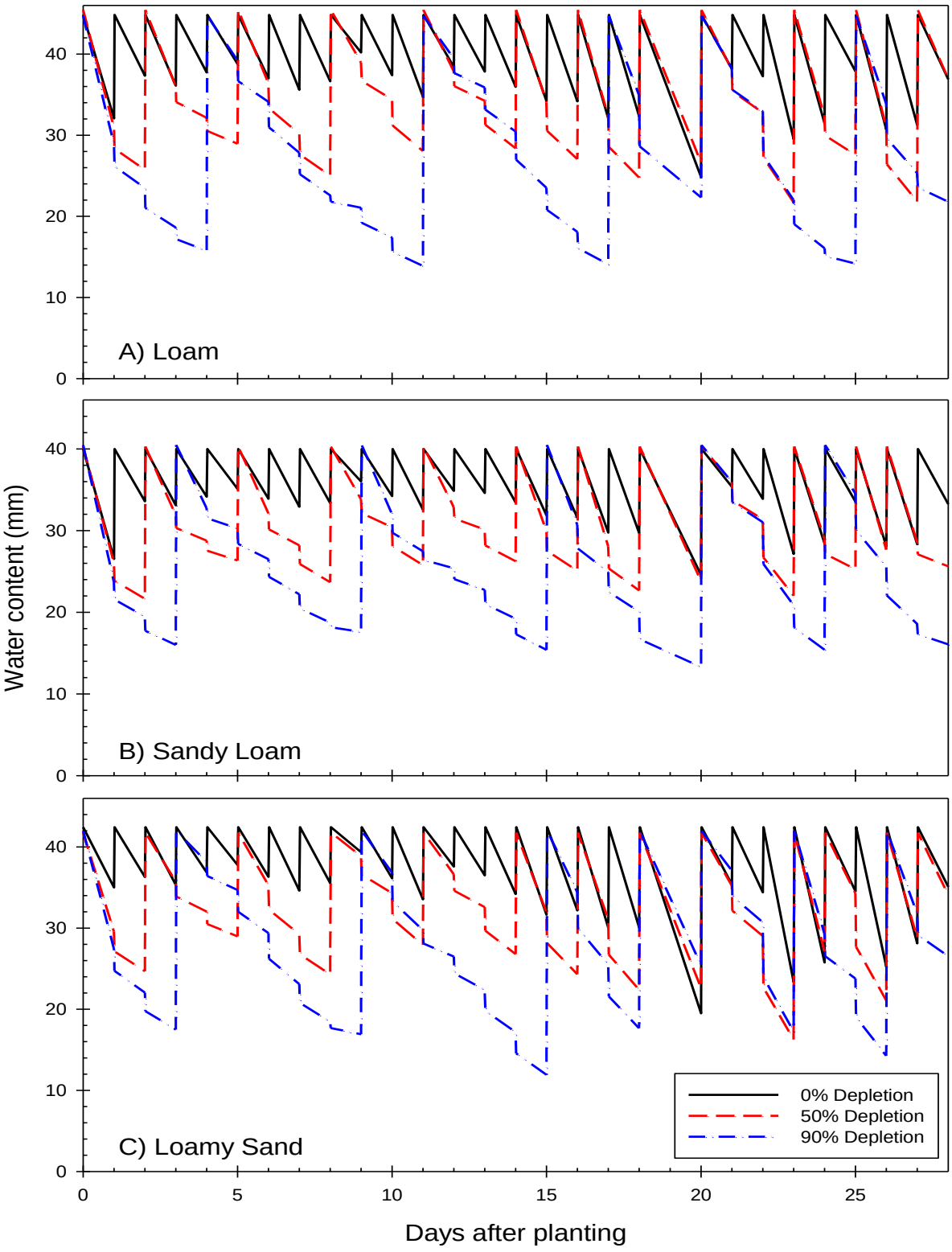

Figure 3.1 Change in average water content of three different soil textures used during the growing season

The slope of the graphs was initially similar and as time accumulated, they started to differ. These differences in daily plant water use were mainly as a result of differences in soil physical properties such as silt and clay content of the soils being investigated. According to Figure 3.1, the solid line represents 0% depletion of plant available water. The dashed line and dashed line with dots represent 50% and 90% depletion of plant available water. During the first day of the experiment, there was a marked difference between the three soils, e.g. at 0%, 50% and 90% depletion of PAWaverages of 13 mm, 19 mm and 29 mm of water were respectively depleted on loam soil. Figure 3.1 further indicates that soil water needed to be replenished daily, on day two and on day four for 0%, 50% and 90%, depletion of PAW respectively on loam soil. On the latter two cycles of water replenishment, an average three-day and six-day period of time was considered for applying irrigation water at 50% and 90% depletion of PAW, respectively. At day four the loam soil had a water content of 16 mm at 90% depletion of PAW. The same water content of 16 mm was recorded for the sandy loam on day three when 90% depletion of PAW had been reached. On day three (90% depletion of PAW) the loamy sand had a water content of 17 mm. Loamy sand contained more water than sandy loam and loam soil. This was attributed to the loamy sand containing more of the fine and very fine sand that gives enough porosity to hold higher PAW.

Soil water depletion on sandy loam appeared to differ from those of loam and loamy sand. Figure 3.1 further shows that, 13 mm, 18 mm and 24 mm of soil water was depleted at 0%, 50% and 90% depletion of PAW respectively from the first to the third day after planting. Water was replenished daily, after two days and after three days on 0%, 50% and 90% depletion of PAW respectively for the first cycle of applying water. On the second cycle of application of soil water, an average of six, two and daily was recorded for 90%, 50% and 0% depletion of PAW. Figure 3.2 shows an average number of times that replenishing soil water was done.

On day one, two and three, water content was 35 mm, 22 mm and 17 mm respectively on loamy sand. This shows that 25 mm, 17 mm and 7 mm was depleted from 90%, 50% and 0% depletion of PAW. The average number of times

water needed to be replenished is shown in Figure 3.2. It was daily, after three and six days for 0%, 50% and 90% depletion of PAW.

On considering the supporting evidence, sandy soil has less total pore space to hold soil water, but more of the soil water that it holds is easily available to plant roots for absorption (Sullivan, 2002). In Figure 3.1 there is a common soil moisture depletion and recharge around day twenty after planting. This marks the period when fertigation was introduced. Yellowing of all plants' leaves showed that they needed added nutrients. A hydroponic nutrient powder – CHEMICULT® at the concentration of 2g l^{-1} was used in fertigation.

Within and throughout the growing season, these three soil textures behaved differently. At 90% depletion of PAW, there was a difference in the number of times water was replenished. It was four, five and six times on loam, sandy loam and loamy sandy respectively. This is shown in Figure 3.2. According to this figure, it was more frequently necessary to replenish soil water to loamy sand than to loam and sandy loam. The reason was that there was a high PAW on this texture. The better the plantroots managed to absorb the water, then more water was needed. This tendency was true in the context of loam soil. This texture had 20.6% clay content and therefore it was able to hold water films tightly in contact. The moisture release pattern then seemed to be much slower.

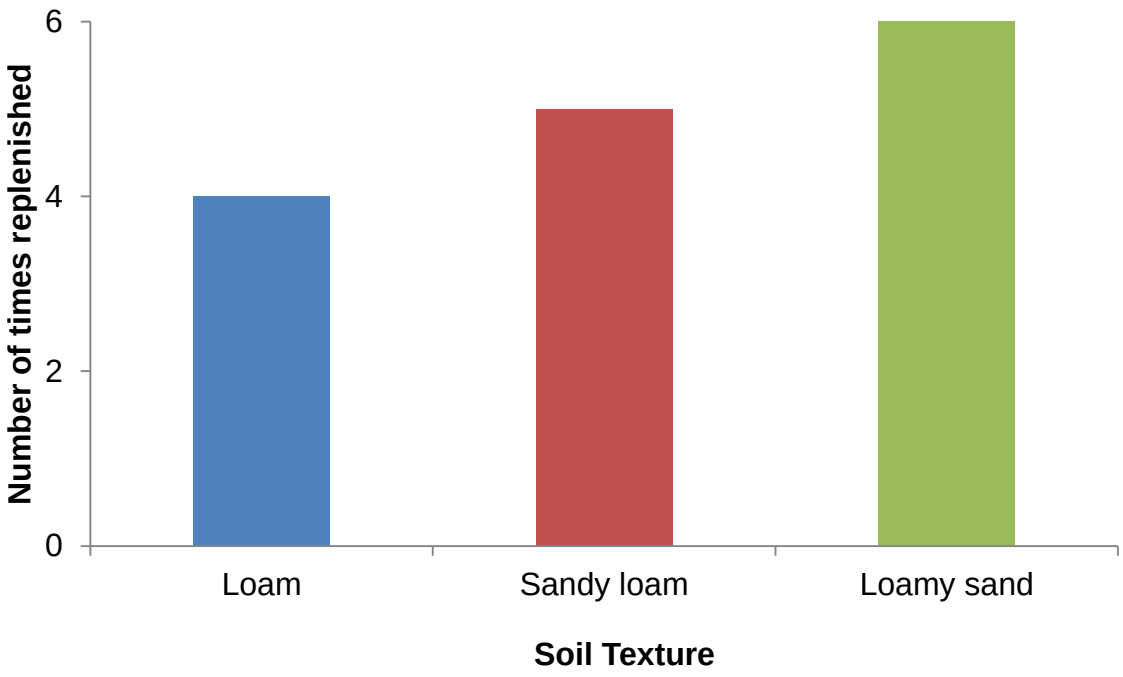

Figure 3.2: Average number of times water was replenished at the 90% depletion of PAW for three soils used during the growing season.

Daily evapotranspiration (ET) expressed in (mm) was considered a good basis for daily water use. It was determined by weighing of the pots to find how much of soil moisture was lost. Water use in terms of consumption of water, is that part of water used by plants for transpiration and growth plus water loss. According to deductions drawn from Figure 3.3, there was a significant difference ($p < 0.05$) in ΣET at different levels of moisture depletion across three soil textures used in the investigation. Generally, ΣET was highest at 0% depletion of PAW in all textures. It was moderate and lower at 50% and 90% depletion of PAW respectively.

Figure 3.3 shows that ΣET at 0% depletion of PAW was highest in loam soil followed by loamy sand and sandy loam respectively. This trend appeared likewise at 50% and 90% depletion of PAW. However, at 90% depletion of PAW loam and loamy sand converged and matched each other. Other parallel analyses such as those on daily water use and ΣET gave similar deductions.

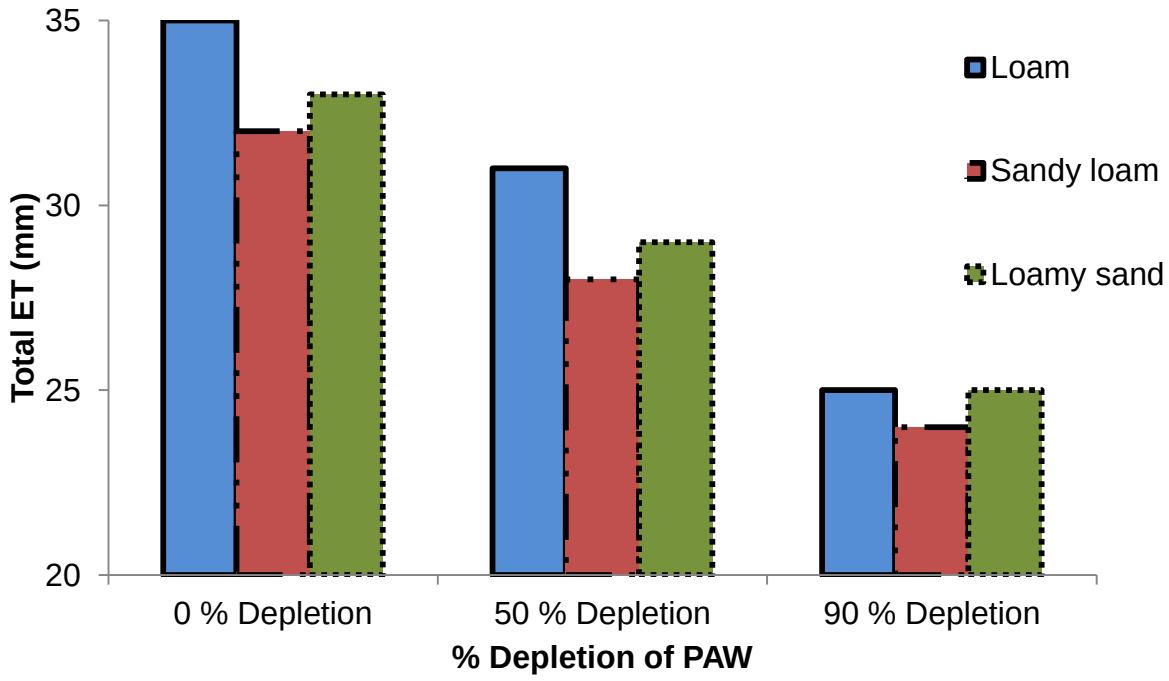

Figure 3.3 Average Total ET of different soil textures determined at harvest

Daily water use

Figure 3.4 has been compiled according to the data of Figure 3.1. This figure shows the scatter plot of daily water used in the three different textures. Daily water use in all the textures was not constant due to differences in daily temperature and humidity, since the experiment was not conducted in a temperature controlled glass house. Table 3.1 shows the records of temperature and humidity. These differences affected the rate of ET from these soils, especially because these soils had different physical properties such as clay, silt and sand fraction content that influenced them to hold and release soil water differently. The mathematical calculations show that an average ET was 10 mm, 8 mm and 9 mm for loam, sandy loam and loamy sand respectively. Figure 3.4 indicates a common higher (13 mm) daily water use on the first day of the experiment on loam and sandy loam. The reason was the presence of some inevitable preferential flow paths created during filling of the pots. The aggregates of loam and sandy loam soil were more stable than those of loamy sand. Table 2.1 shows that loam and sandy loam had higher silt and clay content. These soils separately influence aggregate formation and stability (Brady and Weil,

2002). Loamy sand was well-filled due to its very loose and gritty consistency. Its particles easily settled during packing. In this incidence the loam and sandy loam, daily water use included the water that drained, transpired and was used by the plants. Although, this was not the complete case on loamy sand, daily water use was assumed to have been used mainly by plants, hence as ET. Table 3.1 shows that on the first day of the experiment, the humidity was 45%. This was true for the ET that occurred. When humidity was low, ET was high due to high evaporative demand of the atmosphere. Around day nine of the experiment as shown in Figure 3.4, daily water use was at its lowest. It was 5 mm, 4 mm and 3 mm on loam, sandy loam and loamy sand respectively. This was as the result of a high humidity of 94%. When humidity was high the evaporative demand of the atmosphere was low ET was low as well. There was an outlier value around day twenty of the experiment. It was 20 mm, 15 mm and 23 mm on loam, sandy loam and loamy sand respectively. It indicates the period when all the pots were allowed to dry out in order to cater for the suggested fertigation, since fertilization was exclusive in this part of the experiment. All the pots received equal amounts of fertilizer dissolved in irrigation water.

Table 3.1 Temperature and Humidity records of the growing season

Date - Nov 2010	Temp (°C)	Humidity (%)	Date - Nov 2010	Temp (°C)	Humidity (%)
1st	23	45	**15th**	28	42
2nd	18	86	**16th**	23	61
3rd	17	81	**17th**	22	53
4th	16	84	**18th**	27	41
5th	15	64	**19th**	18	62
6th	14	85	**20th**	18	69
7th	19	48	**21st**	18	77
8th	17	68	**22nd**	16	58
9th	15	94	**23rd**	19	42
10th	17	63	**24th**	22	33
11th	16	70	**25th**	24	44
12th	16	81	**26th**	24	52
13th	17	74	**27th**	16	83
14th	22	40	**28th**	16	67

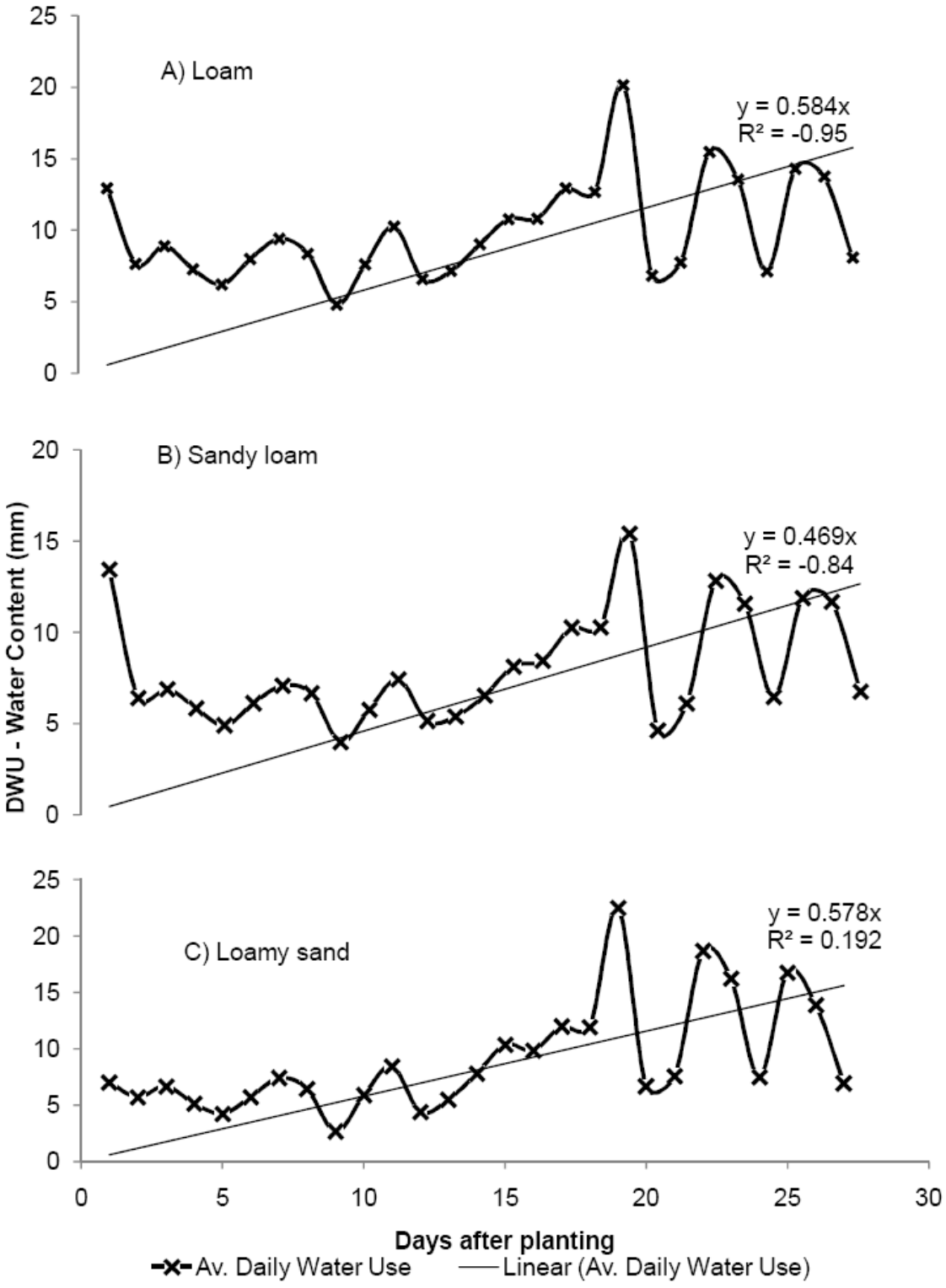

Figure 3.4 Change in Average Daily Water Use of three different soil textures used during the growing season

PAW had an impact on the total evapotranspiration (ΣET). That is, the cumulative water use of each texture (Figure 3.5) depended on the physical properties of soils used. These properties included, *inter alia*, the percentages of clay and silt of the three soils. The obtained data indicate that there were differences in water use efficiency (WUE) due to differences in ET between the three soils. Figure 3.5 depicts the cumulative water use (ΣET) of the three soils. ΣET for the three soils at different levels of PAW depletion seemed very similar at the beginning of the production cycle. The pattern changed with time in each soil type. The ΣET increased with increase in days after planting. The reason was that as the plants grew, there were increases in plant root and leaf development. Therefore, more water was needed for efficient plant growth.

Generally, Figure 3.5 also indicates that ΣET reflected by 0% depletion of PAW was highest in all soil textures. It was then followed by that of 50% and 90% depletion of PAW respectively. Therefore, if more water is applied there will be more ΣET and hence a higher WUE. Table 3.2 indicates the statistical differences drawn from Figure 3.4. According to Table 3.2, average ΣET was high, moderate and low at 0%, 50% and 90% depletion of PAW across the three soil textures. The average ΣET at 0% depletion of PAW across the three textures was statistically not significantly different ($p < 0.05$). However, loam soil had a higher ΣET value followed by loamy sand and sandy loam respectively.

Table 3.2 further shows that an average ΣET at 50% depletion of PAW was significantly lower ($p < 0.05$) on sandy loam. Average ΣET on loam and loamy sand did not differ significantly. There was a significant difference ($p < 0.05$) of average ΣET between all the textures at 90% depletion of PAW with loamy sand being the highest in average ΣET. Turkeys's grouping in Table 3.2 shows that on average loamy sand performed better than loam and sandy loam.

On loam soil as shown in Figure 3.5, there was no difference in ΣET between the three levels of moisture depletion until day three after planting. From day three till the end of the experiment, 0% depletion of PAW had the highest ΣET. This trend was followed by 50% and then 90% depletion of PAW respectively.

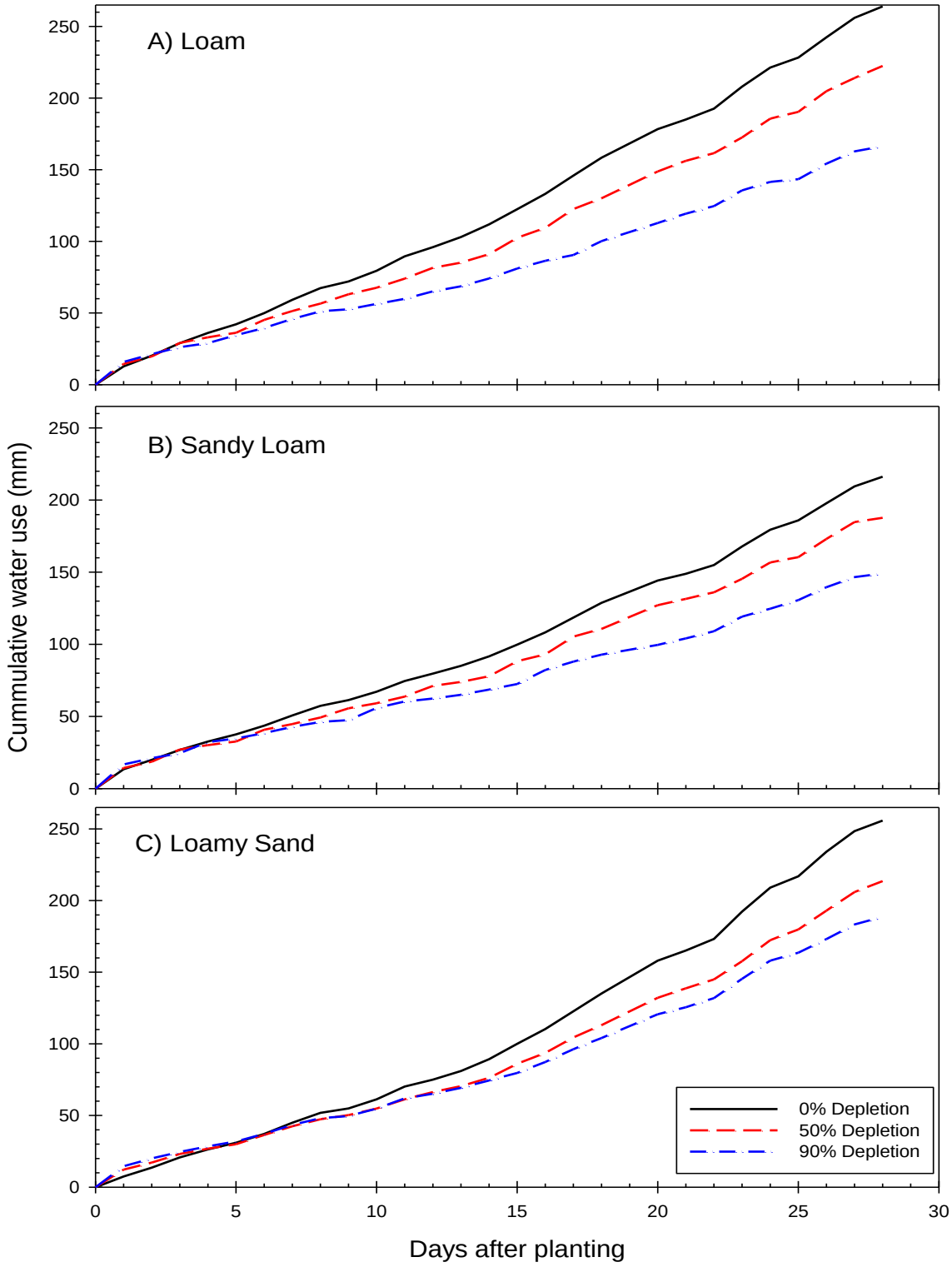

Figure 3.5 Cumulative water use in loam, sandy loam and loamy sand during the growing season

According to Figure 3.5, ΣET on day twenty (the day when fertigation was introduced) was 175 mm, 145 mm and 110 mm for 0%, 50% and 90% depletion of PAW.

The pattern of ΣET on sandy loam for three levels of moisture depletion was similar from day one till day four of the experiment. There was a significant difference (0.05) of ΣET from day four till the end of the experiment. Figure 3.5 shows that the ΣET for 0%, 50% and 90% depletion of PAW was 145 mm, 130 mm and 100 mm respectively. From day one till day four, the ΣET on three levels of moisture depletion behaved differently on loamy sand. However, there was no significant difference ($p < 0.05$) in ΣET from day four till day seven. Figure 3.5 further shows that 0% depletion of PAW had the highest ΣET from day seven onwards. Depletion of PAW (50% and 90%) was lower than 0% depletion of PAW but correlated till day fourteen of the experiment. On day twenty of the experiment, the ΣET 160 mm, 130 mm and 120 mm for 0%, 50% and 90% depletion of PAW respectively.

Table 3.2 Average ΣET at harvest for different soil textures used in the investigation

Soil Texture Class	**Average ΣET (mm)**		
	0%	50%	90%
Loam (L)	263.98 a	222.43 b	166.34 b
Sandy loam (Sa L)	245.33 a	184.11 a	149.10 a
Loamy sand (L Sa)	259.12 a	213.60 b	201.34 c
LSD $_{\text{Turkey (0.05)}}$	32.7	23.6	16.8

Averages followed by the same letter do not differ significantly

3.2.2 The biomass production components, leaf area and leaf area index of three different soil textures at different levels of PAW depletion

Table 3.3 indicates the average (replicated four times) biomass production components, leaf area (LA) and leaf area index (LAI) of the three different soil textures at different levels of PAW depletion. The table reflects that loamy sand produced the highest biomass components, leaf area and leaf area index across three levels of PAW depletion. The productivity of loam soil surpassed that of sandy

loam. Plants in sandy loam revealed the lowest development in all the aspects of biomass production components, leaf area and leaf area index. For example, the average total above-ground wet biomass production was highest in loamy sand (31.27g). It was then followed by that in loam soil. Plants in sandy loam had the lowest average total above-ground biomass production. The tendency of the performance of PAW on biomass production was such that 0% depletion of PAW outperformed 50% and 90% depletion of PAW across the three different soil textures used in this investigation. However, in some instances 50% and 90% depletion of PAW almost correlated, especially with regard to sandy loam in biomass production components. These results support the results obtained by Haileselasie and Hiwot (2012). Haileselasie and Hiwot (2012) reported that sufficient availability of water significantly favours the species diversity of *Mentha longifolia* and other garden herbs. To answer the first and second specific objectives of this investigation, detailed statistical comparisons were incorporated. Statistical comparisons of these results are fully discussed in sections 3.2.3 and 3.2.4.

Table 3.3 Average biomass production components, LA and LAI of three different soil textures at different levels of PAW

Soil Texture Class	% Depletion of PAW	Wet Leaf (g)	Wet Stem (g)	Total Above ground Wet Biomass (g)	Wet Roots (g)	Dry Leaf (g)	Dry Stem (g)	Total Above ground Dry Biomass (g)	LA (cm^2)	LAI
Loam	**0%**	7.35	5.50	12.85	4.07	3.08	1.96	5.04	328	0.72
	50%	6.36	3.73	10.09	3.52	2.66	1.31	3.97	269	0.60
	90%	4.56	1.93	6.49	2.27	1.90	0.73	2.63	186	0.41
Average		6.09	3.73	9.81	3.29	2.55	1.33	3.88	261	0.58
Sandy loam	**0%**	4.45	2.31	6.76	0.90	1.55	0.65	2.20	195	0.43
	50%	1.41	0.36	1.77	0.49	0.68	0.16	0.84	37	0.08
	90%	1.36	0.44	1.80	0.51	0.62	0.19	0.81	35	0.08
Average		2.41	1.04	3.44	0.63	0.95	0.33	1.28	89	0.20
Loamy sand	**0%**	19.58	18.95	38.53	12.36	7.66	6.13	13.79	942	2.09
	50%	14.58	13.30	27.88	6.03	5.51	4.06	9.57	620	1.37
	90%	14.74	12.61	27.35	8.74	5.88	3.96	9.84	539	1.19
Average		16.30	14.95	31.27	9.04	6.35	4.72	11.07	700	1.55

3.2.3 Soil Texture and Biomass Production

To achieve the objective of this investigation, the experiment was carried out according to the design explained in materials and methods in chapter two. The test plants were grown in three different soil textures, *i.e.* loam, sandy loam and loamy sand as described. Water management was implemented to examine in which ways PAW influences the biomass production of the test plants.

At harvest (after three months), fresh plant biomass components of under the ground and above the ground biomass were collected and were naturally dried in order to measure dry biomass as well. Differences in weight per plant biomass component delineated the differences caused by differences in soil texture and PAW. Plant biomass components included leaf, stem, and root weights. Leaf Area Index (LAI) was also determined to investigate the influence of soil texture class on leaf area development.

Table 3.4 shows that there is a significant difference ($p < 0.05$) between the biomass productions obtained from these three differently textured soils. Statistical comparisons were made and the multiple comparisons (Least Significant Difference) were used to determine the significant difference between the treatments. Loamy sand biomass production across all the different PAW treatments surpassed the performance of loam and sandy loam soil. Table 3.4 shows that the total wet and dry biomass production and total leaf area were highest in loamy sand. Loam soil showed the second best results whereas sandy loam had the lowest averages in biomass production components and total leaf area. These three differently textured soils characteristically had different water release paths from FC to PWP. This was due to differences in the percentage of clay in soil of each texture. PAW was principally influenced by this property. Abd EL-Wahab *et al*. (2006) are of the opinion that water availability can be regarded as the key variable in controlling soil productivity. Asgarzadeh *et al*. (2011) indicate that soils with different physical properties, like clay content, have different soil moisture release curves. It was this property that influenced the way that plants accessed

PAW, which in turn exercised an impact on biomass production. This can be related to the fact that plants use more energy to absorb a unit of mass of PAW from clay soil than from sandy soil (Asgarzadeh *et al*., 2011; Hussain, 2009). Their findings support what was found in this investigation (Table 3.4), where loamy sand (releases PAW easily) performed better than the other textures. Other contributions indicate that it is difficult for plants to absorb nutrients from dry soil (Havlin *et al*., 2005). Brady and Weil (2002) contend that soils with more clay have smaller average pore size and greater resistance to root penetration, thereby affecting plant growth. Moreover, plant roots penetrate more easily in moist sandy soil than in moist clayey soil (Brady and Weil, 2002). These findings correlate with this investigation's results, e.g. the influence of soil texture was found as the literature confirms that numerous physical factors influence root growth and absorption of soil water and nutrients sufficient for optimum plant productivity as Havlin *et al*. (2005) reported. Other findings further supported the theory that plants absorb more available nutrients from naturally fertile sandy soils than from fine textured soils. Bretzel *et al.* (2009) reported in their investigation on soil influence on performance of native herbaceous plants that soil texture affected plant growth. That was evidenced by some herbs that developed high biomass production per plant in soils with low nutrient content (Bretzel *et al*., 2009). The results of their research agree with the findings of this investigation.

Table 3.4 Average plant biomass components in different soil textures

Texture	Wet Leaf (g)	Wet Stem (g)	Wet Root (g)	Total Wet Biomass (g)	Dry Leaf (g)	Dry Stem (g)	Total Dry biomass (g)	Total LA (cm^2)
Loam	6.1 b	3.7 b	3.3 b	13.1 b	2.5 b	1.3 b	3.8 b	261 b
Sandy loam	2.4 a	1.0 a	0.6 a	4.0 a	0.9 a	0.3 a	1.2 a	89 a
Loamy sand	16.3 c	15.0 c	9.0 c	40.3 c	6.4 c	4.7 c	11.1 c	700 c
LSD $_{(0.05)}$	1.63	1.89	1.27	4.79	0.64	0.65	1.29	102.8

Averages followed by the same letter do not differ significantly

3.2.4 Percentage Depletion of PAW and Biomass production

The results and discussion on this section are based on the influence of percentage depletion of PAW (0%, 50% and 90% depletion of PAW) on the plant biomass components (root, stem and leaf) of *Mentha longifolia* L. Similarly, differences in weight per plant biomass component were used to determine the influence of percentage depletion of PAW on the biomass production.

When taking into consideration the quantity of all biomass production components (leaf, stem and root) that had been produced, the production of the average plant's biomass components was higher at a 0% depletion of PAW than was the case with 50% and 90% depletion of PAW. Dry leaf and stem biomass production and LAI at 50% and 90% depletion of PAW were likely to match each other. The lower moisture levels and a lack of adequate PAW exercise a diverse effect on biomass production. Table 3.5 indicates the statistical comparisons that were made. LSD values at 95% confidence in Table 3.5 show no significant differences among the treatments.

Lower PAW depletion such as 0% depletion of PAW gave the highest production of wet leaf mass and LAI. Referring to the impact of PAW, Jalota *et al*. (2010) and Marcum and Hanson (2006), indicate that sufficient PAW was beneficial for improving biomass production. This agrees with 0% depletion of PAW that gave better biomass production in this experiment. The results reported from the investigation by Ram *et al.* (2006) substantiate the theory that increased supply of water results in increased crop production, herb and essential oil of *Mentha arvensis* L. Other studies that support this investigation's results are reported by Mitchell *et al.* (1992). They indicate that dry matter and oil yield were affected by irrigation level and fertility level. They show that mint produces optimum yield at highest levels of irrigation (Clark and Menary, 1980 ; Simon and Jolly, 1989, in; Mitchell *et al*., 1992).

The research on the impact of water stress on the growth and yield of Japanese mint, indicated that 100% available FC(0% depletion according to the current

investigation) produced maximum plant height and fresh herb product (Shormin *et al.,* 2009). In other studies it was reported that plant growth and other yield components of a medicinal herb,caraway, were significantly affected by water deficit as well (Laribi *et al*., 2009; Singh and Sharma, 2001). According to Batanouny (1987), soil texture is a major factor that affects distribution, growth and regeneration of herbs. Herbage growth is more responsive to water content in sandy soils than in clayey soils. These findings are in agreement with the results of this study, especially for biomass production obtained from loamy sand. However, sandy loam was surpassed by loam soil.

Leaf Area Index (LAI), which is the leaf area per unit land area, is similarly affected by PAW. Table 3.5 indicates that 0% depletion of PAW produced a higher LAI than did the 50% and 90% depletion of PAW. Therefore, this means sufficient PAW leads to improved LAI which in turn is directly proportional to biomass production. This supports the results of Mahdavi-Damghani (2010) which indicated that increased PAW increases the LAI of crops and biomass production. Brady and Weil (2002) indicated that an increase in LAI leads to an increase in plant production up to the point at which most available radiation is absorbed by the leaf surface.

Table 3.5 Average plant biomass components at different PAW depletion

% Depletion PAW	Wet Leaf (g)	Wet Stem (g)	Wet Root (g)	Total Wet Biomass (g)	Dry Leaf (g)	Dry Stem (g)	Total Dry Biomass (g)	LAI
0% Depletion PAW	10.5 a	8.9 a	5.8 a	25.2 a	4.1 a	2.9 a	7.0 a	1.1 b
50% Depletion PAW	7.5 a	8.5 a	3.3 a	19.3 a	2.9 a	1.8 a	4.7 a	0.7 a
90% Depletion PAW	6.9 a	5.0 a	3.8 a	15.7 a	2.8 a	1.6 a	4.4 a	0.6 a
LSD $_{(0.05)}$	3.84	4.02	2.41	10.27	1.49	1.28	2.77	0.38

Averages followed by the same letter do not differ significantly

3.2.5 Biomass Water Use Efficiency (BWUE) and Biomass Production

Plant dry matter produced by a given amount of water is an important measure of water use efficiency (Brady and Weil, 2002). This efficiency is expressed in terms of dry matter per unit of water loss by evapotranspiration.

A decrease in PAW leads to a decrease in ET (Table 3.6). This occurs because ET depends on the PAW. Therefore, limiting PAW reduces the total ET which, in turn, has an impact on the biomass production. According to Table 3.6, BWUE (expressed in $kg.ha^{-1}.mm^{-1}$) at 0% depletion of PAW was significantly ($p < 0.05$) higher in loamy sand. It was then followed by that in loam soil and sandy loam. This means that sandy loam produced the least in BWUE. At 50% and 90% depletion of PAW loamy sand outperformed loam and sandy loam respectively in BWUE. However, there was a significant difference ($p < 0.05$) of BWUE between the three textures. Sandy loam was significantly lowest. Loam was moderate while loamy sand was the highest. Findings reported by Jalota *et al.* (2006) indicate that the ET and yield of crops decrease with a decreasing number of irrigations that relate to decreasing PAW. Their finding agrees with results in this investigation. Increased moisture depletion leads to a decrease in ET and hence in the yield.

These relationships indicate that to obtain maximum biomass production components of *Mentha longifolia* L, water amounting to 263 mm, 245 mm and 259 mm from loam, sandy loam and loamy sand should be available in soil to produce 0.0129 kg (2842.92 kg ha^{-1}), 0.0068 kg (1495.58 kg ha^{-1}) and 0.0385 kg (8524.34 kg ha^{-1}) respectively. Biomass water use efficiency (BWUE) values observed in loamy sand were significantly higher than those observed in sandy loam and loam. In a study conducted by Katerji and Mastrorilli (2009), WUE was reduced significantly when crops were grown in clay soil. This decrease was coupled with significant reduction in yield and ET (Katerji & Mastrorilli, 2009; Tolk & Howell 2003). These findings are similar to findings in this research: the highest ET was observed in 0% depletion of PAW in loam (20.6% clay), but the highest biomass production was obtained from 0% depletion of PAW in loamy sand (4.6% clay).

Table 3.6 Average dry biomass production per ha and BWUE of three moisture depletion levels in three differently textured soils

Soil Texture Class	BWUE kg.ha-1.mm-1		
	0%	**50%**	**90%**
Loam (L)	10.77 b	10.04 b	8.63 b
Sandy loam (Sa L)	6.92 a	2.09 a	2.67 a
Loamy sand (L Sa)	40.58 c	13.34 c	33.32 c
LSD $_{(0.05)}$	3.77	2.41	4.52

Averages followed by the same letter do not differ significantly

Chapter 4: Influence of Soil Texture and Nitrogen Fertilizer on the Growth Rate and Biomass Production of *Mentha longifolia* L

"When you plant lettuce, if it does not grow well, you don't blame the lettuce.
You look into the reasons it is not doing well.
*It may need **fertilizer**, or more water, or less sun. You never blame the lettuce.*
Yet if we have problems with our friends o" **Thich Nhat Hanh**

4.1 Introduction

The purpose of this chapter is to give a brief discussion on the need and use of fertilizer in the investigation of the interaction of soil texture, water management and nitrogen fertilizer on the biomass production of *Mentha longifolia* L. Fertilizer use involves making appropriate decisions regarding which nutrient elements are required, how much and when to apply them (Havlin *et al*., 2005; Brady and Weil, 2002).

According to this research's requirements, fertigation was chosen as fertilizer application method. Fertigation is the application of liquid fertilizer together with irrigation water (Havlin *et al*., 2005; Brady and Weil, 2002). This practice has been elaborated on in the materials and methods chapter.

The following specific topics are discussed in this chapter: results and discussion about soil texture and biomass production, nitrogen fertilizer and biomass production, growth rate and leaf area measurements. More about the correlation of methods will follow.

4.2 Results and Discussion

4.2.1 Soil texture and Biomass Production under Fertilized conditions

Three differently textured soils, as had been described and discussed in chapter two, were used in this investigation. Numerous soil physical properties influence the plant root growth. Plant root growth is restricted by increased bulk density of the soil. Increased bulk densities are associated with heavy sub-soils (Table 2.2). Sandy soils are not low in oxygen levels. Low oxygen levels constrain root respiration. Root respiration provides the plant with energy needed for absorption of nutrients and water (Brady and Weil, 2002). Absorption of PAW and plant nutrients in quantities sufficient for optimum productivity is a prerequisite for healthy growth (Havlin *et al*., 2005). Plants cannot absorb nutrients from a dry soil. In this experiment, 0% depletion of PAW was maintained as the best soil moisture management practice. Nitrification rates are generally highest at FC (Havlin *et al*., 2005).

Nitrogen is the most frequently deficient and most required plant nutrient in non-legume cropping systems. Therefore, an average of 150 ml water, containing the fertilizer, was added daily in fertigation practice. Fertigation is a management practice that minimises nitrogen losses and maximises the quantity of applied nitrogen to increase production efficiency (Havlin *et al*., 2005; Brady and Weil, 2002). Nitrogen losses are minimised as the plant nutrient is dissolved in irrigation water. Therefore, processes such as denitrification and volatilisation are avoided. The already dissolved plant nutrient is accessed in PAW. Hence, nitrogen efficiency in production is revealed.

It was important to examine the influence of soil texture on the plant biomass components under fertilized conditions. Soil fertility of the soil is the state of the soil at which the quality of the soil enables it to provide essential chemical elements in quantities and proportions essential for the growth of specified plants (Brady and Weil, 2002). The weight differences and or similarities were determined at harvest.

Table 4.1 indicates statistical comparisons made between plant biomass components across three different soil textures. Results shown in Table 4.1, indicate that there is no significant difference (0.05) in plant biomass production components among the three soils that were used in the investigation. However, loamy sand happened to outperform loam and sandy loam on wet roots biomass production. The reason is associated with the opinions stated by Havlin *et al.* (2005) and Brady and Weil (2002). They indicated that sufficient nutrient availability is required to realise maximum yield potential (Brady and Weil, 2002; Havlin *et al*., 2005). The influence of soil texture will remain comparatively unrecognisable if the soil is well fertilized. This consequence suggests the need to look at the influence of the fertilizer rate on the biomass production.

Table 4.1 Average plant biomass components at harvest of different soil textures under fertilized conditions

Texture (fertilized)	Wet Leaf (g)	Wet Stem (g)	Total Above ground Wet biomass (g)	Wet Root (g)	Dry Leaf (g)	Dry Stem (g)	Total Above ground Dry Biomass (g)	Dry Root (g)	LAI
Loam	9.9 a	7.7 a	17.6a	2.4 a	2.7 a	0.8 a	3.50 a	1.1 a	1.5 a
Sandy loam	10.0 a	7.4 a	17.4 a	1.9 a	2.4 a	0.8 a	3.20 a	1.0 a	1.4 a
Loamy sand	13.3 a	11.1 a	24.4 a	4.9 b	3.6 a	1.1 a	4.70 a	1.4 a	1.9 a
$LSD_{(0.05)}$	4.87	4.68	9.55	1.63	1.33	0.46	1.79	0.4	0.68

Averages followed by the same letter do not differ significantly

4.2.2 LAI, Biomass Production and Nitrogen Fertilizer Rate

Plant biomass components were influenced by the nitrogen fertilizer rate, and the degree of influence was determined at the end of the specific production cycle. Nitrogen is a widely used, essential plant nutrient usually applied as Nitrogen fertilizer to improve the yield of agriculturally important plants (Pedraza, 2008). Table 4.2 shows that increasing the nitrogen fertilizer rate led to an increase in total biomass production. That is, remarkable weights in biomass production reflected a

greater number of leaves and stems, and a corresponding abundance of belowground biomass production obtained from 250kg ha^{-1} nitrogen fertilizer.

Table 4.2 shows that all wet plant biomass components were different. In all three nitrogen fertilizer rates, wet leaf component was high. Wet stem was the second in biomass production to wet leaf biomass production. Wet roots biomass ended up with the lowest biomass production. Statistically, there was a significant difference ($p < 0.05$) between biomass production components produced by different nitrogen fertilizer rates. For wet biomass production components 250kg ha^{-1} of nitrogen fertilizer produced the highest biomass production. It was followed by 150kg ha^{-1} nitrogen fertilizer rate. Then 0kg ha^{-1} of nitrogen fertilizer produced the lowest wet biomass. On dry biomass production there was a significant difference ($p < 0.05$) between the three fertilizer rates especially on dry leaf and stem biomass components. Nitrogen fertilizer rates 250kg ha^{-1} and 150kg ha^{-1} matched each other on dry roots biomass. The two fertilizer rates were significantly higher than 0kg ha^{-1} that seemed to produce the lowest plant biomass components. Once more, there was a significant difference ($p < 0.05$) between three nitrogen fertilizer rates on LAI. A nitrogen fertilizer rate of 250kg ha^{-1} outperformed both 150kg ha^{-1} and 0kg ha^{-1} nitrogen fertilizer rates. Furthermore, the 150 kg ha^{-1} was also significantly different ($p < 0.05$) from the 0kg ha^{-1} nitrogen fertilizer rate.

These results are in agreement with those reported by Alsafar and Al-Hassan (2009). The results indicated that LAI, total number of leaves and essential oil of *Mentha longifolia* L increased significantly with increasing the rate of fertilizer application from 75/50 kg ha^{-1} N/P_2O_5 ha^{-1} to 100/75 kg ha^{-1} N/P_2O_5 ha^{-1} as was evident when compared to the control and the lowest rates of fertilizer application. Leleu *et al.* (2000) observed similar trends.

Mitchell *et al*. (1992) indicate that the highest nitrogen application yielded the most biomass, especially if PAW was kept high. They further indicated that at low PAW level, low nitrogen rates produced better results than their counterparts. In other related studies, it was found that increases in N fertilizer rates resulted in increased herbal yield of *Mentha arvensis* (Kiran and Patra, 2003). Hendawy and Khalid

(2011) and Yassen *et al.* (2010) pointed out that improved fertilization increased biomass production components of medicinal plants

In their concluding remarks, Zhu *et al.* (2009) note that proper amounts of fertilizer N and P were important for medicinal plants' production. This is similar to what has been found and reported in our investigations that an increase in nitrogen fertilizer increased biomass production components. According to Abbass (2009), the highest rates of about 100kg ha^{-1} of nitrogen and 150kg ha^{-1} of P_2O_5 fertilizers improved the vegetative growth properties, *i.e.* plant height, total number of leaves, branches, vegetative yield, and chlorophyll content and oil yield. Results reported by Singh and Rao (2009) revealed that an application of 200kg ha^{-1} nitrogen fertilizer produced high patchouli herbage and oil yield when compared to the controls. The findings of the latter authors, together with the findings of this study agree with the results of Jha *et al*. (2011). They claim that biomass production of *A. annua* (a medicinal plant) increased progressively with increase in nutrient content of N – P – K – S. Leaf area and leaf stem ratio were greatest with the highest rates of chemical fertilizer (Jha *et al*., 2011). Chung *et al.* (2010) also conclude that bioactive constituents of medicinal plants were affected by the quantity of nitrogen fertilizer applied.

Praszna (1992), Munsi (2002) and Abbass (2009) provide results that show that an increase in nitrogen fertilizer increases mint leaf size and green yield several fold in comparison to plants raised without nitrogen fertilization. Once more, results of this study are in agreement with the results of Shormin *et al.* (2009). They report that the highest dose of 240kg ha^{-1} of nitrogen fertilizer produced the highest mint biomass production. Other studies report that mint requires high levels of nitrogen fertilizer, between 150kg ha^{-1} to 250kg ha^{-1} depending on the agro-climatic conditions (Ram *et al*., 1987). In the study which investigated the effect of a combined N – P – K fertilizer rate on vegetative growth and yield of *Aroniamelanocarpa*, increased application of fertilizer increased vegetative growth and yield (Jeppsson, 2000). Looking at general and or total biomass production, Sotiropoulou and Karamanos (2010) provide more evidence that an increase in N fertilizer significantly affected

the total number of stems, branches, LAI and the oil yield of *Origanum vulgare ssp* (Zhao *et al*., 2005; Leleu *et al*., 2000). In other parallel studies that investigated the influence of organic mulch and fertilizer N use on herbage yield, it was found that 160kg ha^{-1} nitrogen fertilizer with mulch gave a significant result (Ram, M., Ram, D and Roy, 2003).

Table 4.2 Average plant biomass components averaged over all textures at different nitrogen fertilizer rates

N Fertilizer Rate	Wet Leaf (g)	Wet Stem (g)	Total Above ground Biomass (g)	Wet Root (g)	Dry Leaf (g)	Dry Stem (g)	Total Ab0ve ground Biomass (g)	Dry Root (g)	LAI
0 kg ha-1	2.0 a	0.8 a	2.8 a	0.7 a	0.5 a	0.1 a	0.6 a	0.5 a	0.3 a
150 kg ha-1	12.4 b	9.1 b	21.5 b	3.7 b	3.1 b	1.0 b	4.1 b	1.3 b	1.8 b
250 kg ha-1	18.9 c	16.3 c	35.2 c	4.9 c	5.0 c	1.6 c	6.6 c	1.6 b	2.6 c
$LSD_{(0.05)}$	2.55	2.80	5.35	1.46	0.77	0.29	1.06	0.29	0.35

Averages followed by the same letter do not differ significantly

Collectively and in support of this investigation's results, differences in fresh leaves and stems are shown in Figure 4.1. With 250kg ha^{-1} nitrogen fertilizer, plants showed high branching, and the total number of leaves and the development increased as the growing season progressed. There was an average development of branches and total number of leaves with 150kg ha^{-1} nitrogen fertilizers. At 0kg ha^{-1} nitrogen fertilizer, there was no branching and few leaves developed. Observations and analyses indicated that vegetative development and branching was better off in loamy sand than in loam and sandy loam. Table 4.3 shows the height of selected plants at harvest. According to this table, the height of plants at 250kg ha $^{-1}$ and 150kg ha $^{-1}$ seemed to match each other on loamy sand and sandy loam soils used in the investigation. Plants that were grown at 0 kg ha $^{-1}$ of nitrogen fertilizer seemed to grow stunted hence their height was the least at harvest. These differences were as a result of different nitrogen fertilizer rates. These diferences in height show that there was a significant difference ($p < 0.05$) in the biomass

production components produced by different nitrogen fertilizer rates. Some of these features (plant biomass components such as plant height and vegetative compostion) can be seen in Figure 4.1.

Table 4.3 Height at harvest for selected plants at different nitrogen fertilizer rates on three soil textures

Nitrogen Fertilizer Rates	Soil Texture Class	Height at Harvest for selected plants (mm)
250kg ha $^{-1}$	Loamy sand	234
	Sandy loam	267
	Loam	234
150kg ha $^{-1}$	Loamy sand	234
	Sandy loam	267
	Loam	247
0kg ha $^{-1}$	Loamy sand	160
	Sandy loam	200
	Loam	167

Figure 4.1 Fresh biomass production differences resulting from three nitrogen fertilizer rates few weeks prior to harvest.

One of the important primary factors that determine whether plants are well supplied with water is the rate at which soil water is supplied by the soil to the absorbing roots (Brady and Weil, 2002). Aboveground biomass (stems and roots) rely on belowground biomass (roots). That is, the shoot and root systems are functionally closely related. Like leaves, roots abundance (length, surface or volume) increases with increasing availability of nitrogen fertilizer (Liedgens and Richner, 2001).

Differences in root abundance at three different nitrogen fertilizer rates in three soil textures are shown in Figure 4.2. Root distribution and or abundance was significantly higher ($p < 0.05$) at 250 kg ha^{-1} in loamy sand. Relatively, sandy loam was the second in belowground biomass production. Loam soil at this nitrogen fertilizer rate produced lowest belowground biomass. Altogether, the performance of 250 kg ha^{-1} nitrogen fertilizer on root biomass was significantly higher than those of 150kg ha^{-1} and 0kg ha^{-1} nitrogen fertilizer. However, root biomass production at 150 kg ha^{-1} was higher than that of a 0kg ha^{-1} nitrogen fertilizer rate.

The discussion on the rhizosphere architecture takes the types of agricultural plants into account. The morphology of roots changes according to the developmental stage of the plant. This directly influences the ability of the plant to absorb mineral nutrients from the soil. This fact means that plant growth, biomass and yield will be influenced (Lynch, 1995).

On average, results indicate a significant difference between 250kg ha^{-1}nitrogen fertilizer and 150kg ha^{-1} nitrogen fertilizer in comparison with 0kg ha^{-1} nitrogen fertilizer and a significant difference between 250kg ha^{-1}nitrogen fertilizer and 150kg ha^{-1}nitrogen fertilizer. Figure 4.2 also shows that at 250kg ha^{-1} nitrogen fertilizer, root biomass was high. This correlates with the increase in aboveground (stem and leaf) biomass that has been reported. Moreover, roots were most abundant in loamy sand, followed by those in sandy loam and then roots in loam. In similar observations, Bo *et al*. (2009) substantiate that nutrient supply in soil, in addition to the physical properties of the soil, strongly influences the belowground (root) biomass and its morphology, which in turn affects the aboveground growth (stem and leaf).

These results indicate that when belowground biomass increases, aboveground biomass also increases. The evidence is shown in Table 4.2. The higher the wet root weight the higher the wet stem and leaf. Then, when the nitrogen fertilizer rate is increased, belowground biomass also increases and in turn, aboveground biomass also increases and vice versa.

Figure 4.2 shows that at 150kg ha^{-1} and 0kg ha^{-1} nitrogen fertilizer across three soil textures, root distribution (abundance) differed insignificantly. That is, the response seemed to agree with previous findings that there was no significant difference in root development in plants given high fertilizer load, compared with those given none at all in different soil textures. Other studies show different results. In a study conducted by Li and Shao (2003), they report that increased levels of nitrogen fertilizer in soil resulted in a decrease in root biomass, but increased growth of shoots (Li and Shao, 2003; Zeng *et al*., 2000).

Figure 4.2 Belowground biomass (roots) in three soil textures as affected by nitrogen fertilizer rates

4.2.3 Nitrogen Fertilizer Rate and LAI

Plant growth is a measure of the progressive life of a plant growing on an anchoring material (Havlin *et al.,* 2005). There are several factors that affect plant growth. Soil texture and nitrogen fertilizer are of concern in this investigation. Bretzel *et al*. (2009) support the view that soil properties had little effect on the emergence of seedlings of herbaceous plants, but had a greater effect on their growth and flowering. They further indicate that the highest values of biomass and plant height depended on the fertility of the soil. In this investigation, growth rate was measured as total leaf area. Total leaf area was finally converted into LAI which was then mainly used in reporting. Additionally, daily leaf expansion rate was observed. It was considered with the aim to trace growth rate from another angle. With this property, growth rate was determined in order to differentiate the performance of each soil texture at a given nitrogen fertilizer rate.

Table 2.8 shows how each plant's growth progressed. There was a stunted plant leaf growth (number) at 0 kg ha-1 nitrogen fertilizer rate on sandy loam for three successive weeks. Figure 4.3 shows that the daily leaf expansion rate was constant from the first week to the second week of the experiment. After this period, the daily expansion rate accelerated and differed succinctly. It was observed that the LAI of plants in loam soil was the poorest, whereas those in loamy sand outperformed both those in sandy loam and in loam soil with regard to LAI. At 250 kg ha-1 nitrogen fertilizer the leaf area expansion rate was highest in loam soil followed by sandy loam and loam respectively. Figure 4.4 indicates that the leaf growth rate at 0kg ha^{-1} nitrogen fertilizer treatments in three soil textures was lowest compared to the other nitrogen fertilizer rates. Increase in leaf area was minimal at this nitrogen fertilizer rate. From the first week to some few days of the second week of the experiment, there was no difference in LAI of the different soil textures at 0 kg ha -1 nitrogen fertilizer. There was no difference between loamy sand and sandy loam LAI until the third week of the experiment. Closer examination of the plant leaf growth rate with this treatment shows that over the course of the growing season, especially towards week four of the experiment, leaf growth rate of plants in

loamy sand was greater than that of the plants in sandy loam and therefore greater than those in loam (the least). Growth rate in sandy loam with a treatment of 150kg ha^{-1}nitrogen fertilizer was the lowest though much better than the rate at 0kg ha^{-1}nitrogen fertilizer. Then followed the growth rate of plants in loamy sand and then with loam rendering the best. It was, however, interesting to notice that the growth rate of plants in loamy sand outcompeted that of the loam when 250kg ha^{-1} nitrogen fertilizer was administered. An irregular growth rate pattern was noticed in loam soil where 150kg ha^{-1} nitrogen fertilizer had a stronger influence on plant growth rate than 250kg ha^{-1} nitrogen fertilizer. The reason for this was related to the following findings: Nitrogen fertilizer - 250kg ha^{-1} on this texture could have reached an excessive or toxic nutrient element concentration high enough to reduce plant growth (Havlin *et al*., 2005). Evidence in support of this theory was found in the literature. Choi *et al*. (2011) report that in some cases where N and P fertigation rates were increased, nutrient accumulation resulted in the vegetative organs and such an accumulation impaired plant growth in the current season, noticeably in perennials. Those nutrients would result in better plant growth in the next season (Choi *et al*., 2011). Vegetative growth was high at a lower rate of nitrogen fertilizer applied in fertigation. The growth was curtailed due to some nutrient toxicity caused by short-term induced soil acidity (Haynes, 1988). This is similar to what was experienced in this investigation. Table 4.3 and Figure 4.2 shows this argument. According to Table 4.3, the average height of plants on loam soil at harvest was highest at 150kg ha^{-1} and not at 250kg ha^{-1}. As findings in this experiment are similar to Haynes's, the same explanation seems reasonable. In the last week of this experiment, as shown in Figure 4.4, loamy sand at 250Kg ha^{-1} N fertilizer gave the highest growth rate followed by sandy loam and finally loam. Leleu *et al*. (2000) are of the opinion that increases in nitrogen fertilizer rates lead to high growth rates, producing more leaves and stems or also larger ones in these soil textures. According to the results of the present experiment this seems likely, especially when considering the combination of soil texture and nitrogen fertilizer rate. Therefore, on average, loamy sand could be expected to have given a better growth rate. However, statistical comparisons of least significant (LS) means show no

significant difference (P > 0.05) across all soil textures throughout the experiment period. This statistical inference is indicated in Table 4.1.

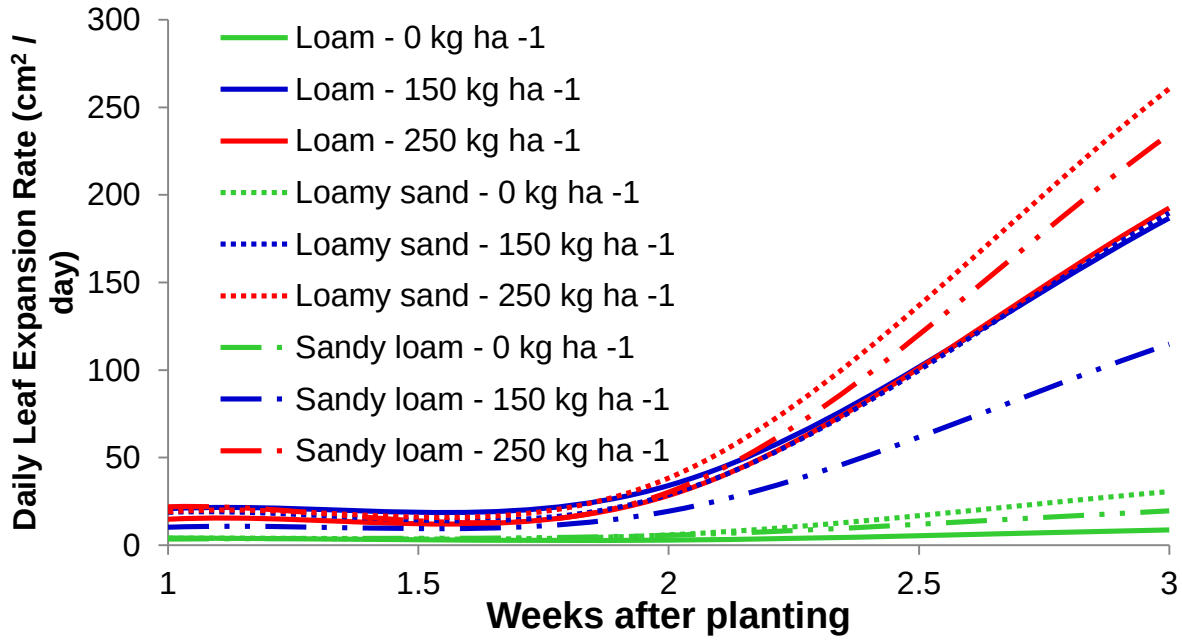

Figure 4.3 Change in leaf area expansion rate of different soil textures and nitrogen fertilizer rates during the growing season

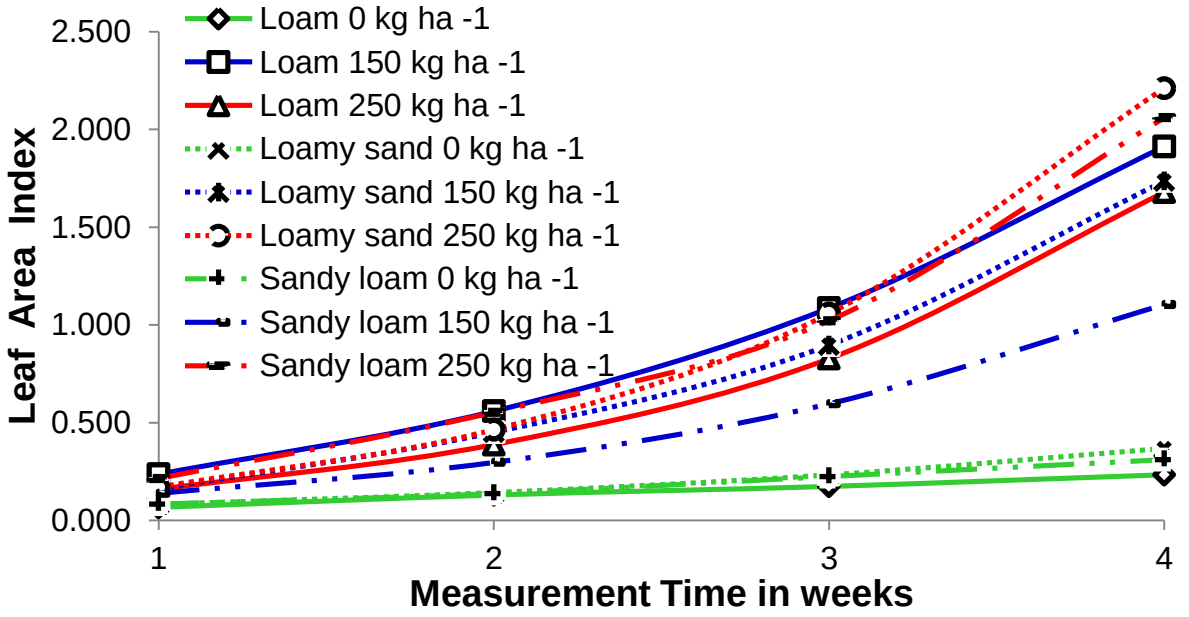

Figure 4.4 Change in LAI of different soil textures and nitrogen fertilizer rates during the growing season

4.2.4 Leaf Area Measurement – Correlation of Methods

Two methods of leaf area (LA) measurement for determining LA were investigated and correlated (Table 4.4). This correlation was done in order to confirm one of the methods as the more relevant and readily available method to use, especially in in-situ and for within growing cycle measurements.

The test method included measurement of the length and width of an individual leaf. The formula of an area of an ellipse (*Mentha longifolia* leaf shape is similar to an ellipse) was then used to calculate the area of a given leaf. This method was employed in determining the growth rate and the LA of the test plant. This method was used throughout the growing season. The second method was using a Leaf Area Meter Model 3100. This method only works at harvest to determine the final LA.

According to the results portrayed in Figure 4.5 and the calculated correlation coefficient value (0.9979) indicated, there is a perfect positive linear correlation between the two methods of determining LA. These results imply that Y (observed or calculated LA) increases as X (actual or leaf area meter LA) increases. That is, the more closely actual values and observed values are linearly related, the more the variability in observed values can be explained by the variability in actual values. This behaviour is indeed relevant to what has been investigated. This investigation's results therefore imply a coefficient of linear correlation that is close to 1. Therefore, the use of an ellipse formula has been of importance. The method can then be used as an alternative to leaf area meter, especially in cases where the area shape is similar to the shape in this investigation.

$$\boldsymbol{Area\ of\ an\ Ellipse} = \pi * \frac{1}{2} * (length) * \frac{1}{2} * (width)$$

Equation 4.1

Table 4.4 Correlation of two methods: Y = LA calculated using equation 4.1. X = LA measured using LA meter model 3100.

Soil Texture Class	Observations	Observed / Calculated LA cm^2 (Y)	Actual / Measured LA cm^2 (X)
Loam	1	82.84	79.24
	2	355.74	377.57
	3	304.30	302.97
Loamy sand	4	96.36	97.72
	5	225.58	226.44
	6	334.56	337.14
Sandy loam	7	35.42	43.46
	8	208.26	223.07
	9	356.30	356.70
	Total	**1999.36**	**2044.31**

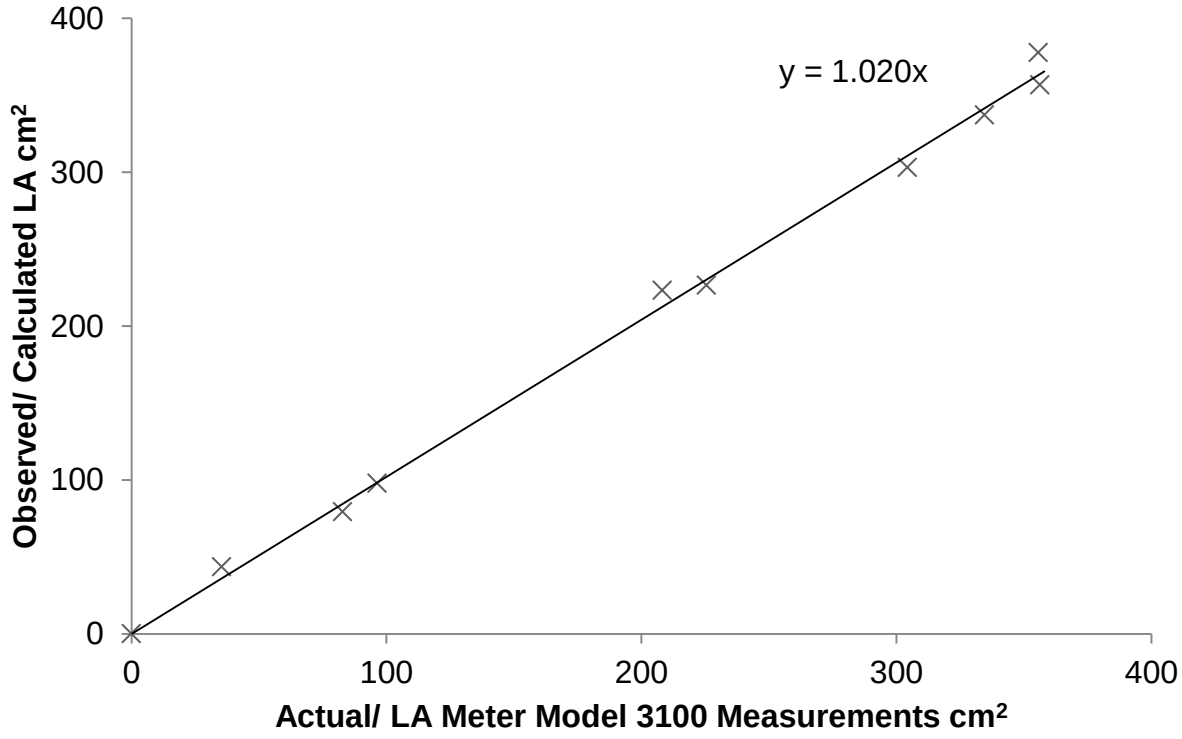

Figure 4.5 Regression plot of data for determination of linear correlation

This chapter provided an overview and discussion of the incorporation of nitrogen fertilizer as a requirement and a specific part in this investigation. The conclusive remarks from this chapter shall be presented in conclusions and recommendations chapter that shall follow immediately after a chapter on antimicrobial properties of Mentha longifolia, which is now to follow.

Chapter 5: Antimicrobial Properties of *Mentha longifolia* L.

"When health is absent Wisdom cannot reveal itself,
Art cannot become manifest, Strength cannot be exerted,
Wealth is useless and Reason is powerless." **Herophilies,** 300 B.C.

5.1 Introduction

In the last decade, there has been a global upsurge in the use of traditional medicine and complementary and alternative medicine, a movement that has been recognised and acknowledged (WHO, 2003; WHO, 2005). This chapter reports on the antimicrobial properties of *Mentha longifolia* L., the test plant that was used in this investigation. With reference to the general aim of this investigation, this section focuses on the results and discussion based on the Minimum Inhibition Concentration of the agent, and the influences of soil texture and Nitrogen fertilizer on the antimicrobial action.

5.2 Results and Discussion

Analyses and findings on the data that were collected are presented as results by means of a template in Table 5.1. Size differences in the inhibition zones were measured and recorded. Only samples from 150kg ha^{-1} and 250 kg ha^{-1}nitrogen fertilizer treatments from three different soil textures were used in the experiment as 0kg ha-1 nitrogen fertilizer produce was not enough to provide required amounts of either fresh or dry leave samples.

Table 5.1 Minimum Inhibition Concentration – inhibition zones

R = Rich Medium, P = Poor Medium, Digits = Replicates and readings are in (mm), L = Loam, Sa L = Sandy loam and L Sa = Loamy sand

L Sa150Kg ha^{-1} N	R1	R2	P1	P2	L Sa 250Kg ha^{-1} N	R1	R2	P1	P2
A Fresh 20g 100ml^{-1}	1	1.2	1.5	1.7	A Fresh 20g 100ml^{-1}	1	1.3	1.6	1.7
B Fresh 30g 100ml^{-1}	1.6	1.3	2.1	2.4	B Fresh 30g 100ml^{-1}	1.3	1	1.9	2.5
C Dry 20g 100ml^{-1}	0	0	0.6	0.5	C Dry 20g 100ml^{-1}	0	0	0.8	0.5
D Dry 30g 100ml^{-1}	0	0	0.9	1.2	D Dry 30g 100ml^{-1}	0	0	0.9	1.4

L 150Kg ha^{-1} N	R1	R2	P1	P2	L 250Kg ha^{-1} N	R1	R2	P1	P2
A Fresh 20g 100ml^{-1}	0.9	1	1.3	1.2	A Fresh 20g 100ml^{-1}	1	1.4	1.9	1.5
B Fresh 30g 100ml^{-1}	1.3	1.1	2	1.9	B Fresh 30g 100ml^{-1}	1.5	0	1.7	1.9
C Dry 20g 100ml^{-1}	0	0	0.6	0.5	C Dry 20g 100ml^{-1}	0	0	1.4	1.6
D Dry 30g 100ml^{-1}	0.5	0.6	1.7	1.3	D Dry 30g 100ml^{-1}	0	0	1.4	0

Sa L 150Kg ha^{-1} N	R1	R2	P1	P2	Sa L 250Kg ha^{-1} N	R1	R2	P1	P2
A Fresh 20g 100ml^{-1}	0	0	0	0	A Fresh 20g 100ml^{-1}	0.5	0.6	0.9	2
B Fresh 30g 100ml^{-1}	0	0	0.5	0	B Fresh 30g 100ml^{-1}	0.6	0	0	2.1
C Dry 20g 100ml^{-1}	0	0	0	0	C Dry 20g 100ml^{-1}	0	0	1	1.2
D Dry 30g 100ml^{-1}	0.5	0	0.5	0.5	D Dry 30g 100ml^{-1}	0	0	0.7	0.6

Minimum Inhibition Concentration (MIC) is the minimum concentration of the agent that inhibits the growth of the pathogen (bacterium) (Collins *et al.,* 1995). Even though the inhibition zones were minimal, with an average of 1mm, 20g 100ml^{-1} had an impact that could be recorded and reported as the MIC of *Mentha longifolia* L against *Staphylococcus aureus*.

Table 5.1 shows that loamy sand contributed positively to the production of the best bioactive compounds of *Mentha longifolia* L. The MIC from this texture gave an average of 1.3mm, followed by that from loam soil. Sandy loam showed the least, with the MIC of 20g 100ml^{-1} resulting in 0.4mm inhibition zone. These findings led to the deduction that soil texture has an influence on the bioactive components of *Mentha longifolia* L. On average, the nitrogen fertilizer rate has no influence on the antimicrobial properties of *Mentha longifolia* L, especially for plants grown in loamy sand and loam. These observations agree with the results reported by Mitchell *et al*. (1992). They showed that nitrogen fertility did not affect oil quality but did affect the

dry matter production. Abbas (2005), Singh *et al.* (1989) and Saxena and Singh (1995) are also of the opinion that significantly higher biomass and essential oil yield of *Mentha spp* were obtained with higher nitrogen application. They also reported that any increase in nitrogen fertilizer did not affect the chemical composition of the essential oil. However, on plants grown in sandy loam, 250kg ha^{-1} nitrogen fertilizer showed a recognisable influence over the same texture's 150kg ha^{-1} nitrogen fertilizer. In summary, this discussion indicates the fact that nitrogen fertilizer does not have an influence on the bioactive components of the *Mentha longifolia* L. Figure 5.1 shows selected and observed reactions of the experiment. It indicates the minimal inhibition zones that were accompanied by microbial contamination.

The foremost challenge of this investigation was microbial contamination, as there were cases of minimum inhibition zones that were accompanied by contamination (Shown in Figure 5.1) (Britz, 2011: personal communications). Good Manufacturing Practices for Pharmaceutical Products (GMPPP) (2003) defines contamination as "the undesired introduction of impurities of chemical or microbiological nature or of foreign nature into the starting material or intermediate during production, sampling, packaging, storage or transport" (GMPPP, 2003). Fennell *et al.* (2004) and WHO (2003) explain that inadvertent contamination by microbial or chemical agents during any of the production stages can lead to deterioration in safety and quality of the medicinal plants (Fennell *et al*., 2004; WHO, 2003).

In this investigation, microbial contamination was experienced. Bacterial contamination was suspected (Britz, 2011: personal communications) With findings similar to those of this experiment, Gulluce *et al*. (2007) in their investigation of the antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L, report that the essential oil had great potential for antimicrobial activity against *Staphylococcus aureus,* because the bioactive components of *Mentha longifolia* possessed antimicrobial properties against the test organisms.

Evidence of antimicrobial activity of *Mentha longifolia* L is showed in the effect on the *Staphylococcus aureus*. The effect of *Mentha longifolia* L essential oil on the morphology of four pathogenic bacteria, including *Staphylococcus aureus,* was reported. *Mentha longifolia* L was reported to have a high antibacterial effect, in that the cell wall of the test bacteria was damaged at MIC concentrations (Hafedh *et al*., 2010). Dzamic *et al*., (2010) agree that *Mentha longifolia* has antimicrobial activity.

In another study by Khattak et al. (2004), it was reported that *Mentha longifolia* L possesses good antifungal activity against Trichophyton longifusus. However, they indicated that the extracts obtained from *Mentha longifolia* L were found to be devoid of any antibacterial properties. Here the argument can be linked to their drying method. Their *Mentha longifolia* L plant parts were oven dried at 70°C for 24 to 26 hours. Higher temperatures affect the chemical composition of the essential oil (Stanisavljevic *et al.,* 2010).

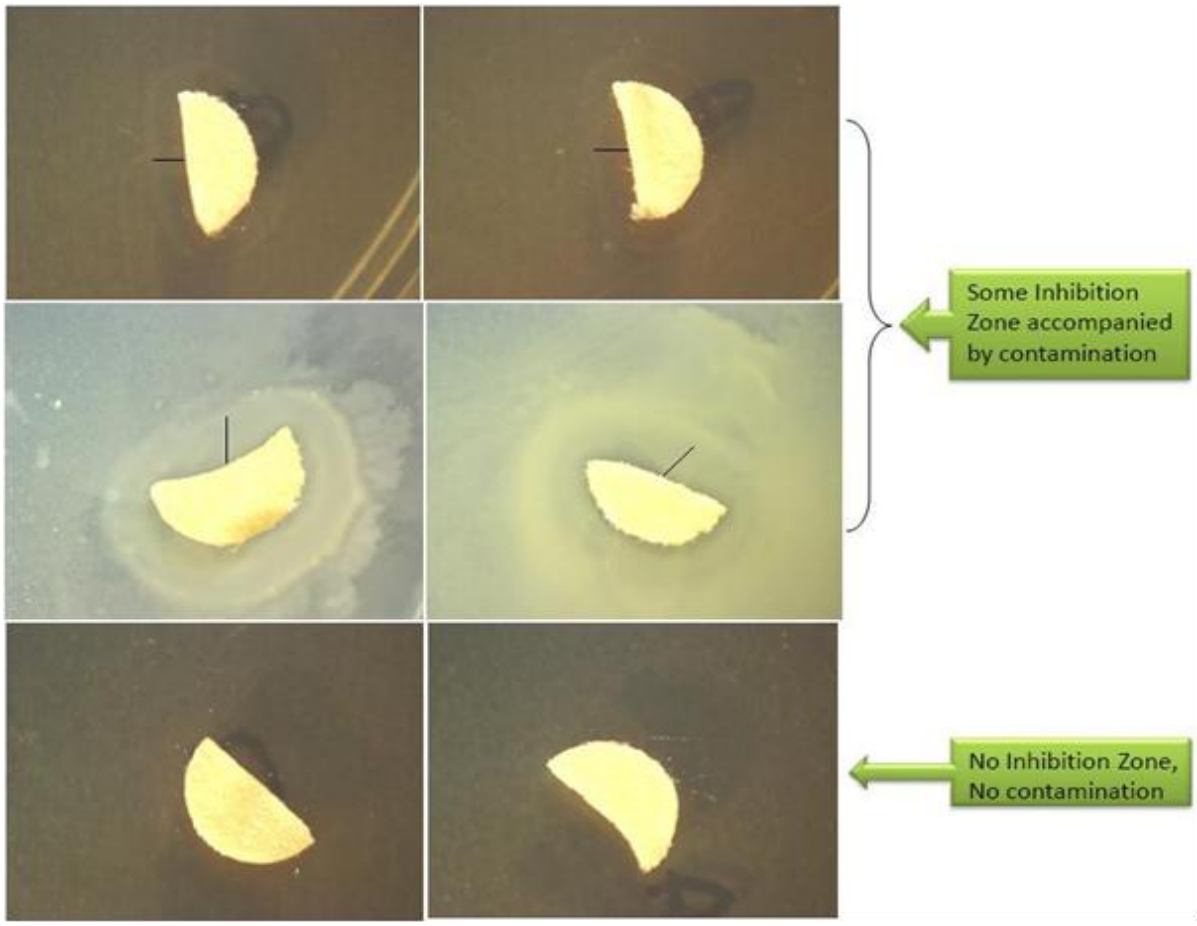

Figure 5.1 This plate indicates inhibition zones on rich and poor mediums. Photographs were captured under a microscope. Inhibition zone was seemingly minimal and accompanied by microbial contamination. First two films are on rich medium – inhibition zone was thin. The middle films show activity on poor medium; the inhibition zone was thin too and microbial contamination was pronounced. **NOTE:** black thin bar on the first four films indicates the extent or the length (*not to scale*) of the inhibition zone.

In summary, this chapter provided results and discussions about the influence of soil texture and nitrogen fertilizer under 0% depletion of PAW on the antimicrobial properties of *Mentha longifolia* L. The crucial findings will be presented in the chapter dealing with the conclusions and research recommendations.

Chapter 6: Conclusions and Research Recommendation

"A wealthy landowner cannot cultivate and improve his farm without spreading comfort and well-being around him. Rich and abundant crops, a numerous population and prosperous countryside are the rewards for his efforts" **Antoine Lavoisier**

6.1 Introduction

Evidently, through literature, people across the world have always used naturally provided plants for medicinal purposes. Noteworthy is that wild medicinal plants, including *Mentha longifolia* L*,* are being eradicated due to the increase in population, settlement, and the over-harvesting of the said plant (Agelet *et al.,* 2000; Mkhize, Msango and Smith, personal communications in; Fennell *et al*., 2004; WHO, 2003). According to literature, previous research has looked at the importance of *Mentha longifolia* L as an antimicrobial agent. Adding to previous statements, alternatives to pharmaceuticals are popular, partly because of the overuse of antibiotics leading to multidrug resistant bacterial strains (Doughari *et al*., 2009). Consequently cultivation will be needed to ensure a continuing supply of *Mentha longifolia* L as proposed by this research*.* This research looked at the best conditions for the cultivation of *Mentha longifolia* L.

In this chapter, the researcher illuminates the main findings from chapters three, four and five. These results will be compared to the relevant sections of the literature, interpreted and synthesised. The researcher will further deduce conclusions from the previous discussions. Relevant recommendations are made accordingly to address future research directions for parallel studies within the domain of knowledge that built these investigation contributions.

6.2 Ideal Flow diagram for most effective production of *Mentha longifolia* L

This flow diagram shows the link of necessary resources required in the production of *Mentha longifolia* L. This flow diagram portrays summative suggestions for application of this research. Figure 6.1 shows the relationship to a central idea, that is, the relationship that occurred between the resources that contributed in the ideal biomass production for antimicrobial properties of *Mentha longifolia* L.

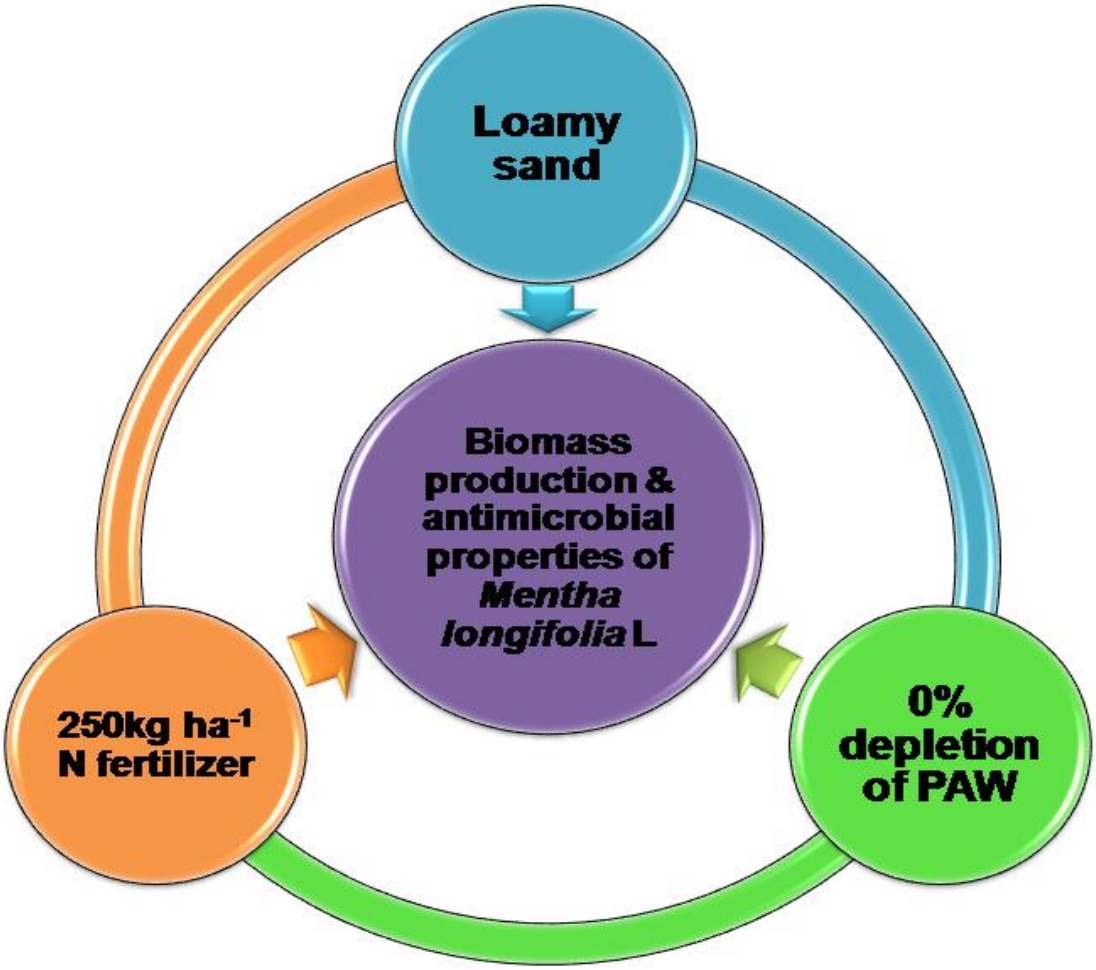

Figure 6.1: Flow diagram of resources involved in *Mentha longifolia* L production

6.3 Summary of Findings and Conclusions

6.3.1 Summary of findings and conclusions from chapter three: soil texture, water management, water use efficiency and biomass production

The first objective of this investigation was to investigate the impact of soil texture on the growth and biomass production of *Mentha longifolia* L. The results indicated that soil texture had an influence on the biomass production of *Mentha longifolia* L. It influenced the total biomass production components (leaves, stems and roots) and leaf area index. At the lowest common soil moisture content, biomass production differed across the three different soil textures. **The highest biomass production of *Mentha longifolia* L was obtained from plants growing in the soil texture classified as loamy sand. Its productivity surpassed both loam and sandy loam. Plants cultivated in sandy loam soil revealed the poorest biomass production components of *Mentha longifolia* L.**

Therefore, it is concluded that in this investigation loamy sand is the best soil texture for cultivating *Mentha longifolia* L. The descriptive statistical comparisons indicated that there was a significant difference ($p < 0.05$) between the biomass productions obtained from three different soil textures. When the results from this investigation are compared to the results of other studies, the correlation becomes evident. The supporting studies by Asgarzadeh *et al.* (2011) and those by Hussain (2009) indicate that they are of the opinion that soil texture does have an influence on the biomass production. Rajeswara Rao (1999) supports the previous authors' findings by stating that soil texture demonstrated the economic feasibility of growing corn-mint. In terms of the antimicrobial and or bioactive components production, loamy sand also performed the best.

The second objective of this investigation was to investigate the influence of plant available water on biomass production of *Mentha longifolia* L. The results prove that *Mentha longifolia* L is a hydrophilic (water-loving) plant. The results of this

investigation indicate that PAW showed a recognisable influence on the biomass production components. **Throughout the investigation, 0% depletion of PAW outperformed both 50% and 90% depletion of PAW in each soil texture. At the interaction of soil texture and depletion of PAW, it is found that biomass production at 90% depletion of PAW on loamy sand surpassed that of 0% depletion of PAW of loam and sandy loam.** This confirms that loamy sand proved to be the best soil texture in this investigation.

It is concluded that 0% depletion of PAW is the best soil moisture content for an outstanding performance of the biomass production components. Hence, in cases of using the other soil textures, this level of moisture depletion of PAW would still be an option in the biomass production of *Mentha longifolia* L. However, statistical comparison shows no significant difference at 95% confidence across three levels of PAW depletion. When the results of this investigation are compared to the results of other investigations, it becomes noticeable that in the presence of statistical comparison, certain findings contradict the findings of this investigation. Tzialla *et al.* (2006) and Rajeswara Rao (1999) support this investigation's finding by stating that total aboveground and belowground biomass of perennial grass such as *Mentha longifolia* L increases with increase in soil moisture.

6.3.2 Summary of findings and conclusions from chapter four: influence of soil texture and nitrogen fertilizer on the growth rate and biomass production of *Mentha longifolia* L

Biomass production, soil texture and fertilization interaction: Fertilization brings about a balance in the influence of soil texture. In this regard the three soil textures performed the same. The results in Table 4.1 show no significant difference in biomass production components of *Mentha longifolia* L. Application of fertilizer can advance the performance of soil texture in general, thereby allowing all soil textures' productive status to become almost equal. Fertilizing the soil through fertigation showed that some plants were without the necessary nutrients, *i.e.* they were suffering from nutrient deficiency, especially nitrogen, throughout the investigation

period. Fertigation was the best in correcting in-season nutrient deficiencies and maintaining the best production.

The third objective of this investigation was to investigate the influence of nitrogen fertilizer on biomass production and the antimicrobial properties of *Mentha longifolia* L. The obtained results were indicative of improvement, though exceptional in the case of loam soil where 250kg ha^{-1}nitrogen fertilizer was suspected to have been excessive or to have caused toxicity that impaired a proper growth rate. **The 250kg ha^{-1} nitrogen fertilizer produced a highly desired growth rate and finally the highest vegetative biomass production.**

According to the results of this investigation it can be concluded that nitrogen fertilizer has a positive influence on the growth rate and biomass production. An increase in the nitrogen fertilizer rate from 0kg ha^{-1} to 150kg ha^{-1} to 250kg ha^{-1} can increase the total biomass production components (leaves, stems and roots) and LAI. Nitrogen fertilizer showed a remarkable performance for increasing the number of leaves, promoting optimum branching and improving the LAI. This ensures good development of biomass of *Mentha longifolia* L. Statistical comparisons indicate that there was a significant difference ($p< 0.05$) between biomass production components of the three different rates of nitrogen fertilizer. When comparing the results of this investigation with the results from other investigations they echo what has been said in common chronicled literature. Most findings support the fact that any increase in the nitrogen fertilizer rate leads to an increase in the biomass production components (Hendawy and Khalid, 2011; Rajeswara Rao, 1999).

6.3.3 Summary of findings and conclusions from chapter five: antimicrobial properties of *Mentha longifolia* L

The fourth specific objective of this investigation was to verify the antimicrobial properties of *Mentha longifolia* L through the determination of the interaction of soil texture, plant available water and nitrogen fertilizer on the growth, biomass production, and the antimicrobial properties of *Mentha longifolia* L. **The results indicate that *Mentha longifolia* L possesses antimicrobial properties against a**

gram positive *Staphylococcus aureus*. On general inferences, 20g 100ml^{-1} can be accepted as the MIC concentration.

It is then concluded that *Mentha longifolia* L plants possess antimicrobial properties. However, it must be noted that the antimicrobial properties are low, therefore Mentha longifoloie L must be used in combination therapy. This conclusion and other subsequent conclusions are supported by the findings of Solorzano-Santos and Miranda-Novales (2012); Kumar *et al.* (2011) and Teixeira *et al.* (2012). The mentioned concentration showed an activity against the test organism. Furthermore, bioactive compounds traced from antimicrobial activity of *Mentha longifolia* L are influenced by soil texture and not the nitrogen fertilizer rate. Plants in loamy sand and loam produced a significant effect, which was not the case with plants in sandy loam. In the case of nitrogen fertilizer, only plants grown in sandy loam showed any influence due to fertilizer rate. However, this cannot rule out the observations drawn from the other two soil textures In that nitrogen fertilizer did not influence the bioactive components of *Mentha longifolia* L. On the other hand *Mentha longifolia* L has shown to be among the species that are prone to microbial contamination. This conclusion arose in relation to the contamination that was observed. Microbial contamination was usually triggered by aromaticity of the plant like mint (Fennell *et al*., 2004; WHO, 2003). *Mentha longifolia* L is recommended for use due to its antimicrobial properties, as verified in this investigation. Traditionally, different communities use *Mentha longifolia* L for different purposes, as shown in the literature. The results of this investigation compare to most findings of the parallel studies.

6.3.4 Unanticipated Findings – Emerging Evidence about leaf area measurement – correlation of methods (calculated versus measured)

Besides the main findings that emerged from the specific objectives of this investigation, the following finding was discovered. ***Mentha longifolia* L leaf shape is similar to an ellipse. This is indicated in Figure 4.5 as well as by the**

calculated correlation coefficient value shown in section 4.2.4. Therefore, it is concluded that the use of an ellipse formula for determination or calculation of leaf area can be used as an alternative method to a leaf area meter. This can be used in other cases, especially in those crops that possess leaves similar to *Mentha*'s leaves. The advantage of this method over the leaf area meter is that it could be used in-situ for in and within-season measurements. In cases where a leaf area meter is inaccessible or in cases where field measurements for leaf area are to be performed, the ellipse formula used in this research is recommended.

6.4 Recommendations based on this Research

The following recommendations area aimed at implementation and application of this research: cultivation of *Mentha longifolia* L should be on loamy sand. Suitable and adequate soil moisture for the production of *Mentha longifolia* L should be applied. It is recommended that a 0% depletion of PAW should be maintained by replenishing soil moisture daily. Fertilizer applications of 250kg ha^{-1} nitrogen fertilizer are recommended for the best and highest biomass production of *Mentha longifolia* L. *Mentha longifolia* L has minimal antimicrobial properties against *S. aureus*. Nevertheless, it is evident that this herb has high anticongestant properties (Manos, 2012; Petkar, 2008; Naseri *et al* 2008; Jan *et al* 2008).

6.5 Recommendations for Future Research

This research was undertaken with the aim to propose recommendations for sustainable cultivation of the indigenous herb *Mentha longifolia* L. for biomass production and antimicrobial properties. However, it was limited to the influence of soil texture (loamy sand, loam and sandy loam), water management (0%, 50% and 90% depletion of PAW) and nitrogen fertilizer rate (0kg ha^{-1}, 150kg ha^{-1} and 250kg ha^{-1} N fertilizer) on the biomass production and antimicrobial properties of *Mentha longifolia* L. Therefore, exploring other soil textural classes is recommended in order to investigate the influence of various soil textural classes (different soil series) on the biomass production, essential oil content and quality.

Future research is recommended on investigating the influence of other major elements besides nitrogen. Moreover, limitation of nitrogen should be avoided while exploring other nutrient elements. Nitrogen is suggested because of its influence on vegetative growth and branching of herbaceous plants (Abbas, 2009; Pedraza, 2008; Kiran and Petra, 2003; Munsi, 2002). Ahmed *et al.* (2011) and Abbas (2005) are of the opinion that the amount and composition of essential oil is strongly dependent on developmental stages of the plant (ontogeny).

Harvesting time is one of the most important factors that have bearing on *Mentha* oil quality. Harvesting the crop early or late, may result in low yield of biomass production and essential oil content (Ahmed *et al.,* 2011; Abbas, 2005). Therefore, further research on harvesting time of *Mentha longifolia* L is recommended.

Further research is suggested to investigate ways to avoid microbial contamination. Furthermore, studies in parallel are necessary to confirm the antimicrobial and antioxidant properties of *Mentha longifolia* L species (Gulluce *et al*., 2007). This could be undertaken by using different pathogens.

An investigation that would conduct research on the combined effect of *Mentha longifolia* L and other herbs with higher antimicrobial properties is recommended.

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Appendices

APPENDIX I – Particle Size Analysis

Particle size distribution: Pipette method (Gee and Bauder, 1986)

Introduction

Soil particles are distinct units constituting the solid phase of the soil. They generally cluster together as aggregates, but can be separated from one another by chemical and mechanical means. Particles have diverse composition and structure and generally differ from one another in both size and shape. The method that was used here possibly applies only on the inorganic particles, typically single crystalline fragments. The particle size distribution of any given soil articulates on the proportions of the various sizes of particles that it contains. The methods of fractionation and particle size analysis described are limited to sieving and sedimentation procedures.

Soil Particle size classes and method of separation

Class Name	Diameter (mm)	Method of separation
Gravel	> 2	Sieve
Coarse sand	2.0 – 0.5	Sieve
Medium sand	0.5 – 0.25	Sieve
Fine sand	0.25 – 0.106	Sieve
Very fine sand	0.106 – 0.05	Sieve
Coarse silt	0.05 – 0.02	Sedimentation
Fine silt	0.02 – 0.002	Sedimentation
Clay	< 0.002	Sedimentation

Table: Particle size classes and method of separation

Principles

Generally, soils do contain OM, sometimes gypsum and often iron oxides or hydroxides, aluminium oxides or hydroxides and carbonates coatings that bind the soil particles together and may practically prevent dispersion. Since the development of this method, pretreatments were used to overcome such difficulties. However, these pretreatments could be destructive and could also cause some dissolution of certain soil minerals. After these procedures, chemical dispersion is normally accomplished by using sodium hexametaphosphate. Sedimentation analysis is often based on the fact that spherical particles in suspension settle at a velocity that can be calculated from the Stokes' equation for settling bodies.

Apparatus

1. Glass sedimentation cylinders, 1 dm^3.
2. Hand stirrer, 50 cm long brass rod joined to the centre of a circular brass plate 1.5 mm thick.
3. Lowy pipette, 25 cm^3 capacity and the pipette stand.
4. Constant temperature room or sedimentation cabinet.
5. Set of sieves 100 mm diameter with the lid and the collecting or receiving pan. Sieve opening (mm): 2.0; 0.5; 0.25; 0.106 and 0.053.
6. Hotplate, water bath, beakers, thermometer, drying oven, high speed stirrer.

Reagents

1. Hydrogen peroxide (H_2O_2): 30 – 35 volume per cent.
2. Sodium acetate (NaOAc), 1 mol dm^{-3}, pH 5: Dissolve 82 g NaOAc in 1 dm^3 deionised water. Adjust to pH 5 with acetic acid.
3. Sodium hydroxide (NaOH), 0.1 mol dm^{-3}: dissolve 4 g NaOH in 1 dm^3 deionised water.

4. Hydrochloric acid (HCl), 0.2 mol dm^{-3}: Dilute 18 cm^3 concentrated HCl to 1 dm^3 of deionised water.

5. Calgon dispersing solution: Dissolve 35.7 g sodium hexametaphosphate [$(NaPO_4)_6$] and 7.94 g sodium carbonate (Na_2CO_3) in 1 dm^3 deionised water.

6. Sodium Citrate or bicarbonate solution: Dissolve 88.4 g sodium citrate ($Na_3C_6H_5O_7.2H_2O$) in 1 dm^3 deionised water and adjust to pH 5. Add 125 cm^3 of 1 mol dm^{-3} sodium bicarbonate (84 g $NaHCO_3$ dissolved in 1 dm^3 deionised water) to each 1 dm^3 of citrate solution

Procedure

Weigh 40 g of soil sample and transfer it into a glass beaker. Thereafter, depending on the properties of the sample, some pretreatments could be followed with the aim of removing the cementing and or the flocculating compounds.

Removal of Organic Matter

- Transfer sample to a 250 cm^3 glass beaker with deionised water.
- Add 5 cm^3 H_2O_2 to the suspension. Stir and cover with a watch glass.
- When frothing has ceased, remove cover and heat on the water bath.
- Evaporate the excess water but not to dryness. Continue to add H_2O_2 and heat until most of the organic material has been destroyed (judged by the frothing and bleached colour of the sample). After final addition of H_2O_2, heat to destroy excess H_2O_2. Dry the sample over night in an oven at 105 °C and determine the mass. The mass of oven dry H_2O_2 treated sample is the base mass.

Dispersion of the sample

Add 10 cm3 Calgon dispersing solution to the pretreated oven dried soil. Quantitatively transfer the suspension to a 250 cm3 centrifuge bottle. Make volume to approximately 150 cm3 with the deionised water, stopper and shake overnight on

a horizontal reciprocating shaker. Alternatively, the suspension could be transferred into a dispersion cup and mixed for 5 minutes with an electric mixer.

Separation of sand sample

Wash the dispersed sample on a 0.053 mm sieve, passing the silt and clay through the sieve through a funnel into a 1000 cm3 measuring cylinder. Continue washing until the percolate is clear. Remove the sieve from the cylinder and quantitatively transfer the sand to a tared evaporating dish or beaker. Dry the sample at 105 C to constant mass. Transfer the dried sand to a nest of sieves arranged from top to bottom with decreasing size in the following order: 0.5; 0.25; 0.106; 0.053 mm and pan. Shake the sieves on a sieve shaker for approximately 10 minutes. Determine the mass of each sand fraction and the residual silt plus clay that passed via the 0.053 mm sieve. A precision of 0.01 g is sufficient.

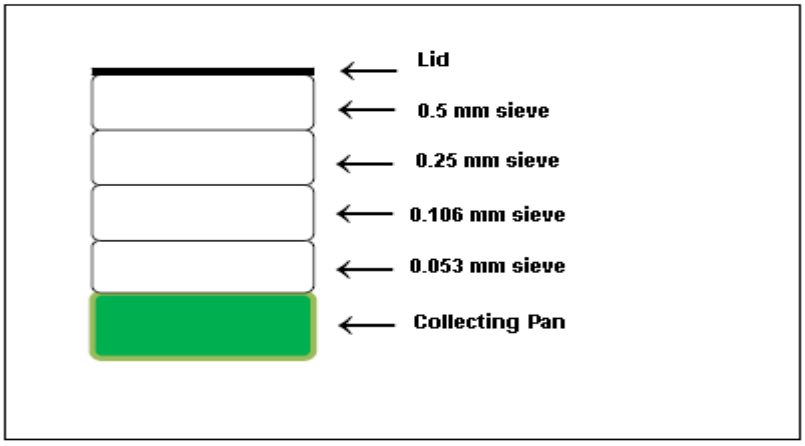

Figure: nest of sieves arranged in decreasing order

Determination of Silt and Clay with Pipette

Fill the cylinder with the silt and clay suspension to the 1 dm^3 mark. Use the watch glass to cover the cylinder. Then the cylinder is placed in a constant temperature water bath or at room temperature of 20 °C. After equilibration, stir the suspension thoroughly with a hand stirrer for 30 seconds in a vertical direction. Immediately after stirring has been terminated, after the appropriate time interval (Table 5) for determining the 0.05 mm fraction (coarse silt + fine silt + clay), lower the closed

Lowy Pipette to a depth of 10 cm into the suspension. Withdraw 25cm^3 sample with the gentle suction (about 12 sec). Discharge a sample into a tared evaporating dish. Rinse the Pipette with deionised water and add to the suspension in the dish. Evaporate the water and dry at 105 °C to constant mass, cool in a desicator and determine the mass. Repeat this procedure at specified times to determine the 0.02 mm fraction (fine silt + clay) and the 0.002 mm fraction (clay). In these two determinations, the sample is withdrawn at a depth of 10 cm for the clay fraction, a sampling depth of 7 cm can be used to reduce settling time in order to complete the determination during an 8 hour working day.

Table: sedimentation times

Temp	0.05 mm (coarse silt)		0.02 mm (fine silt)		0.002 mm (clay)		0.002 mm (clay)	
	30 cm depth		10 cm depth		10 cm depth		7 cm depth	
°C	Min	Sec	Min	Sec	Hrs	Min	Hrs	Min
15	1	31	5	17	8	48	6	10
16	1	29	5	9	8	34	6	1
17	1	27	5	1	8	21	5	51
18	1	25	4	53	8	9	5	42
19	1	22	4	46	7	57	5	34
20	1	20	4	39	7	45	5	26
21	1	18	4	32	7	34	5	30
22	1	16	4	26	7	23	5	17
23	1	15	4	20	7	13	5	3
24	1	13	4	14	7	3	4	56
25	1	11	4	8	6	53	4	49

Raw Data

_Base mass			
			F
N° sample	Glass weight	Glass and sample weight	Sample weight
Sand	149.471	189.288	39.817
Clay	136.329	175.888	39.559
Loam	154.791	194.611	39.820

Sand Fraction									
N° Sample	N° sieve	Sieve wt (g)	Sieve + Sample wt (g)	sample wt (g)	Ttl Sand Fraction sieved		Sand Fraction sieved %	Base Mass	Sand fraction % base mass
Sand	500	120.44	126.144	5.704	33.915	Coarse sand	16.8	39.817	14.3
	250	141.97	150.652	8.685		Medium sand	25.6		21.8
	106	138.22	151.930	13.708		Fine sand	40.4		34.4
	53	157.39	162.788	5.395		Very fine sand	15.9		13.5
	Pot	131.47	131.888	0.423		Coarse silt	1.2		1.1
Clay	500	120.44	124.462	4.022	19.615	Coarse sand	20.5	39.559	10.2
	250	141.97	146.347	4.380		Medium sand	22.3		11.1
	106	138.22	144.570	6.348		Fine sand	32.4		16.0
	53	157.39	161.612	4.219		Very fine sand	21.5		10.7
	Pot	131.47	132.111	0.646		Coarse silt	3.3		1.6
Loam	500	120.44	124.101	3.661	29.188	Coarse sand	12.5	39.820	9.2
	250	141.97	152.045	10.078		Medium sand	34.5		25.3
	106	138.22	148.830	10.608		Fine sand	36.3		26.6
	53	157.39	160.329	2.936		Very fine sand	10.1		7.4
	Pot	131.47	133.370	1.905		Coarse silt	6.5		4.8

Silt fraction						
T°= 20°C Time: 4'39" Depth:10cm		PED = Porcelan evaporation dish				
N° Sample	**PED weight**	**PED + sample weight (g)**	**Fine silt + clay weight (g)**	**Fine Silt weight (g)**	**Base mass**	**Fraction fine silt %**
Sand	41.43	41.493	0.06	0.01	39.82	1.4
Clay	43.64	43.875	0.24	0.03	39.56	3.3
Loam	31.50	31.61	0.11	0.03	39.82	3.3

clay fraction					
T°= 20°C Time: 5hr:26" Depth:7cm		PED = Porcelan evaporation dish Pipette volume: 25ml.			
N° Sample	**PED weight**	**PED + sample weight (g)**	**clay weight (g)**	**Base mass**	**Clay fraction % Base mass**
Sand	42.364	42.41	0.046	39.82	4.6
Clay	40.953	41.157	0.204	39.56	20.6
Loam	42.082	42.158	0.076	39.82	7.6

	Soil particle sizes classes					
N° sample	**Class**	**%**	**Total without coarse silt**		**Summary**	
Sand	Coarse sand	14.3	90.1			
	medium sand	21.8			Sand (0,05 - 2 mm)	84.1
	Fine sand	34.4			Silt (0,002 - 0,05 mm)	11.3
	Very fine sand	13.5			Clay (< 0,002 mm)	4.6
	Fine silt	1.4				
	Clay	4.6			Loamy Sand	
	Coarse silt	9.9				
Clay	Coarse sand	10.2	71.9			
	medium sand	11.1			Sand (0,05 - 2 mm)	48.0
	Fine sand	16.0			Silt (0,002 - 0,05 mm)	31.4
	Very fine sand	10.7			Clay (< 0,002 mm)	20.6
	Fine silt	3.3				
	Clay	20.6			Loam	
	Coarse silt	28.1				
Loam	Coarse sand	9.2	79.5			
	medium sand	25.3			Sand (0,05 - 2 mm)	68.5
	Fine sand	26.6			Silt (0,002 - 0,05 mm)	23.8
	Very fine sand	7.4			Clay (< 0,002 mm)	7.6
	Fine silt	3.3				
	Clay	7.6			Sandy loam	
	Coarse silt	20.5				

$$\%\ \boldsymbol{Sand\ Fraction} = \frac{Sample\ weight}{Base\ mass} * 100$$

$$\%\ \boldsymbol{Fine\ Silt}\ \&\ Clay\ Fraction = \frac{fraction\ weight}{Base\ weight} * \frac{1000\ ml}{25\ ml\ pipette\ vol} * 100$$

APPENDIX II – Bulk Density

Bulk Density – Core Method (Blake and Hartge, 1986)

Introduction

Soil bulk density is the ratio of the mass of dry solids to the bulk volume of the soil. The bulk volume includes the volume of the solids and the pore space. The mass of a given soil is determined after drying to constant weight at 105 °C and the volume is that of the sample taken in the field. Bulk density is a widely used value. It is required for the conversion of water percentage by weigh to water content by volume.

Bulk density varies from soil to soil, with structural conditions of the soil, particularly that related to packing. With the core method, the core should be of the known volume. This method is usually unsatisfactory if more than an occasional stone is present in the soil.

Apparatus and Materials

1. Soil cores of the known volume
2. Disturbed soil samples
3. Oven dryer

Procedure

The exact procedure for obtaining the samples depends on the kind of samplers used. Drive or press the sampler into either a vertical or horizontal soil surface far enough to fill the sampler. Trim the soil extending beyond the end of the core. Transfer the soil sample into a container, place it in an oven at 105 °C until constant weight is reached, and then weigh it. Read and record the values. The bulk density of the soil is the oven-dry mass of the sample divided by the sample volume (volume of the sampler - core).

Raw Data

Bulk Density				
Core / Ring				
	Diameter (mm)	**height (mm)**	**vol core (CM3)**	
Sand	51	45	95.57964	
Loam	51	45	95.57964	
Clay	51	45	95.57964	
sample	**beaker wt (g)**	**wt after oven-dry (g)**	**wt of sample (g)**	**bulk density (g/cm3)**
sand(loamy sand)	58.062	177.8	119.738	1.25275634
sand(loamy sand)	58.091	178	119.909	1.254545424
sand(loamy sand)	57.999	177.1	119.101	1.246091741
loam (sandy loam)	59.587	186.516	126.929	1.327992028
loam (sandy loam)	59.581	186.512	126.931	1.328012953
loam (sandy loam)	59.6	186.521	126.921	1.327908329
clay (loam)	57.857	184.596	126.739	1.326004157
clay (loam)	57.856	184.59	126.734	1.325951845
clay (loam)	57.856	184.594	126.738	1.325993695

$$\textbf{Bulk density} = \frac{Weight\ of\ oven\ dry\ soil}{Volume\ of\ soil\ (solids\ and\ pores)}$$

APPENDIX III – Field Capacity

Field Capacity – Cassel and Nielsen 1986

Introduction

The soil serves as a leaky reservoir, that holds water that may be withdrawn by plants and or other processes such as redistribution. The amount of water that can be held in this condition varies from soil to soil and indeed, from horizon to horizon and is further related to pore size distribution of any given soil. The amount of water that is retained by this reservoir at the upper or full end is referred to as field capacity. The amount of water that is retained at the lower or dry end is the permanent wilting point. The difference between the two ends is the available water capacity.

Principles

The field capacity concept is defined as the amount of water held in the soil after the excess gravitational water has drained away and after the rate of downward movement of soil water has materially decreased. In more practical terms, field capacity is considered as the amount of water remaining in the soil 2 to 3 days after the irrigation episode or rainfall and after free drainage is negligible.

Procedure

The procedure of determining the field capacity of the soil used in the research was not carried out in the laboratory but was done right at the experimental site. Each and every individual pot filled with planting material (soil), especially of the desired texture, was saturated with water and was allowed to stand for 2 days. The pots were then weighed to determine the field capacity.

Raw Data

Sand (loamy sand)

Field Capacity						
Sample	**Initial wt (Kg)**	**wt after 48hrs (Kg)**	**Mass water (Kg)**	**Grav. cont. (kg /kg dry soil)**	**Vol. cont. (m3/m3)**	**Vol. cont. (cm3/cm3)**
SL1R1	7	8.43	1.43	0.2	0.26	25.61
SL1R2	7	8.49	1.49	0.21	0.27	26.67
SL1R3	7	8.47	1.47	0.21	0.26	26.32
SL1R4	7	8.53	1.53	0.22	0.27	27.27
SL2R1	7	8.35	1.35	0.19	0.24	24.13
SL2R2	7	8.49	1.49	0.21	0.27	26.54
SL2R3	7	8.49	1.49	0.21	0.27	26.66
SL2R4	7	8.5	1.5	0.21	0.27	26.84
SL3R1	7	8.48	1.48	0.21	0.27	26.51
SL3R2	7	8.57	1.57	0.22	0.28	27.96
SL3R3	7	8.53	1.53	0.22	0.27	27.29
SL3R4	7	8.51	1.51	0.22	0.27	26.96

Loam (sandy loam)

Field Capacity						
Sample	**Initial wt (Kg)**	**wt after 48hrs (Kg)**	**Mass water (Kg)**	**Grav. cont. (kg/kg dry soil)**	**Vol. cont. (m3/m3)**	**Vol. cont. (cm3/cm3)**
LL1R1	7	8.29	1.29	0.18	0.25	24.58
LL1R2	7	8.31	1.31	0.19	0.25	24.91
LL1R3	7	8.37	1.37	0.2	0.26	26.11
LL1R4	7	8.44	1.44	0.21	0.27	27.33
LL2R1	7	8.33	1.33	0.19	0.25	25.18
LL2R2	7	8.36	1.36	0.19	0.26	25.78
LL2R3	7	8.34	1.34	0.19	0.25	25.43
LL2R4	7	8.29	1.29	0.18	0.25	24.57
LL3R1	7	8.28	1.28	0.18	0.24	24.23
LL3R2	7	8.36	1.36	0.19	0.26	25.81
LL3R3	7	8.31	1.31	0.19	0.25	24.94
LL3R4	7	8.39	1.39	0.2	0.26	26.43

Clay (loam)

Field Capacity						
Sample	**Initial wt (Kg)**	**wt after 48hrs (Kg)**	**Mass water (Kg)**	**Grav. cont. (kg/kg dry soil)**	**Vol. cont. (m3/m3)**	**Vol.cont. (cm3/cm3)**
CL1R1	7	8.52	1.52	0.22	0.29	28.95
CL1R2	7	8.45	1.45	0.21	0.27	27.46
CL1R3	7	8.46	1.46	0.21	0.28	27.82
CL1R4	7	8.48	1.48	0.21	0.28	28.2
CL2R1	7	8.53	1.53	0.22	0.29	29.15
CL2R2	7	8.45	1.45	0.21	0.28	27.58
CL2R3	7	8.51	1.51	0.22	0.29	28.63
CL2R4	7	8.5	1.5	0.21	0.29	28.55
CL3R1	7	8.5	1.5	0.21	0.28	28.43
CL3R2	7	8.39	1.39	0.2	0.26	26.4
CL3R3	7	8.56	1.56	0.22	0.3	29.6
CL3R4	7	8.46	1.46	0.21	0.28	27.69

$$\boldsymbol{Gravimetric\ Water\ Content} = \frac{Wet\ weight - Dry\ weight}{Dry\ weight}$$

$$\boldsymbol{Volumetriv\ Water\ Content} = \frac{Wet\ weight - Dry\ weight}{Dry\ weight} * Bulk\ density$$

APPENDIX IV – Soil pH

Soil pH Determination in Water and KCl – (Thomas, 1996)

Introduction

Soil pH is almost certainly the single informative measurement that can be made to determine soil characteristics. At a single momentary look, soil pH tells more about the nature of the soil than merely indicating whether it is acidic or basic. For example, availability of essential nutrients and toxicity of other elements can be estimated because of their known relationship with soil pH.

Factors that influence soil pH include the effect of dilution *i.e.* the ratio of water to soil. In the suspension there is the effect of increasing pH as the ratio increases. Another major factor that influences pH in the soil solution is the salt content. Such salts may be a natural part of the soil such as NaCl, Na_2SO_4 or $Ca(NO_3)_2$ or they may be added anthropogenically during the application of fertilizer.

Principles

Hydrogen-ion activity in soil is measured potentiometrically in soil-water and or soil-KCl suspensions by using a 1:2.5 (w/v) ratio. The measurement of soil pH in water for hydrogen-ion activity or concentration in the soil solution is being done while the measurement of soil pH is taking place. The KCl solution determines the activity of hydrogen-ions both in the soil solution and on the exchange surfaces. A high hydrogen-ion activity (pH<7) indicates an acidic soil and a low hydrogen-ion activity (pH>7) indicates alkaline soil while pH =7 indicates a neutral soil.

Equipment and Reagents

1. Well-equipped and calibrated pH meter
2. 50 or 100ml beaker or plastic bottles
3. Pipette
4. Standard buffers, pH 7 and pH 4

5. Deionised water

6. 1M KCl solution

Procedure for determination of pH in Water

1. Weigh out 20 g of air-dry soil < 2mm and transfer to a 50ml or 100ml beaker or plastic bottle.

2. Add 50ml of deionised water to the soil in the beaker and shake well on a reciprocal shaker (150 r.p.m) for 30 minutes.

3. Allow the suspension to stand until the soil particles have settled before measuring the pH of the suspension with calibrated pH Meter.

4. Read pH and record as pH water.

5. Between the pH readings, rinse the electrodes with distilled water.

Procedure for determination of pH in 1 M KCl

1. Repeat steps 1 to 3 and 5 but use 1 M KCl instead of water to make the soil suspension.

2. Read pH and record as pH KCl

pH Adjustment using H_2SO_4

In order to achieve this goal, three trials were conducted using different dilution ratios and or molarities. Firstly, three samples – 50 g each, were drawn from each texture and then wetted with different dilutions of diluted H_2SO_4. The wetted soil sample was allowed to stand for two days in an air-drying condition to give it a chance for reaction. The samples were then analysed for pH in water and in KCl.

Raw Data

Soil pH		
Sample	**pH Water**	**pH KCl**
Sand	6.75	6.35
Loam	7.51	7.11
Clay	6.99	6.38

Soil pH	Adj 1		
Sample	**pH Water**	**pH KCL**	**Dilutions in Distilled water**
sand1	6.73	6.35	**0.2 ml H2SO4 = 2000ml Distilled water**
sand2	6.77	6.36	**0.2 ml H2SO4 = 5000ml Distilled water**
sand3	6.87	6.42	**0.2 ml H2SO4 = 7000ml Distilled water**
loam1	7.5	7.19	
loam2	7.52	7.2	
loam3	7.54	7.22	**Could be desired**
clay1	7.13	6.35	
clay2	7.2	6.38	
clay3	7.21	6.43	

Soil pH	Adj 2			
Sample	**pH water**	**pH KCl**		**Dilutions in Distilled water**
sand1	4.32	3.85		0.25M
sand2	4.4	4		0.125M
sand3	5.26	4.88		0.0625M
loam1	5.32	5.2		
loam2	5.64	5.47		**Could be desired**
loam3	6.33	6.12		
clay1	5.55	4.91		
clay2	6.03	5.8		
clay3	6.29	6.07		

Soil pH	Adj 3			
Sample	**pH water**	**pH KCl**		**Dilution into 1000ml distilled water**
sand1	6.65	6.27		**0.2 ml H2SO4**
sand2	6.1	5.79		**0.4 ml H2SO4**
sand3	5.63	5.2		**1.0 ml H2SO4**
sand4	5.14	4.75		**2.0 ml H2SO4**
sand5	4.65	4.23		**3.0 ml H2SO4**
loam1	7.5	7.14		
loam2	7.36	7.07		
loam3	7.16	6.86		
loam4	6.8	6.51		
loam5	6.6	6.38		**Could be desired**
clay1	6.95	6.25		
clay2	6.62	5.75		
clay3	6.36	5.45		
clay4	6.29	5.4		
clay5	6.28	5.43		

APPENDIX V – Antimicrobial Susceptibility

Antimicrobial susceptibility tests (Collins, Lyne and Grange 1995)

The major aim of this type of testing is to guide the clinician with regard to the selection of appropriate agents for therapy. These tests may be reported qualitatively as sensitive, intermediate or resistant or quantitatively in terms of the concentration of the agent that inhibits the growth of the organism – the Minimum Inhibitory Concentration (MIC). In MIC, the susceptibility of organisms to series of dilutions of the agent in agar is determined (Collins, Lyne and Grange, 1995:179).

Factors that affect susceptibility test

According to Collins, Lyne and Grange (1995:180), the most critical factors include the following:

- **Medium:** the medium should support the growth of the organisms normally tested and should not contain antagonists of antimicrobial activity. In cases where supplements are necessary for growth of some organisms, add 5% defibrinated blood of media for testing fastidious organisms such as streptococci.
- **pH**: the activity of aminoglycosides, macrolides, lincosamides and nitrofurantoin increases with increasing pH. Conversely, the activity of fusidic acid, tetracycline and novobiocin increases with decreasing pH.
- **Depth of the agar medium:** the plates should have a definite level depth of 3 – 4 mm that is 18 – 25 ml in a 9 cm petri dish.
- **Inoculation size:** increase in inoculum size reduces the susceptibility to agents in both the diffusion and dilution tests.
- **Pre-incubation and pre-diffusion:** in diffusion tests pre-incubation and pre-diffusion would decrease and increase the size of zones respectively.

- **Antimicrobial agents/ discs:** store the discs in a sealed container in the dark. The containers should include an indicating desiccant. Discs may be kept at < 8°C within the expiry date indicated by the maker.

- **Incubation:** incubate the plates at a temperature range of 35 – 37°C in air. Use alternative conditions only if essential growth of organism is desired. Incubation in an atmosphere containing additional CO_2 may reduce pH which in turn affects the activity of some agents.

- **Reading of results:** unless the test results are read by an automated system, the reading is somewhat subjective unless endpoints are clear.

Agar dilution methods

The agar dilution method for determining MICs has been recognised as the standard against which other methods are evaluated. Its advantage is that, contamination is easily identified and re-isolation of the organism is usually not a difficulty.

Preparation of stock solutions

Use analytical balance and weigh at least 100 mg of powder to ensure the accurate weighing of agents. With balances weigh to five decimal places. The amount of powder weighed may be reduced to 10 mg. Use the following formula to make allowance for the potency of the powder:

$$\boldsymbol{Weight\ of\ powder\ (mg)} = \frac{volume\ (ml) * Concentration\ \left({}^{mg}/_{l}\right)}{potency\ of\ powder\ \left({}^{\mu g}/_{mg}\right)}$$

And or the following:

$$\boldsymbol{Volume\ of\ diluent\ (ml)} = \ (weight\ (mg) * potency\ ((\mu g)\ /\ ml)/(concentration\ (mg\ /\ l)$$

Concentrations of stock solutions should be made up to 1000 mg/l or greater although the insolubility of some agents will prevent this. Sterile water should always be used to dilute and dissolve the agents.

Preparation of the working solution: Twenty millimetre volume units of agar are commonly used in 9 cm petri dishes for agar dilution MICs. Two dilution schemes could be engaged. For example, 19 ml volumes of molten agar are added to 1 ml volume of antimicrobial solution.

Preparation of plates

The agar should be prepared according to the recommendation by the manufacturer. In procedure allow the sterilised agar to cool to 50°C in a water bath. Prepare a dilution series of antimicrobial agents in 1 ml volumes, 20 X final the concentration in 25 ml screw-capped bottles. Include a drug-free control. Add 19 ml molten agar to each bottle, mix thoroughly and pour the agar into pre-labelled sterile petri dishes on a level surface.

Inoculation of plates

The plates should be marked so that the orientation would be obvious. Then, transfer diluted bacterial suspensions to the inoculum wells of an inoculum replicating apparatus. Use the provided apparatus to transfer the inocula to the series of agar plates. Inoculum pins 2.5 mm in diameter will transfer close to 1 µl.

Incubation of plates

Incubate the plates at 35 – 37 °C in air for 18 hours. To avoid uneven heating, do not stack them more than four high. If the incubation period is extended for slow growing organisms, assess the stability of the agent over the incubation period. Avoid incubating in an atmosphere containing 5% CO_2 unless absolutely necessary for the growth of the organisms.

Reading results

The MIC is the lowest concentration of the agent that completely inhibits the growth, disregarding up to four colonies or a thin haze in the incubated spot. Tolerance is the ability of strains to survive but not grow in the presence of an agent and relates particularly to Gram-positive organisms tested against agents acting on the terminal

stags of peptidoglycan synthesis. Strains with an MIC ratio of 32 or greater after 24 hrs of incubation are usually termed tolerant. However, MICs are determined after fixed periods of incubation and tolerance may be a reflection of a lower rate of killing.

APPENDIX VI – Data Collection Checklist

General Information: Date: _____ Time: _____ Temperature: _____ Humidity: _____					
Data Collector: Researcher | | Assistant | |					
Treatme	Rep	Initial Pot	Wt prior to	Amount of water	Days between
1 (Level 1)	1				
	2				
	3				
	4				
2 (Level 2)	1				
	2				
	3				
	4				
3 (Level 3)	1				
	2				
	3				
	4				
Observations:			Action taken:		Solutions:

APPENDIX VII – Water Management: Average % depletion ($mm.mm^{-1}$) L = Loam, SL = Sandy loam and LS = Loamy sand

Date/ Time	L 0%	L 50%	L 90%	SL0%	SL50%	SL90%	LS0%	LS50%	LS90%
31/10/2010 08:00	0.280	0.284	0.279	0.257	0.252	0.253	0.265	0.261	0.272
01/11/2010 08:00	0.200	0.193	0.180	0.166	0.163	0.148	0.276	0.185	0.171
01/11/2010 08:30	0.280	0.177	0.164	0.257	0.149	0.135	0.265	0.169	0.155
02/11/2010 08:00	0.233	0.161	0.147	0.210	0.135	0.122	0.227	0.154	0.138
02/11/2010 08:30	0.280	0.284	0.131	0.257	0.252	0.111	0.265	0.261	0.123
03/11/2010 08:00	0.226	0.225	0.116	0.207	0.199	0.100	0.221	0.223	0.109
03/11/2010 08:30	0.280	0.213	0.107	0.257	0.189	0.253	0.265	0.212	0.272
04/11/2010 08:00	0.236	0.201	0.098	0.213	0.180	0.205	0.230	0.200	0.238
04/11/2010 08:30	0.280	0.191	0.279	0.257	0.172	0.197	0.265	0.191	0.228
05/11/2010 08:00	0.242	0.181	0.245	0.219	0.165	0.189	0.236	0.181	0.217
05/11/2010 08:30	0.280	0.284	0.229	0.257	0.250	0.177	0.265	0.261	0.200
06/11/2010 08:00	0.231	0.228	0.213	0.212	0.201	0.166	0.227	0.221	0.184
06/11/2010 08:30	0.280	0.208	0.194	0.257	0.188	0.152	0.265	0.202	0.164
07/11/2010 08:00	0.222	0.189	0.174	0.206	0.176	0.139	0.216	0.183	0.144
07/11/2010 08:30	0.280	0.172	0.157	0.257	0.162	0.128	0.265	0.167	0.130
08/11/2010 08:00	0.229	0.156	0.141	0.208	0.148	0.117	0.222	0.152	0.115
08/11/2010 08:30	0.280	0.284	0.136	0.257	0.252	0.114	0.265	0.261	0.110
09/11/2010 08:00	0.251	0.243	0.132	0.225	0.212	0.110	0.246	0.243	0.106
09/11/2010 08:30	0.280	0.229	0.120	0.257	0.201	0.253	0.265	0.229	0.272
10/11/2010 08:00	0.234	0.215	0.109	0.214	0.190	0.200	0.226	0.214	0.230
10/11/2010 08:30	0.280	0.195	0.098	0.257	0.175	0.186	0.265	0.194	0.208
11/11/2010 08:00	0.217	0.175	0.087	0.204	0.161	0.172	0.210	0.174	0.186
11/11/2010 08:30	0.280	0.284	0.279	0.257	0.252	0.165	0.265	0.261	0.176
12/11/2010 08:00	0.240	0.237	0.247	0.218	0.205	0.159	0.235	0.229	0.165
12/11/2010 08:30	0.280	0.225	0.235	0.257	0.197	0.150	0.265	0.216	0.153
13/11/2010 08:00	0.237	0.214	0.224	0.216	0.188	0.142	0.228	0.204	0.140
13/11/2010 08:30	0.280	0.196	0.207	0.257	0.176	0.131	0.265	0.185	0.124
14/11/2010 08:00	0.225	0.177	0.190	0.209	0.164	0.120	0.214	0.167	0.107
14/11/2010 08:30	0.280	0.284	0.169	0.257	0.252	0.108	0.265	0.261	0.091
15/11/2010 08:00	0.214	0.213	0.147	0.199	0.187	0.096	0.198	0.200	0.074
15/11/2010 08:30	0.280	0.191	0.130	0.257	0.172	0.253	0.265	0.176	0.272
16/11/2010 08:00	0.214	0.169	0.113	0.197	0.157	0.192	0.201	0.152	0.215
16/11/2010 08:30	0.280	0.284	0.100	0.257	0.252	0.174	0.265	0.261	0.187
17/11/2010 08:00	0.200	0.202	0.088	0.186	0.175	0.156	0.188	0.194	0.159
17/11/2010 08:30	0.280	0.179	0.279	0.257	0.159	0.141	0.265	0.167	0.135
18/11/2010 08:00	0.202	0.155	0.218	0.186	0.142	0.125	0.188	0.140	0.110
18/11/2010 08:30	0.280	0.284	0.179	0.257	0.252	0.104	0.265	0.261	0.272
20/11/2010 08:00	0.155	0.167	0.139	0.154	0.149	0.083	0.122	0.141	0.159
20/11/2010 08:30	0.280	0.284	0.279	0.257	0.252	0.253	0.265	0.261	0.272
21/11/2010 08:00	0.239	0.238	0.239	0.221	0.225	0.225	0.221	0.220	0.232
21/11/2010 08:30	0.280	0.221	0.223	0.257	0.210	0.209	0.265	0.201	0.212
22/11/2010 08:00	0.233	0.205	0.206	0.212	0.196	0.194	0.215	0.181	0.192
22/11/2010 08:30	0.280	0.170	0.172	0.257	0.167	0.162	0.265	0.142	0.150
23/11/2010 08:00	0.184	0.136	0.138	0.170	0.138	0.130	0.146	0.102	0.108

23/11/2010 08:30	0.280	0.284	0.119	0.257	0.252	0.113	0.265	0.261	0.272
24/11/2010 08:00	0.197	0.202	0.100	0.178	0.181	0.096	0.161	0.170	0.183
24/11/2010 08:30	0.280	0.187	0.094	0.257	0.170	0.253	0.265	0.261	0.166
25/11/2010 08:00	0.237	0.172	0.089	0.210	0.158	0.216	0.216	0.214	0.149
25/11/2010 08:30	0.280	0.284	0.279	0.257	0.252	0.188	0.265	0.173	0.119
26/11/2010 08:00	0.192	0.194	0.212	0.176	0.173	0.160	0.158	0.132	0.089
26/11/2010 08:30	0.280	0.165	0.185	0.257	0.252	0.138	0.265	0.261	0.272
27/11/2010 08:00	0.195	0.136	0.158	0.177	0.179	0.116	0.176	0.180	0.198
27/11/2010 08:30	0.280	0.284	0.147	0.257	0.169	0.108	0.265	0.261	0.182
28/11/2010 08:00	0.231	0.231	0.136	0.208	0.160	0.100	0.219	0.213	0.166

Weather Conditions

Date Nov	Average Time (hours)	Temperature (°C)	Humidity (%)
1st	09h00	23	45
2nd	09h00	18	86
3rd	09h00	17	81
4th	09h00	16	84
5th	09h00	15	64
6th	09h00	14	85
7th	09h00	19	48
8th	09h00	17	68
9th	09h00	15	94
10th	09h00	17	63
11th	09h00	16	70
12th	09h00	16	81
13th	09h00	17	74
14th	09h00	22	40
15th	09h00	28	42
16th	09h00	23	61
17th	09h00	22	53
18th	09h00	27	41
19th	09h00	18	62
20th	09h00	18	69
21st	09h00	18	77
22nd	09h00	16	58
23rd	09h00	19	42
24th	09h00	22	33
25th	09h00	24	44
26th	09h00	24	52
27th	09h00	16	83
28th	09h00	16	67

APPENDIX VIII LAI – Growth rate measured as total leaf area: an ellipse formulae was used to calculate total leaf area.

	Week a	Week b	Week c	Week d	Week a	Week b	Week c	Week d
Smpl	Tot Area cm^2	Tot Area cm^2	Tot Area cm^2	Tot Area cm^2	LAI	LAI	LAI	LAI
CL1R1	27.04	46.33	62.07	89.13	0.060	0.103	0.137	0.197
CL1R2	33.39	71.07	95.18	122.10	0.074	0.157	0.211	0.270
CL2R1	134.53	296.64	605.06	1051.62	0.298	0.656	1.339	2.327
CL2R2	79.15	208.34	376.60	677.20	0.175	0.461	0.833	1.498
CL3R1	63.65	159.38	365.28	692.28	0.141	0.353	0.808	1.532
CL3R2	80.03	191.28	382.38	825.38	0.177	0.423	0.846	1.826
SL1R1	26.40	49.76	76.76	125.51	0.058	0.110	0.170	0.278
SL1R2	47.83	79.07	133.88	207.42	0.106	0.175	0.296	0.459
SL2R1	48.11	235.42	252.32	663.78	0.106	0.521	0.558	1.469
SL2R2	97.53	172.02	558.00	905.27	0.216	0.381	1.235	2.003
SL3R1	79.14	245.04	548.50	1167.70	0.175	0.542	1.213	2.583
SL3R2	74.51	174.92	407.78	831.38	0.165	0.387	0.902	1.839
LL1R1	16.03	18.52	23.88	31.86	0.035	0.041	0.053	0.070
LL1R2	59.52	105.52	178.94	249.20	0.132	0.233	0.396	0.551
LL2R1	47.92	118.86	243.44	480.28	0.106	0.263	0.539	1.063
LL2R2	77.21	149.20	295.72	517.88	0.171	0.330	0.654	1.146
LL3R1	122.03	339.66	597.90	1141.96	0.270	0.751	1.323	2.526
LL3R2	71.64	160.50	325.54	719.90	0.158	0.355	0.720	1.593

APPENDIX IX Statistical Analysis

ANOVA wet leaves and Stems (biomass and soil texture)

TEXTURE; LS Means (DATA Biomass and Texture May2011)
Current effect: F(2, 33)=81.321, p=.00000
Effective hypothesis decomposition

Cell No.	TEXTURE	Wet Leaf Weight (g) Mean	Wet Leaf Weight (g) Std.Err.	Wet Leaf Weight (g) -95.00%	Wet Leaf Weight (g) +95.00%	N
1	LOAM	6.08917	0.798244	4.46513	7.71321	12
2	SANDY LOAM	2.40500	0.798244	0.78096	4.02904	12
3	LOAMY SAND	16.30000	0.798244	14.67596	17.92404	12

Bonferroni test; variable Wet Leaf Weight (g) (DATA Biomass and Texture May2011)
Probabilities for Post Hoc Tests
Error: Between MS = 7.6463, df = 33.000

Cell No.	TEXTURE	{1} 6.0892	{2} 2.4050	{3} 16.300
1	LOAM		0.007690	0.000000
2	SANDY LOAM	0.007690		0.000000
3	LOAMY SAND	0.000000	0.000000	

TEXTURE; LS Means (DATA Biomass and Texture May2011)
Current effect: F(2, 33)=63.493, p=.00000
Effective hypothesis decomposition

Cell No.	TEXTURE	Wet Stem Weight (g) Mean	Wet Stem Weight (g) Std.Err.	Wet Stem Weight (g) -95.00%	Wet Stem Weight (g) +95.00%	N
1	LOAM	3.72083	0.926352	1.83616	5.60551	12
2	SANDY LOAM	1.03833	0.926352	-0.84634	2.92301	12
3	LOAMY SAND	14.95167	0.926352	13.06699	16.83634	12

Bonferroni test; variable Wet Stem Weight (g) (DATA Biomass and Texture May2011)
Probabilities for Post Hoc Tests
Error: Between MS = 10.298, df = 33.000

Cell No.	TEXTURE	{1} 3.7208	{2} 1.0383	{3} 14.952
1	LOAM		0.145875	0.000000
2	SANDY LOAM	0.145875		0.000000
3	LOAMY SAND	0.000000	0.000000	

ANOVA wet roots and LAI (biomass and soil texture)

TEXTURE; LS Means (DATA Biomass and Texture May2011)
Current effect: F(2, 33)=47.948, p=.00000
Effective hypothesis decomposition

Cell No.	TEXTURE	Wet Roots Weight (g) Mean	Wet Roots Weight (g) Std.Err.	Wet Roots Weight (g) -95.00%	Wet Roots Weight (g) +95.00%	N
1	LOAM	3.282500	0.620932	2.019204	4.54580	12
2	SANDY LOAM	0.631833	0.620932	-0.631462	1.89513	12
3	LOAMY SAND	9.041667	0.620932	7.778371	10.30496	12

Bonferroni test; variable Wet Roots Weight (g) (DATA Biomass and Texture May2011)
Probabilities for Post Hoc Tests
Error: Between MS = 4.6267, df = 33.000

Cell No.	TEXTURE	{1} 3.2825	{2} .63183	{3} 9.0417
1	LOAM		0.014608	0.000001
2	SANDY LOAM	0.014608		0.000000
3	LOAMY SAND	0.000001	0.000000	

TEXTURE; LS Means (DATA Biomass and Texture May2011)
Current effect: F(2, 33)=46.374, p=.00000
Effective hypothesis decomposition

Cell No.	TEXTURE	Leaf Area Index Mean	Leaf Area Index Std.Err.	Leaf Area Index -95.00%	Leaf Area Index +95.00%	N
1	LOAM	0.577629	0.102391	0.369312	0.785946	12
2	SANDY LOAM	0.197629	0.102391	-0.010688	0.405946	12
3	LOAMY SAND	1.549631	0.102391	1.341315	1.757948	12

Bonferroni test; variable Leaf Area Index (DATA Biomass and Texture May2011)
Probabilities for Post Hoc Tests
Error: Between MS = .12581, df = 33.000

Cell No.	TEXTURE	{1} .57763	{2} .19763	{3} 1.5496
1	LOAM		0.039154	0.000000
2	SANDY LOAM	0.039154		0.000000
3	LOAMY SAND	0.000000	0.000000	

ANOVA interaction of soil texture and fertilizer on LAI

Texture; LS Means (DATA Biomass and Texture August 2011)
Current effect: F(2, 33)=.57261, p=.56956
Effective hypothesis decomposition

Cell No.	Texture	Leaf Area lindex Mean	Leaf Area lindex Std.Err.	Leaf Area lindex -95.00%	Leaf Area lindex +95.00%	N
1	Loam	1.472716	0.331520	0.798233	2.147198	12
2	Sandy loam	1.392815	0.331520	0.718333	2.067298	12
3	Loamy sand	1.861729	0.331520	1.187247	2.536212	12

Bonferroni test; variable Leaf Area lindex (DATA Biomass and Texture August 2011)
Probabilities for Post Hoc Tests
Error: Between MS = 1.3189, df = 33.000

Cell No.	Texture	{1} 1.4727	{2} 1.3928	{3} 1.8617
1	Loam		1.000000	1.000000
2	Sandy loam	1.000000		0.973535
3	Loamy sand	1.000000	0.973535	

ANOVA wet leaves and Stems (biomass and fertilizer)

Fertilizer N Rate; LS Means (DATA Biomass and Fertilizer N August 2011)
Current effect: F(2, 33)=45.867, p=.00000
Effective hypothesis decomposition

Fertilizer N Rate	Wet Leaf Weight (g) Mean	Wet Leaf Weight (g) Std.Err.	Wet Leaf Weight (g) -95.00%	Wet Leaf Weight (g) +95.00%	N
0 Kg/ha N Fertilizer	1.99917	1.255090	-0.55433	4.55267	12
150 Kg/ha N Fertilizer	12.36667	1.255090	9.81317	14.92017	12
250 Kg/ha N Fertilizer	18.85083	1.255090	16.29733	21.40433	12

Bonferroni test; variable Wet Leaf Weight (g) (DATA Biomass and Fertilizer N August 2011)
Probabilities for Post Hoc Tests
Error: Between MS = 18.903, df = 33.000

Cell No.	Fertilizer N Rate	{1} 1.9992	{2} 12.367	{3} 18.851
1	0 Kg/ha N Fertilizer		0.000005	0.000000
2	150 Kg/ha N Fertilizer	0.000005		0.002670
3	250 Kg/ha N Fertilizer	0.000000	0.002670	

Fertilizer N Rate; LS Means (DATA Biomass and Fertilizer N August 2011)
Current effect: F(2, 33)=31.787, p=.00000
Effective hypothesis decomposition

Fertilizer N Rate	Wet Stem Weight (g) Mean	Wet Stem Weight (g) Std.Err.	Wet Stem Weight (g) -95.00%	Wet Stem Weight (g) +95.00%	N
0 Kg/ha N Fertilizer	0.84667	1.375432	-1.95167	3.64500	12
150 Kg/ha N Fertilizer	9.06583	1.375432	6.26750	11.86417	12
250 Kg/ha N Fertilizer	16.34667	1.375432	13.54833	19.14500	12

Bonferroni test; variable Wet Stem Weight (g) (DATA Biomass and Fertilizer N August 2011)
Probabilities for Post Hoc Tests
Error: Between MS = 22.702, df = 33.000

Cell No.	Fertilizer N Rate	{1} .84667	{2} 9.0658	{3} 16.347
1	0 Kg/ha N Fertilizer		0.000530	0.000000
2	150 Kg/ha N Fertilizer	0.000530		0.002080
3	250 Kg/ha N Fertilizer	0.000000	0.002080	

ANOVA wet roots and LAI (biomass and fertilizer)

Fertilizer N Rate; LS Means (DATA Biomass and Fertilizer N August 2011)
Current effect: F(2, 33)=9.0102, p=.00075
Effective hypothesis decomposition

Cell No.	Fertilizer N Rate	Wet Roots Weight (g) Mean	Wet Roots Weight (g) Std.Err.	Wet Roots Weight (g) -95.00%	Wet Roots Weight (g) +95.00%	N
1	0 Kg/ha N Fertilizer	0.690000	0.717930	-0.770640	2.150640	12
2	150 Kg/ha N Fertilizer	3.682500	0.717930	2.221860	5.143140	12
3	250 Kg/ha N Fertilizer	4.872500	0.717930	3.411860	6.333140	12

Bonferroni test; variable Wet Roots Weight (g) (DATA Biomass and Fertilizer N August 2011)
Probabilities for Post Hoc Tests
Error: Between MS = 6.1851, df = 33.000

Fertilizer N Rate	{1} .69000	{2} 3.6825	{3} 4.8725
0 Kg/ha N Fertilizer		0.017531	0.000718
150 Kg/ha N Fertilizer	0.017531		0.748697
250 Kg/ha N Fertilizer	0.000718	0.748697	

Fertilizer N Rate; LS Means (DATA Biomass and Fertilizer N August 2011)
Current effect: F(2, 33)=47.081, p=.00000
Effective hypothesis decomposition

Cell No.	Fertilizer N Rate	Leaf Area lindex Mean	Leaf Area lindex Std.Err.	Leaf Area lindex -95.00%	Leaf Area lindex +95.00%	N
1	0 Kg/ha N Fertilizer	0.301840	0.171790	-0.047669	0.651349	12
2	150 Kg/ha N Fertilizer	1.797635	0.171790	1.448126	2.147143	12
3	250 Kg/ha N Fertilizer	2.627786	0.171790	2.278277	2.977295	12

Bonferroni test; variable Leaf Area lindex (DATA Biomass and Fertilizer N August 2011)
Probabilities for Post Hoc Tests
Error: Between MS = .35414, df = 33.000

Fertilizer N Rate	{1} .30184	{2} 1.7976	{3} 2.6278
0 Kg/ha N Fertilizer		0.000002	0.000000
150 Kg/ha N Fertilizer	0.000002		0.005095
250 Kg/ha N Fertilizer	0.000000	0.005095	