KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES.



# RESPONSE OF CACAO (*Theobroma cacao*) SEEDLINGS TO DIFFERENT SOIL AMENDMENT RATIOS AND WATERING REGIMES.

BY

ERIC KUBI-TETTEH

JUNE, 2015

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ERIC KUBI-TETTEH



THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, FACULTY OF AGRICULTURE OF THE COLLEGE OF AGRICULTURE AND NATURAL RESOURCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN PLANT PHYSIOLOGY

JUNE, 2015

**DECLARATION**

I, Eric Kubi-Tetteh, do hereby declare that this thesis is original and is my own composition under supervision, and that it has neither been produced wholly nor partially for the award of any degree in this university or elsewhere.

Materials from other authors which have served as source of relevant information have been duly acknowledged.



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**DEDICATION**

This thesis is dedicated to the Almighty God for His guidance and protection throughout my study, my dad Mr. Kubi, mum Miss Dora Tekper and my entire family.

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**ABSTRACT**

A study was conducted in a gauze house of the Physiology Division at the Cocoa Research Institute of Ghana (CRIG), New Tafo-Akim, from August 2013 to February 2014 to determine the response of cacao (*Theobroma cacao*) seedlings to different soil amendment ratios and watering regimes.

Sawdust obtained from the plant species Emeri (*Terminalia ivorensis)* was used as the soil amendment. This was mixed with topsoil. Three different sawdust mixing ratios and a control (no sawdust) formed the first treatment (M). The field capacity (F.C) of the topsoil to be used was determined. The field capacity determined, was halved (0.5 F.C) and quartered (0.25 F.C) to obtain the second treatment (W). Reduced water supply (0.25 F.C) significantly affected stomatal conductance, transpiration rate and plant leaf area. This affected the photosynthetic activity of plants especially for the control (no sawdust). The addition of sawdust to the soil helped retain soil moisture and this increased plant growth in those treatments compared to the control (no sawdust). Differences observed in vegetative and physiological parameters measured due to varying watering regimes and the addition of sawdust eventually affected the final total dry matter produced and partitioned. The results suggest that sawdust could be used as a soil amendment during cacao establishment.

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# CHAPTER ONE

## 1.0 INTRODUCTION

*Theobroma cacao* also called the cocoa tree, is a small (4–8 m tall) evergreen tree in the family Malvaceae (EOL, 2012), native to the deep tropical regions of Central and South America. Cacao contains important nutrient elements and several minerals including calcium, copper, magnesium, phosphorus, potassium, sodium and zinc (Bearden *et al*., 2011). Its seeds are used to make cocoa powder and chocolate (Mehta, 2013). Ghana currently produces hebhrehy5raegrabout 632,037 m/t of cacao which represents 14.9% of the world's total production (FAOSTAT, 2012).

Climate changes have greater influence on most processes involved in cacao production. Cacao is highly sensitive to changes in climate- from hours of sun, to rainfall and application of water, soil conditions and particularly to temperature due to effects on evapotranspiration. It is predicted that climate change will remain one of the major drivers of biodiversity patterns in the future (Armesto *et al*., 2000). Uncertain rainfall patterns and soil water deficits are likely to affect most cacao growing regions in Ghana and other West African cacao producing countries which would result in lower pod output. Cacao is highly susceptible to drought and the pattern of cropping of cacao is related to rainfall distribution. Significant correlations between cacao yield and rainfall over varying intervals prior to harvest have been reported. It was found that in Ghana a year with high rainfall is followed by a year with a large crop, though the correlation was not applicable in all years (Skidmore, 1929; Brew, 1991).

Values defining the limits or adequate soil moisture capacities or available moisture contents for cacao cultivation during the dry season in Ghana were found to be variable and under field

conditions depend on many factors such as: shade, air movement, soil texture and structure, age and vigour of the cacao, volume and distribution of active roots and root depth. In considering the suitability of a soil for cacao in relation to soil moisture, it is not the quantity of available soil moisture *per se* which is important; it is rather the rate of release of the available water from the soil to the tree which matters (Wessel, 1971; Ahenkorah, 1981).

The annual total rainfall in the cacao growing regions of Ghana is less than 2000mm. The rainfall distribution pattern is bi-modal from April to July and September to November. There is a short dry period from July to August during which the relative humidity is still high. There is a main dry season from November to February-March. The four to six months of dry weather results in soil water deficit and since irrigation is not part of the farming system, cacao seedling mortality is high during the establishment phase. In bearing plants, the existence of the short dry season during main crop pod filling can affect bean size if it is sufficiently severe. In adult plantings, water deficits result in lower yields and an increase in the level of mirid (capsid) damage. The use of soil amendments in crop cultivation is seen by scientists as a way of improving soil water amounts by having the ability to retain water for plant root absorption.

Soil amendments are materials which are worked into the soil to enhance the soil's properties such as water retention, permeability, aeration etc (Glossary of Soil Science Terms, 2012). Soil compaction is reduced by the addition of soil amendments which add more loft to keep the soil loose. Many soil amendments aside their water retention ability, add nutrients such as nitrogen, phosphorus etc to enrich the soil and allow plants to grow bigger and stronger. HuiLan *et al*., (1998) noted that the application of organic amendments increased water stress resistance of sweet corn leaves. In particular, stomatal and cuticular conductances of the leaves were lower in

these plants than in inorganically-fertilized plants. Researchers have observed that composted organic amendments can improve plant health beyond the nitrogen fertility value (Ayuso *et al.,* 1996; Vadrighi *et al.,* 1996; Buckerfield *et al.,* 1999; Atiyeh *et al.,* 2000a and b; Atiyeh *et al.,* 2001; Atiyeh *et al.,* 2002). Of particular interest is the apparent ameliorating effect of organic amendments on drought-stressed crops. One of the factors affecting cacao production in Ghana is uncertain rainfall patterns and inadequate soil water during the dry season and the issue of high temperatures which result in poor plant growth and lower pod output. The ability of a soil amendment in retaining soil water for plant use will help overcome the issue of crops being stressed during uncertain weather conditions and the minor or dry season.

To address the question of whether sawdust as a soil amendment would be able to retain soil moisture for plant use, this study assessed the response of cacao seedlings to different soil amendment ratios and watering regimes.

The main objective of the study was to investigate the response of cacao under varying soil moisture levels in relation to different soil amendment ratios.

Specific objectives were

i. To determine the effect of sawdust as a soil amendment on seedling growth and performance.
ii. To determine the effect of water and soil amendment interactions on seedling performance.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 TAXONOMY AND ORIGIN OF CACAO

Cacao (*Theobroma cacao)* belongs to the genus Theobroma classified under the subfamily Sterculioidea of the mallow family Malvaceae. Cacao is one of 22 species of *Theobroma*. The generic name is derived from Greek words which mean "food of the gods" (Young, 1994). *Theobroma grandiflorum* is a closely related species found in Colombia, Peru, Bolivia and Brazil. Like cacao, it is also the source for a kind of chocolate known as *cupulate* or cupuaçu chocolate (http://www.fruitipedia.com/cupuacu_theobroma_grandiflorum.html). *T. cacao* is widely distributed from southeastern Mexico to the Amazon basin. There were originally two hypotheses about its domestication.

Brown *et al.,* (2008) conducted a study which identified areas, for example around Iquitos in modern Peru, where representatives of several genetic clusters originated. The result suggested that this is where *T. cacao* was originally domesticated, probably for the pulp that surrounds the beans, which is eaten as a snack and fermented into a mildly alcoholic beverage (Alves Pereira, 2010). Using the DNA sequences obtained by Motomayor *et al.* (2008) and comparing them with data derived from climate models and the known conditions suitable for cacao, Thomas *et al.* (2008) have further refined the view of domestication, linking the area of greatest cacao genetic diversity to a bean-shaped area that encompasses the border between Brazil and Peru and the southern part of the Colombian-Brazilian border (Thomas *et al.*, 2012*).*

## 2.2 BOTANY OF CACAO

Cacao tree grows about 4-8m tall. Leaves are alternate, entire, unlobed, 10–40 cm long and 5–20 cm broad. The flowers are produced in clusters directly on the trunk and older branches; this is known as cauliflory. The flowers are small, 1–2 cm diameter, with pink calyx. While many of the world's flowers of many crops are pollinated by bees (Hymenoptera) or butterflies/moths (Lepidoptera), cacao flowers are pollinated by tiny flies, *Forcipomyia* midges in the order Diptera (Hernández,1965). The fruit, called a cacao pod, is ovoid, 15–30 cm long and 8–10 cm wide, ripening yellow to orange, and weighs about 500 g when ripe. The pod contains 20 to 60 seeds, usually called "beans", embedded in a white pulp (BeMiller, 1993).

## 2.3 IMPORTANCE AND NUTRITIONAL COMPOSITION OF CACAO

Cacao has many important uses. Its seeds are used to make cocoa powder and chocolate. In general chocolate and cacao is considered to be a rich source of antioxidants such as procyanidins and flavanoids, which may impart anti-aging properties (Mehta, 2013). Cacao beans contain up to 10% of phenols and flavenoids which are antioxidants potentially inhibiting cancer or cardiovascular diseases (Taubert *et al.*, 2007 and Schroeter *et al.*, 2006), as well as sodium, phosphorus, zinc, potassium, magnesium, calcium and iron. All of these minerals are found in greater quantities in cacao powder than either cacao butter or cacao liquor (Bearden *et al.*, 2003). Additionally, they contain 1-3% theobromine and caffeine, alkaloids that stimulate the central nervous system (Mehta, 2013).

It is believed that the improved blood flow after consumption of flavanol-rich cacao may help to achieve health benefits in hearts and other organs. In particular, the benefits may extend to the

brain and have important implications for learning and memory (Bayard *et al.*, 2007). The pulp is used in some countries to prepare a refreshing juice. Each seed contains a significant amount of fat (40–50%) as cacao butter. Their most noted active constituent is theobromine, a compound similar to caffeine. Cacao beans possess 45-53.2% fat in the form of cacao butter (also known as theobroma oil) which is made up of a variety of fatty acids. The ingredients for chocolate – cacao powder and cacao butter (solids) – are prepared from fermented and roasted cacao seeds. The distinctive flavour of chocolate develops during the fermentation process (Dimick and Lopez, 1995). Chocolate is more than just a delicacy; evidence suggests that eating between 46 and 105g chocolate a day can have a moderate effect on lowering blood pressure. Cacao has been used for an array of medicinal purposes. Unfermented cacao seeds and the seed coat are used to treat a variety of ailments, including diabetes, digestive and chest complaints. Cacao powder, prepared from fermented cacao beans, is used to prevent heart disease (Corti *et al.*, 2009).

It is also used widely in foods and pharmaceutical preparations, as well as being used as a rich moisturizer for the skin. The crushed shells of cacao beans are used as an alternative to peat mulch. Mulches are layered on to the soil surface to suppress weeds, conserve moisture, improve its visual appearance and minimize erosion.

## 2.4 SOIL AND CLIMATIC REQUIREMENTS

According to International Cocoa Organization (ICCO) in 2013 reported that, cacao plants respond well to relatively high temperatures, with a maximum annual average of 30 - 32ºC and a minimum average of 18 - 21ºC. If the absolute minimum temperature falls below 10°C for

several consecutive nights, the yield is likely to be reduced. Defoliation and dieback occurs between 4-8°C.

Although cacao will grow above 32°C, the upper temperature limit is not well defined and shade cover will influence maximum temperatures for the cacao. High temperatures may affect bean characteristics and yield. Variations in the yield of cacao trees from year to year are affected more by rainfall than by any other climatic factor. Trees are very sensitive to a soil water deficiency. Rainfall should be plentiful and well distributed through the year. An annual rainfall level of between 1,500 mm and 2,000 mm is generally preferred. Dry spells, where rainfall is less than 100 mm per month, should not exceed three months. Annual rainfall greater than 2500 mm may result in a higher incidence of fungal diseases. The cacao tree will make optimum use of any light available and traditionally has been grown under shade. Its natural environment is the Amazonian forest which provides natural shade trees. Shading is indispensable in a cacao tree's early years.

Cacao needs a soil containing coarse particles and with a reasonable quantity of nutrients, to a depth of 1.5 m to allow the development of a good root system. The cacao tree is sensitive to a lack of water, so the soil must have both water retention properties and good drainage. The chemical properties of the topsoil are most important, as the plant has a large number of roots for absorbing nutrients. Cacao can grow in soils with a pH in the range of 5.6-7.2. It can therefore cope with both acid and alkaline soil, but excessive acidity (pH 4.0 and below) or alkalinity (pH 8.0 and above) must be avoided. Cacao is tolerant of acid soils, provided the nutrient content is high enough. The soil should also have a high content of organic matter: 3.5% in the top 15 centimetres of soil. Soils for cacao must have certain anionic and cationic balances.

Exchangeable bases in the soil should amount to at least 35% of the total cation exchange capacity (CEC), otherwise nutritional problems are likely. The optimum total nitrogen / total phosphorus ratio should be around 1.5. The best soils in terms of high cacao production tend to have an average pH 5.6-7.2 in 1:2.5 water: soil, C/N ratio between 10-12, organic carbon not less than 3%, base exchange capacity of 3-15 me/100 g soil available P greater than 20 ppm in the 0-5 cm and 15 ppm in 0-20 cm layer (using buffered 0.002N $H_2SO_4$ extractant), exchangeable K not less than 0.25 /100 g soil, (Ca + Mg) about 8-13 me/100 g soil and no aluminum in the exchange complex (Ahenkorah *et al.,* 1982).



## 2.5 FIELD CAPACITY AND ITS RELATION WITH THE SOIL

"Field capacity is the amount of soil moisture or water content that remains in the soil after excess water has drained and the rate of downward movement has decreased" (Israelson and West, 1922).

Veihmeyer and Hendrickson (1949) suggested that the above method of field capacity determination had a limitation because it is affected by so many factors, precisely, it is not a constant (for a particular soil), yet it does serve as a practical measure of soil water-holding capacity. Leeper and Uren (1993) reported that the spaces that exist between soil particles provide for the passage and/or retention of gasses and moisture within the soil profile. The ability of a particular soil type to retain water is strongly related its particle size.

Conversely, sands provide easier passage or transmission of water through the profile. Clay type, organic content and soil structure also influence soil water retention (Charman and Murphy

1977). The maximum amount of water that a given soil can retain is called field capacity, whereas a soil so dry that plants cannot liberate the remaining moisture from the soil particles is said to be at wilting point (Leeper and Uren 1993). Available water is that which the plants can utilise from the soil within the range of field capacity and wilting point. Field capacity is characterized by measuring water content after wetting a soil profile, covering it (to prevent evaporation) and monitoring the change in soil moisture in the profile. Water content when the rate of change is relatively small is indicative of when drainage ceases and is called Field Capacity, it is also termed drained upper limit (DUL). Oke (1987) argued that "soil moisture has an effect on the thermal properties of a soil profile, including conductance and heat capacity. The association of soil moisture and soil thermal properties has a significant effect on temperature-related biological triggers, including seed germination, flowering and faunal activity". Timbal *et al.,* (2002) suggested "a strong linkage between soil moisture and the persistence and variability of surface temperature and precipitation; further, that soil moisture is a significant consideration for the accuracy of "inter-annular" predications regarding the Australian climate".

## 2.6 IMPORTANCE OF WATER TO PLANTS

Water is important for plants because of the following reasons:

(i) Water helps in the germination of seeds.

(ii) Water helps in the process of photosynthesis by which plants prepare their food.

(iii) Water helps in the transport of nutrients and minerals from the soil to the plants.

(iv) Water helps in the maintenance of the plant structure by providing the appropriate pressure to the plant tissues

(v) Water provides habitat in the form of ponds, rivers, lakes and sea for a large number of plants.

(http://www.preservearticles.com/201101012194/importance-of-water-for-plants.html)

Plants need large quantities of water for growth. Water typically makes up 80 – 95% of the mass of growing plant tissues. Mature woody plant tissue water content ranges from 45 – 50% while herbaceous plant water content ranges from 70 – 95% (Raven *et al.*, 1999 and Burns *et al.*, 1995). Plants have cell walls that allow the build up of turgor pressure within each cell. Turgor pressure contributes to rigidity and mechanical stability of non-woody plant tissue and is essential for many physiological processes including cell enlargement (plant growth), gas exchange in the leaves, transport of water and sugars, and many other processes. The most important factor driving water movement in plants is a process known as transpiration. Transpiration is the loss of water from plants in the form of vapor (evaporation). Plants utilize most of the water absorbed from the soil for transpiration (95%), but a small portion of the water absorbed is used during photosynthesis for producing the carbohydrates necessary for plant growth (5%). The rate of transpiration is dependent on water availability within the plant (and soil) and on sufficient energy to vaporize water. Most energy supporting transpiration is derived directly from the sun (solar radiation). Sunny, hot weather increases the rate of transpiration and thus the risk for wilting if adequate water is not available.

(http://www.clemson.edu/extension/horticulture/nursery/irrigation/why_plants_need_water.html)

"The process of photosynthesis is directly dependent on the supply of water, light**,** and carbon dioxide. Limiting any one of the factors can limit photosynthesis regardless of the availability of

the other factors. An implication of drought or severe restrictions on landscape irrigation is a reduction in photosynthesis and thus a decrease in plant vigor and growth".

(http://www.ext.colostate.edu/mg/gardennotes/141.html)

## 2.7 EFFECTS OF WATER STRESS ON PLANTS

Normally, plants do not grow in optimum conditions during their life cycle, but suffer many adverse situations that cause different types of stress, and prevent them from reaching maximum development. In addition, the physiological optimum for any one species differs from what is known as the ecological optimum, and therefore in each particular case, the plant has to adapt to the environmental conditions prevailing in its habitat.
Stress is considered as a change in any environmental factor that has an impact on the plant by affecting its biochemical and physiological response to such changes, and may on occasions lead to damage or injury. (http://plantstress.bioiberica.com/Training/What_is/Plant_stress.html)

Plants have adapted over time to tolerate extremes in water availability. Plant water availability is influenced by soil moisture. The texture and structure of soils and container substrates influence their relative capacities to retain water. Plant water uptake does not always keep up with transpirational water loss rates, even if soil moisture is adequate. Temporary midday wilting is common during hot, sunny afternoons, but plants can rehydrate over night when lower temperatures result in decreased transpirational water losses. If the soil/substrate dries without addition of water from precipitation or irrigation, permanent wilting may occur, resulting in plant death. Growth is dramatically affected by the timing and amount of water applied during

production. Certain stages of plant growth are more sensitive to water stress than others. Plant vigor and overall resistance to stress from insects and/or disease are influenced by water status. (http://www.clemson.edu/extension/horticulture/nursery/irrigation/why_plants_need_water.html)

Bohnert and Sheveleva (1998) reported that early responses to water stress aid immediate survival, whereas acclimation, calling on new metabolic and structural capabilities mediated by altered gene expression, helps to improve plant functioning under stress. Pereira and Chaves (1993) suggested that these responses occur at the leaf level in the plant and have a negative influence on carbon assimilation and plant growth. Some of the differences among species in growth and survival can be traced to different capacities for water acquisition and transport rather than to drastic differences in metabolism at a given water status. Nevertheless, carbon assimilation at the whole plant level always decreases as a consequence of limitations to $CO_2$ diffusion in the leaf, diversion of carbon allocation to non-photosynthetic organs and defence molecules, or changes in leaf biochemistry that result in the down-regulation of photosynthesis. Acclimatory changes in the root : shoot ratio or the temporary accumulation of reserves in the stem (Rodrigues *et al*., 1995) under water deficit are accompanied by alterations in carbon and nitrogen metabolism, the fine regulation of which is still largely unknown (Pinheiro *et al*., 2001).

Earlier research suggests that subjecting some plants (Sesames and onions) to water stress increase leaf chlorophyll concentrations (Mensah *et al.,* 2006; Beeflink *et al.,* 1985). Studies conducted by Joly and Hahn (1989) and Deng *et al.,* (1990) reported reduced stomatal conductance and transpiration rate under water stress conditions. Huck *et al.,* (1983) and Karamanos *et al.,* (1982) reported low photosynthesis under stressed conditions in the leaves of plants that were studied. Ackerson and Herbert, 1981 and Genty *et al.,* (1987) also reported low

photosynthesis in cucumber leaves under water stress conditions. Ehleringer and Forseth (1980) reported that under drought conditions, plants regulated their rate of photosynthesis by getting rid of excess light and this was done by preventing light absorption through heliotropism.

## 2.8 SOIL AMENDMENTS AND IMPORTANCE

Soil amendments are materials which are worked into the soil to enhance the soil's properties such as water retention, permeability, aeration etc (Glossary of Soil Science Terms, 2012).

Two broad categories of soil amendments are known which are organic and inorganic. Organic amendments include sphagnum peat, wood chips, grass clippings, straw, compost, manure, biosolids, sawdust and wood ash. Inorganic amendments include vermiculite, perlite, tire chunks, pea gravel and sand (Davies and Whiting, 2012).

Soil-based application of organic amendments to field grown crops has shown ameliorating effect on drought stressed crops. Sahs and Lesoing (1985) observed higher sweet corn yields in plots amended with beef feedlot manure than those that were inorganically fertilized during drought years. Heckman *et al.*, (1987) found that field grown soybeans fertilized with sewage sludge had increased drought resistance and nitrogen fixation than the control treatment. Improved drought tolerance of crops grown in organically amended soils has been linked to the maintenance of optimum leaf health. In five-week old water stressed maize seedlings, Xu (2000) measured higher photosynthetic rates when the soils were organically amended.

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 EXPERIMENTAL SITE

The experiment was conducted at the Cocoa Research Institute of Ghana, New Tafo-Akim between August 2013 and January 2014. The soil at the experimental site belonged to the WACRI series.



## 3.2 GROWTH MEDIA PREPARATION

Sawdust was obtained from the tree species Emeri (*Terminalia ivorensis)* and oven dried at a temperature of 100°C for 72hrs. Topsoil to be used was sieved to obtain a finer texture and to get rid of unwanted materials. The dried sawdust was mixed together with the topsoil at different ratios to form the first treatments (M).

## 3.3 DETERMINATION OF FIELD CAPACITY OF THE SOIL

The choice of the second treatment (W) was based on the field capacity (F.C) of the soil which was determined as follows:

Five (5) samples of topsoil placed in perforated polybags to almost full volume were watered until they were completely saturated and then allowed to drain freely until the water ceased to drip out. The surfaces of the polybags were covered with dried grass to prevent evaporation of water from the soil surfaces. At that stage, the water that remained in the soil was assumed to be at field capacity. Measurement of the field capacity was carried out by first weighing, then oven-drying the moist soil at 100°C until a constant weight was obtained and then by subtraction of

weights. Calculations involved in determining the field capacity of the topsoil for this research is illustrated below:

Average weight of topsoil only = 6.30kg

Table 1: Determination of field capacity of soil.

| Soil sample | Wt. of soil + water after dripping (kg) | Wt. of soil after oven drying (kg) | Difference (kg) |
|---|---|---|---|
| 1 | 6.90 | 5.02 | 1.78 |
| 2 | 6.85 | 5.13 | 1.62 |
| 3 | 7.22 | 5.25 | 1.87 |
| 4 | 7.13 | 5.25 | 1.78 |
| 5 | 6.40 | 4.82 | 1.48 |
| Average/Mean | | | 1.71kg |

By mass, the field capacity or the water holding capacity of the soil was 1.71 kg. This was converted into volumetric figure by weighing a 1.71 kg mass of water and pouring it into a volumetric flask to obtain field capacity in volume (ml). Therefore, we have 1710 ml.

The field capacity determined, was halved (0.5 FC) and quartered (0.25 FC) to obtain the second treatments for the research as shown below:

- W1- 0.5 F.C of the soil (855 ml).
- W2- 0.25 F.C of the soil (427.5 ml).

### 3.4 EXPERIMENTAL DESIGN AND TREATMENTS

The research was carried out in a gauze chamber. The cacao used was a PA7 X POUND7 hybrid variety. Seeds were nursed in 01/02/2013 by the Plant Breeding Division of the Institute. Nursed seeds were watered. The seedlings were transplanted on 16/08/2013 into the various treatment media. The experiment was laid out in split-plot arranged in CRD with the different watering regime (W) being the main plot and the soil mixed with sawdust (M) the sub-plot. This was replicated three times.



Factor A

Growth containers (black polybags) of size 28.2 cm in width by 32.6 cm in height was used in the determination of the mixing ratios for factor A as shown below:

- M1- 1 full polybag of topsoil (wt-5.45 kg) : 50% by vol. polybag of sawdust (wt-0.5717 kg) i.e. a ratio of 1: 0.5
- M2- 1 full polybag of topsoil (wt-5.45 kg): 35% by vol. polybag of sawdust (wt-0.4001 kg) i.e. a ratio of 1: 0.35
- M3- 1 full polybag of topsoil (wt-5.45 kg): 25% by vol. polybag of sawdust (wt-0.2859 kg) i.e. a ratio of 1: 0.25
- M4 (Control)- Topsoil with no sawdust (wt-5.45 kg) i.e. a ratio of 1: 0

Factor B

- W1- 0.5 F.C (855 ml).
- W2- 0.25 F.C (427.5 ml).

The experimental area measured 4.64 m x 8.90 m and each treatment table measured 1.77 m x 2.50 m with spacing of 1.10 m between treatment tables and 0.7 m among replications. The spacing between seedlings on treatment tables was 0.3 m by width and 0.4 m by length. Each treatment table had four rows with five plants per row for each sawdust treatment and the control. Records were taken on the three middle plants for each sawdust treatment as well as the control. Seedlings after transplanting were watered at field capacity for one week. Watering treatments was then applied once every ten (10) days for the subsequent weeks.

### 3.5 CULTURAL PRACTICES

Weeds were controlled by handpicking during the research. Seedlings were sprayed once with Metalm 72 WP, a contact and systemic fungicide to check a fungal infection at a rate of 50 g/15 litres of water. Sidalco fertilizer N: P: K, 10:10:10 was applied at a rate of 30 ml/15 litres of water on 06/11/2013. Subsequent application was done once every twenty days (at two (2) watering regime intervals). W2 plants received 427.5 ml of the fertilizer solution while W1 plants received 427.5 ml of the fertilizer solution plus 427.5 ml of normal water making the total of 855 ml.

### 3.6 SOIL SAMPLING AND ANALYSIS

Sampling of soil for analysis was done randomly using hand trowel from five (5) different spots of heaped topsoil. The soil was air dried for one (1) week, ground to obtain finer particles and sieved in a 2mm mesh. The soil was sent to the laboratory for various analyses. Organic carbon, soil pH, total nitrogen, exchangeable bases and other important soil properties were all determined using various protocols as shown below.



#### 3.6.1 DETERMINATION OF AVAILABLE PHOSPHORUS IN THE SOIL USING 0.2 NORMAL SULPHURIC ACID (0.002N IN 3% $(NH_4)_2SO_4$) AS EXTRACTION SOLUTION / EXTRACTANT (TRUOG METHOD*)*

- 5.0 g of air-dried soil samples were weighed into shaker bottles.
- 100 ml of 0.002N $H_2SO_4$ were added to the samples in the shaker bottles, which were then shaken for 2 hours on a mechanical shaker.
- Each shaken sample was filtered through Whatman No. 42 filter paper into a 100 ml volumetric flask.
- 10 ml aliquots of the sample solutions (filtrate) in the 100 ml volumetric flasks were pipetted into 25 ml volumetric flasks.
- 4 ml of 'Reagent B' was added to the sample solutions, followed by distilled water to the 25 ml mark and then shaken by hand to mix well. Blank solutions were also prepared with 4 ml Reagent B and distilled water.
- UV Visible Cecil Spectrophotometer (CE 7400 model) was calibrated using Phosphorus Standards of known concentrations at a wavelength of 882 nm.

- Upon colour development, absorbance readings of the samples were taken on the spectrophotometer at the same wavelength.
- The absorbance readings (nm) were then used to calculate the Available Phosphorus in the samples using the formula below:

**Available Phosphorus (µg / g) = (Absorbance / G.F) x D.F x Volume of extractant (100 ml)**

**Weight of soil (5.0 g)**

Where; G.F is Graph Factor = $\frac{\sum \text{ of Concentrations of Phosphorus Standards}}{\sum \text{ of Absorbance readings of Phosphorus Standards}}$

D.F is Dilution Factor = $\frac{\text{Volume of volumetric flask used (25 ml)}}{\text{Volume of aliquot used (10 ml)}}$

The reagents used were prepared as follow:

**0.2N $H_2SO_4$ in 2 litres:** 11 ml of conc. $H_2SO_4$ was added to a volume of distilled water in a 2 litre volumetric flask. The flask was well shaken by hand and allowed to cool under fume chamber. The volume was made to the 2 litre mark with distilled water and the flask labeled.

**'Reagent A':** 12.0 g of Ammonium molybdate $[(NH_4)_6Mo_7O_{24}.4H_2O]$ was dissolved in about 250 ml distilled water. 0.2908 g of Antimony potassium tartrate ($KSbO.C_4H_4O_6$) was also dissolved in about 100 ml distilled water. Both of the dissolved reagents were added to a litre of 5N $H_2SO_4$ (135.98 ml conc. $H_2SO_4$ / litre). The reagent was mixed thoroughly and made to 2 litres. The prepared reagent was then stored in Pyrex glass bottle in dark, cool compartment.

**'Reagent B':** 1.056 g of L-Ascorbic acid was dissolved in 200 ml reagent A. The flask was shaken by hand to mix the reagents well, and then labeled.

### 3.6.2 DETERMINATION OF ORGANIC CARBON IN SOILS BY WALKLEY-BLACK METHOD AND SUBSEQUENT ESTIMATION OF ORGANIC MATTER (1934)

- 1.0 g of air-dried soil samples were weighed into 500 ml conical flasks, and were placed under fume chamber.
- 10 ml of Potassium dichromate were added to the samples in the flasks, followed by 20 ml of Concentrated Sulphuric acid (Conc. $H_2SO_4$).
- The flasks were swirled vigorously for one minute and were allowed to stand for 30 minutes.
- 200 ml of distilled water was added to each sample, followed by 10 ml of Orthophosphoric acid ($H_3PO_4$).
- 10 drops of diphenylamine indicator were added to the contents in the flask and were swirled to mix well.
- The samples were then titrated with standard Ferrous Ammonium Sulphate until the solutions were purple or blue.
- Small lots of the Ferrous Ammonium Sulphate were added to the solutions until the colour flashed to green.
- Exactly 0.5 ml of standard Potassium dichromate was added to give an excess and then titrated drop by drop with the Ferrous Ammonium Sulphate until the blue colour just disappeared.
- Blank titrations were carried out in an identical way using the same reagents, but omitting the soil.

- The percentage organic carbon in the soil samples were then calculated using the formula below:

**% Organic Carbon = [Dichromate used – (Factor x Titre value of sample)] x Soil factor**

Where; Dichromate used = 10.5

$$\text{Factor} = \frac{\text{Dichromate used}}{\text{Mean Blank titre value}}$$

Soil factor = 0.39

% Organic Matter (OM) was calculated by multiplying the % Organic Carbon (OC) value by a factor of 1.724 (van Bennelen factor). Thus %OM = %OC X 1.724

The reagents used were prepared as follow:

**Ferrous Ammonium Sulphate – $(NH_4)_2\,Fe(SO_4)_2.6H_2O$ in 1L:** 196.07 g of solid Ammonium iron (II) sulphate was weighed and dissolved with distilled water in 1 litre volumetric flask. 15 ml of conc. $H_2SO_4$ was added. The volume was made up to the 1 litre mark with more distilled water and mixed well. The flask was labeled.

**Potassium dichromate – $K_2Cr_2O_7$ in 1L:** 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 labeled.

**Diphenylamine indicator – $(C_6H_5)_2NH$:** 0.5 g of solid Diphenylamine was weighed into a beaker. 20 ml of distilled water followed by 100 ml of conc. $H_2SO_4$ were added and mixed well. The prepared indicator was transferred into 250 ml volumetric flask and labeled. Some quantity was poured into indicator bottle for use.

### 3.6.3 DETERMINATION OF EXCHANGEABLE BASES IN SOILS BY AMMONIUM ACETATE METHOD OF HANWAY AND HEIDEL (1952)

The Exchangeable Bases analyzed were Potassium, Magnesium and Calcium. The procedure used is as follows:

- 5.0 g of air-dried soil samples were weighed into shaker bottles.
- 25 ml of 1M Ammonium acetate (1M $NH_4OAC$) solution were added to the samples in the shaker bottles, which were then shaken for 10 minutes on a mechanical shaker.
- The shaken samples were filtered through Whatman No. 42 filter papers into 50 ml volumetric flasks.
- The sample solutions (filtrates) were analyzed for the concentrations of the various elements on the Atomic Absorption Spectrometer (Spectr AA 220 FS model, Varian Brand )

The extraction solution was prepared by weighing 77.08 g of solid $NH_4OAC$ into a 1 litre beaker and completely dissolved with distilled water. The solution was then poured into a 1 litre volumetric flask and made to volume with distilled water. The flask was labeled.

### 3.6.4 DETERMINATION OF TOTAL NITROGEN IN SOILS BY KJELDAHL METHOD (1965)

- 2.5 g of air-dried soil samples were weighed into smaller digestion tubes, after which about 0.5 g of catalyst was added to the samples in the tubes.
- 12 ml of conc. $H_2SO_4$ of Nitrogen free were added to the samples under fume chamber.

- The tubes were put in a digestor under fume chamber, and the samples were digested for 2 hours at 350 $^{o}$C (the temperature and time were increased when samples were not well-digested). Well-digested samples were either white or colourless.

- The digested samples (digests) in the tubes were allowed to cool under fume chamber until there were no fumes evolving.

- The smaller tubes containing the digests were washed and rinsed about three times with distilled water into bigger tubes for distillation.

- The distilled samples (distillates) which contained the ammonia compounds were then collected in receiver flasks and titrated with 0.02N $H_2SO_4$ which had previously been standardized with borax (Boric acid), till just a colour change was observed (from green to blue).

- The Percentage Nitrogen in the samples was then calculated using the formula below:

**% Nitrogen =** $\frac{\text{Titre value of sample (ml) x Normality of acid (0.02) x 1.401}}{\text{Weight of sample (g)}}$

The reagents used were prepared as follow:

**0.02N $H_2SO_4$ in 1 litre:** 0.54 ml of conc. $H_2SO_4$ 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 labeled.

**40 % Sodium hydroxide (NaOH) in 1 litre:** 400.0 g of solid NaOH was weighed into a 1 litre beaker and completely dissolved with distilled water. The solution was then poured into a 1 litre volumetric flask and made to volume with distilled water. The flask was labeled.

**2 % Boric acid ($H_3BO_3$) in 1 litre:** 20.0 g of solid $H_3BO_3$ was weighed into a 1 litre beaker and completely dissolved with distilled water. The solution was then poured into a 1 litre volumetric flask and made to volume with distilled water. The flask was labeled.

**Indicator:** 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 catalyst container.

### 3.6.5 DETERMINATION OF SOIL pH USING GLASS ELECTRODE / pH METER, AND 1:2.5 SOIL - WATER SUSPENSION (1992)

- 10.0 g of air-dried soil samples were weighed into 100 ml beakers.
- 25 ml of distilled water were added to the samples.
- The beakers containing the samples were stirred and left to stand for 30 minutes. (This was to make sure that the hydrogen ions had been extracted).
- Before taken any measurements or readings, the pH meter was standardized as follows:

- A buffer solution of pH 4 was measured by dipping the electrode into the solution and adjusting the meter to read the pH of 4.
- The electrode was rinsed with distilled water and wiped gently with tissue paper.
- A buffer solution of pH 7 was measured in the same manner after which the electrode was rinsed again with distilled water and wiped gently.
- The test samples were then measured, making sure the electrode dipped into the solution properly, after which the pH of the samples read on the pH meter and recorded.



### 3.6.6 MECHANICAL ANALYSIS (PARTICLE SIZE AND SOIL TEXTURE DETERMINATION) USING HYDROMETER METHOD OF BOUYOUCOS (1951).

- 52.0 g of air-dried soil samples were weighed into 250 ml beakers.
- 20 ml of 20 % Hydrogen peroxide ($H_2O_2$) was added to each of the samples in the beakers, and were left to stand until they got wet, after which they were dried on a hot plate and grinded.
- 100 ml of 5 % Sodium hexametaphosphate / Calgon $(NaPO_3)_6$ were added and mixed thoroughly, after which they were left to stand for between 15 – 20 hours.
- The contents in the beakers were then washed into soil cup with distilled water, and were stirred with dispersing machine for 2 minutes.
- The cup was disconnected, the contents washed into 1 litre soil cylinders and filled to the 1 litre mark with distilled water.

- The mouths of the cylinders were closed with rubber stoppers and turned completely upside down and back about 20 times.
- Few drops of Amyl alcohol ($C_5H_{11}OH$) were quickly added on top of the suspension to dissipate froths where they appeared.
- Hydrometer was gently placed in the soil suspensions and the first reading taken within 40 seconds. The hydrometer was removed and washed with distilled water.
- After exactly 2 hours of continuous sedimentation, the second reading was taken with the hydrometer. The hydrometer was removed and washed with distilled water.
- The relative amounts of sand, silt and clay were then calculated using the formula below:

**% Sand = 100 – 2 (X+2.88); % Clay = 2 (Y+2.88); % Silt = 100 – (A+B)**

Where; X = First corrected hydrometer reading = $1^{st}$ Hyd. Read – 6.5

Y = Second corrected hydrometer reading = $2^{nd}$ Hyd. Read – 6.5

A = % Sand; B = % Clay

Once the relative amounts of sand, silt and clay were known, the soils' textural classes were determined by using a soil textural triangle.

The reagents used were prepared as follow:

**20 % Hydrogen peroxide ($H_2O_2$) in 1 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 labeled.

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

### 3.7 DATA COLLECTION

Data were collected on the following

- Soil moisture ( using a moisture meter- SM300 at every other day prior to watering)
- Humidity and temperature of the experimental area [using a Tinytag data logger]
- Temperature of the growth media (using soil thermometers, taken daily at 9:30am (min.) and 3:30pm (max.))
- Soil nutrient analysis ( before and after the experiment)
- Leaf chlorophyll content [ using a chlorophyll content meter at 3weeks interval]
- Leaf chlorophyll fluorescence [using (FP100; Photon Systems Instruments at 2weeks interval]
- Diurnal profile of the plant ( stomatal conductance, transpiration rate and photosynthesis using Infra-Red Gas Analyzer)
- Stem girth ( using digital vernier calipers taken at 5cm above soil surface at 4weeks interval)
- stem height ( using meter rule at 4weeks interval)

- Leaf number (flushes)
- Leaf Area (using leaf area meter)
- Seedling organ weight analysis (dry) - leaves, stem and roots.

### 3.8 STATISTICAL ANALYSIS

The experimental results were analyzed using analysis of variance (ANOVA) Genstat version 12. Least Significant Difference (LSD) was used to separate the means at 5% probability level.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 HUMIDITY AND TEMPERATURE OF EXPERIMENTAL AREA

Figure 1 shows the temperature and relative humidity of the experimental area recorded from September 2013 to February 2014. Maximum and minimum readings were taken for each parameter. It was observed that maximum (37.4°C) and minimum (22.5°C) temperature and maximum (96%) and minimum (50.7%) relative humidity readings did not change greatly from mid-September through to early December. However, readings from December to February 2014 were greatly interrupted. Minimum relative humidity (41.9%) and temperature readings (18.8°C) dropped greatly. Maximum readings were less affected (temp.: 35.3°C and RH: 93.5%).

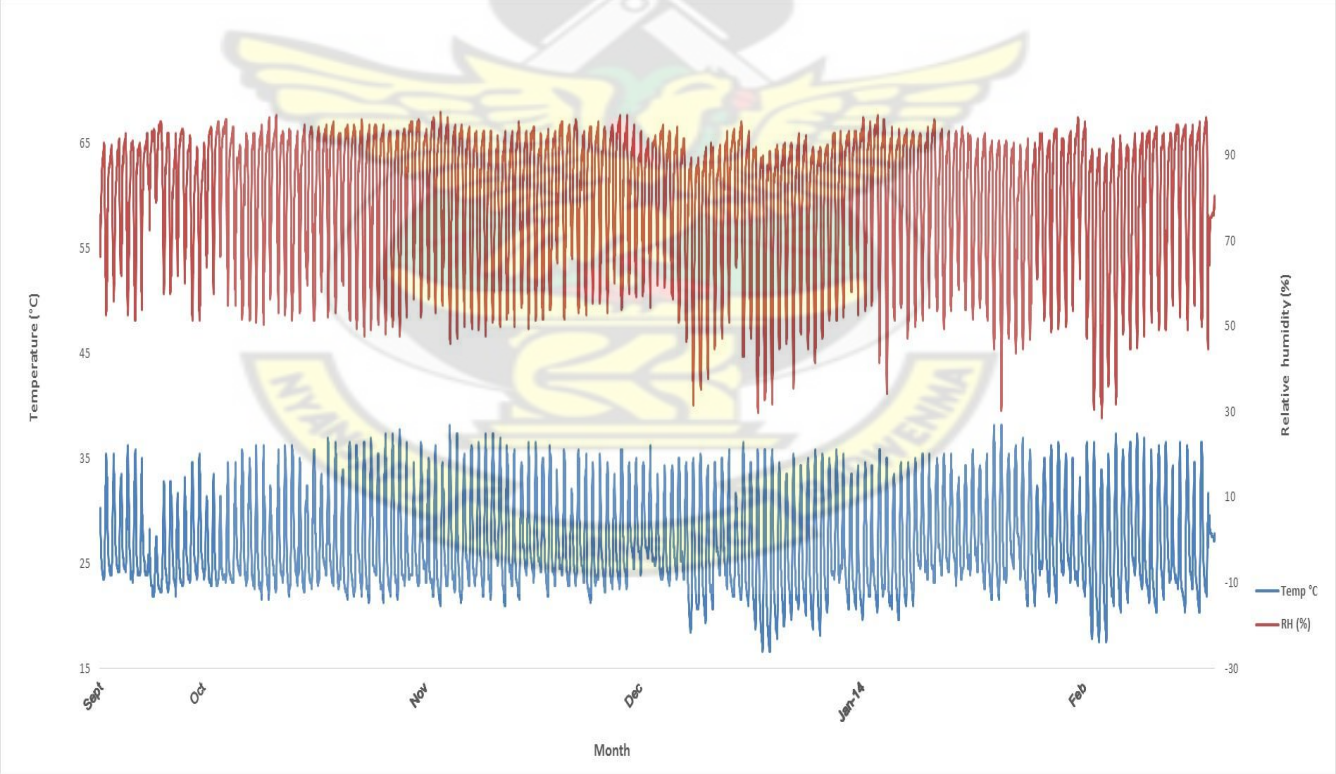


**Figure 1: Temperature and Relative Humidity of experimental area.**

## 4.2 SOIL ANALYSIS

Table 2: Soil analysis.

| SAMPLE | pH | Organic C (%) | O.M (%) | Total N (%) | Available P (µg $g^{-1}$) | Exchangeable K (meq 100 $g^{-1}$) | Exchangeable Mg (meq 100 $g^{-1}$) |
|---|---|---|---|---|---|---|---|
| 1 | 6.45 | 1.18 | 2.03 | 0.148 | 30.042 | 0.644 | 1.862 |
| 2 | 6.47 | 1.16 | 2.00 | 0.146 | 30.040 | 0.642 | 1.860 |
| 3 | 6.44 | 1.20 | 2.07 | 0.143 | 30.047 | 0.645 | 1.865 |
| 4 | 6.43 | 1.18 | 2.03 | 0.147 | 30.044 | 0.643 | 1.863 |
| 5 | 6.45 | 1.19 | 2.05 | 0.146 | 30.043 | 0.641 | 1.862 |
| MEAN | 6.45 | 1.18 | 2.04 | 0.146 | 30.043 | 0.643 | 1.862 |

From the results of soil analysis, the pH ranged from 6.43 to 6.47 with an average value of 6.45, which is suitable for growing cacao. Organic carbon and organic matter contents recorded were 1.18 % and 2.04 % respectively in the top 15 cm of soil sampled. Although these levels slightly fall outside the limits regarded as good soil for growing cacao in the upper horizons, it could still support cacao growth and development because the margin is not so wide. Ahenkorah *et al.*, (1982) gave the limits as 2.03 % for organic carbon, and 3.50 % for organic matter.

The soil analysis also gave a mean nitrogen content of 0.146 % and could therefore support cacao growth. The mean available P content in top 15 cm of soil used to grow the cacao seedlings was 30.043 µg $g^{-1}$, and is sufficient to support the plants. Available P content of 20.000 µg $g^{-1}$ or more in the top layers of soil is considered adequate for cocoa.

Good cacao soils have K and Mg values around 0.34 meq 100 $g^{-1}$ and 1.33 meq 100 $g^{-1}$ respectively in the top 15 cm layer of soil. Values obtained from the soil analysis are adequate enough to support cacao growth.

### 4.3 SOIL MOISTURE CONTENT

Soil moisture content increased with increasing quantity of water supplied (P=0.039)

A significant difference (P=0.004) was observed between the control-no sawdust and the sawdust treatments. 50% sawdust recorded the highest moisture content value of 2.358 with the control recording the least value of 1.036. Soil moisture content increased with increasing sawdust amount.

Figure 2 shows water and sawdust interaction on soil moisture content. There was an increasing trend in soil moisture content from the control to 50% sawdust under 0.5 FC as shown in Figure 2. The trend was almost same under 0.25 FC with the difference been a slight decrease in the mean soil moisture content for 50% sawdust. This shows that increasing the proportion of sawdust increases the amount of moisture retained.

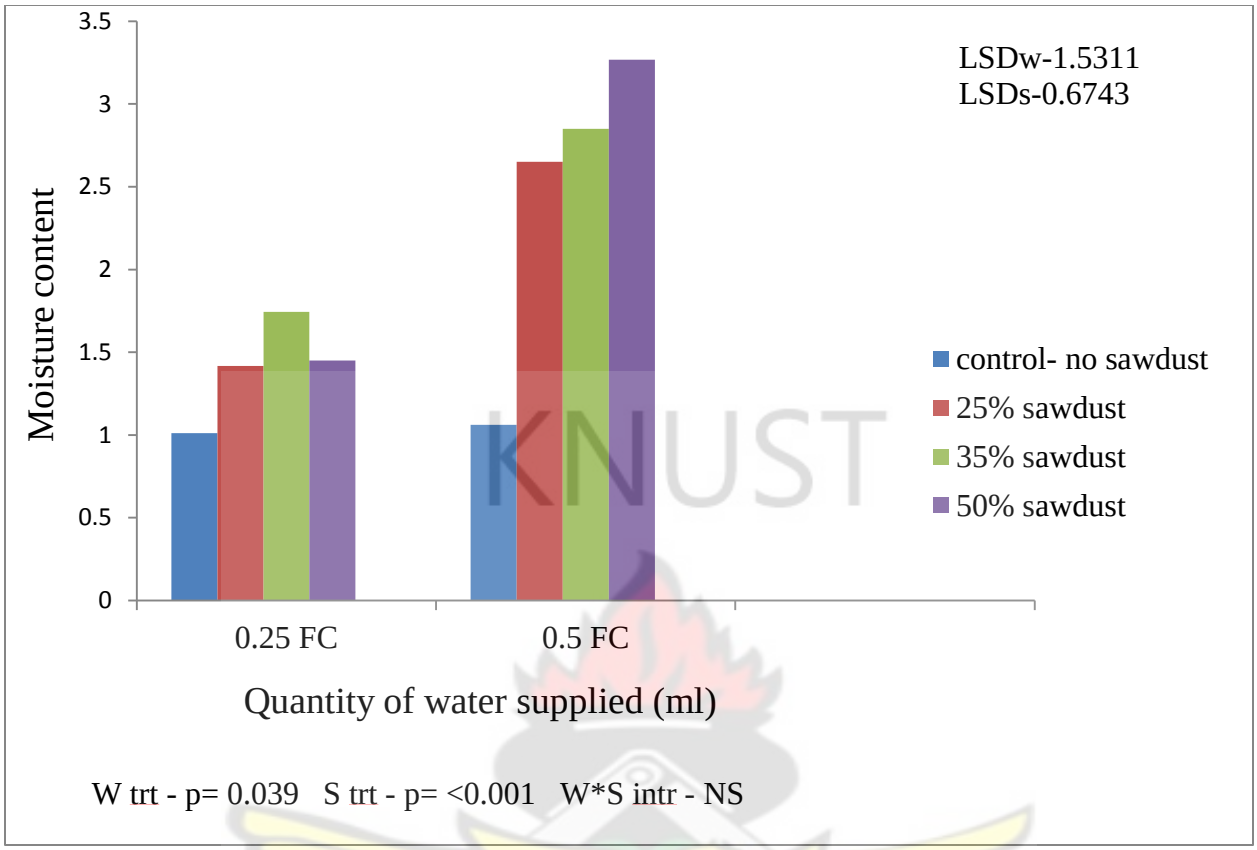


**Figure 2: Mean soil moisture content of cacao seedlings under different sawdust ratios**

## 4.4 LEAF CHLOROPHYLL CONTENT

Differences in mean leaf chlorophyll content of seedlings under both 0.25 FC and 0.5FC was not significant (P=0.678). The difference was small (4.76 and 4.6) for 0.25 FC and 0.5 FC, respectively.

Under the sawdust treatment, a highly significant difference was observed between the control and both 35% and 50% sawdust treatments (P=0.007). It was observed that increasing sawdust amount reduced the leaf chlorophyll content of seedlings thus the control recording the highest

mean leaf chlorophyll content with 50% sawdust recording the least. There was no significant difference between the control and 25% sawdust. Difference in the mean chlorophyll content between 25% sawdust and 35% sawdust was also not significant.

Figure 3 shows the interaction between water and sawdust amendment on leaf chlorophyll content.

Under both water quantities, leaf chlorophyll content increased with decreasing sawdust amount. However under 0.5 FC, the mean chlorophyll content for the control (no sawdust) reduced allowing 25% sawdust to record the highest mean value. Significant difference (P=0.038) was only observed between 25% sawdust and 50% sawdust. Differences amongst means for the remaining treatments were all not significant.

Under 0.25 FC, an interaction which was significant (P=0.038) was observed between the control and all the sawdust treatments but amongst the sawdust treatments, the interaction was not significant.

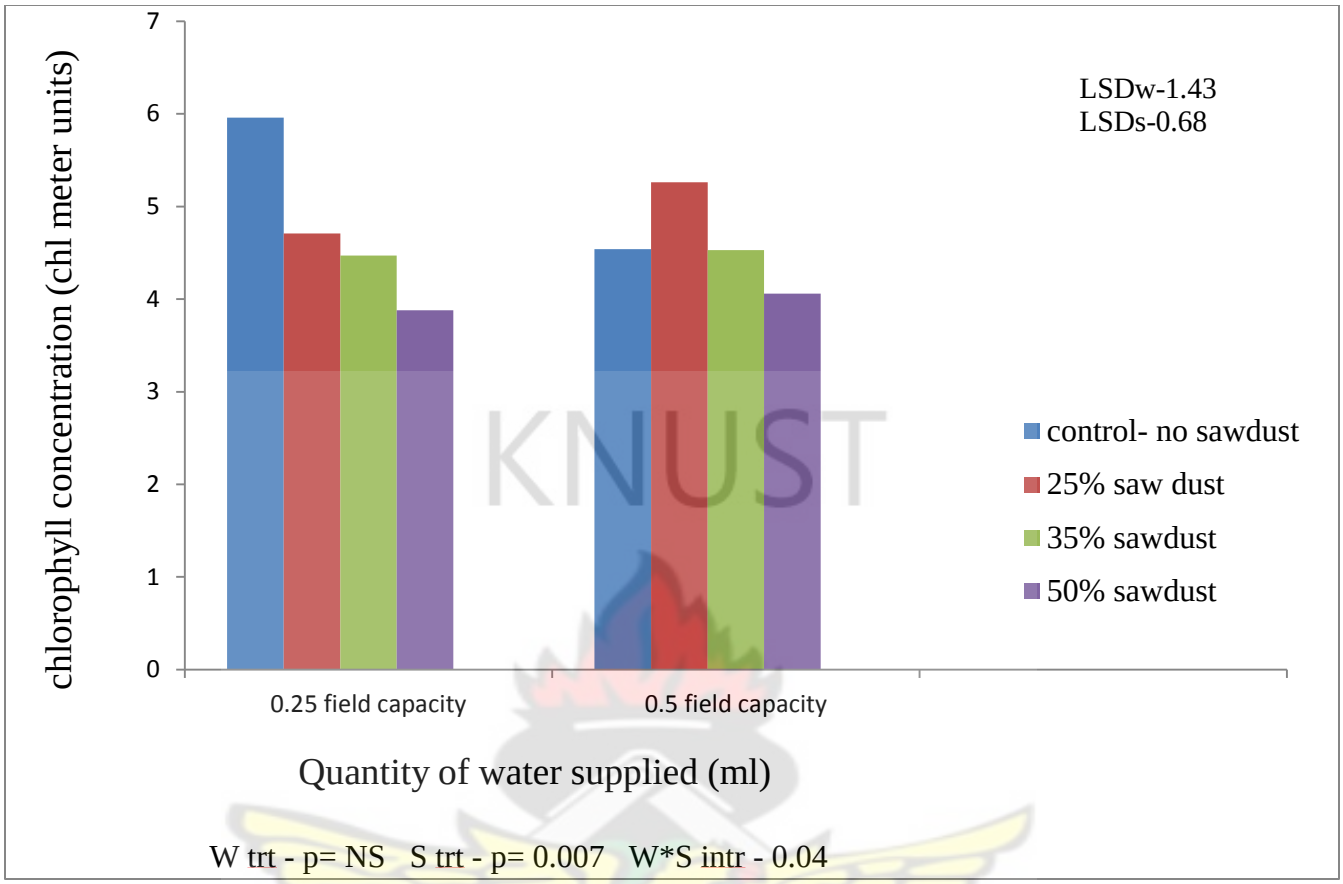


**Figure 3: Mean leaf chlorophyll concentration of cacao seedlings.**

## 4.5 LEAF CHLOROPHYLL FLUORESCENCE

Leaf chlorophyll fluorescence of cacao seedlings under 0.5 FC and 0.25 FC was not significantly affected (P=0.329). Although increasing the quantity of supplied water slightly increased the leaf chlorophyll fluorescence of seedlings (0.4489 Fv/Fm and 0.461 Fv/Fm) under 0.25 FC and 0.5 FC respectively.

Under the sawdust treatment, highly significant difference (P=0.009) was observed between the sawdust treatment and the control (no sawdust) with 35% sawdust recording the highest and the control (no sawdust) recording the least chlorophyll fluorescence. No significant difference was

observed amongst the sawdust treatments. From the mean values obtained, it was observed that increasing the proportion of sawdust from 25% sawdust to 35% sawdust increased the leaf chlorophyll fluorescence from 0.4827 Fv/Fm to 0.4843 Fv/Fm. There was however a decrease in leaf chlorophyll fluorescence as sawdust proportion increased to 50% (mean value=0.4762 Fv/Fm). This indicates that too much of sawdust is not needed as it will negatively affect leaf chlorophyll fluorescence. The control which had no sawdust had the least leaf chlorophyll fluorescence.



Result in Figure 4 shows the interaction between water and sawdust on leaf chlorophyll fluorescence. A highly significant difference (P=0.009) was observed between the control (no sawdust) and the remaining treatments under both 0.25 FC and 0.5 FC. This shows that, there was an interaction between water and sawdust under these treatments. Seedlings growing in media amended with 35% sawdust recorded the highest fluorescence of 0.488 Fv/Fm under 0.25 FC and those growing in media amended with 25% sawdust recorded the highest fluorescence of 0.4883 Fv/Fm under 0.5 FC. The control in both cases recorded the least leaf chlorophyll fluorescence of 0.3656 Fv/Fm and 0.3877 Fv/Fm respectively. Differences amongst the sawdust treatments for both water treatments were however, not significant.

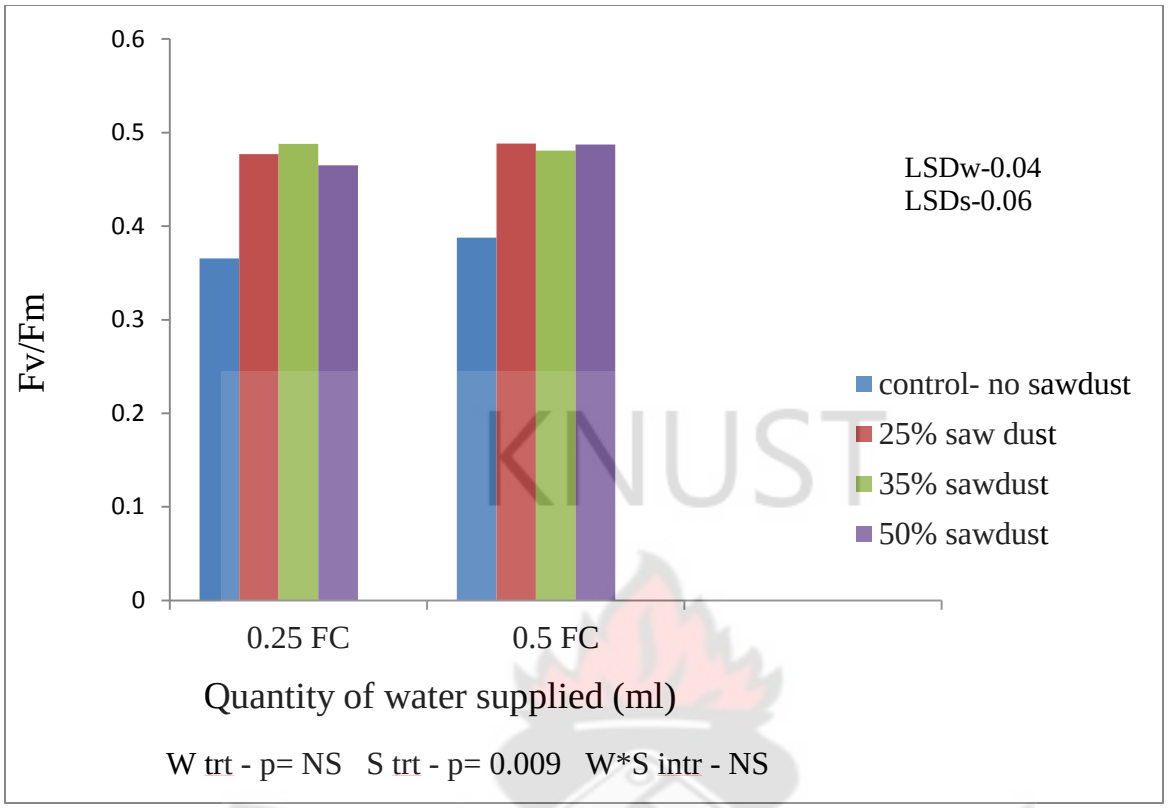


**Figure 4: Mean leaf chlorophyll fluorescence of cacao seedlings.**

## 4.6 MEAN DIURNAL VALUE OF TREATMENTS AT 9AM

### 4.6.1 Stomatal conductance

The watering treatment had no significant effect (P=0.83) on the stomatal conductance of cacao leaves as the difference was marginal (0.0213 mol/m²/s and 0.0194 mol/m²/s for 0.25 FC and 0.5 FC, respectively).

Under sawdust treatment, the control (no sawdust) had a mean stomatal conductance of 0.0213mol/m²/s whiles the remaining sawdust treatments recorded a similar mean stomata conductance of 0.02mol/m²/s. Sawdust amount did not significantly influence stomatal conductance of leaves (P=0.998).

Figure 5 shows water and sawdust interaction on leaf stomatal conductance.

Under 0.25 FC, as sawdust reduces leaf stomata conductance is increased. There was however a deviation as 25% sawdust decreased along the trend.

Under increased water level (0.5 FC), leaf stomatal conductance decreased with decreasing sawdust amount. There was a deviation again as 25% sawdust increased down the trend.



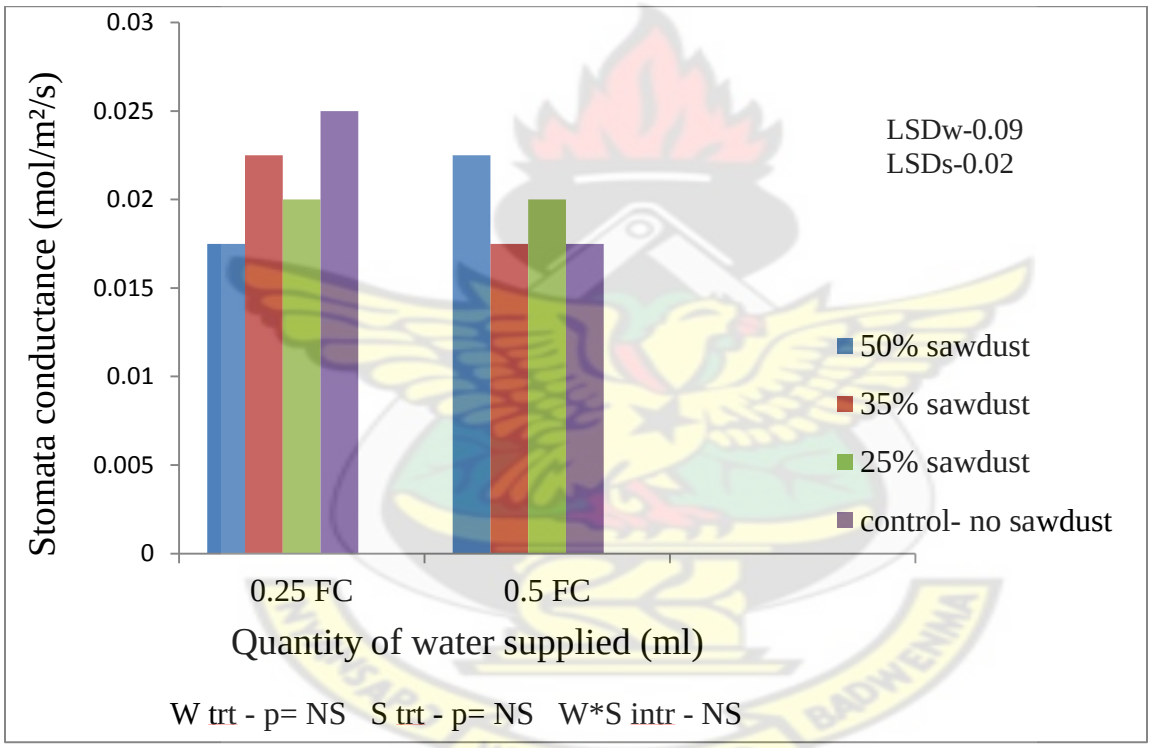


**Figure 5: Mean stomata conductance of cacao seedlings.**

### 4.6.2 Transpiration rate

The rate of transpiration of cacao seedlings under 0.25 FC and 0.5 FC was not significantly affected (P=0.904).

This was same under the sawdust treatment, where no significant difference was seen amongst the various treatments (P=0.943). This shows that sawdust had no effect on the rate of transpiration of the seedlings.



Figure 6 below shows water and sawdust interaction on plant transpiration rate. There was no interactive effect on plant transpiration rate (P=0.915).

It was however observed that, under reduced water level (0.25 FC), the rate of transpiration decreased with increasing sawdust amounts. This implies that under reduced water quantities, sawdust should be increased in order to conserve water and reduce water loss through transpiration.

Under 0.5 FC, the rate of transpiration again decreased with increasing sawdust amount from the control (no sawdust) to 35% sawdust. There was however an increase in the rate of transpiration for 50% sawdust.

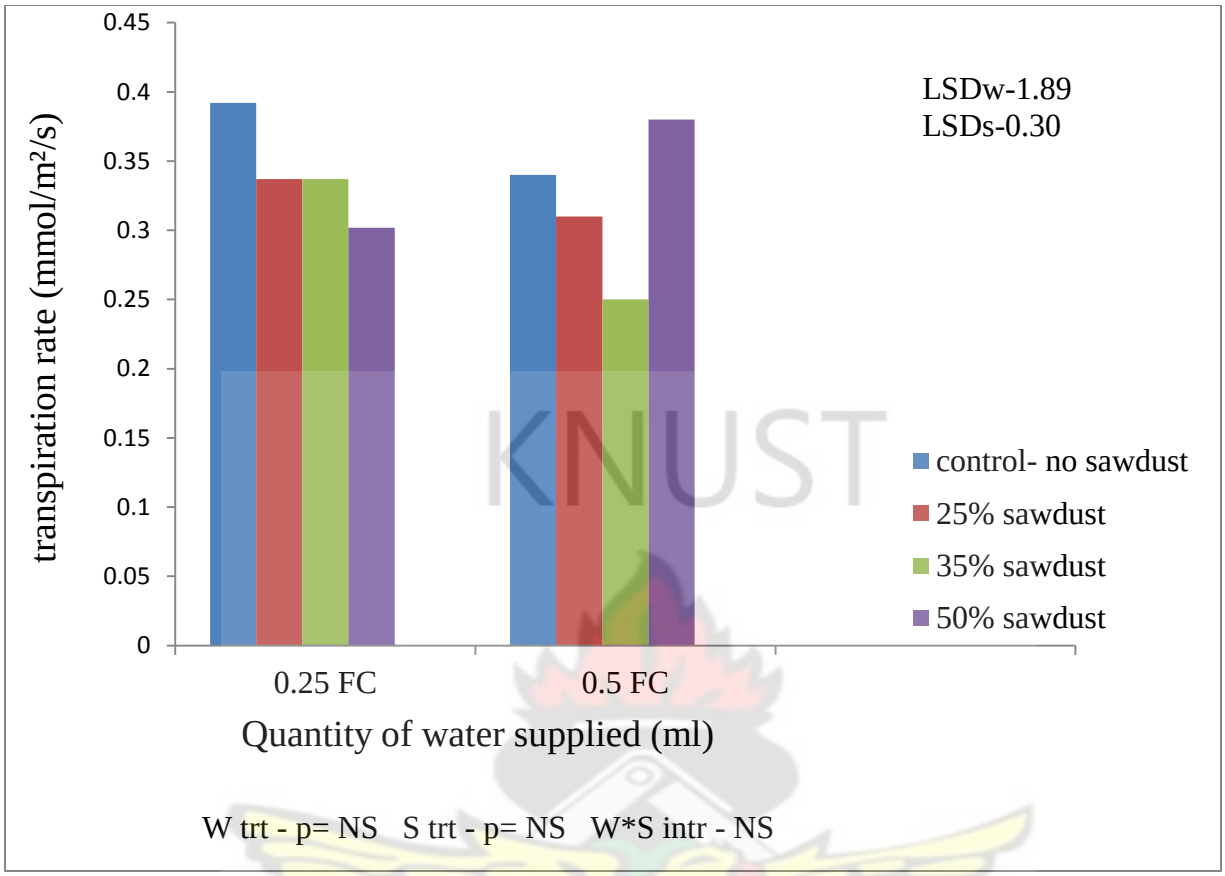


**Figure 6: Mean transpiration rate of cacao seedlings.**

### 4.6.3 Photosynthesis

Although increasing the quantity of water for seedlings increased photosynthesis (0.74 and 1.328 for 0.25 FC and 0.5 FC respectively), the difference was statistically not significant (P=0.62).

This was same under the sawdust treatment, where no significant difference was seen amongst the various treatments (P=0.22). This shows that sawdust had no substantial effect on the photosynthetic activity of the seedlings.

Figure 7 shows water and sawdust interaction on plant photosynthetic activity.

Under 0.25 FC, plant photosynthetic activity increased with increasing sawdust amount (from the control to 25% sawdust) but decreased henceforth (from 35% sawdust to 50% sawdust)

Under 0.5 FC, photosynthetic activity again increased from the control to 25% sawdust but decreased under 35% and 50% sawdust proportions.

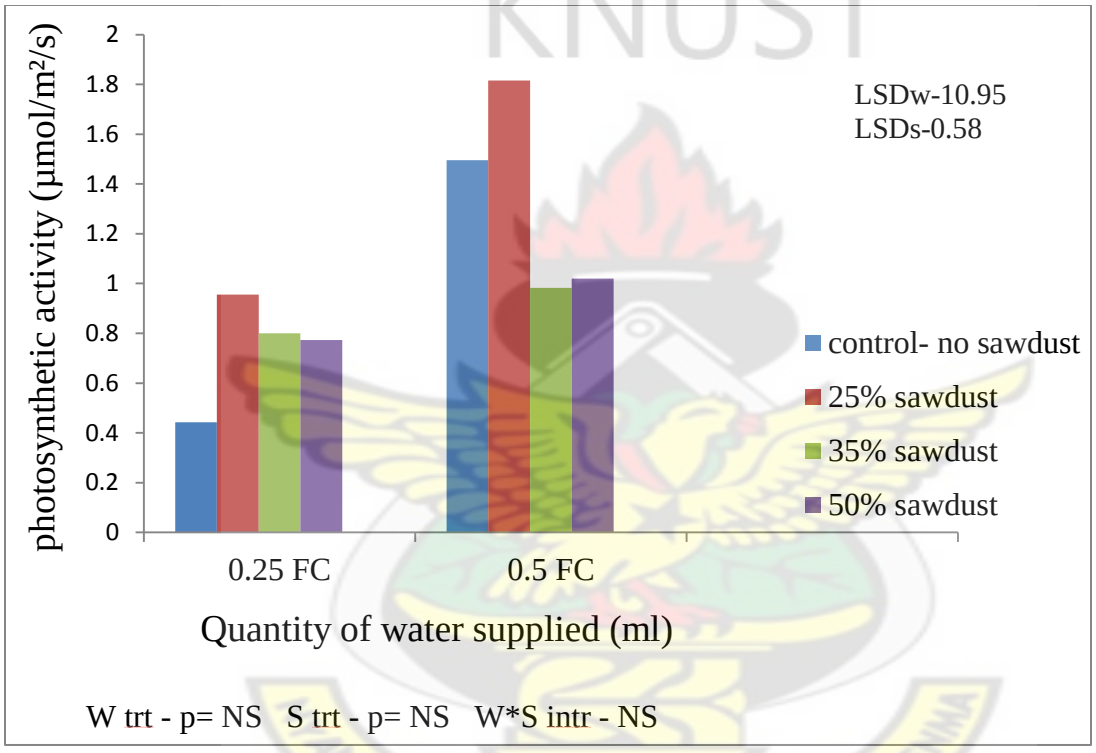


**Figure 7: Mean photosynthetic activity of cacao seedlings.**

## 4.7 STEM GIRTH

There was no significant difference (P=0.213) in the stem girth of cacao seedlings under the two water treatment as the difference was marginal (10.214 mm and 10.765 mm for 0.25 FC and 0.5 FC, respectively).

Under the sawdust treatment, a significant difference (P=0.038) was observed between 50% sawdust and 25% sawdust (mean=10.993 mm and 10.151 mm, respectively). A significant difference (P=0.038) was again observed between 50% sawdust and the control (no sawdust) treatments with the control recording a mean stem girth of 10.225mm. There was no significant difference between 50% sawdust and 35% sawdust treatments. From the mean values obtained, it was observed that increasing sawdust amount increased the stem girth of plants.

Figure 8 shows the interaction between water and sawdust on stem girth.

Under 0.25 FC, mean stem girth decreased with decreasing sawdust amount. Water and sawdust had no interactive effect on the respective treatments under 0.25 FC (P=0.346).

This was same under 0.5 FC with the exception of the control (no sawdust) having a relatively higher mean stem girth to 25% sawdust.

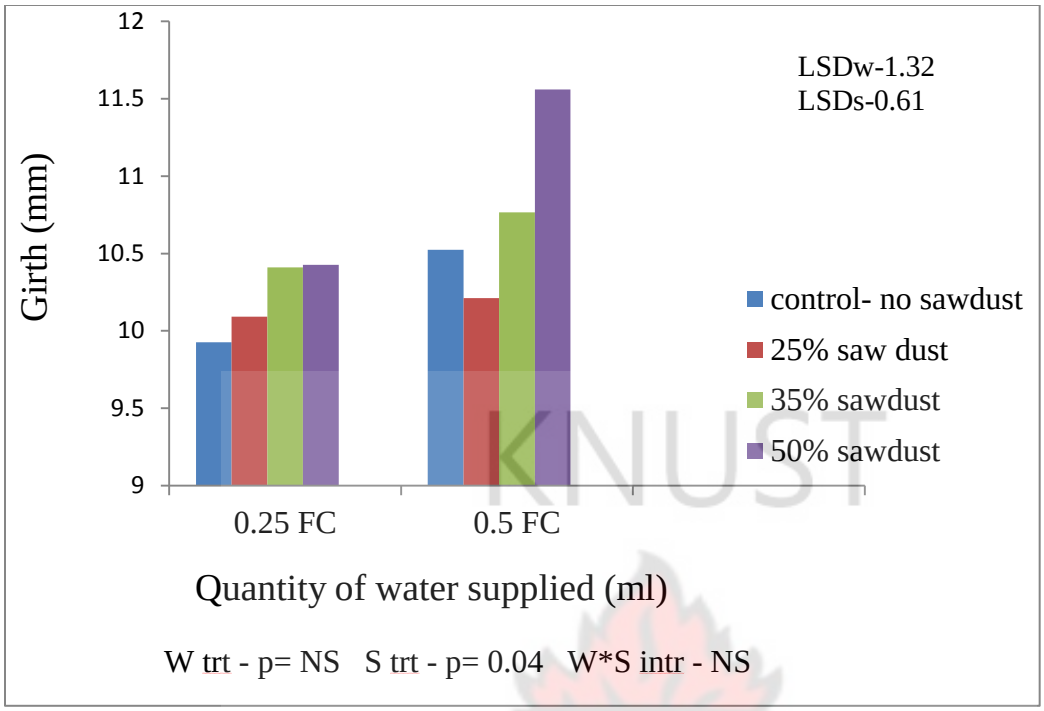


**Figure 8: Mean stem girth of cacao seedlings.**

## 4.8 PLANT HEIGHT

Water and sawdust had no interactive effect on plant height (P=0.581).

Also plant height was not significantly affected (P=0.672) under the two water treatments (0.25 FC and 0.5 FC). 0.5 FC recorded a mean plant height of 70.93cm whiles 0.25 FC recorded 69.19cm. Although a trend was observed in which increasing proportions of sawdust was associated with increases in plant height the differences were not statistically significant (P=0.795). Plants under the control (no sawdust), 25% sawdust, 35% sawdust and 50% sawdust had mean plant heights of 71.11cm, 68.7cm, and 70.19cm and 70.24cm, respectively.

Under 0.25 FC, whereas mean plant height consistently increased from zero sawdust to 35% sawdust it decreased at 50% sawdust (Figure 9). This shows that though increasing the proportion of sawdust increased plant height, the increase becomes minimal as the sawdust goes beyond a threshold.

Under 0.5 FC, plant height decreased when moving from the control (no sawdust) to 25% sawdust but increased henceforth to 50% sawdust. Plants under the control (no sawdust), 25% sawdust, 35% sawdust and 50% sawdust had mean plant heights of 73.75cm, 67.91cm, 70.8cm and 71.26cm, respectively.

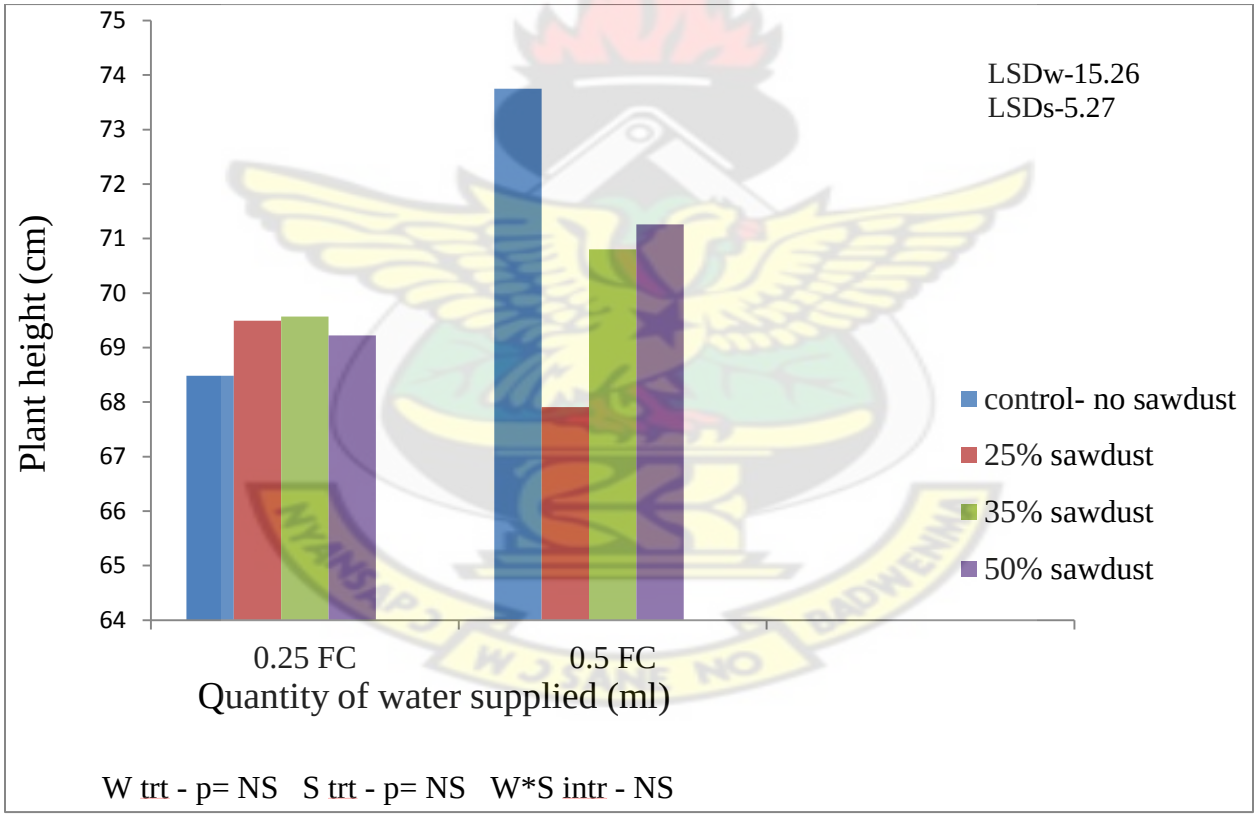


**Figure 9: Mean plant height of cacao seedlings.**

## 4.9 NUMBER OF LEAVES IN A LEAF FLUSH

There was a substantial difference (P=0.007) in the mean number of leaf flushes between the two water treatments. Increasing the quantity of water significantly increased the number of plant leaves. Plants under 0.5 FC had a higher leaf flush compared to plants under 0.25 FC.

Under sawdust treatment, there was no significant difference (P=0.162) amongst the sawdust treatments (zero sawdust, 25%, 35% and 50% sawdust).

Water and sawdust interaction had no effect (P=0.799) on leaf flush as shown in figure 10 below. The control had the highest leaf flush with 35% sawdust having the least for both 0.5and 0.25 FC.

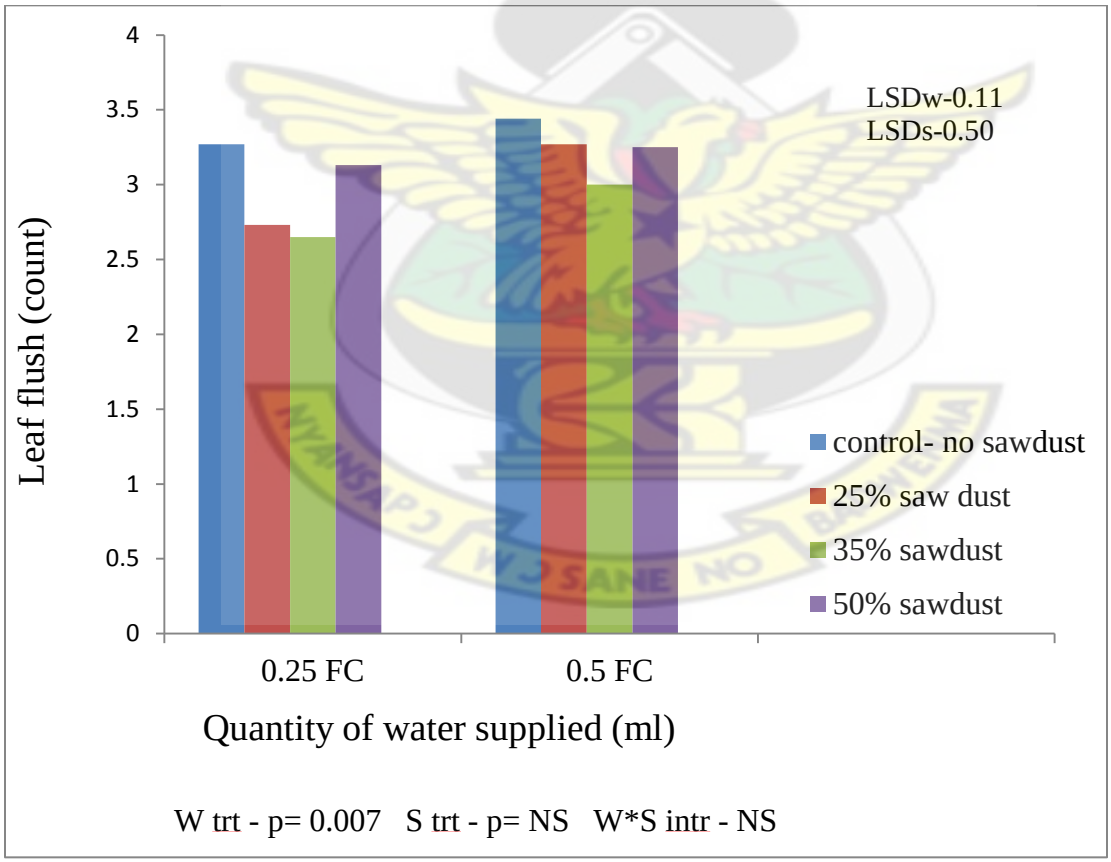


**Figure 10: Mean number of leaves in a leaf flush of cacao seedlings.**

## 4.10 LEAF AREA

Water had a substantial effect on leaf area of cacao seedlings under the two (2) water treatments (P=0.032). 0.5 FC recorded higher leaf area (11040cm²) compared to 0.25 FC (6199cm²). Increasing the quantity of water supplied increased the leaf area of cacao seedlings.

Under sawdust treatment, borderline significance (P=0.089) was observed between the control (no sawdust) and the sawdust treatments. This implies that any factor could trigger the value to be significant.

Figure 11 shows water and sawdust interaction on leaf area. No significant interaction (P=0.903) was observed.

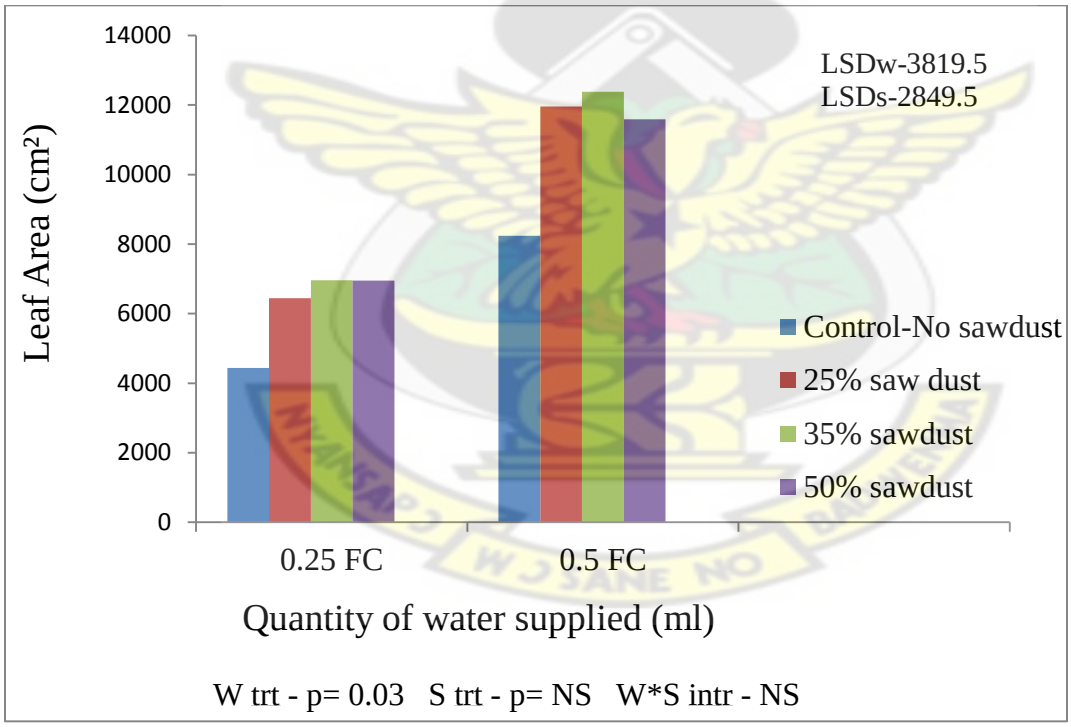


**Figure 11: Mean leaf area of cacao seedlings.**

## 4.11 DRY MATTER ANALYSIS

### 4.11.1 Leaf dry matter analysis

There was a significant difference in the mean dry weight of leaves between the two water treatments (P=0.02). 0.5 FC recorded a mean leaf dry weight of 45.1 g whiles 0.25 FC recorded 24 g. When water was increased, leaf dry matter increased.

Under sawdust treatment, significant difference was observed between the control and the sawdust treatments (P=0.038) but amongst the sawdust treatments, differences were insignificant. The control recorded the least leaf dry weight with 35% sawdust recording the highest. Increasing the amount of sawdust increased leaf dry matter, however when sawdust amount exceeds certain amounts, leaf dry matter is decreased.

Figure 12 shows the effect of water and sawdust on the mean dry weight of leaves.

Under 0.5 FC, although statistically no interaction existed (P=0.858), mean difference between the control and the sawdust treatments was substantial. Under 0.25 FC, there was an increasing order in the mean leaf dry weight from the control to 50% sawdust. This implies that, as sawdust increases leaf dry matter is also increased.

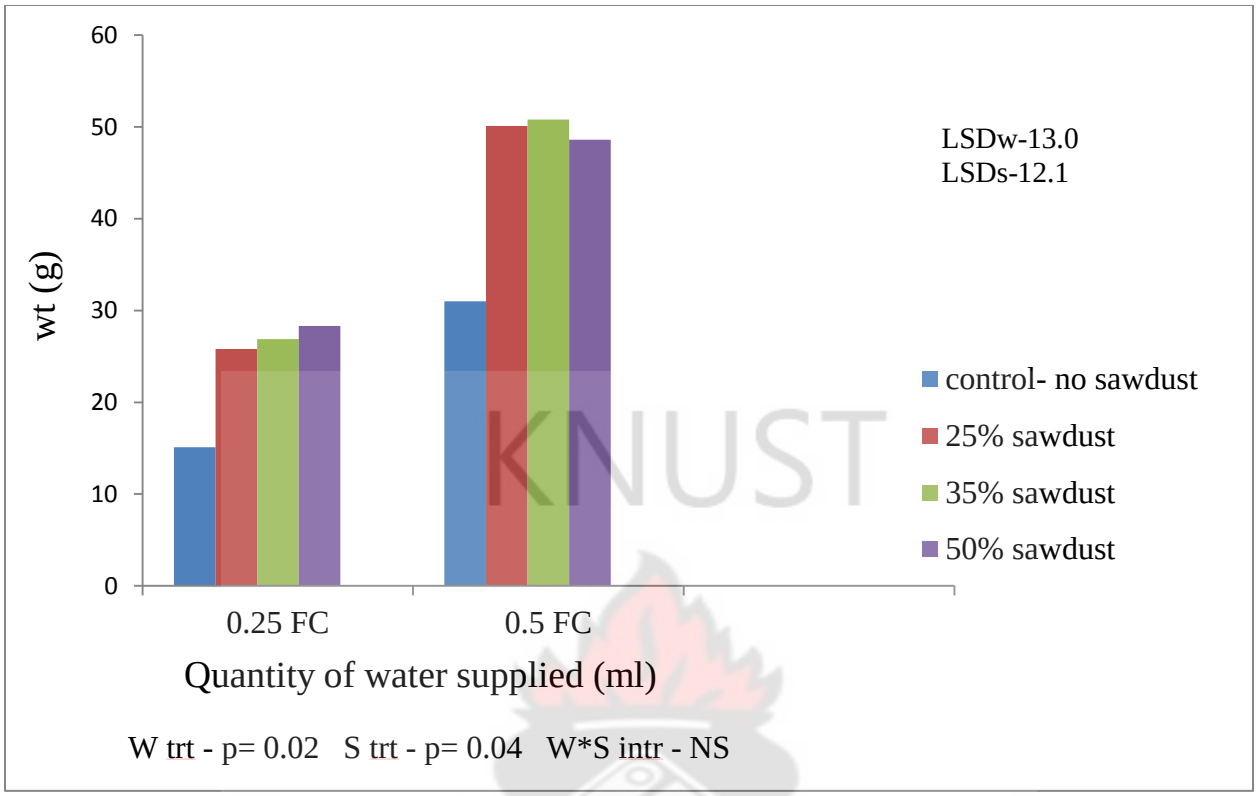


**Figure 12: Mean weight of leaves of cacao seedlings dried at 100°C for 24hrs.**

### 4.11.2 Stem dry matter analysis

There was a significant difference in the mean dry weight of stem between the two water treatments (P=0.036). 0.5 FC record a mean stem dry weight of 49.69g whiles 0.25 FC recorded 36.47g. Stem dry matter is increased with increasing soil water.

Under the sawdust treatment, a highly significant difference was observed between the control and the sawdust treatments (P=0.002) but differences amongst sawdust treatments were not significant. Stem dry matter increased with increasing sawdust amount. The control (no sawdust) recorded the least stem dry matter whiles 50% sawdust recorded the highest.

Figure 13 shows water and sawdust interaction on the mean dry weight of stem.

Under 0.25 FC, there was an increasing trend in the mean leaf dry weights from the control to 35% sawdust. 50% sawdust decreased slightly along the trend.

Under 0.5 FC, there was an increasing trend in stem dry matter from the control to 50% sawdust. When water and sawdust quantities are both increased, stem dry matter also increased.



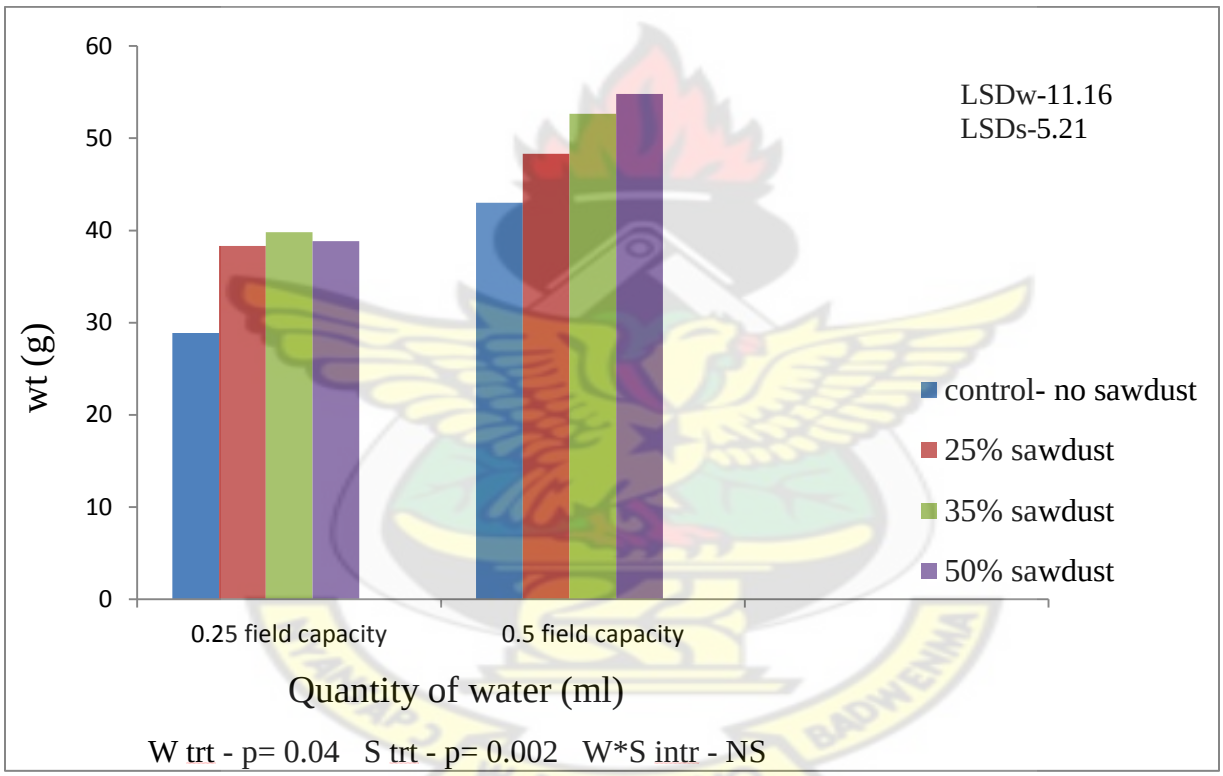


**Figure 13: Mean stem weight of cacao seedlings dried at 100°C for 48hrs.**

### 4.11.3 Root dry matter analysis

Water had a highly significant difference on the mean root dry weight between 0.25 FC and 0.5 FC (P=0.007). 0.5 FC had a higher mean root dry weight than 0.25 FC.

Under sawdust treatment, differences seen amongst treatments were not significant (P=0.255).

Figure 14 shows water and sawdust interaction on plant roots. An increasing trend in mean root weight from control to 50% sawdust was observed under 0.5 FC. Under 0.25 FC, mean root weights for the treatments were almost equal with the exception of 50% sawdust which had a slightly higher mean root dry weight. Mean differences for treatments under both 0.25 FC and 0.5 FC were statistically not significant (P=0.515). This shows that water and sawdust did not have any significant effect on plant roots.

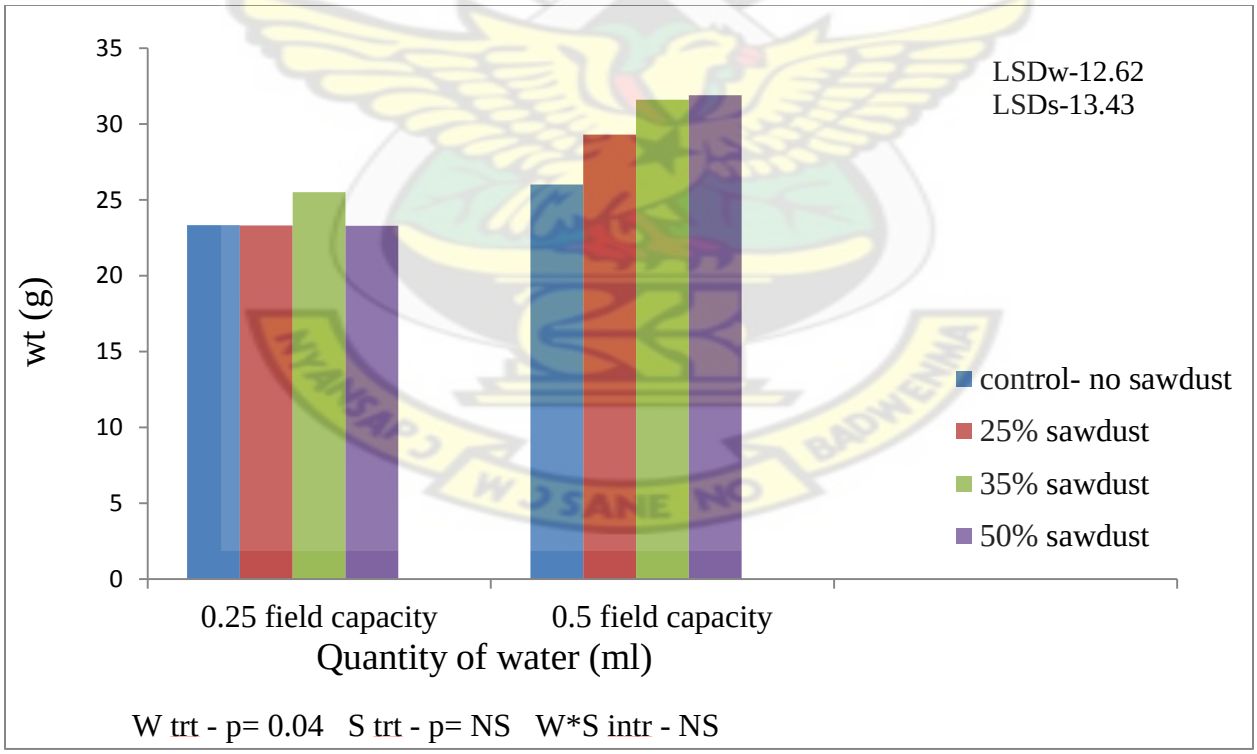


**Figure 14: Mean root weight of cacao seedlings dried at 100°C for 48hrs.**

# CHAPTER FIVE

# 5.0 DISCUSSION

## 5.1 CHLOROPHYLL CONTENT

The observation that leaf chlorophyll concentration was highest for the control (no sawdust) under low water supply (0.25F.C) suggests that probably soil nutrients were not leached under this condition and this made available nutrients especially N for leaf chlorophyll formation. This agrees with observations made on sesame, onions and wheat in which higher leaf chlorophyll was associated with lower soil moisture (Beeflink *et al.* 1985; Cartelat *et al.* 2005; Mensah *et al.* 2006; Nzokou and Cregg, 2010).

It appears that mixing sawdust with the soil reduced nutrient leaching to an extent. It is likely that the sawdust component increased the soil's water holding capacity and interfered with rapid soil water movement thereby preventing rapid leaching of soil nutrients. This is based on the observation that the 25% sawdust mixture had a higher leaf chlorophyll concentration than the control soil. However, it appears also that adding more than 25% sawdust introduces another means of rapid soil N depletion. Earlier work done on blueberry bushes has shown that microbe activity increases with increasing proportions of sawdust in a soil-sawdust mixture (Perry, 2010). It is also on record that high microbe activity leads to increased rate of soil N depletion (Barney and Colt 1991). That may explain why under high water supply (0.5 FC), 25% sawdust recorded higher leaf chlorophyll concentrations than the 35% and 50% sawdust mixtures.

## 5.2 TRANSPIRATION, STOMATAL CONDUCTANCE AND CHLOROPHYLL FLUORESCENCE

The results indicated that reduced supply of water was associated with marginal increase in stomatal conductance and transpiration rate for control (no sawdust) compared to sawdust treatment. The reason for the marginal increase in stomatal conductance and transpiration rate could be attributed to the fact that during the harsh dry season (December-January where relative humidity was as low as 29.895% and temperature as high as 38.177°C) the control plants had most of their leaves dried. Therefore, the total transpiration pull in such plants was very much reduced. This led to persistent soil water stagnation in the growing pots days after watering. Therefore, the remaining active leaves exerted more energy towards transpiration through increased stomatal opening to avoid a condition of suffocation.

It was observed that leaf chlorophyll fluorescence was significantly lower for the control treatment (no sawdust) compared to sawdust treatments under both watering regimes. Leaf fluorescence and plant photosynthetic activity are complementary; therefore an increase in one parameter should result in a decrease in the other (Baker, 2008). The lower leaf fluorescence observed under the control should have resulted in an increase in photosynthesis for control plants. However, the absence of sawdust and the inability of the control treatment to retain moisture resulted in water stress which affected photosynthesis and so although leaf fluorescence was low which should result in high photosynthesis, photosynthesis was also low under the control compared to sawdust treatments. This suggests that the addition of sawdust helped retain much moisture and enhanced the ability of the plants to make proper use of light energy because plants under sawdust treatments were not stressed. This observation agrees with work done by

(Bahar *et al.,* 2011) on grapevine where he recorded increased light use efficiency in less stressed plants compared to stress plants.

## 5.3 PHOTOSYNTHESIS

The results brought to the fore that increasing the quantity of soil water supply increased photosynthesis at the leaf level. This suggests that the stomata of leaves were more open to allow $CO_2$ absorption and the availability of water together aided or increased photosynthesis. Although increased leaf chlorophyll concentration and stomatal conductance under reduced water supply was observed for this experiment, the increased chlorophyll concentration and stomatal conductance did not increase photosynthetic activity under such treatment. Wood *et al.,* (1993) reported that leaf chlorophyll meter readings are essentially a measure of leaf greenness and therefore the greenness of leaves observed under the control did not mean its leaves would be effective in light absorption which would eventually increase photosynthesis. The low chlorophyll fluorescence observed for the control plants shows that although the control plant leaves recorded highest chlorophyll concentrations and were possibly capturing more light energy they had lower light use efficiency and this affected photosynthesis and total dry matter produced.

It was seen that sawdust treatments recorded higher photosynthesis than the control (no sawdust) under 0.25 FC. This may be due to the fact that, under reduced water supply the sawdust was able to retain soil water for plant use and prevent excessive soil surface water loss. The fact that sawdust treatments recorded higher photosynthesis than the control (no sawdust) is consistent with earlier observations made by Xu (2000) who observed higher photosynthetic rates in five-

week old water stressed maize seedlings after the soils were organically amended with poultry litter and agronomic yard waste compost.

The low photosynthesis observed in the control (no sawdust) under 0.25 FC again confirms earlier observations made in this experiment where control plants had most of their leaves drying and thereby reducing leaf chlorophyll fluorescence and eventually plant photosynthetic activity. Huck *et al.*, (1983) and Karamanos *et al.*, (1982) also reported low photosynthesis under stressed conditions in the leaves of cucumber plants that were studied. Findings in this study again agree with work done by (Ackerson and Herbert, 1981; Genty *et al.*, 1987) where low photosynthesis was observed in cucumber leaves under water stress conditions. Again the low photosynthesis observed under the control (no sawdust) could be attributed to the angle of leaf inclination during the dry season. It was seen that control plants which were more stressed had their leaves almost parallel to the stem (larger leaf angle) whereas plants under soils amended with sawdust which were less stressed had their leaves inclined at right-angle with the stem for maximum light absorption (smaller leaf angle). This observation agrees with earlier work done by Ehleringer and Forseth (1980) on *Lupinus arizonicus* and *Malvastrum rotundifolium* where under drought conditions, they reported that plants regulated their rate of photosynthesis by getting rid of excess light and this was done by preventing light absorption through heliotropism. The angle of inclination of the leaves ensured that plants under sawdust treatments had higher leaf chlorophyll fluorescence and this increased photosynthesis compared to the control plants.

## 5.4 LEAF AREA DEVELOPMENT

It was observed that the control (no sawdust) recorded the highest leaf flush under both watering regimes. The increase in leaf flush for the control was probably as a result of drying of leaves of plants under the control due to water stress which led to the formation of new leaves from the nodes of the stem thereby increasing leaf flush. The size of leaves under the control was however small compared to the leaves of plants that received 25% sawdust, 35% sawdust and 50% sawdust treatment. Although the control had the highest leaf flush, total leaf area measured for the control was least due to the small leaf size. Hutcheon (1977) explained that leaf production, leaf expansion, leaf fall, cambial growth, flowering as well as other cacao growth and yield parameters are all affected by the plant-water potential. He further explained that amount of water in plants and the availability of soil moisture for plant use is important for plant growth. The reduced leaf surface area for the control (no sawdust) could also explain why lower photosynthetic activity was observed under that treatment.

## 5.5 PLANT GROWTH

It appears that under increase water supply, plant physiological processes increase which eventually lead to an increase in plant vegetative growth. Keltjens and Nelemans (1998) noted that under water stress conditions, nutrient uptake decreases, P-availability becomes low and therefore growth is decreased.

It was observed that increasing sawdust proportion generally increased stem girth under both watering regimes. Katsuru (1987) reported that application of sawdust on tomato farms increased the vegetative growth of the plant compared to the control (no sawdust). The higher values

recorded for the organically amended soils (sawdust treatments) over the control indicated that the use of the soil amendment is of benefit to cacao production, but may require variation in the amount supplied from the results or mean values obtained. Akanbi (2002) reported that higher plant growth as a result of organic amendment application may be associated with the fact that the materials release considerable amount of nutrients for plant use.

It appears that under increase water supply (0.5 FC), the control recorded the highest plant height. This was due to the fact that plants under sawdust treatment jorquetted (branching at the apex of cacao plants) and increase in plant height reduces after jorquetting.

## 5.6 DRY MATTER PARTITIONING TO LEAVES, STEM AND ROOTS

The fact that increasing water supply and addition of sawdust to some treatments was associated with higher vegetative growth suggests that dry matter produced and partitioned to various plant parts was increased under such conditions. Reducing water supply affect normal plant activities and this reflected in the dry matter produced for the control and other sawdust treatments under 0.25 FC. The observation agrees with an earlier work done by Rajagopal *et al.,* (1989) where reproductive dry matter production was reduced under severe moisture stress.

It appears that the low leaf area observed under the control affected the total dry matter or assimilates produced and partitioned to various plant parts. The control recorded the least leaf and stem dry matter under both watering regimes. This observation is consistent with earlier observations made by Jones (1992) and Campbell and Norman (1998) who noted that the

development of crop leaf area is controlled by the amount of assimilates allocated to the leaves and determines radiation interception and assimilate production.

Brouwer (1983), Wilson (1988) and Setter (1990) also reported that drought stress mostly reduce leaf growth and increase at least relatively dry matter allocation into the root fraction, leading to a declining shoot/root-ratio. This confirms findings in this research where the control recorded the least leaf area but root mean dry matter was almost equal to that of sawdust treatments under 0.25 FC

The observation that soil temperature increased with reduced water supply suggests that under extreme water shortage, soil water viscosity, surface tension as well as soil biological processes are interrupted due to high soil temperatures. This observation to an extent explains the reason behind the low dry matter produced under the control and confirms work done by Willis *et al.,* (1957), Radke and Bauer (1961), Power *et al.,* (1970) and Cooper (1973) who reported that dry matter produced by tops and roots is materially increased by increasing soil temperature to an optimum level but decreases when temperature is further increased.

# CHAPTER SIX

# 6.0 CONCLUSION AND RECOMMENDATION

## 6.1 CONCLUSION

From the results of this study, the following conclusions could be drawn:

1. Topsoil+Sawdust media significantly retained moisture for seedling usage until the next watering period (10 days interval), which helped enhance seedling growth.

2. Though sawdust retained moisture, the amount retained differed due to differences in the sawdust proportions and quantity of water supplied.

3. The leaves of the plants without sawdust application dried up due to water stress whiles seedlings under sawdust treatments remained unaffected.

4. There was an increase in vegetative growth of cacao seedlings under sawdust treatments as a result of increased plant physiological activities compared to the control (no sawdust) and this was evident in the dry matter analysis performed.

## 6.2 RECOMMENDATION

Based on the findings of this study, it is recommended that

1. Sawdust from the tree species Emeri (*Terminalia ivorensis)* should be used as a soil amendment during the establishment of cacao to help retain moisture for plant use during adverse weather conditions (dry season).
2. It is recommended that under conditions or areas where water available or rainfall is less, more sawdust (35% sawdust) should be used and less sawdust (25% sawdust) should be used when rainfall or water available is quite enough.

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# APPENDICES

## ANALYSIS OF VARIANCE TABLES

### Appendix 1: Soil moisture

Analysis of variance

Variate: S_moisture

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 2 | 25.838 | 12.919 | 2.21 | |
| rep.water stratum | | | | | |
| water | 1 | 140.375 | 140.375 | 24.03 | 0.039 |
| Residual | 2 | 11.683 | 5.842 | 0.16 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 1490.78 | 496.927 | 13.54 | <.001 |
| water.s_dust | 3 | 88.398 | 29.466 | 0.8 | 0.516 |
| Residual | 12 | 440.55 | 36.712 | 5.64 | |
| rep.water.s_dust.*Units* stratum | | | | | |
| | 336 | 2186.324 | 6.507 | | |
| Total | 359 | 4383.949 | | | |

**Appendix 2: Chlorophyll content**

Analysis of variance

Variate: chl_Cont

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 2 | 20.19 | 10.1 | 0.73 | |
| rep.water stratum | | | | | |
| water | 1 | 3.19 | 3.19 | 0.23 | 0.678 |
| Residual | 2 | 27.69 | 13.84 | 2.25 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 119.82 | 39.94 | 6.5 | 0.007 |
| water.s_dust | 3 | 71.24 | 23.75 | 3.86 | 0.038 |
| Residual | 12 | 73.76 | 6.15 | 0.6 | |
| rep.water.s_dust.*Units* stratum | | | | | |
| | 480 | 4918.17 | 10.25 | | |
| Total | 503 | 5234.06 | | | |

## Appendix 3: Leaf fluorescence

Analysis of variance

Variate: leaf_flr

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 2 | 0.123372 | 0.061686 | 6.42 | |
| rep.water stratum | | | | | |
| water | 1 | 0.01572 | 0.01572 | 1.64 | 0.329 |
| Residual | 2 | 0.019217 | 0.009609 | 0.2 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 0.886979 | 0.29566 | 6.17 | 0.009 |
| water.s_dust | 3 | 0.015815 | 0.005272 | 0.11 | 0.953 |
| Residual | 12 | 0.575411 | 0.047951 | 4.93 | |
| rep.water.s_dust.*Units* stratum | | | | | |
| | 408 | 3.968162 | 0.009726 | | |
| Total | 431 | 5.604675 | | | |

## Appendix 4: Stomata conductance (gs)

Analysis of variance

Variate: gs

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 1 | 0.000138 | 0.000138 | 0.26 | |
| rep.water stratum | | | | | |
| water | 1 | 3.15E-05 | 3.15E-05 | 0.06 | 0.847 |
| Residual | 1 | 0.000527 | 0.000527 | 3.58 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 0.000249 | 8.29E-05 | 0.56 | 0.659 |
| water.s_dust | 3 | 0.00019 | 6.35E-05 | 0.43 | 0.738 |
| Residual | 6 | 0.000883 | 0.000147 | 2.07 | |
| rep.water.s_dust.Time stratum | | | | | |
| Time | 5 | 0.015873 | 0.003175 | 44.72 | <.001 |
| water.Time | 5 | 0.000386 | 7.71E-05 | 1.09 | 0.383 |
| s_dust.Time | 15 | 0.000565 | 3.77E-05 | 0.53 | 0.907 |
| water.s_dust.Time | 15 | 0.001074 | 7.16E-05 | 1.01 | 0.466 |
| Residual | 40 | 0.00284 | 7.1E-05 | | |
| Total | 95 | 0.022756 | | | |

## Appendix 5: Transpiration rate (E)

Analysis of variance

Variate: E

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 1 | 0.0172 | 0.0172 | 0.06 | |
| rep.water stratum | | | | | |
| water | 1 | 0.00025 | 0.00025 | 0 | 0.981 |
| Residual | 1 | 0.27041 | 0.27041 | 2.86 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 0.24749 | 0.0825 | 0.87 | 0.505 |
| water.s_dust | 3 | 0.05543 | 0.01848 | 0.2 | 0.896 |
| Residual | 6 | 0.5664 | 0.0944 | 3.45 | |
| rep.water.s_dust.Time stratum | | | | | |
| Time | 5 | 2.30586 | 0.46117 | 16.83 | <.001 |
| water.Time | 5 | 0.19023 | 0.03805 | 1.39 | 0.249 |
| s_dust.Time | 15 | 0.5263 | 0.03509 | 1.28 | 0.259 |
| water.s_dust.Time | 15 | 0.41753 | 0.02784 | 1.02 | 0.459 |
| Residual | 40 | 1.09594 | 0.0274 | | |
| Total | 95 | 5.69304 | | | |

## Appendix 6: Photosynthetic activity

Analysis of variance

Variate: A

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 1 | 0.0541 | 0.0541 | 0.04 | |
| rep.water stratum | | | | | |
| water | 1 | 0.8012 | 0.8012 | 0.65 | 0.567 |
| Residual | 1 | 1.224 | 1.224 | 7.21 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 0.157 | 0.0523 | 0.31 | 0.819 |
| water.s_dust | 3 | 0.4843 | 0.1614 | 0.95 | 0.474 |
| Residual | 6 | 1.0185 | 0.1698 | 0.52 | |
| rep.water.s_dust.Time stratum | | | | | |
| Time | 5 | 6.4307 | 1.2861 | 3.95 | 0.005 |
| water.Time | 5 | 1.4779 | 0.2956 | 0.91 | 0.485 |
| s_dust.Time | 15 | 2.9371 | 0.1958 | 0.6 | 0.856 |
| water.s_dust.Time | 15 | 3.8143 | 0.2543 | 0.78 | 0.69 |
| Residual | 40 | 13.0191 | 0.3255 | | |
| Total | 95 | 31.4181 | | | |

## Appendix 7: Girth

Analysis of variance

Variate: girth

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 2 | 16.491 | 8.246 | 0.81 | |
| rep.water stratum | | | | | |
| water | 1 | 32.868 | 32.868 | 3.25 | 0.213 |
| Residual | 2 | 20.249 | 10.125 | 2.42 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 48.359 | 16.12 | 3.86 | 0.038 |
| water.s_dust | 3 | 15.256 | 5.085 | 1.22 | 0.346 |
| Residual | 12 | 50.156 | 4.18 | 2.2 | |
| rep.water.s_dust.*Units* stratum | | | | | |
| | 408 | 774.343 | 1.898 | | |
| Total | 431 | 957.723 | | | |

## Appendix 8: Plant height

Analysis of variance

Variate: height

| Source of variation | d.f. | (m.v.) | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|---|
| rep stratum | 2 | | 8066.01 | 4033.01 | 2.23 | |
| rep.water stratum | | | | | | |
| water | 1 | | 435.57 | 435.57 | 0.24 | 0.672 |
| Residual | 2 | | 3623.58 | 1811.79 | 4.3 | |
| rep.water.sawdust stratum | | | | | | |
| sawdust | 3 | | 432.44 | 144.15 | 0.34 | 0.795 |
| water.sawdust | 3 | | 858.35 | 286.12 | 0.68 | 0.581 |
| Residual | 12 | | 5053.54 | 421.13 | 6.53 | |
| rep.water.sawdust .*Units* stratum | | | | | | |
| | 535 | -17 | 34517.42 | 64.52 | | |
| Total | 558 | -17 | 51956.55 | | | |

## Appendix 9: Leaf flushes

Variate: flush

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 2 | 20.79 | 10.395 | 134.33 | |
| rep.w stratum | | | | | |
| w | 1 | 11.161 | 11.161 | 144.23 | 0.007 |
| Residual | 2 | 0.155 | 0.077 | 0.02 | |
| rep.w.sd stratum | | | | | |
| sd | 3 | 20.101 | 6.7 | 2.04 | 0.162 |
| w.sd | 3 | 3.323 | 1.108 | 0.34 | 0.799 |
| Residual | 12 | 39.468 | 3.289 | 0.54 | |
| rep.w.sd.*Units* stratum | | | | | |
| | 480 | 2905.619 | 6.053 | | |
| Total | 503 | 3000.617 | | | |

## Appendix 10: Leaf area

Analysis of variance

Variate: leaf_area_cm

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 2 | 8797967 | 4398984 | 0.93 | |
| rep.water stratum | | | | | |
| water | 1 | 140577801 | 140577801 | 29.73 | 0.032 |
| Residual | 2 | 9456226 | 4728113 | 0.92 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 42377288 | 14125763 | 2.75 | 0.089 |
| water.s_dust | 3 | 2885121 | 961707 | 0.19 | 0.903 |
| Residual | 12 | 61574029 | 5131169 | | |
| Total | 23 | 265668433 | | | |

## Appendix 11: Leaf dry weight

Analysis of variance

Variate: leaf_dwt_g

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 2 | 94.08 | 47.04 | 0.86 | |
| rep.water stratum | | | | | |
| water | 1 | 2668.31 | 2668.31 | 48.71 | 0.02 |
| Residual | 2 | 109.57 | 54.78 | 0.6 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 1068.07 | 356.02 | 3.88 | 0.038 |
| water.s_dust | 3 | 69.74 | 23.25 | 0.25 | 0.858 |
| Residual | 12 | 1101.86 | 91.82 | | |
| Total | 23 | 5111.62 | | | |

## Appendix 12: Stem dry weight

Analysis of variance

Variate: stem_dwt_g

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 2 | 82.37 | 41.19 | 1.02 | |
| rep.water stratum | | | | | |
| water | 1 | 1049.4 | 1049.4 | 26.01 | 0.036 |
| Residual | 2 | 80.69 | 40.35 | 2.35 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 451.24 | 150.41 | 8.77 | 0.002 |
| water.s_dust | 3 | 28.47 | 9.49 | 0.55 | 0.656 |
| Residual | 12 | 205.82 | 17.15 | | |
| Total | 23 | 1898 | | | |

## Appendix 13: Root dry weight

Analysis of variance

Variate: root_dwt_g

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| rep stratum | 2 | 3.3 | 1.65 | 1.19 | |
| rep.water stratum | | | | | |
| water | 1 | 204.93 | 204.93 | 147.11 | 0.007 |
| Residual | 2 | 2.79 | 1.39 | 0.13 | |
| rep.water.s_dust stratum | | | | | |
| s_dust | 3 | 50.92 | 16.97 | 1.54 | 0.255 |
| water.s_dust | 3 | 26.63 | 8.88 | 0.8 | 0.515 |
| Residual | 12 | 132.39 | 11.03 | | |
| Total | 23 | 420.96 | | | |