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RESPONSE OF COCOA (*Theobroma cacao* L.) SEEDLINGS TO FOLIAR FERTILIZER APPLICATION AND FERTIGATION

A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE MASTER OF PHILOSOPHY DEGREE IN SOIL SCIENCE

BY

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DECLARATION

"I declare that I have wholly undertaken this study reported herein under the supervision of Dr. Andrews Opoku, Prof. Vincent Logah and Dr. Richard Asare, and that, except portions where references have been duly cited, this thesis is the outcome of my research".

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DEDICATION

This research work is dedicated to my late mother, Mad. Florence Ama Akyaa. May her soul continue to rest in perfect peace in the bosom of the Almighty God, Amen!

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ABSTRACT

The production of quality and vigorous seedlings is important for sustainable cocoa production. A greenhouse nursery study was conducted in 2019/2020 at CRIG to assess the effects of foliar fertilizer application and fertigation on soil chemical properties, nutrient uptake and growth of cocoa seedlings. The study was 4x3 factorial experiment, laid out in completely randomized design with 13 treatments and three replicates. Two liquid fertilizers (NPK 24:17:18 and NPK 10:10:10) and two granular fertilizers (ammonium sulphate and calcium nitrate) were applied at 42, 84 and 126 mg N per pot. The liquid fertilizers were applied through foliar spraying, while the granular fertilizers were applied through fertigation. Topsoil was collected from an uncultivated land at CRIG and used for the experiment. The soil was processed and analyzed to assess the pre and post experiment physico-chemical properties using standard protocols. Growth and nutrient uptake by the cocoa seedlings were determined using standard procedures. Data from the experiment were subjected to ANOVA and Pearson's correlation analysis. The initial topsoil was sandy clay loam; had pH of 7.04; 1.94 % organic C; 0.21 % total N; 8.01 mg kg^{-1} available P; and 0.32 $cmol_c$ kg^{-1}, 2.41 $cmol_c$ kg^{-1}, 10.20 $cmol_c$ kg^{-1} exchangeable K, Mg, Ca respectively. The initial topsoil had normal levels of physico-chemical characteristics suitable for cocoa cultivation, except available P which was lower than the threshold level of 20 mg kg^{-1}. The study showed that high ammonium sulphate fertigation rate (126 mg N per pot) significantly reduced soil pH from 7.04 to 5.09, compared with the other treatments, due to the nitrification effect of ammonium. Foliar fertilizer application using NPK 10:10:10 at high concentration (1.26 % v/v) significantly improved soil available P content from 8.01 mg kg^{-1} to 12.27 mg kg^{-1}, compared with the other treatments, due to drippings from the foliar sprays which improved P availability. The highest calcium nitrate fertigated pot significantly increased exchangeable Ca level in the soil from 10.20 $cmol_c$ kg^{-1} to 10.69 $cmol_c$ kg^{-1} than the other treatments. The study indicated that the highest fertigated rates of ammonium sulphate and calcium nitrate fertilizers had depressive effects on cocoa seedling growth (stem diameter, plant height, number of leaves, total leaf area, total dry matter). The study revealed that foliar application of NPK 10:10:10 at moderate and high concentrations produced seedlings with significantly higher P content than those from the other treatments

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LIST OF ABBREVIATIONS AND ACRONYMS

%	Percent
<	Less than
>	Greater than
±	Plus or minus
µg g^{-1}	Microgram per gram
µg ml^{-1}	Microgram per millilitre
µg $plant^{-1}$	Microgram per plant
AAS	Atomic Absorption Spectrometer
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
cm	Centimetre
cm^2	Square centimetre
cm^2 g^{-1}	Square centimetre per gram
cm^3	Cubic centimetre
$cmol_c$ kg^{-1}	Centimole of charge per kilogram
COCOBOD	Ghana Cocoa Board
CRIG	Cocoa Research Institute of Ghana
EDTA	Ethylenediaminetetraacetic acid
et al.	And others
etc.	And the rest
EU	European Union
FAO	Food and Agriculture Organization
g $plant^{-1}$	Gram per plant
g	Gram
GPS	Global Positioning System
H_2O	Water
i.e.	That is
ICCO	International Cocoa Organization
ID	Identity
IITA	International Institute of Tropical Agriculture

kg	Kilogram
KNUST	Kwame Nkrumah University of Science of Technology
m	Metre
mg g^{-1}	Milligram per gram
mg kg^{-1}	Milligram per kilogram
Mg m^{-3}	Megagram per cubic metre
mg $plant^{-1}$	Milligram per plant
mg	Milligram
ml	Millilitre
mm	Millimetre
nm	Nanometre
^{o}C	Degree Celsius
pH	Power of hydrogen
ppm	Parts per million
UNCTAD	United Nations Conference on Trade and Development
USDA	United States Department of Agriculture
v/v	Volume per volume
viz.	Namely
w/v	Weight per volume
WACRI	West Africa Cocoa Research Institute
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Cocoa production generates employment, income and export revenues for producing countries worldwide. For instance, between 1995 and 2014, cocoa accounted for more than 30 % of export earnings for the two major producer countries (Cote d'Ivoire and Ghana) (UNCTAD, 2015). In Ghana, cocoa production supports the economy by generating external revenues and domestic incomes (COCOBOD, 1995; Osei, 2007). The cash crop generates about 2 billion dollars annually from foreign earnings for the nation (Danquah, 2015).

There have been several efforts by governments over the years to ensure yield increase and sustainability of cocoa production in Ghana. These include the production of hybrid and quality cocoa seedlings, and incorporation of fertilizer application in the production system. Since cocoa plantations in Ghana are mostly established from 3–6 months old seedlings raised from mixed hybrid seeds in nurseries (Amoah *et al.,* 1999; Oppong *et al.,* 2007; Opoku-Ameyaw *et al.,* 2010), it is important to maintain the quality and vigour of seedlings for successful field establishment. Fertilizer application in cocoa production is reported to be an essential agronomic component, especially in the establishment phase. It ensures adequate nutrient supply, enhances cocoa seedling survival and growth, and gives optimum field establishment (Egbe, 1968; Smith, 1994; Famuwagun and Oladitan, 2016). According to Weih *et al.* (2011), mineral nutrition is one of the most important areas of agriculture that increases the quality and yield potentials of plants.

Advances in agriculture, with regards to fertilizer application have led to the development of technologies such as foliar fertilizer application (foliar fertilization) and fertigation to ensure

efficient use of applied nutrients, improve crop nutrition and growth. There are several reports that these fertilizer application methods (foliar fertilization and fertigation) ensure efficient absorption, uptake and utilization of water and nutrients better than the conventional method (broadcasting) (Veeranna *et al.,* 2001; Kinaci and Gulmezoglu, 2007; Fageria *et al.,* 2009; Bozorgi *et al.,* 2011; Jeyajothi *et al.,* 2017). However, information on their effectiveness on soil fertility, nutrient uptake and growth of cocoa seedlings in Ghana is limited. It is therefore important to assess the effectiveness of these two methods and determine the most appropriate one that will help improve the quality of cocoa seedlings produced in Ghana.

1.2 Problem statement

Several studies have reported that nutrients supplied either by foliar application or through fertigation are more advantageous than soil application (broadcasting) (Veeranna *et al.,* 2001; Sathya *et al.,* 2008; Fageria *et al.,* 2009; Jadhav, 2017). However, not much work has been done on evaluating the effectiveness and impact of these two fertilizer application methods (i.e., foliar fertilization and fertigation) on cocoa seedlings in Ghana. Furthermore, there is inadequate information on the use of granular fertilizers for fertigation in the cocoa production system in Ghana, because the granular fertilizers are usually broadcasted. With the broadcasting method, nutrients may take longer to be absorbed by the plant; some immobile nutrients may not get to the root at the required time; there could be high salt build-up which may burn the plant.

1.3 Justification

Technological advancement in agriculture has resulted in different techniques of supplying essential nutrients to plants for improved plant nutrition and sustainable crop production. Some of such techniques are applying fertilizers in liquid formulations directly onto the foliage of plants

(foliar fertilization), or applying nutrients directly to the roots of plants through water (fertigation). Since fertilizer application has been incorporated into the cocoa production system in Ghana, there is the need for more and diverse research to study the effectiveness of these technologies, and assess their impacts on soil fertility, biomass nutrient content, and growth of cocoa seedlings.

Understanding the effects of foliar fertilization and fertigation on the growth and nutrient uptake by cocoa seedlings, as well as on the nutrient levels of soil used to raise the seedlings will contribute substantially to existing knowledge gap on the most appropriate form of fertilizer for sustainable cocoa seedling production in Ghana. It will reduce try and error practices of cocoa seedling producers that could lead to loss of resources.

1.4 Main objective

The general objective of the study was to compare the effectiveness or the response of cocoa seedlings to two different fertilizer application methods.

1.5 Specific objectives

The specific objectives were to:

i. evaluate the effect of foliar fertilizer application and fertigation on soil pH, organic carbon, N, P, K, Mg, Ca, Fe, Zn;
ii. assess the growth of cocoa seedlings as influenced by foliar fertilizer application and fertigation;
iii. assess the effect of foliar fertilizer application and fertigation on N, P, K, Mg, Ca, Fe, Zn uptake by cocoa seedlings.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Cocoa production in Ghana

An area of approximately 1.5 million hectares is used for cocoa production by about 800,000 families in the cocoa-growing regions of Ghana (Afoakwa *et al.,* 2011). Globally, Ghana is known to produce high quality cocoa beans for the international markets. The country produced one million metric tonnes of cocoa in the 2010/2011 crop season, which was a remarkable achievement. It is estimated that Ghana produced 778000, 970000, and 880000 metric tonnes of cocoa beans in the 2015/2016, 2016/2017 and 2017/2018 crop seasons respectively (Statista, 2019). Cocoa contributes greatly to the economy of Ghana by generating foreign incomes, supporting the socio-economic development of the country, and improving the livelihoods of most farmers. Helena and Pärssinen (2009) simply reported the enormous contribution of cocoa to the Ghanaian economy as "Cocoa is Ghana; Ghana is cocoa".

Until the introduction of hybrid varieties in 1984, cocoa was previously cultivated from varieties such as Amazonica and Amelonado. The hybrid cocoa varieties produce more beans per pod and yield earlier than the older varieties (Kolavalli and Vigneri, 2011). In Ghana, cocoa is produced in the forest zones and in ten regions, which are Western, Western North, Ashanti, Eastern, Bono, Bono East, Ahafo, Central, Oti, and part of Volta. The Ghana Cocoa Board has stipulated two seasons to produce cocoa in the country as main crop season (October - May) and light or minor crop season (June - September).

2.2 Importance of cocoa production in Ghana

Cocoa contributes immensely to the socio-economic development of the country, and also provides health and nutritional benefits to consumers.

2.2.1 Economic importance of cocoa

Cocoa production in Ghana has been the backbone of the country's economy for some decades now and still contributes greatly to the economy. It generates about 2 billion dollars annually from foreign earnings (Danquah, 2015). Cocoa production also supports the agricultural gross domestic product in the country and Government's revenue for infrastructural development. Cocoa takes about 25% of the total export earnings in Ghana and is considered the second most important export commodity after gold (Nartey *et al.,* 2012). Many farmers in Ghana obtain their main source of income from cocoa cultivation (Lowor and Shiloh, 2013), thus, contributing to the socio-economic development of the country. According to Afoakwa (2014), the cocoa industry provides sources of livelihoods for over 800,000 families in the cocoa-growing areas of Ghana.

2.2.2 Health and nutritional benefits of cocoa

Cocoa and cocoa products are known to provide several health and nutritional benefits to humans. According to Takrama *et al.* (2015), cocoa and cocoa products contain high levels of antioxidants. Antioxidants improve blood circulation and reduce the risk of stroke. Cocoa is also rich in magnesium which helps reduce the occurrence of heart attacks (Aikpokpodion *et al.,* 2013). The concentrations of essential polyphenols in cocoa are relatively higher than in red wine and tea (Lee *et al.,* 2003). The polyphenols, including catechins and epicatechins contain good antioxidants which help lower the risk of cardiovascular diseases (Amankwaah *et al.,* 2015). Cocoa products such as chocolates contain vitamins and minerals which are good for human health.

2.3 Essential plant nutrients

Several elements are present in the soil, but not all are essential for plant growth and development. Sixteen (16) chemical elements are considered essential nutrients, which plants require for growth and to complete their life cycles (Brady and Weil, 1999). Among these elements, carbon (C) is obtained from carbon dioxide (CO_2) from the air, while hydrogen (H) and oxygen (O) are obtained from water (H_2O) (Brady and Weil, 1999). The remaining 13, grouped into major nutrients (macronutrients) and minor nutrients (micronutrients or trace elements) are taken from the soil by plants (Brady and Weil, 1999). The macronutrients are required by plants in relatively larger quantities. These include nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and sulphur (S) (Brady and Weil, 1999). The micronutrients are required by plants in relatively smaller quantities. These include iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), boron (B), molybdenum (Mo) and chlorine (Cl) (Brady and Weil, 1999).

2.3.1 Nitrogen (N)

Nitrogen is one of the essential primary macronutrients needed by plants in relatively larger amounts for proper growth, development, and the formation of plant tissues and chlorophyll for photosynthesis. After carbon, N is the nutrient required by plants in largest quantities (Hajiboland, 2018; Akhimien and Omonigho, 2019). In the soil, N is present in both organic (about 98 % N) and inorganic or mineral (about 2 % N) forms. According to Bremner (1965), the inorganic N in most soils is in the form of ammonium (NH_4^+) and nitrate (NO_3^-). Other inorganic forms of N that occur in soils are nitrite (NO_2^-) and free ammonia (NH_3) dissolved in soil solution. Most of the N in soils occur in organic forms, humic and non-humic fractions, and are concentrated principally in the top layers of soil (Altomare and Tringovska, 2011).

Plants absorb N from the soil solution in mineral forms, mostly as nitrate and ammonium ions (Brady and Weil, 1999). Depending on the plant part, the concentration of total N in plant tissues ranges from 1 to 7 % of dry weight (Altomare and Tringovska, 2011). Nitrogen is necessary for synthesis of proteins, nucleic acids, chlorophyll, coenzymes, phytohormones and secondary metabolites in plants which are essential to the structure and metabolism of plants (Barker and Bryson, 2007; Altomare and Tringovska, 2011; Hawkesford *et al.,* 2012). Nitrogen also helps in regulating plant's ability to utilize P and K, which facilitate green pigmentation to absorb light energy for photosynthesis; stimulates rapid and vigorous vegetative growth; and enhances dark green leaves (Bergmann, 1992; Ogunrinde, 2006). However, when too much N is supplied, plants become susceptible to lodging; delayed maturity; diseases and insect pests attack; fruit and seed crops failure (Bennett, 1993; Brady and Weil, 1999). Conversely, plants deficient in N may have light green to yellow appearance of leaves, especially older leaves (chlorosis); stunted growth; poor fruit development; thin and spindly stems (Bennett, 1993; Brady and Weil, 1999).

2.3.2 Phosphorus (P)

Phosphorus is an essential primary major nutrient required in relatively larger amounts by plants, for normal growth and development. Phosphorus plays important roles in various biochemical, physiological, and metabolic processes in plants. Phosphorus is involved in energy transfer processes as a component of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) in plant metabolism (Altomare and Tringovska, 2011). Phosphate ions are also essential structural components of phospholipids, phosphoproteins, nucleic acids, nucleotides, and coenzymes in plants' biochemical functions (Hajiboland, 2018). Phosphorus is needed by plants to increase root growth, seed and fruit formation, flower development, and maturation (Brady and Weil, 1999; Ayeni *et al.,* 2011).

Phosphorus is present in soils in both organic and inorganic forms. Some organic forms of P in the soil include inositol phosphate which is the most stable form; phospholipids and nucleic acids which are readily bio-available (Rodriguez and Fraga, 1999; Soon, 2008). Inorganic P in the soil exists as free phosphate ions in soil solution; labile P adsorbed on soil surfaces and released by anion exchange; and non-labile P which is not readily available as a result of precipitation or diffusion processes (Soon, 2008; White and Hammond, 2008). Phosphorus is available and absorbed by plants from the soil solution as orthophosphate ions ($H_2PO_4^-$ or HPO_4^{2-}), depending on the soil pH. Plant tissues normally contain about 0.1-1 % total P (Sanchez, 2007). Inorganic P forms (relatively insoluble phosphate) complexes with Fe and Al in acidic soils with pH below 5, or with Ca in alkaline soils with pH above 7 (Altomare and Tringovska, 2011), thus making P unavailable for plant use.

Phosphorus is a major plant growth-limiting nutrient, because most of it is fixed or complexed as insoluble phosphates (Fernandez *et al.,* 2007). Phosphorus-deficient plants may exhibit red or purple leaf and stem colourations, stunted growth, delayed maturity, and smaller-sized fruits (Bennett, 1993; Hajiboland, 2018). However, excess P in soils may cause micronutrient (Zn or Fe) deficiencies (Bennett, 1993).

2.3.3 Potassium (K)

Potassium is an essential primary macronutrient which plays vital roles in the nutrition, physiology and growth of plants. Potassium in soils can be categorized into four forms based on the level of availability to plants. These forms are: soil solution K (1-10 mg kg^{-1}) considered the primary source of K absorbed by plant roots; exchangeable K (40-600 mg kg^{-1}) held by negatively charged clay particles and organic matter at the exchange sites; non-exchangeable K (50-750 mg kg^{-1}) held as fixed ions on clay minerals; and mineral K (5000-25000 mg kg^{-1}) present in K-bearing minerals in

soils (Tisdale *et al.,* 1993; Pal *et al.,* 1999; Yawson *et al.,* 2011). The first two forms are considered labile or available which meet the immediate K requirements of growing plants, while the last two which are not directly available to plants are considered non-labile responsible for long-term supply of K to plants (Askegaard *et al.,* 2003). Potassium is absorbed by plants mainly as a univalent cation (K^+) from the soil solution.

It is reported that in plants, K activates over 60 different enzymes, promotes photosynthesis, regulates stomata opening, promotes transport of ions and assimilates to plant organs, aids in protein synthesis, and enhances cell extension in leaves and roots (Bennett, 1993; Yawson *et al.,* 2011; Hajiboland, 2018). Potassium deficiency may reduce turgor, cell size and leaf area in expanding leaves; retard plant growth; cause leaves and stems to become chlorotic and necrotic under severe deficiencies (Hajiboland, 2018). Excessive concentrations of K may affect plant growth by causing Mg deficiency (Bennett, 1993).

2.3.4 Magnesium (Mg)

Magnesium is an essential secondary macronutrient needed by plants in relatively larger amounts for normal growth and development. Magnesium has vital physiological and molecular roles in plants, such as being an essential component of the chlorophyll molecule for photosynthesis; a co-factor for many enzymatic processes associated with phosphorylation, dephosphorylation and hydrolysis of various compounds; and a structural stabilizer for various nucleotides (Merhaut, 2007; Hawkesford *et al.,* 2012; Hajiboland, 2018).

In the soil, Mg is present in water-soluble, exchangeable, and fixed forms in minerals such as magnesite, olivine, dolomite, biotite, etc. Weathering and dissolution of the minerals release appreciable quantities of Mg into the soil solution where it is absorbed by plants. It is also reported

that, the toxic effects of Al are reduced when Mg is released into the soil nutrient solution (Keltjens, 1995). Magnesium-deficient plants show initial yellowing of older leaves between leaf veins (interveinal chlorosis), which spreads to younger leaves; poor development and production of fruits (Bennett, 1993). Inadequate Mg supply also decreases plant's enzymatic function and photosynthetic capacity as a result of leaf necrosis and defoliation (Hermans and Verbruggen, 2005). High concentrations of Mg may cause imbalances with Ca and K, which can reduce plant growth (Bennett, 1993).

2.3.5 Calcium (Ca)

Calcium is one of the essential secondary macronutrients required by plants in relatively larger quantities for normal growth and development. It exists in the soil as exchangeable and non-exchangeable (fixed) forms. Calcium is relatively abundant in soils, making up about 3.6 % of the earth's crust, and soils normally have large amounts of exchangeable Ca (300-5000 ppm) than Mg and K (Kelling and Schulte, 2004). Calcium is present in soil minerals such as amphibole, apatite, calcite, dolomite, feldspar, gypsum, and pyroxene, and usually made available in the soil for plant use through weathering or dissolution of the minerals (Kelling and Schulte, 2004).

Calcium is absorbed by plants as divalent cation (Ca^{2+}) from the soil solution. It is important for structural roles in cell wall and membrane, cell division and elongation, and nitrogen metabolism (Bennett, 1993; Marschner, 1995; Kelling and Schulte, 2004). Calcium is immobile in plants, and when deficient, may cause malformation of young leaves, reduced growth or death of growing tips or young tissues, poor fruit development and appearance, poor development of terminal buds and roots (Bennett, 1993; Kelling and Schulte, 2004). Excessive Ca in the soil may cause deficiency

in either Mg or K, prevent the germination of seeds, and reduce plant growth rates (Bennett, 1993; White and Broadley, 2003).

2.3.6 Iron (Fe)

Iron is the fourth most abundant element in the earth's crust, mostly in the form of ferromagnesium silicates, and the total Fe concentrations in soils typically range from 1 to 5 % (Schulte, 2004). Majority of the Fe in soils are found in silicate minerals or iron oxides and hydroxides, which impart reddish and yellowish colourations to soils (Schulte, 2004). Iron in soils exists in ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms, but it is mainly and readily absorbed by plants in the ferrous form (Lindsay and Schwab, 1982; Schulte, 2004). The ferric forms in soils are relatively unavailable for plant use.

Iron is an essential micronutrient in the soil needed by plants in relatively smaller quantities, therefore, excessive concentrations can become toxic to plants. Physiologically, Fe is involved in chlorophyll synthesis and maintenance, protein synthesis, and in the growth of root tip meristem (Bennett, 1993; Fahad *et al.,* 2014). Plants deficient in Fe may exhibit initial distinct yellowing between veins (interveinal chlorosis) of young leaves, and other physiological abnormalities such as scorching (Bennett, 1993; Singh *et al.,* 2012). Excessive concentrations of Fe (Fe toxicity) may result in possible bronzing of plant leaves with tiny brown spots (Bennett, 1993).

2.3.7 Zinc (Zn)

Zinc is an essential micronutrient in the soil, which is vital for plant growth, though required by plants in relatively smaller amounts. The concentration of Zn in soils generally ranges from 10 to 300 ppm and occurs in mostly as the mineral sphalerite (ZnS) (Lindsay, 1972). According to

Alloway (2008), the total concentration of Zn in soils is distributed over five main fractions namely, water-soluble fraction (Zn ions present in the soil solution); exchangeable fraction (Zn ions bound to soil particles by electrical charges); organically bound fraction (Zn ions complexed with organic ligands); non-exchangeable fraction (Zn ions sorbed onto clay minerals and insoluble metallic oxides); and fraction of weathering primary minerals.

Zinc in the soil is absorbed by plants largely or entirely as divalent ion (Zn^{2+}) found in the soluble fraction (Alloway, 2008). Zinc is needed by plants for biochemical processes such as sugar regulation, enzyme activation, phosphorus absorption, auxin metabolism, as well as protein and starch synthesis (Bennett, 1993; Graham and McDonald, 2001; Alloway, 2008; Khosa *et al.,* 2011; Hussain *et al.,* 2019). However, excessive Zn concentrations (Zn toxicity) may cause Fe deficiency in some plants and affect their growth (Bennett, 1993). Zinc deficiency is reported to cause interveinal chlorosis on young leaves, reduced leaf size, small and abnormally shaped leaves, and reduced plant height (Bennett, 1993; Alloway, 2008).

2.4 Foliar fertilization, and its effect on nutrient uptake and plant growth

Foliar fertilization is a technique of supplying growing plants with nutrients by spraying fertilizer solutions containing one or more nutrient elements directly onto their foliage for absorption and utilization (Fageria *et al.,* 2009; Patil and Chetan, 2018). It is reported that the mechanism of nutrient uptake by plant leaves involves three stages, facilitated by the energy from photosynthesis (Middleton and Sanderson, 1965; Franke, 1967). These stages are described as: (i) substances applied to the leaf surface penetrate the cuticle and cellulose cell wall; (ii) these substances are adsorbed to the surface of the plasma membrane; (iii) the absorbed substances are taken up into the cytoplasm of plants. Other studies also suggested that foliar-applied nutrients can be absorbed by plants through the stomata of leaves (Eichert *et al.,* 1998; Eichert and Burkhardt, 2001). Opened

stomata usually facilitate easy absorption of nutrients applied through foliar sprays (Burkhardt *et al.,* 1999).

However, factors such as concentration of nutrient solution and time of application may affect the rate of absorption of foliar-applied nutrients and efficiency of utilization by plants. Foliar sprays can supply higher plants with essential mineral elements for absorption when applied at suitable concentrations (Fageria *et al.,* 2009). Higher dosages of foliar fertilizer solutions can result in leaf burning due to salt effects or phytotoxic effects of ammonia in the fertilizer solution (Ahenkorah *et al.,* 1987). Generally, macronutrient concentrations of less than 2 % are used in foliar fertilizations to avoid leaf burning or scorching, and prevent serious damage to plants (Fageria *et al.,* 2009). The nutrient concentration of the foliar fertilizer should also be selected based on the age of the plant, since older plants can tolerate higher concentrations of salts than younger plants (Fageria *et al.,* 2009).

The time of application of foliar fertilizers is another important factor to consider in order to ensure efficient absorption of the applied nutrient, and to avoid burning of plant foliage which will eventually affect growth. Thus, foliar fertilizers should be applied in the morning (before 10:00 a.m.) or evening (after 3:00 p.m.) when leaves' stomata are opened and temperature is not too high (Poole *et al.,* 1983; Noordiana *et al.,* 2007; Fageria *et al.,* 2009; Yap, 2012). Again, for good absorption and efficient utilization of applied nutrients, foliar spraying should not be done during windy or rainy hours of the day to prevent spray solutions from drifting or washing off.

The technique of foliar fertilization comes with some advantages over conventional soil application of solid or dry fertilizers. Fageria *et al.* (2009) reported that, at early growth stages of plants when their roots are not well developed, foliar fertilization is more advantageous in nutrient

absorption, compared with soil application. Foliar fertilizer application allows for more rapid and effective utilization of applied nutrients and enhances the correction of observed deficiencies in less time than will be required by soil application, hence, crop response occurs in short time in foliar application, compared with soil application (Alexander, 1986; Kerin and Berova, 2003; Fageria *et al.,* 2009; Patil and Chetan, 2018). Foliar fertilization is very effective in correcting nutritional disorders, especially micronutrient deficiencies in plants, because very small amounts are sufficient to produce optimum effect (Saleh, 1977; Ahenkorah *et al.,* 1987; Fageria *et al.,* 2009; Jadhav, 2017).

Foliar spraying facilitates better uptake of nutrients (Bozorgi *et al.,* 2011), and can be more effective about 6 to 20 times than conventional soil application (Jadhav, 2017). In perennial crops, foliar fertilization has been a more rapid method of supplying micronutrients, especially when there are restrictions to root absorption, such as soils with low water availability, low root activity during reproductive stage, and for situations in which soil fertilization has lower efficiency (Weinbaum, 1988; Wójcik, 2004; Faquin, 2005; Eichert and Fernández, 2012; Fernández *et al.,* 2013). Foliar application of nutrients is very helpful and better than conventional soil application, when plant roots cannot absorb essential nutrients due to very low or very high soil pH, or heavy-textured soil properties (Kinaci and Gulmezoglu, 2007; Jadhav, 2017). Foliar fertilization can help correct micronutrient deficiencies. It is reported that, iron deficiency in calcareous soils can be effectively corrected by foliar application of ferrous sulphate or iron chelates solution than applying these iron sources directly to the soil (Fageria *et al.,* 2009). Also, foliar application of zinc sulphate is reported to be effective in correcting Zn deficiency in cocoa (Ahenkorah, 1969; Malavolta, 1987; Chepote *et al.,* 2013). Foliar fertilizer application can also enhance soil quality.

For instance, Shashikumar *et al.* (2013) and Jadhav (2017) found improvements in soil properties following foliar application of nutrients.

In terms of plant growth, foliar application of fertilizers produced thicker seedling stem girth (Famuwagun and Oladitan, 2016) and increased the root dry weights of cocoa seedlings (Arthur *et al.,* 2019). Again, the effects of organic and foliar fertilizers on cocoa seedlings, as reported by Fasiaben *et al.* (1982) showed that, the organic fertilizer inhibited root increase and total dry matter weight, while foliar fertilization showed no effect. Foliar fertilizer application improved the total dry matter produced by soybean, as reported by Hiwale (2015). It also increased the height of soybean at harvest (Eman *et al.,* 2014).

Foliar fertilization has profound effect on crop yield. Early yield response by young cocoa was observed following foliar application of K (Ojeniyi, 1981). Foliar fertilizer application gave the highest number of fruits, fruit length, fruit weight and maximum yield of mango (Singh *et al.,* 2005; Vashistha *et al.,* 2010; Nehete *et al.,* 2011; Sankar *et al.,* 2013) and citrus (Thirugnanavel *et al.,* 2007; Meena-Kumari *et al.,* 2009). Foliar spraying also produced the highest number of nuts per cashew tree (Nanthakumar *et al.,* 1997). Foliar applications on soybean have been shown to increase yields (Garcia and Hanway, 1976; Poole *et al.,* 1983) and nutrient concentrations (Boote *et al.,* 1978). Foliar N application on wheat resulted in higher grain protein contents than when N was broadcasted as dry granular fertilizer at the late growth stages (Alkier *et al.,* 1972; Strong, 1982; Bly and Woodard, 2003).

2.5 Fertigation, and its effect on nutrient uptake and plant growth

Fertigation is the application of fertilizers through irrigation or watering system, therefore, providing the potential for precise control of nutrients and water, which are the major resources

for plant growth (Boman and Obreza, 2002). This technique of fertilizer application supplies both water and essential nutrients to plants timely and precisely. The nutrients are supplied directly to the root zone for effective and efficient uptake and utilization by plants. Water-soluble fertilizers are normally used for fertigation, because they are highly soluble in nature and in readily available forms, hence plants can absorb the nutrients more efficiently and at higher rate (Jeyajothi *et al.,* 2017). Water-soluble fertilizers used for fertigation also improve the quality of plants (Krishnamoorthy *et al.,* 2015).

With this technology, essential nutrients are mainly applied in the wet root zone, where they can be easily taken up by plants, thus, causing more flexibility in nutrient supply and better synchronization with crop uptake (Incrocci *et al.,* 2017). This method of application may be effective for better nutrient uptake when the plant's leaf area is not enough for nutrient absorption. Fertigation improves water and nutrient use efficiencies (Veeranna *et al.,* 2001; Jeyajothi *et al.,* 2017). Tanaskovik *et al.* (2011) reported that, fertigation treatments produced almost 87 % more water use efficiency (WUE) in comparison to the treatments with conventional application of fertilizer. Veeranna *et al.* (2001) also reported that WUE was higher with fertigation, and lower with soil application of normal fertilizers. Fertigation gave the highest fertilizer use efficiency compared with other treatments (Hebbar *et al.,* 2004; Kumar *et al.,* 2007). Fertigation with N fertilizer increased the nitrogen use efficiency over soil application, by improving nutrient distribution and minimizing leaching beyond the root zone (Bharamble *et al.,* 1997). Fertigation significantly reduced fertilizer application rate up to 40 % without affecting crop yield, compared with conventional method of nutrient application (Sathya *et al.,* 2008), and helped minimize looses of water and applied nutrients through leaching and runoff (Nadagouda, 2011; Jeyajothi *et al.,* 2017).

Fertigation enhances the quality of crops (Ashok and Alva, 2008; Krishnamoorthy *et al.,* 2015), and produces optimum crop yields when essential nutrients are supplied precisely (Godara *et al.,* 2013). Krishnamoorthy and Rajamani (2013) reported that maximum vegetative growth parameters such as trunk girth increment, canopy spread, leaf fresh and dry weights, etc. of cocoa were obtained under fertigation with water-soluble NPK fertilizers compared with soil application. Nutrient supply through fertigation improved the yield of cocoa by 52 % over control treatment (Sujatha and Ravi, 2013). Water-soluble fertilizers used in fertigation performed better than conventional application in terms of growth, yield, and nutrient uptake of maize (Anitta-Fanish and Muthukrishnan, 2013). Fertigation in pigeonpea saved 30 - 70 % of water, increased yield by 20 - 90 % and resulted in higher WUE from 35.5 % to 50.8 % compared with conventional method (Jeyajothi *et al.,* 2017). Shinde *et al.* (2000) reported that growth parameters and yield of sugarcane were significantly improved in fertigation treatments compared with conventional method of soil application. Dinesh and Ahmed (2014) reported that the maximum tree height, nut weight, nut yield, and leaf nutrient content of almond were recorded through fertigation.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted in a greenhouse at the Cocoa Research Institute of Ghana (CRIG), located in New Tafo in the Abuakwa North Municipality of the Eastern Region of Ghana. New Tafo is geographically sited around latitude 06º 13'N, longitude 00º 22' W, with an altitude of 222 m above mean sea level. The average annual rainfall of the area is about 1,750 mm (Dogbatse *et al.,* 2019). The greenhouse at CRIG used for the study is specifically positioned on latitude 06º13' 28" N and longitude 00º 21' 49" W.

3.2 Experimental design and treatments application

The research was a pot experiment carried out in greenhouse for six months (September 2019 to March 2020). Standard black polythene bags measuring 17.5 x 25 cm, perforated at the base were each filled with 2 kg of sieved topsoil to raise the cocoa seedlings. Each polybag was packed with the topsoil to a bulk density of 1.23 Mg m^{-3}, which is the bulk density of the land where the topsoil was taken. The perforations at the base of the polybags were done to facilitate drainage of excess water. For the fertigation treatments, the filled polybags were put in another polybag to collect leachates after each application and then poured back. All the filled polybags were arranged on trestle tables. Two seeds were sown in each polybag and later thinned out to one seedling per polybag at emergence (i.e., 11 - 14 days after sowing). The experiment was 4x3 factorial treatment structure, laid out in completely randomized design (CRD). The experiment consisted of 13 treatments including absolute control and three replications, making a total of 39 experimental units. Each unit had 24 cocoa seedlings (6x4 rows), making a total of 936 seedlings for the study.

The fertilizer treatments were two foliar (liquid) and two water-soluble inorganic granular fertilizers applied at three different rates (i.e. low, moderate and high) (Table 3.1). The foliar fertilizers used were NPK 24:17:18 and NPK 10:10:10, whereas the water-soluble inorganic granular fertilizers used were ammonium sulphate (NPK 21:0:0) and calcium nitrate (NPK 15.5:0:0). These fertilizers have been approved by COCOBOD for use on cocoa. The foliar fertilizers were diluted with water and sprayed directly onto the leaves of cocoa seedlings as foliar fertilization, while the water-soluble inorganic granular fertilizers were dissolved completely in water and applied directly to the soil as fertigation. Details of quantities applied are presented in Table 3.1. The application rates used in this study were adjusted to supply equivalent amounts of N for each fertilizer type. Thus, the three application rates per fertilizer type were equivalent to 42 mg N, 84 mg N, and 126 mg N (Table 3.1). The application rates were selected on the basis of recommendations by Moyin-Jesu and Atoyosoye (2002), Ayeni *et al.* (2011), and Famuwagun and Oladitan (2016).

Treatments application commenced 4 weeks after emergence (WAE) and continued at bi-weekly intervals for 16 weeks (4 months). The treatments were applied manually using pneumatic knapsack sprayer for the foliar application and graduated measuring cylinder for the fertigation. During the foliar applications, adjacent treatments were protected with plywood barriers to avoid contact with the spraying solutions. Regarding the fertigation, two days after each application, leachates collected in the additional polybags were poured back into the respective polybags to help reduce nutrient losses through leaching. All the fertilizer treatments were applied in the morning, before 10:00 a.m., throughout the application period of the study. At that time, the stomata may be opened for efficient and effective absorption, and temperatures were not too high

to cause leaf burn or serious damage to the cocoa seedlings (Poole *et al.,* 1983; Noordiana *et al.,* 2007; Fageria *et al.,* 2009; Yap, 2012).

Table 3.1. Description of the treatments used for the experiment

Fertilizer options (Treatments)	Fertilizer type	N rate (mg pot^{-1})	Actual application rate	Concentration
NPK24/N42	Foliar 1 (NPK 24:17:18)	42	0.17 ml per 100 ml water per plant	0.17 % (v/v solution)
NPK24/N84	Foliar 1 (NPK 24:17:18)	84	0.34 ml per 100 ml water per plant	0.34 % (v/v solution)
NPK24/N126	Foliar 1 (NPK 24:17:18)	126	0.51 ml per 100 ml water per plant	0.51 % (v/v solution)
NPK10/N42	Foliar 2 (NPK 10:10:10)	42	0.42 ml per 100 ml water per plant	0.42 % (v/v solution)
NPK10/N84	Foliar 2 (NPK 10:10:10)	84	0.84 ml per 100 ml water per plant	0.84 % (v/v solution)
NPK10/N126	Foliar 2 (NPK 10:10:10)	126	1.26 ml per 100 ml water per plant	1.26 % (v/v solution)
NPK21/N42	Granular 1 (Ammonium sulphate)	42	0.20 g per 100 ml water per plant	0.20 % (w/v solution)
NPK21/N84	Granular 1 (Ammonium sulphate)	84	0.40 g per 100 ml water per plant	0.40 % (w/v solution)
NPK21/N126	Granular 1 (Ammonium sulphate)	126	0.60 g per 100 ml water per plant	0.60 % (w/v solution)
NPK15.5/N42	Granular 2 (Calcium nitrate)	42	0.27 g per 100 ml water per plant	0.27 % (w/v solution)
NPK15.5/N84	Granular 2 (Calcium nitrate)	84	0.54 g per 100 ml water per plant	0.54 % (w/v solution)
NPK15.5/N126	Granular 2 (Calcium nitrate)	126	0.81 g per 100 ml water per plant	0.81 % (w/v solution)
Control	No fertilization	-	-	-

Foliar (liquid) fertilizers 1 and 2 were applied by foliar fertilization

Granular fertilizers 1 and 2 were applied through fertigation

3.3 Collection of topsoil and cocoa pods for planting

3.3.1 Topsoil collection and preparation

Topsoil was collected from a depth of 0-15 cm from an uncultivated land at CRIG. The soil belongs to WACRI series according to the soil classification system of Ghana (Brammer, 1962; Dwomo and Dedzoe, 2010). The WACRI series is reported to be one of the best cocoa-growing soils in Ghana (Appiah *et al.,* 1997). Topsoil was used to raise the cocoa seedlings, because that is where most essential plant nutrients are concentrated (Soil Survey Laboratory Staff, 1992). The topsoil was air-dried, sieved to get rid of debris and stones, before filling into the polybags.

3.3.2 Cocoa pod collection and preparation

Matured cocoa pods were harvested from a 7-year old mixed hybrid cocoa plantation at CRIG. Fresh seeds were carefully removed from the pods and mucilage removed before planting.

3.4 Cultural practices and growth conditions in the greenhouse

Watering was done immediately after sowing, and continued at two days intervals throughout the period of the experiment. Weeds in the polythene pots were regularly hand-picked to minimize competition for nutrients, water and other growth resources, and to prevent them from serving as alternative host for insect pests and diseases. There were no serious occurrences of insect pests and diseases, so no insecticides and fungicides were applied throughout the study period.

The average temperature and relative humidity recorded in the greenhouse during seedling growth were 28.5 ^{o}C and 73.7 % respectively. These figures were within suitable ranges for cocoa growth recommended by the International Cocoa Organization (Konlan *et al.,* 2018). The temperature and relative humidity measurements were taken at three hourly intervals throughout the period of the experiment and the averages calculated. These climatic parameters were recorded with a Tinytag

data logger device (TGP-4500 model) centrally placed in the greenhouse throughout the experiment.

3.5 Sample collection for physico-chemical analysis

3.5.1 Soil sampling

Initial soil samples were taken from heaped topsoil before filling them in the polybags for planting. Three samples each were collected from six different points, mixed thoroughly, bulked together and sub-sampled to obtain six composite samples for baseline soil analysis. At the end of the experiment, the soils in each experimental unit/treatment were also mixed thoroughly, bulked together and sub-sampled to obtain 39 composite samples for final soil analysis. The soil samples were taken with the aid of a hand trowel and put into polybags with labels attached, for preparation and laboratory analysis.

3.5.2 Plant tissue sampling

At the end of the experiment, plant tissues were sampled from the six months old seedlings (Plate 3.1) to assess nutrient concentrations in the leaves by chemical analysis in the laboratory. Three tagged seedlings were uprooted from each experimental unit, making a total of 117 samples. These were further separated into leaves, stems and roots with the aid of scissors and put into well-labelled paper bags. Leaf samples were used for the nutrient analysis, because nutrients concentrations in plant leaves are better indicators of plant nutrition status (Barber, 1995; Marschner, 1998; Baligar *et al.,* 2001).

Plate 3.1. Six months old cocoa seedlings ready for final sampling

3.6 Sample preparation for physico-chemical analysis

3.6.1 Soil preparation

The soil samples were spread on clean polyethylene sheets and air-dried for three to five days at room temperature. The dried samples were ground with hand-held wooden roller, sieved through a 2 mm mesh to obtain fine earth fractions, and kept in well-labelled plastic containers for physico-chemical analysis in the laboratory.

3.6.2 Leaf tissue preparation

The leaf samples of the final harvest were carefully washed with water, cleaned and oven-dried at 75 ºC for 72 hours to attain constant weight. The dried leaf samples were ground using a stainless-steel grinding machine and stored in well-labelled plastic containers for nutrient concentration analysis in the laboratory.

3.7 Physico-chemical analysis of soil samples

The soil samples were analyzed for some physico-chemical properties at the Soil Science Laboratory of CRIG using standard laboratory protocols. The physical properties determined were bulk density, particle size distribution (i.e., amounts of sand, clay, and silt) and textural class for only the initial soil samples. However, chemical properties such as pH, organic carbon, total nitrogen, available phosphorus, exchangeable bases (potassium, magnesium and calcium), micronutrients *viz.,* iron and zinc were analyzed in both the initial and final soil samples.

3.7.1 Determination of soil pH

Soil pH was determined in a 1:2.5 (w/v) soil:water suspension using an electronic pH meter with glass electrode (Van Reeuwijk, 1992). Ten grams of air-dried soil sample was weighed into 100 ml Pyrex beaker, followed by the addition of 25 ml of distilled water. The soil-water suspension was stirred and allowed to stand for 30 minutes. An electronic pH meter (Mettler Toledo brand) was calibrated with buffer solutions at pH 4 and 7 after which the test sample was measured. The beaker containing the test sample was swirled by hand for about 5 seconds before the reading was taken. In all the readings, it was ensured that the electrode was correctly and properly dipped into the supernatant (upper part of the mixture). Distilled water was used to rinse the electrode and wiped gently with soft tissue material after each reading for both buffers and test samples.

3.7.2 Organic carbon

Organic carbon in the soil was determined using the wet combustion and rapid titration procedure described by Walkley and Black (1934). Exactly 1.0 g of air-dried soil sample was weighed into 500 ml Pyrex conical flask. The sample was mixed with 10 ml of 1.0 *N* potassium dichromate solution and 20 ml of sulphuric acid, swirled vigorously for a minute, and allowed to settle under fume hood for about half an hour. The mixture was cooled and diluted with 200 ml of distilled

water, and 10 ml of orthophosphoric acid (H_3PO_4) was added. Ten drops (about 0.5 ml) of diphenylamine indicator were added and titrated against 0.5 *N* ferrous ammonium sulphate until the solution turned green. About 0.5 ml of 1.0 *N* potassium dichromate solution was added again and back-titrated against the 0.5 *N* ferrous ammonium sulphate until a green colour was observed. Two blank samples (without soil) were also analyzed in the same manner. The organic carbon content was calculated using the formula below:

$$Organic\ carbon\ (\%)\ = \frac{(B - S)\ \times\ N\ \times\ 0.39}{W}$$

where: B = Mean titre value of blank (ml)

S = Titre value of soil sample (ml)

N = Normality of titrant concentration

W = Weight of soil sample (g)

0.39 = Correction factor

3.7.3 Total nitrogen

Total nitrogen in the soil was determined using the Kjeldahl method of Bremner (1965), which involves sample digestion, distillation and titration. Exactly 2.5 g of air-dried soil sample was weighed into digestion tube, after which about 0.5 g of catalyst (selenium, copper sulphate, and potassium sulphate) was added. The sample was placed under fume chamber and 12 ml of concentrated sulphuric acid added. Digestion tube containing the sample was fixed in a block digester and digested for 2 hours at 350 ºC. The digested sample (digest) was cooled under the fume chamber until there were no fumes evolving, and then subjected to distillation with 50 ml of 40 % sodium hydroxide for 4 minutes using Kjeldahl distillation apparatus (Kjeltec™ 2100 model). The distillate was collected in a receiver flask containing 20 ml of 2 % boric acid and 2 drops of indicator (methyl blue, methyl red, and alcohol) and then titrated with 0.02 *N* sulphuric

acid till just a colour change was observed from green to blue. Percent total nitrogen in the soil sample was calculated using the formula below:

$$Total\ nitrogen\ (\%)\ =\ \frac{T\ \times\ N\ \times\ 1.401}{W}$$

where: T = Titre value of soil sample (ml)

N =Normality of titrant concentration

W = Weight of soil sample (g)

1.401 = Milliequivalent weight of nitrogen x 100

3.7.4 Available phosphorus

Available phosphorus in the soil was determined by Mehlich-3 extraction procedure using 1:10 (w/v) soil:extractant ratio (Mehlich, 1984), and colorimetrically on spectrophotometer using ascorbic acid for colour development. A 2.5g of air-dried soil sample was weighed into plastic extraction bottle and 25 ml of Mehlich-3 (ammonium fluoride, EDTA, ammonium nitrate, acetic acid, and nitric acid) extracting solution added. The mixture was shaken on a mechanical shaker for 10 minutes at 200 revolutions per minute. The mixture was filtered through Whatman No. 42 filter paper into 50 ml volumetric flask and 2 ml aliquot of filtrate taken into 25 ml volumetric flask. Exactly 4 ml of ascorbic acid colouring reagent was added, made to the 25 ml volume mark with distilled water and shaken by hand to mix well. A blank sample was also prepared with 4 ml of ascorbic acid colouring reagent and distilled water only. Ultraviolet visible spectrophotometer (CE 7400 model, Cecil brand) was calibrated using the blank sample and phosphorus standards of known concentrations at a wavelength of 845 nm. Upon blue colour development, absorbance reading of the sample solution was taken and used for calculation with the formula below:

$$Available\ phosphorus\ (mg\ kg^{-1})\ =\ \frac{(A\ \div\ G)\ \times D\ \times\ V}{W}$$

where: A = Absorbance reading of sample (nm)

$$Graph\ factor\ (G)\ =\ \frac{Sum\ of\ absorbance\ readings\ of\ phosphorus\ standards\ (nm)}{Sum\ of\ concentrations\ of\ phosphorus\ standards\ (ppm)}$$

$$Dilution\ factor\ (D)\ =\ \frac{Volume\ of\ volumetric\ flask\ used\ for\ reading\ (ml)}{Volume\ of\ aliquot\ taken\ from\ filtrate\ (ml)}$$

V = Volume of extracting solution (ml)

W = Weight of soil sample (g)

3.7.5 Exchangeable bases

Exchangeable bases (potassium, magnesium and calcium) in the soil were determined by ammonium acetate extraction method using 1:5 (w/v) soil:extractant ratio (Hanway and Heidel, 1952), followed by atomic absorption spectrometry. Exactly 5.0 g of air-dried soil sample was weighed into plastic extraction bottle and 25 ml of 1.0 *M* ammonium acetate solution (pH 7) added. The mixture was shaken on a mechanical shaker for 10 minutes at 200 revolutions per minute, and filtered through Whatman No. 42 filter paper into 50 ml volumetric flask. Ionization suppressants such as caesium, lanthanum and potassium were added to the filtrate (analyte solution) to minimize interferences. The filtrate was analyzed for the concentrations of potassium (K), magnesium (Mg) and calcium (Ca) on Atomic Absorption Spectrometer (Spectr AA 220 FS model, Varian Brand). The exchangeable bases were calculated using the formulae below:

$$Exchangeable\ potassium\ (cmolc\,kg^{-1}) = \left(\frac{C\ \times\ V}{W}\right) \times 0.003$$

$$Exchangeable\ magnesium\ (cmolc\,kg^{-1}) = \left(\frac{C\ \times\ V}{W}\right) \times 0.008$$

$$Exchangeable\ calcium\ (cmol_c\,kg^{-1}) = \left(\frac{C\ \times\ V}{W}\right) \times 0.005$$

where: C = Concentration read on AAS (µg ml^{-1})

V = Volume of extracting solution (ml)

W = Weight of soil sample (g)

0.003, 0.008 and 0.005 = Factors for K, Mg and Ca respectively

3.7.6 Available micronutrients

Micronutrient elements such as iron (Fe) and zinc (Zn) in the soil were determined by Mehlich-3 extraction method using 1:10 (w/v) soil:extractant ratio (Mehlich, 1984), followed by atomic absorption spectrometry. Exactly 2.0 g of air-dried soil sample was weighed into plastic extraction bottle and 20 ml of Mehlich-3 extractant added. The mixture was shaken on a mechanical shaker for 10 minutes at 200 revolutions per minute, and filtered through Whatman No. 42 filter paper into 25 ml volumetric flask. The concentrations of Fe and Zn in the filtrate were determined on Atomic Absorption Spectrometer (Spectr AA 220 FS model, Varian Brand), and each element calculated using the formula below:

$$Micronutrient\ element\ (mg\ kg^{-1}) = \frac{C \times V}{W}$$

where: C = Concentration read on AAS (µg ml^{-1})

V = Volume of extracting solution (ml)

W = Weight of soil sample (g)

3.7.7 Particle size distribution

The relative proportions of the three soil separates (i.e., sand, clay, and silt) were determined by the hydrometer method of Bouyoucos (1951), and subsequently estimated the textural class of the soil using the USDA's soil textural triangle. Exactly 52.0 g of air-dried soil sample was weighed into 250 ml Pyrex beaker and 20 ml of 20 % hydrogen peroxide added to wet the sample. The content in the beaker was dried on a hot plate and ground. Exactly 100 ml of 5 % sodium hexametaphosphate (calgon) was added, mixed thoroughly, and left to stand for 15 - 20 hours. The

content in the beaker was washed with distilled water into stainless steel soil dispersing cup and stirred vigorously with soil dispersing machine for 2 minutes. The content in the cup was washed with distilled water into 1000 ml graduated measuring cylinder and made to the mark with more distilled water. The measuring cylinder was stoppered and shaken vigorously by hand for about a minute. Few drops of amyl alcohol ($C_5H_{11}OH$) were quickly added on top of the suspension to dissipate froths. Hydrometer was gently placed in the soil suspension and first reading taken within 40 seconds. After 2 hours of continuous sedimentation, the second reading was taken with the hydrometer. The relative amounts of sand, clay, and silt were then calculated using the formulae below:

$$Sand\ (\%) = 100 - 2 \times (A + 2.88)$$
$$Clay\ (\%) = 2 \times (B + 2.88)$$
$$Silt\ (\%) = 100 - (\%\ Sand + \%\ Clay)$$

where: A = 1st corrected hydrometer reading

B = 2nd corrected hydrometer reading

2.88 = Correction factor

The relative percentages of sand, clay, and silt were used to determine the soil's textural class using the soil textural triangle of USDA.

3.7.8 Bulk density

Bulk density of field slightly disturbed soil was determined by the core sampler method described by Sarkar and Haldar (2005), before the topsoil was excavated. Three core samples were taken at 15 cm, and the mean calculated. A cylindrical core sampler (sample can) was pushed vertically into the soil, deep enough to fill the sampler. With the aid of hand trowel, the sampler was carefully removed without disturbing the soil core contained therein. Extra soil was removed from both ends of the sampler by levelling with a sharp knife. The weight of moist core sample was immediately

taken, after which the sample was oven-dried at 105 °C for 24 hours to attain constant weight and re-weighed. The height and inner diameter of the sample can was measured to determine its volume, which represented the total volume of soil. The bulk density of the soil was calculated using the formula below:

$$Bulk\ Density\ (Mg\ m^{-3}\ ) = \frac{W}{V}$$

where: W = Weight of oven dry core sample (g)

V = Total volume of soil (cm^3) = $\pi r^2 h$

π = 3.142

r = radius = diameter (cm) divided by 2

h = height of sample can (cm)

3.8 Chemical analysis of leaf tissue

Leaf tissue samples of the six months old cocoa seedlings were analyzed for some major and minor nutrient concentrations at the Soil Science Laboratory of CRIG. Standard laboratory protocols were followed in the analysis. Macronutrients *viz.,* nitrogen, phosphorus, potassium, magnesium and calcium, as well as micronutrients such as iron and zinc were analyzed in the samples.

3.8.1 Determination of total nitrogen in leaf tissue

Total nitrogen in the leaf samples was determined using the Kjeldahl method of Bremner (1965), which involves sample digestion, distillation and titration. Exactly 0.5 g of oven-dried and ground leaf tissue sample was weighed into digestion tube, after which about 0.5 g of catalyst added. The tube containing the sample was placed under fume chamber and 12 ml of concentrated sulphuric acid added. The tube was fixed in a block digester and digested for 3 hours at 350 ºC. The digested sample (digest) was cooled under the fume chamber until there were no fumes evolving, and then subjected to distillation with 50 ml of 40 % sodium hydroxide for 4 minutes in the Kjeldahl

distillation unit (Kjeltec^{TM} 2100 model). The distillate was collected in a receiver flask containing 20 ml of 2 % boric acid and 2 drops of indicator, and then titrated with 0.02 *N* sulphuric acid till just a colour change was observed from green to blue. Percent total nitrogen in the leaf tissue was calculated using the formula below:

$$Total\ nitrogen\ (\%) = \frac{T \times N \times 1.401}{W}$$

where: T = Titre value of leaf tissue sample (ml)

N = Normality of titrant concentration

W = Weight of leaf tissue sample (g)

1.401 = Milliequivalent weight of nitrogen x 100

The percent total nitrogen value was multiplied by 10 to convert it to mg g^{-1}

3.8.2 Total phosphorus

Total phosphorus in the leaf samples was determined by double acid wet digestion method using 2:1 (v/v) nitric:perchloric acids ratio (AOAC, 1990), and colorimetrically on spectrophotometer using ascorbic acid for colour development. Exactly 0.5g of oven-dried and ground leaf tissue sample was weighed into digestion tube and placed under fume chamber. The sample was mixed with 20 ml of 70 % concentrated nitric acid and 10 ml of 70 % concentrated perchloric acid, and digested at 250 ºC in a block digester for 1 hour, 30 minutes. Dense white fume was produced, indicating complete digestion. The digest was cooled under the fume chamber until there were no fumes evolving. The digest was filtered through Whatman No. 42 filter paper into 200 ml volumetric flask and made to the mark with distilled water. Exactly 1 ml aliquot was taken into 25 ml volumetric flask and 4 ml of ascorbic acid colouring reagent added. The content was made to the 25 ml mark with distilled water and shaken by hand to mix well. Blank sample was also prepared with 4 ml ascorbic acid colouring reagent and distilled water only. Ultraviolet visible

spectrophotometer (CE 7400 model, Cecil brand) was calibrated using the blank sample and phosphorus standards of known concentrations at a wavelength of 882 nm. Upon blue colour development, absorbance reading of the sample solution was taken and used for calculation with the formula below:

$$Total\ phosphorus\ (mg\ kg^{-1}) = \frac{(A \div G) \times D \times V}{W}$$

where: A = Absorbance reading of leaf tissue sample (nm)

$$Graph\ factor\ (G) = \frac{Sum\ of\ absorbance\ readings\ of\ phosphorus\ standards\ (nm)}{Sum\ of\ concentrations\ of\ phosphorus\ standards\ (ppm)}$$

$$Dilution\ factor\ (D) = \frac{Volume\ of\ volumetric\ flask\ used\ for\ reading\ (ml)}{Volume\ of\ aliquot\ taken\ from\ final\ sample\ solution\ (ml)}$$

V = Final volume of sample solution after digestion (ml)

W = Weight of leaf tissue sample (g)

The total phosphorus value was multiplied by 0.001 to convert it to mg g^{-1}

3.8.3 Total potassium and other elements

Total K, Mg, Ca, Fe and Zn in the leaf samples were determined by double acid wet digestion method using 2:1 (v/v) nitric:perchloric acids ratio (AOAC, 1990), followed by atomic absorption spectrometry. Exactly 0.5 g of ground leaf tissue sample was weighed into digestion tube and placed under fume chamber. The sample was mixed with 20 ml of 70 % concentrated nitric acid and 10 ml of 70 % concentrated perchloric acid, and digested at 250 ºC in a block digester for 1 hour, 30 minutes. Dense white fume was produced, indicating complete digestion. The digest was cooled under the fume chamber until there were no fumes evolving. The digest was filtered through Whatman No. 42 filter paper into 200 ml volumetric flask and made to the mark with distilled

water. Ionization suppressants such as caesium, lanthanum and potassium were added to the analyte solution to minimize interferences. The concentrations of K, Mg, Ca, Fe and Zn in the final analyte solution were then determined on Atomic Absorption Spectrometer (Spectr AA 220 FS model, Varian Brand), and each element calculated using the formula below:

$$Element\ (\mu g\ g^{-1}) = \frac{C \times V}{W}$$

Where: C = Concentration read on AAS (μg ml^{-1})

V = Final volume of sample solution after digestion (ml)

W = Weight of leaf tissue sample (g)

The total K, Mg and Ca values were multiplied by 0.001 to convert them to mg g^{-1}

3.9 Growth measurements

The growth of cocoa seedlings was measured at monthly intervals for six months (i.e., from October 2019 to March 2020), beginning from one month after emergence (MAE). Three plants in each treatment were randomly selected and tagged for monthly measurement of height, stem diameter and number of leaves. At the end of the experiment, those tagged plants were destructively sampled for the measurement of total leaf area and dry weights of leaves, stems and roots. Apart from the tagged plants, destructive sampling of other three randomly sampled plants per treatment was also performed monthly for the measurement of total leaf area and dry matter.

The destructive sampling was done by tearing apart the polybag containing each of the sampled seedlings and the whole seedling carefully removed and washed with water, ensuring the roots were intact. The seedlings were partitioned into leaves, stems and roots (Plate 3.2) after which the fresh weights and total leaf area were taken before oven-drying them.

Plate 3.2. Cocoa seedling separated into plant parts after destructive sampling

3.9.1 Plant height

The height of the cocoa seedlings was measured from the base of plant on soil surface to the tip of the terminal bud using standard meter rule. This was performed on the three tagged plants per treatment per replication and the mean calculated.

3.9.2 Stem diameter

The diameter of the stem of cocoa seedlings was measured 5 cm from the base of the plant using digital vernier caliper (Plate 3.3). This was performed on the three tagged plants per treatment per replication and the mean calculated.

Plate 3.3. Measurement of stem diameter using a digital vernier caliper

3.9.3 Number of leaves

The total number of leaves produced by each seedling was counted. This was performed on the three tagged plants per treatment per replication and the mean calculated.

3.9.4 Total leaf area

The total leaf area per seedling was measured with the aid of a leaf area meter. The area of individual leaves was taken after which the total area of leaves produced by each seedling was calculated. This was performed on three uprooted plants per treatment per replication and the mean calculated.

3.9.5 Dry matter production

After taking the fresh weights of plant parts and total leaf area, all the plant parts were oven-dried to constant weight at 75 ^{o}C for 72 hours. The dry weight of each part (leaf, stem and root) was measured, after which they were summed up to obtain the total dry weight (total dry matter) of the plant. These were carried out on three uprooted plants per treatment per replication and the mean calculated. The biomass allocated to the respective plant parts at the end of the study was calculated as follows:

$$Biomass\ allocation\ to\ plant\ part\ (\%) = \left(\frac{Dry\ weight\ of\ plant\ part}{Total\ dry\ weight}\right) \times 100$$

3.10 Plant nutrient uptake

The uptake of a nutrient by plant indicates the amount of that nutrient accumulated in the plant. Nutrient concentrations in plant leaves are considered better indicators of plant nutrition status (Barber, 1995; Marschner, 1998; Baligar *et al.,* 2001). Hence, in this study, the uptake of macronutrients (N, P, K, Mg and Ca) and micronutrients (Fe and Zn) by six months old cocoa

seedlings were determined in the leaf tissues at the end of the experiment. The uptake or accumulation of each nutrient element was quantified using the equations of Baligar *et al.* (2005); Ribeiro *et al.* (2008); and Li *et al.* (2015) below:

$$U = C \times W$$

Where: U = Nutrient uptake (mg plant^{-1} for macronutrients and µg plant^{-1} for micronutrients)

C = Concentration of nutrient element in leaf tissue (mg g^{-1} for macronutrients and µg g^{-1} for micronutrients)

W = Dry weight of leaf tissue (g plant^{-1})

3.11 Statistical analysis of data

Results of the various parameters obtained from the experiment were compiled and summarized into Microsoft-Excel spreadsheet. The data were subjected to statistical analysis by Analysis of Variance (ANOVA) using the linear model procedure of Statistix software, version 9.1 (Analytical software, Tallahassee, FL, USA). Where the ANOVA was significant, treatment means were compared using Least Significant Difference (LSD) method of the same software. Significance level was set at 5 % ($p < 0.05$). Graphs were drawn using Microsoft-Excel and R software. Mean values and standard deviations were also calculated with Microsoft-Excel. Pearson's correlation analysis was also performed with the Statistix software.

3.12 Quality control and quality assurance

High quality glassware and sample containers which were carefully washed with chromic mixture and thoroughly rinsed with distilled water were used for all the laboratory analyses. Blanks, standards, and reagents were prepared with pure distilled water. In all the chemical analyses, high purity analytical grade reagents were used. Stock standard solutions (concentration of 1000 mg/l) of K, Mg, Ca, Fe and Zn were used for the preparation of working standards after appropriate dilution with distilled water. Three standard solutions of each element were prepared for the

calibration of the Atomic Absorption Spectrometer (AAS), before concentrations of the test samples were determined. In ensuring that the working standard solutions were correctly prepared, their concentrations were read on the AAS for precision before reading the samples. To ensure accuracy of results, standards, samples and parameters were analyzed in triplicates. In handling the leaf tissue samples, clean gloves were worn to prevent any external contamination which could affect the analytical results.

CHAPTER FOUR

4.0 RESULTS

4.1 Initial soil physico-chemical properties

The results of the initial analysis of some physical and chemical characteristics of the topsoil are presented in Table 4.1.

Table 4.1. Initial physico-chemical properties of the topsoil before sowing

Soil property	Mean ± SD (N=6)
pH (1:2.5, H_2O)	7.04 ± 0.08
Organic carbon (%)	1.94 ± 0.03
Total nitrogen (%)	0.21 ± 0.00
Available phosphorus (mg kg^{-1})	8.01 ± 1.56
Exchangeable potassium ($cmol_c$ kg^{-1})	0.32 ± 0.02
Exchangeable magnesium ($cmol_c$ kg^{-1})	2.41 ± 0.07
Exchangeable calcium ($cmol_c$ kg^{-1})	10.20 ± 0.54
Available iron (mg kg^{-1})	11.05 ± 0.20
Available zinc (mg kg^{-1})	10.95 ± 0.77
Bulk density (Mg m^{-3})	1.23 ± 0.01
Sand (%)	63.91 ± 4.68
Clay (%)	24.43 ± 5.85
Silt (%)	11.67 ± 2.94
Textural class (USDA)	Sandy clay loam

SD = Standard deviation

4.2 Effect of foliar fertilization and fertigation on selected soil chemical properties

At the end of the experiment, the effects of foliar fertilizer application and fertigation on the levels of the studied soil chemical properties are indicated in Table 4.2. The results show some variations in the levels of the soil chemical properties.

4.2.1 Effect of foliar fertilization and fertigation on soil pH

Soil pH ranged between 5.09 in NPK21/N126 and 6.82 in the control (Table 4.2). The initial pH of 7.04 reduced to 5.09 under NPK21/N126 treatment. Among the treatments, soil pH significantly decreased with increasing fertigation rates of the ammonium sulphate fertilizer. Also, soil pH in the control pot was significantly higher than in all the fertigation treatments for ammonium sulphate and calcium nitrate fertilizers.

4.2.2 Effect of foliar fertilization and fertigation on soil organic carbon

Soil organic carbon content ranged from 2.04 % in NPK21/N126 to 2.15 % in NPK10/N84 (Table 4.2). However, the fertilizer options had no significant ($p > 0.05$) effect on soil organic carbon content.

4.2.3 Effect of foliar fertilization and fertigation on soil total nitrogen

Similar to the organic carbon, the fertilizer options had no significant ($p > 0.05$) effect on the levels of total nitrogen in soil. The range was 0.26 – 0.29 % across the treatments (Table 4.2).

4.2.4 Effect of foliar fertilization and fertigation on soil available phosphorus

Soil available P content ranged from 4.61 mg kg^{-1} in NPK21/N42 to 12.27 mg kg^{-1} in NPK10/N126 (Table 4.2). Significant differences ($p < 0.05$) were observed among the treatments. There was improvement in the soil available P content from the initial 8.01 mg kg^{-1} to 12.27 mg kg^{-1} under NPK10/N126 treatment. Available P content in soil under NPK 10:10:10 pots were significantly ($p < 0.05$) higher than those under the fertigated and control pots.

4.2.5 Effect of foliar fertilization and fertigation on exchangeable bases in soil

Exchangeable K content was between 0.21 $cmol_c\ kg^{-1}$ in the control and 0.35 $cmol_c\ kg^{-1}$ in NPK21/N126, with significant differences ($p < 0.05$) among the treatments (Table 4.2). The soil K content in all the foliar fertilizer treatments were significantly ($p < 0.05$) higher than in the control. Exchangeable Mg content ranged from 2.56 $cmol_c\ kg^{-1}$ in NPK10/N84 to 2.85 $cmol_c\ kg^{-1}$ in NPK21/N126, while exchangeable Ca content ranged from 9.52 $cmol_c\ kg^{-1}$ in NPK10/N84 to 10.69 $cmol_c\ kg^{-1}$ in NPK15.5/N126 (Table 4.2). There were significant differences ($p < 0.05$) in soil Mg and Ca among the treatments. Exchangeable Ca level in the soil improved from the initial 10.20 $cmol_c\ kg^{-1}$ to 10.69 $cmol_c\ kg^{-1}$ under NPK15.5/N126 treatment, and was significantly ($p < 0.05$) higher than the remaining treatments, including control.

4.2.6 Effect of foliar fertilization and fertigation on some micronutrients in soil

Each of the studied micronutrients did not show significant differences ($p > 0.05$) among the treatments. Available Fe concentration ranged from 13.26 mg kg^{-1} in the control to 15.49 mg kg^{-1} in NPK21/N126, while available Zn ranged between 11.35 mg kg^{-1} in NPK15.5/N126 and 13.33 mg kg^{-1} in NPK24/N42 (Table 4.2).

Table 4.2. Effect of foliar fertilization and fertigation on selected soil chemical properties

Fertilizer options	pH (1:2.5, H_2O)	Organic C (%)	Total N (%)	Available P (mg kg^{-1})	Exchangeable bases ($cmol_c$ kg^{-1})			Micronutrients (mg kg^{-1})	
					K	Mg	Ca	Fe	Zn
NPK24/N42	6.74^{ab}	2.05^{a}	0.26^{a}	5.84^{cd}	0.30^{bcd}	2.63^{bcd}	9.54^{ef}	14.12^{a}	13.33^{a}
NPK24/N84	6.69^{ab}	2.09^{a}	0.27^{a}	5.78^{cd}	0.31^{abcd}	2.68^{abcd}	9.68^{ef}	14.06^{a}	11.64^{a}
NPK24/N126	6.57^{bc}	2.14^{a}	0.29^{a}	6.07^{cd}	0.29^{cd}	2.59^{cd}	9.55^{ef}	13.85^{a}	11.89^{a}
NPK10/N42	6.56^{bc}	2.11^{a}	0.29^{a}	8.41^{b}	0.28^{cd}	2.69^{abcd}	9.65^{ef}	13.77^{a}	12.19^{a}
NPK10/N84	6.58^{bc}	2.15^{a}	0.28^{a}	12.21^{a}	0.33^{abc}	2.56^{d}	9.52^{f}	13.96^{a}	12.23^{a}
NPK10/N126	6.49^{cd}	2.06^{a}	0.27^{a}	12.27^{a}	0.34^{ab}	2.60^{cd}	9.58^{ef}	14.23^{a}	12.35^{a}
NPK21/N42	5.99^{e}	2.07^{a}	0.28^{a}	4.61^{e}	0.22^{ef}	2.75^{abcd}	9.97^{cd}	14.64^{a}	11.98^{a}
NPK21/N84	5.44^{f}	2.09^{a}	0.28^{a}	6.54^{c}	0.31^{abcd}	2.76^{abc}	9.81^{de}	15.14^{a}	12.56^{a}
NPK21/N126	5.09^{g}	2.04^{a}	0.29^{a}	6.08^{cd}	0.35^{a}	2.85^{a}	10.11^{bc}	15.49^{a}	11.89^{a}
NPK15.5/N42	6.42^{cd}	2.06^{a}	0.28^{a}	5.20^{de}	0.29^{cd}	2.82^{ab}	10.27^{b}	14.24^{a}	11.50^{a}
NPK15.5/N84	6.37^{d}	2.08^{a}	0.29^{a}	5.73^{cde}	0.28^{d}	2.83^{a}	10.36^{b}	13.62^{a}	11.76^{a}
NPK15.5/N126	6.47^{cd}	2.12^{a}	0.29^{a}	5.96^{cd}	0.27^{de}	2.81^{ab}	10.69^{a}	14.53^{a}	11.35^{a}
Control	6.82^{a}	2.11^{a}	0.28^{a}	6.08^{cd}	0.21^{f}	2.69^{abcd}	9.59^{ef}	13.26^{a}	13.29^{a}
LSD (0.05)	0.18	0.20	0.03	1.16	0.05	0.19	0.27	2.94	2.34
CV (%)	1.71	5.79	7.25	9.93	9.93	4.20	1.65	12.33	11.46

Results are mean values

Within the same column, means followed by different superscript letter(s) are significantly different at $p < 0.05$

4.3 Effect of foliar fertilization and fertigation on growth of cocoa seedlings

The results of the studied growth parameters at the end of the experiment (6^{th} month) are presented in Table 4.3. Generally, significant differences ($p < 0.05$) were observed among the treatments. The growth trends with time measured for the six months are shown in Figure 4.1. The results generally indicate that the fertilizer options (treatments) from foliar fertilization and fertigation had some effect on cocoa seedling growth.

4.3.1 Effect of foliar fertilization and fertigation on stem diameter of cocoa seedlings

At 6 MAE, mean stem diameter ranged from 6.54 mm in NPK21/N126 to 7.96 mm in NPK21/N42, with the former being significantly the lowest and the latter being the highest among the treatments (Table 4.3). Fertilizer option NPK21/N42 was significantly ($p < 0.05$) higher than NPK21/N84, NPK21/N126 and control at 2 MAE, 4 MAE and 6 MAE (Figure 4.1a). Treatments NPK10/N84 and NPK10/N126 each had significantly ($p < 0.05$) higher stem diameter than NPK21/N84 and NPK21/N126 options at 2 MAE and 6 MAE (Figure 4.1a).

4.3.2 Effect of foliar fertilization and fertigation on cocoa seedling height

On the sixth month, the seedlings attained mean heights between 49.36 cm in NPK21/N126 and 64.14 cm in NPK24/N42, with the former and NPK15.5/N126 being significantly the lowest among the treatments (Table 4.3). Seedling height at 2 MAE, 4 MAE and 6 MAE under NPK24/N42 was significantly ($p < 0.05$) higher than those under NPK10/N84, NPK21/N126 and NPK15.5/N126 (Figure 4.1b). Treatment NPK21/N42 had significantly higher plant height than NPK21/N126 at 2 MAE, 4 MAE and 6 MAE (Figure 4.1b).

4.3.3 Effect of foliar fertilization and fertigation on number of leaves of cocoa seedlings

At 6 MAE, the number of leaves produced under NPK21/N84 and NPK21/N126 were significantly lesser than those produced under all the foliar fertilizer treatments, NPK21/N42, NPK15.5/N42, and control (Table 4.3). Generally, the mean number of leaves produced at 6 MAE were more than 20 across treatments (Table 4.3). Treatment NPK24/N84 had significantly ($p < 0.05$) more leaves than the remaining treatments at 2 MAE, except NPK24/N42, NPK10/N42 and NPK10/N126 (Figure 4.1c). At 4 MAE, the number of leaves under NPK21/N84 were significantly lesser than those under the remaining treatments, apart from NPK21/N126 (Figure 4.1c).

4.3.4 Effect of foliar fertilization and fertigation on total leaf area of cocoa seedlings

The mean total leaf area at 6 MAE ranged from 1280.64 cm^2 in NPK21/N126 to 2030.80 cm^2 in NPK21/N42, with the former and NPK15.5/N126 being significantly the lowest among the treatments (Table 4.3). The total leaf area of the seedlings at 2 MAE were significantly ($p < 0.05$) higher under NPK24/N42 and NPK21/N42 fertilizer options than under NPK24/N84, NPK21/N126, NPK15.5/N126, and control (Figure 4.1d). The treatments did not significantly ($p > 0.05$) affect the total leaf area of the seedlings at 4 MAE (Figure 4.1d).

4.3.5 Effect of foliar fertilization and fertigation on total dry matter production

The mean total dry matter production on the sixth month ranged between 6.98 g $plant^{-1}$ in NPK21/N126 and 11.83 g $plant^{-1}$ in NPK21/N42, with the former being significantly the lowest and the latter being the highest among the treatments (Table 4.3). The total dry matter produced by the seedlings at 2 MAE and 4 MAE were significantly ($p < 0.05$) more under NPK21/N42 than

those produced under the remaining treatments, except NPK24/N42, NPK10/N126 and NPK15.5/N126 (Figure 4.1e).

Considering biomass allocation to the leaf, stem and root components at the end of the study, significant differences ($p < 0.05$) were observed among the treatments. Treatment NPK15.5/N126 had significantly ($p < 0.05$) lower leaf biomass allocation than the remaining treatments, including control. Generally, more than 40 % of the total plant biomass was allocated to the leaves across treatments (Figure 4.2). Biomass allocated to the stem and root parts under NPK15.5/N126 were significantly ($p < 0.05$) more than the other treatments.

Table 4.3. Effect of foliar fertilization and fertigation on cocoa seedling growth at 6 MAE

Fertilizer options	Stem diameter (mm)	Plant height (cm)	Number of leaves	Total leaf area (cm^2)	Total dry matter (g $plant^{-1}$)
NPK24/N42	7.41^{cd}	64.14^{a}	24.44^{bc}	1868.81^{ab}	9.15^{bc}
NPK24/N84	7.51^{bc}	59.51^{c}	25.33^{abc}	2016.40^{a}	10.00^{b}
NPK24/N126	7.52^{bc}	64.04^{a}	25.61^{ab}	1947.54^{a}	9.71^{bc}
NPK10/N42	7.34^{d}	62.24^{ab}	26.00^{ab}	1943.73^{a}	8.76^{bcd}
NPK10/N84	7.29^{de}	58.77^{cd}	26.00^{ab}	1996.87^{a}	9.14^{bc}
NPK10/N126	7.29^{de}	61.78^{b}	26.72^{a}	1903.64^{ab}	9.28^{bc}
NPK21/N42	7.96^{a}	63.94^{a}	25.56^{ab}	2030.80^{a}	11.83^{a}
NPK21/N84	6.78^{f}	57.62^{cd}	21.78^{d}	1531.63^{d}	7.81^{de}
NPK21/N126	6.54^{g}	49.36^{e}	22.11^{d}	1280.64^{e}	6.98^{e}
NPK15.5/N42	7.65^{b}	56.68^{d}	25.56^{ab}	1863.09^{ab}	9.27^{bc}
NPK15.5/N84	7.30^{d}	59.19^{c}	24.44^{bc}	1734.59^{bc}	9.54^{bc}
NPK15.5/N126	7.66^{b}	49.68^{e}	21.89^{d}	1314.94^{e}	8.95^{bcd}
Control	7.13^{e}	58.61^{cd}	23.89^{c}	1659.28^{cd}	8.55^{cd}
LSD (0.05)	0.16	2.11	1.60	200.53	1.29
CV (%)	1.32	2.14	3.87	6.73	8.42

Results are mean values

Within the same column, means followed by different superscript letter(s) are significantly different at $p < 0.05$

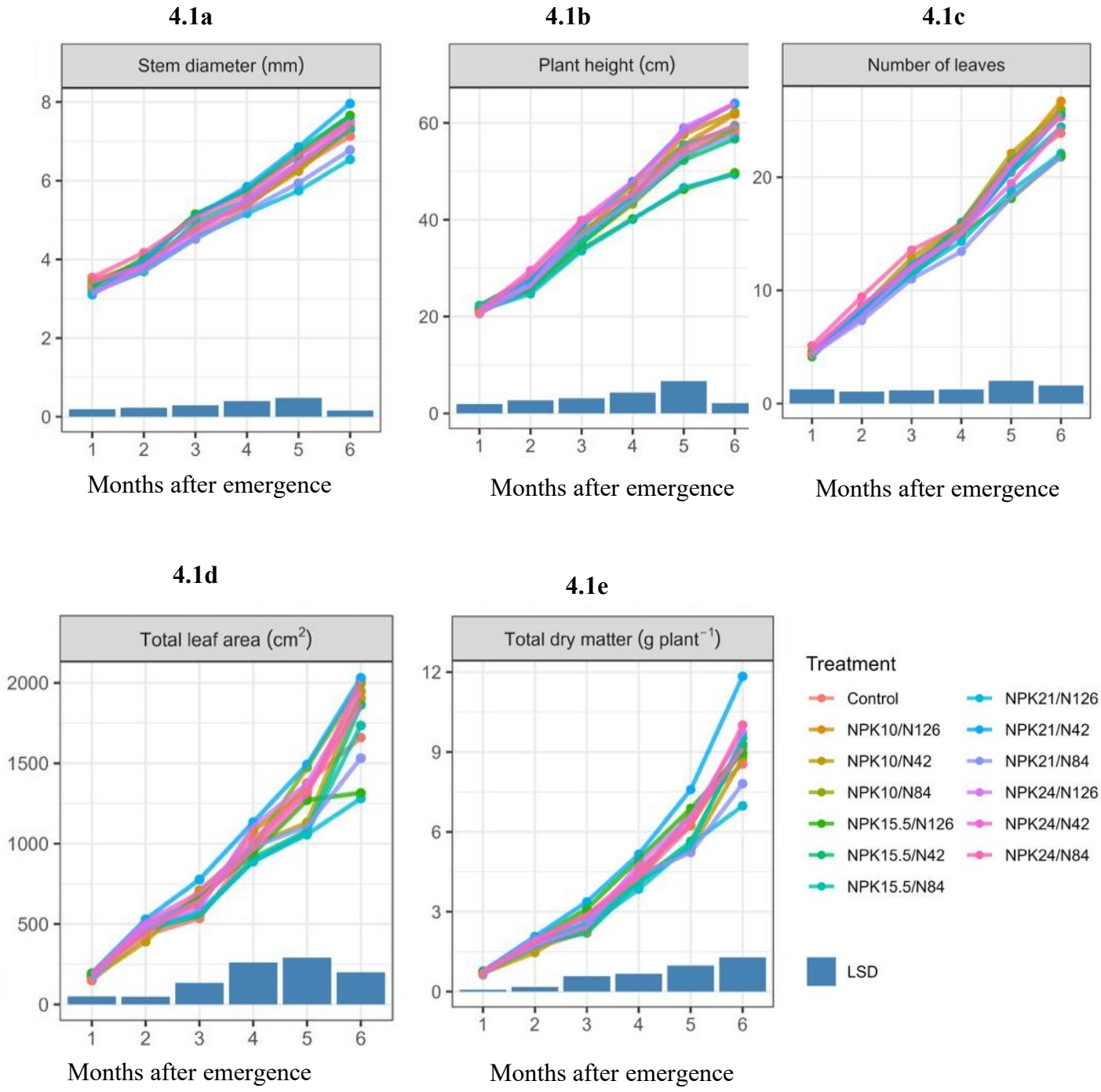

Figure 4.1. Effect of foliar fertilization and fertigation on monthly seedling growth

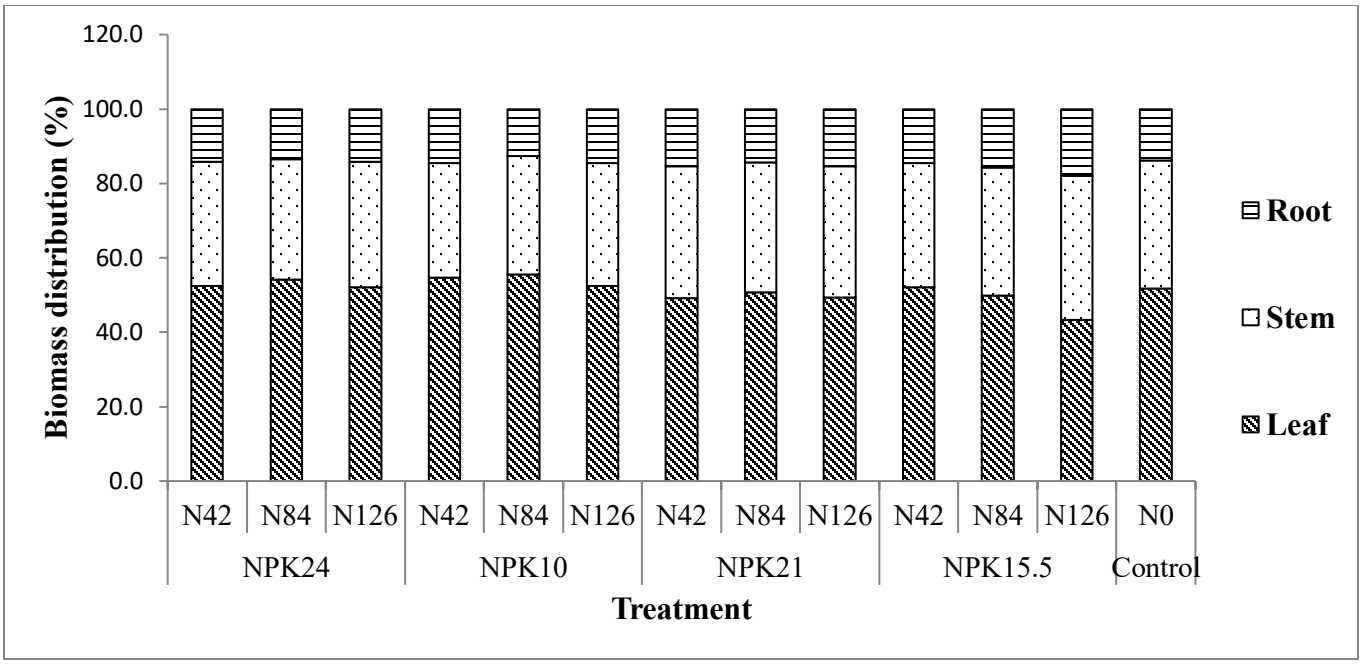

Figure 4.2. Allocation of biomass to leaf, stem and root parts of the cocoa seedlings at the end of the study

4.4 Effect of foliar fertilization and fertigation on nutrient uptake by cocoa seedlings

At the end of the experiment, significant differences ($p < 0.05$) were generally observed among the treatments in the uptake of the studied nutrients. The results are shown in Table 4.4.

4.4.1 Effect of foliar fertilization and fertigation on nitrogen uptake

The uptake of N ranged from 92.49 mg plant^{-1} in NPK21/N126 to 143.58 mg plant^{-1} in NPK21/N42 (Table 4.4). The uptake of N under the highest dose of the fertigated ammonium sulphate was significantly ($p < 0.05$) lower than those under the foliar fertilizer treatments.

4.4.2 Effect of foliar fertilization and fertigation on phosphorus uptake

The uptake of P ranged from 3.39 mg plant^{-1} in NPK21/N126 to 9.69 mg plant^{-1} in NPK10/N84 (Table 4.4). All the foliar treatments and control produced seedlings with significantly ($p < 0.05$) higher P uptake than NPK21/N84, NPK21/N126 and NPK15.5/N126 options (Table 4.4). Again, treatments NPK10/N84 and NPK10/N126 each had significantly ($p < 0.05$) higher P uptake, compared with the other treatments, including control.

4.4.3 Effect of foliar fertilization and fertigation on potassium uptake

The uptake of K ranged from 58.54 mg plant^{-1} in NPK15.5/N126 to 104.01 mg plant^{-1} in NPK10/N84 (Table 4.4). Treatments NPK10/N84 and NPK10/N126 each had significantly ($p < 0.05$) higher K uptake, compared with NPK21/N84, NPK21/N126, calcium nitrate fertigated treatments and control.

4.4.4 Effect of foliar fertilization and fertigation on magnesium uptake

Magnesium uptake ranged between 13.76 mg plant^{-1} in NPK21/N126 and 26.25 mg plant^{-1} in NPK21/N42 (Table 4.4). The uptake of Mg under the foliar fertilizer treatments were significantly ($p < 0.05$) higher than that under the highest ammonium sulphate fertigation rate.

4.4.5 Effect of foliar fertilization and fertigation on calcium uptake

Calcium uptake ranged between 72.12 mg plant^{-1} in NPK10/N84 and 102.94 mg plant^{-1} in NPK21/N42 (Table 4.4).

4.4.6 Effect of foliar fertilization and fertigation on micronutrient uptake

The uptake of Fe ranged from 239.53 µg plant^{-1} in NPK21/N126 to 489.34 µg plant^{-1} in NPK21/N42, while Zn uptake ranged from 95.47 µg plant^{-1} in NPK21/N126 to 227.46 µg plant^{-1} in NPK21/N42 (Table 4.4). The uptake of Fe and Zn under low ammonium sulphate rate were significantly ($p < 0.05$) higher than those under moderate and high rates, calcium nitrate rates and control. Also, the uptake of Zn under the foliar fertilizer treatments were significantly ($p < 0.05$) higher than that under the highest ammonium sulphate fertigation rate.

Table 4.4. Effect of foliar fertilization and fertigation on nutrient uptake by cocoa seedlings at 6 MAE

Fertilizer options	Macronutrient uptake (mg plant^{-1})					Micronutrient uptake (µg plant^{-1})	
	N	P	K	Mg	Ca	Fe	Zn
NPK24/N42	119.95^{bcde}	6.90^{cde}	74.96^{efg}	23.53^{abc}	82.76^{bcde}	364.61^{bcdef}	173.00^{bc}
NPK24/N84	127.19^{abcd}	7.83^{bc}	89.87^{bc}	26.01^{ab}	90.34^{abcd}	416.10^{ab}	191.22^{ab}
NPK24/N126	139.28^{ab}	7.66^{bc}	95.14^{ab}	22.44^{abcd}	98.41^{ab}	448.93^{ab}	181.12^{abc}
NPK10/N42	125.56^{abcd}	8.12^{b}	88.89^{bcd}	21.15^{abcde}	82.10^{cde}	264.73^{efg}	161.88^{bc}
NPK10/N84	133.33^{abc}	9.69^{a}	104.01^{a}	21.92^{abcd}	72.12^{e}	398.74^{abc}	177.72^{abc}
NPK10/N126	131.32^{abc}	9.63^{a}	93.52^{ab}	20.41^{bcde}	91.91^{abcd}	385.05^{abcd}	167.47^{bc}
NPK21/N42	143.58^{a}	7.14^{bcd}	83.94^{bcde}	26.25^{a}	102.94^{a}	489.34^{a}	227.46^{a}
NPK21/N84	108.29^{def}	4.52^{f}	63.70^{gh}	15.59^{ef}	77.82^{de}	258.50^{fg}	170.04^{bc}
NPK21/N126	92.49^{f}	3.39^{f}	66.31^{fgh}	13.76^{f}	72.42^{e}	239.53^{g}	95.47^{d}
NPK15.5/N42	129.30^{abcd}	5.86^{e}	75.97^{defg}	21.71^{abcd}	101.22^{a}	286.37^{defg}	154.73^{bc}
NPK15.5/N84	128.73^{abcd}	5.93^{e}	71.56^{efgh}	21.98^{abcd}	95.40^{abc}	373.19^{bcde}	137.66^{cd}
NPK15.5/N126	101.62^{ef}	3.53^{f}	58.54^{h}	17.19^{def}	81.98^{cde}	300.03^{cdefg}	129.41^{cd}
Control	111.57^{cdef}	6.30^{de}	79.09^{cdef}	19.36^{cdef}	77.24^{de}	352.76^{bcdef}	134.47^{cd}
LSD (0.05)	21.80	1.14	13.76	5.67	16.14	111.00	53.20
CV (%)	10.60	10.22	10.20	16.19	11.10	18.78	19.61

Results are mean values

Within the same column, means followed by different superscript letter(s) are significantly different at $p < 0.05$

4.5 Correlation analysis of soil chemical properties, seedling growth, and nutrient uptake under foliar fertilization and fertigation

Significant positive correlations were observed between soil organic carbon and total nitrogen under foliar fertilization (Table 4.5). There was a significant negative correlation between N rate and soil pH under fertigated ammonium sulphate (Table 4.6). Correlation between N rate and exchangeable Ca in soil under fertigated calcium nitrate was significant and positive (Table 4.6).

Significant negative correlation was observed between N rate and total leaf area under fertigation with ammonium sulphate. Also, total leaf area correlated significantly and positively with soil pH under fertigated ammonium sulphate (Table 4.8). Correlations between leaf dry matter and stem dry matter, total dry matter, total leaf area were significant and positive under both foliar fertilization and fertigation. Significant correlation was observed between seedling height and stem dry matter under the two application methods.

There were significant correlations between the uptake of P and N, Mg, Fe under foliar fertilization (Table 4.9). Under fertigation, the correlations between the uptake of Ca and N, K, Mg, Fe, Zn were significant (Table 4.10). Significant negative correlations were observed between N rate and P, Mg uptake under fertigation with ammonium sulphate (Table 4.10).

Table 4.5. Correlation coefficient (r) relating N rate and selected soil chemical properties under foliar fertilization

NPK 24:17:18 foliar fertilization

	C	Ca	Fe	K	Mg	N	N rate	P	Soil pH
Ca	-0.32NS								
Fe	0.55NS	0.42NS							
K	-0.75*	0.71*	-0.18NS						
Mg	-0.31NS	0.75*	0.35NS	0.64NS					
N	0.88*	-0.36NS	0.50NS	-0.69*	-0.37NS				
N rate	0.37NS	0.03NS	-0.10NS	0.04NS	-0.19NS	0.37NS			
P	0.24NS	0.49NS	0.71*	0.32NS	0.53NS	0.22NS	0.12NS		
Soil pH	-0.73*	0.12NS	-0.33NS	0.34NS	0.13NS	-0.77*	-0.76*	-0.34NS	
Zn	-0.27NS	-0.55NS	-0.29NS	-0.06NS	-0.09NS	-0.09NS	-0.55NS	-0.08NS	0.32NS

NPK 10:10:10 foliar fertilization

	C	Ca	Fe	K	Mg	N	N rate	P	Soil pH
Ca	0.46NS								
Fe	0.73*	0.53NS							
K	0.30NS	0.33NS	-0.01NS						
Mg	0.05NS	0.56NS	0.18NS	-0.20NS					
N	0.84*	0.47NS	0.65NS	0.07NS	0.34NS				
N rate	-0.19NS	-0.17NS	0.12NS	0.43NS	-0.40NS	-0.32NS			
P	0.36NS	0.42NS	0.39NS	0.75*	-0.20NS	0.06NS	0.60NS		
Soil pH	-0.06NS	0.11NS	-0.41NS	0.19NS	-0.32NS	-0.32NS	-0.38NS	-0.00NS	
Zn	-0.40NS	-0.54NS	-0.32NS	-0.46NS	0.29NS	-0.11NS	0.05NS	-0.46NS	-0.53NS

***** = *Significant at $p < 0.05$; NS = Not significant*

Table 4.6. Correlation coefficient (r) relating N rate and selected soil chemical properties under fertigation

Ammonium sulphate fertigation									
	C	**Ca**	**Fe**	**K**	**Mg**	**N**	**N rate**	**P**	**Soil pH**
Ca	0.05NS								
Fe	0.46NS	0.07NS							
K	0.11NS	0.51NS	0.05NS						
Mg	-0.00NS	0.87*	-0.26NS	0.58NS					
N	0.46NS	0.62NS	0.45NS	0.47NS	0.62NS				
N rate	-0.18NS	0.22NS	0.23NS	0.66*	0.26NS	0.49NS			
P	0.61NS	0.18NS	0.67*	0.59NS	0.16NS	0.66*	0.47NS		
Soil pH	0.03NS	-0.18NS	-0.39NS	-0.60NS	-0.20NS	-0.59NS	-0.97*	-0.60NS	
Zn	-0.17NS	-0.01NS	-0.62NS	-0.17NS	0.34NS	0.17NS	-0.02NS	-0.38NS	0.04NS

Calcium nitrate fertigation									
	C	**Ca**	**Fe**	**K**	**Mg**	**N**	**N rate**	**P**	**Soil pH**
Ca	0.25NS								
Fe	0.61NS	0.50NS							
K	-0.10NS	-0.04NS	-0.16NS						
Mg	0.24NS	-0.19NS	-0.29NS	0.53NS					
N	0.28NS	0.66*	0.28NS	0.24NS	0.09NS				
N rate	0.19NS	0.76*	0.07NS	-0.36NS	-0.06NS	0.58NS			
P	0.87*	0.34NS	0.65NS	0.17NS	0.42NS	0.44NS	0.19NS		
Soil pH	0.01NS	-0.13NS	-0.05NS	-0.68*	0.06NS	-0.57NS	0.15NS	-0.12NS	
Zn	0.16NS	-0.46NS	-0.61NS	0.34NS	0.57NS	0.03NS	-0.09NS	0.03NS	-0.24NS

***** = *Significant at $p < 0.05$; NS = Not significant*

Table 4.7. Correlation coefficient (r) relating N rate, soil pH and seedling growth at 6 MAE under foliar fertilization

NPK 24:17:18 foliar fertilization

	N rate	Soil pH	LDM	NL	SH	RDM	SD	SDM	TDM
Soil pH	-0.76*								
LDM	0.13NS	-0.25NS							
NL	0.21NS	-0.21NS	0.46NS						
SH	-0.01NS	-0.15NS	0.49NS	0.65NS					
RDM	0.10NS	-0.18NS	0.90*	0.26NS	0.46NS				
SD	0.06NS	-0.30NS	0.85*	0.46NS	0.39NS	0.83*			
SDM	0.13NS	-0.24NS	0.94*	0.57NS	0.67*	0.92*	0.88*		
TDM	0.13NS	-0.24NS	0.98*	0.47NS	0.56NS	0.95*	0.87*	0.98*	
TLA	0.14NS	-0.30NS	0.96*	0.47NS	0.54NS	0.81*	0.82*	0.90*	0.94*

NPK 10:10:10 foliar fertilization

	N rate	Soil pH	LDM	NL	SH	RDM	SD	SDM	TDM
Soil pH	-0.38NS								
LDM	0.06NS	-0.07NS							
NL	0.15NS	-0.39NS	0.57NS						
SH	-0.04NS	-0.22NS	0.60NS	0.66*					
RDM	0.25NS	-0.65NS	0.45NS	0.32NS	0.53NS				
SD	-0.06NS	0.05NS	-0.04NS	-0.43NS	-0.29NS	0.28NS			
SDM	0.42NS	-0.28NS	0.82*	0.69*	0.75*	0.53NS	-0.08NS		
TDM	0.20NS	-0.21NS	0.97*	0.64NS	0.70*	0.59NS	-0.02NS	0.92*	
TLA	-0.07NS	0.16NS	0.96*	0.39NS	0.56NS	0.40NS	0.06NS	0.71*	0.90*

LDM = Leaf dry matter; NL = Number of leaves; SH = Seedling height; RDM = Root dry matter; SD = Stem diameter; SDM = Stem dry matter; TDM = Total dry matter; TLA = Total leaf area

** = Significant at $p < 0.05$; NS = Not significant*

Table 4.8. Correlation coefficient (r) relating N rate, soil pH and seedling growth at 6 MAE under fertigation

Ammonium sulphate fertigation									
	N rate	**Soil pH**	**LDM**	**NL**	**SH**	**RDM**	**SD**	**SDM**	**TDM**
Soil pH	-0.97*								
LDM	-0.59NS	0.54NS							
NL	-0.48NS	0.40NS	0.95*						
SH	-0.64NS	0.53NS	0.90*	0.89*					
RDM	-0.48NS	0.46NS	0.98*	0.93*	0.82*				
SD	-0.59NS	0.52NS	0.99*	0.96*	0.88*	0.97*			
SDM	-0.49NS	0.41NS	0.98*	0.97*	0.88*	0.96*	0.98*		
TDM	-0.53NS	0.47NS	1.00*	0.96*	0.88*	0.99*	0.99*	0.99*	
TLA	-0.70*	0.66*	0.97*	0.90*	0.93*	0.93*	0.94*	0.91*	0.94*

Calcium nitrate fertigation									
	N rate	**Soil pH**	**LDM**	**NL**	**SH**	**RDM**	**SD**	**SDM**	**TDM**
Soil pH	0.15NS								
LDM	-0.34NS	-0.42NS							
NL	-0.61NS	-0.53NS	0.91*						
SH	-0.32NS	-0.64NS	0.82*	0.87*					
RDM	0.20NS	-0.50NS	0.77*	0.55NS	0.61NS				
SD	0.01NS	-0.47NS	0.80*	0.68*	0.68*	0.92*			
SDM	0.15NS	-0.54NS	0.83*	0.65NS	0.70*	0.97*	0.95*		
TDM	-0.05NS	-0.51NS	0.93*	0.78*	0.77*	0.94*	0.93*	0.97*	
TLA	-0.58NS	-0.30NS	0.94*	0.92*	0.72*	0.53NS	0.60NS	0.60NS	0.77*

LDM = Leaf dry matter; NL = Number of leaves; SH = Seedling height; RDM = Root dry matter; SD = Stem diameter; SDM = Stem dry matter; TDM = Total dry matter; TLA = Total leaf area

** = Significant at $p < 0.05$; NS = Not significant*

Table 4.9. Correlation coefficient (r) relating N rate, soil pH and nutrient uptake by cocoa seedling at 6 MAE under foliar fertilization

NPK 24:17:18 foliar fertilization

	N rate	Soil pH	Ca	Fe	K	Mg	N	P
Soil pH	-0.76*							
Ca	0.37NS	-0.20NS						
Fe	0.27NS	-0.38NS	0.57NS					
K	0.70*	-0.74*	0.55NS	0.42NS				
Mg	-0.08NS	0.14NS	0.77*	0.73*	0.27NS			
N	0.43NS	-0.25NS	0.93*	0.67*	0.55NS	0.76*		
P	0.19NS	-0.23NS	0.85*	0.79*	0.63NS	0.89*	0.86*	
Zn	0.11NS	0.13NS	0.65NS	0.48NS	0.27NS	0.62NS	0.50NS	0.62NS

NPK 10:10:10 foliar fertilization

	N rate	Soil pH	Ca	Fe	K	Mg	N	P
Soil pH	-0.38NS							
Ca	0.20NS	-0.71*						
Fe	0.68*	-0.36NS	0.37NS					
K	0.11NS	0.27NS	0.31NS	0.32NS				
Mg	-0.08NS	-0.06NS	0.50NS	0.32NS	0.58NS			
N	0.10NS	0.14NS	0.50NS	0.32NS	0.82*	0.83*		
P	0.46NS	-0.29NS	0.63NS	0.79*	0.68*	0.75*	0.77*	
Zn	0.07NS	0.09NS	0.36NS	0.40NS	0.49NS	0.11NS	0.43NS	0.47NS

* = *Significant at p < 0.05; NS = Not significant*

Table 4.10. Correlation coefficient (r) relating N rate, soil pH and nutrient uptake by cocoa seedling at 6 MAE under fertigation

Ammonium sulphate fertigation								
	N rate	**Soil pH**	**Ca**	**Fe**	**K**	**Mg**	**N**	**P**
Soil pH	-0.97*							
Ca	-0.41NS	0.35NS						
Fe	-0.55NS	0.53NS	0.86*					
K	-0.39NS	0.34NS	0.81*	0.95*				
Mg	-0.67*	0.62NS	0.89*	0.96*	0.90*			
N	-0.57NS	0.53NS	0.86*	0.90*	0.84*	0.92*		
P	-0.70*	0.65NS	0.87*	0.96*	0.89*	0.99*	0.94*	
Zn	-0.52NS	0.44NS	0.84*	0.90*	0.87*	0.91*	0.94*	0.94*

Calcium nitrate fertigation								
	N rate	**Soil pH**	**Ca**	**Fe**	**K**	**Mg**	**N**	**P**
Soil pH	0.15NS							
Ca	-0.25NS	-0.40NS						
Fe	0.05NS	-0.51NS	0.66*					
K	-0.42NS	-0.42NS	0.82*	0.82*				
Mg	-0.25NS	-0.46NS	0.71*	0.93*	0.94*			
N	-0.28NS	-0.47NS	0.82*	0.89*	0.90*	0.93*		
P	-0.58NS	-0.33NS	0.59NS	0.73*	0.90*	0.86*	0.82*	
Zn	-0.15NS	-0.36NS	0.88*	0.83*	0.84*	0.85*	0.96*	0.67*

***** *= Significant at $p < 0.05$; NS = Not significant*

CHAPTER FIVE

5.0 DISCUSSION

5.1 Initial soil physico-chemical properties

The soil used for the experiment was sandy clay loam and had bulk density of 1.23 Mg m^{-3}. These soil physical properties were suitable to support growth of the cocoa seedlings (USDA, 1987; Lerner, 2000).

Regarding the chemical properties, the recorded pH of 7.04 was neutral (Jones Jr., 2001; Kumar *et al.,* 2014) and within optimum range of 5.6 - 7.2 reported to be suitable for cocoa (Ahenkorah, 1981; Opeke, 1987). The organic carbon content in the soil was 1.94 % which was slightly lower than the recommended level of 2.03 % necessary for cocoa cultivation as reported by Ahenkorah (1981). Total N in the soil (0.21 %) was higher than the critical level of 0.09 % (Ahenkorah, 1981; Opoku-Ameyaw *et al.,* 2010) required for cocoa cultivation. The available P content in the soil was 8.01 mg kg^{-1} which was below the threshold level of 20 mg kg^{-1} (Ahenkorah, 1981; Opoku-Ameyaw *et al.,* 2010) considered adequate for optimum cocoa production. The levels of exchangeable K, Mg, and Ca were 0.32 $cmol_c$ kg^{-1}, 2.41 $cmol_c$ kg^{-1}, and 10.20 $cmol_c$ kg^{-1} respectively, which were above the established critical limits of 0.25 $cmol_c$ kg^{-1} for K, 1.33 cmolc kg^{-1} for Mg, and 7.50 $cmol_c$ kg^{-1} for Ca required for cocoa cultivation (Ahenkorah, 1981; Opoku-Ameyaw *et al.,* 2010). The concentrations of available Fe and Zn in the soil were 11.05 mg kg^{-1} and 10.95 mg kg^{-1} respectively, which were higher than the threshold level of 1.33 mg kg^{-1} each for Fe and Zn necessary for cocoa in Ghana, as recommended by Ahenkorah (1981).

The nutrient sufficiency observed in the topsoil from the uncultivated land may partly be attributed to high litter accumulation on the soil surface over the years, which might have undergone mineralization to release essential nutrient.

5.2 Effect of foliar fertilization and fertigation on soil chemical properties

At the end of the study, there were some variations in the levels of the studied soil chemical properties following the application of fertilizers.

The initial soil pH (7.04) reduced to 5.99, 5.44 and 5.09 under the ammonium sulphate fertilizer treatments. These pH values were significantly lower than those recorded under the other treatments and control. This could probably be attributed to the acidifying effect of the ammonium sulphate due to nitrification process which generated hydrogen ions (H^+) and increased acidification. It is reported that, inputs of N fertilizer to soil result in increased acidification of the soil through oxidation of ammonium ions to nitrate ions, which generates hydrogen ions in the process and lowers the soil pH (Tang *et al.,* 2002; Garvin and Carver, 2003). This also agrees with the findings of Bouman *et al.* (1995) and Arthur *et al.* (2019) that the application of NPK fertilizer containing ammonium sulphate as N source decreases soil pH. Furthermore, soil pH significantly decreased with increasing N rate of the ammonium sulphate from 42 mg to 126 mg, suggesting that higher doses of ammonium sulphate fertilizer increased the rate of nitrification of NH_4^+ to release more H^+ ions to the soil. Also, soil pH had significant negative correlation with N rate of fertigated ammonium sulphate, indicating that soil pH decreases as N rate of ammonium sulphate fertilizer increases. This corroborates the studies by He *et al.* (1999) and Fageria *et al.* (2010) who reported that soil pH decreases with increasing rates of ammonium sulphate fertilizer.

There was significant improvement in the soil available P content following foliar application of NPK 10:10:10 at concentrations of 0.84 % (v/v) and 1.26 % (v/v), by supplying 84 mg and 126 mg of P respectively. They increased the available P content by more than 50 % above the baseline value. This could be attributed to drippings from the foliar sprays to the soil which might have optimized soil conditions, enhanced mineralization and solubilization, and ultimately improved the available P content. This gives foliar fertilization a potential advantage in improving soil fertility status. This assertion is justified by the fact that the concentrations of available P in soil under those two levels of NPK 10:10:10 foliar application were significantly higher than the rest of the treatments, including control. Shashikumar *et al.* (2013) and Jadhav (2017) also reported a significant increase in soil available P due to higher microbial activity in the rhizosphere and improved soil properties following foliar application of nutrients.

The exchangeable K contents in soils under the foliar fertilizer treatments were significantly higher than that under the control. This could partly be due to the elemental K concentrated in those fertilizers, which might have been solubilized in the soil through drippings, since all the foliar fertilizers applied were potassium-containing. The highest rate of calcium nitrate applied through fertigation increased exchangeable Ca level in the soil, which was significantly higher than those in the remaining treatments, including control. This increment might have originated from the soluble calcium oxide (CaO) contained in the fertilizer formulation. The high calcium nitrate fertilizer rate supplied 152.28 mg of Ca to the soil. Significant positive correlation was observed between exchangeable Ca in soil and application rate of calcium nitrate, suggesting that Ca content in soil increases with increasing application rate of calcium nitrate fertilizer. Ahn (1979) and

Famuwagun and Oladitan (2016) reported that fertilizer material containing reasonable amount of Ca increases the elemental Ca content of soils following soil amendment with such material.

5.3 Effect of foliar fertilization and fertigation on growth of cocoa seedlings

The highest fertigated ammonium sulphate rate had significantly smaller stem diameter and shorter plants than the lowest rate at the second, fourth and sixth months after emergence. Also at the same months, the lowest foliar application rate of NPK 24:17:18 had significantly taller seedlings than the highest fertigation of ammonium sulphate and calcium nitrate fertilizers. At 6 MAE, stem diameter and total dry matter of seedlings obtained from the highest ammonium sulphate fertigated pot were significantly the lowest among the treatments. The highest ammonium sulphate and calcium nitrate fertigated pots had significantly shorter seedlings with lesser leaf area, compared with the rest of the treatments, and fewer number of leaves, compared with the foliar treatments and control at 6 MAE.

The negative impact of the highest rate of the ammonium sulphate fertigation on seedling growth may be attributed to the reduction of soil pH below the optimum range suitable for plant growth. Shamshuddin (2022) reported that the long-term application of ammonium sulphate fertilizer can lower soil pH to level that affects the growth and production of crops. Significantly, total leaf area correlated negatively with N rate, and positively with soil pH under ammonium sulphate fertigation. Studies by Ofori-Frimpong *et al.* (2010), Famuwagun and Oladitan (2016) and Arthur *et al.* (2019) reported that reduction in soil pH resulting from the application of inorganic NPK fertilizer has depressive effects on cocoa seedling growth. The negative growth effect observed in the highest calcium nitrate rate also confirms the observation made by Puentes-Paramo *et al.* (2014) that high N doses can have inhibitory effects on cocoa seedlings.

\Generally, the distribution of dry matter was higher in the leaf mass than in the remaining plant parts, with more than 40 % of the total plant biomass being allocated to the leaf fraction. This suggests that more carbohydrates or photosynthates were allocated to the leaves than either the stems or the roots. This finding was similar to that of Arthur *et al.* (2019) who stated that the greater proportion of dry matter production is in the leaves, followed by the stems and then the roots. The fraction of biomass allocated to the leaf component under the highest rate of fertigated calcium nitrate was significantly lower, compared with the other treatments. Also, regarding the allocation of biomass to the roots, the highest rate of fertigated calcium nitrate had significantly more than the remaining treatments. This may be attributed to increased nitrate concentration in the rhizosphere, which might have enhanced the allocation of more assimilates to the below-ground biomass to improve root development, in order to exploit larger soil volumes for nutrients. Laine *et al.* (1995) and Scheible *et al.* (1997) reported that high concentrations of nitrate in the rooting zone stimulate local root growth of plants to enhance nutrient acquisition.

5.4 Effect of foliar fertilization and fertigation on nutrient uptake by cocoa seedlings

The uptake of N, Mg and Zn under the foliar fertilizer treatments were significantly higher than those under the highest rate of the fertigated ammonium sulphate, suggestion an enhanced absorption and utilization of those essential nutrients supplied by the foliar fertilizers.

Additionally, all the foliar fertilizer treatments and control produced seedlings with significantly higher P content than those produced under the highest rate of the fertigated ammonium sulphate. This suggests that the highest dose of the ammonium sulphate fertilizer which had significantly reduced soil pH might have reduced phosphorus availability, absorption and translocation, and inhibited biomass production. Altomare and Tringovska (2011) and Mahdi *et al.* (2012) noted that, under acidic soil conditions, inorganic P forms insoluble complexes with Al, Fe and Mn, resulting

in phosphorus fixation, thus rendering P unavailable for plants. Also, P uptake had significant negative correlation with N rate of fertigated ammonium sulphate, indicating that, as N rate of ammonium sulphate fertilizer increases, the uptake of P decreases. Gorissen *et al.* (1993) reported that, at the highest ammonium sulphate rate, soil chemical and biological status change, resulting in reduced uptake of essential nutrients, especially P.

Foliar application of NPK 10:10:10 at 0.84 % (v/v) and 1.26 % (v/v) concentrations under which soil available P was significantly improved, produced seedlings with significantly higher P uptake than the other treatments, including control, indicating varying responses to P from the treatments. This suggests that the cocoa seedlings might have responded well to P, which is the most limiting nutrient in the production of cocoa (Smith and Acquaye, 1963; Ahenkorah, 1981; Arthur *et al.,* 2017), and was low in the soil. This could also be attributed to increased availability of P to the cocoa seedlings, and subsequently, efficient absorption, translocation and utilization by the seedlings. Similar observations were made by Ravi *et al.* (2008), Yadav and Choudhary (2012), Shashikumar *et al.* (2013) and Jadhav (2017) who found increments in plant nutrient uptake superior to untreated control, following foliar application of nutrients. The uptake of K under NPK 10:10:10 moderate and high concentrations was significantly higher than the moderate and high ammonium sulphate and calcium nitrate rates, and control, suggesting that the K contained in the NPK 10:10:10 foliar fertilizer was efficiently utilized by the seedlings. Reickenberg and Pritts (1996) and Afrifa *et al.* (2009) observed that, drops from foliar sprays to the soil may be absorbed and utilized by plants through their root system.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. The application of ammonium sulphate fertilizer through fertigation caused a reduction in the soil pH. Fertigation with calcium nitrate fertilizer increased exchangeable Ca content of the soil. Foliar application of NPK 10:10:10 at moderate and high concentrations improved soil available P and exchangeable K contents. These indicate that fertilizer applications generally have some effects on soil fertility.

ii. The highest fertigation rates of ammonium sulphate and calcium nitrate fertilizers had some depressive effects on seedling growth. However, assimilates allocated to below-ground biomass were more under the highest rate of fertigated calcium nitrate.

iii. Foliar application of NPK 10:10:10 at moderate and high concentrations which improved soil available P level, produced seedlings with higher P content than the remaining treatments. This suggests that plants may normally respond to the most limiting nutrient applied.

6.2 Recommendations

Based on the findings from this study, the following recommendations are proposed:

1. Foliar fertilizer application on cocoa seedlings should be encouraged in the nursery production system, especially in soils with limiting or deficient nutrients, in order to improve nutrient availability and uptake. Also, fertigation with low rates of N-fertilizers should be used in the nurseries to enhance growth. This would eventually ensure quality and vigorous seedling production and their successful field establishment.

2. In future, similar research should be conducted on different soil types with contrasting properties to compare the effectiveness of these fertilizer application methods on soil fertility and performance of cocoa seedlings.
3. In future, a comparative assessment of foliar fertilizer application, fertigation and broadcasting methods should be undertaken in nursery and subsequently on field to evaluate their long-term effects on nutrient uptake, growth, and yield of cocoa.

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APPENDICES

Appendix 1. Reagents used for the analysis of soil and leaf samples

- **Organic carbon analysis**

***0.5 N* ferrous ammonium sulphate [$(NH_4)_2Fe(SO_4)_2.6H_2O$] solution in one litre:** 196.07 g of solid ammonium iron (II) sulphate was weighed and dissolved with distilled water into 1 litre volumetric flask. 15 ml of concentrated H_2SO_4 was added. The volume was made up to the1 litre mark with more distilled water and mixed well. The flask was labelled.

***1.0 N* potassium dichromate ($K_2Cr_2O_7$) solution in one litre:** 49.04 g of solid $K_2Cr_2O_7$ was weighed and dissolved with distilled water into 1 litre volumetric flask. The volume was made up to the 1 litre mark with more distilled water and mixed well. The flask was labelled.

Diphenylamine indicator [$(C_6H_5)_2NH$] for titration: 0.5 g of solid diphenylamine was weighed into a beaker. 20 ml of distilled water, followed by 100 ml of concentrated H_2SO_4 were added and mixed well. The prepared indicator was transferred into 200 ml volumetric flask, labelled and stored. Some quantity was poured into indicator bottle for use.

- **Total nitrogen analysis**

***0.02 N* sulphuric acid (H_2SO_4) solution in one litre:** 0.54 ml of concentrated H_2SO_4 was added to some distilled water in a 1 litre volumetric flask. The flask was well shaken by hand and allowed to cool under fume chamber. The volume was made to the 1 litre mark with distilled water and the flask labelled.

40 % sodium hydroxide (NaOH) solution in one litre: 400.0 g of solid NaOH was weighed into a 1 litre beaker and completely dissolved with about 800 ml distilled water. The solution was then

poured into a 1 litre volumetric flask and made to volume with distilled water. The flask was labelled.

2 % boric acid (H_3BO_3) solution in one litre: 20.0 g of solid H_3BO_3 was weighed into a 1 litre beaker and completely dissolved with about 800 ml distilled water. The solution was then poured into a 1 litre volumetric flask and made to volume with distilled water. The flask was labelled.

Indicator for titration: 1.0 g each of methyl blue and methyl red were dissolved in 50 ml of 95 % alcohol.

Catalyst: 1:5:25 g selenium (Se), copper sulphate ($CuSO_4$), potassium sulphate (K_2SO_4) ratio, prepared by grinding separately 4 g Se, 20 g $CuSO_4$, and 100 g K_2SO_4, and put together in a labelled catalyst container.

- **Available phosphorus and trace elements analysis**

Mehlich–3 extractant in one litre: 20.0 g of solid ammonium nitrate (NH_4NO_3) was weighed and dissolved into 1litre volumetric flask. 11.5 ml of glacial acetic acid (CH_3COOH) was measured into the flask. 840 µl of concentrated nitric acid (HNO_3) was measured into the flask. 40 ml of Mehlich–3 stock solution was measured into the flask. The content was made to volume with distilled water and the flask labelled.

Mehlich–3 stock solution in 100 ml: 1.389 g of solid ammonium fluoride (NH_4F) was weighed and dissolved into 100 ml volumetric flask. 0.7306 g of solid EDTA ($C_{10}H_{16}N_2O_8$) was weighed and dissolved into the 100 ml volumetric flask. The content was made to volume with dissolved water and the flask labelled.

Ascorbic acid ($C_6H_8O_6$) colouring reagent: 1.056 g of L-ascorbic acid was dissolved in 200 ml ‘reagent A’. The flask was shook by hand to mix the reagents well, and then labelled.

'Reagent A': 12.0 g of solid ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] was dissolved in about 250 ml distilled water. 0.2908 g of solid antimony potassium tartrate ($KSbO.C_4H_4O_6$) was also dissolved in about 100 ml distilled water. Both dissolved reagents were added to a litre of *5.0 N* H_2SO_4 (135.98 ml conc. H_2SO_4 / litre). The reagent was mixed thoroughly in 2 litres volumetric flask, and made to volume with distilled water. The prepared reagent was then stored in dark compartment in refrigerator.

- **Exchangeable bases analysis**

***1.0 M* ammonium acetate (NH_4OAc) solution in one litre:** 77.08 g of solid NH_4OAc was weighed into a 1 litre beaker and completely dissolved with about 800 ml distilled water. The solution was then poured into a 1 litre volumetric flask and made to volume with distilled water. The flask was labelled.

- **Particle size analysis**

20 % hydrogen peroxide (H_2O_2) solution in one litre: 200 ml of H_2O_2 was measured into a 1 litre volumetric flask and made to volume with distilled water. The flask was labelled.

5 % sodium hexametaphosphate $(NaPO_3)_6$ solution in one litre: 50.0 g of solid $(NaPO_3)_6$ was weighed into a 1 litre beaker and completely dissolved with about 800 ml distilled water. The solution was then poured into a 1 litre volumetric flask and made to volume with distilled water. The flask was labelled.

Appendix 2. Analysis of variance (ANOVA)

Soil ANOVA

Completely Randomized AOV for P

Source	DF	SS	MS	F	P
Trt	12	222.208	18.5173	38.55	0.0000
Error	26	12.490	0.4804		
Total	38	234.698			

Grand Mean 6.9828 CV 9.93

Observations per Mean	3
Standard Error of a Mean	0.4002
Std Error (Diff of 2 Means)	0.5659

Completely Randomized AOV for K

Source	DF	SS	MS	F	P
Trt	12	0.05881	4.901E-03	5.88	0.0001
Error	26	0.02167	8.333E-04		
Total	38	0.08048			

Grand Mean 0.2908 CV 9.93

Observations per Mean	3
Standard Error of a Mean	0.0167
Std Error (Diff of 2 Means)	0.0236

Completely Randomized AOV for C

Source	DF	SS	MS	F	P
Trt	12	0.04546	0.00379	0.26	0.9912
Error	26	0.38033	0.01463		
Total	38	0.42579			

Grand Mean 2.0905 CV 5.79

Observations per Mean	3
Standard Error of a Mean	0.0698
Std Error (Diff of 2 Means)	0.0988

Completely Randomized AOV for Ca

Source	DF	SS	MS	F	P
Trt	12	5.19736	0.43311	16.36	0.0000
Error	26	0.68813	0.02647		
Total	38	5.88550			

Grand Mean 9.8703 CV 1.65

Observations per Mean	3
Standard Error of a Mean	0.0939
Std Error (Diff of 2 Means)	0.1328

Completely Randomized AOV for Fe

Source	DF	SS	MS	F	P
Trt	12	13.3468	1.11223	0.36	0.9660
Error	26	79.9918	3.07661		
Total	38	93.3386			

Grand Mean 14.223 CV 12.33

Observations per Mean 3
Standard Error of a Mean 1.0127
Std Error (Diff of 2 Means) 1.4322

Completely Randomized AOV for Mg

Source	DF	SS	MS	F	P
Trt	12	0.34566	0.02880	2.22	0.0428
Error	26	0.33673	0.01295		
Total	38	0.68239			

Grand Mean 2.7128 CV 4.20

Observations per Mean 3
Standard Error of a Mean 0.0657
Std Error (Diff of 2 Means) 0.0929

Completely Randomized AOV for pH

Source	DF	SS	MS	F	P
Trt	12	9.64724	0.80394	68.91	0.0000
Error	26	0.30333	0.01167		
Total	38	9.95057			

Grand Mean 6.3251 CV 1.71

Observations per Mean 3
Standard Error of a Mean 0.0624
Std Error (Diff of 2 Means) 0.0882

Completely Randomized AOV for N

Source	DF	SS	MS	F	P
Trt	12	0.00241	2.009E-04	0.49	0.9024
Error	26	0.01067	4.103E-04		
Total	38	0.01308			

Grand Mean 0.2792 CV 7.25

Observations per Mean 3
Standard Error of a Mean 0.0117
Std Error (Diff of 2 Means) 0.0165

Completely Randomized AOV for Zn

Source	DF	SS	MS	F	P
Trt	12	13.6174	1.13478	0.59	0.8342

```
Error    26   50.4164   1.93909
Total    38   64.0338

Grand Mean 12.150    CV 11.46

Observations per Mean            3
Standard Error of a Mean    0.8040
Std Error (Diff of 2 Means) 1.1370
```

LSD All-Pairwise Comparisons Test of P by Trt

Trt	Mean	Homogeneous Groups
NPK10/N126	12.267	A
NPK10/N84	12.207	A
NPK10/N42	8.4100	B
NPK21/N84	6.5433	C
Control	6.0767	CD
NPK21/N126	6.0767	CD
NPK24/N126	6.0733	CD
NPK15.5/N1	5.9600	CD
NPK24/N42	5.8433	CD
NPK24/N84	5.7800	CD
NPK15.5/N8	5.7267	CDE
NPK15.5/N4	5.2000	DE
NPK21/N42	4.6133	E

```
Alpha              0.05     Standard Error for Comparison  0.5659
Critical T Value  2.056     Critical Value for Comparison  1.1633
There are 5 groups (A, B, etc.) in which the means
are not significantly different from one another.
```

LSD All-Pairwise Comparisons Test of K by Trt

Trt	Mean	Homogeneous Groups
NPK21/N126	0.3500	A
NPK10/N126	0.3433	AB
NPK10/N84	0.3267	ABC
NPK24/N84	0.3100	ABCD
NPK21/N84	0.3067	ABCD
NPK24/N42	0.2967	BCD
NPK24/N126	0.2933	CD
NPK15.5/N4	0.2900	CD
NPK10/N42	0.2833	CD
NPK15.5/N8	0.2767	D
NPK15.5/N1	0.2667	DE
NPK21/N42	0.2233	EF
Control	0.2133	F

```
Alpha              0.05     Standard Error for Comparison  0.0236
Critical T Value  2.056     Critical Value for Comparison  0.0484
There are 6 groups (A, B, etc.) in which the means
are not significantly different from one another.
```

LSD All-Pairwise Comparisons Test of C by Trt

Trt	Mean	Homogeneous Groups
NPK10/N84	2.1533	A
NPK24/N126	2.1433	A
NPK15.5/N1	2.1200	A
Control	2.1133	A
NPK10/N42	2.1067	A
NPK24/N84	2.0933	A
NPK21/N84	2.0867	A
NPK15.5/N8	2.0800	A
NPK21/N42	2.0733	A
NPK10/N126	2.0600	A
NPK15.5/N4	2.0600	A
NPK24/N42	2.0467	A
NPK21/N126	2.0400	A

Alpha 0.05 Standard Error for Comparison 0.0988
Critical T Value 2.056 Critical Value for Comparison 0.2030
There are no significant pairwise differences among the means.

LSD All-Pairwise Comparisons Test of Ca by Trt

Trt	Mean	Homogeneous Groups
NPK15.5/N1	10.693	A
NPK15.5/N8	10.360	B
NPK15.5/N4	10.267	B
NPK21/N126	10.113	BC
NPK21/N42	9.9700	CD
NPK21/N84	9.8067	DE
NPK24/N84	9.6767	EF
NPK10/N42	9.6467	EF
Control	9.5933	EF
NPK10/N126	9.5800	EF
NPK24/N126	9.5500	EF
NPK24/N42	9.5367	EF
NPK10/N84	9.5200	F

Alpha 0.05 Standard Error for Comparison 0.1328
Critical T Value 2.056 Critical Value for Comparison 0.2730
There are 6 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of Fe by Trt

Trt	Mean	Homogeneous Groups
NPK21/N126	15.490	A
NPK21/N84	15.137	A
NPK21/N42	14.640	A
NPK15.5/N1	14.527	A
NPK15.5/N4	14.240	A
NPK10/N126	14.227	A
NPK24/N42	14.120	A
NPK24/N84	14.060	A
NPK10/N84	13.957	A
NPK24/N126	13.850	A
NPK10/N42	13.767	A
NPK15.5/N8	13.623	A
Control	13.260	A

Alpha 0.05 Standard Error for Comparison 1.4322
Critical T Value 2.056 Critical Value for Comparison 2.9438
There are no significant pairwise differences among the means.

LSD All-Pairwise Comparisons Test of Mg by Trt

Trt	Mean	Homogeneous Groups
NPK21/N126	2.8533	A
NPK15.5/N8	2.8333	A
NPK15.5/N4	2.8200	AB
NPK15.5/N1	2.8067	AB
NPK21/N84	2.7567	ABC
NPK21/N42	2.7467	ABCD
Control	2.6900	ABCD
NPK10/N42	2.6900	ABCD
NPK24/N84	2.6800	ABCD
NPK24/N42	2.6300	BCD
NPK10/N126	2.6033	CD
NPK24/N126	2.5933	CD
NPK10/N84	2.5633	D

Alpha 0.05 Standard Error for Comparison 0.0929
Critical T Value 2.056 Critical Value for Comparison 0.1910
There are 4 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of pH by Trt

Trt	Mean	Homogeneous Groups
Control	6.8167	A
NPK24/N42	6.7400	AB
NPK24/N84	6.6900	AB
NPK10/N84	6.5833	BC
NPK24/N126	6.5700	BC
NPK10/N42	6.5600	BC
NPK10/N126	6.4900	CD
NPK15.5/N1	6.4733	CD
NPK15.5/N4	6.4200	CD
NPK15.5/N8	6.3667	D
NPK21/N42	5.9900	E
NPK21/N84	5.4367	F
NPK21/N126	5.0900	G

Alpha 0.05 Standard Error for Comparison 0.0882
Critical T Value 2.056 Critical Value for Comparison 0.1813
There are 7 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of N by Trt

Trt	Mean	Homogeneous Groups
NPK24/N126	0.2900	A
NPK10/N42	0.2867	A
NPK15.5/N1	0.2867	A
NPK15.5/N8	0.2867	A
NPK21/N126	0.2867	A
NPK21/N84	0.2833	A

Control	0.2767	A
NPK10/N84	0.2767	A
NPK15.5/N4	0.2767	A
NPK21/N42	0.2767	A
NPK10/N126	0.2700	A
NPK24/N84	0.2700	A
NPK24/N42	0.2633	A

Alpha 0.05 Standard Error for Comparison 0.0165
Critical T Value 2.056 Critical Value for Comparison 0.0340
There are no significant pairwise differences among the means.

LSD All-Pairwise Comparisons Test of Zn by Trt

Trt	Mean	Homogeneous Groups
NPK24/N42	13.327	A
Control	13.287	A
NPK21/N84	12.563	A
NPK10/N126	12.353	A
NPK10/N84	12.230	A
NPK10/N42	12.190	A
NPK21/N42	11.977	A
NPK24/N126	11.890	A
NPK21/N126	11.887	A
NPK15.5/N8	11.757	A
NPK24/N84	11.637	A
NPK15.5/N4	11.500	A
NPK15.5/N1	11.353	A

Alpha 0.05 Standard Error for Comparison 1.1370
Critical T Value 2.056 Critical Value for Comparison 2.3371
There are no significant pairwise differences among the means.

6 MAE Growth ANOVA

Completely Randomized AOV for TDM6

Source	DF	SS	MS	F	P
Trt	12	46.2964	3.85803	6.50	0.0000
Error	26	15.4391	0.59381		
Total	38	61.7355			

Grand Mean 9.1503 CV 8.42

Observations per Mean 3
Standard Error of a Mean 0.4449
Std Error (Diff of 2 Means) 0.6292

Completely Randomized AOV for TLA6

Source	DF	SS	MS	F	P
Trt	12	2383648	198637	13.91	0.0000
Error	26	371174	14276		
Total	38	2754822			

Grand Mean 1776.3 CV 6.73

Observations per Mean 3
Standard Error of a Mean 68.983
Std Error (Diff of 2 Means) 97.557

Completely Randomized AOV for SD6

Source	DF	SS	MS	F	P
Trt	12	4.94956	0.41246	43.78	0.0000
Error	26	0.24493	0.00942		
Total	38	5.19449			

Grand Mean 7.3377 CV 1.32

Observations per Mean 3
Standard Error of a Mean 0.0560
Std Error (Diff of 2 Means) 0.0792

Completely Randomized AOV for NL6

Source	DF	SS	MS	F	P
Trt	12	101.530	8.46082	9.35	0.0000
Error	26	23.519	0.90459		
Total	38	125.049			

Grand Mean 24.564 CV 3.87

Observations per Mean 3
Standard Error of a Mean 0.5491
Std Error (Diff of 2 Means) 0.7766

Completely Randomized AOV for PH6

Source	DF	SS	MS	F	P
Trt	12	846.223	70.5186	44.54	0.0000
Error	26	41.167	1.5834		
Total	38	887.391			

Grand Mean 58.889 CV 2.14

Observations per Mean 3
Standard Error of a Mean 0.7265
Std Error (Diff of 2 Means) 1.0274

LSD All-Pairwise Comparisons Test of TDM6 by Trt

Trt	Mean	Homogeneous Groups
NPK21/N42	11.830	A
NPK24/N84	9.9967	B
NPK24/N126	9.7067	BC
NPK15.5/N8	9.5367	BC
NPK10/N126	9.2800	BC
NPK15.5/N4	9.2733	BC
NPK24/N42	9.1467	BC
NPK10/N84	9.1367	BC
NPK15.5/N1	8.9467	BCD
NPK10/N42	8.7567	BCD

```
Control     8.5467    CD
NPK21/N84   7.8133     DE
NPK21/N126  6.9833      E

Alpha              0.05     Standard Error for Comparison  0.6292
Critical T Value  2.056     Critical Value for Comparison  1.2933
There are 5 groups (A, B, etc.) in which the means
are not significantly different from one another.
```

LSD All-Pairwise Comparisons Test of TLA6 by Trt

Trt	Mean	Homogeneous Groups
NPK21/N42	2030.8	A
NPK24/N84	2016.4	A
NPK10/N84	1996.9	A
NPK24/N126	1947.5	A
NPK10/N42	1943.7	A
NPK10/N126	1903.6	AB
NPK24/N42	1868.8	AB
NPK15.5/N4	1863.1	AB
NPK15.5/N8	1734.6	BC
Control	1659.3	CD
NPK21/N84	1531.6	D
NPK15.5/N1	1314.9	E
NPK21/N126	1280.6	E

```
Alpha              0.05     Standard Error for Comparison  97.557
Critical T Value  2.056     Critical Value for Comparison  200.53
There are 5 groups (A, B, etc.) in which the means
are not significantly different from one another.
```

LSD All-Pairwise Comparisons Test of SD6 by Trt

Trt	Mean	Homogeneous Groups
NPK21/N42	7.9567	A
NPK15.5/N1	7.6633	B
NPK15.5/N4	7.6533	B
NPK24/N126	7.5167	BC
NPK24/N84	7.5133	BC
NPK24/N42	7.4100	CD
NPK10/N42	7.3367	D
NPK15.5/N8	7.3033	D
NPK10/N126	7.2933	DE
NPK10/N84	7.2900	DE
Control	7.1333	E
NPK21/N84	6.7767	F
NPK21/N126	6.5433	G

```
Alpha              0.05     Standard Error for Comparison  0.0792
Critical T Value  2.056     Critical Value for Comparison  0.1629
There are 7 groups (A, B, etc.) in which the means
are not significantly different from one another.
```

LSD All-Pairwise Comparisons Test of NL6 by Trt

Trt	Mean	Homogeneous Groups
NPK10/N126	26.720	A

NPK10/N42	26.000	AB
NPK10/N84	26.000	AB
NPK24/N126	25.610	AB
NPK15.5/N4	25.557	AB
NPK21/N42	25.557	AB
NPK24/N84	25.333	ABC
NPK24/N42	24.443	BC
NPK15.5/N8	24.440	BC
Control	23.887	C
NPK21/N126	22.113	D
NPK15.5/N1	21.890	D
NPK21/N84	21.777	D

Alpha	0.05	Standard Error for Comparison	0.7766
Critical T Value	2.056	Critical Value for Comparison	1.5963

There are 4 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of PH6 by Trt

Trt	Mean	Homogeneous Groups
NPK24/N42	64.140	A
NPK24/N126	64.043	A
NPK21/N42	63.943	A
NPK10/N42	62.243	AB
NPK10/N126	61.777	B
NPK24/N84	59.510	C
NPK15.5/N8	59.190	C
NPK10/N84	58.767	CD
Control	58.610	CD
NPK21/N84	57.623	CD
NPK15.5/N4	56.677	D
NPK15.5/N1	49.677	E
NPK21/N126	49.357	E

Alpha	0.05	Standard Error for Comparison	1.0274
Critical T Value	2.056	Critical Value for Comparison	2.1119

There are 5 groups (A, B, etc.) in which the means
are not significantly different from one another.

Biomass allocation ANOVA

Completely Randomized AOV for Rootpart

Source	DF	SS	MS	F	P
Trt	12	58.138	4.84480	2.72	0.0159
Error	26	46.313	1.78126		
Total	38	104.450			

Grand Mean 14.662 CV 9.10

Observations per Mean	3
Standard Error of a Mean	0.7706
Std Error (Diff of 2 Means)	1.0897

Completely Randomized AOV for Leafpart

Source	DF	SS	MS	F	P
Trt	12	347.935	28.9946	4.26	0.0010
Error	26	176.994	6.8075		
Total	38	524.929			

Grand Mean 51.352 CV 5.08

Observations per Mean 3
Standard Error of a Mean 1.5064
Std Error (Diff of 2 Means) 2.1303

Completely Randomized AOV for Stempart

Source	DF	SS	MS	F	P
Trt	12	140.146	11.6788	5.61	0.0001
Error	26	54.149	2.0827		
Total	38	194.295			

Grand Mean 33.987 CV 4.25

Observations per Mean 3
Standard Error of a Mean 0.8332
Std Error (Diff of 2 Means) 1.1783

LSD All-Pairwise Comparisons Test of Rootpart by Trt

Trt	Mean	Homogeneous Groups
NPK15.5/N1	17.940	A
NPK15.5/N8	15.650	B
NPK21/N126	15.430	B
NPK21/N42	15.353	B
NPK15.5/N4	14.520	BC
NPK10/N126	14.510	BC
NPK10/N42	14.483	BC
NPK21/N84	14.380	BC
NPK24/N126	14.220	BC
NPK24/N42	14.120	BC
Control	13.827	BC
NPK24/N84	13.447	BC
NPK10/N84	12.720	C

Alpha 0.05 Standard Error for Comparison 1.0897
Critical T Value 2.056 Critical Value for Comparison 2.2400
There are 3 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of Leafpart by Trt

Trt	Mean	Homogeneous Groups
NPK10/N84	55.560	A
NPK10/N42	54.677	AB
NPK24/N84	54.150	ABC
NPK10/N126	52.410	ABCD

NPK24/N42	52.410	ABCD
NPK24/N126	52.133	ABCD
NPK15.5/N4	52.090	ABCD
Control	51.700	ABCD
NPK21/N84	50.697	BCD
NPK15.5/N8	49.820	CD
NPK21/N126	49.400	D
NPK21/N42	49.217	D
NPK15.5/N1	43.307	E

Alpha 0.05 Standard Error for Comparison 2.1303
Critical T Value 2.056 Critical Value for Comparison 4.3790
There are 5 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of Stempart by Trt

Trt	Mean	Homogeneous Groups
NPK15.5/N1	38.753	A
NPK21/N42	35.430	B
NPK21/N126	35.170	B
NPK21/N84	34.923	B
NPK15.5/N8	34.533	BC
Control	34.470	BC
NPK24/N126	33.647	BCD
NPK24/N42	33.470	BCD
NPK15.5/N4	33.390	BCD
NPK10/N126	33.080	BCDE
NPK24/N84	32.403	CDE
NPK10/N84	31.717	DE
NPK10/N42	30.843	E

Alpha 0.05 Standard Error for Comparison 1.1783
Critical T Value 2.056 Critical Value for Comparison 2.4221
There are 5 groups (A, B, etc.) in which the means
are not significantly different from one another.

Nutrient uptake ANOVA

Completely Randomized AOV for Feuptake

Source	DF	SS	MS	F	P
Trt	12	216808	18067.3	4.13	0.0012
Error	26	113726	4374.1		
Total	38	330534			

Grand Mean 352.15 CV 18.78

Observations per Mean 3
Standard Error of a Mean 38.184
Std Error (Diff of 2 Means) 54.000

Completely Randomized AOV for Znuptake

Source	DF	SS	MS	F	P
Trt	12	38572.4	3214.37	3.20	0.0063

Error	26	26128.2	1004.93
Total	38	64700.6	

Grand Mean 161.66 CV 19.61

Observations per Mean 3
Standard Error of a Mean 18.302
Std Error (Diff of 2 Means) 25.883

Completely Randomized AOV for Cauptake

Source	DF	SS	MS	F	P
Trt	12	4115.40	342.950	3.71	0.0025
Error	26	2405.12	92.505		
Total	38	6520.52			

Grand Mean 86.667 CV 11.10

Observations per Mean 3
Standard Error of a Mean 5.5529
Std Error (Diff of 2 Means) 7.8530

Completely Randomized AOV for Kuptake

Source	DF	SS	MS	F	P
Trt	12	6615.37	551.281	8.20	0.0000
Error	26	1748.47	67.249		
Total	38	8363.84			

Grand Mean 80.423 CV 10.20

Observations per Mean 3
Standard Error of a Mean 4.7346
Std Error (Diff of 2 Means) 6.6957

Completely Randomized AOV for Mguptake

Source	DF	SS	MS	F	P
Trt	12	487.663	40.6386	3.56	0.0032
Error	26	296.620	11.4085		
Total	38	784.283			

Grand Mean 20.868 CV 16.19

Observations per Mean 3
Standard Error of a Mean 1.9501
Std Error (Diff of 2 Means) 2.7578

Completely Randomized AOV for Nuptake

Source	DF	SS	MS	F	P
Trt	12	8105.4	675.451	4.00	0.0015
Error	26	4385.2	168.660		
Total	38	12490.6			

Grand Mean 122.48 CV 10.60

Observations per Mean 3
Standard Error of a Mean 7.4980
Std Error (Diff of 2 Means) 10.604

Completely Randomized AOV for Puptake

Source	DF	SS	MS	F	P
Trt	12	147.569	12.2974	26.60	0.0000
Error	26	12.019	0.4623		
Total	38	159.587			

Grand Mean 6.6532 CV 10.22

Observations per Mean 3
Standard Error of a Mean 0.3925
Std Error (Diff of 2 Means) 0.5551

LSD All-Pairwise Comparisons Test of Feuptake by Trt

Trt	Mean	Homogeneous Groups
T7	489.34	A
T3	448.93	AB
T2	416.10	AB
T5	398.74	ABC
T6	385.05	ABCD
T11	373.19	BCDE
T1	364.61	BCDEF
T13	352.76	BCDEF
T12	300.03	CDEFG
T10	286.37	DEFG
T4	264.73	EFG
T8	258.50	FG
T9	239.53	G

Alpha 0.05 Standard Error for Comparison 54.000
Critical T Value 2.056 Critical Value for Comparison 111.00
There are 7 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of Znuptake by Trt

Trt	Mean	Homogeneous Groups
T7	227.46	A
T2	191.22	AB
T3	181.12	ABC
T5	177.72	ABC
T1	173.00	BC
T8	170.04	BC
T6	167.47	BC
T4	161.88	BC
T10	154.73	BC
T11	137.66	CD
T13	134.47	CD
T12	129.41	CD
T9	95.466	D

Alpha 0.05 Standard Error for Comparison 25.883
Critical T Value 2.056 Critical Value for Comparison 53.204
There are 4 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of Cauptake by Trt

Trt	Mean	Homogeneous Groups
T7	102.94	A
T10	101.22	A
T3	98.413	AB
T11	95.402	ABC
T6	91.912	ABCD
T2	90.336	ABCD
T1	82.759	BCDE
T4	82.103	CDE
T12	81.982	CDE
T8	77.819	DE
T13	77.238	DE
T9	72.420	E
T5	72.122	E

Alpha 0.05 Standard Error for Comparison 7.8530
Critical T Value 2.056 Critical Value for Comparison 16.142
There are 5 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of Kuptake by Trt

Trt	Mean	Homogeneous Groups
T5	104.01	A
T3	95.141	AB
T6	93.524	AB
T2	89.868	BC
T4	88.888	BCD
T7	83.935	BCDE
T13	79.088	CDEF
T10	75.973	DEFG
T1	74.957	EFG
T11	71.559	EFGH
T9	66.314	FGH
T8	63.704	GH
T12	58.542	H

Alpha 0.05 Standard Error for Comparison 6.6957
Critical T Value 2.056 Critical Value for Comparison 13.763
There are 8 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of Mguptake by Trt

Trt	Mean	Homogeneous Groups
T7	26.253	A
T2	26.012	AB
T1	23.527	ABC
T3	22.437	ABCD
T11	21.976	ABCD

T5	21.915	ABCD
T10	21.713	ABCD
T4	21.146	ABCDE
T6	20.413	BCDE
T13	19.358	CDEF
T12	17.190	DEF
T8	15.592	EF
T9	13.755	F

Alpha 0.05 Standard Error for Comparison 2.7578
Critical T Value 2.056 Critical Value for Comparison 5.6688
There are 6 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of Nuptake by Trt

Trt	Mean	Homogeneous Groups
T7	143.58	A
T3	139.28	AB
T5	133.33	ABC
T6	131.32	ABC
T10	129.30	ABCD
T11	128.73	ABCD
T2	127.19	ABCD
T4	125.56	ABCD
T1	119.95	BCDE
T13	111.57	CDEF
T8	108.29	DEF
T12	101.62	EF
T9	92.491	F

Alpha 0.05 Standard Error for Comparison 10.604
Critical T Value 2.056 Critical Value for Comparison 21.796
There are 6 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pairwise Comparisons Test of Puptake by Trt

Trt	Mean	Homogeneous Groups
T5	9.6927	A
T6	9.6307	A
T4	8.1187	B
T2	7.8323	BC
T3	7.6580	BC
T7	7.1400	BCD
T1	6.8963	CDE
T13	6.2963	DE
T11	5.9273	E
T10	5.8600	E
T8	4.5187	F
T12	3.5290	F
T9	3.3910	F

Alpha 0.05 Standard Error for Comparison 0.5551
Critical T Value 2.056 Critical Value for Comparison 1.1411
There are 6 groups (A, B, etc.) in which the means
are not significantly different from one another.