INVESTIGATION OF THE EFFECT OF LONG TERM EXPOSURE OF MAGNETIC FIELD ON THE ELEMENTAL COMPOSITION AND CHLOROPHYLL CONCENTRATION IN SPINACH BEET (*Beta vulgaris subsp. vulgaris*)

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EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This is my original work and has not been submitted for any examination in any institution for any academic award whatsoever.

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Recommendation

This Thesis has been submitted with our approval as Supervisors for examination according to Egerton University regulations.

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ABSTRACT

Visible light plays an important role in the survival of species in our ecosystem. Light signals control seed germination, leaf expansion and stem elongation. Visible light consists of electric and magnetic fields. Earlier studies have focused on the exposure of magnetic field for a short span on the growth of plants, this study will investigate the effect of magnetic field on the whole lifespan of spinach with emphasis on its effect on chlorophyll and elemental composition. In this study the focus was on the effect of magnetic flux density on spinach. Spinach is an important nutritional crop in Kenya, an excellent source of vitamin K, Vitamin A, manganese, folate, magnesium, iron, copper, vitamins B2, Vitamin E and calcium and a good source of dietary fiber. The specific objective was to determine the effect of magnetic flux density and exposure time on the growth of spinach and the concentration of chlorophyll, iron, sodium, zinc, magnesium, potassium and calcium in the leaves of spinach. A Completely Randomized Design (CRD) pot experiment with 13 treatments was set up in a greenhouse. The pots were sowed with four spinach seeds which were thinned to two immediately after germination, the plants were exposed to magnetic flux density generated by a current. There were two variables, intensity of magnetic flux density and exposure duration. Magnetic flux density of varying intensities of 0 mT, 0.5 mT, 1.0 mT, 1.5 mT and 2.0 mT and exposure time of 10 minutes, 30 minutes and 60 minutes. The plants were exposed to magnetic field at the specified duration daily for 60 days. The results indicated that the magnetic flux increases the concentration of chlorophyll a by up to 50%, chlorophyll b by up to 20%, the total chlorophyll content increased by 38% while the concentration of zinc, sodium, potassium, iron, magnesium and calcium rose by 97%, 26%, 74%, 78%, 20% and 98% respectively. The data was analyzed using R statistical software (R version 3.3.4), the results were subjected to ANOVA, the effects were significant, the means were separated using Tukey’s HSD test at $p<0.05$. These results showed that magnetic field can be used to enhance the growth of spinach that have high mineral concentration and that can therefore be used by groups of people that need high amounts of these elements like vegetarians, expectant women and young children.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAS	Atomic absorbance spectrophotometer
ANOVA	Analysis of variance
DNA	Deoxyribonucleic acid
EMF	Electromagnetic fields
FAO	Food and Agricultural Organization
He-Ne	Helium Neon laser
pH	Potential of hydrogen
ICR	Ion cyclotron resonance
IQ	Intelligence quotient
MFD	Magnetic flux density
mT	Militesla
Nd-YAG	Neodymium-doped Yttrium Aluminium Garnet laser
SHG	Second harmonic generation
SMF	Sinusoidal magnetic field
SSA	Sub Saharan Africa
TDS	Total dissolved solids
UV	Ultra violet
UV-VIS	Ultra violet and visible
MF	Magnetic field

LIST OF SYMBOLS

B	Magnetic flux density
H	Magnetic field intensity
Ca	Calcium
f	Frequency
Fe	Calcium
K	Calcium
m	Mass
Mg	Magnesium
Na	Sodium
q	Charge
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 Background information

More than 2 billion people suffer from micro-nutrient deficiencies, 1.62 Billion people globally suffer from anemia, caused by iron deficiency, of which 52 percent are pregnant women and 39 percent are children under five (Jaroz and Rychlik, 2012). Zinc deficiency causes poor pregnancy outcome, impaired growth, genetic disorder and decreased resistance to infectious diseases. Mineral deficiency not only cause diseases but they act as exacerbating factors in infectious and chronic diseases such as osteoporosis due to calcium deficiency, osteomalacia, corolectal cancer and cardiovascular diseases (Allen *et al.,* 2006). They increase the severity of infectious diseases, such as measles, HIV/AIDS and tuberculosis.

Methods such as food fortifications have been used to come up with foods that have high mineral concentration; however they are not affordable to majority of the population due to their high costs (Horton, 2006). Therefore, this necessitates the research for ways to increase production of plant foods with high mineral concentration through ways that are environment- friendly, non-chemical and affordable. However even with such methods, it’s important to determine how they affect the health of plant. One of the most effective ways of measuring how external factors affect plants is through measuring chlorophyll concentration. Leaf chlorophyll content is important because :the solar energy absorbed by a plant is a function of chlorophyll content, and therefore low levels of chlorophyll can limit primary production (Zhani *et al.,* 2012). Since nitrogen is incorporated in chlorophyll, its measurement also gives an indirect measurement of nitrogen content in plants (Bojovic and Markovic, 2009). The chlorophyll content can serve as an indicator of physiological stress as carotenoids and chlorophyll content decrease during stress (Zhani *et al.,* 2012). Since the quantity of chlorophyll changes with abiotic factors such as light, its quantification can provide valuable information about the relationship between plants and the environment .A way of production of food with high mineral concentration through a method that is affordable and environmentally friendly can be through the exposure of plants to magnetic field.

The exposure of magnetic field on plants can be achieved in three ways, through exposure of irrigation water to magnetic field, by exposure of seeds to magnetic field or by exposure of plants to magnetic field. Application of magnetic field sought to and irrigation water has been shown to lead to an alteration of the growth and yield of plants depending on the duration of exposure, intensity of magnetic field and the frequency (Aladjadjiyan, 2002). Although it is clear that magnetic water and exposure of magnetic field to seeds have an effect on plants; there is a lack of adequate information about the effect of direct exposure of magnetic field to plants (Kordas, 2002). Research on the effect of magnetic fields on plants is very active. Among the common conclusions of many researchers in this field is that there is a minimum, optimum and maximum magnetic field levels for stimulating plant growth (Aladjadjiyan, 2002). These levels change with the type of magnetic field used, species of the plant, stage of growth of the plant and external conditions (Celestino *et al.*, 2000). Extensive research has been conducted on the effect of magnetic field exposure on seeds and early growth of plants and some results have shown alteration in biomass and mineral concentration. However, there is scanty information on its effect on exposure beyond the early growth stages of plants. This study seeks to seek to address this gap, by investigating if exposing spinach plants to magnetic flux density in later growth stages can lead to an increase in concentration of chlorophyll and selected mineral elements.

Magneto reception by plants has been explained by three theories that suggest mechanisms of how magnetic field affects plants: the radical pair mechanism theory, Ion cyclotron Resonance theory and magneto hydrodynamics. According to the radical pair theory, magnetic fields alter the yield of biochemical reactions which are preceded by intermediates which are radicals (Ritz and Schulten, 2000; Parola *et al.*, 2005). Therefore, a process like photosynthesis, which involves formation of radicals, is affected by magnetic fields (Rochalska, 2005). The Ion Cyclotron Resonance (ICR) theory proposes that ions that respond to magnetic fields can be excited by an external magnetic field of specific magnetic field induction and with specific frequencies; these excited ions can easily cross the cytoplast even against the electrochemical gradient (Liboff, 2005). The influx of these minerals act as messengers in the plant cell, thereby altering growth and the elemental composition (Belyavskaya, 2001). However, this theory has faced criticism because studies using magnetic field with frequencies and strength other than those of ICR have shown to have effects on elemental composition of plants (Liboff *et al.*, 2005). The effect of magnetic field on water such as its ability to increase solubility of ions has also been used to explain the effect of magnetic field on plants (Fujimura and Lino, 2009).

1.2 Statement of the problem

Mineral deficiency is a nutrition problem that afflicts more than 2 billion people in the world. It causes the impairment of hundreds of millions of growing minds and the lowering of intelligence quotient. It means wholesale damage to immune systems, deaths of more than a million children a year, 250,000 serious birth defects annually, deaths of approximately 50,000 young women a year during pregnancy and childbirth and large-scale loss of national energies, intellects, productivity, and growth, anemia alone is associated with a 2.5% drop in adult wages. Based on these impacts mineral deficiency, programs have been put in place to solve this problem. Unfortunately, currently available data on the magnitude of deficiencies and program coverage levels indicate that only a small part of the vulnerable populations has been reached with effective interventions because of their inability to afford them. Conventional approaches to improve mineral content are expensive. The use of fertilizers to increase the mineral content is expensive and also increases toxicity. There is a lack of an affordable source of plants with high mineral concentration that is affordable to a certain segment of the population. Exposure of magnetic field to plants can result to plants with high mineral concentration that is affordable.

1.3 Objectives

1.3.1 General objectives

To investigate the nutritional quality of spinach vegetables through exposure of magnetic field

1.3.2 Specific objectives

i. To investigate the effect of magnetic flux density and exposure duration on the elemental composition of spinach.
ii. To investigate the effect of magnetic flux density and exposure duration on the chlorophyll content of spinach.

1.4 Hypotheses

i. Magnetic flux density and exposure duration does not significantly affect the concentration of Iron, Calcium, Zinc, sodium, magnesium and Potassium in spinach.
ii. Magnetic flux density and exposure duration does not significantly affect spinach's chlorophyll content of spinach.

1.5 Justification

The use of magnetic field in crop production is affordable and environmentally friendly. According to the World Bank (2008), about 982 million people live below the poverty line of which 850 million people are undernourished therefore affordable vegetables with high mineral concentration will help this category of people. Increased mineral content in spinach by exposure to magnetic field will ensure an affordable source of minerals for people such as expectant mothers and young children who need a diet with high mineral concentration. This will reduce incidences mineral deficiency in pregnant mothers and children. It will also improve the health of persons previously with poor access to nutritious foods. This will save lives of women dying from micronutrient deficiency, will improve the lives of children with micronutrient deficiency and will also improve the adult wages in the country.

CHAPTER TWO
LITERATURE REVIEW

2.1 Effect of mineral deficiency on health

Mineral deficiency affects the health of majority of people in the developing world especially the sub-Saharan Africa. Accordingly, micronutrient malnutrition is recognized as one of the most serious hindrance to human development and survival by World Health Organization. (Allen *et al.*, 2006). The table below (Table 1) shows the mineral deficiency in the world.

Table 1: Mineral deficiency

Micronutrient	Deficiency prevalence	Major deficiency Disorders
Iron	2 billion	Iron deficiency, anemia, reduced learning and work capacity, increased maternal and infant mortality, low birth weight
Zinc	Estimated high in developing countries	Poor pregnancy outcome, impaired growth (stunting), genetic disorders, decreased resistance to infectious diseases
Calcium	Insufficient data, estimated to be widespread	Decreased bone mineralization, rickets, osteoporosis
selenium	Insufficient data, common in Asia, Scandinavia, Siberia	Cardiomyopathy, increased cancer and cardiovascular risk

Source: Adapted from Allen *et al.* (2006)

2.2 Magnetism

Magnetism in materials arises from the magnetic dipoles of its atoms. At the atomic scale, moment of electrons produce small currents which generate magnetic fields. However the application of Maxwell equations to the movement of electrons led to a lot of contradictions hence the quantum theory was applied to resolve this contradiction. According to this theory, magnetic moment is derived from the orbit of the electron around the nucleus and its orbit along its own axis. Orbital motion is considered a current loop and results in formation of orbital motion dipole magnetic moment. The spin motion also generates spin dipole moment, which is spin of electron. The total magnetic moment is derived from the sum of all the magnetic moments of the individual electrons. For each pair of electrons, their magnetic moments can cancel each other and therefore for a complete electron shell its total magnetic moment is zero because they cancel out. Magnetic moments will therefore result from partially filled electron shells and its strength will be proportional to the number of unpaired electrons (Sami and Abdel Rahman, 2011).

2.2.1 Magnetic field, Magnetic field strength and Magnetic flux density

Magnetic field strength, given the symbol H, is measured in Amperes per meter (Am^{-1}). Magnetic flux density, is given the symbol B, is measured in teslas (Wbm^{-2}). In electromagnetic theory, it's absolutely clear that magnetic flux density and magnetic field strength are different quantities and therefore there is a need to be clear that this study will be measuring magnetic flux density. Magnetic flux density and magnetic field strength are related by the relationship given in equation 2.1.

$$\vec{B} = \overrightarrow{\mu H} \qquad 2.1$$

Because of this relationship, whether we are measuring B, the magnetic flux density, in militesla (mT) or H, the magnetic field strength in Amperes per meter. The term magnetic field is perfectly a description of both magnetic flux density and magnetic field strength, because both are examples of magnetic field. In this study the term magnetic field will mean magnetic flux density.

2.2.2 Magnetic field due to Helmholtz coils

In order to set up a Helmholtz coil, two identical coils with radius R have to be placed in the distance R from each other, this is to ensure a uniform magnetic field is created, this is shown in diagram 2.1. When the coils are set in such a manner, a current is passed through the coils the same direction, the Helmholtz coils will therefore produce a fairly uniform region of magnetic field. The magnetic field at the middle position of the coils is proportional to the current passed through, as shown by equation 2.2.

$$B = \mu \frac{8.I.N}{\sqrt{125}.R} \qquad 2.2$$

Where I is the current, μ is the permeability of free space, N the number of windings in the coil and R is the distance between the coils.

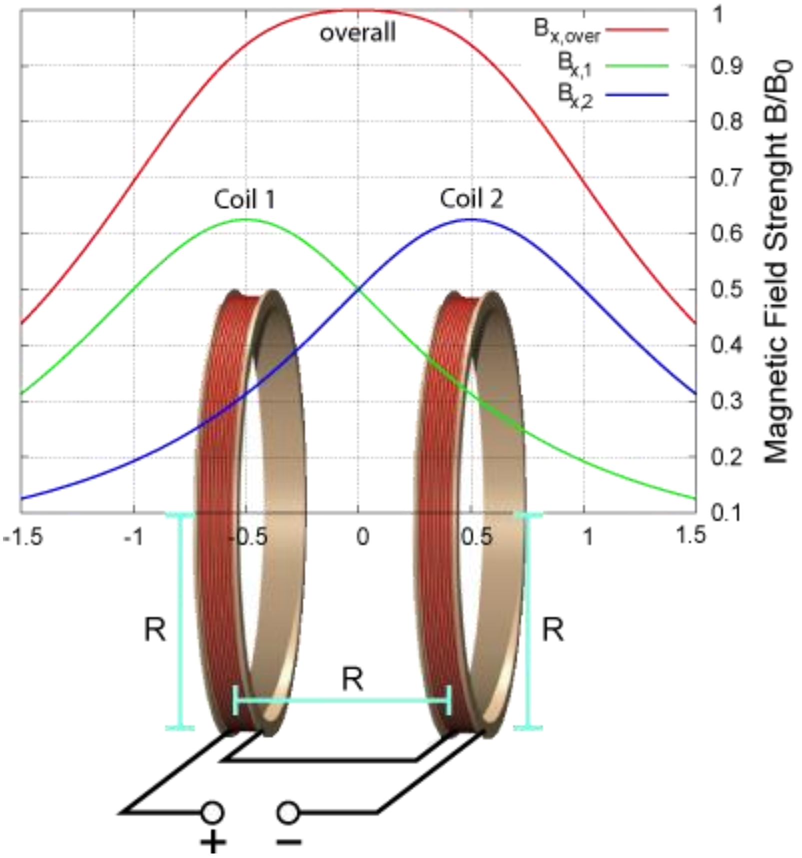

Figure 2.1: Helmholtz set up and distribution of magnetic field

2.3 Theories of magneto-reception in plants

Three main theories have been advanced to explain how magnetic flux density affects plants. They include the principles of magneto –hydrodynamics, which proposes that magnetic field decreases the surface tension and increases the viscosity of water and this has effects on plants, the ion cyclotron theory which proposes that magnetic field of certain frequencies and intensity accelerate the entry of certain ions into the cell thereby altering biochemical processes (Liboff, 2005) and the radical pair mechanism which uses the concept of Zeeman effect to explain the influence of magnetic field on radicals that are involved in biochemical processes (Ritz and Schulten, 2000). They are explained in detail in 2.3., 2.4 and 2.5 respectively.

2.3.1 Radical pair mechanism theory

A radical is an atom or a molecule with an unpaired electron. Reactions involving radical pairs as intermediates are sensitive to magnetic field (Schulten, 1986). In such reactions, the magnetic field in resonance with the electron Zeeman splitting perturbs the singlet –triplet interconversion of the radical pair thereby affecting the reaction yield (Plenio and Huelga, 2008). The Hamiltonian of a radical pair's electron nuclear spins motion is given by:

$$H = \sum a_{1k} S_1 I_K + \sum a_{2l} s_2 I_l + a_0 B (g_1 S_1 + g_2 S_2) - J (\frac{1}{2} + 2 S_1 S_2) \qquad 2.3$$

where a_{1K} ,a_{2l} are the hyperfine interaction constant ,S_1, S_2 are the electron spins, I_k ,I_l are the nuclear spins, a_0 is Bohr's constant, B is the magnetic field density,g_1 and g_2 are the g factors of the two radicals and J is the exchange interaction (Werner *et al.,* 1977).

The interaction between the spins of the nucleus and the unpaired electron is shown by the first two terms of equation 2.2; it's called the hyperfine interaction (Schulten, 1986). The interaction between the electron and the external magnetic field (Zeeman Effect) is represented by the third term.

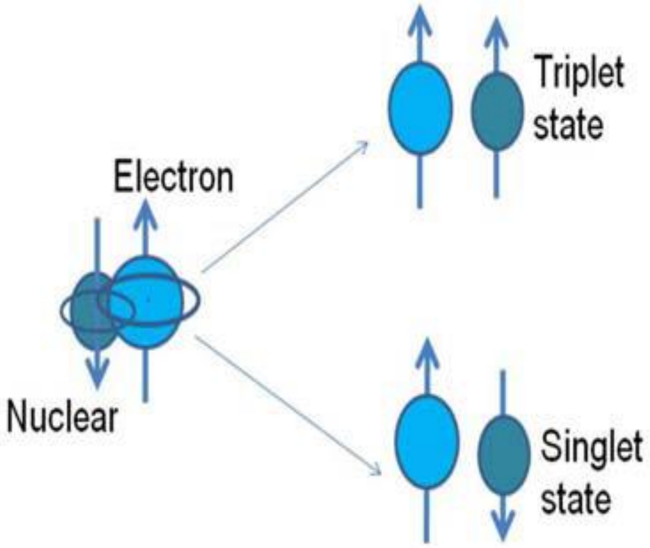

Figure 2.2: Hyperfine interaction **(**Chathurika, 2010)

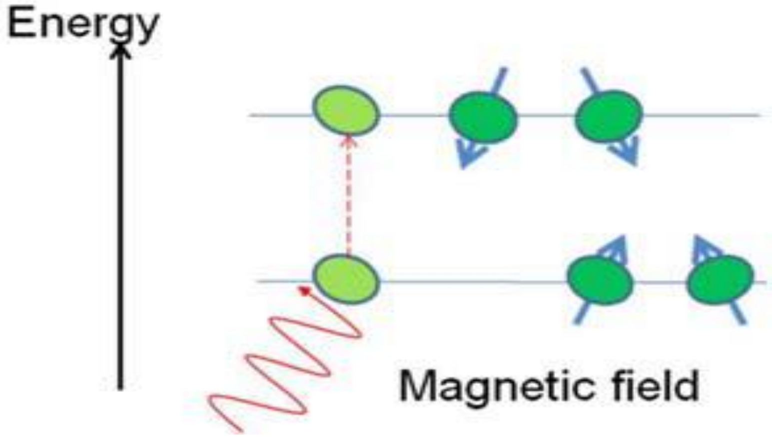

Figure 2.3: The spilt of energy levels to sublevels due to the presence of magnetic field (Zeeman Effect)

2.3.2 Hyperfine interaction and magnetic flux density

Refers to the interaction between the magnetic moment of the nucleus and that of the unpaired electron, and the orientation depends on the external magnetic field as shown in figure 2.3.When the spins of the unpaired electron and the nucleus are anti-parallel it results in a singlet state, if they are parallel, it results in triplet states (T_0 ,T_{-1} , T_1) (Timmel and Henblest, 2004). The interconversion between the singlet and triplet states is facilitated and driven by the hyperfine coupling (Plenio and Huelga, 2008). The recombination kinetics are sensitive to applied magnetic field more than the hyperfine interaction (~ 1mT) .

2.3.3 Zeeman Effect and magnetic flux density

An atom moves from one energy level to another by either absorbing or emitting energy, this energy is the deference between the energy level it's moving from and the one it's moving to. Magnetic field causes a split in these spectral lines; this process is called Zeeman Effect. The strength of magnetic field determines the distance between these sub-levels (Wang and Ritz, 2006).

2.3.4 Exchange interaction

Refers to the interaction between the unpaired electron spins of two radicals. The exchange interaction reduces the singlet-triplet interconversion by lifting the triplet states away from the singlet states (Engstro, 2006). As the distance between the radicals increases, there is a decrease in exchange interaction. At a certain distance, J becomes negligible and therefore the singlet-triplet interconversion becomes possible. The exchange interaction between the singlet and triplet state is 2J (Gould and Zimmt, 1984).

2.3.5 Magnetic flux density and Singlet–triplet interconversion

In normal reactions, a radical is created by a molecule that is photo-excited. The strength of hyperfine interaction determines the frequency of singlet-triplet interconversion of the radical formed (Timmel and Henbest, 2004). The singlet and triplet radicals formed form different products and therefore reducing the amount of radicals. Magnetic field affects the frequency of the singlet –triplet interconversion through the introduction of sublevels in the energy levels (Zeeman Effect), thereby affecting the reaction rates and the products formed from the singlet and triplet states. When the magnetic field is smaller than the hyperfine coupling strength, then the singlet-triplet interconversion is increased hence an increase in triplet yield, however when the magnetic field is higher than the hyperfine coupling, then only the T_0 to S interconversion is possible, this is because the Zeeman interaction shifts the energy of the triplet states away from the singlet states and therefore reduces the number of triplet states that can be converted to singlet states (Ritz and Schulten, 2000). When the exchange interaction is large, the singlet-triplet interconversion is not possible. At specific magnetic field, the singlet-triplet interconversion is possible, if the strength of this magnetic field enables the matching of Zeeman energy and the electron exchange energy, hence making the hyperfine interaction feasible. Difference in g factors of the two radicals only lead to S to T_0 interconversion and its independent of the hyperfine coupling, this interconversion becomes significant when magnetic field is large because the difference in g factors are very small (Engstro, 2006).

2.3.6 Magnetic field and chlorophyll content

The interconversion between the singlet and triplet state is higher in a static magnetic field than in alternating magnetic field (Chathurika *et al.*, 2010). This might explain the different physiological changes resulting from exposure of plants to both alternating and static magnetic field. For instance, there is a decrease in chlorophyll a and chlorophyll b after exposure of alternating magnetic field to chick pea (*Cicer areitinum*) (Singh and Singh, 2015), while there is an increase in chlorophyll content in most experiments involving static magnetic field (Dhawi and Essam, 2009; Racuciu, 2012).

Since the lifetime of a radical is in the microsecond range, according to the radical pair mechanism theory, it would imply that only alternating magnetic field of frequency up to a few megahertz would affect the radicals. However, experimental results have shown that even static magnetic field and alternating magnetic field of 50 or 60 Hertz have an effect on the radicals. The frequency of alternating magnetic field has an effect on the changes that results from exposure to plants, because of its effect on the interconversion between singlet and triplet states (Illia *et al.*, 2009). The ratio of AC to DC magnetic field on radicals is shown in the figure 2.4.

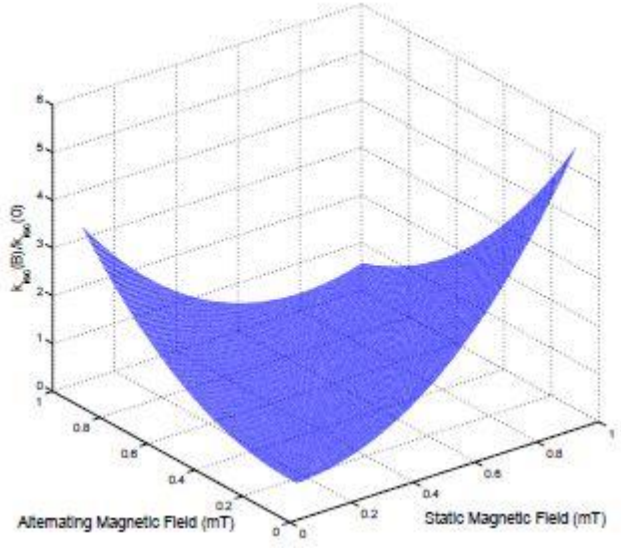

Figure 2.4: Variation of reaction rates with increase in alternating and static magnetic field

Source: Chathurika *et al. (*2010)

The reaction rate is also influenced by the strength of the magnetic field. When the magnetic field is more than the hyperfine constant, the reaction rate increases with increasing magnetic field. However the reaction rate is affected more by static magnetic field than alternating magnetic field (Henbes *et al.*, 2008). The effects of the magnetic field on the radicals and hence the physiological effects on plants, like chlorophyll content in this case do not act in isolation. Some factors like hyperfine constant, the exchange interaction and also other environmental factors contribute to this process (Ritz and Schulten, 2000).

The combination of magnetic field strength and durations of exposure have varied effects on plants. This is a fact that is supported by varied results that arise by exposure of plants to different combinations .Most studies have focused on using magnetic field strength with wide ranges between them. Racuciu (2012) used magnetic field of 50, 100, 150 and 200 mT on maize seedlings whereas Dhawi and Essam (2009) used magnetic field strength of 10, 50 and 100 mT on Date palms. Both studies have used a range of 50mT. It’s therefore important to carry out a study on the effect over a small range of magnetic flux density and determine its effect on plants.

Most studies on the effect of magnetic field on radicals in solvents have focused on magnetic field strength of between 0.5 mT and 5 mT (Ritz and Schulten, 2000). However, the results of these studies are mostly used to explain the effect of magnetic field much higher than that used in biological processes that result from exposure to magnetic field. Most of the studies on the effect of magnetic field on chlorophyll content have focused on the early stages of growth. This study therefore aims to study the effect of magnetic field on the whole lifespan of plants.

2.4 Ion cyclotron resonance (ICR) theory

This theory proposes that magnetic fields increases the movement of ions into and out of the cell , thereby altering the signaling mechanism and cellular function of the biological systems which in turn leads to changes in the physiological processes (Liboff, 2005). This is manifested by changes in physiochemical characteristics of both plants and animals exposed to magnetic fields (Aladjadjiyan, 2002). It explains that biological systems are sensitive to extremely low frequency magnetic fields that are tuned resonantly to various biological ions. Therefore if the frequency of magnetic field used resonates with the frequency of specific ions then, there will be an increased amount of the ion in the cell, this resonant frequency is given by:

$$f = \frac{1}{2\Pi}\left(\frac{q}{m}\right) B \qquad 2.4$$

Where f is the frequency of magnetic field used, q is the ionic charge, m is the mass of an ion and B is the magnetic flux density.

Cells convert one type of stimuli into another using a series of biochemical reactions involving enzymes that are first activated by specific molecules that serve as second messengers. One such ionic second messenger is the cellular calcium ion Ca^{2+} (Rasmussen *et al.,* 1976). A large amount of calcium is found in the extracellular space, where it plays a very active role in conveying information to the cells, however, the amount of calcium ions in the cells is so small as compared to the amount in the extracellular space resulting is an enormous difference in concentration between the inside and outside of the cell and this difference allows slight changes in the interior calcium that do occur to be used as signals (McAinsh *et al.*, 1998).

The frequencies proposed by the ICR theory have been supported by experiments that have led to an accumulation of elements when appropriate frequencies are applied. An application of magnetic flux density of 65 microtesla with a frequency of 50 Hz to mousear cress (*Arabidopsis thaliana*) lead to an increased amount of calcium ions in its cells (Alexander and Valentina, 2009). However, studies using magnetic field and frequencies out of the set of those proposed by ICR have also been shown to have an effect on biological organisms as far as concentration of ions is concerned. Exposure of Date palm to static magnetic field had an effect of increasing the concentration of Calcium, sodium and potassium, while it reduced the concentration of phosphorous (Racuciu *et al.,* 2008).

Most studies have used magnetic flux density above 10mT when exposing plants to magnetic field ,not only when investigating its effect on mineral concentration but also on many other parameters such as yield, plant height and maturity period. Magnetic field intensity of (10-100) mT with a frequency of (50-60) Hz changed the plasma membrane permeability of broad bean (*Vicia faba* L.) tip cells, and altered the movement of ions across the membrane (Dhawi *et al.*, 2009). Dhawi and Al-Khayri (2008) reported that magnetic fields of 120mT altered the content of Manganese, Sodium, Zinc, Copper, Magnesium, Potassium, nitrogen, iron and Phosphorous in strawberry leaves (*Fragaria xanannassa*) and date palm seedlings (*Phoenix dactylifera* L.). The range of 0-10 mT is rarely used, unless when investigating the effect on magnetic field directly on biological processes such as enzyme activity.

The exposure time is critical in experiments involving the exposure of magnetic field to plants, so is the cumulative period of the exposure. To be able to simulate the natural environment that plants grow in, it's important that magnetic field is exposed to plants for the entire period of growth. Studies that have been conducted on the effect of magnetic field on plants have focused on exposing plants for a short period of growth and generalizing the results to represent the effect of magnetic field on the entire period of growth. Racuciu (2012) exposed maize (*Zea mays L.*) to magnetic field for 10 days and determined its effect on chlorophyll. Dhawi and Essam (2009) exposed Date palms to magnetic field for 20 days and investigated its effect on elemental composition. Racuciu (2009) exposed maize to magnetic field for 14 days and investigated its effect on chlorophyll content. Yano (2004) exposed radish seedlings for 15 days while investigating its effect on CO_2 uptake. There is a need to investigate the effect of magnetic field on plants for the whole period of growth.

2.5 Principle of magneto-hydrodynamics

Magnetic fields affect the physical properties of water such as activation energy, viscosity, evaporation rate and surface tension of water. These effects are attributed to the fact that MF stabilizes the hydrogen bonds of water (Cai *et al.*, 2009). This theory proposes that these changes in the physical properties of water accelerates the entry of water into the plant and therefore accelerate plant growth. Some of these changes include increasing the surface tension of water (Fujimura and Lino, 2009), increasing the evaporation rate (Guo *et al.*, 2012) and increasing the solubility of water (Cai *et al.*, 2009). The increase in the interfacial tension implies that the MF strengthens the hydrophobic bonds in biological molecules such as enzymes (Fujimura *et al.*, 2009). Since biological systems include water–air or water-molecule interfaces, it would relate to the surface thermodynamic properties of water including surface tension.

It has been highlighted that magnetized water can remove 50% to 80% of soil salinity, compared to a removal of 30% by normal irrigation water. Laboratory tests have shown that desalination of a saline soil was 29% greater in the first leaching and 33% greater in the second leaching with magnetized water compared to untreated water (Campbell and Norman, 1977; Tanwar, 2003; Selim, 2008). After exposure of water to magnetic field, it was found that pH value changed from ~7to 7.6 and conductivity to 4.29 while the surface tension reduced by 2% (Samir, 2008). Maheshwari and Grewal (2009) concluded that Snow pea irrigated with magnetized water had higher yields and also the soil properties changed as a result of using magnetized water, such as a decrease in soil PH, increase in soil electrical conductivity and increase in availability of Ca, Mg, Na and P in the soil. Ahmed and Bassem (2013) concluded that magnetic water increased the entry of potassium (20%), nitrogen (15%) and calcium (50%) elements into the roots of faba bean. It’s important to study how exposure of magnetic field to the plant environment, which includes the plant itself and the water in the plant and soil, will affect the composition of elements in the plant and the chlorophyll content.

2.6 Summary

From earlier studies, it is evident that: the response of plants to magnetic field will vary according to the species of plant, magnetic flux density and exposure duration. The selection of the exposure duration and magnetic flux density used did not have any backing from theoretical approaches, partly because there is insufficient data on that aspect, therefore most studies used considerations such as, selection of magnetic flux densities that have recorded positive results and affordability to the end user.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The study site

The study was carried out in a greenhouse at Egerton University (0° 22'11.0'' S, 35°55' 58.0'' E) which is 2250 meters above the sea level, between January and November 2016 in a greenhouse where the maximum humidity levels were 80% and the maximum and minimum temperatures were 30°C and 10°C respectively

3.2 Sample preparation

The test plant was Fordhook Giant Swiss Chard (*Beta vulgaris subsp vulgaris*), a variety of spinach, which is well adapted to local environmental conditions and is also a good source of vitamins and fiber. Pots were filled with 2kg of soil then seeds without visible defect, insect damage and malformation were selected and sown at a rate of 4 seeds per pot. After germination thinning was done based on the criteria of the health of the seedlings to leave with two seedlings per pot. Phosphorus (P) application of 112 kg P/ha, nitrogen quantities was applied at a rate of 224 kg N/ha. Watering was done twice a day, in the morning and evening, and the quantity of water used depended on the age and the field capacity of the soil. The potted spinach plants were exposed to various levels of magnetic flux density and duration daily for 60 days consecutively. The treatments were imposed on the plants after germination. The treatments were as shown in Table 2.The exposure took place during the day because the spinach plants were active physiologically during the day. After 60 days of exposure to magnetic field the plants were sampled for analysis. The youngest fully opened leaves samples of different sizes were collected on the 60th day from each pot of each treatment. The samples were sorted to remove any foreign matter. The leaves were then washed with de-ionized water to remove the dust particles. All these procedures were carried out in a clean bench so as to avoid contamination. Then they were stored in clean labeled plastic bags. They were then stored in refrigerators before analysis.

3.3 Experimental design

The experimental design was a Completely Randomized Design (CRD) with 13 treatments replicated four times. Each treatment consisted of four plants. The youngest four leaves of each plant were plucked and taken for analysis for each replication. Since it was a completely random design, all other external factors were the same for all groups accepts the exposure to magnetic field and exposure duration. The treatments were made up of two variables, the magnetic flux density applied at four levels and the duration of exposure of the magnetic field applied at three levels (Table 2).

Table 2: Treatments

Treatment	Magnetic flux density (mT)	Duration of exposure (minutes)
Treatment 1 (control)	0	0
Treatment 2	0.5	10
Treatment 3	0.5	30
Treatment 4	0.5	60
Treatment 5	1.0	10
Treatment 6	1.0	30
Treatment 7	1.0	60
Treatment 8	1.5	10
Treatment 9	1.5	30
Treatment 10	1.5	60
Treatment 11	2.0	10
Treatment 12	2.0	30
Treatment 13	2.0	60

3.4 Exposure to magnetic field

The magnetic flux density was generated by a Helmholtz coil. The Helmholtz coil system consisted of 2 coils, each of 1000 turns of 1mm copper wire, a mean diameter of 260mm and thickness of 25mm. The coils were placed co-axially and placed at a distance of 130 mm from each other. The coils were connected to a power source and current was adjusted to get the required magnetic flux density. When current passed through the coils, a vertical magnetic field was generated in the center of the coil. The plants were placed at the center of the coil to be exposed to the magnetic field. Exposure time was controlled by an automatic timer. Magnetic flux density was measured by a Digital Gauss meter with a Hall probe by placing it at the center of the coils. The set-up is shown in figure 3.1 below;

Figure 3.1: Magnetic flux density exposure designs showing the arrangement of Helmholtz coils

3.5 Elemental analysis

The leaves were dried at 70°C in an oven for 48 hours, and then crushed in a mortar. Dry ashing method was adopted by placing the properly dried sample into the versatile crucible overnight in an electric muffle furnace at a temperature between 400°C to 440°C. The ash was removed from crucible and dried in desiccators. A 1 gram sample of ash was taken and digested using conc. HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 10:6:3. This procedure is according to the method described by (Rowell *et al.*, 1993). Digested ashes were stored in sterilized bottles and used for the determination of Ca, Zn, K, Mg, Fe and Na by flame atomic absorption spectroscopy. Then using the standard curves, the concentration of these elements were obtained .The actual concentrations of elements in the samples was worked out from the results obtained from AAS read out using equation 3.1 below (Black,1982).

$$\text{Actual concentration of minerals } (\mu g/g) = \frac{\text{concentration } (\mu g/ml)\ \times \text{volume of digest (ml)}}{\text{(weight of dried sample taken in g).}} \qquad 3.1$$

3.6 Determination of chlorophyll content

The extraction of chlorophyll was done using pure acetone. Freshly plucked spinach leaves were ground using 2ml of acetone in a mortar and pestle. After grinding, 1.5 ml of acetone was then used to clean the pestle. The homogenate was then centrifuged at 2500 r.p.m for 10 minutes. The volume of the homogenate was adjusted to 8ml using acetone. Absorbance values at 644nm and 661 nm of the resulting solution was recorded using Atomic Absorbance Spectrophotometer. The samples were analyzed in replicates under the same condition as blanks. For better precision, blanks were measured before and after the sample solutions to ensure stability. The concentration of chlorophyll a, chlorophyll b was calculated using equations 3.2 and 3.3. The method used to measure chlorophyll was according to Litchtenthalar (1987).

$$\text{Chlorophyll a } (\mu g/ml) = 11.24\, A_{661} - 2.04\, A_{644} \qquad 3.2$$

$$\text{Chlorophyll b } (\mu g/ml) = 20.13\, A_{644} - 4.19\, A_{661} \qquad 3.3$$

$$\text{Total chlorophyll content} = (\text{chlorophyll a} + \text{chlorophyll b}) \qquad 3.4$$

3.8 Data analysis

Data was analyzed using R statistical software (R version 3.3.4) (R, 2013). The parameter analyzed were the effect of treatment on the concentration of chlorophyll a, chlorophyll b, and the ratio of chlorophyll a/chlorophyll b and the concentration of Na, K, Fe, Mg, Zn and Ca. The results were subjected to ANOVA and where the effects were significant the means were separated using Tukey's HSD test at $p<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of magnetic field on elemental content in spinach leaves

4.1.1 Effect on concentration of zinc

The variation of the concentration of zinc in the leaves of spinach as a function of magnetic flux density and time of exposure is shown in Figure 4.1. Magnetic flux density and time of exposure had a statistically significant effect on the concentration of zinc. The trend shows a slight change in Zinc content between 0.0 mT and 1.0 mT and an increase from 1.0 mT to 2.0 mT. Other studies that have reported an increase in the concentration of zinc after exposure to magnetic field include an increase in 24% in tomato and 18% in sunflower after exposure of their seeds before germination (Abdul-Aziz *et al.*, 2015).

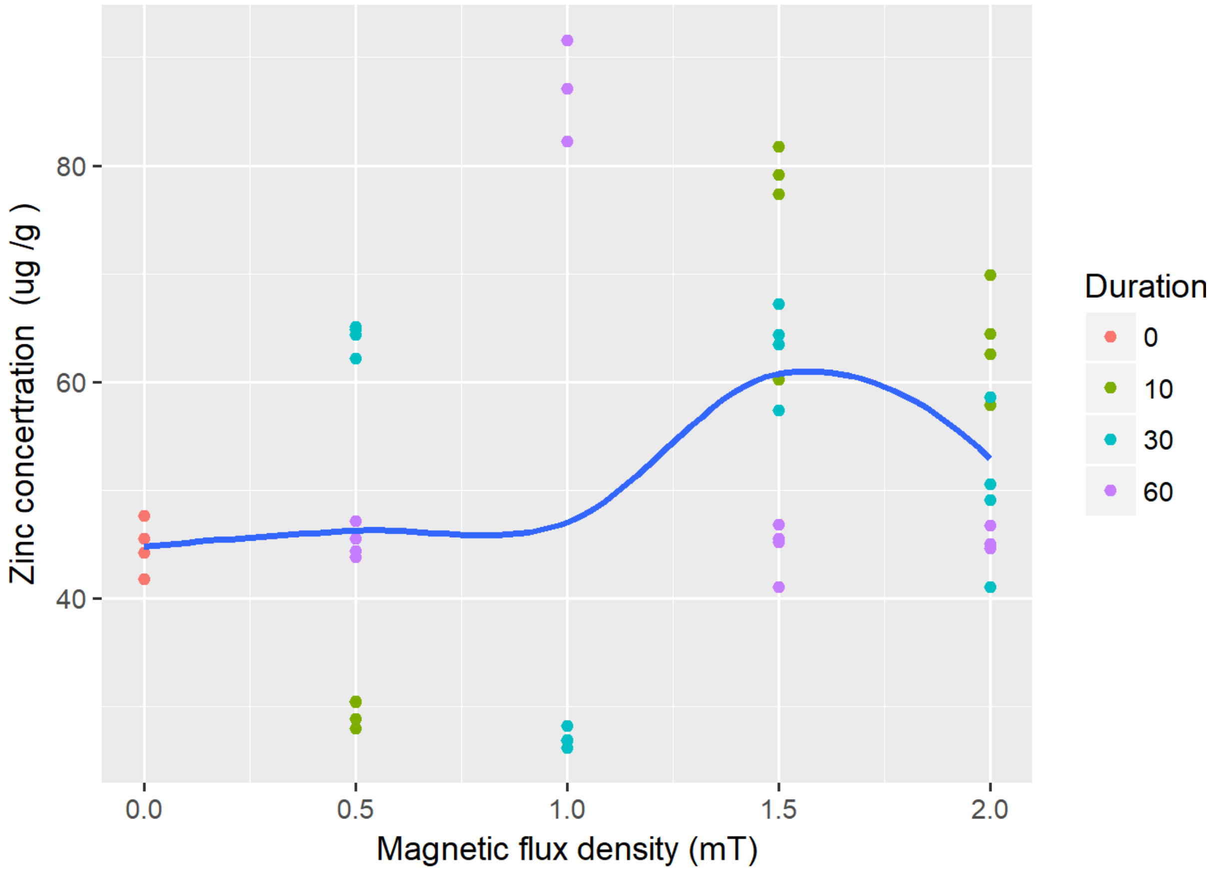

Figure 4.1: Variation of zinc concentration in spinach leaves with magnetic field dose

4.1.2 Effect on concentration of sodium

Figure 4.2 shows the effect of magnetic field and exposure period on the concentration of sodium in the leaves of spinach. Magnetic field and exposure duration had a statistically significant effect on the concentration of sodium in the leaves. The trend shows a decrease in the concentration of sodium from 0.0 mT to 2.0 mT, with the lowest concentration being at 0.5 mT. Shahin *et al.* (2016) reported an increase of 51% in sodium concentration after using magnetized water to irrigate cucumber (Shahin *et al.*, 2016).

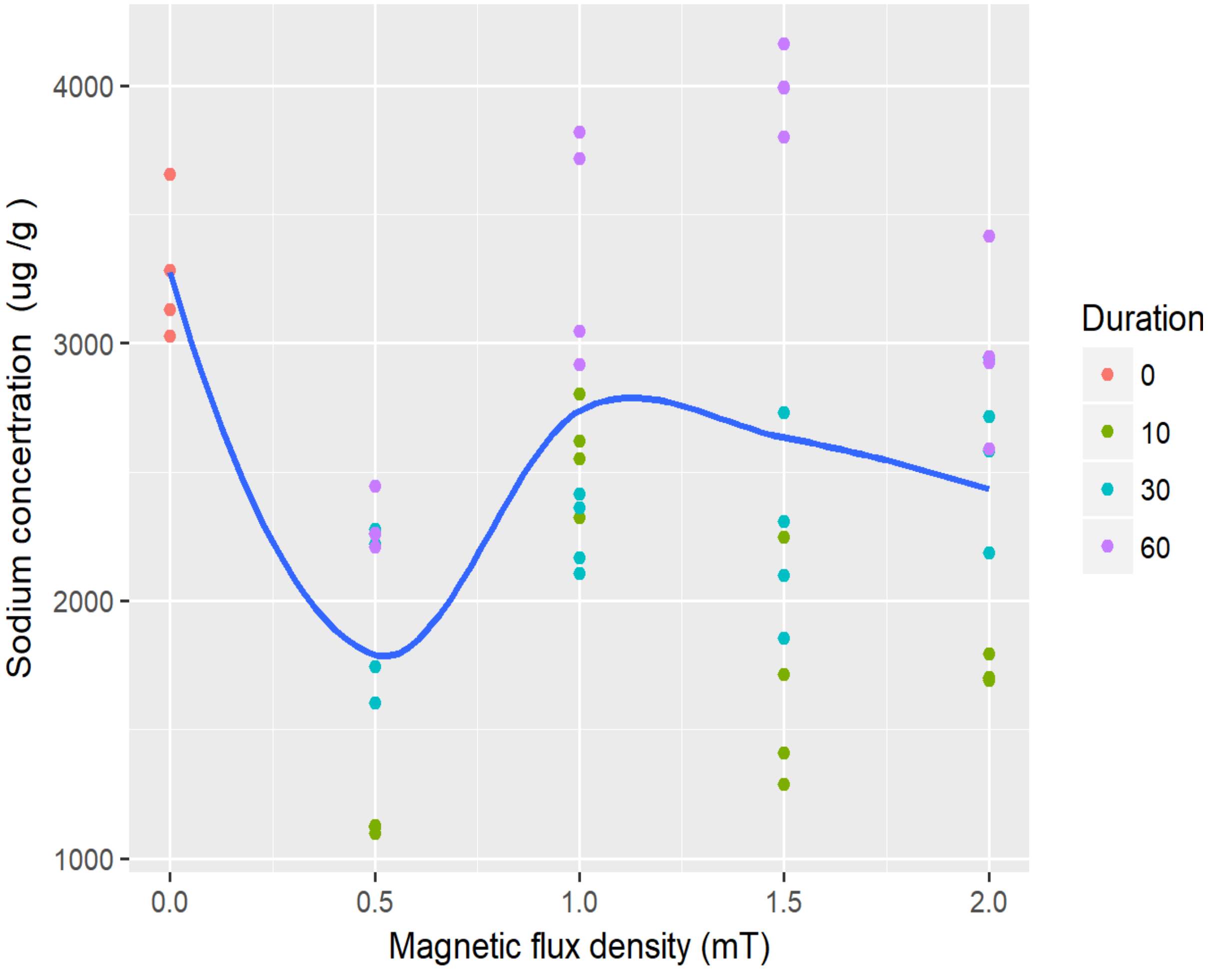

Figure 4.2: Variation of sodium concentration with magnetic field dose

4.1.3 Effect on concentration of potassium

The variation of the concentration of potassium in the leaves of spinach with magnetic flux density and exposure time is shown in Figure 4.3 below. Both magnetic flux density and exposure time had a significant effect on the concentration of potassium. There is an increase in the concentration of potassium at 0.5 mT and 1.5 mT. Ahmed (2013) reported an increase in the concentration of potassium in the faba beans after irrigation with magnetic water.

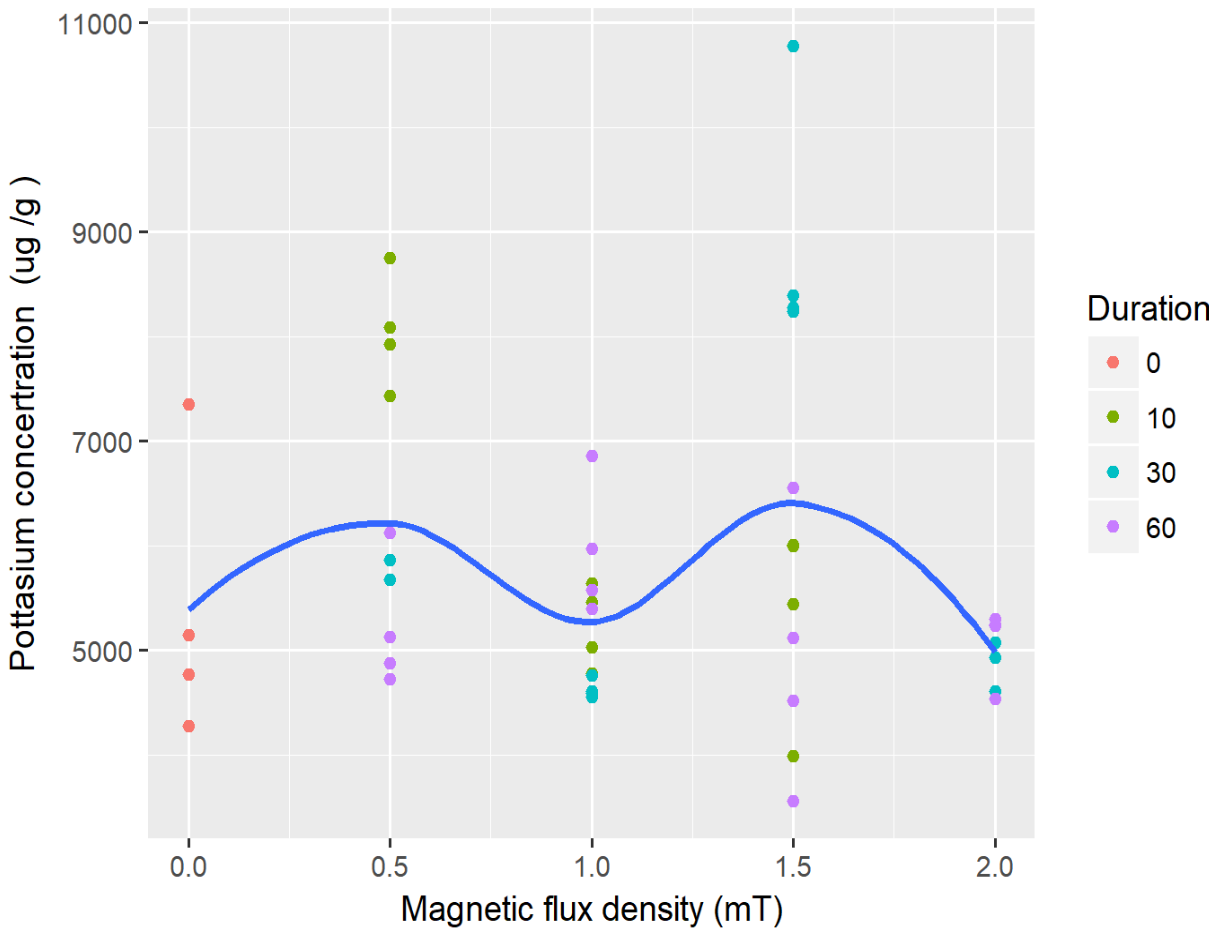

Figure 4.3: Variation of potassium concentration with magnetic field dose

4.1.4 Effect on concentration of iron

The variation of the concentration of iron in the leaves of spinach after exposure to various levels of magnetic flux densities and exposure times is shown in figure 4.4 shown below. Magnetic flux density and exposure time had a statistically significant effect on the concentration of iron. There is a decrease in the concentration of iron from 0.00 mT to 0.5 mT and an increase from 0.5 mT to 2.0 mT. The increase in the content of iron has also been recorded by Maheshwari (2009) after irrigation of chickpea with magnetic water. Hozayn *et al.* (2013) reported an increase of 7% in the content of iron after exposure of oilseed to magnetic field.

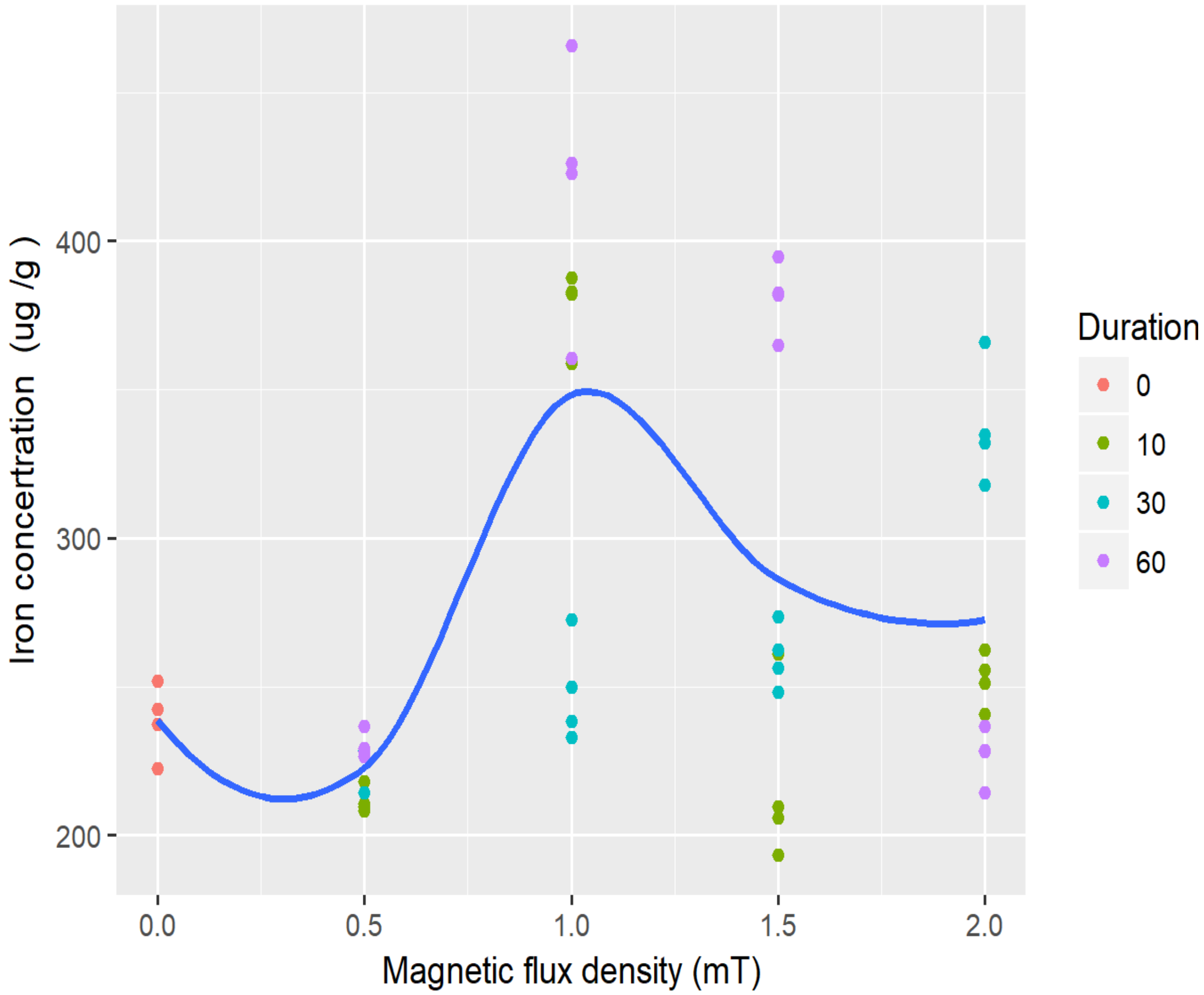

Figure 4.4: Variation of iron concentration with magnetic field

4.1.5 Effect on concentration of magnesium

The effect of magnetic field on the content of magnesium is shown in figure 4.5. The effect of magnetic flux density and exposure time on the content of magnesium was statistically significant. From the trend, there is an increase in increase in the concentration of magnesium around 1.0 mT and a decrease from 1.0 mT to 2.0 mT. Hilal (2002) reported an increase of magnesium content by 80% in citrus fruits after exposure to magnetic field. These results are consistent with studies carried out earlier. Exposure of magnetic field to oilseed increased magnesium content by 6.7% (Hozayn *et al.*, 2013).

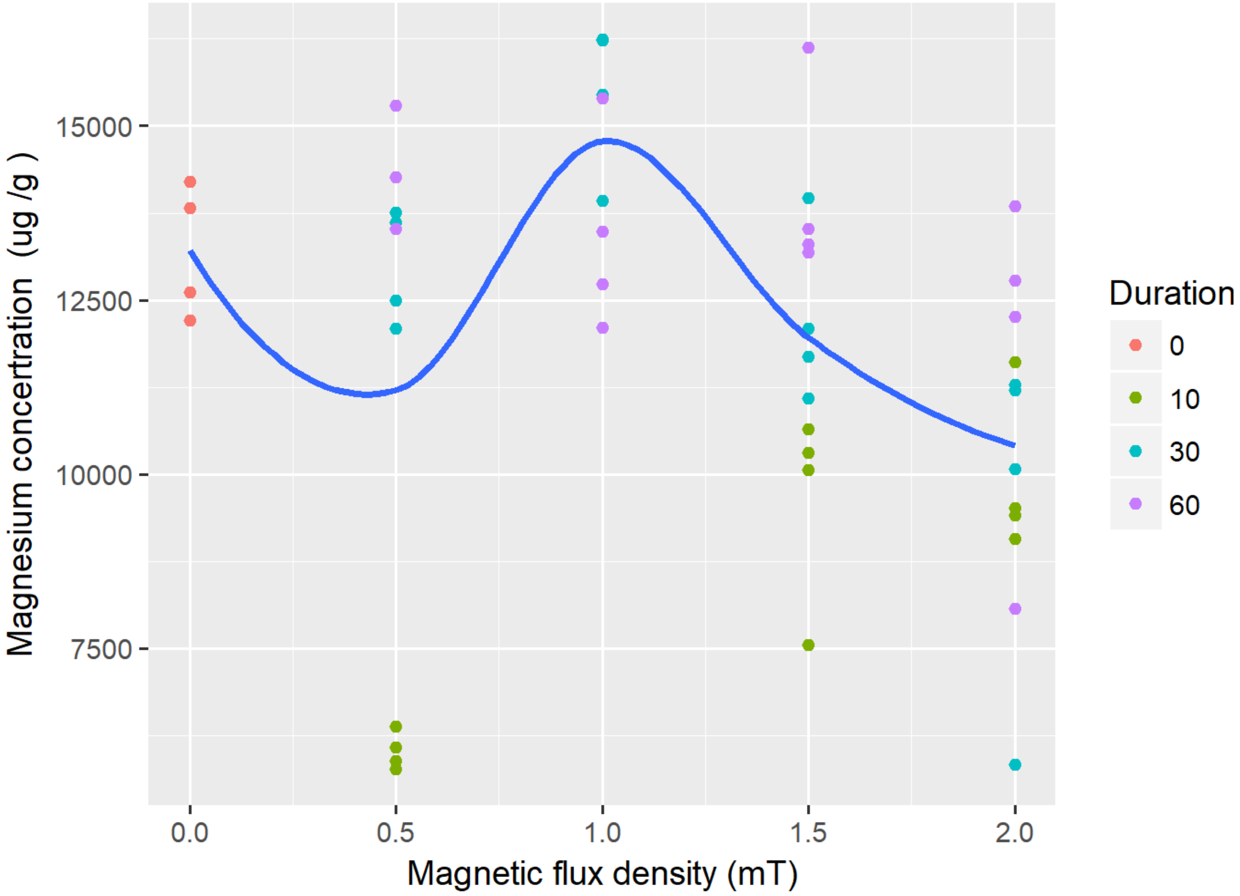

Figure 4.5: Variation of magnesium concentration with magnetic field dose

4.1.6 Effect on concentration of calcium

Figure 4.6 shows the effect of magnetic field on calcium content. Magnetic flux density and exposure time had a significant effect on calcium content. From the trend, there is a decrease in calcium concentration from 0.0 mT to 0.5 mT and there is an increase from 0.5 mT to 2.0 mT. Exposure of magnetic field to date palm also led to an increase in the concentration of magnetic flux density from 50 mT to 150 mT, there was an increase in the concentration of calcium with increase in the magnetic flux density (Dhawi *et al.*, 2009).

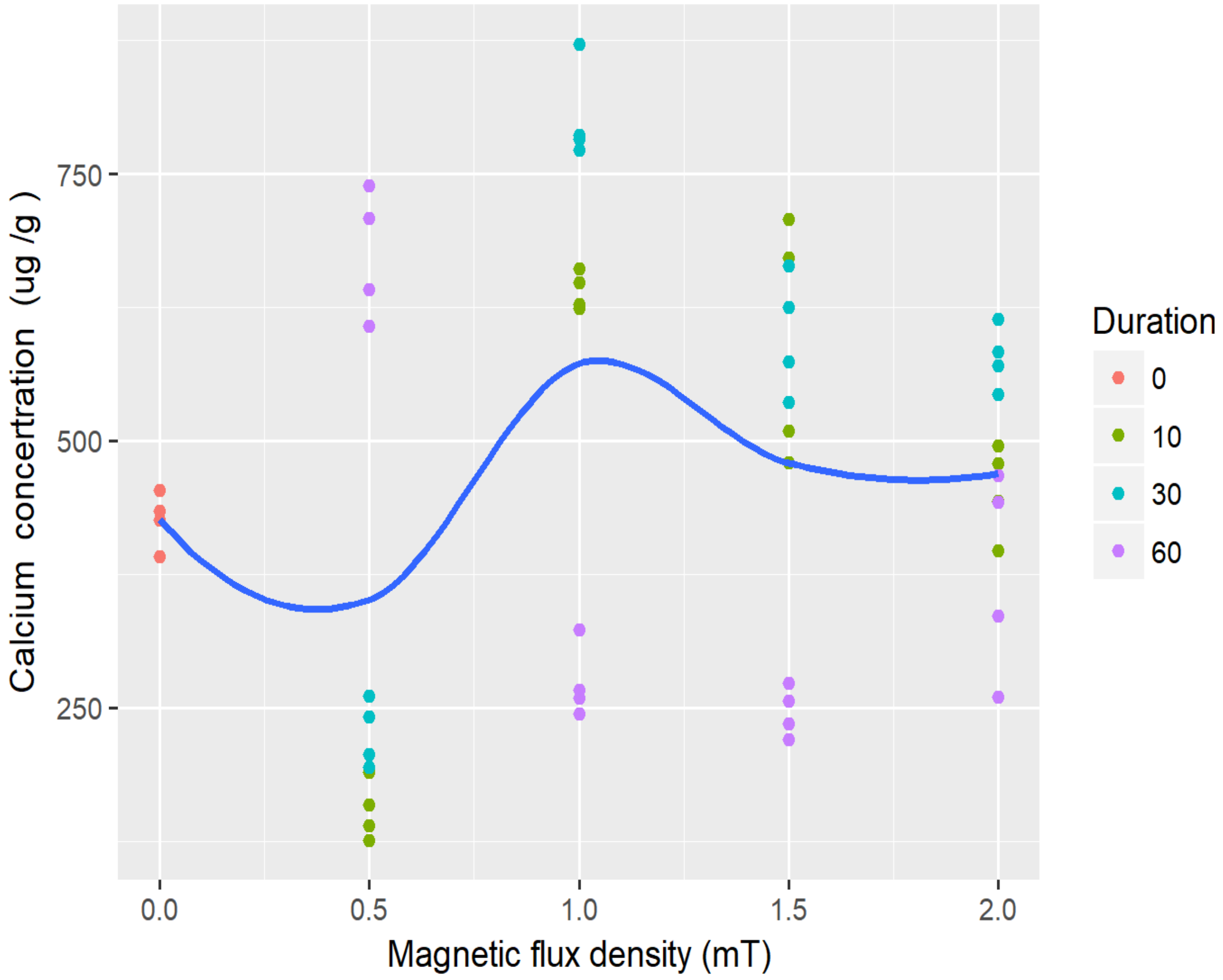

Figure 4.6: Variation of calcium concentration with magnetic field dose

4.2 Effect of magnetic field on chlorophyll

4.2.1 Chlorophyll a

Figure 4.7 below, depicts the effect of magnetic flux density and time of exposure has on the concentration of chlorophyll a. The means between the groups was statistically different (*p*<0.05). Magnetic flux density, exposure time and the interaction of magnetic flux density and exposure time had a significant effect on chlorophyll a. From the trend that emerges, there is an increase in chlorophyll content from the control to 0.5 mT and decreases from 0.5 mT to 2.0 mT. Earlier studies that have also reported similar results include: exposure of chickpea (Ci*cer areitum*) to magnetic field also leads to decrease in chlorophyll a (Singh *et al*., 2015). An increase in chlorophyll a has also been recorded after irradiating maize (*Zea mays*) and date palm seedlings with magnetic field (Racuciu, 2012).

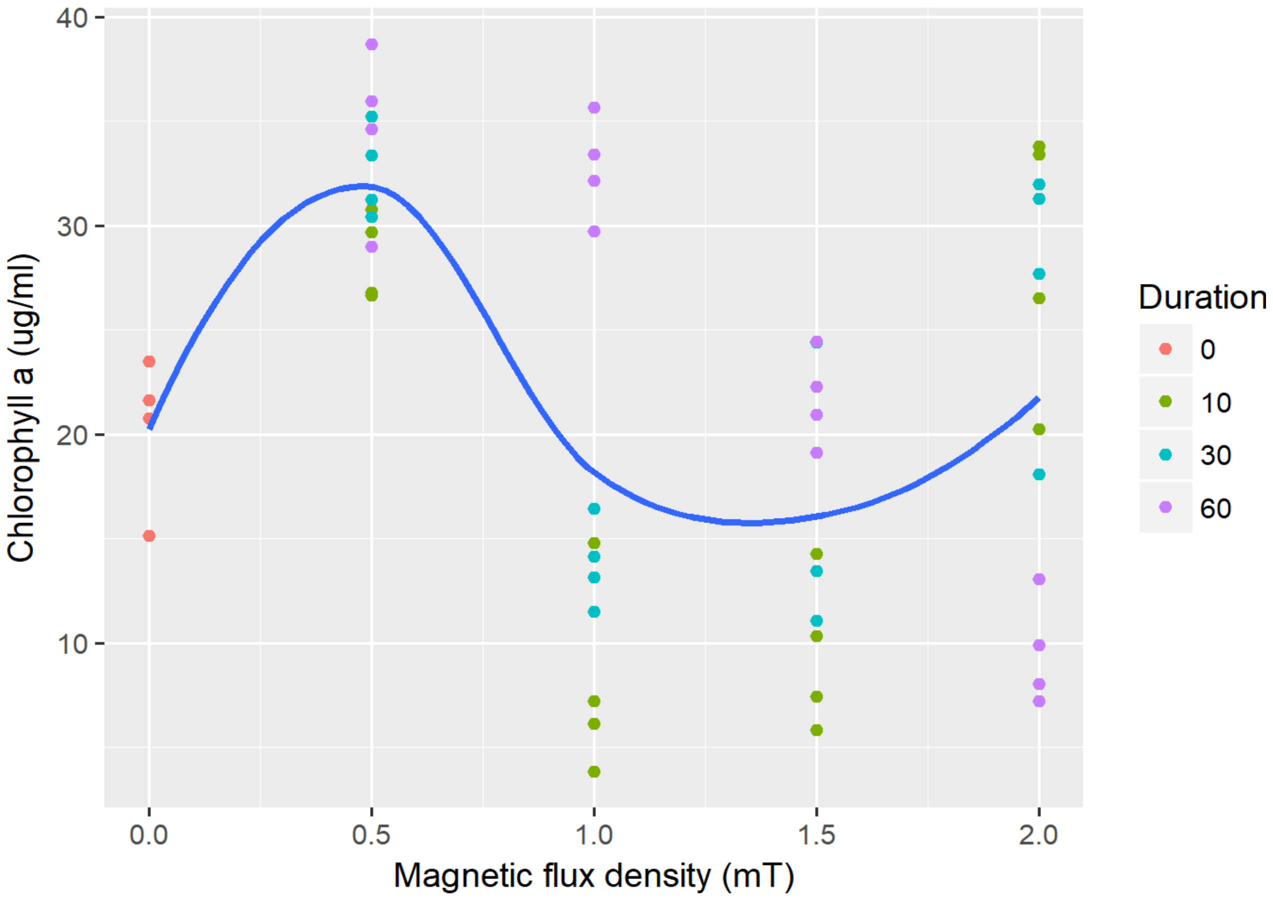

Figure 4.7: Variation of chlorophyll a with magnetic field

4.2.2 Chlorophyll b

Figure 4.8 shows the variation of the concentration of chlorophyll b with magnetic flux density and exposure time. Both magnetic flux density and exposure time had a significant effect on chlorophyll b (*p*<0.05). From figure 6, there is a decrease in chlorophyll b from control to 1.5 mT .There is an increase in chlorophyll b from 1.5 mT to 2.0 mT. Earlier studies that have been conducted also agree with the trend that emerges from above both from static and alternating magnetic. An increase in chlorophyll b with increase in magnetic flux density of 10 mT daily at 1, 2 and 4 hours for 10 days was also observed in maize (*Zea mays*) seedlings and pumpkin (*Curcubita pepo*) seedlings (Rucuciu , 2012).

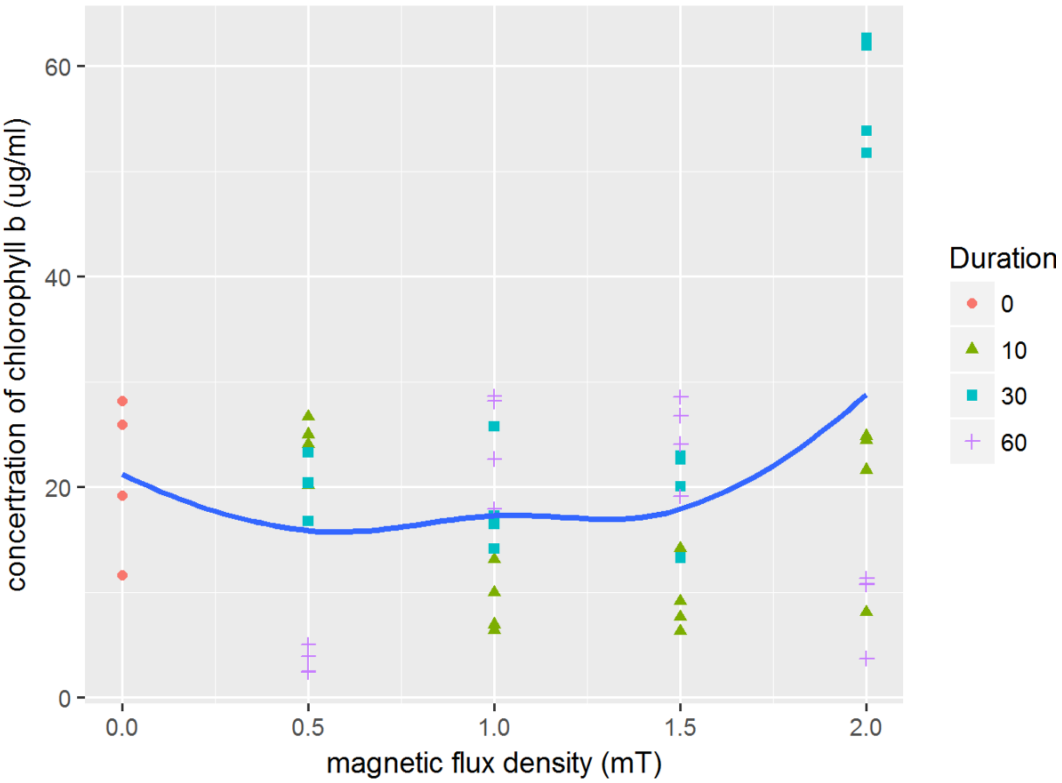

Figure 4.8: Variation of chlorophyll b with increase in magnetic field

4.2.3 Ratio of chlorophyll a to chlorophyll b

Figure 4.9 shows the effect of magnetic flux density on the ratio of chlorophyll a to chlorophyll b. Magnetic flux density and time of exposure had a significant effect on the ratio of chlorophyll a to chlorophyll b. There is an increase in the ratio of chlorophyll a to chlorophyll b from control to 1.0 mT. However, there is no change from 1.0 mT to 2.0 mT. The ratio of chlorophyll a to chlorophyll b is an indicator of the photosynthetic efficiency of plants, which implies that magnetic field affects the photosynthetic efficiency of spinach plants. Racuciu (2012) reported a slight increase in the ratio of chlorophyll a to chlorophyll b with increase in exposure time (1-4 hours) in maize plants after exposure to magnetic field an increase in the ratio of chlorophyll a to chlorophyll b from 1-2 hours and a decrease from 2-4 hours inpumpkin (*Cucurbita pepo*).

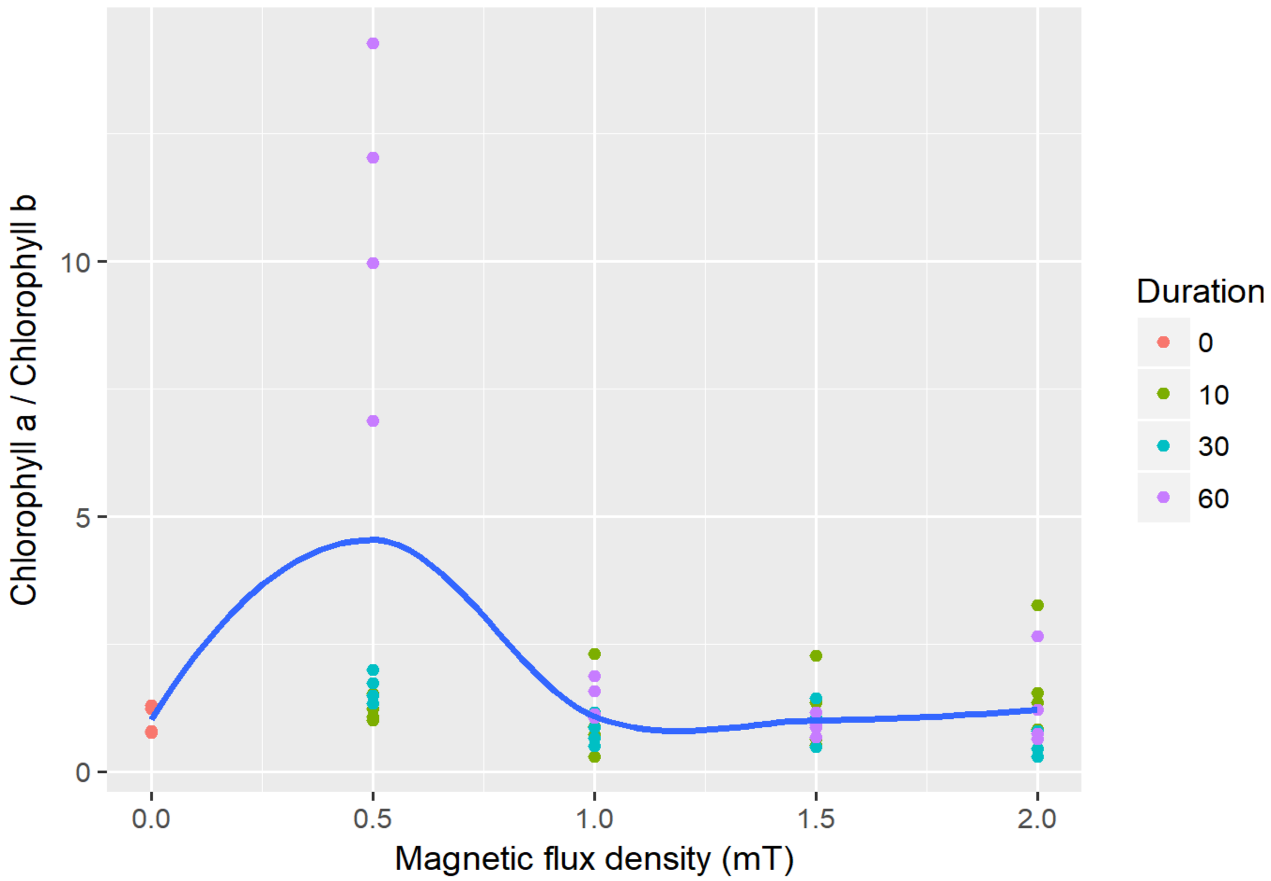

Figure 4.9: Variation of the ratio of chlorophyll a: chlorophyll b with increase in magnetic field

4.2.3 Total chlorophyll content

Figure 8 shows the effect of magnetic field on the total chlorophyll content. Magnetic flux density and exposure time had a significant effect on the total chlorophyll content. From figure 8, there is an increase in the total chlorophyll content from 0.0 mT to 0.5 mT. However there was a decrease in total chlorophyll content from 0.5 mT up to 2.0 mT. Dhawi (2009) reported an increase in the chlorophyll content in steps of 50, 100, 150 mT and an increase with time from 30 minutes, 60 minutes and 1 hour. The presence of magnetic field in the soil where beans were planted lead to a decrease in chlorophyll content in the leaves; moreover there was a decrease in the chlorophyll content as the strength of the magnetic field increased (Jovanic and Jevtovic, 2009).

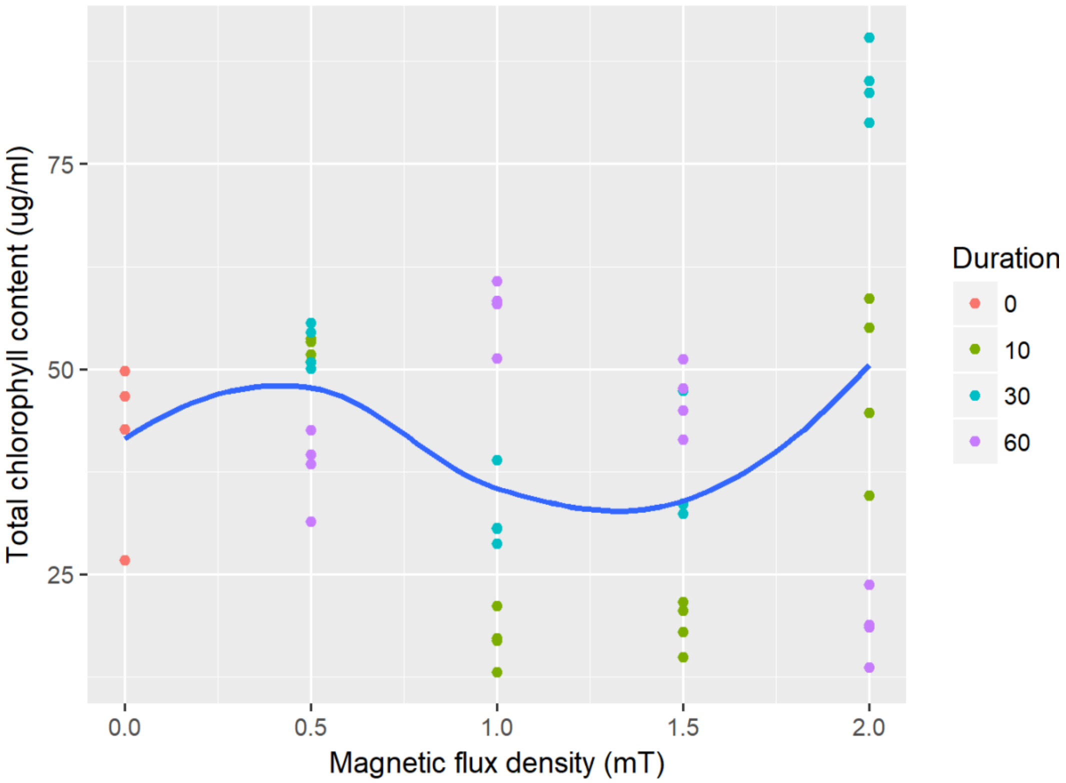

Figure 4.10: Variation of total chlorophyll content with increase in magnetic field

4.3 Discussions

4.3.1 Effect of magnetic field on elemental composition

From the results in section 2, it can be concluded that magnetic field affects the elemental composition of spinach. Exposure of magnetic field to spinach increases its elemental concentration and this therefore can help solve the mineral deficiency problems. The magnetic field doses that increases the mineral concentration is shown in table 3.

Table 3: Beneficial magnetic field doses on elements

ELEMENT	MAGNETIC FIELD DOSE
Calcium	(1 mT, 30 minutes), (0.5 mT, 60 minutes), (1 mT, 10 minutes), (1.5 mT, 30 minutes), (2 mT, 30 minutes)
Magnesium	(1 mT, 10 minutes), (1 mT, 30 minutes)
Iron	(1 mT, 60 minutes), (1.5 mT, 60 minutes), (1 mT, 10 minutes), (2 mT, 30 minutes)
Potassium	(1.5 mT, 30 minutes), (0.5 mT, 10 minutes)
Sodium	(1.5 mT, 60 minutes)
Zinc	(1 mT, 60 minutes), (1.5 mT, 10 minutes), (0.5 mT, 30 minutes), (2 mT, 10 minutes), (1.5 mT, 30 minutes)

One explanation on the observed changes in the concentration of the elements is the fact that magnetic field increases the permeability of cells in the roots of spinach. According to the ion cyclotron resonance theory, when magnetic field resonates with the ions, then this leads to an increased influx of ions into cells, and there is a reduction in the inflow of ions when they do not resonate (Liboff, 2005). However this theory does not consider the fact that cells have charges which are affected by magnetic field according to the Lorenz law. This can explain why applications of magnetic field out of those proposed by ICR have shown to affect the concentration of ions in plants. Therefore the increased permeability of cells for certain magnetic field and exposure times might explain the change in concentration of nutrient ions in the cells.

The second explanation of the observed changes in the concentration of elements is based on the effect of magnetic field on water and soil properties (Duarte *et al.*, 1997). Magnetic field has an effect on water because of the partial positive and negative charges of water which form a hydrogen bond (Shahim *et al.*, 2016). Magnetic field causes the two opposite charges to move in opposite directions due to the force that is created according to Lorenz law (Baker and Judd, 1996). There is also weakened *van der waals* force between the water molecules and therefore the hydrogen bond in water is strengthened. This leads to an increased solubility of salts and minerals which may lead to an alteration of the absorption of elements (Sharaf el-Deen, 2016). Ahmed. (2013) reported an increase in soluble soil K^+, Mg^{2+} and a decrease in Ca^{2+}. Magnetic field also increases the pH, electrical conductivity and decreases the TDS (Shahim *et al.,* 2016).

The increase in the concentration of elements could also be explained by the fact that magnetic field exposure to soils increases the availability of available elements in the soil and also affects water and ion absorption by plants (Taia *et al.,* 2007). Ahmed (2013) reported that magnetic field increases the available N, P and K.

4.3.2 Effect of magnetic field on chlorophyll content

Exposure of spinach to magnetic field for a long time has an effect on chlorophyll. Time of exposure and magnetic flux density have an impact on chlorophyll. Magnetic flux density of 0.5 mT has a positive effect on chlorophyll a, chlorophyll b, total chlorophyll content and the ratio of chlorophyll a to chlorophyll b. Most conclusions from previous studies have been that high values of magnetic field combined with short exposure times and low values of magnetic field combined with long exposure times. A good example, magnetic flux density of 160 mT and exposure time of 5 minutes enhanced chlorophyll a and chlorophyll b in pea (Iqbal *et al.*, 2010). Compare to these studies, the magnetic flux density and exposure times used in this study are both low.

The effect of magnetic flux density less than 10 mT on the singlet born radicals is the suppression of the S-T_{+-} interconversion , thereby reducing the yield of the triplet state of the primary electron donor by 30% to 40% and increase in the lifetime of the pair by 20% to 70% (Norris *et al.,* 1982). In some cases an increase in the triplet yield is observed (1-2 mT), but this raises from the 2J response that results when predominantly singlet energy levels become degenerate with triplet levels that are mostly T_- or T_+ in character (Till and Hore., 1997). The dependence of the triplet yield in chlorophyll formation and also photosynthesis is confirmed by Blankenship *et al*. (1977) who reported that the triplet yield in the reaction centers of *Rhodopseudomonas sphaeroides* was dependent on magnetic field up to 200 mT. This is consistent with the gradual energetic isolation of the T_{+-} spin states of the radical pair by the Zeeman interaction leaving only the S –T_0 interconversion. Energetic isolation of the T_{+-} from the spin state mixing regime limits the radical pair population that is able to interconvert from the initial singlet state therefore decreasing the triplet yield, theoretically from 75% to 50% (Alex, 2016). This lifting of the T_{+-} states away from the singlet states might affect chlorophyll formation leading to the variations in chlorophyll concentration observed.

The variation of chlorophyll content may also be explained through the variation of the concentration of magnesium and potassium in spinach. Magnesium ions are needed for the chlorophyll synthesis and potassium ion may lead to photosynthetic efficiency possibility by increasing the number of chloroplasts per cell (Hozayn *et al.,* 2013). The findings of this study show that there is an increase in magnesium content with increase in time, and therefore this might also explain the changes in chlorophyll content. There is also slight deviation in the concentration of potassium for most magnetic field doses. There is a general decrease in chlorophyll efficiency.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Magnetic field had an effect on both the chlorophyll content and the mineral concentration. The magnitude of the effect seems to be dependent on the exposure time and magnetic flux density. There are there are patterns that emerge for each element after exposure to magnetic field. There are “windows “or regions of magnetic field dose that increase the concentration of elements in spinach: magnetic flux density of (1-2)mT increased the concentration of most elements while magnetic flux density of (0.5-1)mT increased the concentration of chlorophyll . Generally there seems to be specific magnetic field doses that singularly increase the concentration of elements without following a particular pattern. The identification and verification of these element specific magnetic field doses can be used for production of spinach with high elemental composition that can help solve mineral deficiency problems in people, they can also be used as a case study for the ICR theory.

Magnetic field of 1.0, 1.5 and 2.0mT, increased the elemental composition for zinc, iron, magnesium and calcium for all exposure periods. Therefore exposure of spinach to such magnetic flux densities can be used to grow spinach that have a high concentration of the above mentioned elements that can accessed by people because of their affordability. There was a reduction in the concentration of sodium for all exposure times and magnetic flux densities. Therefore it’s important to note that whereas the other elements were increased, sodium concentration diminished.

5.2 Recommendations

Magnetic field in the region of (1-2) mT can be used to grow spinach that has high mineral content. It’s an affordable, environment friendly and non-chemical method that does not affect the health of the plant. However future research should focus on the health safety of the food products produced using this method. It’s also important for other studies to focus on the effect of magnetic flux density beyond 2mT on spinach growth,

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APPENDICES

APPENDIX A

Summary tables for chlorophyll

1. ch.a (means)

```
Power Duration N      ch.a       sd       se        ci
1     0        0 4 20.253950 3.610665 1.805333  5.745374
2   0.5       10 4 28.467900 2.088102 1.044051  3.322637
3   0.5       30 4 32.551825 2.160266 1.080133  3.437466
4   0.5       60 4 34.552800 4.074772 2.037386  6.483872
5     1      10 4  7.990200 4.741694 2.370847  7.545094
6     1      30 4 13.806275 2.079346 1.039673  3.308703
7     1      60 4 32.737900 2.475615 1.237808  3.939256
8   1.5       10 4  9.449300 3.722114 1.861057  5.922714
9   1.5       30 4 17.004200 5.974568 2.987284  9.506871
10   1.5       60 4 21.692525 2.237000 1.118500  3.559566
11     2       10 4 28.485650 6.436365 3.218182 10.241693
12     2       30 4 27.242467 6.406742 3.203371 10.194557
13     2       60 4  9.552375 2.586453 1.293226  4.115623
```

2. ch.b

```
  Power Duration N      ch.b       sd       se        ci
1     0        0 4 21.234572 7.457740 3.728870 11.866928
2   0.5       10 4 23.983038 2.759034 1.379517  4.390239
3   0.5       30 4 20.212592 2.675526 1.337763  4.257359
4   0.5       60 4  3.459349 1.243210 0.621605  1.978225
5     1      10 4  9.110559 3.109247 1.554623  4.947505
6     1      30 4 18.422845 5.079003 2.539501  8.081826
7     1      60 4 24.335117 5.075719 2.537860  8.076602
8   1.5       10 4  9.317922 3.445620 1.722810  5.482750
9   1.5       30 4 19.737485 4.496834 2.248417  7.155466
10   1.5       60 4 24.648067 4.111981 2.055990  6.543079
11     2       10 4 19.755273 7.891852 3.945926 12.557697
```

```
12     2       30 4 57.573483 5.575871 2.787936  8.872455
13     2       60 4  9.145520 3.626360 1.813180  5.770348
```

3. ch.a.ch.b

```
Power Duration N  ch.a.ch.b        sd        se        ci
1      0        0 4  1.0232970 0.2784582 0.1392291 0.4430892
2    0.5       10 4  1.2069657 0.2341925 0.1170962 0.3726525
3    0.5       30 4  1.6370120 0.2841309 0.1420654 0.4521156
4    0.5       60 4 10.7893830 3.1455131 1.5727566 5.0052133
5      1       10 4  1.0521932 0.8742878 0.4371439 1.3911870
6      1       30 4  0.7989870 0.2821350 0.1410675 0.4489397
7      1       60 4  1.4049170 0.3844728 0.1922364 0.6117820
8    1.5       10 4  1.1926788 0.8033046 0.4016523 1.2782370
9    1.5       30 4  0.9149723 0.4236267 0.2118134 0.6740847
10   1.5       60 4  0.9036485 0.2036281 0.1018140 0.3240177
11     2       10 4  1.7502537 1.0570173 0.5285087 1.6819505
12     2       30 4  0.5851891 0.2515909 0.1257954 0.4003372
13     2       60 4  1.3152915 0.9340633 0.4670317 1.4863032
```

4. Tot.ch

```
Power Duration N   Tot.ch        sd        se        ci
1      0        0 4 41.48852 10.251263 5.1256313 16.312046
2    0.5       10 4 52.45094  1.300048 0.6500239  2.068666
3    0.5       30 4 52.76442  2.702984 1.3514918  4.301050
4    0.5       60 4 38.01215  4.731383 2.3656916  7.528687
5      1       10 4 17.10076  3.313023 1.6565115  5.271759
6      1       30 4 32.22912  4.538719 2.2693593  7.222114
7      1       60 4 57.07302  4.047326 2.0236628  6.440198
8    1.5       10 4 18.76722  2.980261 1.4901305  4.742260
9    1.5       30 4 36.74169  7.115573 3.5577866 11.322465
10   1.5       60 4 46.34059  4.149784 2.0748920  6.603233
11     2       10 4 48.24092 10.837888 5.4189442 17.245499
12     2       30 4 84.81595  4.294201 2.1471003  6.833031
```

```
13     2       60 4 18.69789  4.145410 2.0727051  6.596273
```

ANOVA

1. Chlorophyll a

MODEL: chlo.a ~ Rep + Power * Duration, data = chlorophyll

```
Analysis of Variance Table
Response: ch.a
              Df  Sum Sq Mean Sq F value    Pr(>F)
Rep            3   64.03   21.34  1.3203 0.2828365
Power          4 1777.72  444.43 27.4938 1.658e-10 ***
Duration       2  302.86  151.43  9.3679 0.0005303 ***
Power:Duration  6 2316.20  386.03 23.8812 3.508e-11 ***
Residuals     36  581.93   16.16
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
> #Tukey HSD
> outFactorial <-HSD.test (outAOV, c("Power", "Duration"),
+                          main = "ch.a ~ Rep + Power + Duration + Power:Duration",
+                          console=TRUE)

Study: ch.a ~ Rep + Power + Duration + Power:Duration
HSD Test for ch.a
Mean Square Error:  16.16476
Power:Duration,  means
           ch.a      std r     Min     Max
0.5:10 28.467900 2.088102 4 26.6303 30.7829
0.5:30 32.551825 2.160266 4 30.4119 35.2113
0.5:60 34.552800 4.074772 4 28.9935 38.6732
0:0    20.253950 3.610665 4 15.1173 23.5071
1.5:10  9.449300 3.722114 4  5.8013 14.2828
```

```
1.5:30 17.004200 5.974568 4 11.0400 24.3832
1.5:60 21.692525 2.237000 4 19.1317 24.4316
1:10    7.990200 4.741694 4  3.8263 14.7786
1:30   13.806275 2.079346 4 11.4777 16.4496
1:60   32.737900 2.475615 4 29.7355 35.6648
2:10   28.485650 6.436365 4 20.2315 33.7849
2:30   27.242467 6.406742 4 18.0548 31.9608
2:60    9.552375 2.586453 4  7.2156 13.0447
alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141
Honestly Significant Difference: 10.07174
Means with the same letter are not significantly different.
Groups, Treatments and means
a          0.5:60     34.55
a          1:60       32.74
a          0.5:30     32.55
ab         2:10       28.49
ab         0.5:10     28.47
ab         2:30       27.24
bc         1.5:60     21.69
bc         0:0        20.25
cd         1.5:30     17
cd         1:30       13.81
d          2:60       9.552
d          1.5:10     9.449
d          1:10       7.99

>

>
```

2. Chlorophyll b

```
Analysis of Variance Table
Response: ch.b
               Df Sum Sq Mean Sq F value    Pr(>F)
Rep             3   36.0   11.99  0.5199    0.6713
Power           4 1284.6  321.14 13.9274 5.845e-07 ***
Duration        2 1949.1  974.57 42.2656 3.578e-10 ***
Power:Duration  6 5151.3  858.55 37.2340 5.227e-14 ***
Residuals      36  830.1   23.06
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
> #Tukey HSD
> outFactorial <-HSD.test (outAOV, c("Power", "Duration"),
+                          main = "ch.b ~ Rep + Power + Duration + Power:Duration",
+                          console=TRUE)

Study: ch.b ~ Rep + Power + Duration + Power:Duration
HSD Test for ch.b
Mean Square Error:  23.05818
Power:Duration,  means

            ch.b      std r       Min       Max
0.5:10 23.983038 2.759034 4 20.177830 26.683150
0.5:30 20.212592 2.675526 4 16.753600 23.284170
0.5:60  3.459349 1.243210 4  2.408403  5.030137
0:0    21.234572 7.457740 4 11.622900 28.159730
1.5:10  9.317922 3.445620 4  6.303907 14.186330
1.5:30 19.737485 4.496834 4 13.283950 22.999400
1.5:60 24.648067 4.111981 4 19.131900 28.572670
1:10    9.110559 3.109247 4  6.401653 13.131750
1:30   18.422845 5.079003 4 14.128510 25.769070
```

```
1:60   24.335117 5.075719 4 17.891570 28.610170
2:10   19.755273 7.891852 4  8.112550 24.853570
2:30   57.573483 5.575871 4 51.747000 62.705567
2:60    9.145520 3.626360 4  3.718390 11.294990

alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141
Honestly Significant Difference: 12.02908
Means with the same letter are not significantly different.

Groups, Treatments and means
a        2:30     57.57
b        1.5:60   24.65
b        1:60     24.34
b        0.5:10   23.98
bc       0:0      21.23
bcd      0.5:30   20.21
bcd      2:10     19.76
bcd      1.5:30   19.74
bcd      1:30     18.42
cde      1.5:10   9.318
de       2:60     9.146
de       1:10     9.111
e        0.5:60   3.459

>

>
```

3. Ratio of chlorophyll a to chlorophyll b

```
> anova (outAOV)
Analysis of Variance Table
Response: ch.a.ch.b
               Df  Sum Sq Mean Sq F value    Pr(>F)
Rep             3   1.717   0.572  0.5107    0.6774
Power           4 110.189  27.547 24.5845 7.202e-10 ***
Duration        2  65.406  32.703 29.1856 2.926e-08 ***
Power:Duration  6 172.687  28.781 25.6857 1.254e-11 ***
Residuals      36  40.338   1.121
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
> #Tukey HSD
> outFactorial <-HSD.test (outAOV, c("Power", "Duration"),
+                          main = "ch.a.ch.b ~ Rep + Power + Duration + Power:Duration",
+                          console=TRUE)

Study: ch.a.ch.b ~ Rep + Power + Duration + Power:Duration
HSD Test for ch.a.ch.b
Mean Square Error:  1.120513
Power:Duration,  means

         ch.a.ch.b       std r       Min        Max
0.5:10  1.2069657 0.2341925 4 0.9980180  1.5255820
0.5:30  1.6370120 0.2841309 4 1.3416900  1.9902590
0.5:60 10.7893830 3.1455131 4 6.8793700 14.2755400
0:0     1.0232970 0.2784582 4 0.7679430  1.3006480
1.5:10  1.1926788 0.8033046 4 0.5222860  2.2657020
1.5:30  0.9149723 0.4236267 4 0.4881810  1.4401520
1.5:60  0.9036485 0.2036281 4 0.6695820  1.1649440
1:10    1.0521932 0.8742878 4 0.2913800  2.3085640
```

```
1:30    0.7989870 0.2821350 4 0.5100280  1.1642860
1:60    1.4049170 0.3844728 4 1.0555370  1.8673010
2:10    1.7502537 1.0570173 4 0.8284470  3.2690830
2:30    0.5851891 0.2515909 4 0.2934007  0.7977284
2:60    1.3152915 0.9340633 4 0.6388350  2.6647550

alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141
Honestly Significant Difference: 2.651724
Means with the same letter are not significantly different.

Groups, Treatments and means
a          0.5:60     10.79
b          2:10       1.75
b          0.5:30     1.637
b          1:60       1.405
b          2:60       1.315
b          0.5:10     1.207
b          1.5:10     1.193
b          1:10       1.052
b          0:0        1.023
b          1.5:30     0.915
b          1.5:60     0.9036
b          1:30       0.799
b          2:30       0.5852

>
```

4. Total chlorophyll

```
Analysis of Variance Table
Response: Tot.ch
               Df  Sum Sq Mean Sq F value    Pr(>F)
Rep             3    88.8   29.61  0.9239    0.4391
Power           4  2570.4  642.60 20.0482 9.291e-09 ***
Duration        2  2536.5 1268.26 39.5680 8.159e-10 ***
Power:Duration  6 11633.8 1938.96 60.4930 < 2.2e-16 ***
Residuals      36  1153.9   32.05
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
Study: Tot.ch ~ Rep + Power + Duration + Power:Duration
HSD Test for Tot.ch
Mean Square Error:  32.05264
Power:Duration,  means

         Tot.ch       std r      Min      Max
0.5:10 52.45094  1.300048 4 50.96073 53.74170
0.5:30 52.76442  2.702984 4 50.09760 55.59467
0.5:60 38.01215  4.731383 4 31.40190 42.55444
0:0    41.48852 10.251263 4 26.74020 49.78483
1.5:10 18.76722  2.980261 4 14.93762 21.59563
1.5:30 36.74169  7.115573 4 32.41485 47.38260
1.5:60 46.34059  4.149784 4 41.41950 51.22230
1:10   17.10076  3.313023 4 13.06880 21.18025
1:30   32.22912  4.538719 4 28.77675 38.91197
1:60   57.07302  4.047326 4 51.30047 60.75257
2:10   48.24092 10.837888 4 34.63315 58.63847
2:30   84.81595  4.294201 4 80.02170 90.38330
2:60   18.69789  4.145410 4 13.62699 23.77442
```

```
alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141
Honestly Significant Difference: 14.18247
Means with the same letter are not significantly different.

Groups, Treatments and means
a       2:30     84.82
b       1:60     57.07
bc      0.5:30   52.76
bc      0.5:10   52.45
bcd     2:10     48.24
bcde    1.5:60   46.34
cde     0:0      41.49
de      0.5:60   38.01
de      1.5:30   36.74
ef      1:30     32.23
fg      1.5:10   18.77
fg      2:60     18.7
g       1:10     17.1
```

APPENDIX B

Mineral data

1. K

Means table

```
sum <- summarySE(mineral22, measurevar="K", groupvars=c("Duration", "Power"))
> sum
   Duration Power N        K        sd       se        ci
1         0     0 4 5387.172 1357.8823 678.9411 2160.6937
2        10   0.5 4 8050.387  543.7386 271.8693  865.2094
3        10     1 4 5229.700  393.2325 196.6163  625.7207
4        10   1.5 4 5360.448  953.5813 476.7906 1517.3606
5        10     2 4 5080.368  361.9759 180.9879  575.9844
6        30   0.5 4 5387.207  459.0059 229.5030  730.3808
7        30     1 4 4626.750   90.7342  45.3671  144.3784
8        30   1.5 4 8921.895 1237.9638 618.9819 1969.8766
9        30     2 4 4788.570  256.5479 128.2740  408.2250
10       60   0.5 4 5211.688  628.1281 314.0640  999.4919
11       60     1 4 5951.380  651.5753 325.7876 1036.8016
12       60   1.5 4 4937.515 1254.7899 627.3949 1996.6507
13       60     2 4 5080.368  361.9759 180.9879  575.9844
```

```
Analysis of Variance Table
Response: K
              Df   Sum Sq Mean Sq F value    Pr(>F)
Rep            3  4235123 1411708  2.7041   0.05979 .
Power          4 17974825 4493706  8.6077 5.558e-05 ***
Duration       2  4306874 2153437  4.1249   0.02438 *
Power:Duration  6 57998357 9666393 18.5160 1.113e-09 ***
Residuals     36 18794021  522056
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> #Tukeys HSD Example
> outFactorial <-HSD.test (outAOV, c("Power", "Duration"),
+                        main = "K ~ Rep + Power + Duration + Power:Duration",
+                        console=TRUE)

Study: K ~ Rep + Power + Duration + Power:Duration
HSD Test for K
Mean Square Error:  522056.1
Power:Duration,  means


              K       std r     Min      Max
0.5:10 8050.387  543.7386 4 7430.00  8747.65
0.5:30 5387.207  459.0059 4 4880.15  5860.03
0.5:60 5211.688  628.1281 4 4723.74  6120.19
0:0    5387.172 1357.8823 4 4281.17  7353.69
1.5:10 5360.448  953.5813 4 3987.15  6011.60
1.5:30 8921.895 1237.9638 4 8238.31 10776.13
1.5:60 4937.515 1254.7899 4 3558.71  6552.70
1:10   5229.700  393.2325 4 4782.68  5644.48
1:30   4626.750   90.7342 4 4554.52  4759.12
1:60   5951.380  651.5753 4 5397.72  6859.68
2:10   5080.368  361.9759 4 4538.98  5299.20
```

```
2:30   4788.570  256.5479 4 4538.40  5075.32
2:60   5080.368  361.9759 4 4538.98  5299.20

alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141
Honestly Significant Difference: 1810
Means with the same letter are not significantly different.

Groups, Treatments and means
a        1.5:30    8922
a        0.5:10    8050
b        1:60      5951
b        0.5:30    5387
b        0:0       5387
b        1.5:10    5360
b        1:10      5230
b        0.5:60    5212
b        2:10      5080
b        2:60      5080
b        1.5:60    4938
b        2:30      4789
b        1:30      4627

>
```

2. Na

Means table

```
sum <- summarySE(mineral22, measurevar="Na", groupvars=c("Duration", "Power"))
> sum
   Duration Power N       Na        sd         se        ci
1         0     0 4 3274.785 275.19371 137.596853 437.89460
2        10   0.5 4 1116.528  13.28862   6.644308  21.14515
3        10     1 4 2574.807 198.25949  99.129745 315.47509
```

```
4        10   1.5 4 1665.665 427.08588 213.542940 679.58894
5        10     2 4 1721.327  49.68071  24.840357  79.05310
6        30   0.5 4 1961.800 338.58160 169.290800 538.75888
7        30     1 4 2262.858 149.63240  74.816198 238.09853
8        30   1.5 4 2247.825 370.56570 185.282849 589.65272
9        30     2 4 2607.870 318.81599 159.407994 507.30738
10       60   0.5 4 2294.145 103.90033  51.950165 165.32861
11       60     1 4 3375.830 458.92223 229.461115 730.24768
12       60   1.5 4 3987.720 147.07681  73.538403 234.03202
13       60     2 4 2969.307 340.09917 170.049583 541.17367

>
```

```
Analysis of Variance Table

Response: Na
              Df   Sum Sq Mean Sq  F value    Pr(>F)
Rep            3   342319  114106   1.4873    0.2344
Power          4  9324415 2331104  30.3835 4.300e-11 ***
Duration       2 15791542 7895771 102.9130 1.289e-15 ***
Power:Duration  6  4770475  795079  10.3630 1.163e-06 ***
Residuals     36  2762019   76723
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
> #Tukeys HSD Example
> outFactorial <-HSD.test (outAOV, c("Power", "Duration"),
+                           main = "Na ~ Rep + Power + Duration + Power:Duration",
+                           console=TRUE)

Study: Na ~ Rep + Power + Duration + Power:Duration
```

```
HSD Test for Na
Mean Square Error:  76722.76
Power:Duration,  means

            Na       std r     Min     Max
0.5:10 1116.528  13.28862 4 1097.17 1126.60
0.5:30 1961.800 338.58160 4 1602.24 2279.72
0.5:60 2294.145 103.90033 4 2209.54 2445.70
0:0    3274.785 275.19371 4 3027.73 3656.34
1.5:10 1665.665 427.08588 4 1289.80 2247.19
1.5:30 2247.825 370.56570 4 1854.65 2729.51
1.5:60 3987.720 147.07681 4 3801.77 4161.60
1:10   2574.807 198.25949 4 2323.14 2802.64
1:30   2262.858 149.63240 4 2105.36 2416.61
1:60   3375.830 458.92223 4 2918.54 3819.10
2:10   1721.327  49.68071 4 1690.37 1795.52
2:30   2607.870 318.81599 4 2186.21 2946.11
2:60   2969.307 340.09917 4 2589.38 3416.63

alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141
Honestly Significant Difference: 693.8762
Means with the same letter are not significantly different.

Groups, Treatments and means
a          1.5:60     3988
ab         1:60       3376
bc         0:0        3275
bcd        2:60       2969
cde        2:30       2608
de         1:10       2575
def        0.5:60     2294
```

```
ef          1:30       2263
ef          1.5:30     2248
ef          0.5:30     1962
fg          2:10       1721
fg          1.5:10     1666
g           0.5:10     1117

>
```

3. Fe

Means table

```
> sum <- summarySE(mineral22, measurevar="Fe", groupvars=c("Duration", "Power"))
> sum
   Duration Power N       Fe        sd        se        ci
1         0     0 4 238.6100 12.261802  6.130901 19.511264
2        10   0.5 4 211.6150  4.425460  2.212730  7.041895
3        10     1 4 377.8075 12.954658  6.477329 20.613751
4        10   1.5 4 217.4450 29.979124 14.989562 47.703477
5        10     2 4 252.5125  9.085246  4.542623 14.456654
6        30   0.5 4 226.9725  9.313225  4.656612 14.819419
7        30     1 4 248.3500 17.546506  8.773253 27.920406
8        30   1.5 4 260.1225 10.719861  5.359931 17.057691
9        30     2 4 337.7125 20.201317 10.100659 32.144804
10        60   0.5 4 230.1575  4.451302  2.225651  7.083015
11        60     1 4 418.8025 43.421277 21.710638 69.092941
12        60   1.5 4 380.9625 12.267033  6.133516 19.519586
13        60     2 4 226.9725  9.313225  4.656612 14.819419
```

```
Analysis of Variance Table
Response: Fe
              Df Sum Sq Mean Sq F value    Pr(>F)
Rep            3   1070   356.7  1.0643    0.3764
Power          4 103075 25768.9 76.8765 < 2.2e-16 ***
Duration       2  24320 12160.2 36.2778 2.353e-09 ***
Power:Duration  6 124240 20706.6 61.7742 < 2.2e-16 ***
Residuals     36  12067   335.2
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
> #Tukeys HSD Example
> outFactorial <-HSD.test (outAOV, c("Power", "Duration"),
+                         main = "Fe ~ Rep + Power + Duration + Power:Duration",
+                         console=TRUE)

Study: Fe ~ Rep + Power + Duration + Power:Duration
HSD Test for Fe
Mean Square Error:  335.198
Power:Duration,  means

            Fe       std r    Min    Max
0.5:10 211.6150  4.425460 4 208.23 218.08
0.5:30 226.9725  9.313225 4 214.24 236.63
0.5:60 230.1575  4.451302 4 226.54 236.63
0:0    238.6100 12.261802 4 222.59 252.00
1.5:10 217.4450 29.979124 4 193.35 261.21
1.5:30 260.1225 10.719861 4 248.05 273.51
1.5:60 380.9625 12.267033 4 364.83 394.68
1:10   377.8075 12.954658 4 358.70 387.47
1:30   248.3500 17.546506 4 232.84 272.40
1:60   418.8025 43.421277 4 360.59 465.69
```

```
2:10   252.5125  9.085246 4 240.70 262.39
2:30   337.7125 20.201317 4 317.93 365.90
2:60   226.9725  9.313225 4 214.24 236.63

alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141
Honestly Significant Difference: 45.86386
Means with the same letter are not significantly different.

Groups, Treatments and means
a        1:60      418.8
ab       1.5:60    381
ab       1:10      377.8
b        2:30      337.7
c        1.5:30    260.1
cd       2:10      252.5
cd       1:30      248.4
cd       0:0       238.6
cd       0.5:60    230.2
cd       0.5:30    227
cd       2:60      227
cd       1.5:10    217.4
d        0.5:10    211.6

>
```

4. Zn

Means table

```
sum <- summarySE(mineral22, measurevar="Zn", groupvars=c("Duration", "Power"))
> sum
   Duration Power N      Zn        sd        se        ci
1         0     0 4 44.7975 2.4539679 1.2269839  3.904810
2        10   0.5 4 29.4500 1.2106472 0.6053236  1.926410
3        10     1 4 27.0250 0.8412094 0.4206047  1.338552
4        10   1.5 4 74.5900 9.7668419 4.8834209 15.541225
5        10     2 4 63.7150 4.9750477 2.4875239  7.916411
6        30   0.5 4 64.1225 1.3494783 0.6747391  2.147321
7        30     1 4 27.0250 0.8412094 0.4206047  1.338552
8        30   1.5 4 63.0975 4.1161420 2.0580710  6.549700
9        30     2 4 49.8250 7.1973166 3.5986583 11.452537
10       60   0.5 4 45.2200 1.4686275 0.7343137  2.336914
11       60     1 4 87.0250 3.7908706 1.8954353  6.032121
12       60   1.5 4 44.6200 2.4911978 1.2455989  3.964052
13       60     2 4 45.2650 1.0057336 0.5028668  1.600347

>

Analysis of Variance Table


Response: Zn
               Df  Sum Sq Mean Sq  F value    Pr(>F)
Rep             3    91.7   30.56   1.9021    0.1467
Power           4  1800.4  450.11  28.0156 1.290e-10 ***
Duration        2   386.8  193.42  12.0386 9.924e-05 ***
Power:Duration  6 14191.8 2365.31 147.2219 < 2.2e-16 ***
Residuals      36   578.4   16.07
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```

```
> #Tukeys HSD Example
> outFactorial <-HSD.test (outAOV, c("Power", "Duration"),
+                     main = "Zn ~ Rep + Power + Duration + Power:Duration",
+                     console=TRUE)

Study: Zn ~ Rep + Power + Duration + Power:Duration
HSD Test for Zn
Mean Square Error:  16.06628
Power:Duration,  means

            Zn       std r   Min   Max
0.5:10 29.4500 1.2106472 4 28.01 30.52
0.5:30 64.1225 1.3494783 4 62.16 65.12
0.5:60 45.2200 1.4686275 4 43.78 47.13
0:0    44.7975 2.4539679 4 41.77 47.63
1.5:10 74.5900 9.7668419 4 60.19 81.71
1.5:30 63.0975 4.1161420 4 57.40 67.17
1.5:60 44.6200 2.4911978 4 41.03 46.78
1:10   27.0250 0.8412094 4 26.18 28.19
1:30   27.0250 0.8412094 4 26.18 28.19
1:60   87.0250 3.7908706 4 82.27 91.55
2:10   63.7150 4.9750477 4 57.89 69.92
2:30   49.8250 7.1973166 4 41.03 58.60
2:60   45.2650 1.0057336 4 44.63 46.75

alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141
Honestly Significant Difference: 10.04101
Means with the same letter are not significantly different.
Groups, Treatments and means
a          1:60       87.02
b          1.5:10     74.59
```

```
c       0.5:30   64.12
c       2:10     63.72
c       1.5:30   63.1
d       2:30     49.82
d       2:60     45.26
d       0.5:60   45.22
d       0:0      44.8
d       1.5:60   44.62
e       0.5:10   29.45
e       1:10     27.02
e       1:30     27.02

>
```

5. Ca

Means table

```
sum <- summarySE(mineral22, measurevar="Ca", groupvars=c("Duration", "Power"))
> sum
   Duration Power N       Ca        sd       se        ci
1         0     0 4 426.1550  25.80538 12.90269  41.06212
2        10   0.5 4 153.6200  27.78835 13.89418  44.21747
3        10     1 4 640.3700  17.45024  8.72512  27.76723
4        10   1.5 4 591.8400 114.14145 57.07072 181.62451
5        10     2 4 453.4050  43.20204 21.60102  68.74408
6        30   0.5 4 225.8375  30.88657 15.44328  49.14742
7        30     1 4 803.1075  45.71603 22.85801  72.74440
8        30   1.5 4 599.6050  56.11147 28.05574  89.28587
9        30     2 4 577.9750  29.23005 14.61502  46.51153
10       60   0.5 4 674.0650  60.04394 30.02197  95.54331
11       60     1 4 273.2800  34.58561 17.29280  55.03342
12       60   1.5 4 246.0450  22.89964 11.44982  36.43844
13       60     2 4 376.6300  96.50370 48.25185 153.55892
```

```
>
```

```
Analysis of Variance Table

Response: Ca
               Df  Sum Sq Mean Sq F value    Pr(>F)
Rep             3    2074     691  0.2205    0.8815
Power           4  302179   75545 24.0898 9.359e-10 ***
Duration        2  204172  102086 32.5535 8.460e-09 ***
Power:Duration  6 1429872  238312 75.9934 < 2.2e-16 ***
Residuals      36  112894    3136
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
> #Tukeys HSD Example
> outFactorial <-HSD.test (outAOV, c("Power", "Duration"),
+                          main = "Ca ~ Rep + Power + Duration + Power:Duration",
+                          console=TRUE)

Study: Ca ~ Rep + Power + Duration + Power:Duration

HSD Test for Ca

Mean Square Error:  3135.956

Power:Duration,  means
             Ca       std r    Min    Max
0.5:10 153.6200  27.78835 4 125.65 189.71
0.5:30 225.8375  30.88657 4 194.29 261.18
0.5:60 674.0650  60.04394 4 607.59 738.53
0:0    426.1550  25.80538 4 391.50 453.18
1.5:10 591.8400 114.14145 4 479.65 707.47
```

```
1.5:30 599.6050  56.11147 4 535.94 663.71
1.5:60 246.0450  22.89964 4 220.59 272.65
1:10   640.3700  17.45024 4 624.31 661.37
1:30   803.1075  45.71603 4 772.04 871.06
1:60   273.2800  34.58561 4 244.11 323.19
2:10   453.4050  43.20204 4 397.32 494.93
2:30   577.9750  29.23005 4 543.82 614.25
2:60   376.6300  96.50370 4 259.76 467.65

alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141

Honestly Significant Difference: 140.283

Means with the same letter are not significantly different.

Groups, Treatments and means
a          1:30      803.1
ab         0.5:60    674.1
b          1:10      640.4
b          1.5:30    599.6
bc         1.5:10    591.8
bc         2:30      578
cd         2:10      453.4
d          0:0       426.2
de         2:60      376.6
ef         1:60      273.3
ef         1.5:60    246
f          0.5:30    225.8
f          0.5:10    153.6
```

6. Mg

Means table

```
sum <- summarySE(mineral22, measurevar="Mg", groupvars=c("Duration", "Power"))
> sum
   Duration Power N        Mg        sd        se        ci
1         0     0 4 13208.555  947.4134  473.7067 1507.5462
2        10   0.5 4  6031.320  269.6280  134.8140  429.0384
3        10     1 4 15459.092 1088.3516  544.1758 1731.8102
4        10   1.5 4  9645.073 1412.9931  706.4965 2248.3873
5        10     2 4  9903.073 1151.3608  575.6804 1832.0719
6        30   0.5 4 12987.675  824.4746  412.2373 1311.9230
7        30     1 4 15459.092 1088.3516  544.1758 1731.8102
8        30   1.5 4 12206.657 1240.8118  620.4059 1974.4085
9        30     2 4  9599.478 2571.2779 1285.6389 4091.4769
10       60   0.5 4 14589.570  862.3665  431.1833 1372.2176
11       60     1 4 13429.142 1429.0578  714.5289 2273.9499
12       60   1.5 4 14031.583 1402.3062  701.1531 2231.3821
13       60     2 4 11740.333 2537.2140 1268.6070 4037.2737

>

Analysis of Variance Table
Response: Mg
               Df    Sum Sq  Mean Sq F value    Pr(>F)
Rep             3   2519793   839931   0.390    0.7609
Power           4 134948773 33737193  15.666 1.633e-07 ***
Duration        2  86678314 43339157  20.125 1.359e-06 ***
Power:Duration  6 139490317 23248386  10.796 7.472e-07 ***
Residuals      36  77526606  2153517
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
> #Tukeys HSD Example
```

```
> outFactorial <-HSD.test (outAOV, c("Power", "Duration"),
+                          main = "Mg ~ Rep + Power + Duration + Power:Duration",
+                          console=TRUE)

Study: Mg ~ Rep + Power + Duration + Power:Duration
HSD Test for Mg
Mean Square Error:  2153517
Power:Duration,  means

              Mg       std r      Min      Max
0.5:10  6031.320  269.6280 4  5768.31  6386.74
0.5:30 12987.675  824.4746 4 12086.47 13755.05
0.5:60 14589.570  862.3665 4 13520.33 15289.17
0:0    13208.555  947.4134 4 12209.53 14195.00
1.5:10  9645.073 1412.9931 4  7556.60 10652.43
1.5:30 12206.657 1240.8118 4 11085.76 13960.64
1.5:60 14031.583 1402.3062 4 13180.28 16124.18
1:10   15459.092 1088.3516 4 13925.74 16237.06
1:30   15459.092 1088.3516 4 13925.74 16237.06
1:60   13429.142 1429.0578 4 12105.96 15400.36
2:10    9903.073 1151.3608 4  9077.21 11607.20
2:30    9599.478 2571.2779 4  5833.42 11280.11
2:60   11740.333 2537.2140 4  8065.93 13851.82

alpha: 0.05 ; Df Error: 36
Critical Value of Studentized Range: 5.010141
Honestly Significant Difference: 3676.157
Means with the same letter are not significantly different.
Groups, Treatments and means
a        1:10      15460
a        1:30      15460
ab       0.5:60    14590
```

```
ab      1.5:60   14030
abc     1:60     13430
abcd    0:0      13210
abcd    0.5:30   12990
abcd    1.5:30   12210
bcd     2:60     11740
cd      2:10     9903
de      1.5:10   9645
de      2:30     9599
e       0.5:10   6031

>
```