USE OF MAGNETIC FIELD NUTRIENT SOLUTION AMENDED WITH MYCORRHIZAL AND RATIO OF CALCIUM TO MAGNESIUM ON YIELD AND OIL QUALITY OF ROSE GERANIUM (*Pelargonium graveolens* L.)

By

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DECLARATION WITH REGARD

TO INDEPENDENT WORK

I, **Neo Edwin Nyakane**, student number , do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree MASTER OF AGRICULTURE, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

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ABSTRACT

Holistic improvements of oil yield and quality could be realised through integrating the knowledge of mycorrhizae and magnetic field stimulation. The main objective of this study was to determine the effect of Ca:Mg ratios and the inclusion of mycorrhizal fungi in the root media on the yield and oil quality of rose geranium exposed to static magnetic field (facing south). The experiment was structured as a 3×2 factorial experimental design, with three levels of Ca:Mg ratios (2.40:6.78, 4.31:4.39, and 6.78:2.40 meq L^{-1}), two levels of MF (zero MF, denoted by 0MF, and 110.1 mT, denoted by 1MF), and two levels of mycorrhizae amendment (none, denoted by 0Myco, and 20 ml of mycorrhizae amendment per plant, denoted by 1Myco) as a split-plot factor. The results showed that the application of Ca and Mg in approximately equal proportions of 4.31 and 4.39 meq L^{-1} had significant effects on leaf Zn and stem Mg content. The application of Ca and Mg in equal proportions and in combination with either an MF or mycorrhizae had significant effects on plant height, chlorophyll content, stem N, stem Cu, and stem Zn contents. Furthermore, high Ca and low Mg (6.78:2.40 meq L^{-1}) in the nutrient solution significantly reduced the concentration of Mg in the leaves. On the other hand, a ratio of high Ca to low Mg (6.78:2.40 meq L^{-1}) in the nutrient solution caused a small increase in the Zn concentration in comparison to that resulting from a ratio of high Mg to low Ca (6.78:2.40 meq L^{-1}) in the nutrient solution. Although plant height and the number of leaves per plant were affected by equal proportions of Mg and Ca with 1MF and mycorrhizae use, this effect did not increase the oil yield and C:G ratio which favour essential oil of rose geranium, but the oil quality was within the acceptable international range for perfume industry. A multivariate analysis was further used to determine eigenvalue of the correlation matrix. Amongst the observed variables, only two principal components, PC1 (27.23%) and PC2 (23.78%) accounted for more than half of the total variance. Multivariate analysis revealed the antagonistic and synergetic effects between mineral elements subjected to Ca to Mg ratio, MF and mycorrhizal fungi.

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CHAPTER 1

1.1 MOTIVATION AND BACKGROUND OF THE STUDY

Rose geranium (*Pelargonium graveolens* L.) is an essential oil crop that belongs to the family *Geraniacea* (Gebremeskel, 2014). *Pelargonium* species are endemic to South Africa and are predominantly confined to the Western Cape and Limpopo provinces of the South Africa (Sedibe, 2012). However, the major producing provinces in South Africa are Mpumalanga, KwaZulu-Natal, and Eastern Cape (DAFF, 2017). According to Motsa (2006) the first *Pelargonium* material was harvested from the wild but, it was off late, domesticated and cultivated and used for the production of essential oil.

Subsequently, the harvested material of this crop in South Africa was introduced to other countries such as Britain and Holland through spice trade and medicinal plant collectors (Khetsha, 2013). Currently, the most leading geranium oil producing countries are China, Egypt, Algeria, Morocco, Madagascar, South Africa and Réunion Island (DAFF, 2017). Nevertheless, the oil extracted from the crop is an income earner for South Africa through exporting of the oil to other countries such as the USA, Japan, and several countries in Europe with the estimated income between R60 and R100 million (DAFF, 2011).

The essential oil of *P. graveolens* is mainly used for its essence and its therapeutic or odoriferous properties in perfumes, cosmetics, pharmaceuticals and aromatherapy (Sedibe & Allemann, 2012). The oil is also useful in the food, beverage and soft drink industries (Gebremeskel, 2014; DAFF, 2012; Sedibe, 2012; Rajeswara *et al*., 1996). Rose geranium oil has also been found to have use as the main component of skin care products that are predominantly used by women to cleanse and exfoliate dead skin cells (Gebremeskel, 2014). However, South African producers of rose geranium oil are battling in producing oil of good quality (Motsa 2006). This is due to the fact that cultivation conditions in South Africa are not always favourable for the production of high-quality oil (Sedibe & Allemann, 2012).

Using an improved production practices and technologies enhances crop growth (Shine & Guruprasad, 2012; Shine *et al.*, 2011a; 2011b; Vashisth & Nagarajan, 2010) and these techniques mitigate the effect of poor growing conditions (Khetsha, 2013; Sedibe, 2012). Example of improved production practices is crop stimulation, which is complementary to crop nutrition. These stimulants operate through different mechanisms from that of fertilizers because they only influence plant vigour (Yakhin *et al.*, 2017; Du Jardin, 2015; Sharma *et al.*, 2014).

Lately, the use of magnetic field (MF) and mycorrhizae in crop production caught attention as an enhancer and a relatively cheap way of improving yield, growth and seed germination of sweet pepper (*Capsicum annuum* L.) (Ahamed *et al.*, 2013). The MF is a physical field generated from a piece of iron or other material that has ability to run an electrical current through a wire or similar device that conduct electrons (Kocaman, 2014; Leo *et al.*, 2013; Habte, 2000). The effect of MF on living organisms depends on the size and nature of exposure and it affects plant cells and tissues (Belyavskaya, 2004; Occhipinti, 2000).

There is conflicting information on the target components of MF exposure in the biological system of plants. However, Ca ions constitute a possible target for transmission of MF (Pazur & Rassadina, 2009; Baureus *et al.*, 2003; Gartzke, & Lange, 2002). In particular, it was documented that a static MF can improve the transportation of Ca across the cell membrane and, as a consequence, adjust pollen germination (Germanà *et al.*, 2003).

Both Ca and Mg are essential plant nutrient elements after N, P and K required by plants in modest amounts. A deficiency of these elements in crops occurs in three ways i) due to under supply, ii) oversupply iii) antagonistic effect that these elements have on one another (Nzanza, 2006). The uptake of K, Ca and Mg is affected by the over supplying one or more cations (K, Ca and Mg) as it interferes and compete with each other's uptake (El-Dissoky, 2017; Maria, 2017; Ertiftik, & Zengin, 2016). According to Chang-Tsern *et al.* (2018) inadequate supply of K reduces the node number, plant height and leaf area, whereas small chlorotic specks and marginal necrosis were observed on the lower part of the *Eustoma* plant. On the other hand,

Hao & Papadopoulos (2003) reported that Ca deficiency causes a decline in growth of merismatic tissue, reduces leaf size, yield and causes necrosis of young leaves in extreme cases. In some instances, volatile oil content is not entirely dependent on the application of Ca and Mg (Dordas, 2009). For instance, high application of calcium carbonate in sweet basil contributed to decreased oil yield. However, linalool and methyl chavicol level were increased by calcium (Dzida, 2010). Carvacrol content of saturn (*Satureja hortensis* L.) was enhanced by calcium carbonate (Mumivand *et al.*, 2011). Lack of Mg may seriously affect the production and supply of photo assimilates to other parts of the plant (Hao & Papadopoulos, 2003; Sonneveld & Voogt, 1991).

Mycorrhizal fungi amendments ameliorate plant yield due to its ability to increase water and nutrient uptake (Gamal *et al*., 2016; Pharudi, 2010). According to Azcon-Aguilar & Barea (1997) soil microbe's activity is affected by rhizosphere conditions, including the level of salinity, moisture content and fertility. Mycorrhizal fungi tend to increase the effective absorptive area of roots by forming an extensive extra-radical hyphae network that enhances efficiency in the absorption of nutrients (Abdel-Rahman *et al*., 2011). This hypha grows in close association with plant root hair and plays an important symbiotic role in the uptake and transfer of water and nutrients by the root system. In exchange, the plant supplies the fungal organism with carbon compound (Farahani *et al*., 2008).

1.2 PROBLEM STATEMENT

Growers in South Africa are facing challenges of producing rose geranium oil that meets international standards. This scenario hampers competitiveness of these growers against other global role players such as China, Egypt, Morocco, Crimea, Ukraine, Georgia and India. These demands are difficult to meet due to inconsistent climatic condition and inadequate nutrient supply (Sedibe, 2012). Most notably, poor development in plants and the amount of oil produced by rose geranium may be affected by the antagonistic effect of Ca and Mg ratios and the impairment of polarity due to the lack of MF gradients.

1.3 HYPOTHESIS

Since it is conceivable that plants sense different light wavelengths, respond to gravity, react to touch and electrical signalling; it is, therefore, hypothesised that plants exposed to MF amended with Ca and Mg ratios and mycorrhizae may enhance yield and oil composition of rose geranium. Mycorrhizae can then be the important root fungus used to enhance nutrient uptake, plant growth and yield, as it also increases the resilience of the plant. This enables the plant to strive under severe conditions, such as high salinity, droughts and low fertility supply (Azcon-Aguilar & Barea, 1997). These above observations and succinct discussion clearly show that more research need to be conducted on rose geranium to study the effects Ca and Mg ratios, mycorrhizal fungi and different polarities and strengths of magnetic fields on the yield and quality of oil.

1.4 OBJECTIVE

The main objective of this study was to determine the effect of Ca and Mg ratios and the inclusion of mycorrhizal fungi in the root media on the yield and oil quality of rose geranium exposed to magnetic fields. Therefore, the specific objectives of this study are:

- To study the effect of different levels of Ca:Mg ratios, MF and mycorrhizal fungi on oil yield and quality of rose geranium;
- To study the effect of Ca:Mg ratios, MF and mycorrhizal fungi on mineral utilisation by rose geranium and
- To evaluate the antagonistic or synergetic relationship between mineral and agronomic attributes affected by Ca:Mg ratios and MF, amendment with mycorrhizal fungi.

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CHAPTER 2

LITERATURE REVIEW

This chapter covers: topics on calcium and magnesium`s functions in plants, their uptake from the soil by the roots, movement through the membrane and the effects of their deficiencies. Interaction between calcium and magnesium will be part of the review while the importance of mycorrhizal fungi and the magnetic field in crop production will also be discussed.

Calcium and magnesium are secondary macronutrient elements required to maintain plant health. These macro elements play a vital role in various metabolic processes of plants and, are required in a relatively large quantity for the development of a healthy plant (Tripathi *et al*., 2014). Generally, application of these two elements increases growth, yield and quality of crops (Morgan & Connolly, 2013; Mikkelsen, 2010). The most common sources of Ca are liming materials, mainly $CaCO_3$, (Ca $(H_2PO_4)_2$ H_2O, (Ca $(NO_3)_2$), ($CaCl_2$) and (CaS_2O_3). On the other hand, Ca is mostly applied as gypsum to improve soil chemical (Jaime & Judith 2014; Huang *et al*., 1993). Whereas, the most common source of Mg is $MgCO_3$. $CaCO_3$, MgO. CaO/MgO. $Ca(OH)_2$, $MgCl_2$, $Mg(NO_3)_2$. 6H2, MgO and $MgSO_4$. $7H_2O$ (Mikkelsen, 2010).

2.1 CALCIUM AND MAGNESIUM FORMS AND FUNCTIONS

2.1.1 Calcium

Calcium is part of every plant cell. It is one of the most essential nutrient elements after nitrogen, phosphorus and potassium in crop production. Calcium does not only play an important role in its organic form (Ca) as a structural component of the cell wall and cell membrane. It also plays a fundamental role as a cytoplasmic second messenger, meditating several hormonal and environmental cues that aggravate appropriate physiological response and as a counter cation for inorganic and organic anions in the vacuole (Supanjani *et al*., 2005).

Although indirectly, in its function as the second messenger, it promotes pollen germination and growth; activating several enzymes for cell mitosis, division, and elongation; possibly

detoxify the presence of heavy metals in plant tissue (Motsa, 2006; Nzanza, 2006; Roberts & Harmon 1992). However, its role is elusive because it is also involved in numerous cellular functions that are regulated in plant cells by changes in cytosolic Ca concentrations (Bush, 1995). These include ionic balance, gene expression, and carbohydrate metabolism (Hao & Papadopoulos, 2003).

According to the citations reviewed the total Ca in a healthy plant tissue normally range between 0.1-1 percent of dry matter (White, 2000). This high Ca concentration is a result of high Ca levels in the soil solution rather than from the efficiency of Ca uptake by root cells (Dordas, 2009; White & Broadley, 2003). Furthermore, many reports from the literature show that in crops, Ca deficiency has a fundamental effect on yield, even without vegetative signs of deficiency. This can also be observed when Ca concentration is in low, supply, it causes a reduction in the growth of the meristematic tissues and the youngest leaves become deformed and chlorotic (Dordas, 2009; Prasand *et al*., 2008; Brennan *et al*., 2007; Murillo-Amador *et al*., 2006).

Slak & Morrill (1972) have shown that, adequate Ca content in the soil leads to increased yield, high oil and protein content of kernel in peanuts. Gashti *et al*. (2012) also stated that Ca increases the growth and survival of the symbiotic bacteria in peanut, especially in acidic soil and thus, has a positive effect on nitrogen fixation. They further stated that the presence of adequate Ca content in the soil leads to the prevention of black hallow and cracked pods. Their results also revealed that optimum Ca content decreases the chances of having aflatoxin problems, the production of immature pods, black embryo in seed and weak germination of seeds.

2.1.2 Calcium uptake from the soil to the roots

Plants take up calcium in the ionized form (Ca^{2+}) from the soil solution that is in contact with the root surface. This is determined by nutrient interception during root growth, mass flow and diffusion of nutrients towards the root surface (Rajni *et al*., 2014).

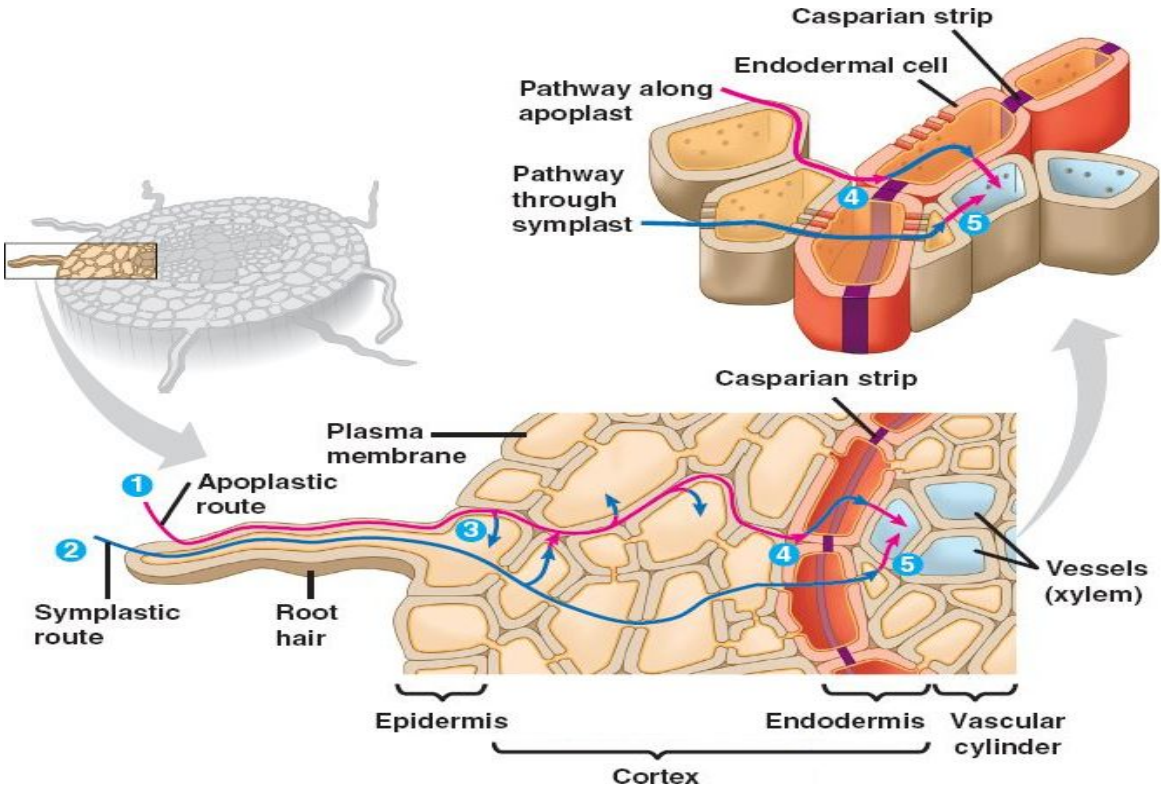

Figure 2.4 *Schematic representation of calcium transport through symplastic route and apoplastic route across the root (White, 1998).*

Calcium solutes move across the root cortex either by diffusion or more likely by displacement exchange in the free space (Figure 2.1) (Modisane, 2007; White, 1998). Calcium enters the root apoplast along with mass flow of water (Barber, 1995). It then follows apoplastic or symplastic pathways to the xylem where the exchange between xylem parenchyma and the xylem sap will alter the ion concentrations in ascending sap (Bangerth, 1979). However, Ca cannot enter the stele via the apoplastic when the Casparian band is present. To circumvent the Casparian band, it is proposed that Ca might be entering the cytoplasm of the endodermal cell through Ca-permeable channels (Modisane, 2007). Calcium is then actively effluxed from the symplast by the plasma membrane Ca ATPases or Ca/H antiporters of the cell within the stele (Gilliham *et al*., 2011).

This theory is based on three perspectives, namely; (i) the hydrophobic chemistry of the Casparian band, (ii) the rapid penetration of divalent cation such as Ca and Sr and of cell impairment trace (hydrophilic dyes) into the cortex but not the stele of plant roots and (iii) the observation that a Ca delivery to the xylem increases in regions of the root where the Casparian band is still developing or damaged (Modisane, 2007; White & Broadley, 2003). In this case, the apoplastic Ca is taken up by cells on the cortical side of the endodermis through Ca-permeable channels (White, 2001).

However, there is a belief that Casparian bands are impermeable to Ca and therefore, Ca must bypass Casparian band through the symplast prior to its delivery into the xylem (Taylor & Locascio, 2004). It is further believed that, since Ca mobility in the symplast is restricted by low cytosolic Ca concentration $[Ca]_{cyt}$ most radial movement of Ca across that root will occur through the apoplast up to the Casparian band (Dodd *et al*., 2010).

Bearing in mind that Ca is only a friction of cytoplasmic Ca pool, Ca chelates might move in symplastic pathway. Since the shortest symplastic route that circumvents the Casparian band is through the cytoplasm of the endodermal cell, it is debatable whether Ca utilises this pathway in order to minimize any compromise of $[Ca]_{cyt}$ signalling mechanization (White, 2001; Clackson, 1994).

Nevertheless, the relative contribution of the apoplastic and symplastic pathways to the delivery of Ca to the xylem is still shrouded in mystery (White, 2001). Hence, the movement of Ca must be well balanced to allow root cell signal $\{Ca\}_{cyt}$ to control the rate of Ca delivery to the xylem, and to prevent the accumulation of toxic cations in the shoot (White & Broadley, 2003).

Generally, the Ca concentration of the soil is about 10 times higher than that of K whereas the uptake rate of Ca is usually lower than that of K (Clarkson & Sanderson, 1978). Calcium uptake can also be competitively depressed by the presence of other cations such as N and P since roots usually take these up more rapidly than Ca (Mengel & Kirkby, 2001). However,

monocotyledonous plants require less Ca in their tissue than dicotyledonous plants do. This has been attributed to a larger cation exchange capacity of their cell wall membrane (White, 2000).

2.1.3 Calcium movement through the membrane

It is well documented that Ca exists as a gradient across the plasma membrane extracellular concentration that is about 10,000 times higher than the intracellular concentration (Hsieh *et al*., 1991). The concentration of Ca inside the cell can vary between different organelles, the transport of Ca between the cytoplasm and organelles such the sarcoplastic and endoplastic reticulum acting to control $\{Ca\}_{cyt}$ (White, 2001).

Different types of efflux transporters responsible for restoring and maintenance of low $\{Ca\}_{cyt}$ have been identified in higher plant parts (Bush, 1995; Askerlund & Sommarin, 1996). These are P-type ATPase located in the plasma membrane, the endoplasmic reticulum, vacuolar membrane and chloroplast envelope membranes (Gilroy & Jones, 1993; Huang *et al.*, 1993; Hsieh *et al.*, 1991; Graf & Weiler, 1989). However, Ca ATPase is a member of the P-type family of ion pumps, which are responsible for the ATP-dependent active transport of ions across a wide variety of cellular membranes (Hirschi, 2001).

Calcium enters plant cells through Ca permeable ion channels in their plasma membranes which, is driven large by electrochemical gradient from intercellular store or extracellular space in cytoplasm (White, 2000). Calcium is efflux from the cytoplasm to the apoplast, the endodermis reticulum and vacuole antiporters and extrusion is accomplished by Ca/H^+ antiporter present in the vascular membrane (Hirschi, 2001; Sze *et al*., 2000). However, the movement of Ca in apoplast is limited by the Casparian band (Gilliham *et al*., 2011). In this case, the apoplastic Ca is taken up by cell on the cortical side of the endodermis through a Ca-permeable channel (White, 2001). Therefore, Ca is actively effluxed across the plasma membrane from the symplast by Ca transporter (Ca-ATPases) in the stele (Gilliham *et al*., 2011; Cholewa & Peterson, 2004; White, 2001).

Nevertheless, there is some doubt as to whether Ca-ATPases could catalyse enough Ca to the xylem to meet nutritional demands of the high plant parts (White, 1998). Moreover, if the Ca transportation to the xylem occurred exclusively through endodermal cell, the density of Ca-ATPases needed would exceed the protein packing capacity of the plasma membrane. Even, if many cell-types within the steele contributed to Ca efflux from the symplast to the xylem, the mechanism would still be kinetically challenged (White, 2001.) Therefore, it is unlikely that a symplastic pathway alone could deliver the necessary physiological Ca flux to the xylem. Hence, White (2001) proposed that Ca might also traverse the symplast as Ca chelates to balance low $[Ca]_{cyt}$.

2.1.4 Calcium deficiency

Calcium deficiency often occurs in tissues that have low relative rates of respiration compared with other parts of the plant. This clearly highlights the role of transpiration in the supply of Ca and the lower rate of symplastic of Ca within most tissues (Gilliham *et al*, 2011), and a clear indication that Ca cannot be mobilized from older tissues and redistribute itself via the phloem.

Calcium deficiency often occurs in most acidic soil, and also from the soil where one nutrient competes with other nutrients due to undersupply or over supply of Ca to the fruits or storage tissue or the developing organ (Dordas, 2009). Lower level of Ca in the soil increase the frequency of Ca deficiency in a crop, but most often problems observed are rather a matter of poor Ca translocation within the plant than one of low soil Ca levels (Camberato & Pan, 2000).

Calcium deficiency symptoms appear in the meristem regions (new growth) of leaves, stems, buds, and roots. Younger leaves are affected first and are usually deformed. In extreme cases, the growing tips die. The leaves of some plants hook downward and exhibit marginal necrosis (Ray, 1999). Calcium deficiency causes a reduction in the growth of the meristematic tissues and the youngest leaves become deformed and chlorotic (Mengel & Kirkby, 2001; Marschner, 1995). Lack of Ca in the cell causes holes or cracks in the cell walls, which allows the salt concentrations in the cytoplasm to flow out from the cell (Tanany *et al*., 2011). However, insufficient Ca in the plant is usually alleviated where the surrounding media have adequate

Ca, plant absorb enough Ca and transport the mineral to the Ca deficient organs (Supanjani, 2005).

2.2 Magnesium

Magnesium is an essential plant macro nutrient which serves as a cofactor with ATP in a number of enzymatic reactions. Magnesium plays a fundamental role in the process of photosynthesis, which is very important for the life of the plant in general (Kobayashi & Tanoi, 2015; Bergmann, 1992). Magnesium also plays a critical role in phloem loading and phloem transportation of photo assimilates into sink organs. Apart from its function in the chlorophyll molecule, Mg is involved in the cation balance, where it regulates the pH and turgor adjustment on the cell (Zhang & Turgeon, 2009). Nonetheless, Mg has also been documented as an essential element for a vast number of fundamental biochemical processes in all living cells of plants (Yao *et al*., 2014; Maguire & Cowan, 2002).

Besides its functions in photosynthesis, Mg further plays a vital role in stepping up the growth (both quantitative as well as qualitative features) of the plant (NF, 2011). According to Carvajal *et al.* (1999), Mg has an effect on leaf osmotic potential, which decreases with increasing concentrations of this element in the nutrient solution. Increased Mg levels in the nutrient solution in the soil have been found to increase Mg levels in the plant, but decrease dry matter in fruits (Gunes *et al*., 1998). The functions of Mg in plants are mainly related to its capacity to interact with strongly nucleophilic ligands through ionic bonding, and to act as a bridging element and form complexes of different stabilities (Marschner, 1995). Magnesium also appears to stabilize the ribosomal particles in the configuration necessary for protein synthesis and is believed to have a similar stabilizing effect in the matrix on the nucleus (Mengel & Kirkby, 2001).

2.2.1 Magnesium uptake from the soil to the roots

The amount of Mg absorbed by plants through root interception depends on the soil volume occupied by the roots, the concentration of Mg in the soil and the root morphology (Legong *et al.*, 2001).

The absorption of Mg has not been studied as extensively as N, P, K and Ca in plant cell, hence, there are ample uncertainties where Mg absorption is concerned (Mikkelsen, 2010). In earlier studies, it was theorised that Mg absorption is an "active" process consuming metabolic energy (Shaul *et al.*, 1999). Thus, Mg was thought to be carrier-mediated at the low K concentration (i.e., <1 mM) and also followed enzyme kinetics (Michaelis-Menten equation). Others, however, proposed a high and low affinity absorption system and Mg transport, absorption as passive progress, having many characteristics in common with Ca absorption (Cowan, 1995).

Subsequently, Mg influx was proposed to be passive and active efflux pump that controls the movement of free Mg ions in the cytoplast (Maguire, 2002). On the other hand, the absorption of Mg component was observed as active, but then there was passive absorption, which was occupied by Ca efflux (Karley & White, 2009). Thus, there is no consensus concerning the basic mechanism of Mg absorption by root cells (Karley & White, 2009). These observations beg for further studies that are required for clarification.

2.2.2 Magnesium movement through the membrane

Since cytosolic Mg is around 0.4 mM, it is then possible that Mg entry into root cells is through Mg-permeable channels. The movement of Mg across biological membranes is reviewed from the perspectives of primary active transport and secondary active transport (Karley & White, 2009; Li *et al.*, 2001). Since all cells maintain intracellular Mg at a lower electrochemical potential than extracellular Mg, so active transport pumps bringing Mg into the cell have neither been hypothesised nor been confirmed (Deng, 2006). Most evidence points to influx leaks, presumably via membrane channels and carriers which do not perfectly exclude Mg but

members of the MRS2 family of transport proteins (MGT1, MGT10) appear to dominate Mg influx across the plasma membrane (Deng, 2006; Shaul, 2002).

The MHX Mg/H^+ antiporter, which is encoded by a single gene is perceived to dominate Mg influx to the vacuole (Berezin *et al*., 2008; *Qi et al*., 2008; David *et al*., 2007). Mg is permeable from vacuoles through Mg-permeable cation channels, including the SV channel (Pottosin & Scho, 2007). It must be transported against its electrochemical gradient from living cells into the xylem sap, where it is present at concentrations between 0.5 and 1.0 mM as either Mg or complexes with organic acids (Buxten *et al*., 2007). Mg is frequently stored in root cells and released to the xylem if the shoots become Mg deficient (Karley & White, 2009).

2.2.3 Magnesium deficiency

Magnesium deficiency often occurs in acidic soils, mainly sandy soils; where there is competition of cations such as H, K, Ca and P (Kobayashi & Tanoi, 2015). Many researchers have pointed out the symptoms of Mg deficiency in crops. Under normal conditions, the Mg content is within the range of 0.15%-0.30% of the dry mass of the vegetative parts (Wanlu *et al*., 2016). When Mg content is below this range, symptoms emerge in the leaves. Symptoms tend to occur during the period of grain filling and fruit expansion, which influence the quantity and quality of agricultural products (Merle, 2016).

Mg deficiency causes chlorosis of fully expanded leaves, impairment of the export of carbohydrates from source to sink sites and decrease in starch content of storage tissue, such as tubers and the single grain-mass of cereals (Dordas, 2009, Mengel *et al*., 2001). In rice, Mg deficient leaves have a yellowish, bronze, orange-yellow, or reddish tissues between the green veins and sometimes they have brown interveinal necrosis (Cakmak & Kirkby, 2008). Plants under low Mg supply are very sensitive to high light intensity and heat stress as they rapidly become chlorotic and necrotic, probably due to extensive production of reactive oxygen species (Merle, 2016).

In general, mature leaves senesce faster than juvenile leaves when Mg availability is limited (Kobayashi & Tanoi, 2015; Dordas, 2009; Cakmak & Kirkby, 2008). More than one week is generally required to produce these symptoms after the removal of Mg from the nutrient solution in greenhouse production.

2.3.4 Interaction between calcium and magnesium

The interaction between nutrients in higher plants occur when the supply of one nutrient affects the absorption, distribution or function of another nutrient. Thus, depending on nutrient supply, interaction between nutrients can either induce deficiency or toxicity and can also modify the growth response of the plant (Husa & Mclrath 1965). However, where nutrient supply is neither deficient nor toxic for plant growth, interaction can be assessed from the perspective of nutrient concentration and content within the plant (Wenzl *et al*., 2003).

There are basically two ways in which nutrients can interact, either within the soil or plant, to affect nutrient absorption or utilisation. Thus, synergism is a positive effect between nutrients and antagonism is a negative effect between nutrients (Ranade-Malviu, 2011). The interaction between Ca and Mg is well known, hence; the rate of Mg uptake can be depressed by Ca and vice versa (Hao & Papadopoulos, 2003).

Calcium is strongly competitive more than the Mg whereby the bonding sites on the root plasma membrane appear to have less affinity for the highly hydrated Mg than for Ca (Marschner, 1986). In general, increased levels of external Ca results in decreased uptake of Mg due to cationic antagonism or interactions. The decreases in Mg could also be attributed to the withdrawal of Mg from the soil or nutrient solution in order to maintain the balance between cations against the increasing Ca (Carvajal *et al.*, 1999). Calcium is also reported as an inhibitor of enzymes that require Mg. The high activity of Ca also counteracts the function of Mg (Clarkson & Sanderson, 1978). According to Jakobsen (1993) Mg exerts a more depressing effect on the Ca uptake than Ca levels does on the uptake of Mg. Grattan & Grieve (1999) further reported that excessive leaf Ca concentrations might interfere with CO_2 fixation by

inhibition of stroma enzymes, particularly those that are Mg activated. However, Ca has the most damaging effect on several key micro-element's availability for plant uptake.

2.4 MYCORRHIZAL FUNGI

Mycorrhizal fungi are the structure that exists on mutualistic association that is formed between soil fungi and roots of higher plants (Habte, 2000). Mycorrhizal fungi are classified according to their fungal associations, extent of root penetration, the presence or lack of an external mantle and/or sheath, as well as the intra and intercellular structures produced inside the root of the host (Srivastava *et al*., 1996).

Seven different types of mycorrhizae associations have been discovered and documented, involving different groups of fungi and host plants and distinct morphology patterns (Harley, 1989). The most common associations being Ectomycorrhizal, Ectendomycorrhizae, Arbutoid, Monotropoid, Orchidoid, Endomycorrhizae or the vesicular-arbuscular mycorrhizae that is usually referred to as arbuscular mycorrhizae (AM), and Ericoid mycorrhizae involving hyphal coils in the outer cells of the narrow "hair roots" of plants in the Ericales (Habte, 2000). The hyphae of a mycorrhizal fungus originating from one entry point in roots or one propagule in soil are referred to as colonies. As a result, colonization refers to the degree of root occupation by mycorrhizal fungi (Bagyaraj, 1991).

The (Figure 2.2) below illustrates the roots with and without mycorrhizae to highlight some of these properties. Mycorrhizal fungi are known to stimulate nutrient uptake capacity by extending far beyond root surfaces and proliferating in soil pores that are too small for root hairs to enter. Mycelia networks of mycorrhizal fungi often connect plant root systems over broad areas. These fungi frequently make up the largest portion of soil microbial biomass.

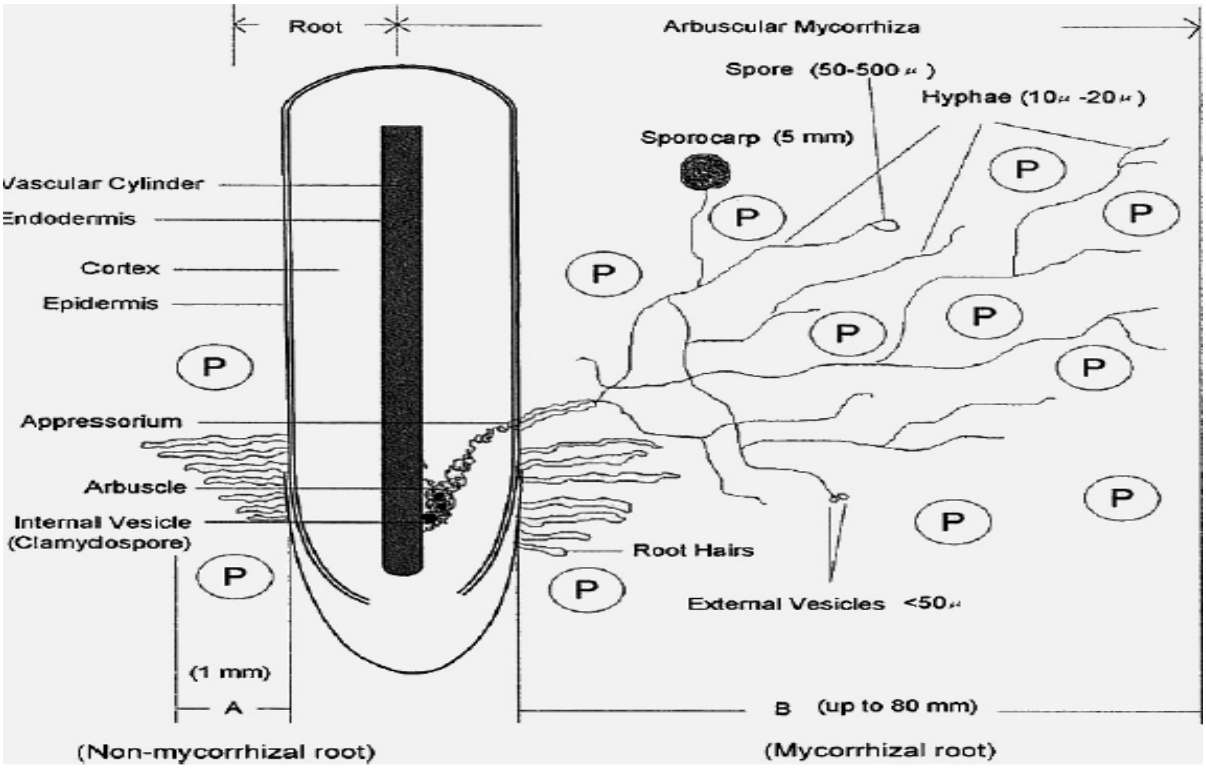

Figure 5.2 *Schematic representation of rhizosphere versus mycorrhizosphere (Cardon & Whitbeck, 2007).*

2.4.1 Colonization of mycorrhizal fungi

The fungus colonises the root cortex and develop an extra-mycelium that helps the plant to acquire mineral nutrients from the soil (Harley & Smith, 1983). As the internal colonisation spreads, the extra-radical hyphae ramify, and grow along the root surface, forming more penetration points (Habte, 2000). They also grow outwards, into the surrounding soil, thus developing an extensive tri-dimensional network of mycelium which interfaces with soil particles (Smith & Smith, 1990). The mycelia network can extend several centimetres outward from the root surface, bridging over the zone of nutrient depletion around roots to absorb low mobile elements from the bulk soil (Azcon & Barea, 1997).

Once the hyphae reach the inner cortex, they will grow into the cells and, by means of repeated dichotomous branching, form tree-like structures called ‘arbuscules’. Arbuscule formation, therefore, represents a large surface of cellular contact between symbionts (host plant and mycorrhizal fungus) (Smith & Smith, 1990). This formation facilitates the exchange of metabolites between host and fungus as the arbuscules are the main transfer site of mineral nutrients from fungus to plant and C compounds to the fungus (Azcon & Barea, 1997, Smith & Smith, 1990; Smith & Gianinazzi-Pearson, 1988).

2.4.2 Factors affecting mycorrhizae growth and development

2.4.2.1 Soil temperature

The effect of soil temperature on mycorrhizae function has already been reported by many authors as it is an important factor influencing mycorrhizae development and function (Liu *et al*., 2004; Jakobsen & Andersen, 1982). Mycorrhizae development is usually optimal in cool temperature climates, with temperatures ranging from 20 to 25°C (Matsubara *et al*., 2000). According to Van Sangyan (2015) soil temperature alters the physiology of mycorrhizae symbiosis to stimulate greater inoculum production by influencing root morphology and host plant nutrition. Wu & Zou (2010) also observed less mycorrhizae root colonisation at the temperature of 15°C as compared to the roots grown at a warmer temperature of 25°C on three-month seedling of citrus.

2.4.2.2 Soil pH

The efficiency of the mycorrhizae is directly determined by its ability to adapt to soil pH (Elmmore, 2006). Mycorrhizae fungi vary in their soil pH tolerance; some grow in low pH and others grow after adding the amount of lime for soil pH modification (Van Sangyan, 2015). Soil pH affects both spore germination and hyphae development (Angle & Heckman, 1986; Green *et al*., 1976).

Mycorrhiza development in connection to soil pH differs with soil types, plant and fungal species. Typically, mycorrhizae are able to colonize and grow well in soils of pH 5.6 to 7.0, but not in soils of pH 3.3 to 4.4, as reported by Hayman & Mosse (1971).

2.4.2.3 Soil salinity

Mycorrhizae have been known to occur naturally in saline environments. Salinity not only affects the plant, but also the mycorrhizal fungi. It hampers colonization percentage growth of hyphae and spore germination of fungus. High salinity may, however, have negative effects on arbuscular mycorrhizal fungi (AMF) growth and hyphae extension (Asghari *et al*., 2008; Juniper & Abbott, 1993). Asghari *et al*. (2008) reported that high soil salinity (45 dS m^{-1}) inhibited mycorrhizae root colonization, possibly due to inhibition of spore germination, hyphae growth and hyphae spreading after initial 20 infections (McMillen *et al*., 1998). Reduction of arbuscules in high saline environment has also been observed (Pfeiffer & Bloss, 1988).

2.4.2.4 Fertilizer and organic matter

Fertilizer and organic matter (OM) content play vital roles in AMF survival and development. Mycorrhizae hypha acts as binding agents within and between aggregate (Van Sangyan, 2015). In the mycorrhizosphere, mycorrhizae hyphae may contribute further to the aggregates effect as they grow into small pores and bind soil particles together (Elmmore, 2006). Organic matter and root residue are important ecologically as part of the three-way soil, plant and mycorrhizae relationship. Organic root debris may act as a reserve for soil inoculant (Warner & Mosse, 1980).

Phosphorous (P) fertilizers are known to inhibit colonization of roots and spore production by AMF. Hayman (1975) demonstrated that fertilizers such as P and N could potentially reduce spore number and fungal colonization with N is having a more detrimental effect than phosphorus. Hayman *et al*. (1976) stated that these other factors such as host species, soil type, and management practices also influence fungal survival and development.

2.4.2.5 Drought stress and soil moisture

The abiotic components of the environment in which a mycorrhizae plant grows are known to influence mycorrhizae functioning (Fitter & Garbaye, 1994; Hetrick & Wilson, 1991). The mechanisms involved are often elusive, but drought stress has shown a greater yield loss than any other single biotic or abiotic factor (Johnson & Gehring, 2007). According to Staddon *et al*. (2003), mycorrhizal fungi are affected by long term climatic manipulation in the field, including a change in both soil temperature and soil moisture. Thus, Michelsen & Rosendahl (1990) reported that drought reduced the biomass and nodulation of *Acacia nilotica* and *Leucocephala* seedling. There is however difference in the growth of plant species subjected to drought and inoculated with arbuscular mycorrhiza and/ or supplied P fertilizer (Tahat & Sijam, 2012).

2.4.2.6 Pesticides

Several interactions between mycorrhizal fungi, their host and the environment must be recognized to understand the impact of pesticides. Cause and effect can be difficult to determine, because what influences the fungi may thereby indirectly influence the host and vice versa (Van Sangyan, 2015).

Management practices such as pesticide applications, in particular, fungicides, may inhibit the effect of AMF sporulation and colonization (Nemec & O'Bannon, 1979). One study conducted by Kelly & South (1978) found that orzalin and trifluralin inhibited the growth of certain species of mycorrhizal fungi.

However, roundup has been shown to be toxic to mycorrhizal fungi in laboratory studies and some damaging effects were seen at concentrations lower than those found in soil following typical application (Estok *et al*., 1989). Triclopyr was also found to be toxic to several species of mycorrhizal fungi and oxidation reduced the number of mycorrhizal fungal spores (Tahat & Sijam, 2012).

2.4.2.7 Crop rotation

Crop rotation has a critical effect on soil microbial communities, soil structure and organic matter (Tahat & Sijam, 2012). Hence, crop rotation is known to affect AMF on the field together with species diversity. Crop rotation was found to h affect the spore population of AMF in trials utilizing a maize-vegetable-small grain rotation and chemical fertiliser or organic amendments as source mineral nutrients (Douds & Millner, 1999).

2.5 MAGNETIC FIELD

Magnetic field (MF) is a physical field generated from a piece of iron or other material which has the property of running an electrical current which can be macroscopic currents in wires or microscopic current associated with electrons in atomic objects (Kocaman, 2014; Leo *et al*., 2013). It occurs when there is a current flowing along a conductor and its strength increases as soon as a device like inductor or coiled wire is switched on and current moves through it. However, the magnitude of the magnetic strength decreases with distance from the source (Leo *et al*., 2013).

2.5.1 Historical background of magnetic field use in agriculture

Before the year 1862, scientists were not aware that magnetism has an effect on the biological activity of the plant (Abdulla, 2012). Until the year 1862 when Louis Pasteur observed that magnetism has an effect on plant growth (Kocaman, 2014). However, the study was not accepted by the scientific community as it was classified as pseudoscience (Lower & Stephen, 2011). In 1930 Savostine conducted the first study on wheat seedling under the influence of MF and he observed 100% increases in the rate of elongation. Six years later, Albert Roy Davis (1936) theorized that the north and the south poles of a magnet consist of different energies with opposite effects on the plant.

From then on, there is no definite conclusion on how MF achieves such a change on plants. However, several theories had been proposed to explain this action. MF treatments are assumed

to enhance plant vigour by influencing the biochemical process that involves free radicals, and by stimulating the activity of proteins and enzymes (Kordas, 2002). In the same manner, Radhakrishnan & Kumari (2013) Dhawi *et al.* (2009) Eşitken & Turan, (2004) Phirke *et al.* (1996) associated the mechanism of MF with the activation of phyto-hormone such as gibberellic acid-equivalents, indole-3-acetic acid and trans-zeatin as well as activation of the bio-enzyme systems. Furthermore, magnets altered the membrane structure of the plant cells so that the plants absorb more water and nutrients (Shawanroy, 2012; Lower & Stephen, 2011). In addition, the vast majority of biological substances are proteins that contain metal ions, such as hemoglobin, cytochrome or ferritin, which can be paramagnetic (Turker, 2007).

On the other hand, the external application of electromagnetic fields in plant science and microbiology has been reported to influence both the activation of ions and the polarization of dipoles in living cells (Moon & Chung, 2000). However, some authors argue about this inclusion because they believe that permanent magnetic fields are not biologically active. Whereas, the results obtained by Andrei *et al.* (2014) Mercedes *et al.* (2005) and by Belyavskaya (2004) have shown the high sensitivity of plants to permanent magnetic fields, particularly, in the intensity range from higher MF level to very low ones.

The effect of MF depends on its strength, intensity and on the value of magnetic induction squared (Massimo, 2014). A number of studies have been conducted to prove the viability of magnets on plants development, enhancement, enzyme activity, germination and water uptake in seeds (Massimo, 2014; Occhipinti *et al.*, 2014 Vanderstraeten & Burda, 2012)., but the effects vary due to a number of factors, such as strength, frequency, intensity and form of magnets used, the placement of the magnet in relation to the plant or seed, the type of plant used and time exposure also plays a vital role (Andrei, 2014; Shine & Guruprasad, 2012; Shine *et al.*, 2011a; 2011b; Vashisth & Nagarajan, 2010).

In Table 2.1 below, different works of research are reviewed and summarized. These provide the source authors, a summary of the research focus, MF technology covered and comment on the key results of the work reviewed. These modulations in appropriate conditions can have

useful outcomes such as treatment or inducing the desired characteristics in different compounds.

Table 2.1 Review of magnetic field applied to enhance plant growth and development.

Authors	Method	Focus of the Review	Comments on key results
Shabrangi, A. & Majd, A. 2009.	Pre-treatment of seedling	The study focused on a seed of Lentil (*Lens culinaris L.*) which contains significantly Fe as a ferromagnetic element) which were magnetically pretreated by different magnetic field intensities from 0.06 to 0.36 tesla (T) using Zeeman system for different periods of time 5, 10 and 20 minutes. Under natural light cycle 14-h light/10-h darkness and 25±3°C daily and night temperature.	Magnetic treatment improves first stages of growth in higher plants and increases stress enzyme like APX in seedling which grown from pretreated seeds.
El-Gizawy, A.M. Ragab, M. E. Nesreen A. S. Helal, A. El-Satar & Osman, I. H. 2016.	Seed germination	The study investigated the effect of MF on germination of true potato seeds (TPS), vegetative growth and potato tubers characteristics of potato plants (*Spunta cv.*) exposed to three MF strengths: 20-30-40 mT for three periods: 5, 10 and 15 minutes.	Pre-sowing MF treatments for true potato seeds improved germination of potato tubers, vegetative growth, potato yield and potato tubers and its components.
Kataria, S Baghel, L, & Guruprasad, K.N. 2017.	Pre-treatment of seedling	Evaluation of magnetopriming on germination and early growth characteristics of seeds under saline conditions exposed to static MF of 200mT for 1hour.	The adverse effect of salinity on germination and seedling vigour can be alleviated by magnetoprimig with SMF of 200mT for 1h and it can also be used to increase water uptake and higher activity of hydrolytic enzymes (α amylase and protease).
Hozayn, M. & Qados, A.M.S.A. 2010.	Plant growth and development	Looking at a way of producing more food from less water and optimization unit area for improvement of water productivity and yield crop using	Treatment of magnetic water could be used to improve the quantity and quality traits of faba bean and water productivity under sandy soil conditions.

		magnetic water technology.	
Mridha, N. Chattaraj, S. Chakraborty, D. Anand, A. Aggarwal, P. & Nagarajan, S. 2016.	Pre-treatment of seedling	Looking at the possible involvement of MF pretreatment in physiological factors of (*Phaseolus vulgaris*) in three different treatments; (T1) control, (T2) MF with B=1.8 mT for 30 min per day in 10 days, (T3) MF with B=1.8 mT for 60 min per day in 10 days.	Using a magnetic time exposure for 30-60min led to a higher generation of ROS and garden to decrease the biosynthesis of chlorophyll, carotenoid, phenolic and flavonoid compounds.
Sadeghipour, O. 2016.	Plant growth and development	Evaluation of the effects of irrigation with magnetized water on some agronomic and physiological traits.	Using structured irrigation system (magnetized irrigation) has shown a remarkable increase in the yield, yield components, chlorophyll content, net photosynthetic rate, WUE, RWC, HI, plant height and biomass production. Nevertheless, magnetized water can be considered as one of the most valuable, safe, practical and economical technologies that can help in improving yield, WUE and thus saving water resources.
Podleśny, J. Pietruszewski, S. & Podleśna, A. 2005.	Pre-treatment of seedling	The study focused on seed modification and the course of some physiological and biochemical processes in the seeds, increasing.	Using magnetic fields to stimulate seeds favorably influences the sprouting, plant uniformity and also had a positive influence on plant emergence as it took place 2-3 days earlier than the emergence of plants in the control object.
Kuznetsov, O.A. & Hasenstein, K.H. 1997.	Seed germination	Studying the Physical characteristics (density p and magnetic susceptibility x) of amyloplasts and the ratio of the velocities of individual amyloplasts in the presence and absence of a high gradient MF.	Using uniform magnetic fields causes neither curvature nor changes in growth rate and emerging root and shoot of germinating plants adjust to the gravity vector by changing the rate of elongation of the top and bottom of the root or shoot.
Mroczek-Zdyrska, M. Kornarzyń, K.	Plant growth and development	Focused on the stationary MF (130 mT) on the mitotic activity and	The data presented in this paper concludes that 130-mT MFS enhances

Pietruszewski, S. & Gagos, M. 2016.		selected biochemical parameters of lupin (*Lupinus angustifolius L.*).	the growth and development of aboveground parts, which is manifested by an increase in the length and mass of the shoots and an increase in the photosynthetic pigment content. Nevertheless, the study concluded that, MFS is beneficial for the improvement of growth and productivity of economically important.
Rochalska, M. 2005.	Plant growth and development	Looking at seed improvement of three varieties of sugar beet growth using low frequeny MF acting independently and in combination with other method of seed improvement.	Low frequent MF used independently or in combination with other methods of post- harvest influenced the chlorophyll content. However, the best results were obtained using the MF together with conditioning. MF treatment also had a positive influence on content of nitrogen in plants through the better uptake of nitrogen from the soil.
Huang, H.H. & Wang, S.R., 2007.	Plant growth and development	Looking at the biological effects of both a 60Hz sinusoidal MF and a 60Hz pulsed MF on the early growth of plants.	Sinusoidal and pulsed MFs affect the plant growth in different ways (with respect to different kinds of plant). However, the 60Hz sinusoidal, has an apparent enhancing effect on growth, but with the occurrence of some morbid state phenomena. Nevertheless, the pulsed MF harm plant growth rather than enhancing growth as the sinusoidal MF does.
Novitskii, Yu.I. Novitskaya, G.V. Molokanov, D.R. Serdyukov, Yu. A. & Yusupova, I.U. 2015.	Plant growth and development	Looking at the influence of weak horizontal permanent MF produced by Helmholtz coils of 400 A/m strength under controlled conditions of illumination and temperature in phytothrone.	Using a weak horizontal PMF of a strength of 400 A/m had a positive influence on headstone due to a significant reduction of the polar and neutral lipids and the content of glycolipids decreased among PL, in particular, of MGDG, which are structural components of the reaction

			center of photosystem. Hence, the content of PG, PC, PE, and PI decreased among phospholipids.
Shahin M. M. Mashhour A. M. A. & bd-Elhady, E. S. E. A. 2016.	Plant growth and development	The aim of the study was to evaluate the effect of different MF strengths (0.0, 20.0, 40.0 and 60.0 m T), at different time intervals ranging from 0 to 300 minutes on some properties of irrigation water the pH, electric-conductivity (EC), and total dissolved salts (TDS).	The magnetic treatment of irrigation water had a positive effect on growth parameters, yield, increasing germination, absorption of the nutrients (N, P, K, Fe, Mn, Zn, and Cu) and decreasing ion toxicity.
Rashmi, S. Sunita, T. Pandey, Omvati, V. Neha, J. & Sriastava, R.C. 2014.	Seed germination	The study focused on the physical energy through static magnetic fields of 100 to 250 milli Tesla intensity with the intervals of 50 milli Tesla for 1-4 hour, and to metaphysical energy through BK Rajyog meditation (BKRYM, a positive thought energy-based meditation) with an interval of 1 hour.	Exposure of seeds to physical energy treatments through a static MF of strength below 250 mT for one hour and metaphysical energy treatment through BK RYM, (a positive thought energy-based treatments) for two and four hours could be a suitable, cheap and easy seed invigoration method for improving germination and seedling vigour indices of poor-quality seed. Seed enhancing energy treatments not only increase the growth and vigor parameters of seedlings but also play a significant role in enhancing biochemical properties of seeds.
Ramírez, Ma. E. Carballo, A.C. & Aguilar, C.H. 2016.	Seed germination	This study provides insight into a new way of improving maize productivity under rainy season conditions under variable MF of 560 mT for 30 min prior to sowing.	By treating seed with magnetic fields, it is possible to improve short cycle varieties, adapted to rainy season zones.
Hasenstein, K.H. John, S. Scherp, P. Povinelli, D. & Mopper, S. 2013.	Seed germination	The examination of corn, wheat and potato starch grains suspension movement with video microscopy under parabolic flights of 20–25s of	The advantage of using magnetic gradient is that it can move diamagnetic compounds under weightless or microgravity conditions and serve as a

		weightlessness.	directional stimulus during seed germination in the low-gravity environment.
Bilalis, D.J. Katsenios, N. Efthimiadou, A. & Karkanis, A. 2012.	Pre-treatment of seedling	Treatment of corn seeds with pulsed EMFs for 0, 15, 30 and 45min.	Using pre-magnetic treatment has been found to perform the best results with economic impact on producer's income in a context of a modern, organic, and sustainable agriculture.
Kordyum, E.L. Bogatina, N.I. Kalinina, Y.M. & Sheykina, N.V. 2005.	Plant growth and development	Evaluating the structure and functional organization of cap statocytes under combined MF at the resonance frequency of Ca ions inside a μ-metal shield and the altered gravitropic reaction of cress (*Lepidium sativum*).	The experimented conditions were observed to change normally positively gravitropic cress root to exhibit negative gravitropism.
Fariba, R. V. Ahmad, M. Taher, N. & Sedighe, A. 2013.	Seed germination	The study examined the effect of electromagnetic fields on seed germination and seedling growth of (*Satureja bachtiarica*), seeds were soaked in water for 5 hours and placed on the screen, between the two parallel coils electromagnetic generating device and were under electromagnetic irradiation with one mT intensity with connecting to the electricity for two hours.	Germination speed showed a significant increase in the treated samples, whereas morphological comparison of the treated and control samples showed a significant decrease in the mean shoot length, leaf area, fresh and dry mass.
Racuciu, M. Creanga, D & Amoraritei, C	Plant growth and development	The study presented in this paper was focused on the biochemical changes induced by extremely low frequency MF in maize plants during their early ontogenetic stages (first 12 days of growth). Under five different magnetic doses 1-2 -4 – 8 -10 mT) at 50 Hz frequency.	Electromagnetic energy could trigger complex synergetic cellular mechanisms that can lead to the growth disturbing.
Rostami, Z.E. Majd, A. & Arbabian, S. 2014.	Seed germination	The seed of (*Urtica dioica L*) were put under electromagnetic field by 0.8 and	The interaction of MF and exposure time indicates that a certain combination of

		1.6mT for duration of 5, 10, 20 minutes.	MF and duration, such as 0.8 mT for 20 min is highly effective in enhancing germination.
Seghatoleslami, M. Feizi, H. Mousavi, G. & Berahmand, A. 2015	Plant growth and development	The study focused on two irrigation treatments (control and water stress) and six methods of fertilizer treatment (control, NPK-F, using magnetic band- M, using silver nano particles-N, M+N and M+N+50% F) on the performance of (*Carum copticum*).	Magnetic field exposure, probably by encouraging nutrient uptake efficiency could be applied to reduce the fertilizer requirement. On the other hand, the cultivation of plants under low MF could be an alternative way of WUE improving.
Massimo, E.M. 2014.	Plant growth and development	This review describes the effects of altering MF conditions on plants by considering plant responses to M Values either lower or higher than those of the GMF. The possible role of GMF on plant evolution and the nature of the magneto receptor was also discussed.	Understanding GMF effects on life will provide the fundamental background necessary to under- stand evolution of life forms on our planet and will help us to develop scientific recommendations for the design of life- support systems and their biotic components for future space exploration.
Abou El-Yazied, A. El-Gizawy, A.M. Khalf, S.M. El-Satar, A. & Shalaby O.A. 2012.	Pre-treatment of seedling	The work aimed to determine the effects of magnetic bio-stimulation on tomato plants (*Lycopersicon esculentum cv.*) under NPK fertilizer levels.	Treated tomato seeds (cultivar Castle Rock) with MF by 100 gauss for 15 minutes with a magnetically treated irrigated water improved vegetative growth, increased total phosphorus content of tomato leaves and total yield while reduced pH value in soil extraction.
Betti, L. Trebbi, G. Fregola, F. Zurla, M. Mesirca, P. Brizzi, M & Borghini, F. 2011.	Seed germination	This study concerns the effects of a weak static MF at 10 μT oriented downward, combined with a 16-Hz sinusoidal MF (10 μT), on in vitro pollen germination of kiwifruit (*Actinidia deliciosa*).	Using ELF-MF treatment might partially remove the inhibitory effect caused by the lack of Ca in the culture medium, inducing a release of internal Ca stored in the secretory vesicles of the pollen plasma membrane. Although preliminary, findings seem to indicate the in vitro pollen performance as adequate to study the effects of ELF-MFs on living matter.

Khonsari, A. Gorji, K. Alirezaei, M & Akbar, A. 2015.	Plant growth and development	The aim of this study was to examine the different level of intensities from weak MF (20, 40, 60, 80 gauss) on percentage of seed germination and seedling growth of (*Myrtus communis L.*).	The exposure of (*Myrtus communis L.*) seeds to weak MFs (20, 40, 60 and 80 gauss) reduces germination of seeds, and exposure of 60 gauss for 10 consecutive days, showed a significant reduction in seed germination.
Elfadil, A. G. & R. A. Abdallah. 2013.	Plant growth and development	This study was conducted to determine the impact of sunlight, UV and magnetized water on Dura Sorghum sp. variety plants the Sudanese main staple food.	The effect of magnetized water on the plant indicated that magnetized water has a positive effect on Dura plant growth and development.
Aguilar, C.H. Pacheco, A.D, Carballo, A.C. Cruz-Orea, A. & Tsonchev, R.I. 2009.	Pre-treatment of seedling	In this study alternating MF treatments at low frequency (60 Hz) with combinations of three magnetic flux densities (20, 60 and 100 mT) and three exposure times (7.5, 15 and 30 minutes) were used as pre-sowing seed treatments in three maize (*Zea mays L.*).	The results show that electromagnetic treatment provides a simple and ecologically well compatible method to improve seed vigour in maize but is necessary to find the optimal irradiation parameters to induce a positive bio stimulation in the maize seeds which also depends on the seed genotype.
Sara, F. Ahmad, M. Sedigheh, A. Davoud, D. & Mehrdad, H .2013.	Seed germination	Investigated the effects of electromagnetic field strengths (1 and 2mT) on seed germination, seedlings ontogeny, and protein content and catalase activity in valerian seeds (*Valeriana officinalis L.*).	Electromagnetic fields probably degenerate proteins in the early stages of seedlings ontogeny and the treated valerian seedlings can increase catalase activity by decreasing free radicals against electromagnetic fields tension.
Jaime A. T.S. & Judit, D. 2014.	Plant growth and development	This review focuses on the use of MW in a bid to alter plant growth and development.	The application of MW might be a very practical way to improve the quantitative and qualitative attributes of agronomic and horticultural production under greenhouse or field conditions.
Andrei, P.C. Jonas, B. & Gabriel, B. 2014.	Plant growth and development	The purpose of this study was to observe the effects of Electromagnetic Fields on the growth and photosynthesis of the Lima bean plant (*Phaseolus lunatus*).	No significant difference was observed between the plants grown under the MF and the free ones, but the seeds under electric field didn't grow up at all.

Leo C. R. & Marites, M.R. 2013.	Plant growth and development	The study aimed to determine the effect of electro-magnetic field on the growth characteristics of okra, Tomato and Eggplants and set to establish baseline data for farmers on the possible utilization of electromagnetic Field (EMF).	The study proved that Okra, Tomato and Eggplant plants when exposed to EMF are less susceptible to insects and pests. Thus, occurrence of pest and insects lessen in plants that are exposed to EMF.
Nimmi, V. & Madhu, G. 2009.	Pre-treatment of seedling	Investigated the effect of a weak permanent MF with a strength of 62 µT on hot pepper (*Capsicum annum. L.*) under Four pre-sowing treatments at different times (4, 8, 12, and 24h exposure) of hot pepper seeds were carried out in the experiment to compare with the untreated control.	A static MF of 62 µT has a stimulating effect on the first stages of growth of hot pepper seeds. Both magnetic poles with different treatment times reveal increases in germination speed and total length of plants.
Turker, M. Temirci, C. Battal, P. and Erez, M.E. 2006.	Plant growth and development	Looking at the effects of a continuous static MF on growth and concentration of phytohormones and chlorophylls in maize and sunflower plants SMF in two directions; parallel to gravity force (field-down) and anti-parallel (field-up).	The negative effect of SMF in both directions was seen on dry mass of maize plants, whereas a prominent increase in the roots of sunflowers was detected. However, the field-up direction caused a significant increase in IAA and t-Z contents.
Aksyonov, S.I. Grunina, T. Yu. & Goryache, S. N. 2007.	Germination	The study focused on the mechanisms of stimulation and inhibition of wheat seed germination by low-frequency magnetic field.	Stimulation of wheat seed germination by brief exposure in a 50-Hz electromagnetic field is shown to depend on the extent of membrane stretching upon the seed swelling in sucrose solutions.
Taher. O.A.M.E. 2012.	Germination	Looking at different source of the MF flux from six different sizes of NdFeB ring permanent magnets with (100) mT magnetic flux density and magnetic fluxes (37.7, 32.9, 21.2, 18.8, 15.1, 12.1)×10-5Wb.	The varying MF flux is a very significant factor in influencing the germination process of the (*E. sativa*), (*C. olitorius*), (*P. oleracea*), (*Z. mays*) and (*S. bicolor*). It must be remembered, however, that this influence is varied and depends on the MF flux and the plant type.

Aguilar, J.O. Rivero, D.S. Puentes. A.E. Perilla, P.E.V. & Navarro, A.M.S. 2015	Germination	The study focuses on the comparison of the effects on seeds when exposed to magnetic fields of high and low intensities, and two electric and electromagnetic fields.	By applying electromagnetic fields was evident improvement in germination rates, growth of coleoptiles and thus the decrease in the time of dormancy.

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CHAPTER 3

MATERIAL AND METHODS

3.1 RESEARCH SITE DESCRIPTION

The experimental trial was carried out in a 40 m x 15 m greenhouse at Glen College of Agriculture located under the Mangaung Municipality in the Free State Province of the Republic of South Africa. The roof structure of the tunnel is divided into ten bays or spans, each being 5 m wide, allowing each span to create a designated growing area which is also suitable for replications. The roof and sides of the greenhouse are covered in a single-layered plastic (polyethylene). The geographical position of the college is at 28°55'S and 26°19'E at an altitude of 1307m above sea level. The study was conducted during the 2015/2016 growing season and was repeated during the 2017/2018 growing season.

3.2 PLANTING AND IRRIGATION

Rooted cuttings of rose geranium (± 12cm length) were obtained from Pretty Garden nursery in Bloemfontein, Free State. The cuttings were transplanted in a five litre-potting bags filled with sterile silica sand. Whereas, mycorrhizae used in this study was obtained from Mycoroot^{TM} SuperGo product (Mycoroot^{TM} (PTY) Ltd, South Africa). It consists of five different species of arbuscular mycorrhizae isolates, which are *Rhizophagus clarus*, *Gigaspora gigantean*, *Funneliformis mosseae*, *Claroideoglomus etunicatum* and *Paraglomus occulum*.

Wortex® fountain FP 15 water pumps of 230 volts, 18 W with a flow rate of 900 L/hr were used to fertigate experimental plants. It was fitted into feeding tubes that drain into plastic pots (with a height of 28.5 cm and a diameter of 15 cm). The irrigation systems had four dripper tubing with a flow rate of 900 L hour $^{-1}$; these drippers were allocated to four potted plants. The plants were irrigated twice a day for the first month, scheduled at 11:30 and 14:00 hrs in a closed recirculating irrigation system. Afterwards, the irrigation volume was gradually increased to three times a day scheduled at 08:00, 12:00 and 16:00 hrs due to increase in plants

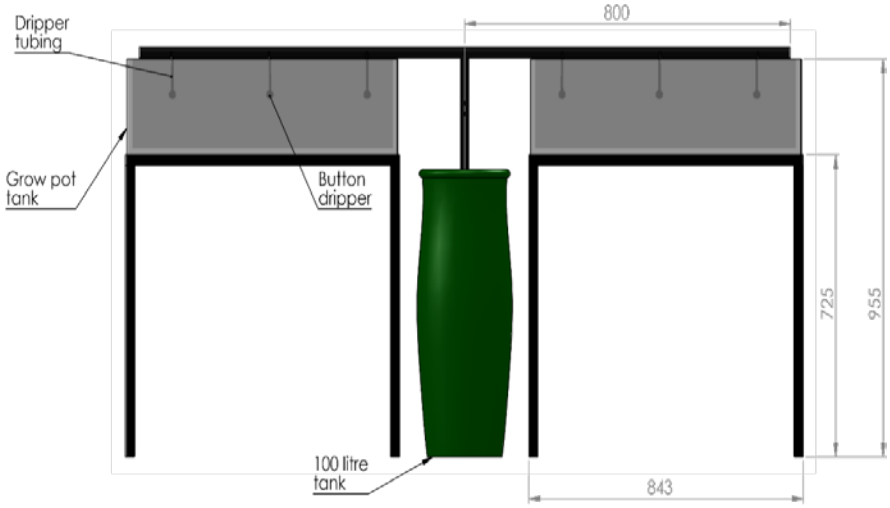
demands as they developed and to ensure that 10-15% of water leached out to reduce salt build-up in the growing medium. A balanced nutrient in the solution was maintained at a pH of 5.5.

3.3 EXPERIMENTAL DESIGN

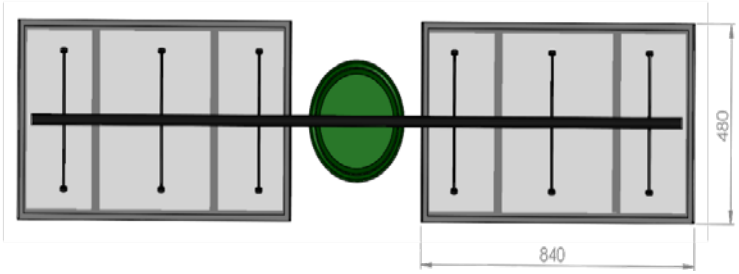
The experiment was structured as a 3×2 factorial experimental design, with three levels of Ca:Mg ratios (2.40:6.78, 4.31:4.39 and 6.78:2.40 meq L^{-1}), two levels of MF (zero MF, denoted by 0MF, and 110.1 mT, denoted by 1MF), and two levels of mycorrhizae amendment (none, denoted by 0Myco, and 20 ml of mycorrhizae amendment per plant, denoted by 1Myco) as a split-plot factor.These treatment effects were evaluated for agronomic attributes and mineral composition of rose geranium.

All treatments were replicated three times using a closed system described by Sedibe & Allemann (2013) shown in Figure 3.1. Additionally, experimental units were kept 2m apart to avoid exposing non-magnified units to magnetized pots. Two south facing magnets weighing ±56.4g with a length and width of 4.5 and 2cm respectively (110.1mT) were placed at the bottom of five litre-potting bag of MF treatments. Figure 3.2 illustrates how the magnets were placed under the pots to expose plants to magnetic field.

a) Side view



b) Aerial view



C) 3D View

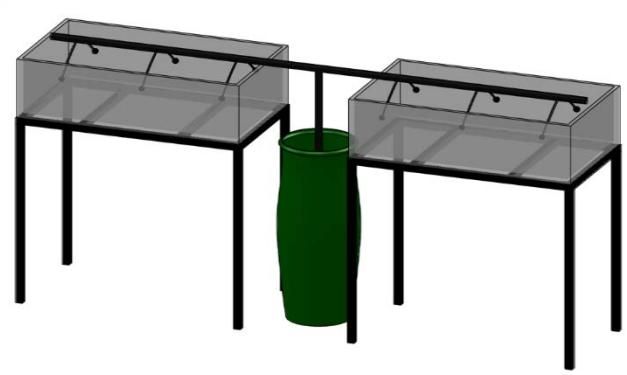


Figure 3.1 *Schematic representation of small-scale growing unit and irrigation system used to grow rose geranium plants adapted from Sedibe & Allemann (2013).*

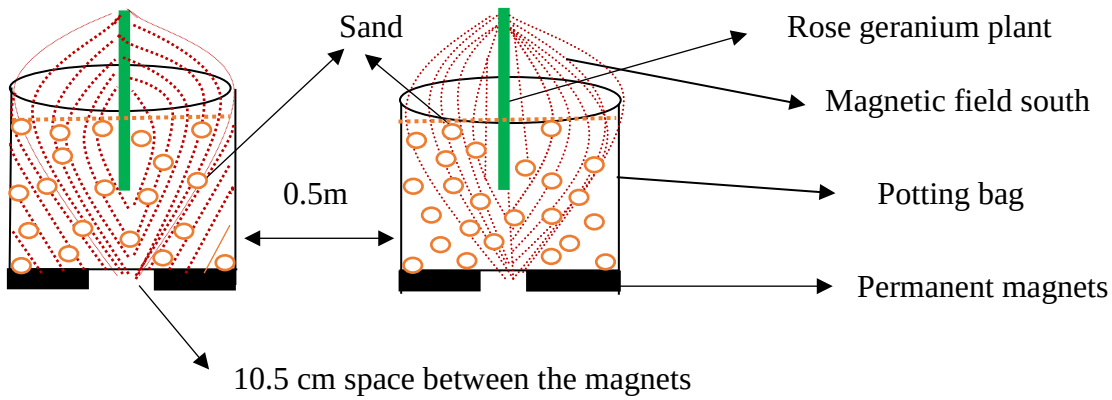


Figure 6.2 *Schematic representation of rose geranium subjected to magnetic field south.*

3.4 CROP MANAGEMENT

Rooted cuttings of rose geranium (±12cm) were selected based on plant vigour and uniformity. A fresh nutrient solution was prepared and replaced in a three-week cycle for six months. All nutrient solutions, treatments were kept in a 100 L reservoir. The irrigation systems had eight dripper tubings per treatment which was divided into two, making four drippers per treatment unit. The irrigation pumps had flow rate of 900 L $hour^{-1}$ which was mounted to a 20 mm tubing pipe distributed to the pot-holding tank of the growing unit; these drippers were allocated to eight potted plants.

A white oil emulsion insecticide (Oleum, Efekto) was applied repeatedly once per month for seven days to control aphids. The rate of application was 20 ml insecticide per litre of water applied in a full cover spray as recommended for ornamental plants.

Micronutrients applied are presented in Table 3.1. Rose geranium plants were grown for six months before harvest (September 2017 to March, 2018).

Table 3.1 Micronutrients applied in the nutrient solution used to fertigate rose geranium plants.

Mineral	Fertilizer	Application (g/1000 L of water)
Iron (Fe)	Libfer, 13%Iron-EDTA	6.54
Boron (B)	Boric acid	1.89
Molybdenum (Mo)	Ammonium molybdate	0.13
Cupper (Cu)	Copper sulphate	0.03
Zinc (Zn)	Zinc sulphate	1.16

3.5 PARAMETERS

3.5.1 Plant harvest and processing

Plant height, foliar fresh mass, number of branches and leaves, root length were measured at harvesting six months after transplanting. Folia materials were oven dried at 60^{o}C for 96 hours. The dried leaves and stems samples used for mineral analyses were milled to 0.30 mm diameter using a micro hammer mill (Sedibe & Allemann, 2013).

3.5.2 Chlorophyll content

The chlorophyll content was determined randomly from the upper six mature leaves on the crop using a portable non-destructive chlorophyll meter (Optisciences CCM 200, USA), following a procedure described by Chen & Black (1992). The chlorophyll content was determined at harvesting.

3.5.3 Mineral content

Stems and leaves of harvested materials of rose geranium were oven dried and used for determination of mineral contents (N, P, K, Ca, Mg, S, Fe, Zn, Cu and B) using a Dumas combustion method (Etheridge *et al*., 1998; Matejovic, 1996) with Leco FP-528 combustion nitrogen analyser (LecoCorp. St. Joseph, MI, USA). Porcelain weighed samples were loaded in the combustion chamber (1300°C) using an automated sample loader (CNS 2000, Operation Manual, Leco, St. Joseph, MI, USA). The N_2 contents were measured by a thermal conductivity cell.

Total concentration of S, Fe, Zn, B, and Cu in leaves and stems were determined using atomic emission spectrometry with Inductive Coupled Plasma Optical Emission Spectrometric (ICP-OES) (Optima 4300 DV, ICP-OES, PerkinElmer Inc. USA). The ICP-OES is a collective instrument used for analysing all essential plant elements, with only a few seconds between each element. Each element was measured at an appropriate emission wavelength, chosen for high sensitivity and lack of spectral interference. The wavelengths set were 383.826 nm and 422.673 nm for all the elements analysed (Zasoski & Burau, 1977).

Calcium was determined with finely milled samples of 0.8g rose geranium dry mass, stirred in 40 ml of 0.5 N Hydrochloric acid for five minutes using a reciprocating shaker. A Calcium hollow cathode lamp was used at 15 Mao Air and acetylene flow rates were set to reduce (rich, slightly yellow) flame. Calcium standard stock solution was prepared by dissolving calcium carbonate in hydrochloric acid. A final concentration of 1% Lanthanum was used to prevent interferences from P and Al in the sample (Sahrawat, 1980).

Magnesium concentration was determined using an ICP emission spectrometry (PerkinElmer Optima 7300, Waltham, Massachusetts, USA) by weighing the dried sample of 0.8g after drying at 105^{o}C and wet-combusted in a mixture of nitric acid/perchloric acid.

3.5.4 Essential oil extraction

Rose geranium oil was extracted from the leaves of a fresh plant material using a custom-built steam distillation (Sedibe & Allemann, 2012). The material was distilled at the temperature of ± 90°C for one hour using the method of Sedibe & Allemann (2012).

3.5.5 Oil composition

Oil quality was determined using a gas chromatography (GC) using an Aligent GC (FID) model 6890N fitted with 30 m x 0.25 mm DB-5 fused silica capillary column and film thickness of 0.25 µm. Samples of 1.0 µl of a 10% solution of the respective oil in hexane were introduced by split injection with a split ratio of 100. Both the injection port and oven temperatures were set at 250 and 70^{o}C, respectively. Helium was a gas carrier at a linear velocity of 37 cm sec^{-1}. The temperature programs started at 70^{o}C isothermally for 3 minutes, increasing gradually by 3^{o}C min^{-1} to 280^{o}C and were held for 10 min (Adams, 2004).

3.6 DATA ANALYSIS

Analysis of variance was conducted using General Linear Model (GLM), statistical analysis software program (SAS inst., 2002). The Tukey's Student Range Test was used to separate means that were significantly different at P=0.05 as described by Steel & Tourie (1980). The principal component analysis (PCA) based on the linear correlation between variables and loading factors was used in multivariate analysis. Thereafter, a stepwise method was used for multivariate analyses using a derived PC factor with eigenvalues greater than 1 (Kaiser, 1960).

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CHAPTER 4

EFFECT OF CALCIUM AND MAGNESIUM RATIOS EXPOSED TO MAGNETIC FIELD AND MYCORRHIZAL FUNGI ON OIL AND AGRONOMIC ATTRIBUTES AND MINERAL UPTAKE OF ROSE GERANIUM

Abstract

The objective of this study was to evaluate the effects of the Ca:Mg ratio, magnetic field (MF), and mycorrhizal amendment on the yield and mineral composition of rose geranium. The experiment was structured as a 3×2 factorial experimental design, with three levels of Ca:Mg ratios (2.40:6.78, 4.31:4.39, and 6.78:2.40 meq L^{-1}), two levels of MF (zero MF, denoted by 0MF, and 110.1 mT, denoted by 1MF), and two levels of mycorrhizal amendment (none, denoted by 0Myco, and 20ml of mycorrhizae amendment per plant, denoted by 1Myco) as a split-plot factor. The results show that the plant height and branch dry mass were significantly ($p < 0.05$) affected by the Ca:Mg ratio. No significant effect of Ca:Mg ratio, MF, or mycorrhizae on the number of leaves, foliar mass, leaf dry mass, or yield was detected. Phosphorus, K, S, Fe, and B accumulation in the stem were unaffected, as were leaf N, P, K, Ca, S, Fe, B, and Cu. However, some agronomic attributes (plant height, number of branches, root length, and chlorophyll content) and mineral composition (Stem-N) were optimized when the 1MF exposed nutrient solution was used with approximately equal proportions of Ca and Mg (4.31:4.39 meq L^{-1}). This Ca:Mg ratio in the nutrient solution, together with the exposure of rose geranium plants to 1MF, yielded positive results. The findings of this study can be applied to improve the production of rose geranium by enhancing the growth and mineral utilization of this crop.

4.1 INTRODUCTION

The fundamental goal of applying fertilizers is to supply nutrients that are essential for crop growth and yield increment. Crop yield is the basic factor that determines optimal fertilization (Ju & Christie, 2011). Therefore, it is important to apply fertilizers in an efficient way to minimize loss and to improve nutrient use efficiency of crops (Muhammad *et al*., 2017). Wise application of nutrients may enhance crop quality, however, oversupply has harmful effects, for instance, it can lead to reduction of seed formation by encouraging excessive vegetative growth (Patel *et al*., 2015). Mengel & Kirkby (2001) reported that fertiliser applied during vegetative stage must be well balanced to provide nutrition for optimum growth of the plant.

Rose geranium responds well to nutrient application; however, the response varies with time and growth stage of the application (Alveiro *et al*., 2017; Araya, 2012; Taiz & Zeiger, 2002). Also, if the crop requirements are not met, shortages lead to negative impact on rose geranium oil yield and quality (Araya, 2012). For instance, lack of nitrogen led to poor yield and reduced chlorophyll content (Sedibe & Allemann, 2012; Welch *et al*., 1993). Nonetheless, rose geranium may require a well-balanced fertiliser application more especially, in fertigation systems (Sedibe, 2012). Rose geranium responds well to N, P and K application (Sedibe & Allemann, 2013; Sedibe 2012; Araya *et al*., 2006). Amongst the essential elements, calcium and magnesium plays a fundamental role in improving the quantitative and qualitative attributes as well as agronomic attributes of crops grown in greenhouses or field conditions.

Calcium plays an important role in the physiological development of the plant, such as, cell elongation, cell maturation, meristematic tissue development and protein synthesis. Calcium is also required for the stability and functions of cell walls (White & Broadley, 2003). Inadequate application of Ca has a negative impact on plants, leading to the collapse of the cell wall (Gilliham *et al*., 2011; Sander & Andren, 1997).

Similarly, an adequate Mg nutrition is required for root and shoot growth, in terms of biosynthesis and translocation of photo assimilates. Magnesium plays a critical role in phloem loading and transportation of photo assimilates into sink organs (Zhang & Turgeon, 2009).

Plants under low Mg supply are very sensitive to light intensity and heat stress and can easily become chlorotic and necrotic, probably due to extensive production of reactive oxygen (Cakmak & Kirkby, 2008). There is limited information available in connection to the effect that Ca and Mg has on rose geranium production.

Biostimulation of crops with external application of MF as a way to improve plant physiology together with oil quantity and oil composition to meet Bourbon standard has caught the interest of many scientists around the world. The external application of MF on nutrient's modification, microorganism and biological system has become more and more important as new evidence reveals the ability of plants and microorganisms to perceive and respond quickly to MF (Occhipinti *et al*., 2014; Vanderstraeten & Burda, 2012; Kordas, 2012). However, the biological effects of MF treatments depend on the strength and exposure period of the plant. The interaction of MF and exposure time indicated that a certain combination of MF and duration are highly effective in enhancing growth characteristics.

There is also a growing interest from hydroponic rose growers in biologically based approaches to plant production in order to reduce the utilisation of high amounts of fertilisers and pesticides. Various studies show that application of mycorrhizae can improve; growth and yield through improved nutrient uptake, particularly under conditions of limited water supply, low-quality irrigation water, low soil fertility, high daytime temperatures with high evapotranspiration rates, or soil salinity (Abdel-Rahman *et al*., 2011; Al-Karaki, 2000). Amongst numerous benefits to plants, mycorrhiza increases the absorption of mineral elements, enhances defence against pathogens and drought conditions (Jeffries *et al*., 2003).

Therefore, the objective of this study was to evaluate the effect of Ca:Mg ratio, MF amended with mycorrhizae on yield and mineral composition of rose geranium.

4.2 MATERIAL AND METHODS

The description of the trial and treatments, experimental design and agronomic practices employed in this experiment are fully detailed in chapter 3; this section only focuses on the relevant material and method used throughout the experimental trial of this chapter.

4.2.1 Experimental treatments

Nutrient solution was prepared to meet all the three levels of Ca:Mg ratios (Table 4.1). Micronutrients' concentration used in this study is tabulated in Table 4.2. However, the rest of the cations and anions were applied at 5.30 K^+, 1.00 NH_4, 10.18 NO^{3-}, 1.60 $H_2PO_4^-$, 3.12 $SO_4^=$, 0.28 Cl^- and 0.4 HCO_3^- meq L^{-1}. A pH level of 5.5 was maintained by adding 79 ml of nitric acid to 1000 L of water and the final EC was maintained at 1.55 mS cm^{-1}. Water analyses of the feeding municipality water used for formulation of the nutrient solution are given in Table 4.2.

Table 4.1 Fertilizers formulation used to meet the ratio of Ca:Mg of 2.40:6.78, 4.31:4.39 and 6.78:2.40 meq L^{-1}.

	Application (g 1000L^{-1} of water)		
Ratio (meq L^{-1})	LCa:HMg 2.40:6.78	SCa:SMg 4.31:4.39	HCa:LMg 6.78:2.40
Fertilizers			
KNO_3	493	471	341
K_2SO_4	0	0	85
KH_2PO_4	102	129	156
$NH_4H_2PO_4$	98	75	52
$5Ca(NO_3)_2.NH_4NO_310H_2O$	182	418	655
$MgSO_4.7H_2O$	365	365	241
$Mg(NO_3)_2$	384	129	0

Table 4.2 Mineral concentration analyses of the Mangaung municipality water used to prepare the nutrient solution used in this study.

Mineral concentration (mmol L^{-1})											
Na^+	NH_4^+	K^+	Ca^{++}	Mg^{++}	NO_3^-	P	$SO_4^=$	Cl^-	HCO_3^-	Ph	EC (mS/cm)
0.54	0	0.07	0.79	0.41	0	0	0.23	0.28	1.42	7.8	0.21

4.2.2 PARAMETERS

4.2.2.1 Agronomic attributes

Plant height, foliar fresh mass, number of branches and leaves, root length were measured at harvesting six months after transplanting. Folia materials were oven-dried at 60ºC for 96 hours. The dried leaves and stems samples used for mineral analyses were milled to 0.30 mm diameter using a micro hammer mill (Sedibe & Allemann, 2013).

4.2.2.2 Chlorophyll content

The chlorophyll content was determined randomly from the upper six mature leaves on the crop using a portable nondestructive chlorophyll meter (Optisciences CCM 200, USA), following a procedure described by Chen & Black (1992). The chlorophyll content was determined at harvesting.

4.2.2.3 Mineral content

Oven dried materials were used for determination of mineral contents (N, P, K, Ca, Mg, S, Fe, Zn, Cu and B) using a Dumas combustion method (Etheridge *et al*., 1998; Matejovic, 1995) with Leco FP-528 combustion nitrogen analyzer (LecoCorp. St. Joseph, MI, USA).

4.2.2.4 Essential oil extraction

Rose geranium oil was extracted from the fresh plant material using a custom-built steam distillation (Sedibe & Allemann, 2012). The material was distilled at the temperature of ± 90°C for one hour using the method of Sedibe & Allemann (2012).

4.2.2.5 Oil composition

Oil quality was determined using a gas chromatography (GC) using an Agilent GC (FID) model 6890N fitted with 30 m x 0.25 mm DB-5 fused silica capillary column and film thickness of 0.25 µm. Samples of 1.0 µl of a 10% solution of the respective oil in hexane were introduced by split injection with a split ratio of 100. Both the injection port and oven temperatures were set at 250 and 70^{o}C, respectively. Helium was a gas carrier at a linear velocity of 37 cm sec^{-1}. The temperature programs started at 70^{o}C isothermally for 3 minutes, increasing gradually by 3^{o}C min^{-1} to 280^{o}C and were held for 10 min (Adams, 2004).

4.2.2.6 Data Analysis

Analysis of variance was conducted using General Linear Model (GLM), statistical analysis software program (SAS inst., 2002). The Tukey's Student Range Test was used to separate means that were significantly different at P=0.05 as described by Steel & Tourie (1980).

4.3 RESULTS

The results of plant height, number of branches, number of leaves, foliar fresh mass, root length, chlorophyll, leaf dry mass, root dry mass and oil yield are shown in Table 4.3.

Table 4.3 Summary of the analyses of variance table depicting the $P_{0.05}$ value of agronomic attributes of rose geranium subjected to ratio of Ca:Mg, magnetic field and mycorrhizal.

	Agronomic attributes $P_{0.05}$ value								
Treatments	**Plant height**	**Number of branches**	**Number of leaves**	**Foliar fresh mass**	**Roots length**	**Chlorophyll**	**Leaf dry mass**	**Roots dry mass**	**Oil yield**
Ratio	**0.05***	0.20^{NS}	0.33^{NS}	0.08^{NS}	0.27^{NS}	0.06^{NS}	0.13^{NS}	0.10^{NS}	1.00^{NS}
Magnetic field	**0.01****	**0.01****	0.79^{NS}	0.83^{NS}	**0.05***	0.67^{NS}	0.35^{NS}	0.13^{NS}	0.20^{NS}
Ratio x magnetic field	**0.01****	0.10^{NS}	0.92^{NS}	0.96^{NS}	0.14^{NS}	0.53^{NS}	0.31^{NS}	0.07^{NS}	0.30^{NS}
Mycorrhizae	0.19^{NS}	0.36^{NS}	0.30^{NS}	0.55^{NS}	0.20^{NS}	0.21^{NS}	0.38^{NS}	0.49^{NS}	0.45^{NS}
Ratio x mycorrhizae	0.69^{NS}	0.40^{NS}	0.35^{NS}	0.83^{NS}	0.38^{NS}	**0.05***	0.39^{NS}	0.75^{NS}	0.67^{NS}
Magnetic field x mycorrhizae	0.35^{NS}	0.48^{NS}	0.93^{NS}	0.72^{NS}	0.92^{NS}	0.11^{NS}	0.73^{NS}	0.86^{NS}	0.64^{NS}
Ratio x magnetic field x mycorrhizae	0.45^{NS}	0.87^{NS}	0.56^{NS}	1.00^{NS}	0.22^{NS}	0.83^{NS}	0.23^{NS}	0.16^{NS}	1.00^{NS}

*Values with NS= Not significant at the P- value of 0.05, *significant at the P-value ≤0.05, and **significant at the P-value ≤0.01*

4.3.1 Agronomic attributes

Number of leaves, foliar fresh mass, root dry mass and oil quality were not affected by the different Ca:Mg ratios, MF exposure and mycorrhizae application. However, the interaction between Ca:Mg ratio's and exposure of rose geranium plants to 1MF had a significant (p=0.01) effect on plant height (Table 4.3). Figure 4.1 show that applying Ca and Mg in concentrations of 4.31 meq L^{-1} respectively, for each element in the nutrient solution exposed to MF resulted in plants that were much taller but not significantly taller than plants exposed to 1MF and grown at a relatively high Ca, 6.78 meq L^{-1} with low Mg, 2.40 meq L^{-1}.

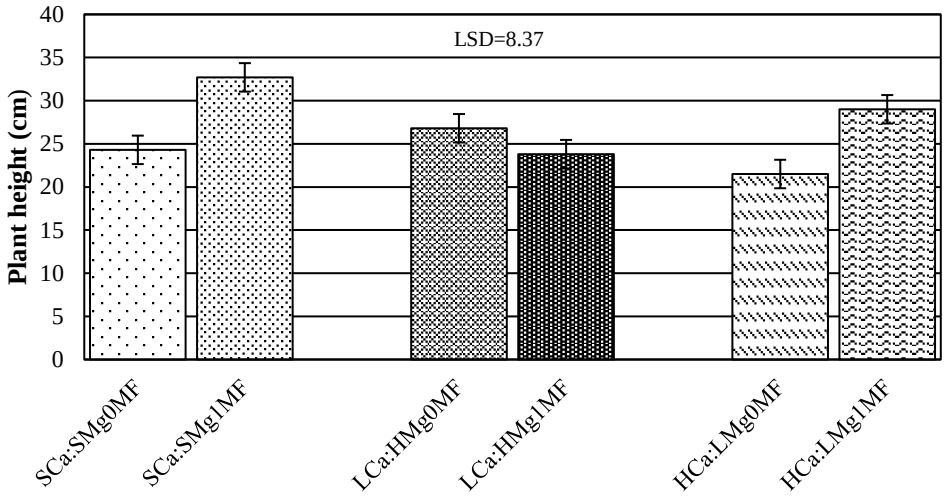


Figure 4.1 *Effect of the interaction of Ca:Mg ratio, and magnetic field on the height of rose geranium. LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg=High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39 meq L^{-1}, 0MF = zero magnetic field, 1MF=110.1mT magnetic field. Vertical lines on bars represent SE (n=3).*

Magnetic field significantly affected the number of branches (p=0.01) and the length of rose geranium roots (p=0.05) (Table 4.3). The exposure of rose geranium plants to 1MF promoted the formation of more branches and resulted in longer roots Figures 4.2 and 4.3.1MF

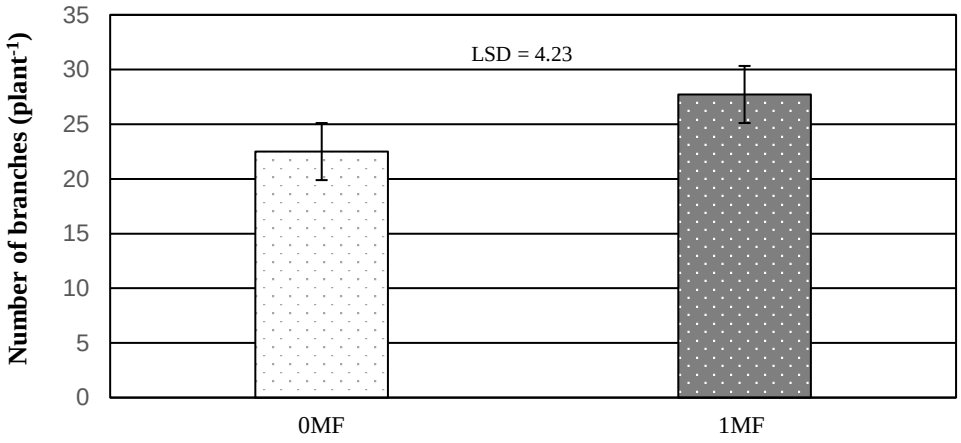


Figure 4.2 *Effect of magnetic field on number of branches of rose geranium. 0MF= zero magnetic field, 1MF=110.1mT magnetic field. Vertical lines on bars represent SE (n=3).*

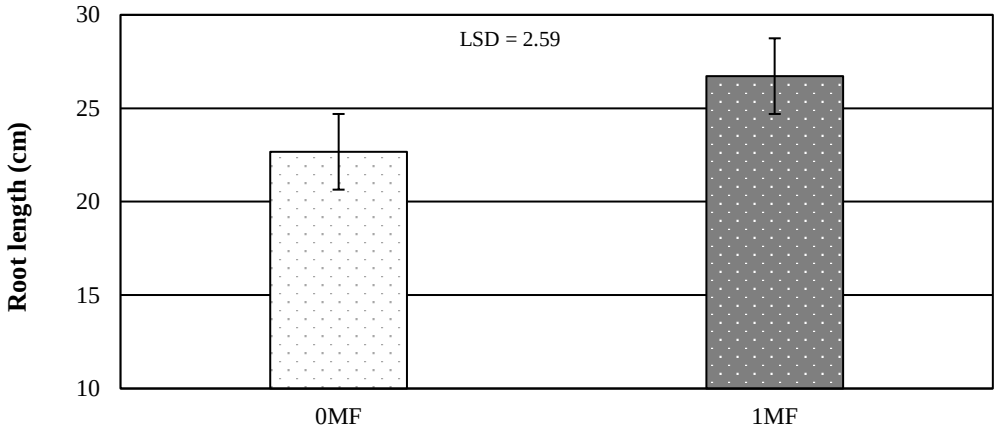


Figure 4.3 *Effect of magnetic field on the length of rose geranium roots. 0MF=zero magnetic field, 1MF=110.1mT magnetic field. Vertical lines on bars represent SE (n=3).*

The interaction between Ca:Mg ratio and MF had a significant (p= 0.05) effect on chlorophyll content. Increased chlorophyll content was found on plants exposed to 1MF and where the equal proportion of Ca:Mg (4.31:4.39meq L^{-1}) was used (Table 4.3; Figure 4.4).

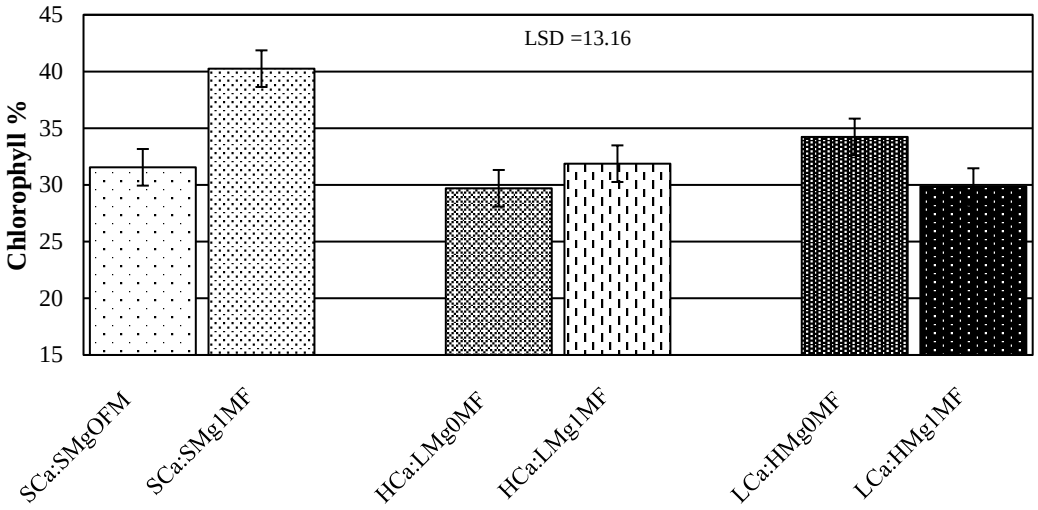


Figure 4.4 *Effect of the interaction between Ca:Mg ratio and magnetic field on chlorophyll content of rose geranium. LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg=High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39 meq L^{-1}, 0MF= zero magnetic field, 1MF= 110.1mT magnetic field. Vertical lines on bars represent SE (n=3).*

4.3.2 Stem mineral composition

Table 4.4 Summary of analyses of variance table depicting the $P_{0.05}$ value of stem mineral composition of rose geranium subjected to ratio of Ca:Mg, magnetic field and mycorrhizal.

	Mineral composition $P_{0.05}$ value									
Treatments	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Fe**	**Zn**	**B**	**Cu**
Ratio	0.55^{NS}	0.30^{NS}	0.09^{NS}	$\mathbf{0.01}^{**}$	$\mathbf{0.01}^{**}$	0.96^{NS}	0.30^{NS}	0.25^{NS}	0.60^{NS}	$\mathbf{0.01}^{*}$
Magnetic field	0.07^{NS}	0.88^{NS}	0.56^{NS}	0.93^{NS}	0.51^{NS}	0.83^{NS}	0.10^{NS}	$\mathbf{0.05}^{*}$	0.20^{NS}	0.21^{NS}
Ratio x magnetic field	0.76^{NS}	0.78^{NS}	0.41^{NS}	0.18^{NS}	0.46^{NS}	0.84^{NS}	0.35^{NS}	0.06^{NS}	0.72^{NS}	0.30^{NS}
Mycorrhizae	0.41^{NS}	0.44^{NS}	0.81^{NS}	0.47^{NS}	0.46^{NS}	0.90^{NS}	0.95^{NS}	0.23^{NS}	0.39^{NS}	0.14^{NS}
Ratio x mycorrhizae	0.07^{NS}	0.63^{NS}	0.56^{NS}	0.25^{NS}	0.93^{NS}	0.84^{NS}	0.08^{NS}	$\mathbf{0.05}^{*}$	0.25^{NS}	0.44^{NS}
Magnetic field x mycorrhizae	0.45^{NS}	0.85^{NS}	0.76^{NS}	0.36^{NS}	0.19^{NS}	0.60^{NS}	0.77^{NS}	$\mathbf{0.01}^{**}$	1.00^{NS}	$\mathbf{0.01}^{**}$
Ratio x magnetic field x mycorrhizae	$\mathbf{0.05}^{*}$	0.91^{NS}	0.69^{NS}	0.56^{NS}	0.47^{NS}	0.16^{NS}	0.23^{NS}	0.19^{NS}	0.22^{NS}	$\mathbf{0.01}^{**}$

*Values with NS= Not significant at the P- value of 0.05, *significant at the P-value ≤0.05, and **significant at the P-value ≤0.01*

Nitrogen

The interaction between Ca:Mg ratio, MF and mycorrhizae had a significant (p=0.05) effect on N accumulation in the stem (Table 4.4). Figure 4.5 shows 2.3% N accumulated in the rose geranium stem that were fertigated with nutrient solution that had equal ratios of Ca and Mg of 4.31:4.39 and MF.

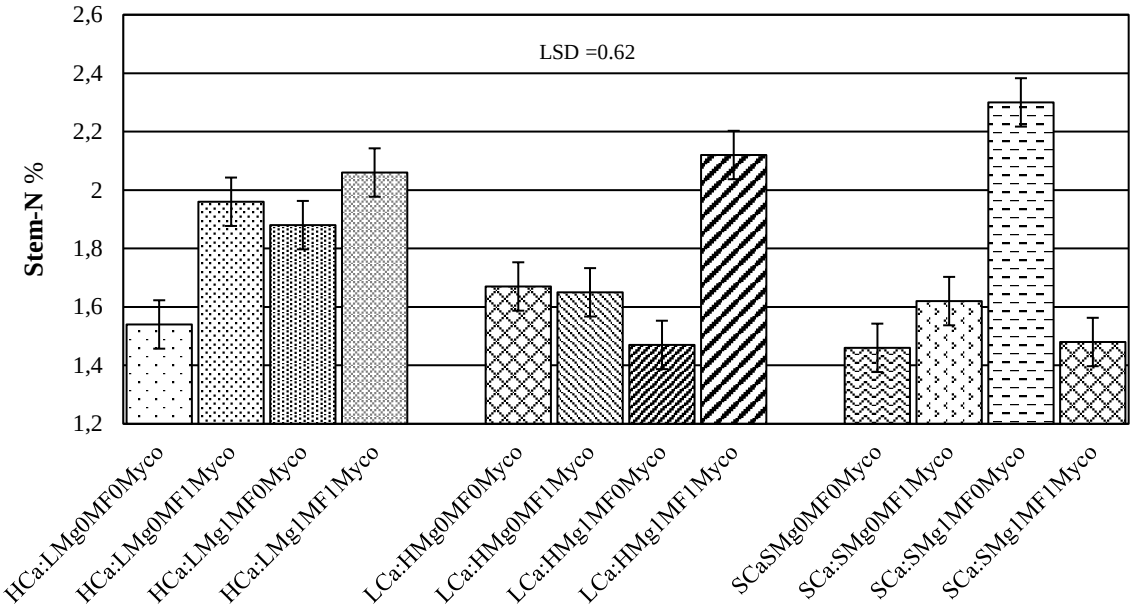
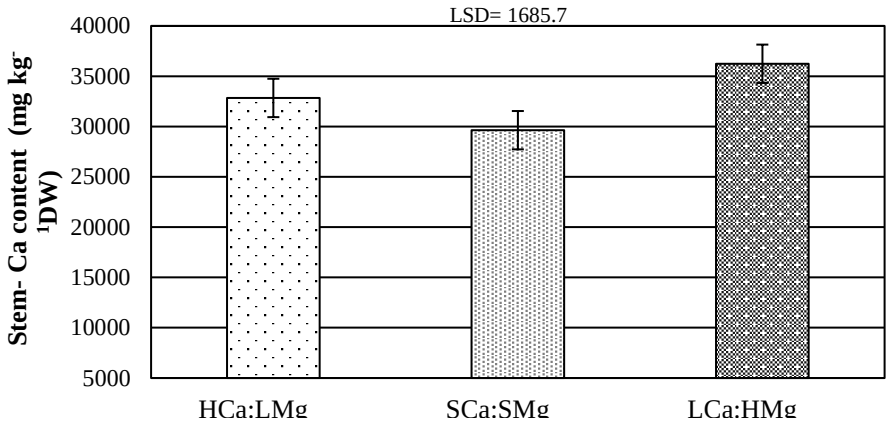


Figure 4.5 *Effect of the interaction between ratio, magnetic field and mycorrhizae on stem N concentration of rose geranium. LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg=High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39 meq L^{-1}, 0MF= zero magnetic field, 1MF=110.1mT magnetic field, 0Myco =zero mycorrhizae, 1Myco= 20ml per plant mycorrhizae. Vertical lines on bars represent SE (n=3).*

Calcium and magnesium

The concentrations of Ca and Mg in the stems of the tested rose geranium plants were affected by the ratio between Ca to Mg at p=0.01 (Table 4.4). As illustrated on Figures 4.6 A and B, both mineral accumulation on the stem were increased by the supply of 2.40 Ca meq L^{-1} and 6.78 Mg meq L^{-1}.

A



B

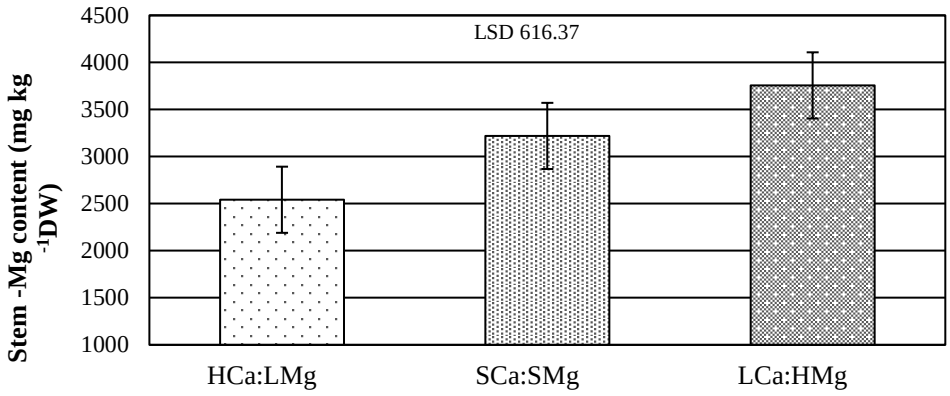


Figure 4.6 *Effect of Ca:Mg ratio on the A) Ca and B) Mg content in the stems of geranium. LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg=High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39 meq L^{-1}. Vertical lines on bars represent SE (n=3).*

Zinc

Interaction between Ca:Mg ratio and mycorrhizae had a significant (p=0.05) effect on Zn accumulation in the stem (Table 4.4). Application of equal proportion of Ca and Mg (4.31:4.39 meq L^{-1}) in the applied nutrient solution amended with mycorrhizae increased Zn accumulation in the stem when compared to all the treatments tested in this study (Figure 4.7).

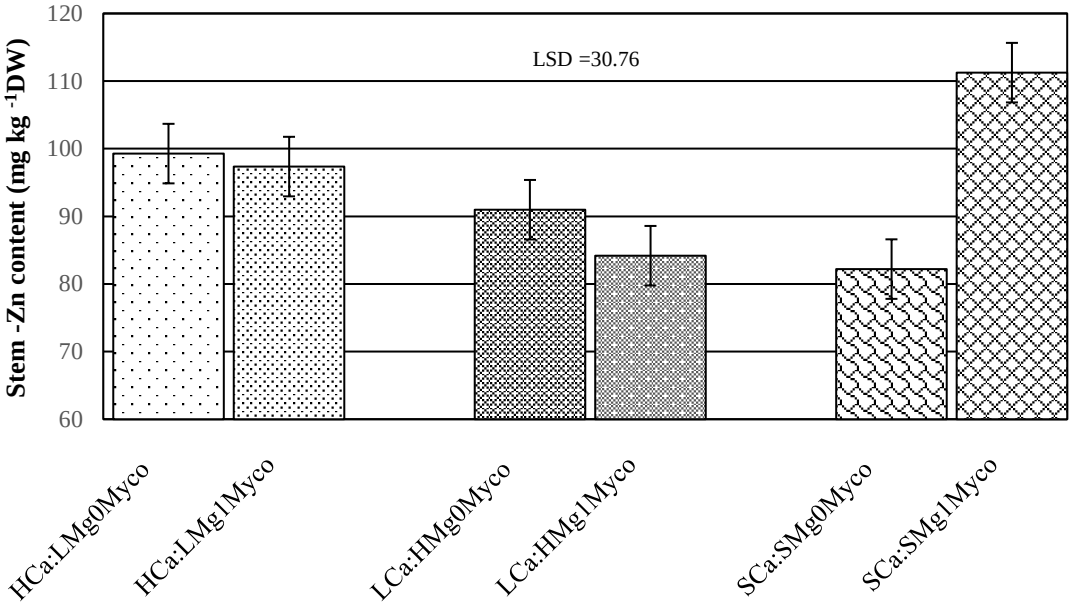


Figure 4.7 *Effect of the interaction between Ca:Mg ratio and Mycorrhizae on Zn concentration of rose geranium stems. LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg=High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39 meq L^{-1}, 0Myco=zero mycorrhizae, 1Myco=20ml per plant mycorrhizae. Vertical lines on bars represent SE (n=3).*

Copper

The interaction between Ca:Mg ratios, MF and mycorrhizae treatments had a significant (p=0.01) effect on the Cu accumulation in the stem (Table 4.4). Figure 4.8 shows that application of Ca and Mg, 6.78:2.40 meq L^{-1} with a zero MF and with application of 20 ml of mycorrhizae per plant and also with 110.1mT MF increased the level of Cu in the same way as 4.31:4.39 meq L^{-1} of Ca:Mg without MF but with a mycorrhizae amendment (Figure 4.8).

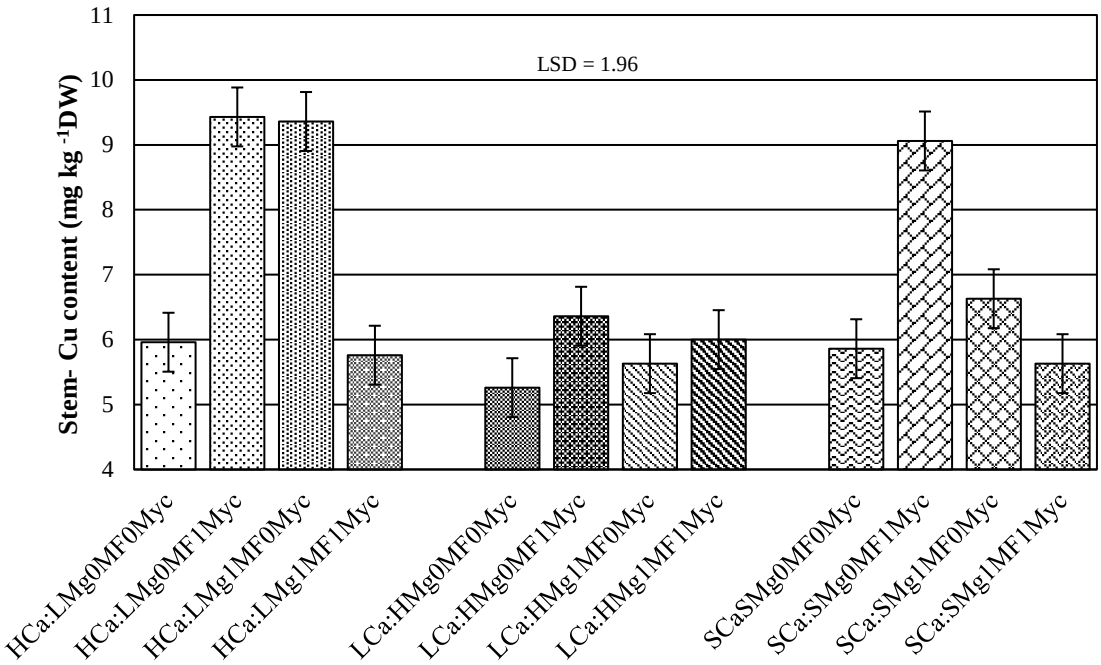


Figure 4.8 *Effect of the interaction between Ca:Mg ratio, magnetic field and mycorrhizae on Cu concentration of rose geranium stem. LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg=High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39 meq L^{-1}, 0MF= zero magnetic field, 1MF=110.1mT magnetic field, 0Myco =zero mycorrhizae, 1Myco= 20ml per plant mycorrhizae. Vertical lines on bars represent SE (n=3).*

Table 4.5 Summary of analyses of variance table depicting the $P_{0.05}$ value of variation in mineral composition in leaves of rose geranium supplied with Ca:Mg, magnetic field and mycorrhizal.

	Leaf mineral composition $P_{0.05}$ value									
Treatments	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Fe**	**Zn**	**B**	**Cu**
Ratio	0.33^{NS}	0.65^{NS}	0.08^{NS}	0.10^{NS}	$\mathbf{0.01}^{**}$	0.72^{NS}	0.38^{NS}	$\mathbf{0.05}^{*}$	0.51^{NS}	0.19^{NS}
Magnetic field	0.79^{NS}	0.63^{NS}	0.86^{NS}	0.90^{NS}	0.54^{NS}	0.45^{NS}	0.27^{NS}	0.48^{NS}	0.78^{NS}	0.10^{NS}
Ratio x magnetic field	0.92^{NS}	0.23^{NS}	0.15^{NS}	0.25^{NS}	0.33^{NS}	0.70^{NS}	0.24^{NS}	0.36^{NS}	0.72^{NS}	0.26^{NS}
Mycorrhizae	0.30^{NS}	0.41^{NS}	0.46^{NS}	0.94^{NS}	0.42^{NS}	0.81^{NS}	0.99^{NS}	0.17^{NS}	0.66^{NS}	0.88^{NS}
Ratio x mycorrhizae	0.35^{NS}	0.87^{NS}	0.78^{NS}	0.80^{NS}	0.12^{NS}	0.28^{NS}	0.08^{NS}	0.40^{NS}	0.23^{NS}	0.13^{NS}
Magnetic field x mycorrhizae	0.93^{NS}	0.86^{NS}	0.20^{NS}	0.81^{NS}	0.25^{NS}	0.70^{NS}	0.86^{NS}	0.50^{NS}	0.17^{NS}	0.87^{NS}
Ratio x magnetic field x mycorrhizae	0.56^{NS}	0.47^{NS}	2.00^{NS}	0.13^{NS}	0.41^{NS}	0.28^{NS}	0.68^{NS}	0.09^{NS}	0.67^{NS}	0.54^{NS}

*Values with NS= Not significant at the P- value of 0.05, *significant at the P-value ≤0.05, and **significant at the P-value ≤0.01*

4.3.3 Leaf mineral composition

Magnesium

The concentration of Mg in leaves of rose geranium was significantly (p= 0.01) affected by the ratio of Ca:Mg (Table 4.5; Figure 4.9). When calcium and Mg were applied at a ratio of 2.40:6.78 meq L^{-1} it enhanced the concentration of Mg in the leaves, but this was similar to the effect of supplying Ca:Mg at equal proportion . This study revealed that, the concentration of Mg in the leaves decreased significantly with the reduced Mg and increased Ca (2.40:6.78 meq L^{-1}) in the nutrient solution.

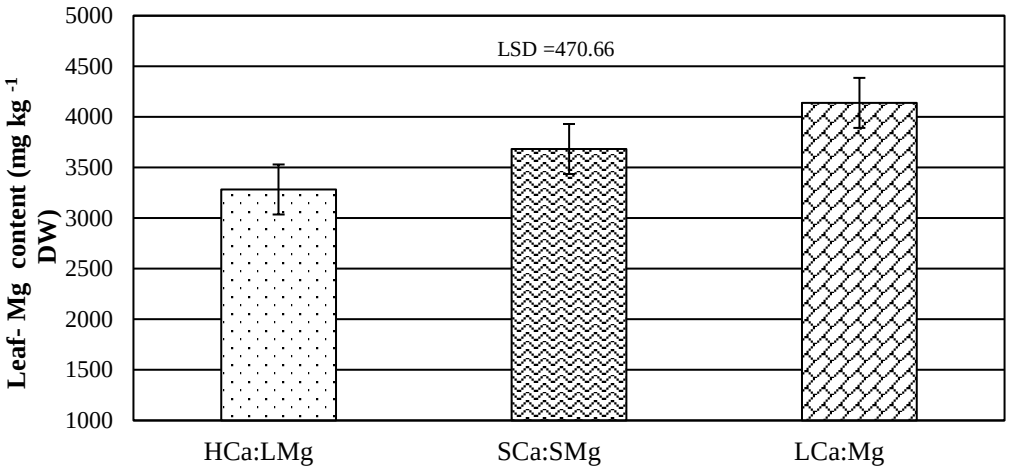


Figure 4.9 *Effect of ratios of Ca:Mg concentration on Mg in rose geranium leaves. LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg=High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39meq L^{-1}. Vertical lines on bars represent SE (n=3).*

Zinc

There was no significant effect of Ca:Mg ratios on B, Fe and Cu contents in the leaves of rose geranium. However, a significant effect of ratios was found on leaf -Zn content (p= 0.05). Equal ratio of Ca:Mg (4.31:4.39 meq L^{-1}) in the nutrient solution had significant influence on

Zn concentration in the leaf when compared to other treatments (Figure 4.10) of LCa:HMg and HCa:LMg.

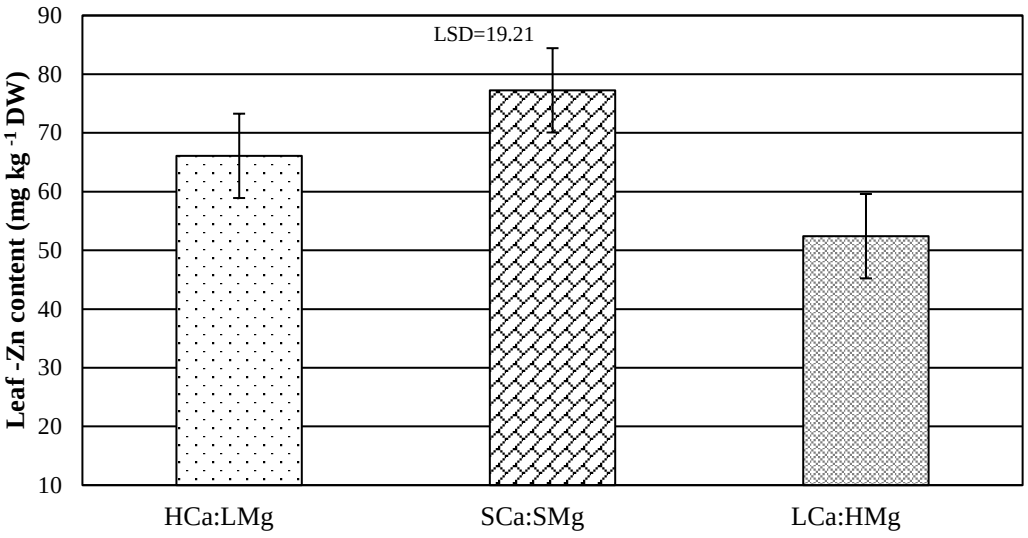


Figure 4.10 *Effect of ratios of Ca:Mg on Zn concentration in rose geranium leaves. LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg= High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39 meq L^{-1}. Vertical lines on bars represent SE (n=3).*

4.3.4 Oil quality

There was no significant effect of ratios, magnetic field, mycorrhizae and the interaction between the ratios, MF and mycorrhizae. However, the Citronellol/Geraniol (C:G) ratio, which favour essential oil of rose geranium obtained under these treatments were within the desired ratio of the perfume industry which is below 3.0.

4.3.5 Multivariate analysis on agronomic attributes

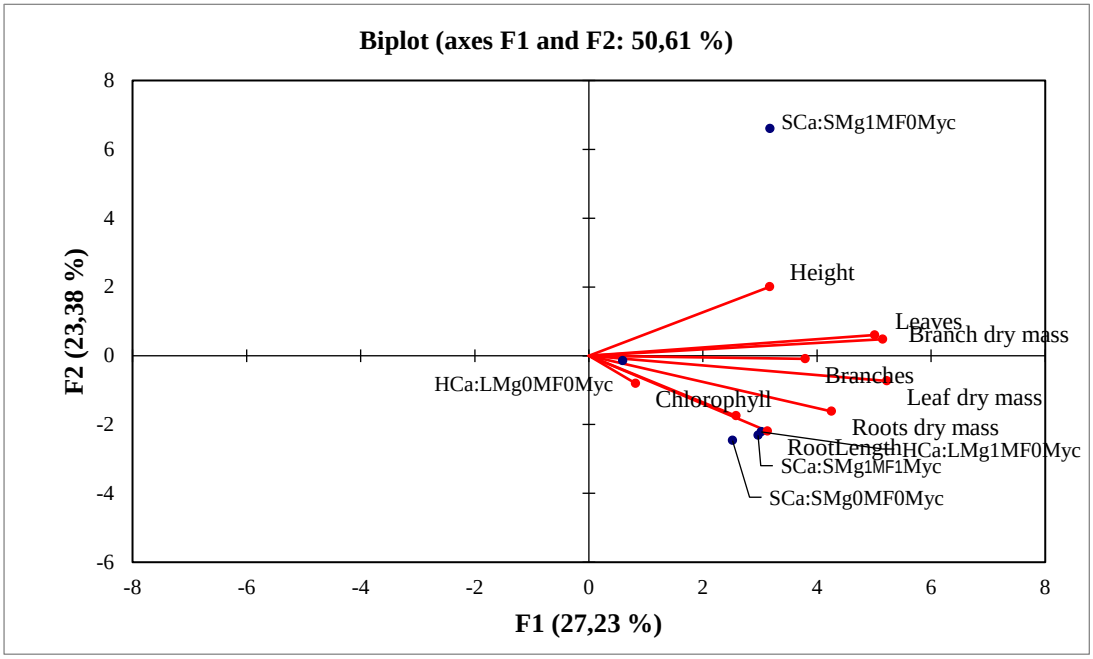


Figure 4.11 *Rotated principal component loadings of agronomic attributes. LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg=High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39 meq L^{-1}, 0MF= zero magnetic field, 1MF=110.1mT magnetic field, 0Myco =zero mycorrhizae, 1Myco= 20ml per plant mycorrhizae.*

To further understand the effect of Ca:Mg ratios, MF and the use of mycorrhizae on agronomic attributes of rose geranium cultivation, a PCA was conducted. Figure 4.11 showed that plant height, number of leaves per plant and the branch dry mass were positively loaded on PC2, where SCa:SMg1MF0Myco treatment was used. This effect of the number of leaves per plant is essential, as this is where most of trichomes containing oil gland are located (Sedibe, 2012). However, SCa:SMg0MF0Myco on PC1 showed a negative loading on the number of branches, leaf dry mass and root length (Figure 4.11).

4.4 DISCUSSION

It was a hypothesis of this study that Ca:Mg ratio, MF exposure, and amendment with mycorrhizae has a positive effect on growth and mineral composition of rose geranium. Application of Ca and Mg in approximately equal proportions of 4.31 and 4.39 meq L^{-1} had significant effects on leaf Zn and stem Mg content. The application of Ca and Mg in equal proportions and in combination with either an MF or mycorrhizae had significant effects on plant height, chlorophyll content, stem N, stem Cu, and stem Zn contents. Steiner's universal solution provided 9.00 and 4.00 meq L^{-1} of Ca and Mg, respectively, contributing to 45% and 20% of the total cations (Combrink, 2013). Most of the nutrient solutions developed after Steiner's solution was developed have had the same ratio, even though these concentrations are adjusted to suit specific crop requirements (Combrink, 2013).

Various studies showed a positive relationship between chlorophyll and N content of the leaves (Wang *et al*., 2004; Scheepers *et al*., 1992). Chlorophyll content is used as an alternative measure for the nitrogen status of most plant species (Sedibe & Allemann, 2013; Fontes & de Araujo, 2006). The accumulation of N observed in this study in the case of equal proportions of Ca and Mg, as well as with high Ca and low Mg in combination with no MF or mycorrhizae, could be attributable to the beneficial effect of mycorrhizae or a magnetic field, which are said to improve root structure and N assimilation (Hozayn *et al*., 2016; Brady & Weil, 2002; Tisdale *et al*., 1993). A MF affects the biochemical processes of plants that control free radicals. Magnetic field gradients activate phytohormones such as gibberellic acid, indole-3-acetic acid, and trans-zeatin and also activate proteins and enzymes responsible for stem elongation and branching of plants (Hozayn & Abdul, 2010; Maheshwari & Grewal, 2009). An increase in root length, root surface area and root volume were reported in chickpea exposed to 1MF of 250 mT (Vashisth & Nagarajan, 2008). In the same conditions, seedlings of sunflower showed higher seedling dry mass, root length, root surface area and root volume (Vashisth & Nagarajan, 2010).

The beneficial effects of applying Ca and Mg in approximately equal proportions were observed in this study regardless of whether it interacted with a MF or mycorrhizae. Furthermore, high Ca and low Mg (6.78:2.40 meq L^{-1}) in the nutrient solution significantly reduced the concentration of Mg in the leaves. Spiers & Braswell (2002) reported observing an increase in leaf Ca and a decrease in leaf Mg as a result of Ca application to blueberry plants. They also observed that increasing Mg fertilization resulted in increased leaf Mg content and decreased leaf Ca and leaf K (Spiers & Braswell, 2002). However, Shaul (2002) postulated that the Mg concentrations in different parts of a plant could be small, depending on the amount of Mg in the soil, the plant growth stage, and water stress. Kadir *et al*. (2004) found that a low application dose of Mg (< 2%) reduced leaf concentration. However, no deficiency symptoms of Ca and Mg were observed when Mg and Ca were both added at lower doses (White, 2001).

On the other hand, a ratio of high Ca to low Mg (6.78:2.40 meq L^{-1}) in the nutrient solution caused a small increase in the Zn concentration in comparison to that resulting from a ratio of high Mg to low Ca (6.78:2.40 meq L^{-1}) in the nutrient solution. In contrast, Kawasaki & Moritsugu (1987) documented that application of a Ca nutrient solution significantly reduced Zn absorption and drastically inhibited the translocation of Zn. However, there was no significant relation between the Ca:Mg ratio with 0MF and without mycorrhizae use.

Although plant height and the number of leaves per plant were affected by equal proportions of Mg and Ca with 1MF and mycorrhizae use, this effect did not increase the oil yield of rose geranium. Oil yield and quality data was not presented on this study; however, it was not affected by the Ca:Mg ratio, magnetic field, or mycorrhizae use. These results suggest that the essential oil content is not dependent on the ratio of Ca to Mg, the magnetic field, or mycorrhizae use. In another study, application of calcium carbonate in basil decreased its oil content and linalool concentration; however, methyl chavicol content was increased (Dzida, 2010). In summer savoury, the application of calcium carbonate increased its oil content and carvacrol concentration (Mumivand *et al*., 2011). With oregano, oil yield was increased to 31% by application of calcium and magnesium (Dordas, 2009).

4.5 CONCLUSION

The results of this study show that neither combinations of high Ca and low Mg nor low Ca and high Mg in the nutrient solution, together with mycorrhizae amendment, contribute to increased oil yield, mineral accumulation, or translocation within rose geranium plants. However, agronomic attributes (plant height and chlorophyll content) and mineral composition (Stem-N,) were optimized when approximately equal proportions of Ca and Mg were applied (Ca:Mg = 4.31:4.39 meq L^{-1}) in combination with exposure to a magnetic field. This treatment can be useful in the production of rose geranium by enhancing the growth and mineral utilization of this crop.

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CHAPTER 5

MULTIVARIATE ANALYSIS OF MINERAL COMPOSITION ON THE STEM AND LEAF OF ROSE GERANIUM (*Pelargonium graveolens* L.) AFFECTED BY CALCIUM TO MAGNESIUM RATIO, AMENDED WITH MYCORRHIZAL FUNGI AND EXPOSURE TO MAGNETIC FIELD

Abstract

The objective of this study was to evaluate the effect of Ca:Mg ratios, magnetic field (MF) and mycorrhizal fungi on mineral utilisation by rose geranium. Multivariate analysis was used to reduce redundancy of the data and to find eigenvalue of the correlation matrix. Amongst the observed variables only two principal components accounted for most of the variabilities. The first principal component (PC1) accounted for 27.23%, while the second principal component (PC2) accounted for 23.78% of the total variance. Using the multivariate analysis, we were able to reveal and to understand the antagonistic or synergetic relationship between minerals of rose geranium affected by Ca:Mg ratio and the impairment of polarity due to the lack of MF gradients as well as the use of mycorrhizae.

5.1 INTRODUCTION

Rose geranium oil is one of the most important essential oils, known for its chemical composition used by various industries including cosmetic, aromatherapy, and food (Jade, 2013; Sedibe, 2012). The only existing standard of rose geranium oil is set by the perfume industry that require a citronellol and geraniol ratio of less than three (Sedibe, 2012). To study the accumulation and translocation of minerals within a plant is important as most of the plant minerals are said to have significant influence on the metabolic activities of plants (Rascio & Navari, 2011; Masarovičova, 2010; McCutcheon & Schnoor, 2003; Baker *et al*., 2000).

The challenge with accumulation of minerals in plants is the relationships which one element have with the others. Various authors reported these associations in the past (Mukhomorov & Anikina, 2011). Nevertheless, the accumulation properties of essential oil plants could be exploited to provide key sources of elements. The essential element composition has inherent nutritional value and the deficiencies of these elements (Chan *et al.*, 2012; Passwater & Cranton, 1983).

Principal component analysis and cluster analysis are one of the most used techniques in agricultural sector for dimensional reduction in many disciplines. These components are applied where there is a larger number of variables which may not be sufficient for the discriminatory power of samples evaluation and management. In such cases, multivariable technique is used to set patterns, to eliminate redundancy in data sets, and to distinguish significant relationships between variables (Adams, 2000; Maji & Shaibu, 2012). A PCA (principal component analysis) is often used to explain the correlation between larger set of variation in terms of a small number of underlying independent factors, whereas, cluster analyses are used to cluster these original variables (Abbe *et al.*, 2017; Losif, 2012).

In this study PCA was used to further understand the correlation between plant nutrients synergism and antagonism on rose geranium. The analysis was based on eigenvalue of the correlation matrix and covariance. The model component had a loading factor that showed the performance of the variable (Yu, 2005; Kara, 2009). According to Losif (2012) the size of residual variance plays an important role in the PC model for the selection of variables. Hence, the closer the similarity between the object, the fewer terms are needed in the expansion to achieve certain approximation goodness.

The objective of this study was to assess the complex phenomenon of MF and Ca:Mg ratio amended with mycorrhizae on rose geranium.

5.2 MATERIAL AND METHOD

The experimental tools and method of MF and Ca:Mg ratio used in this study are well explained and described in chapter three of the study.

5.3 RESULTS AND DISCUSSION

5.3.1 Multivariate analysis

A principal component analysis was used to reduce the redundancy of the data and to find eigenvalue of the correlation matrix. Out of eleven principal components used, the first two principal components (PC1 and PC2) accounted for most of the variability, it showed accumulative variability greater than 50.61%. The PC1 accounted for 27.23%, while PC2 accounted for 23.78% of the total variance. The first PC showed that, P, K, Mg, S, Zn in the stem and N, K, S in the leaves were negatively loaded in LCa:HMg1MFMyco, LCa:HMg0MF0Myco, LCa: HMg1MFMyco, while PC2 showed that N, B, Fe, in the stem and P, Ca, Zn in the leaves were positively loaded where SCa:SMg1MF0Myco treatment was applied. However, SCa:SMg1MF0Myco on PC2 showed a negative loading of Fe, Cu in the stem, whereas HCa:LMg showed a negative loading on Cu in the stem. PC1 loadings, show that the decrease in Ca and increased of Mg in the nutrient solution has a negative influence on the mineral accumulation and translocation (Figure 5.1).

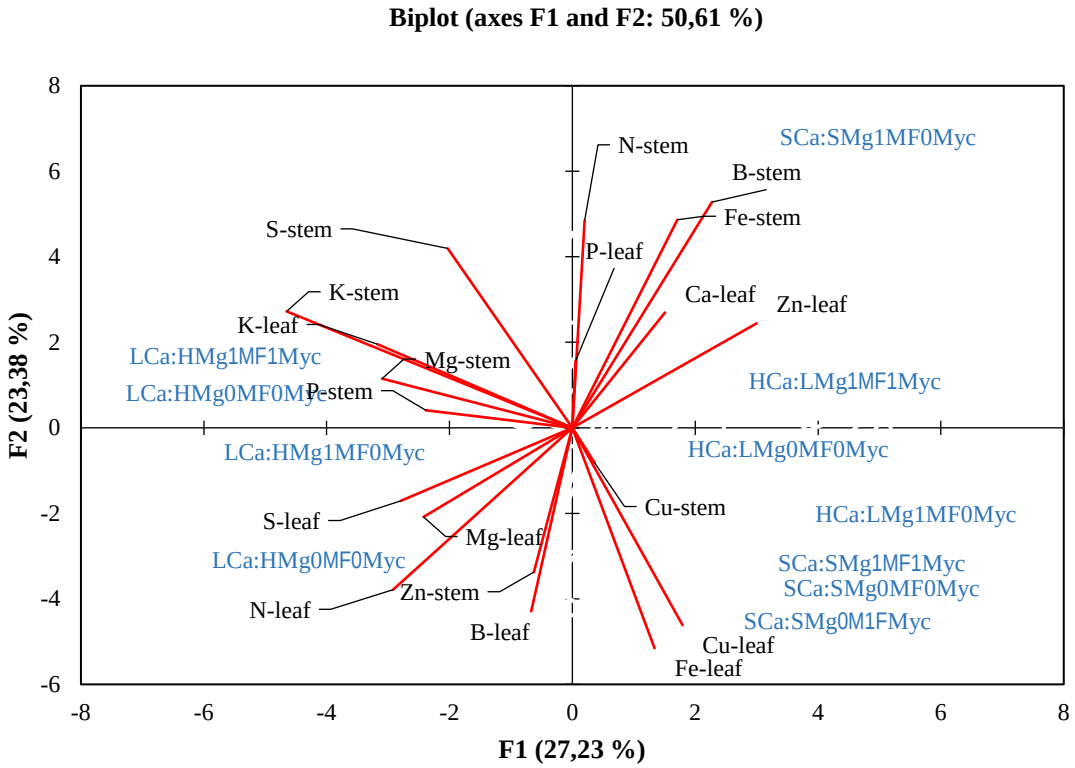

Figure 5.1 *Rotated principal component loadings (mineral content in the stem and leaves of rose geranium). Keys*: *LCa:HMg= Low Ca: High Mg, 2.40:6.78 meq L^{-1}, HCa:LMg=High Ca: Low Mg, 6.78:2.40 meq L^{-1}, SCa:SMg= Equal proportion of Ca and Mg, 4.31:4.39 meq L^{-1}, 0MF= zero magnetic field, 1MF=110.1mT magnetic field, 0Myco =zero mycorrhizae, 1Myco= 20ml per plant mycorrhizae.*

5.3.2 Synergism and antagonism of chemical elements on the stem of rose geranium

Table 5.1 Correlation matrix of minerals showing synergetic and antagonistic relationship on the stem of rose geranium subjected to the ratio of Ca:Mg, magnetic field and mycorrhizal fungi.

Variables	N	P	K	Ca	Mg	S	Fe	Zn	B	Cu
N	**1**									
P	0.381^{NS}	**1**								
K	0.400^{NS}	**0.611******	**1**							
Ca	-0.085^{NS}	-0.172^{NS}	**-0.554*****	**1**						
Mg	0.210^{NS}	0.322^{NS}	**0.464*****	**-0.467*****	**1**					
S	**0.679******	0.430^{NS}	**0.463*****	-0.026^{NS}	**0.452*****	**1**				
Fe	**0.599*****	-0172^{NS}	-0.148^{NS}	0.183^{NS}	-0.011^{NS}	0.411^{NS}	**1**			
Zn	**-0.506*****	-0.076^{NS}	-0.307^{NS}	**0.476*****	-0.137^{NS}	-0.270^{NS}	-0.275^{NS}	**1**		
B	**0.709******	-0.057^{NS}	-0.009^{NS}	0.144^{NS}	0.062^{NS}	**0.647******	**0.792******	**-0.485*****	**1**	
Cu	0,024^{NS}	-0.043^{NS}	-0.251^{NS}	**0.588*****	-0.135^{NS}	0.002^{NS}	0.290^{NS}	**0.666******	0.016^{NS}	**1**

*NS= Not significant at the P- value of 0.05, * significant at the P-value ≤0.05, ** significant at the P-value ≤0.01, *** significant at the P-value ≤0.001, **** significant at the P-value ≤0.0001*

As shown in Table 5.1 and Figure 5.2, mineral content measured on the stem showed that N had a positive and significant correlation with S (r^2 = 0.679), Fe (r^2 = 0.599) and B (r^2 = 0.709). In contrast, increased stem-N accumulation reduced stem-Zn depicting the antagonistic relationship between these minerals (r^2 = -0.506). Similarly, Maruyama *et al*. (2004) and Leustek *et al*. (2000) reported that synergism might be caused by the induction of sulfate transporters 5SULTR1 and SULTR1 depending on the N and C supply. There was a positive and significant correlation between N and B in the leaves (r=0.709).

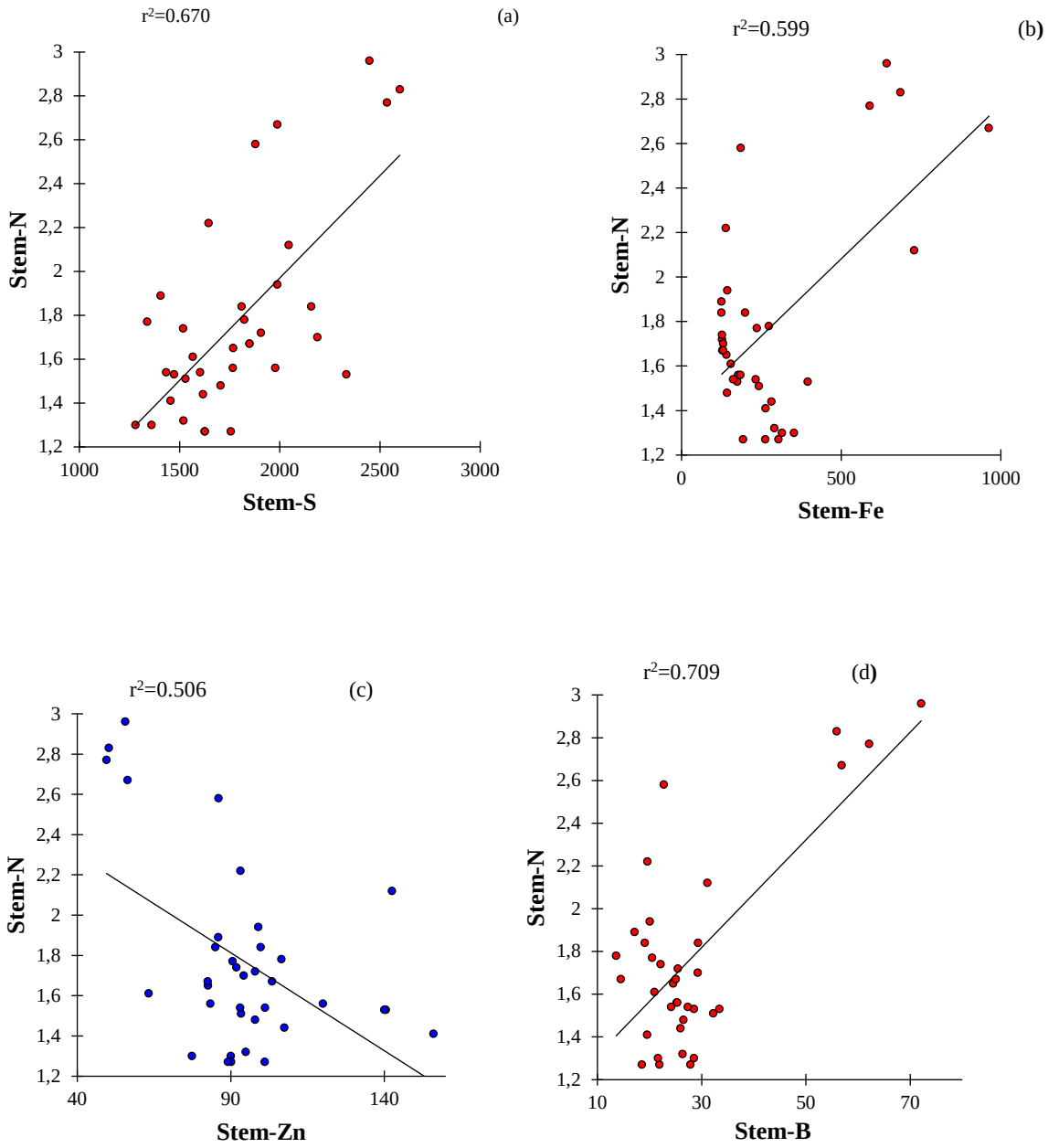

Figure 5.2 *Relationship between nitrogen and sulphur (a), relationship between nitrogen and iron (b), relationship between nitrogen and zinc (c), and, the relationship between nitrogen and boron (d) nutrient elements in the stem.*

A synergism occurred between stem- P and stem-K with the positive and significant correlation (r^2 = 0.611), the amount of P increased significantly with an increase of the K in the stem,

whilst other elements did not show any correlation with P in the stem. Nevertheless, a relationship between P and K in higher plant parts was formerly investigated and well reported by Burovina *et al*. (1978) and by Williams & Lipsett (1970) (Table 5.1; Figure 5.3)

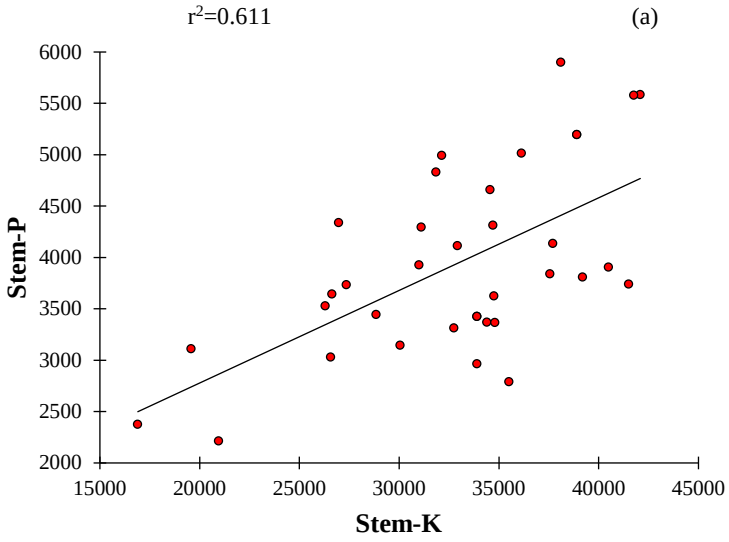

Figure 5.3 *Relationship between phosphorus and potassium nutrient elements in the stem.*

Table 5.1 and Figure 5.4a; show a negative correlation between stem-K and stem- Ca (r^2 = -0.55). An increase in K concentration significantly depressed the availability of Ca accumulation in the stem. The reduction or antagonism effect of monovalent cation on Ca is confined to the top parts of the plant and occurs normally at a higher nutrient solution application of monovalent cation 5000- 600, 000µm K + N (Lazaroff & Pitman, 1966). Nevertheless, (Table 5.1; Figure 5.4b) K accumulation showed a relatively small but significant correlation with Mg accumulation in the stem (r^2 = 0.464). Furthermore, K had generally a small and significant correlation with S (r^2 = 0.463), whereas other elements did not show any correlation with K. Increasing K in the nutrient solution significantly depressed S shoot concentration, but significantly increased the efficiency of these elements (Antonia *et al.*, 2016).

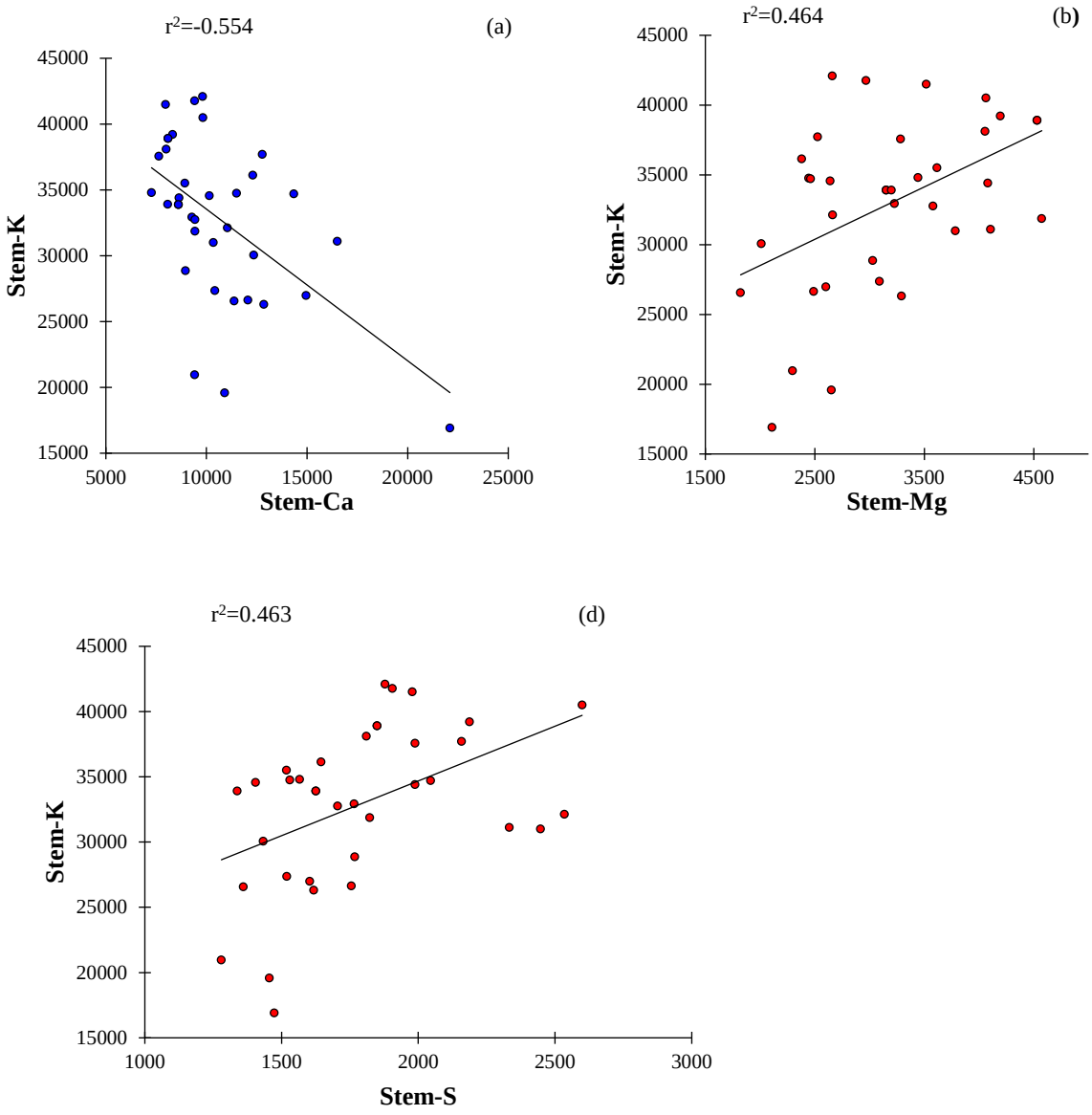

Figure 5.4 *Relationship between potassium and calcium (a), relationship between potassium and magnesium (b), and the relationship between potassium and sulphur nutrient elements in the stem.*

There was a negative correlation between stem-Mg and stem-Ca (r^2 = -0.467) (Figure 5.5a; Table 5.1), suggesting that the increase in Ca reduced the accumulation of the Mg nutrient element. According to Behar, (1975) the supply of higher Ca concentration in the plant part

significantly hampers Mg uptake by a nonspecific reduction in membrane permeability to solutes that induce the net water flow (Behar, 1975). However, the application of Ca nutrient element showed a small positive correlation with Zn accumulation in the stem (r^2 = 0.476) (Table 5.1; Figure 5.5b). Furthermore, the Ca application had a positive and significant correlation with Cu accumulation in the stem (r^2 = 0.588) (Table 51; Figure 5.5c).

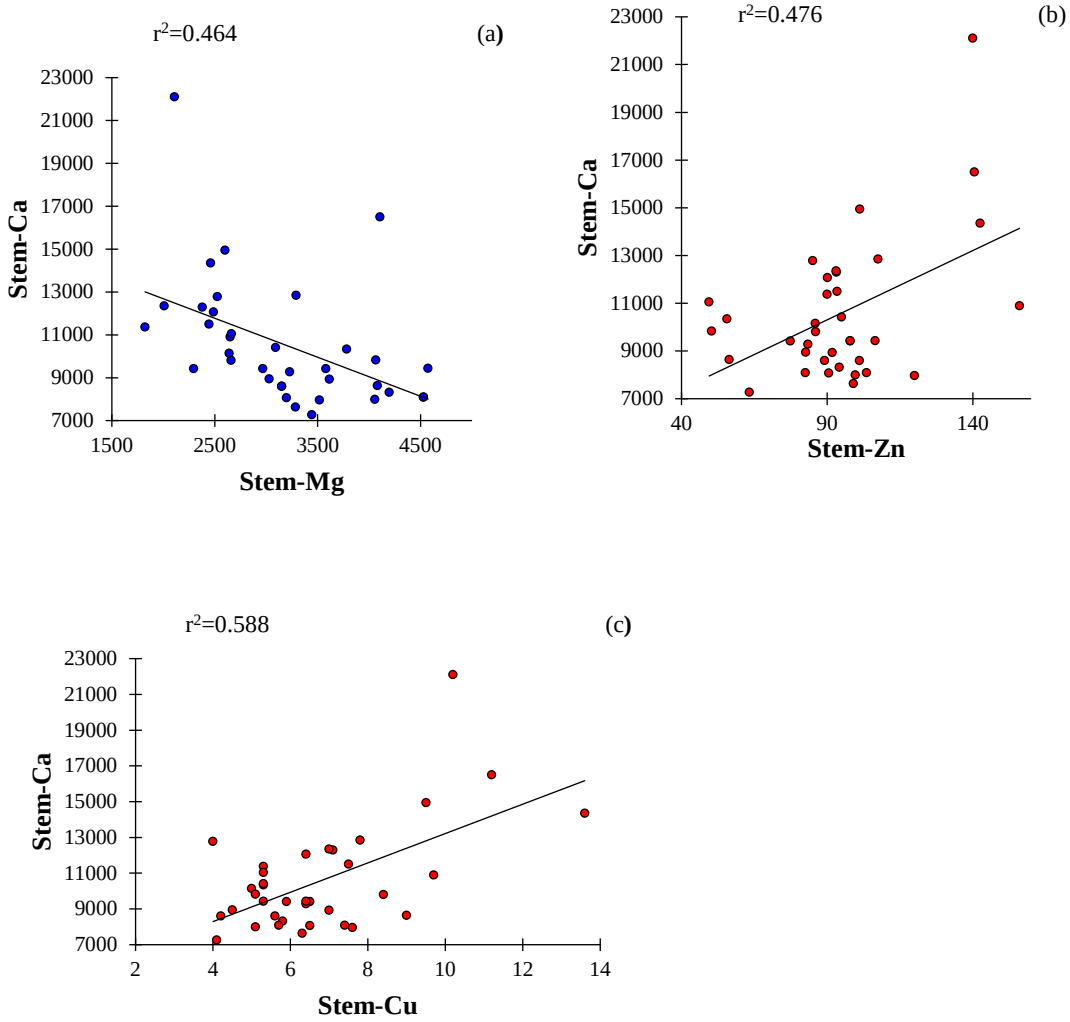

Figure 5.5 *Relationship between calcium and magnesium (a), relationship between calcium and zinc (b), and the relationship between calcium and copper (c) nutrient elements in the stem.*

The application of Mg showed a small positive correlation with S accumulation in the stem (r^2 = 0.452) (Table 5.1; Figure 5.6), whereas other elements did not show any correlation with Mg. Similarly, to the current study Thangavelu & Chiranjivi (2004) reported that Mg application did not show any correlation with N, P, and K in leaves, but Mg correlation was found in the soil. Whereas, Babu (1979) observed a significant accumulation of S content with an increase of Mg which led to higher sugar yield.

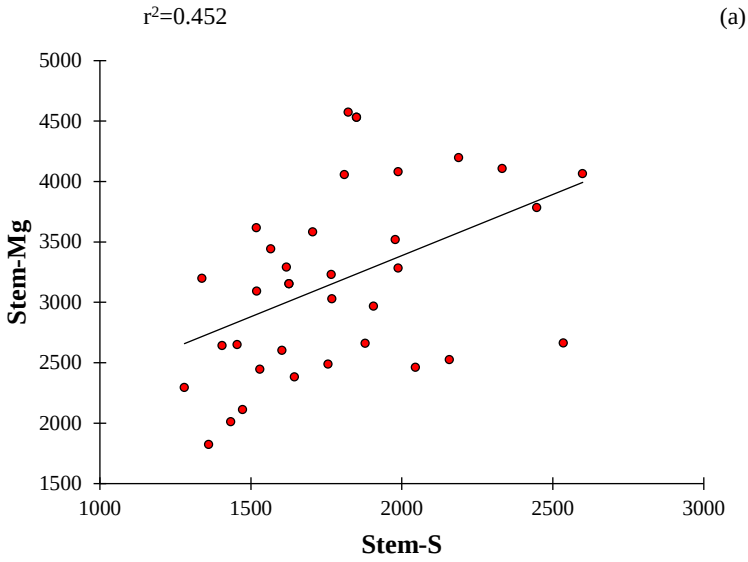

Figure 5.6 *Relationship between magnesium and sulphur nutrient elements in the stem.*

Referring to (Table 5.1; Figure 5.7) there was a positive and significant correlation between stem-S and stem-B (r^2 =0.647), suggesting that the increase of S significantly increases the B uptake in the stem. In line to the current study, Jaiswal *et al*. (2015) reported that the absorption of S in mustard seed increased significantly with S supply which further accumulated with an addition of B concentration.

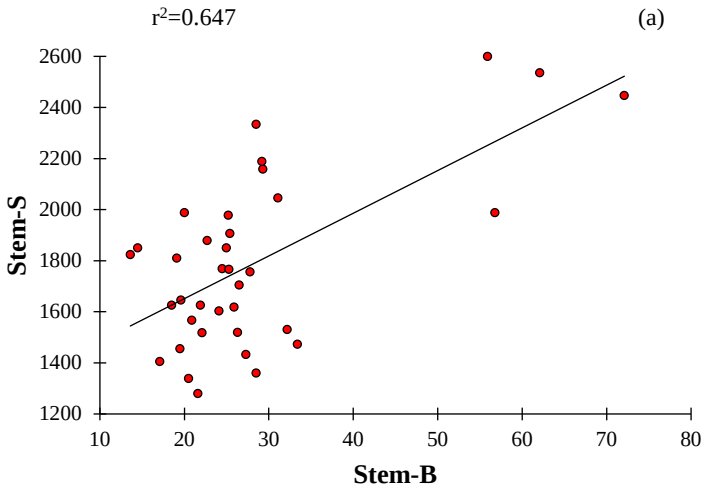

Figure 5.7 *Relationship between sulphur and boron nutrient elements in the stem.*

There was a positive and significant correlation between stem-Fe and stem B (r^2= 0.792). Referring to (Figure 5.8; Table 5.1) the amount of Fe increased significantly with an increase of B. However, there is little information available that shows this effect.

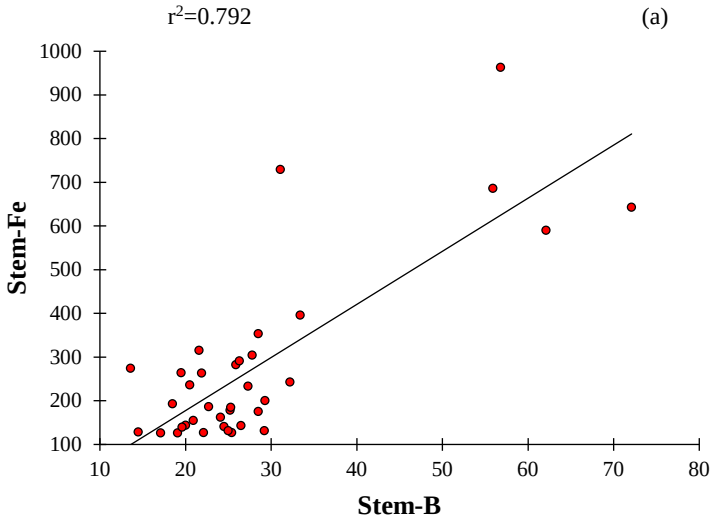

Figure 5.8 *Relationship between iron and boron nutrient elements in the stem.*

There was a small negative correlation between stem-Zn and stem-B accumulation in the stem (r^2 = -0.485) (Table 5.1; Figure 5.9), suggesting that the increase in Zn, alleviates the suppressing accumulation of boron. This decrease of B concentration with an increase of Zn was also reported on maize, mustard and orange (Matin *et al.*, 2012; Sinha *et al.*, 2000; Swietlik, 1995). According to Loneragan & Webb (1993) the antagonistic relationship between Zn and other cations (Fe, Mn and Cu) appears as a result of competing at the absorption sites of plant root. However, there was a significant increase of stem-Cu with an increase of stem-Zn (r^2 = 0.666) (Table 5.1; Figure 5.9).

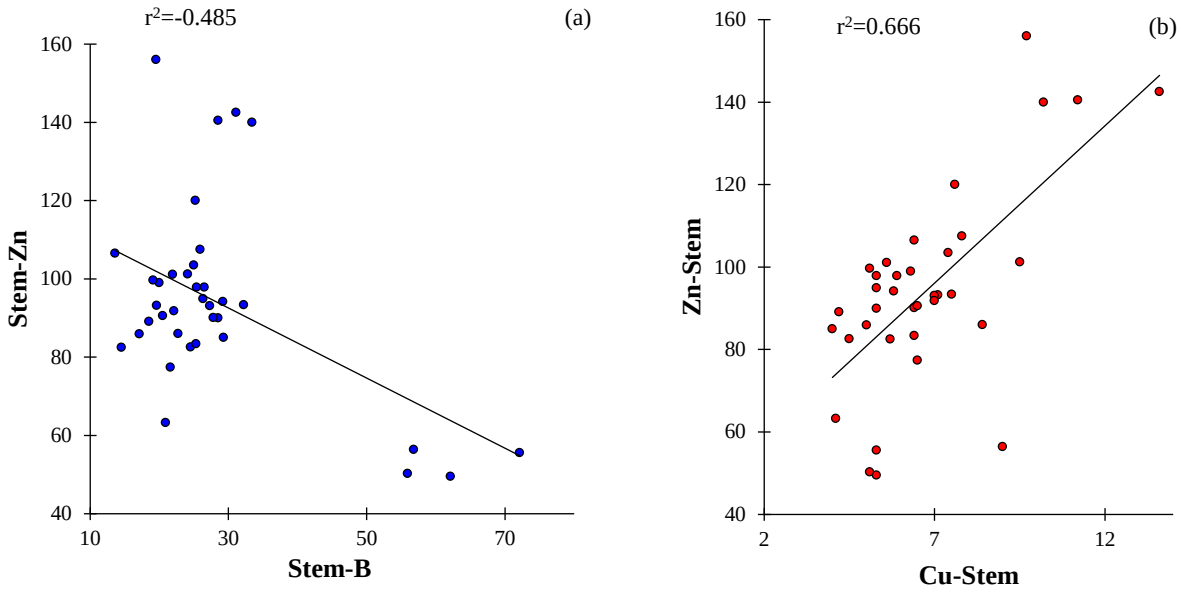

Figure 5.9 *Relationship between zinc and boron (a) and the relationship between zinc and copper (b) nutrient elements in the stem.*

5.3.3 Synergism and antagonism of chemical elements on the leaf of rose geranium

Table 5.2 Correlation matrix of minerals showing synergetic and antagonistic relationship on the leaves of rose geranium subjected to the ratio of Ca:Mg, magnetic field and mycorrhizal fungi.

Variables	N	P	K	Ca	Mg	S	Fe	Zn	B	Cu
N	**1**									
P	**0,418****	**1**								
K	**0,417****	**0,640******	**1**							
Ca	-0,259^{NS}	0,044^{NS}	-0,004^{NS}	**1**						
Mg	0,249^{NS}	0,022^{NS}	0,132^{NS}	-0,163^{NS}	**1**					
S	**0,751******	**0,607******	**0,505*****	0,032^{NS}	0,081^{NS}	**1**				
Fe	-0,135^{NS}	**-0,395****	**-0,539*****	-0,126^{NS}	**0,406****	**-0,460*****	**1**			
Zn	**-0,626******	-0,135^{NS}	-0,212^{NS}	0,241^{NS}	0,147^{NS}	**-0,530*****	**0,470*****	**1**		
B	**-0,630******	0,109^{NS}	-0,093^{NS}	0,158^{NS}	0,002^{NS}	**-0,453*****	0,083^{NS}	**-0,465*****	**1**	
Cu	-0,246^{NS}	-0,315^{NS}	**-0,508*********	-0,115^{NS}	**0,390****	**-0,507*****	**0,950******	**0,630******	-0,005^{NS}	**1**

*NS= Not significant at the P- value of 0.05, * significant at the P-value ≤0.05, ** significant at the P-value ≤0.01, *** significant at the P-value ≤0.001, **** significant at the P-value ≤0.0001*

There was a positive and significant correlation between leaf-N and leaf S (r^2 = 0.751) (Table 5.2; Figure 5.10a). The amount of N translocation increased significantly with an increase of S, this result was highly significant compared to N accumulation on the stem. On the other hand, an increase of leaf-Zn significantly reduced the leaf-N translocation (r^2 = -0.626) (Table 5.2; Figure 5.10b), this reduction of Zn in the leaves may be due to the synergism that occurred in the stem and lack of Zn translocation. Damjanovich *et al*. (1983) reported that Zn in the root cell membrane prevents excessive N uptake and its transport from roots to leaves. Nonetheless, N translocation in the leaves showed a positive and significant correlation with B translocation (r^2 = 0.630) (Table 5.2; Figure 5.10c). This might be due to its higher mobility in the plant which allows contact between ions and roots (Fageria & Baligar, 2008).

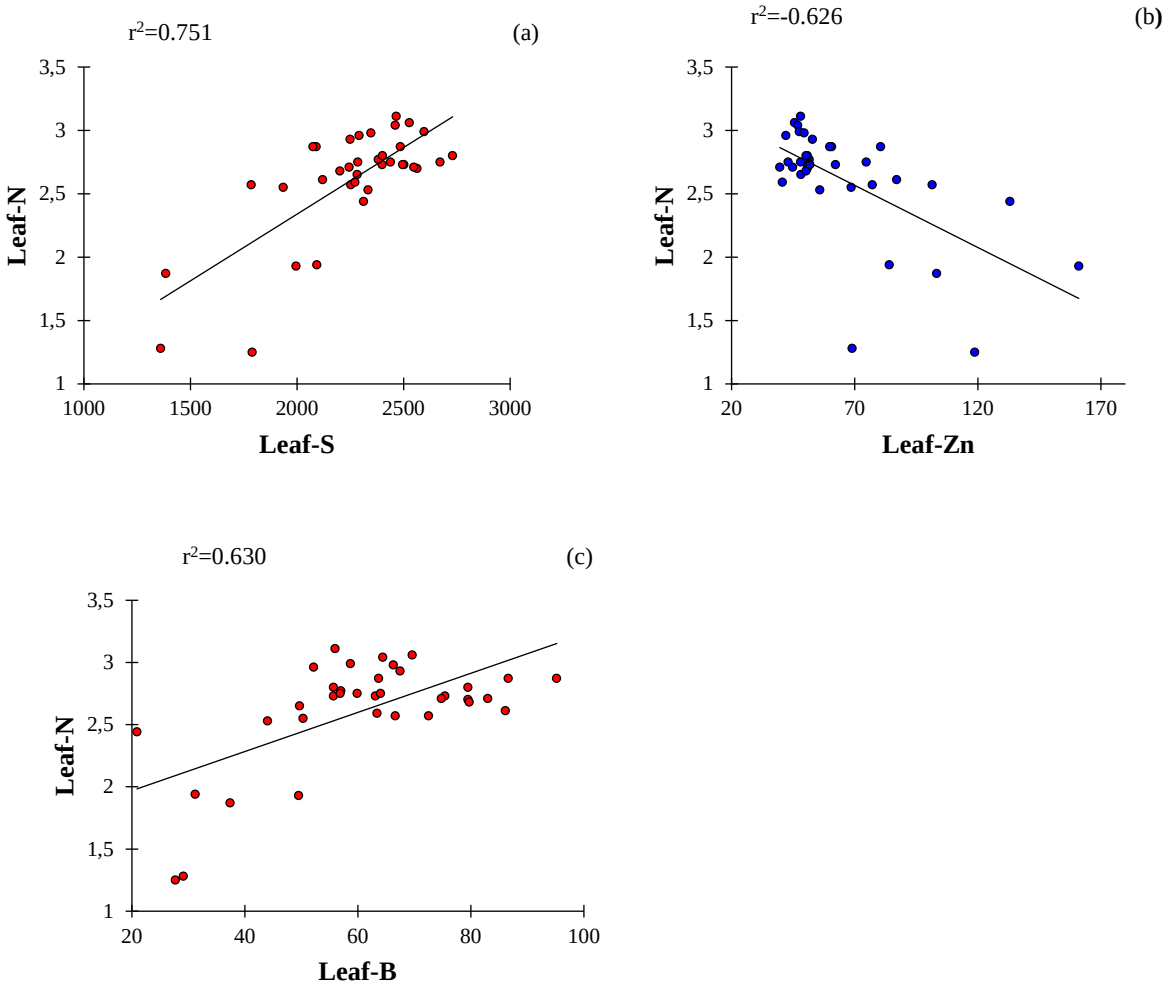

Figure 5.10 *Relationship between nitrogen and sulphur (a), relationship between nitrogen and zinc (b), and the relationship between nitrogen and boron (c) nutrient elements in the leaves.*

There was a positive and significant correlation between leaf-K and leaf-P (r^2 = 0.640) (Table 5.2; Figure 5.11a). The total amount of K translocation in the leaves increased significantly and positively with an increase of P translocation. Furthermore, K nutrient had a positive and significant correlation with boron uptake (r^2 = 0.607). The significant correlation between leaf-P and leaf-K might be due to well-known specific K transporter mechanisms in the root membrane cells, K uptake in some species, such as rose geranium is very high (Sedibe, 2012), and the availability of this nutrient in the soil plays an important role in the P accumulation in these plants (Kaminski *et al*., 2007) (Table 5.2; Figure 5.11b).

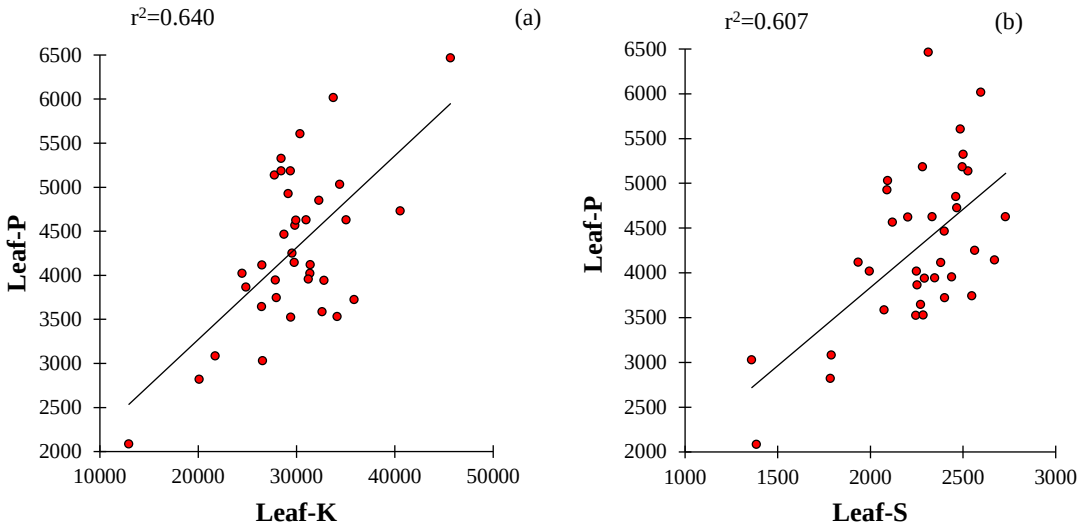

Figure 5.11 *Relationship between phosphorus and potassium (a), and the relationship between phosphorus and sulphur (b) nutrient elements in the leaves.*

There was a significant correlation between leaf-K and leaf-S translocation in the leaves (r^2 = 0.505) (Table 5.2; Figure 5.12a). The amount of K concentration in the leaves increased significantly with an increase of sulphur. There was a negative correlation between K and Fe translocation in the leaves (r^2 = -0.539) (Table 5.2; Figure 5.12b), suggesting that the increase of K concentration significantly and negatively reduces the amount of Fe concentration in the leaves. Furthermore, the total translocation of Cu concentration was significantly reduced by an increase of K nutrient elements in the leaves (r^2 = -0.539) (Table 5.2; Figure 12c).

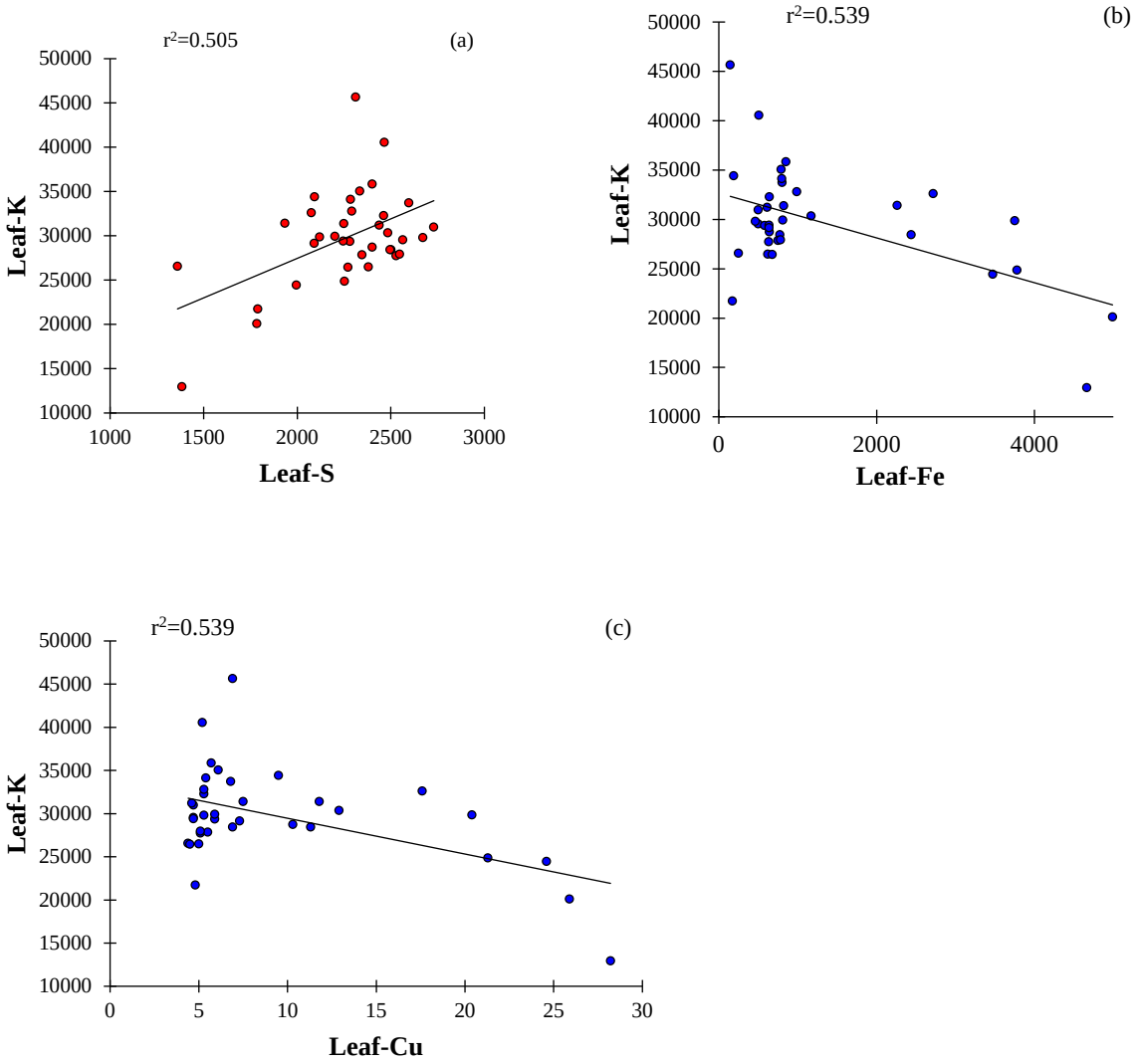

Figure 5.12 *Relationship between potassium and sulphur (a), relationship between potassium and iron (b), and the relationship between potassium and copper (c) nutrient elements in the leaves.*

There was a negative correlation between leaf-S and leaf-Fe translocation in the leaves (r^2 = -0.460). In referring to (Table 5.2; Figure 5.13a) increased S concentration significantly reduced the Fe translocation in the leaves. According to the literature reviewed, the significant demand of Fe in the stoichiometric concentration of Fe-S cluster constellation indicates the development of cross-regulatory interactions between two pathways (Sara *et al*., 2013).

Furthermore, there was a significant and negative decrease of total concentration of Zn in the leaves with the increase in S nutrient elements (r^2 = -0.460) (Table 5.2; Figure 5.13b). Furthermore, there was a small positive correlation between S and B translocation in the leaves (r^2 = 0.453) (Table 5.2; Figure 5.13c).

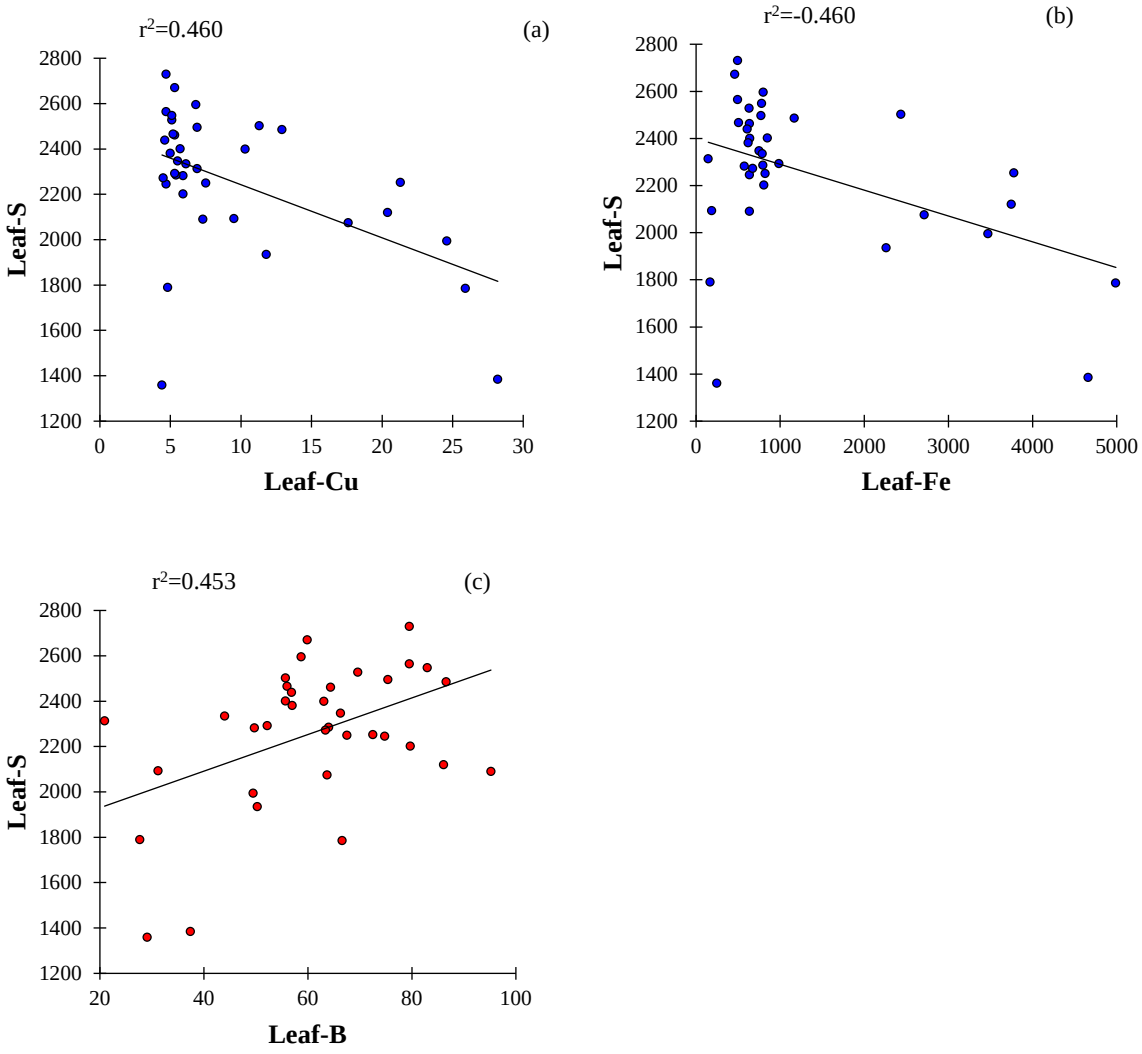

Figure 5.13 *Relationship between sulphur and iron (a), relationship between sulphur and zinc (b), and the relationship between sulphur and boron (c), nutrient element in the leaves.*

There was a small positive correlation between Fe and Zn translocation in the leaves (r^2 = 0.470) (Table 5.2; Figure 5.14a). Furthermore, Fe concentration in the leaves showed highly significant correlation with Cu translocation in the leaves (R^2 = 0.950) (Table 5.2 Figure 5.14b). Furthermore, there was a significant variation of Fe in the leaves and Fe in the stem, the amount of Fe in leaves increased progressively with the increase of Cu concentration in the leaves compared to the stem correlation. Results obtained by Welch *et al*. (1993) showed how Cu plays a fundamental role in the plasma membrane in enhancing Fe absorption and regulating the sulfhydryl groups in transport protein involved in divalent cation transport across the root cell (Welch *et al*., 1993).

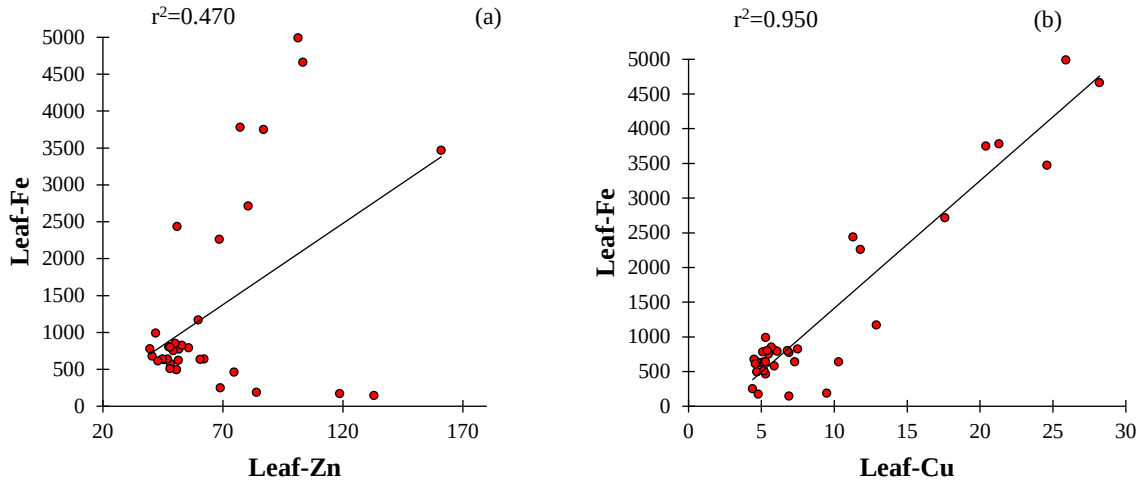

Figure 5.14 *Relationship between iron and zinc (a), and the relationship between iron and copper (b) nutrient elements in the leaves.*

There was a negative correlation between Zn and B in the leaves (r^2 = -0.465) in referring to (Figure 5.15a) Zn translocation significantly reduced the total translocation of B in the leaves. Similarly, to the current study, Rajaiea *et al*. (2008) postulated that the application of Zn concentration significantly leads to a progressive decrease of B accumulation and the linear linkage decreased the plant growth. However, there was a positive correlation between Zn and

Cu (r^2 = 0.630), an increase of Cu nutrient element significantly influenced the accumulation of Zn in the leaves (Table 5.2; Figure 5.15b).

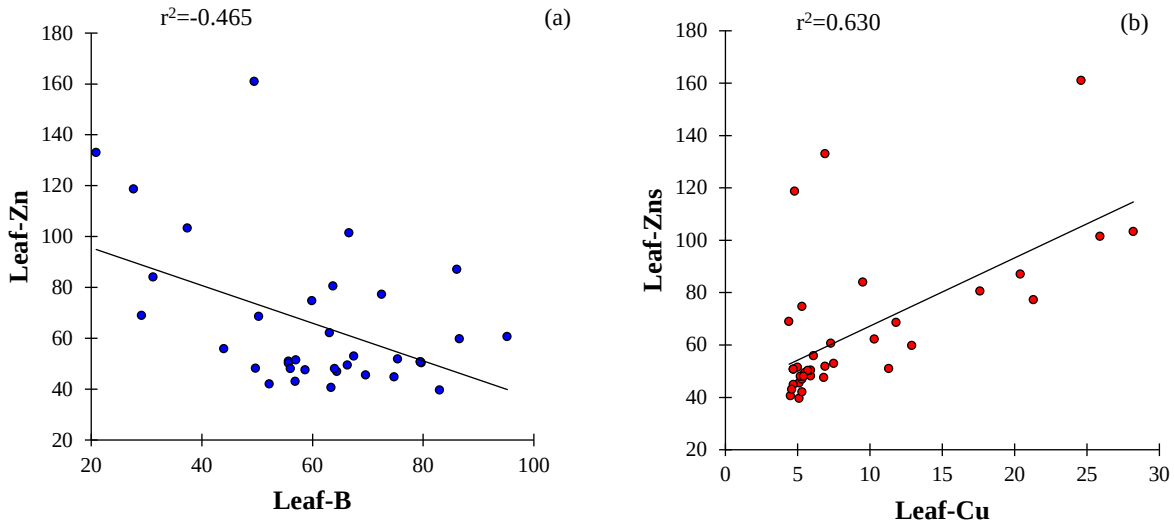

Figure 5.15 *Relationship between zinc and boron (a), and the relationship between zinc and copper (b) nutrient elements in the leaves.*

5.4 CONCLUSION

Multivariate analysis revealed the relationship between mineral composition and agronomic attributes. Using the multivariate analysis, we were able to reveal and to understand the antagonistic or synergetic relationship between mineral and agronomic attributes of rose geranium affected by Ca and Mg and the impairment of polarity due to the lack of MF gradients as well as the use of mycorrhizal fungi.

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CHAPTER 6

SUMMARY AND RECOMMENDATIONS

Rose geranium growers are looking for ways to improve the production, therefore the study was carried out to evaluate the effect of magnetic field, Ca:Mg ratio, and with mycorrhizal fungi to contribute the knowledge of rose geranium production. This study was carried out in the tunnel at Glen College of Agriculture, in Bloemfontein.

To better the production of rose geranium, knowledge of agronomic attributes and physiological response as affected by exposure to magnetic field, mycorrhizae inoculation, and calcium to magnesium ratio could make an indispensable contribution to South African producers. It has long been confirmed and documented that herbal plants like rose geranium require the supply of larger quantities of essential macronutrients for increased growth and yield. Calcium and magnesium play fundamental role in stepping up the growth, quantitative as well as qualitative features of the plant. When supplied or available in quantities that are low, the plant exhibit reduced production and supply of photo assimilates to other parts of the plants (Hao & Papadopoulos, 2003).

In this study, three levels of Ca:Mg ratios (2.40:6.78, 4.31:4.39 and 6.78.2.40 meq L^{-1}) were used to prepare a nutrient solution which was exposed to magnetic fields and mycorrhizal. The growth parameters of rose geranium were evaluated afterwards. Of these treatments, when the equal proportion of Ca:Mg (4.31:4.39 meq L^{-1}) nutrient solution was applied to plants exposed to magnetic field, the combination had a positive and significant effect on plant height, number of branches, number of leaves and root length. However, a nutrient solution made of increased Mg and low Ca (6.78:2.40 meq L^{-1}) did not contribute significantly to the growth parameters of rose geranium. Furthermore, the results showed that low Ca and high Mg (2.40:6.78 meq L^{-1}) in the nutrient solution had the shortest plants and the least number of branches and leaves. These results could be due to the fact that Ca is not easily translocated in the plant. On the other hand, it can be postulated that an insufficient supply of Ca in the nutrient solution, negatively affect rose geranium yield.

Interestingly, plant scientists have explained the interaction between different concentrations of nutrient solutions and growth-related parameters of plants, however, there does not seem to be a clear explanation and trend. Some of these relationships might be seeing straight forward but, most are not. According to Mehra & Jacson (2013) the concentration of essential elements depends on the pH, soil type, geographical location, translocation and accumulative capabilities of the herbal plant. In the present study, increased Mg and low Ca (6.78:2.40 meq L^{-1}) in the nutrient solution resulted in a significant increase of Mg concentration in the leaves. Furthermore, the increased Ca and low Mg (6.78:2.40 meq L^{-1}) in the nutrient solution significantly reduced the translocation of Mg in the leaves. In addition, equal proportion of Ca:Mg (4.31:4.39 meq L^{-1}) in the nutrient solution showed positive and significant translocation of Zn in the leaves. However, the increase of Ca and low Mg (6.78:2.40 meq L^{-1}) in the nutrient solution had small but significant influence in the translocation of Zn.

The current results clearly show that there was a significant synergism and antagonism between nutrient elements. However, other elements were weakly correlated and decoupled. The concentration of micronutrients Fe and Cu were influenced positively by N concentration, but the Zn concentration was negatively influenced by N concentration. Phosphorus accumulated progressively and positively with an increase of K concentration in the stem and in the leaves. The increase of K concentration had small but positive correlation with Mg in the stem, S in the stem and S in the leaves. However, it caused a significant decrease in Ca in the stem, Fe in the leaves and Cu translocation in the leaves. There was a significant accumulation of Ca concentration with an increase of Zn and Cu in the stem. Furthermore, Mg concentration had small but positive correlation with S in the stem. The increase in S concentration had small positive correlation with B concentration, but the increase in S concentration caused a gradual decline of Fe and Cu translocation in the leaves. The increase in Fe concentration had a slight positive correlation with B accumulation in the stem and Zn translocation in the leaves, however, the increase in Fe concentration had a strong positive correlation with Cu translocation in the leaves. Lastly, the increase in Zn concentration had a positive correlation with Cu concentration, but Zn concentration caused significant decrease of B concentration.

For further investigation, it is recommended that studies should focus on the intensity, duration and polarities when investigating the effect of MF on mineral uptake and translocation for the improvement of oil yield and quality of rose geranium.

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