INFLUENCE OF *Trichoderma harzianum* FROM SEMI-ARID SOILS ON MAIZE SEED GERMINATION AND EARLY SEEDLING GROWTH UNDER WATER STRESS

BY

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A THESIS SUBMITTED TO THE SCHOOL OF SCIENCE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY OF UNIVERSITY OF ELDORET, KENYA

2015

DECLARATION

Declaration by the candidate

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Declaration by supervisors

This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

To my parents, siblings and my best friends Francis A. Anyira and Janet Laboso

ABSTRACT

Water stress is one of the major constraints on plant productivity worldwide and is projected to aggravate with climatic changes while human population is at its exponential phase. On the other hand, *Trichoderma spp*. are plant symbionts that have been widely used as seed treatments to control diseases and to enhance plant growth and yield. However, few recent works have been published with regards to their abilities to improve abiotic stresses in plants. The present study sought to assess the effect of *T. harzianum* from semiarid soils on maize seed germination and seedling growth under water stress. *Trichoderma harzianum* from semiarid soils (Marigat rangeland) was isolated using macro and micro-morphological characteristics. The present study employed a three-factor factorial (3×4×4) design, arranged in a completely randomized design (CRD) with three replications. Three maize varieties (H614, H629 and H62 10) were treated with four concentrations of *T. harzianum* (0, $1x10^5$, $1x10^7$and $1x10^{10}$ spore/ml), and thereafter grown under 0, -0.3, -0.6 and -0.9 MPa osmotic potentials. Results showed that, 10^5, $10^7$and 10^{10} spore/ml concentrations of *T. harzianum* had a significant effect ($p<0.05$) on seed germination, seedling length, seedling fresh weight, root dry weight and shoot dry weight compared to control under water stress. Precisely, 10^7 spore/ml of the *T. harzianum* recorded maximum seed germination and seedling growth under all water stress levels. However, there were no significant ($p>0.05$) differences between 10^7 and 10^{10} spore/ml of *T. harzianum* in terms of maize seed germination and seedling growth. It was observed that under normal conditions (0MPa), *T. harzianum* did not enhance either maize seed germination or seedling growth. The activity of superoxide dismutase (SOD) and catalase (CAT) was also significantly enhanced by *T. harzianum* in all the three varieties of maize. Optimum SOD and CAT activity was recorded in seeds treated with 10^7 spores/ml of *T. harzianum*. Under normal growth conditions (0MPa), SOD and CAT activities were not enhanced by *T. harzianum*. However, under severe water stress (-0.9MPa), maximum activity of the enzymes was registered in all the three varieties of maize. It was also noted that the three concentrations of the *T. harzianum* (10^5, $10^7$and 10^{10} spores/ml) significantly promoted growth in all the three varieties of maize. However, in response to the fungus, there was no significant interaction ($p>0.05$) between maize variety and the concentration of *T. harzianum* in terms of seed germination and early seedling growth under water stress. Therefore, the study ascertained that the activity of *T. harzianum* was not dependent on the genotype of the maize plant. Taken together, the study recommends that for enhanced maize seed germination and seedling growth, 10^7 spores/ml of *T. harzianum* isolated from semi-arid soils should be used as seed treatment regardless of the maize variety, since the beneficial, activity of *T. harzianum* is not dependent on the maize plant variety.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABA	Abscisic Acid
ACC	1- Aminocyclopropane-1-Carboxylate
ACCD	1- Aminocyclopropane-1-Carboxylate Deaminase
ANOVA	Analysis of Variance
APX	Ascorbate Peroxidase
CAT	Catalase
DNA	Deoxyribonucleic acids
FAO	Food and Agriculture Organization
GOK	Government of Kenya
GP	Germination Percentage
GST	Glutathione-S-transferase
IAA	Indole Acetic Acid
IPCC	Intergovernmental Panel on Climate Change
LSD	Least Significant Difference
MDGs	Millennium Development Goals
Na_2CO_3	Sodium Carbonate
PDA	Potato Dextrose Agar
PEG	Polyethylene Glycol
POD	peroxidase
RDW	Root Dry Weight
ROS	Reactive Oxygen Species
S L	Seedling Length
SDW	Shoot Dry Weight
SFW	Seedling Fresh Weight
SOD	Superoxide Dismutase

ACKNOWLEDGEMENT

I thank God almighty who has brought me this far and provided me with the strength and endurance, knowledge and vitality that has helped me to finally come up with this thesis project and made it a reality. I would like to express my deepest gratitude to my supervisors, Dr. L.A. Mwamburi and Prof. E.K. Kiprop for their excellent guidance, patience, and providing me with conducive atmosphere for doing this research. I would like to thank the technical staff from Chemistry and Biological departments for their technical support.

I would also like to thank National Council of Science, Technology and Innovation (NACOSTI) for their financial support. Finally I am grateful to all people of goodwill that helped me in carrying out this project.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Agriculture is the mainstay of the Kenyan economy. Unquestionably, the long-term economic development blueprint for Kenya, the "Vision 2030", which is in agreement with the millennium Development Goals (MDGs), has identified agriculture as one of the economic pillars (Alila & Atieno, 2006). Maize (*Zea mays* L.) also known as queen of cereals is an important cereal crop grown all over the world (Verma *et al.,* 2012) and is central to Kenya's agriculture and food security. It is produced by 98% of the 3.5 million small-scale farmers, providing more than a third of caloric intake. Furthermore, it accounts for about 56% of cultivated land in Kenya (Kirimi *et al.,* 2011). Despite its economic importance, the production of maize crop has lagged behind human population growth, leading to a huge discrepancy between food supply and demand (Khush, 1999). In Kenya, the yields of maize are far below their potential. These low yields have resulted from numerous production constraints including abiotic factors such as recurrent adverse weather conditions like water stress, high temperature and salinity (Mohammed & Tarpley, 2009). These abiotic factors have been recently catalyzed by global climate change that has been observed over the past decades and is anticipated to continue in the future (IPCC, 2007).

Water stress or drought stress is an inevitable and recurring feature of global agriculture. It is one of the most devastating environmental stresses. Water stress limits growth and productivity of main crop species, reducing yields to less than half (Bayoumi *et al.,*

2008). Also it has been reported that, about one-third of the world's potentially arable land suffers from water shortage (Kramer 1980).

Seed germination is termed as the first stage of plant growth, and therefore this stage must perform well since effects that occur early in the life of the plant continue throughout the life of most annual plants. In addition, during this stage, plants have a high vulnerability to injury, disease and environmental stress (Rajjou *et al*., 2012). Lack of adequate soil moisture during planting, leads to; poor and unsynchronized seedling emergence, poor establishment of crop stand, a reduction in crop yield and/or total crop failure per unit area (Khan *et al.,* 2004). This effect has been seen even in maize crops, where the dehydration of seedlings was associated with the relative small size of their roots (Casanovas *et al.,* 2002).

Harman *et al.* (2004) showed that *Trichoderma spp*. are cosmopolitan fungi found in agricultural, forest, desert soils. They also colonize roots of various plants found in different ecosystems including maize. They have been defined as plant symbiont opportunistic avirulent organisms, able to colonize plant roots and to produce compounds that stimulate growth and plant defense mechanisms under suboptimal conditions (Harman *et al.*, 2004). *Trichoderma spp*. are the most common research tools as microbial inoculants which have been mostly used as biocontrol agents. However, in the recent years, they have become popular as plant growth promoters (Hermosa *et al*., 2012). For *Trichoderma* to effectively augment plant development, it must be able to establish in the spermosphere of germinating seeds, distribute on the emerging radicle and colonize the developing root (Orr & Knudsen, 2004).

Studies clearly show that seeds do respond to *T. harzianum* very early in germination, that is, before the radicle protrudes (Hermosa *et al*., 2012). Harman (2000) reported that adding conidia of T22 as a seed treatment profits the seed by enhancing phase III imbibition (cell elongation, followed by radicle protrusion). The author further revealed that response of seeds to the fungus is hasty and it is said to begin before the fungus penetrates into the living portions of the seed. In addition, when seeds were exposed to abiotic stresses, mainly water stress in the presence of T22 higher percentages of germination and improved seedling vigor was recorded than the untreated tomato seeds (Mastouri *et al.,* 2010). This is so because the fungus is said to induce the synthesis of auxin hormone in plants. This hormone plays a vital role in regulating the architecture of plant root system (Sofo *et al.,* 2012). Moreover inoculation of maize seeds with *Trichoderma spp.* affected root system architecture, which was related to increased yield of plants (Harman *et al.,* 2004). However, other studies show that *Trichoderma spp*. enhances seed germination by alleviating the damage caused by reactive oxygen species (ROS) (Shoresh *et al.,* 2010).

Harsh environments are one of the most challenging ecosystems for plants and microorganisms. Globally, many researchers are attempting to isolate microorganisms from unexplored sites, habitats and substrates, particularly in extreme environmental conditions (Ilyas *et al*., 2009). Water stress tolerance conferred by some fungal endophytes is through habitat-specific fungal adaptations (Redman *et al.,* 2002). Generally, when recognized in soils exposed to abiotic stresses, microorganisms become adapted to such stressed conditions thus developing tolerance and further they can be isolated and used as inoculum to support crops grown in correspondingly stressed

environments (Khan *et al*., 2012). In that connection, they can protect plants against harmful effects of different environmental stresses to which crop plants are sporadically exposed, such as heavy metals, flooding, salt and drought (Mayak *et al*., 2004). The present study sought to determine whether *T. harzianum* isolated from semi-arid soils had the potential to positively influence maize seedling emergence and growth under water stress.

1.2 Statement of the Problem

FAO (2009) points out that one major challenge towards global agriculture includes production of 70% more food crop for an additional 2.3 billion people by 2050 worldwide. In Kenya, agriculture has been identified as a key driver for achieving vision 2030. Nevertheless, abiotic stresses mainly water stress has been reported as a major stress limiting the increase in the demand for food crops. Moreover, Headey and Fan (2008) reported that water stress may reduce crop production by as much as 50%. Furthermore, maize crop which is central to Kenya's economy and food security is not grown under irrigation in most maize growing areas in Kenya. This means that maize relies nearly completely on rainfall during growth under both lowland and upland conditions. On the other hand, IPCC (2007) reported that global climate change is a pronounced challenge, and that precipitation patterns cannot be predicted with much confidence, with precipitation increasing in some regions and seasons, and decreasing in others. Moreover, projections of climate change will further exacerbate the ability to ensure food security and foster economic growth in maize producing areas (IPCC, 2007). Therefore, utilization of effective *Trichoderma spp.* to meet the needs of future

generations in light of climate change and population growth is of the topmost importance.

1.3 Justification of the Study

With rapid increase in human population, coupled with global climate change, there is need to devise a cheap and safe option to increase the production of food crops. Maize crop plays an essential part in providing food for millions in Kenya, and their role for food security will undoubtedly grow due to population growth and climate change. Use of *T. harzianum* in enhancing plant growth has been characterized as an innovative, cost-effective, less toxic and environmentally friendly means of increasing crop yields (Orr & Knudsen, 2004). Increasing the knowledge about the ability of *T. harzianum* in promoting plant growth precisely maize under stress is highly important as this will improve the performance of food and agriculture and will play an important role in agricultural production. Furthermore, the seed–*Trichoderma spp.* relationship contributes to the plant biotechnology sector by providing insight into a potential tool for increasing food security even under water stress. Besides, use of *Trichoderma spp.* as seed treatment is referred to as ‘’green” which is an alternative to Genetically Modified Organisms (GMOs) which have raised a lot of disagreements amongst scholars. Although *T. harzianum* has been reported to promote plant growth (Harman *et al.,* 2004; Shoresh *et al.,* 2006), few studies have been done on its ability to promote growth under water stress in plants (Mastouri *et al.,* 2010). As far as we know, the capacity of *T. harzianum* to enhance growth under water stress has not been investigated on maize. Therefore, the purpose of the study was to evaluate the effect of *T. harzianum* isolated from semi-arid soils on maize seed germination and seedling growth under water stress.

1.4 Objectives

1.4.1 Broad objective

To assess the influence of *Trichoderma harzianum* isolated from semi-arid soils on maize seedling emergence and growth under drought stress.

1.4.2 Specific objectives

1. To determine whether *T. harzianum* isolated from semi-arid soils has an effect on maize seed germination and seedling growth under water stress.
2. To determine the minimum and optimum concentrations of *Trichoderma harzianum* spores for enhanced seedling emergence and growth under water stress.
3. To determine the ability of *T. harzianum* to improve the activity of antioxidant enzymes of maize seedlings grown under water stress.
4. To find out whether the beneficial effect of *T. harzianum* in enhancing maize seed germination and seedling growth under water stress is dependent on the maize variety.

1.5 Hypotheses

1. There is no significant difference in maize seed germination and seedling growth in seeds treated with *T. harzianum* has no effect on maize seed germination and seedling growth under water stress.
2. There is no minimum and optimum concentration of *T. harzianum* for maize enhanced seed germination and seedling growth under water stress.

3. There is no significant difference in antioxidant enzymes activity between treated and untreated maize seedlings grown under water stress.
4. H_0: The beneficial effect of *T. harzianum* in enhancing maize seed germination and seedling growth under water stress is not dependent on the maize variety.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Trichoderma spp.*

Trichoderma spp. (Hyphomycetes) has been isolated from a wide range of environments, such as crops, native grasslands, forests, salt marshes, and desert soils of all climatic zones, as well as from lake water, dead plant material, and living roots of virtually every plant species examined. It is known to benefit many plant species by increasing shoot and foliar area, plant biomass, fruit production, the exploration capacity of the root system, and tolerance to water deficit and to low levels of photosynthetically active radiation (Harman *et al.*, 2004).

Trichoderma spp. has been well-known for years as biocontrol fungi; nevertheless, some strains are endophytic plant symbionts, while others are rhizosphere competents. As root symbionts, they establish chemical communication with plant which results in reprogramming of plant gene expression and changes plant physiology (Vargas *et al.*, 2009).

Trichoderma spp. is used as seed treatments to control diseases and to enhance plant growth and yield. However, recent work has revealed their abilities to alleviate abiotic stresses through induced systemic resistance that is mediated by alterations in plant gene expression (Shoresh & Harman, 2008). There also are reports of enhanced plant growth as a result of the association of *Trichoderma* strains with plants but the effects, as with other plant-growth-promoting microbes (Gamalero *et al.*, 2009) and these effects are greater when plants are under suboptimal conditions or biotic, abiotic, or physiological

stresses (Bae *et al.,* 2009). Yildirim *et al.* (2006) revealed that the fungi augments tolerance to abiotic stresses during plant growth via; improved root growth, improvement in water-holding capacity of plants, or enhancement in nutrient uptake (potassium and nitrogen), whereas, in the absence of stress, plant growth may or may not be enhanced.

2.2 Taxonomy of *Trichoderma spp.*

The genus *Trichoderma* are common soil saprophytic fungi found in all climates in the world. The genus is complex and polyphyletic. Nine "aggregates species" were accepted in the genus by Rifai, (1969) namely *Trichoderma aureoviride* Rifai, *T. hamatum* (Bonard) Bain, *T. harzianum* Rafai, *T. kongii* Oudem, *T. longibrachiatum* Rifai, *T. piluliferum* Rifai, *T. polysporum* (Link Fr.) Rifai, *T. pseudokongii* Rifai, *T. viride* Pers. 17.

He defined "aggregate species" as morphologically very similar and often hardly separable species. Bisset (1991), like Rifai adopted a morphological approach to taxonomy of *Trichoderma*. Bisset (1991) came up with a classification that recognizes four sections; *T.* section *Hypocreanum* Bisset, *T.* section *Longibrachiatum* Bisset, and *T.* section P*achybasium* (Sacc.) Bisset and *T*. section *Trichoderma*. An additional section, *T.* section *Saturnisporium* Yoshim has since been added (Samuels *et al.,* 2002). The sections were distinguished by differences in conidiophores branching patterns, phialides, and conidia. One of the main characteristics of *T*. section P*achybasium* is that phialides are clustered in heads often arising from a broad cell. *Trichoderma* section P*achybasium* has been divided into two distinct phylogenetic groups termed A and B (Kubicek *et al.,* 2003). *Trichoderma piluliferum* Webster and Rifai, *T. polysporum (*Link: Fr.) Rifai, *T.*

pubescens Bisset are grouped in A while *T. harzianum* Rifai falls into group B. Molecular work has substantiated a new species concept now being used in the genus and also led to the discovery of new strains (Zimand *et al.*, 1994).

2.3 Morphology of *Trichoderma spp.*

Since 1969, morphological characteristics have been used to characterize and distinguish *Trichoderma* species (Bissett *et al.*, 2003). Besides that, Samuels *et al.* (2002) also provided detailed observations on the morphological characters of defined species of *Trichoderma*.

2.3.1 Macroscopic features of *Trichoderma spp.*

Certain colony characters like growth rate, pigmentation, pustules formation and odours can be characteristics of a species. According to Samuels *et al.* (2002), majority of the *Trichoderma* cultures grow optimally at 25 to 30°C and typically minimally at 35°C. Yet, some species grow well at 35°C. This has served as an important distinguishing criterion between morphologically similar species. For example, *T. harzianum* can be distinguished from morphologically similar species such as *T. aggressivum* and *T. atroviride* by growing them at 35°C. After 96 hours, neither *T. aggressivum* nor *T. atroviride* can have colony radius more than 5 mm while *T. harzianum* grows well and sporulates at 35°C (Samuels *et al.*, 2002). Characteristics of mycelia development and pigmentation can be better observed in rich medium like Potato Dextrose Agar (PDA). *Trichoderma harzianum* bear colonies that are pale, thinly cottony, soon giving rise to white sporodochial tufts which turn green as conidia develop. Conidia are smooth, rounded and small in size usually a diameter of 3.6-4.5µm (Bisset, 1991).

2.3.2Microscopic features *Trichoderma spp.*

Trichoderma species usually form vegetative hyphae that are septate, hyaline and smooth-walled (Bissett *et al.,* 2003*).* Conidiophores are highly branched. Lateral side branches produced from main branches may or may not be paired, and sometimes may rebranch. Normally, the branches will form at or near 90° with respect to the main branch.

2.4 Mechanism of plant- *Trichoderma spp*. interaction

Research has shown that, the mechanism of plant drought tolerance involves the promotion of root extension, allowing an efficient water uptake (Ruiz-Lozano *et al.,* 2012). Thus, an alternate plant strategy for coping with water deficiencies is the interaction with beneficial soil microorganism.

Studies have shown also that, plants in nature do not function as independent individuals; however, they house diverse communities of symbiotic microbes. The role played by these microbes in plant development and protection cannot be overlooked. These symbiotic microbial interactions are substantial for the existence of both the host and microbe in both abiotic and biotic stressed environments. A good number of fungi are known to form a symbiotic interaction with various plants without causing disease. For example, mycorrhizas, *Piriformaspora indica,* a range of plant growth-promoting microbes and, the focus of this present study, *Trichoderma spp*. have been shown to form significant plant- fungi interactions (Shoresh *et al.,* 2010). For decades, these organisms have been known as agents that can control diseases in plants. Nevertheless, recent researches have clearly revealed other useful attributes of these organisms.

Harman *et al.* (2004) described *Trichoderma spp*. as plant symbionts. Many *Trichoderma* strains colonize roots of dicot and monocot plants. During this process *Trichoderma* hyphae coil around the roots, form appresoria-like structures (Figure 2.1), and finally penetrate the root cortex. *Trichoderma* grows intercellularly in the root epidermis and cortex and induces the surrounding plant cells to deposit cell wall material and establish chemical communication with the plant compounds. When inside plant roots, fungi have access to plant nutrients, which allow them to proliferate. Moreover, they significantly enhance root growth in many cases thus, providing more niches for growth of the fungi. On the other hand, the plant benefits from this relationship through increased root and shoot growth, increased macro- and micronutrient uptake, and protection from diseases (Harman *et al.*, 2004).

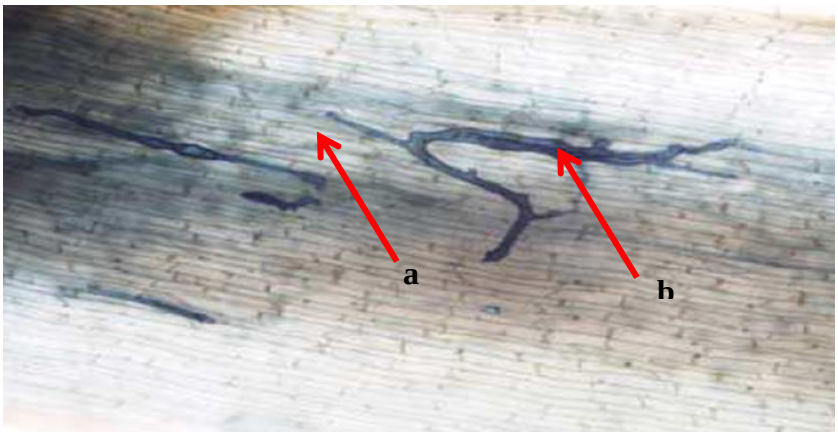

Figure 2.1: Roots of *Prunus* rootstocks (a) with *T. harzianum* (T22) appresorium (b) at 200 x magnification

(Source: Sofo *et al.*, 2011).

The chemical communicants (effectors/elicitors) produced by the *T. harzianum* include, small proteins, peptides and other metabolites, including volatile ones. Once the contact has been initiated, the beneficial responses to plants can last for at least the growing

season for an annual plant because the fungi grow and continue to colonize the roots as they, in turn, also grow and increase (Harman, 2000). There exist signaling that induces systemic effects in plants, so that while only roots are typically colonized, the effects also occur in leaves and in stems. The proteome and transcriptome of plants change as a consequence of the interaction of *Trichoderma* metabolites (Marra *et al.,* 2006) or plant colonization (Bae *et al.,* 2011). Thus, the fungi reprogramme plant gene expression, resulting in alteration of plant physiology and responses to their environment.

2.5 Water stress in plants

There exist a number of abiotic stresses that affect plants and other living organisms in the face of the earth. Examples of these stresses include; heavy metals, flooding, salt and drought. Among such abiotic stresses, drought is becoming more common where it sternly influences the crop yields (Hamayun *et al*., 2010). Agricultural water stress is the lack of ample moisture required for normal plant growth and development to complete the life cycle (Manivannan *et al.,* 2008). Water stress severely affects plant growth and development with substantial reductions in crop growth rate and biomass accumulation. The main consequences of water in crop plants are reduced rate of cell division and expansion, leaf size, stem elongation and root proliferation, and disturbed stomatal oscillations, plant water and nutrient relations with diminished crop productivity, and water use efficiency (WUE) (Li *et al,*. 2007).

Soil water deficit is normally the environmental factor in lots of natural settings that compels the furthermost hold back on plant growth (Wahbi & Sinclair, 2007). It sways more or less all aspects of plant physiology, biochemistry and growth metabolism and

thereby reducing yield (Li *et al.,* 2007). Decisively, Shao *et al.* (2008) pinpointed that sufficient availability of water was critical to growth and development of plants. Crucial changes in water homeostasis escort to osmotic stress, and are amid primary effects of water stress.

Plant microbe interactions intercede to the plant fitness in a variety of ways (Mascher, 2013). Beneficial, symbiotic interactions of plants with microbes can shield plants from biotic and abiotic stresses (Mascher, 2013). Microorganisms have the potential to alter the plant health status and productivity and can elevate crop yield to a remarkable level. The soil microbial communities have definite interactions with plants and can play remarkably important roles in plant growth and development. Microbial strains, isolated from arid or semi-arid soils have not been only well adapted to such environments, but also can abet plants to mitigate the effects of restricted water availability by improving the plant water status through amplified osmolytes production, when used as inoculants. *Trichoderma* is one such competent species that can bring about believable outcomes in context of plant growth promotion and augmenting the drought stress tolerance, when isolated from soils with low water content (Harman *et al.*, 2004).

2.6 Water stress creation using polyethylene glycol (PEG) 6000

Several experiments involving microbial isolates for agricultural use have been largely based on laboratory assays. Polyethylene glycol (PEG) has many applications and is widely used in laboratory studies since this compound has a high molecular weight, hence it cannot pass through the cell wall of plants and is therefore used to regulate water potential in germination tests (Gharoobi *et al.,* 2012). Establishing conditions of water

stress using PEG to create the osmotic potential is considered as one of the best methods to study the effects of drought stress on germination and early seedling growth parameters (Gharoobi *et al.,* 2012.The present study therefore used PEG 6000 to create water stress in maize seeds grown in the laboratory.

2.7 Effect of water stress on root and shoot growth

Several researches that have been done in the laboratory, greenhouse and field show that drought stress affects both root and shoot weight. In limited water availability root size and architecture are the factors which determine yield performance of plants (Price *et al.*, 2002). It was found that drought stress reduced fresh and dry shoot and root weight by 40 and 58 %, respectively. Also, drought stress decreased the length and fresh weight of shoot in maize (Thakur and Rai, 1984). Wu and Cosgrove (2000) observed that the root-shoot ratio of plants is enhanced under limiting availability of water. The increase in ratio was due to the reason that roots are comparatively less susceptible to water deficit condition than shoots growth. Furthermore, *in vitro* findings showed that, the adaptation to low water potential was due to the role of xyloglucan endotransglcosylase, peroxidase and some other wall enzymes in roots of plants. Hu *et al.* (2007) reported that drought and salinity stress decreased the seedling fresh weight and these results were also same for maize crop plants. Another study by Price *et al.* (2002) demonstrated that root size and architecture are factors that determine yield performance of plants under water stress condition. Therefore, measurements of dry and fresh weights of roots and shoots are used to depict the effect of the stress on plants. However, root dry weight was identified as the major criterion for selection of maize genotypes under drought conditions. Studies show that microbes are also known to be affected by abiotic stress conditions. However,

successful deployment of these organisms in stressed ecosystems depend on their ability to withstand and proliferate under adverse environments such as drought, high temperatures, salt stress, mineral deficiency, chemical and heavy metal toxicity which are major problems in rain fed agro-ecosystems (Price *et al.*, 2002).

Several studies have shown that under water deficit conditions *Trichoderma spp*. improve seed germination, seedlings' length and weight (Bae *et al*., 2009). Furthermore, *Trichoderma spp.* enhanced the architect and the morphology of tomato root (Mastouri *et al.,* 2012). Also experimental studies on rice showed that *Trichoderma spp*. enhanced root and shoot growth of the plant, however, the degree of augmentation differed with the plant genotype and the fungal concentration (Gusain *et al.,* 2014). In addition, under conditions of water deficit in presence of *Trichoderma spp.* increased the wheat plant fecundity and root volume to levels similar to those observed under conditions of no abiotic constraints (Donoso *et al.,* 2008). *Trichoderma spp*. stimulated lateral root development and reduced primary root length in rice plant by producing indole-3-acetic acid (IAA) and auxin-like compound (Tucci *et al.*, 2011). Another strain of *T. atroviride* stimulated tomato root growth in a 'controlled manner' by balancing the synthesis and degradation of IAA and/or by limiting Ethylene synthesis through hydrolysis of its precursor molecule 1-aminocyclopropane-1-carboxylic acid (ACC) (Gravel *et al.,* 2007). An experiment on soybean plant showed that, seeds treated with T22 had larger roots than similar seedlings in the absence of T22 (Figure 2.3). Thus, if added as a seed treatment, the best strains colonized root surfaces even when r oots were a meter or more below the soil surface (Harman *et al.,* 2004).

Figure 2.3: Root colonization of soybean by the rhizosphere competent strain *T. harzianum* T22. (**Source: Harman *et al.*, 2004)**

2.8 Habitat-adapted symbiosis

Microbial strains, isolated from arid or semi-arid soils have not only adapted well to such environments, but can also help plant alleviate the effects of restricted water availability by improving the plant water status through amplified osmolytes production, when used as inoculants. *Trichoderma harzianum* is one such competent fungi species that can bring about incredible outcomes in context of plant growth promotion and augmenting the drought stress tolerance, when isolated from soils with low water content. Studies have shown that, plant growth promoting microbes that have been isolated from water stressed conditions mitigate drought effects in plants (Mayak *et al.*, 2004; Bano *et al.*, 2013).

The model that fungal symbionts acclimatize to stress in a habitat-specific way has been established with different fungal and plant species, and different environmental stresses (Rodriguez *et al*., 2009). In the authors experiment carried out both in laboratory and field, they found out that endophytes from stressed habitats confer habitat-specific stress tolerance to plants. Most *Trichoderma spp*. have the ability to overcome extreme

environments hence their presence in very diverse geographical locations (Hermosa *et al*., 2004). Due to their ubiquity and rapid substrate colonization, they are commonly used as biocontrol and plant growth promoters under different environmental conditions. Therefore, the present study hypothesize that *Trichoderma spp*. isolated from arid areas has an immense potential in influencing maize seedling emergence and growth under drought stress.

2.9 Mechanisms employed by *Trichoderma spp*. in alleviating water stress in germinating seeds and seedlings

In their study, Donoso *et al.* (2008) pointed out that there exist very little information concerning the way in which the fungus enhances plant seed germination and seedling growth to water deficit. Nevertheless, since the interaction between the plant and the fungus happens primarily at the rhizosphere, such a mechanism is probably related to an increase in the water absorption effectiveness, which apparently is related to the increased root volume leading to increased water absorption.

Earlier on, *Trichoderma spp*. have been shown to efficiently help plants overcome abiotic stresses, such as salinity and drought, in both field crops and trees (Yildirim *et al.*,2006). Water stress has been reported to be the most important stress factor in the field. *Trichoderma harzianum* has been added as seed treatment mainly in tomatoes or as a soil treatment (*Arabidopsis*) and has improved the germination at osmotic potentials of up to 0.3 MPa (Mastouri *et al.,* 2010). Plants grown from these *Trichoderma* treatments proved to be much more resistant to water deficit conditions. The effects of *Trichoderma* treatments are pretty large and perhaps account for at least a significant part of the

increase in growth of *Trichoderma*-treated versus untreated plants in the field. The capacity of maize plants grown from seeds treated with *T. harzianum* to counterattack water deficit has been demonstrated both in the laboratory and field, and the improved rooting system evidently pays (Harman, 2000). Furthermore, cocoa seedlings inoculated with *Trichoderma spp*. resulted in delayed drought-induced changes such as stomatal closure and reduction of net photosynthesis under drought compared with control plants, permitting plants to stay growing (Bae *et al*., 2009). Mechanisms employed by *T. harzianum* in enhancing growth in plants under water stress are summarized in Table 2.1.

Table 2.1: Summary of the mechanisms employed by *Trichoderma spp*. in alleviating water stress in germinating seeds and seedlings

Mechanisms of *T. harzianum* in alleviating water stress in plants	**Plant-type(s)**	**References**
Amelioration of oxidative damage induced by stress	Cucumber Panic grass Tomato Rice	Bjorkman, 1998 Rodriguez *et al.,* 2009 Mastouri *et al.,* 2010 Gusain *et al.*, 2014.
Enhance the secretion of plant hormones.	Maize Soybean Melon Arabidopsis Thaliana and Prunus	Harman, 2000 Harman *et al.,* 2004 Christmann *et al.,* 2006 Contreras-Cornejo *et al.,* 2009 Sofo *et al.,* 2010.
Increase water use efficiency (WUE)	Dune grass	Sherameti *et al.,* 2005; Rodriguez *et al.,* 2009
Enhance photosynthetic capability and/or efficiency.	Maize Bean Cocoa	Harman, 2000 ; Han & Lee, 2005 Harman& Shoresh, 2008 Bae *et al.,* 2009

Among the above mentioned mechanisms, enhanced production of phyto-hormones has been reported to be the most researched mechanism (Chowdappa *et al.,* 2013). Linkies *et*

al. (2009) shows that auxin, cytokinin, abscisic acid, and ethylene are major players in plant growth and in fitness. IAA is the most abundant naturally occurring auxin in vascular plants, and it is known to play a major role in lateral and adventitious root initiation and emergence and in shoot development (Simon and Petrasek, 2011). In a study conducted by Martinez-Medina *et al.* (2014), IAA levels were shown to be increased by the *Trichoderma* isolates that also promoted plant growth. According to Martinez-Medina *et al.* (2011) another growth-promoting strain *T. harzianum* T-78 is said to have the capacity to increase IAA levels in plant. Another study by Contreras-Cornejo *et al.* (2006) demonstrated that *T. virens* promote growth of Arabidopsis thaliana through the classical auxin response pathway, due to production of IAA and auxin-like compounds. A good number of *Trichoderma* isolates have been reported to produce and release auxin-related compounds in culture medium (Gravel *et al.,* 2007). However, Sofo *et al.* (2012) did not detect hormone production by *T. harzianum* T-22 in axenic growing media, suggesting that T-22 is able to induce hormone synthesis *ex novo* in the plants, probably through the up-regulation of plant genes for hormone biosynthesis or the down-regulation of the genes involved in hormone catabolism. Abscisic acid has been related to plant development and defense against abiotic stress. It is known to modify growth and development under stress conditions, such as cold, drought, and salinity (Christmann *et al.,* 2006). On the other hand, Martinez-Medina *et al.* (2011) elucidates that *Trichoderma* isolates that promoted plant growth also decreased the concentration of ABA in the melon shoots, therefore, suggesting that the promotion of plant growth provided by these *Trichoderma* isolates might be accompanied by an improvement in plant fitness, as has been suggested for mycorrhizal associations regarding drought stress (Aroca *et al.*, 2013).

2.10 Ability of *Trichoderma spp.* to enhance the activity of antioxidative enzymes in plants under water stress

It is well documented that water put forth, at least part of their effects by causing oxidative damage. Oxidative damage is caused by reactive oxygen species (ROS) that can react with a large variety of biomolecules causing irreversible damage and leading to cell necrosis and death (Rivero *et al.,* 2007). Since ROS are toxic but at the same time participate in signaling events, living organisms are equipped with at least two different mechanisms to regulate their intracellular ROS concentrations by scavenging ROS: one enabling the fine modulation of low levels of ROS for signaling purposes [peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT)], and one regenerating the oxidized antioxidants (ascorbate peroxidase (APX) (Mittler, 2002).

Plants develop a variety of mechanisms to acclimatize themselves to forever changing environments. These mechanisms are facilitated through multiple signal transduction pathways acting in a global signal network (Yu *et al.,* 2012). *Trichoderma spp*. colonizes roots and remains restricted to the cortex and outer layers of the root epidermis of host plant. However, it modulates gene expression in both shoots and roots (Yedidia *et al.,* 2001). When plants are subjected to abiotic stresses, the content of reactive oxygen species may increase to toxic concentrations. Several pathways in plants convert toxic ROS to a lesser toxic form (Mittler, 2002). On the other hand, *Trichoderma* strains enhance the activity of these pathways, through enhanced expression of genes encoding the component enzymes (Mastouri *et al.,* 2010). For example, if these pathways are enhanced in the chloroplasts, then it is expected that the photosynthetic efficiency will

increase by reducing damage by the superoxide anion and other reactive species involved in photosynthesis (Bae *et al.,* 2009).

Sharma and Dubey (2005) demonstrate that under drought stress condition ROS like superoxide radical, hydrogen peroxide and hydroxyl radicals greatly affect the membrane and DNA of cells. Mittler (2002) also showed that under severe stress, ROS production can surpass the scavenging aptitude and amass to levels that can harm cell components for example, through lipid peroxidation. *Trichoderma spp.* augments protection against ROS perhaps by increasing ROS scavenging capacities. Proteomics of roots inoculated with *Trichoderma* showed an increase in levels of anti-oxidative enzymes mainly Superoxide dismutase (SOD) as well as increased levels of peroxidase, glutathione-reductase and Glutathione-S-transferase (GST), and other detoxifying enzymes in leaves (Shoresh and Harman, 2008). Superoxide dismutase is the main scavenger of superoxide radicals,which converts the toxic superoxide ($O2^{-}$) to hydrogen peroxide and oxygen, through a process called *dismutation reaction*: $2O_2^{-} + 2H+ \longrightarrow H_2O_2 + O_2$. The enzyme embodies the first line of cell defence against ROS generated abiotic stresses like drought in plants, therefore, preventing the tissue damage due to oxidative stress. Blokhina *et al.* (2003) explained that CAT and POD enzymes are able to convert toxic H_2O_2 to water and oxygen. under water stress only elevated SOD activity cannot protect the plants from toxic effect of oxygen free radical hence CAT and POD is needed to remove toxicity of H_2O_2 (Arora *et al.*, 2002).

Furthermore, Rodriguez *et al.* (2009) have shown that, when both symbiotic and nonsymbiotic plants were exposed to abiotic stress; panic grass and tomato were subjected to heat stress while dune grass and tomato were subjected to salt stress, the

results showed that in the absence of stress, both nonsymbiotic and symbiotic plant leaf tissues for all remained green demonstrating the absence of ROS generation and therefore lack of stress response. On the other hand, when these plants were exposed to stress, nonsymbiotic tissues bleached white indicating the generation of ROS while symbiotic tissues remained green. This suggests that plant symbionts aid either in scavenging ROS or inducing plants to more efficiently scavenge ROS, or prevents ROS production when exposed to abiotic stress.

An earlier experiment conducted by Bjorkman (1998) showed that those seeds that were subjected to oxidative stress had much reduced vigor. Nevertheless, subsequent treatment with *Trichoderma*-T22 restored their vigor. Furthermore, above and beyond, peroxidase gene was primed in cucumber plants inoculated with *Trichoderma* (Shoresh *et al.*, 2006). Another study by Mastouri *et al.* (2010) clearly revealed that treating seeds of tomato with *T. harzianum* T22 enhanced germination percentage under osmotic stress. The study further found an increase in lipid peroxide content in young seedlings with an increase in the water potential of media, whereas T22- treated seedlings had significantly less lipid peroxide than untreated seedlings. In a recent experiment performed by Gusain *et al.* (2014), *T. harzianium* (T-35) benefited rice plants by increasing their tolerance to severe drought stress through the reduction of oxidative stress by enhancing the production of SOD, CAT and POD anti-oxidative enzymes.

2.11 Variation of beneficial effects of *Trichoderma spp*. by the plant genotype

There is no doubt that in both academic research and commercial practice, *T. harzianum* has been revealed to increase seedling emergence and growth in maize and numerous

other plants. The effect of the fungus has been reported to last for the entire life of most annual plants. However, Harman *et al.* (2004) found out that, there was a strong genetic component to the yield and plant-growth enhancement that is drawn out by *T. harzianum* strain T-22. The study using maize plant further revealed that inbred line Mo17 responded to T-22 most, other lines responded only weakly, while a few in fact showed a reduction in growth and yield. This difference in response is owed to variation in the transcriptome or proteome level. Tucci *et al.* (2011) through an experiment on tomato seedlings revealed that the level of seed germination and seedling growth stimulation was mostly dependent on the tomato genotype, signifying that the response to *Trichoderma* spp. was genetically controlled. In the same study most lines responded to *T. harzianum*. Genetic analysis has demonstrated that the maize response is largely conditioned by dominant genes (Harman, 2006). Moreover, Liu *et al.* (1995), further revealed that genetic background affected the response of different cucumber varieties to PGPR species which really are considered to share with *Trichoderma* spp. similar mechanisms of (ITR) (Harman *et al*., 2004).

2.12 Related studies on the beneficial effect of *Trichoderma spp*. in seed germination and seedling growth under water stress

Under optimum conditions, seeds absorb water leading to activation of metabolic processes which result in germination. However, disturbances in seed germination are experienced when compounds with low osmotic properties are present (Hardegree and Emmerich, 1990). These compounds reduce the osmotic potential. Inadequate moisture led to decrease in maize seed germination rate and percentage (Gupta *et al.*, 2003). An experiment on vetch showed that water stress decreased all the germination features (De

and Kar, 1995). Water stress also decreased seed germination percentage and the length of radicle and plumule (Gharoobi *et al.,* 2012). Furthermore, the findings from the study showed that, osmotic potential of -0.50 reduces germination percentages of corn see ds. This led to a conclusion that osmotic potential of -0.40 was the borderline for the reduction of germination features in seeds.

On the other hand, studies have also showed that, *Trichoderna spp*. are utilized worldwide to enhance seed germination and seedling growth even in optimal growth conditions. However, the response is highly positive under suboptimal conditions (Mastouri *et al*., 2010). *Trichoderma harzianum* was shown to enhance germination percentage in chili seeds both in laboratory and field conditions (Asaduzzaman *et al.,* 2010). Experiments on bitter gourd, loofah and cucumber also showed that *Trichoderma* strains significantly increased from 26 to 61 % in seedling height, 85-209 % in root exploration, 27-38% in leaf area and 38 to 62 % in root dry weight after 15 days of sowing Chaur-Tsuen Lo and Chien-Yih Lin (2002). Recently, *Trichoderma spp.* was shown to significantly increase millet seedling height, root length and root dry weight (Hassan *et al.,* 2014). A similar study on tomato showed that *T. harzianum* T22 enhanced the speed of germination. This means that, those seed treated with T22 germinated faster and more uniformly compared with untreated seed at all water stress levels (Mastouri *et al.,* 2010). Colonization of cocoa seedlings by *T. hamatum* isolate resulted to a delay in many aspects of the drought response (Bae *et al.*, 2009). The contributing factor to this was hypothesized to be through enhanced root growth, resulting in an improved water status allowing cacao seedlings to tolerate drought stress. Studies have also shown that *T. harzianum* when added as seed treatment in tomato plant

or as a soil treatment in *Arabidopsis* mainly enhanced the germination at osmotic potentials of up to 0.3 MPa (Mastouri *et al.,* 2010). Furthermore, microbial strains isolated from soils with moisture stressed conditions have even more potential to induce tolerance to host plant, when inoculated (Ilyas *et al*., 2012).

Traditional selection methods and genetic engineering are some of the methods that have been used in curbing water stress in plants (Fleury *et al.*, 2010). However, water stress tolerance has been shown to be a composite trait, and finding of suitable phenotypes is difficult. Even though, engineering technology has been reported to be an environmentally friendly strategy in improving crop tolerance to abiotic stresses, there are several factors to be considered for this method to be effective hence, a longer time is needed to implement it yet the global population is rapidly growth. Therefore, there is need to utilize other effective and cheap methods to address the problem of food insecurity.

Although seed treatment with *Trichoderma spp.* provides an innovative, cost-effective, less toxic and environmentally friendly means of increasing crop yields through improving seedling emergence and growth under drought stress, to date; related studies on maize are very few. Curiously, the effect of fungi from adverse environments is an issue that remains to be addressed. Thus, the system composed of *Trichoderma spp.* from semi- arid soils and maize seedling was worth carrying out so as to explore the influence of the fungus on maize seedling emergence and growth under drought through laboratory analysis of some maize plant growth parameters such as germination percentage, root and shoot elongation, as well as fresh and dry weight of roots and shoots. Seedling length and weight are the most important parameters for drought stress because roots are in

direct contact with growing medium and absorb water from it and shoots supply it to the rest of the plant (Jamil and Rha, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Soil samples were collected from the semi- arid rangeland of Marigat area, Baringo County. Much of the region receives low to average annual rainfall. Rainfall variability is very high in Marigat area with only one rainy season from April to August, and a prolonged dry season. Rainfall patterns are strongly influenced by local topography. Long-term average annual rainfall ranges from 600mm in the lowland flats to 1000-1500mm in the highlands (GOK, 2009).

Marigat is located between latitude 00^{o} 26-00^{o}32‘N and longitude 36^{o} 00‘36^{o} 09' E. The climate is semi-arid with an average altitude of 900m above the sea level and a mean annual temperature of 25.7- 31.4^{0} C (Wasonga, *et al.*, 2011).

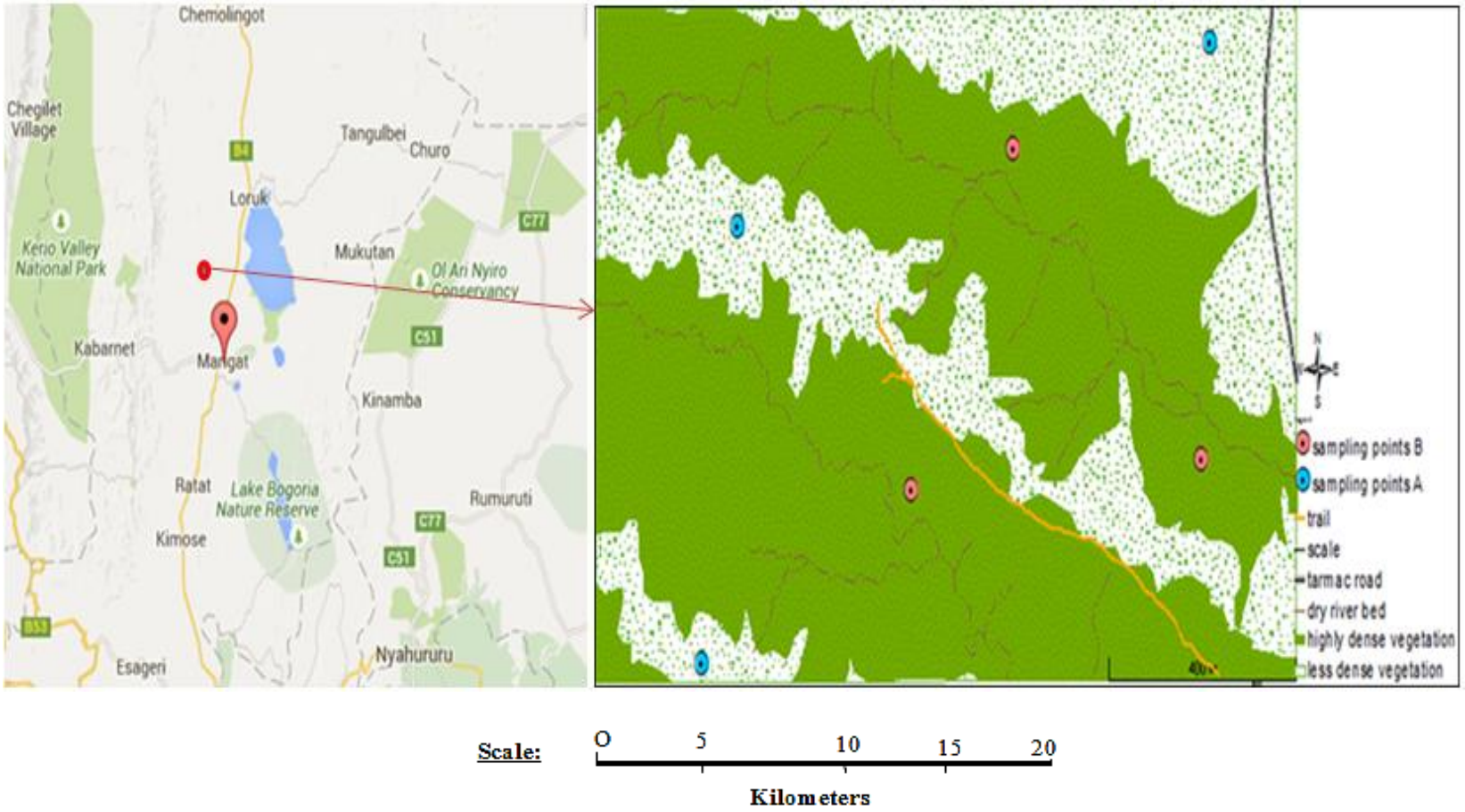

Figure 3.1: Map of the study area (Marigat rangeland. (Source: Author, 2015; Google Maps, 2015)

3.2 Collection of soil samples

Soil samples were collected in January 2014 from 6 sites of Marigat rangeland. The rangeland was divided into two plots (A and B) based on the density of vegetation. Plot A was characterized by low density of vegetation while plot B was categorized by high density of grass plants (Fig. 3.1). A total of six soil samples 10g each were collected, of which three of the six soil samples were randomly obtained from rhizosphere of grass plants in plot A. The other three samples each weighing 10g were randomly obtained from bare soil in 10cm depth under plot B using a sterile soil auger. All the six different soil samples were then transferred into sterile polyethylene bags and transported to the laboratory of Microbiology, University of Eldoret within 24 hours of collection. These samples were used for isolation of *T. harzianum*.

3.3 Isolation of T. *harzianum* from the soil

Isolation *Trichoderma harzianum* from soil was done by using a modified method of Papavizas & Lumsden (1982). Ten grams of the six different soil samples were thoroughly mixed together to make a composite and thereafter made up to 1000ml using sterile distilled water in a sterile conical flask. The soil suspension was left for one hour at room temperature to release conidia and hyphae adhering to soil particles. Serial dilutions up to 10^{-3} were prepared. One ml aliquots were spread-plated onto Potato Dextrose agar (PDA) medium supplemented with 50 mg/l of streptomycin antibiotic to inhibit bacterial growth. The plates were then incubated at 28°C and 35^{o}C for seven days. Distinct colonies of *T. harzianum* were picked based on their on their morphological

characteristics as described by Rifai (1969). Commercially available T22 was used as a confirmatory isolate to ensure that the isolated spp. of *Trichoderma* was *T. harzianum*. To obtain pure cultures of *T. harzianum,* streaking was done on fresh PDA medium twice. Microscopic examination and measurements of conidiophores and conidia were made from slide preparations stained with lactophenol-cotton blue and observed under a light microscope under ×400. Pure cultures of T. *harzianum* were then taken to KARI Njoro for confirmation.

3.4 Inoculum Production of *Trichoderma spp.*

The Hassan *et al.* (2014) procedure was adopted for production of *T. harzianum* inoculum. However, slight modification was made to suit the present study. The pure cultures obtained in Section 3.3 above were sub-cultured aseptically in eight 90 mm diameter Petri plates each containing 15 ml of a freshly autoclaved PDA media. Incubation of the eight plates was done at 28°C for ten days. On the tenth day, spore suspensions from the fungus inoculum were prepared by flooding the surface of the agar slant with 10ml sterile distilled water and the culture surface gently scraped to extricate the spores. The spore suspensions derived from the eight Petri plates were transferred separately to 500ml flask containing 400ml distilled water. Flasks were then shaken for 2 minutes to ensure that the spores are appropriately mixed. Four concentrations of the fungal spore were prepared (0, $1x10^5$, $1x10^7$and $1x10^{10}$ spore/ml) (Appendix x) using a haemocytometer under a light microscope. The control was made up of autoclaved spores of *T. harzianum*. Autoclaving process was done at 121^0C for 15 minutes (Doherty *et al.*, 2010).

3.5 Seed selection and treatment

Maize seeds with no cracks or any visible deformations were obtained from Kenya Seed Company Kitale. It is the leading seed company in Kenya and a good number of farmers acquire their seeds from here. Maize varieties (H614, H629 and H6210) were used in the study because they have been reported to be highly susceptible to drought stress. More so, these varieties are being planted by most farmers within Uasin Gishu and Trans Nzoia counties which are the main maize producing counties in Kenya. Surface sterilization was done for 5 minutes with 1% sodium hypochlorite solution, followed by rinsing with distilled water three times and finally air dried. Wet seed treatment method was adopted, where seed coating was done by applying 2% of starch (adhesive) on the maize seeds. Subsequently, maize seeds were dipped in seed coating suspension of 0, 1×10^{5}, $1x10^{7}$ and 1×10^{10}spores/ml *Trichoderma harzianum* for 2 minutes.

3.6 Preparation of Polyethylene Glycol concentrations

Polyethylene glycol 6000 (PEG) at different concentrations was prepared to establish different levels of osmotic potential. Approximately 0, 143.18, 213.64 and 267.97 g of PEG were dissolved in 1000ml distilled water to generate four osmotic stress levels (0, -0.3, -0.6 and -0.9 MPa, respectively) (Guo *et al.*, 2013). The control was made up of only distilled water with no PEG.

3.7 Effect of Trichoderma spp. on maize seedling emergence and growth under water stress

3.7.1 Determination of Seedling Emergence

The experimental design that the present study employed was a three - factor factorial (4x3x4) design, arranged in a completely randomized design (CRD) with three replications each. The first factor was the concentration of *Trichoderma harzianum* (0, 1×10^5, $1x10^7$ and 1×10^{10}spores/ml). The second factor was the maize seed varieties (H614, H629 and H6210) and the third factor was the osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa).

Seedling emergence assays was performed based on Achakzai (2009). Sterilized maize seeds belonging to H614, H629 and H6210 varieties were treated with *Trichoderma harzianum* at (0, 1×10^5, $1x10^7$ and 1×10^{10}spores/ml) concentrations for 2 minutes. Ten seeds were evenly distributed in each sterile Petri dish lined with two layers of Whatmann filter paper saturated with 8 ml of Polyethylene glycol (PEG) solution to mimic drought stress. The plates were then incubated at 25^o C. The plates were kept moist throughout the experiment by adding 8ml of the appropriate concentration of PEG to each plate after every 48hrs. Observations regarding germination were made after every 24 hours, and continued till the completion of germination. The emergence of radical and plumule was taken as an indicator or measure of germination. After 7 days the % germination was determined using the formula by Achakzai (2009).

3.7.2 Determination of Seedling growth

After 10 days of germination, three seedlings from each Petri dish were taken out randomly. Washing was done gently to avoid losing some parts of the seedling and also to remove any form of particle that would interfere with the readings. The following growth measurements were taken.

a) Seedling length –SL (cm)

The length of seedlings (root-shoot) was measured in centimeters by using a measuring ruler.

b) Seedling fresh we1ight- SFW (mg)

The seedlings were weighed in milligrams by using an electronic balance

c) Root dry weight- RDW (mg**)**

Roots were dried in an oven at 65±5°C for 72 hours. After drying, roots dry weight per seedling was recorded in milligrams by using an electronic balance.

d) Shoot dry weight- SDW (mg)

The shoots were also dried as described above and their weights recorded in milligrams.

3.8 Statistical analysis

Maize seed germination and seedling growth experiments had factorial designs with three factors (Concentration of *T. harzianum*, Maize variety and Osmotic potential). Analysis of variance (ANOVA) on maize seed germination percentage data was performed using statgraphics programme, after transforming the percent germination data to logarithm for normal distribution and homogeneity of variances. Both transformed and untransformed data were used in statistical analysis. For maize seedling length, fresh weight, shoot dry

weight and root dry weight, data was subjected to ANOVA using statgraphics programme without transformation. Means for both seed germination and seedling growth were separated using Tukey's test.

3.9 To determine the capability of *Trichoderma harzianum* to enhance the antioxidant defense in maize seedlings under drought stress

3.9.1 Enzyme extraction from plant samples

Extraction of (SOD and CAT) enzymes from plant samples was done according to Higuchi *et al.* (2008). Both water stressed and control maize seedlings were evaluated for antioxidative enzymes' activity after 10 days of germination. Fresh weight of 0.5g leaf sample was taken and then placed in a freezer at -10^{o}C for 24hrs. The frozen leaf sample was then finely ground by pestle in a frozen motor to prevent the loss of enzymes' activities. The frozen powder was added to 10 mL of phosphate buffer (pH 7.5). The homogenate was centrifuged at 15000 × *g* for 10 min at 25^{o}C and supernatant was used as enzyme source for catalase (CAT) and superoxide dismutase (SOD) (Higuchi *et al.*, 2008).

3.9.2 Assay of Superoxide dismutase (SOD) activity

Superoxide dismutase activity was done according to Kong *et al.* (2012). A 3ml sample of the reaction mixture was made up 0.1 ml of 1.5 M Na_2CO_3, 0.2 ml of 200 kmM methionine, 0.1 ml of 3 mM EDTA, 0.1 ml of 2.25 mM *p*-nitroblue tetrazolium chloride (NBT), 1.5 ml of 100 mM potassium phosphate buffer (pH 7.5), 1 ml of distilled water and 0.05 ml of enzyme samples. A tube without enzyme was used as control. The

reaction was started by adding 0.1 ml 60 μM riboflavin and placing the tubes below a light source for 15 minutes. The reaction was stopped by switching off the light and covering the tubes with black cloth. Absorbance was recorded at 560 nm. An illuminated blank without protein gave the maximum reduction of NBT, and therefore, the maximum absorbance at 560 nm. Superoxide dismutase (SOD) activity was presented as absorbance of blank minus absorbance of sample, giving the total inhibition, calculated per microgram protein. The activity of SOD was expressed as U mg^{-1} protein. One unit of activity is the amount of protein required to inhibit 50 % initial reduction of NBT under light (Kong *et al.,* 2012).

3.9.3 Assay of Catalase (CAT) activity

Determination of CAT activity was done according to Lum *et al.* (2014). A total of 3ml of the assay mixture (0.5 ml of 0.2 M phosphate buffer (pH 7.5), 0.3 ml of H_2O_2, 0.1 ml of the reaction mixture and 2.1ml of distilled water was prepared. Change in optical density was measured at 240 nm at 0 min and 3 min on UV- spectrophotometer. The molar extinction coefficient of H_2O_2 at 240 nm was taken as 36 μmol^{-1} cm^{-1} and the results were expressed as μmol H_2O_2 min^{-1} g^{-1} protein (Higuchi *et al.,* 2008).

3.10 Statistical analysis

The experiment for the activity of SOD and CAT enzymes was carried out using factorial design with three replications. The mean values of SOD and CAT enzymes activity were taken from measurements of the three replicates and standard error (SE) of the means was calculated. The mean values were then analyzed by three way analysis of variance

(ANOVA) using statgraphics programme to determine the activity of antioxidant enzymes (SOD and CAT). Means were separated using Tukey's test.

CHAPTER FOUR

RESULTS

4.1 Isolation of *T. harzianum*

4.1.1 Cultural characteristics

Cultural characteristics of 5day old *Trichoderma harzianum* incubated at 28 and 35^{o} C comprising of color and colony appearance are shown in Plates 4.1(a, b), 4.2 (a, b) and 4.3 (a, b).

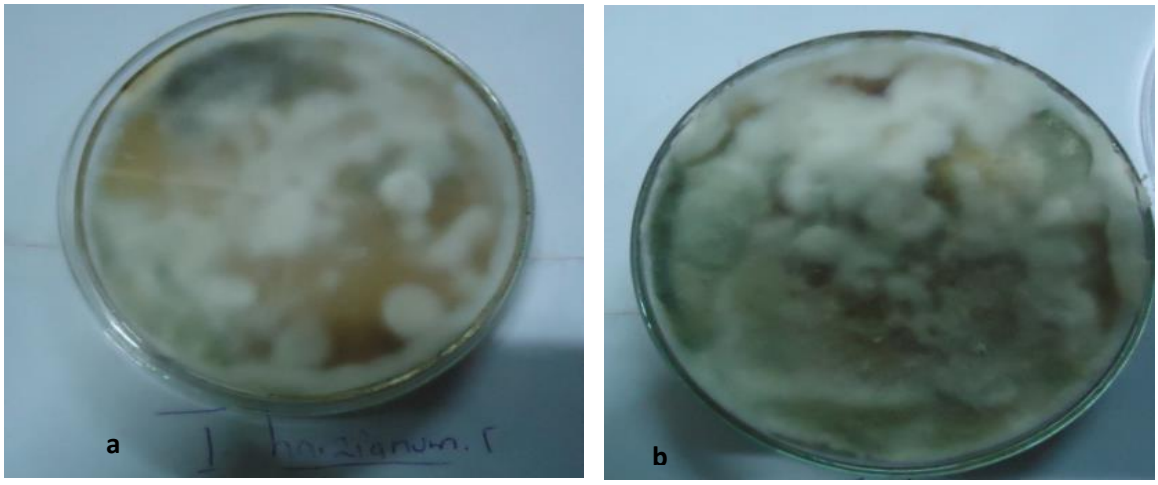

Plate 4.1: Growth of 5day old *T. harzianum* on PDA at (a) 28^{o} C and (b) 35^{o} C

(Source: Author, 2015)

At 28°C and 35^{o} C, *T. harzianum* grew uniformly and formed white mycelia within five days (Plate 4.1).

After seven days of growth, the fungus displayed green conidia, at both 28 and 35^{o}C. The conidia production was dense at the center and towards the margins (Plate 4.2). It was also observed that, conidia production by the fungus was not different at 28 and 35^{o} C.

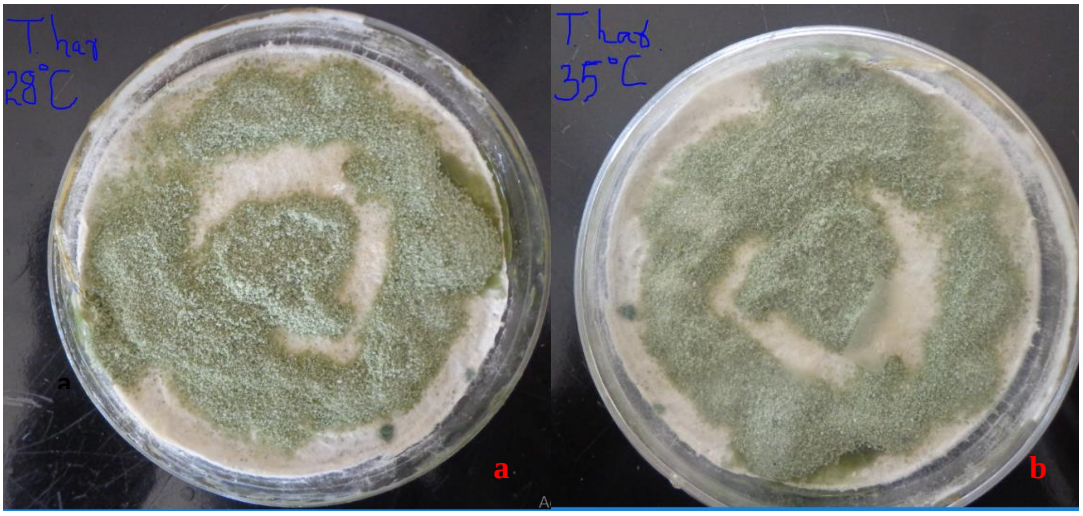

Plate 4.2: Conidia of 7day old *T. harzianum* on PDA at (a) 28 and (b) 35^{0}C

(Source: Author, 2015)

4.1.2 Micro-morphological characteristics

Microscopic examination of the ten day old culture of *T. harzianum* grown on PDA under a light microscope at ×400 magnifications showed globose to subglobose conidiophores. Conidia aggregated at the end of the conidiophores (Plates 4.3a, b).

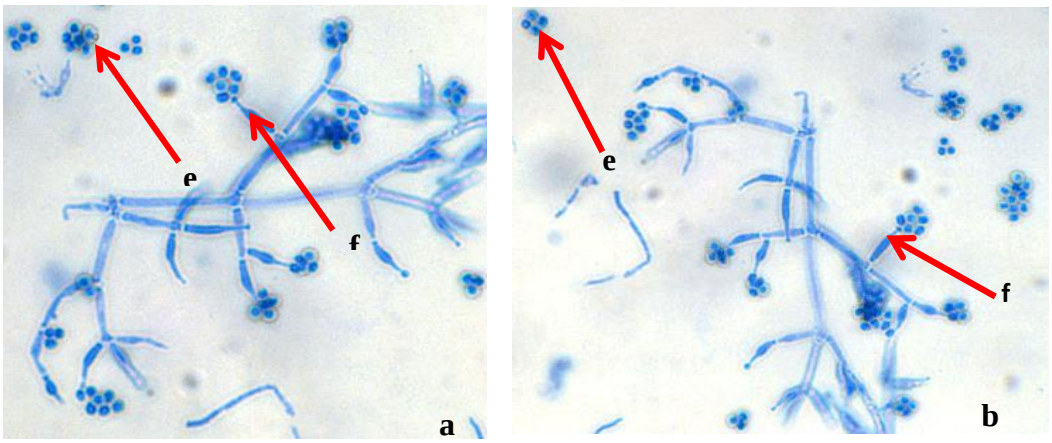

Plate 4.3: (e) Conidia and (f) Conidiophore of *T. harzianum* under a light microscope at (a) 28^0 C and (b) 35^0 C under ×400 magnifications

(Source: Author, 2015)

4.2 Effect of *T. harzianum* from semi-arid soils and its minimum &optimum concentrations for enhanced maize seed germination and seedling growth under water stress

Results on the effects of main factors and their interactions on maize seed germination and seedling growth are summarized in Table 4.1.

Concentration of *T. harzianum* (CT) and osmotic potential (OP) had a significant ($p<0.05$) effect on maize seed germination and seedling length, fresh weight, shoot dry weight and root dry weight. Interactions between concentration of *T. harzianum* by osmotic potential (CT×OP) and maize variety by osmotic potential (V×OP) also had a significant effect on early seedling growth parameters. However, maize variety (V), Maize variety by *T. harzianum* concentration (V×CT) and maize variety by *T. harzianum* concentration by osmotic potential (V×CT×OP) interactions had no significant effect

($p>0.05$) on maize seed germination and seedling growth parameters (Table 4.1; Appendix i, ii, iii, iv and v).

Table 4.1: Effects of main factors and their interactions on maize seed germination and seedling growth parameters

	Germination %			Seedling length (cm)			Seedling fresh weight (g/seedling)			Shoot dry weight (g/seedling)			Root dry weight (g/seedling)		
Source of variation	**F-ratio**	**P-value**	**Effect**	**F-ratio**	**P-value**	**Effect**	**F-ratio**	**P-value**	**Effect**	**F-ratio**	**P-value**	**Effect**	**F-ratio**	**P-value**	**Effect**
Concentration of *T.harzianum*(CT)	100.8	<0.05	**	3055.2	<0.05	**	2206.7	<0.05	**	1378.3	<0.05	**	121.3	<0.05	**
Osmotic Potential (OP)	854.9	<0.05	**	6488.1	<0.05	**	1329.1	<0.05	**	7462.9	<0.05	**	580.7	<0.05	**
Maize .Variety (V)	0.4	>0.05	NS	4.8	>0.05	NS	22.1	>0.05	NS	0.7	>0.05	NS	7.6	>0.05	NS
CT×OP	25.2	<0.05	**	155.9	<0.05	**	117.7	<0.05	**	123.5	<0.05	**	4.7	<0.05	**
CT×V	2.4	>0.05	NS	0.9	>0.05	NS	10.3	>0.05	NS	2.0	>0.05	NS	1.1	>0.05	NS
OP×V	1.2	<0.05	**	44.1	<0.05	**	11.8	<0.05	**	4.9	<0.05	**	8.4	<0.05	**
CT×OP×V	0.6	>0.05	NS	2.6	>0.05	NS	7.3	>0.05	NS	1.5	>0.05	NS	1.2	>0.05	NS

***Significant at p < 0.05. NS denotes not significant at P< 0.05*

4.2.1 Maize seed germination

There was no significant ($p>0.05$) difference in percentage germination among the three varieties of maize at the same osmotic potential with the same spore concentration of *T. harzianum* (Table 4.2). Generally, percentage germination decreased as osmotic potential decreased (Table 4.2). Percentage germination increased as the spore concentration of *T. harzianum* increased (Table 4.2).

At 0MPa, optimum percentage germination (95%) was recorded in both *T. harzianum* treated and untreated maize seeds but it decreased as the moisture stress increased in all the three varieties of maize (Table 4.2 and Appendix viii). Minimum percentage germination (13%) was recorded in untreated maize seeds at -0.9MPa, while treated maize seeds showed significant ($p<0.05$) percentage germination from untreated seeds (Table 4.2 and Appendix ix).

At -0.3, -0.6 and -0.9 osmotic potentials, untreated maize seeds showed lowest percentage germinations (85, 24 and 13%) respectively across the three varieties of maize (Table 4.2). However, seeds treated with 10^5 spores/ml showed significant ($p<0.05$) % germination (88-92, 43-46 and 33-35%) from control at -0.3, -0.6 and -0.9MPa respectively (Table 4.2). At the same osmotic potentials, seeds treated with 10^7 spores/ml of *T. harzianum* recorded highest percentage germination (91, 61 and 54%) respectively. This was followed by stabilization of percentage germination (91, 56 and 54%) in seeds treated with 10^{10} spores/ml of *T. harzianum* at -0.3, -0.6 and -0.9MPa respectively (Table 4.2).

Table 4.2: Effects of four concentrations of *T. harzianum* (0, 10^5, 10^7 and 10^{10} spores/ml) on the germination % of three varieties of maize (H614, H629 and H6210) at four osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa)

Conc. of *T. harzianum* (spores/ml)	Osmotic potential (MPa)j	Germination % of maize seeds (%)		
		Maize variety		
		H614	H629	H6210
0	0	95.5(1.98)^{h}	95.2(1.97)^{h}	96.7(1.98)^{h}
	-0.3	85.4(1.93)^{g}	85.3(1.93)^{g}	85.1(1.93)^{g}
	-0.6	24.1(1.38)^{b}	23.9(1.37)^{b}	23.7(1.37)^{b}
	-0.9	13.4(1.12)^{a}	13.7(1.13)^{a}	13.3(1.12)^{a}
10^5	0	93.1(1.96)^{h}	94.2(1.97)^{h}	95.7(1.98)^{h}
	-0.3	88.7(1.94)^{g}	89.1(1.94)^{g}	92.0(1.96)^{g}
	-0.6	46.5(1.66)^{d}	43.9(1.64)^{d}	46.9(1.66)^{e}
	-0.9	33.3(1.52)^{c}	34.1(1.53)^{c}	35.1(1.54)^{c}
10^7	0	95.5(1.98)^{h}	96.1(1.98)^{h}	96.8(1.98)^{h}
	-0.3	91.3(1.96)^{h}	91.7(1.96)^{h}	90.7(1.95)^{gh}
	-0.6	61.0(1.78)^{f}	60.9(1.78)^{f}	62.3(1.78)^{f}
	-0.9	54.3(1.73)^{e}	54.8(1.73)^{e}	54.4(1.73)^{e}
10^{10}	0	94.3(1.97)^{h}	94.9(1.97)h	95.9(1.98)^{h}
	-0.3	90.0(1.95)^{gh}	91.7(1.96)g	91.7(1.96)^{g}
	-0.6	57.8(1.76)^{ef}	56.5(1.75)^{ef}	56.7(1.75)^{f}
	-0.9	53.2(1.72)^{e}	54.1(1.73)^{e}	55.6(1.74)^{e}
F-ratio		0.58	0.61	0.57
P value		<0.05	<0.05	<0.05
Effect		**	**	**

Values in parenthesis are log transformed values. Means followed by the same letter within the same column are not significantly different at $p < 0.05$.

The % of germination of seeds treated with 10^7 and 10^{10} spores/ml concentrations of *T. harzianum* were not significantly different ($p>0.05$) irrespective of the fact that maize varieties were different (Table 4.2). However, they were significantly different from those seeds treated with 10^5spores/ml of *T. harzianum* and control as shown in Table 4.2. Seeds treated with 10^5 spores/ml of *T. harzianum* showed significant percentage germination from control in all the three varieties of maize (Table 4.2)

4.2.2 Seedling length

There were no significant ($p>0.05$) differences in seedling length among the three varieties of maize at the same osmotic potential within the same spore concentration of *T. harzianum* (Table 4.3). Seedling length increased as the spore concentration of *T. harzianum* increased. Untreated maize seeds recorded lower seedling length compared to treated seeds at -0.3, -0.6 and -0.9 osmotic potentials (Table 4.3).

Maximum seedling length was recorded at -0.3MPa, but further increase in PEG concentration decreased seedling length significantly ($p<0.05$) (Table 4.3). At -0.3MPa, seedling length si4ignificantly ($p<0.05$) increased from 13.7 - 13.9cm in control to 15.0, 15.6 and 15.7cm in seedlings treated with 10^5, 10^{10} and 10^7 spores/ml of *T. harzianum* respectively in all the three varieties of maize (Table 4.3).

At -0.9 MPa, seedling length increased significantly ($p<0.05$) from 1.6 cm in control to 3.4cm in seedlings treated with 10^5 spores/ml of *T. harzianum*. Maximum (5.7cm) seedling length was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum,*

before stabilizing (5.5cm) in seedlings treated with 10^{10} spores/ml of the fungus a cross the three varieties of maize at -0.9MPa (Table 4.3).

Seeds treated with 10^{7} and 10^{10} spores/ml concentrations of *T. harzianum* were not significantly different ($p>0.05$) in seedling length irrespective of the maize variety (Table 4.3). However, they were significantly different ($p<0.05$) from seeds treated with 10^{5} spores/ml of *T. harzianum* and control (Table 4.3). Seeds treated with 10^{5} spores/ml of *T. harzianum* showed significant ($p<0.05$) seedling length from control in all the three varieties of maize (Table 4.3).

Table 4.3: Effects of four concentrations of *T. harzianum* (0, 10^5, 10^7 and 10^{10} spores/ml) on the seedling length (cm) of three varieties of maize (H614, H629 and H6210) at four osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa)

Conc. of *T. harzianum* (spore/ml)	Osmotic potential (MPa)	Seedling length (cm)		
		Maize variety		
		H614	H629	H6210
0	0	12.722±0.03^{f}	12.729±0.02^{f}	12.727±0.04^{f}
	-0.3	13.932±0.04^{g}	13.735±0.07^{g}	13.915±0.01^{g}
	-0.6	4.776±0.01^{c}	4.729±0.01^{c}	4.741±0.05^{c}
	-0.9	1.633±0.01^{a}	1.618±0.04^{a}	1.673±0.04^{a}
10^5	0	13.855±0.05^{g}	13.847±0.03^{g}	13.852±0.07^{g}
	-0.3	15.055±0.04^{i}	15.027±0.02^{i}	15.062±0.04^{i}
	-0.6	5.633±0.08^{d}	5.608±0.09^{d}	5.676±0.03^{d}
	-0.9	3.489±0.01^{b}	3.481±0.01^{b}	3.493±0.09^{b}
10^7	0	14.567±0.04^{h}	14.582±0.03^{h}	14.5970±0.01^{h}
	-0.3	15.733±0.07^{j}	15.729±0.01^{j}	15.753±0.03^{j}
	-0.6	7.144±0.06^{e}	7.111±0.04^{e}	7.174±0.02^{e}
	-0.9	5.644±0.01^{d}	5.621±0.04^{d}	5.667±0.02^{d}
10^{10}	0	14.512±0.01^{h}	14.493±0.03^{h}	14.572±0.06^{h}
	-0.3	15.656±0.02^{j}	15.637±0.03^{j}	15.666±0.02^{j}
	-0.6	7.144±0.02^{e}	7.137±0.01^{e}	7.198±0.04^{e}
	-0.9	5.556±0.04^{d}	5.544±0.07^{d}	5.573±0.02^{d}
F-ratio		2.64	2.63	2.68
P value		<0.05	<0.05	<0.05
Effect		**	**	**

*Means followed by the same letter within the same column are not significantly different at P < 0.05. ** denotes significant at p<0.05*

4.2.3 Seedling fresh weight (SFW)

The results of the study revealed that seedling fresh weights did not differ significantly among the maize varieties at the same osmotic potentials within the same spore concentration of *T. harzianum* (Table 4.4). Increase in PEG concentrations significantly decreased SFW beyond -0.3MPa in both treated and untreated seedlings. Seedling fresh weight increased significantly with increase in *T. harzianum* concentrations upto 10^7 spores/ml. However, SFW became stabilized in seedlings treated with 10^{10} spores/ml of *T. harzianum* in all the three varieties of maize (Table 4.4).

Higher SFW was recorded at -0.3MPa compared to control (0MPa) in both treated and untreated seeds a cross the three varieties of maize. SFW increased significantly ($p<0.05$) from 4.1-4.2 in control to 4.7-4.8 mg/seedlings in seedlings treated with 10^5spores/ml of *T. harzianum* in all the three varieties of maize at -0.3MPa. Maximum SFW (5.8-5.9mg/seedling) was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum* and became stabilized in seedlings treated with 10^{10} spores/ml of *T. harzianum* across the three varieties of maize at -0.3MPa (Table 4.4).

At -0.9 MPa, reduced SFW was recorded in both treated and untreated seedlings across the three maize varieties (Table 4.4). However, SFW increased significantly ($p<0.05$) from 0.5mg/seedling in control to 0.8-0.9 mg/seedling in seedlings treated with 10^5 spores/ml of *T. harzianum*. Further increase (1.3-1.4mg/seedling) in SFW was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum*, before stabilizing (1.2-1.3mg/seedling) in seedlings treated with 10^{10} spores/ml of the fungus at -0.9MPa a cross the three varieties of maize (Table 4.4).

Table 4.4: Effects of four concentrations of *T. harzianum* (0, 10^5, 10^7 and 10^{10} spores/ml) on the fresh seedling weight (mg/seedling) of three varieties of maize (H614, H629 and H6210) at four osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa)

Conc. of *T. harzianum* (spore/ml)	Osmotic potential (MPa)	Fresh seedling weight (mg/seedling)		
		Maize variety		
		H614	H629	H6210
0	0	2.48±0.001^{f}	2.51±0.004^{f}	2.57±0.001^{f}
	-0.3	4.11±0.003^{i}	4.25±0.001^{i}	4.25±0.004^{i}
	-0.6	1.98±0.005^{e}	2.14±0.002^{e}	2.19±0.007^{e}
	-0.9	0.50±0.001^{a}	0.53±0.003^{a}	0.51±0.003^{a}
10^5	0	3.07±0.002^{g}	3.06±0.005^{g}	3.10±0.005^{g}
	-0.3	4.75±0.003^{j}	4.87±0.004^{j}	4.88±0.004^{j}
	-0.6	2.67±0.003^{g}	2.93±0.001^{g}	2.98±0.007^{g}
	-0.9	0.89±0.005^{b}	0.96±0.002^{b}	0.96±0.008^{b}
10^7	0	3.57±0.001^{h}	3.61±0.004^{h}	3.67±0.001^{h}
	-0.3	5.88±0.007^{k}	5.93±0.002^{k}	5.98±0.003^{k}
	-0.6	4.29±0.001^{i}	4.35±0.001^{i}	4.31±0.002^{i}
	-0.9	1.31±0.001^{d}	1.39±0.003^{d}	1.42±0.001^{d}
10^{10}	0	3.62±0.002^{h}	3.60±0.001^{h}	3.65±0.009^{h}
	-0.3	5.64±0.001^{k}	5.82±0.003^{k}	5.81±0.004^{k}
	-0.6	4.27±0.003^{i}	4.33±0.005^{i}	4.34±0.001^{i}
	-0.9	1.24±0.002^{c}	1.27±0.008^{c}	1.39±0.002^{c}
F-ratio		7.39	7.41	7.49
P value		<0.05	<0.05	<0.05
Effect		**	**	**

*Means followed by the same letter within the same column are not significantly different at P <0.05. ** denotes significant at p<0.05*

Irrespective of maize variety, seeds treated with 10^7 and 10^{10} spores/ml concentrations of *T. harzianum* were not significantly different ($p>0.05$) in SFW (Table 4.4). However, they were significantly different ($p<0.05$) from seeds treated with 10^5 spores/ml of *T. harzianum* and control (Table 4.4). Seeds treated with 10^5 spores/ml of *T. harzianum* showed significant ($p<0.05$) SFW from control in all the three varieties of maize (Table 4.4).

4.2.4 Shoot and Root dry weights

The results of the study further showed that maize varieties did not differ significantly in shoot dry weight (SDW) and root dry weight (RDW) at the same osmotic potentials within the same spore concentration of *T. harzianum* (Table 4.5 and 4.6). Increase in PEG concentrations significantly decreased both SDW and RDW in seedlings grown beyond -0.3MPa in both treated and untreated seedlings (Table 4.5 and 4.6) respectively. SDW and RDW increased significantly with increase in *T. harzianum* concentrations upto 10^7 spores/ml. However, they became stabilized in seedlings treated with 10^{10} spores/ml of *T. harzianum*, across the three varieties of maize (Table 4.5 and 4.6).

At -0.3MPa, maximum SDW was recorded in both treated and untreated seeds across the three varieties of maize (Table 4.5). SDW increased significantly ($p<0.05$) from 0.43-0.45mg/ seedling in control to 0.45-0.47mg/seedlings in seedlings treated with 10^5 spores/ ml of *T. harzianum* across the three varieties of maize at -0.3MPa. Maximum SDW (0.53-0.57mg/ seedling) was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum* and became stabilized (0.53-0.55mg/ seedling) in seedlings treated with 10^{10} spores/ml of *T. harzianum* across the three varieties of maize at -0.3MPa (Table 4.5).

At -0.9 MPa, SDW increased significantly ($p<0.05$) from 0.05-0.06mg/seedling in control to 0.08-0.09 mg/seedling in seedlings treated with 10^5 spores/ml of *T. harzianum*. Further increase (0.17-0.19mg/seedling) in SDW was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum* with stabilization (0.17-0.18mg/seedling) recorded in seedlings treated with 10^{10} spores/ml of the fungus at -0.9MPa a cross the three varieties of maize (Table 4.5).

Similarly, irrespective of maize variety, maximum RDW was recorded in both treated and untreated seedlings at -0.3MPa (Table 4.6). RDW significantly ($p<0.05$) increased from 0.81-0.86mg/ seedling in control to 0.95-0.98mg/seedlings in seedlings treated with 10^5spores/ml of *T. harzianum* across the three varieties of maize at -0.3MPa. Maximum RDW (1.39-1.41g/ seedling) was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum* and became stabilized (1.38-1.41mg/ seedling) in seedlings treated with 10^{10} spores/ml of *T. harzianum* across the three varieties of maize at -0.3MPa (Table 4.6).

Likewise, RDW was significantly reduced at -0.9MPa in both treated and untreated seedlings in all the three varieties of maize (Table 4.6). Nevertheless, RDW increased significantly ($p<0.05$) from 0.05-0.06 mg/ seedling in control to 0.17-0.19mg/ seedling in seedlings treated with 10^5 spores/ ml of *T. harzianum.* Maximum increase (0.39-0.42mg/ seedling) in RDW was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum* while stabilization (0.38-0.42mg/ seedling) was recorded in seedlings treated with 10^{10} spores/ml of the fungus at -0.9MPa across the three varieties of maize (Table 4.6).

Irrespective of maize variety, seeds treated with 10^7 and 10^{10} spores/ml concentrations of *T. harzianum* were not significantly different ($p>0.05$) in SDW and RDW (Table 4.5,

4.6). However, they were significantly different ($p<0.05$) from seeds treated with 10^5 spores/ml of *T. harzianum* and control (Table 4.5, 4.6). Seeds treated with 10^5 spores/ml of *T. harzianum* showed significant ($p<0.05$) in SDW and RDW from control in all the three varieties of maize (Table 4.5, 4.6) respectively.

Table 4.5: Effects of four concentrations of *T. harzianum* (0, 10^5, 10^7 and 10^{10} spores/ml) on the shoot dry weight (mg/seedling) of three varieties of maize (H614, H629 and H6210) at four osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa)

Conc. of *T. harzianum* (spore/ml)	Osmotic potential (MPa)	Shoot dry weight (mg/seedling)		
		Maize variety		
		H614	H629	H6210
0	0	0.34±0.002^{e}	0.31±0.001^{e}	0.33±0.003^{e}
	-0.3	0.44±0.001^{i}	0.43±0.001^{i}	0.45±0.006^{i}
	-0.6	0.13±0.002^{c}	0.11±0.003^{c}	0.13±0.009^{c}
	-0.9	0.05±0.004^{a}	0.05±0.001^{a}	0.06±0.001^{a}
10^5	0	0.38±0.002^{f}	0.37±0.002^{f}	0.38±0.005^{f}
	-0.3	0.47±0.002^{j}	0.45±0.004^{j}	0.46±0.001^{j}
	-0.6	0.17±0.003^{d}	0.18±0.002^{d}	0.18±0.004^{d}
	-0.9	0.09±0.001^{b}	0.09±0.008^{b}	0.08±0.001^{b}
10^7	0	0.42±0.003^{g}	0.41±0.003^{g}	0.43±0.003^{g}
	-0.3	0.55±0.006^{k}	0.53±0.003^{k}	0.57±0.007^{k}
	-0.6	0.38±0.002^{f}	0.37±0.007^{f}	0.38±0.003^{f}
	-0.9	0.17±0.004^{d}	0.18±0.001^{d}	0.19±0.005^{d}
10^{10}	0	0.41±0.009^{g}	0.40±0.002^{g}	0.43±0.001^{g}
	-0.3	0.55±0.005^{k}	0.53±0.003^{k}	0.55±0.007^{k}
	-0.6	0.37±0.004^{f}	0.36±0.002^{f}	0.36±0.001^{f}
	-0.9	0.18±0.006^{d}	0.17±0.001^{d}	0.18±0.002^{d}
F-ratio		1.7	1.5	1.7
P value		<0.05	<0.05	<0.05
Effect		**	**	**

*Means followed by the same letter within the same column are not significantly different at P < 0.05. ** denotes significant at p>0.05*

Table 4.6: Effects of four concentrations of *T. harzianum* (0, 10^5, 10^7 and 10^{10} spores/ml) on the root dry weight (mg/seedling) of three varieties of maize (H614, H629 and H6210) at four osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa)

Conc. of *T. harzianum* (spores/ml)	Osmotic potential (MPa)	Root dry weight (mg/seedling)		
		Maize variety		
		H614	H629	H6210
0	0	0.41±0.017^{d}	0.40±0.015^{d}	0.42±0.021^{d}
	-0.3	0.86±0.009^{g}	0.85±0.002^{g}	0.81±0.019^{g}
	-0.6	0.30±0.005^{c}	0.28±0.016^{c}	0.25±0.002^{c}
	-0.9	0.06±0.016^{a}	0.05±0.009^{a}	0.05±0.020^{a}
10^5	0	0.50±0.018^{e}	0.48±0.014^{e}	0.47±0.008^{e}
	-0.3	0.98±0.007^{h}	0.97±0.012^{h}	0.95±0.011^{h}
	-0.6	0.32±0.015^{c}	0.30±0.001^{c}	0.32±0.011^{c}
	-0.9	0.19±0.010^{b}	0.17±0.011^{b}	0.17±0.003^{b}
10^7	0	0.69±0.005^{f}	0.65±0.017^{f}	0.61±0.004^{f}
	-0.3	1.41±0.019^{i}	1.41±0.009^{i}	1.39±0.021^{i}
	-0.6	0.54±0.002^{e}	0.52±0.012^{e}	0.57±0.012^{e}
	-0.9	0.40±0.007^{d}	0.42±0.015^{d}	0.39±0.017^{d}
10^{10}	0	0.69±0.004^{f}	0.67±0.008^{f}	0.65±0.018^{f}
	-0.3	1.40±0.016^{i}	1.41±0.011^{i}	1.38±0.011^{i}
	-0.6	0.52±0.013^{e}	0.51±0.014^{e}	0.49±0.019^{e}
	-0.9	0.38±0.009^{d}	0.42±0.012^{d}	0.41±0.012^{d}
F-ratio		1.29	1.24	1.26
P value		<0.05	<0.05	<0.05
Effect		**	**	**

*Means followed by the same letter within the same column are not significantly different at P <0.05. ** denotes significant at p<0.05*

From the results, SDW was found to be numerically less than RDW in both treated and untreated at all osmotic potentials irrespective of maize variety (Table 4.5and 4.6

4.3 Effect of *T. harzianum* on the antioxidative enzyme activity

Results on the effect of *T. harzianum* on the activity of antioxidative enzymes (SOD and CAT) in maize seedlings grown at different osmotic potential levels are shown in Table 4.7, 4.8 and 4.9.

Table 4.7 showed that concentration of *T. harzianum* and osmotic potential affected SOD and CAT activities significantly ($p<0.05$). Concentration of *T. harzianum* by osmotic potential and maize variety by osmotic potential interactions were also significant ($p<0.05$) for SOD and CAT activities. However, maize variety, interactions for maize variety by *T. harzianum* concentration and maize variety by *T. harzianum* concentration by osmotic potential had no significant ($p>0.05$) effect on SOD and CAT activities (Table 4.7; Appendix VI and VII).

Table 4.7: Effects of main factors and their interactions on SOD and CAT activities

	SOD Activity			**CAT Activity**		
Source of variation	F-ratio	P-value	Effect	F-ratio	P-Value	Effect
Concentration *T. harzianum*(CT)	1.4E+07	<0.05	**	13440.00	<0.05	**
Osmotic potential (OP)	5.4E+07	<0.05	**	33327.53	<0.05	**
Maize variety (V)	1049.00	>0.05	NS	10.01	>0.05	NS
CT×OP	6071030.00	<0.05	**	3354.41	<0.05	**
CT×V	38.10	>0.05	NS	0.97	>0.05	NS
OP×V	799.74	<0.05	**	5.442	<0.05	**
CT×OP×V	178.68	>0.05	NS	2.38	>0.05	NS

***Significant at p < 0.05. NS denotes not significant at p<0.05*

SOD activity increased significantly ($p<0.05$) with decrease in osmotic potential in both treated and untreated maize seedling across the three varieties of maize (Table 4.8). SOD activity increased with increase in concentration of *T. harzianum* upto 10^7 spores/ml of the fungus before stabilizing with 10^{10} spore/ml of the fungus. Maize varieties did not differ significantly in SOD activity at the same osmotic potentials with the same spore concentration of *T. harzianum* (Table 4.8).

At 0MPa, SOD activity increased significantly ($p<0.05$) from 15.0 U g^{-1} protein in control to 15.2 U g^{-1} protein in seedlings treated with 10^5 spores/ml of *T. harzianum*. Further increase (15.5 U g^{-1} protein) in SOD activity was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum*. Stabilization of SOD activity (15.5 U g^{-1} protein) was recorded in seedlings treated with 10^{10} spores/ml of the fungus at 0MPa a cross the three varieties of maize (Table 4.8).

At -0.9MPa, SOD activity increased significantly ($p<0.05$) from 194 U g^{-1} protein in control to 337 U g^{-1} protein in seedlings treated with 10^5 spores/ml of *T. harzianum*. Maximum SOD activity (893 U g^{-1} protein) was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum* with stabilization of SOD activity (892U g^{-1} protein) recorded in seedlings treated with 10^{10} spores/ml of the fungus at 0MPa a cross the three varieties of maize (Table 4.8).

Table 4.8: Effects of four concentrations of *T. harzianum* (0, 10^5, 10^7 and 10^{10} spores/ml) on the SOD activity (U g^{-1} protein) of three varieties of maize (H614, H629 and H6210) at four osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa)

Conc. of *T. harzianum* (spore/ml)	Osmotic potential (MPa)	SOD activity (U g^{-1} protein)		
		Maize variety		
		H614	H629	H6210
0	0	15.054±0.06[a]	15.079±0.08[a]	15.023±0.08[a]
	-0.3	47.338±0.01[d]	47.369±0.07[d]	47.319±0.06[d]
	-0.6	150.440±0.04[g]	178.478±0.05[g]	150.378±0.02[g]
	-0.9	194.379±0.06[h]	194.378±0.04[h]	194.311±0.04[h]
10^5	0	15.212±0.03[b]	15.219±0.09[b]	15.209±0.01[b]
	-0.3	97.579±0.02[e]	97.583±0.10[e]	97.573±0.06[e]
	-0.6	194.112±0.03[h]	194.117±-0.07[h]	194.102±0.07[h]
	-0.9	337.777±0.01[k]	337.781±0.09[k]	337.729±0.07[k]
10^7	0	15.560±0.07[c]	15.572±0.06[c]	15.557±0.10[c]
	-0.3	126.801±0.01[f]	126.825±0.02[f]	126.791±0.04[f]
	-0.6	336.769±0.05[j]	336.772±0.07[j]	336.760±0.09[j]
	-0.9	893.564±0.06[l]	893.570±0.1[l]	893.555±0.07[l]
10^{10}	0	15.520±0.01[c]	15.527±0.05[c]	15.518±0.06[c]
	-0.3	126.616±0.03[f]	126.631±0.06[f]	126.609±0.07[f]
	-0.6	336.358±0.09[i]	336.367±0.07[i]	336.351±0.01[i]
	-0.9	892.912±0.08[l]	892.926±0.05[l]	892.905±0.08[l]
F-ratio		177.68	178.59	178.66
P value		<0.05	<0.05	<0.05
Effect		**	**	**

*Means followed by the same letter within the same column are not significantly different at P < 0.05. ** denotes significant at p<0.05*

Seedlings treated with 10^7 and 10^{10} spores/ml concentrations of *T. harzianum* were not significantly different (p>0.05) in SOD activity in all the three varieties of maize (Table 4.8). However, they were significantly different (p<0.05) from seeds treated with 10^5

spores/ml of *T. harzianum* and control (Table 4.8). Seedlings treated with 10^5 spores/ml of *T. harzianum* showed significant ($p<0.05$) SOD activity from control irrespective of maize variety (Table 4.8).

Similarly, CAT activity increased significantly ($p<0.05$) with decrease in osmotic potential in both treated and untreated maize seedling across the three varieties of maize (Table 4.8). CAT activity increased with increase in concentration of *T. harzianum* upto 10^7 spores/ml of the fungus before stabilizing with 10^{10} spore/ml of the fungus. Maize varieties did not differ significantly in CAT activity at the same osmotic potentials with the same spore concentration of *T. harzianum* (Table 4.8).

CAT activity increased significantly ($p<0.05$) from 0.01μmol H_2O_2 min^{-1} g^{-1} protein in control to 0.06 μmol H_2O_2 min^{-1} g^{-1} protein in seedlings treated with 10^5 spores/ml of *T. harzianum* at 0MPa. Seedlings treated with 10^7 spores/ml of *T. harzianum* recorded highest CAT activity (0.09μmol H_2O_2 min^{-1} g^{-1} protein), while stabilization of CAT activity (0.09 μmol H_2O_2 min^{-1} g^{-1} protein) was recorded in seedlings treated with 10^{10} spores/ml of the fungus at 0MPa a cross the three varieties of maize (Table 4.8).

At -0.9MPa, CAT activity increased significantly ($p<0.05$) from 1.0 μmol H_2O_2 min^{-1} g^{-1} protein in control to 1.3 μmol H_2O_2 min^{-1} g^{-1} protein in seedlings treated with 10^5 spores/ml of *T. harzianum*. Maximum CAT activity (4.0 μmol H_2O_2 min^{-1} g^{-1} protein) was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum*. Stabilization of CAT activity (4.0 μmol H_2O_2 min^{-1} g^{-1} protein) was recorded in seedlings treated with 10^{10} spores/ml of the fungus at 0MPa a cross the three varieties of maize (Table 4.8).

Table 4.9: Effects of four concentrations of *T. harzianum* (0, 10^5, 10^7 and 10^{10} spores/ml) on the CAT activity (µmol H_2O_2 min^{-1} g^{-1} protein) of three varieties of maize (H614, H629 and H6210) at four osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa)

Conc. of *T. harzianum* (spore/ml)	Osmotic potential (MPa)	CAT activity (µmol H_2O_2 min^{-1} g^{-1} protein)		
		Maize variety		
		H614	H629	H6210
0	0	0.016±0.009[a]	0.015±0.011[a]	0.017±0.007[a]
	-0.3	0.076±0.013[bc]	0.076±0.016[bc]	0.081±0.009[c]
	-0.6	0.874±0.011[f]	0.873±0.003[f]	0.883±0.010[f]
	-0.9	1.071±0.008[g]	1.070±0.009[g]	1.075±0.014[g]
10^5	0	0.066±0.007[b]	0.075±0.017[b]	0.079±0.016[b]
	-0.3	0.117±0.008[c]	0.117±0.020[c]	0.119±0.011[c]
	-0.6	1.147±0.008[h]	1.174±0.016[h]	1.150±0.019[h]
	-0.9	1.333±0.009[i]	1.332±0.008[i]	1.335±0.007[i]
10^7	0	0.097±0.012[c]	0.095±0.009[c]	0.097±0.008[c]
	-0.3	0.504±0.013[d]	0.500±0.015[d]	0.509±0.017[d]
	-0.6	3.623±0.010[j]	3.619±0.011[j]	3.626±0.017[j]
	-0.9	4.083±0.009[k]	4.079±0.007[k]	4.085±0.011[k]
10^{10}	0	0.094±0.011[c]	0.097±0.008[c]	0.094±0.018[c]
	-0.3	0.502±0.012[d]	0.497±0.014[d]	0.502±0.015[d]
	-0.6	3.637±0.011[j]	3.623±0.017[j]	3.638±0.017[j]
	-0.9	4.082±0.014[k]	4.078±0.010[k]	4.080±0.009[k]
F-ratio		2.38	2.41	2.38
P value		<0.05	<0.05	<0.05
Effect		**	**	**

*Means followed by the same letter within the same column are not significantly different at P < 0.05. ** denotes significant at p<0.05*

4.4 Effect of maize variety on *T. harzianum* activity

Maize seed germination and early seedling growth were significantly ($p<0.05$) enhanced by *T. harzianum* in all the three varieties of maize grown at different levels of water stress (Table 4.2, 4.3, 4.4, 4.5 and 4.6).

Maize variety by *T. harzianum* concentration and maize variety by *T. harzianum* concentration by osmotic potential interactions were not significant ($p>0.05$) for maize seed germination and seedling growth (Table 4.1). Results also showed that activities of SOD and CAT enzymes were significantly ($p<0.05$) enhanced by 10^5, 10^7 and 10^{10} spores/ml of *T. harzianum* in all the three varieties as shown in Table 4.5 and 4.6. However, interactions for maize variety by *T. harzianum* concentration and maize variety by *T. harzianum* concentration by osmotic potential were found to be insignificant for CAT and SOD enzymes activities (Table 4.7).

CHAPTER FIVE

DISCUSSION

5.1 Isolation of *T. harzianum* from the soil

In this study, *T. harzianum* grew well and uniformly at both 28 and 35° C. There is no doubt that the isolated fungus was *T. harzianum* since growth at 35^{o}C was recorded. Samuels *et al.* (2002) found that the capability of *T. harzianum* to grow at 35°C is useful in distinguishing it from other *Trichoderma* species. Furthermore, morphological characterization has conventionally used in the identification of *T. harzianum* and it remains as a prospective method to identify *Trichoderma* species (Samuels *et al.,* 2002; Bissett *et al.,* 2003; Anees *et al.,* 2010).

5.2 Effect of *T. harzianum* on maize seed germination and seedling growth under water stress

Treatment of seed or plants that could simultaneously confer resistance to abiotic stresses would be of importance to agricultural plant production. *Trichoderma* spp. are among the most extensively used organisms for biological disease control and for plant promotion (Harman *et al*., 2004). The present study showed that the fungus induces tolerance to water stress in maize seedlings, therefore, an opportunity to improve plant agriculture by fully utilizing its capabilities.

Findings from the study showed that concentration of *T. harzianum* had a significant ($p<0.05$) effect on maize seed germination and seedling growth under water stress. This could be attributed to the fact that increase in fungal concentration leads to increase in its

activity. It is worth noting that at low osmotic potential, increase in concentration of the fungus increased seed germination and seedling growth until 10^7 spores/ml of the fungus. Further increase in concentration (10^{10} spores/ml of *T. harzianum*) led to a stabilization of maize seedlings growth under water stress. This could be due to the fact that *T. harzianum* works best at an optimum concentration and beyond this, no significant activity of the fungus is expected. Similar findings have been reported by other authors. For instance, increase in concentration of *Trichoderma spp.* was reported to increase seedling growth in millet only to an optimum concentration of 1.2×10^7spores/ml of *T. viride* (Hassan *et al.*, 2014). Similarly, *Trichoderma spp*. was reported to enhance root and shoot growth of rice plant, however, the degree of augmentation differed with the fungal concentration (Gusain *et al.,* 2014).

Osmotic potential affected maize seed germination and seedling growth under water stress significantly. Results showed that increase in water stress (-0.6 and -0.9MPa) led to a decrease in maize seed germination and seedling growth. Reduction in germination with water stress is attributed to lower infusibility of water through the seed coat and initial water imbibition of the seed under stress condition and decreased external water potential (Bahrami & Razmjoo, 2012). Decrease in seed germination with increase in water stress could also be due to metabolic disorders such as slow hydrolysis of substrate compounds in endosperm or cotyledons and/or slower transportation of hydrolyzed material to developing embryo axis (Ayaz *et al.,* 2000). Furthermore, studies have shown that water stress sways more or less all aspects of plant physiology, biochemistry and growth metabolism (Li *et al.,* 2007). Similar results have been reported by several authors. For example, a study carried out by Gupta *et al.* (2003) recorded a decrease in

maize seed germination under water stress. Another study on tomato seedlings showed reduced plant growth under water stress (Mastouri *et al.,* 2010). Further, an experiment on vetch reported that water stress decreased all germination and early seedling features (De & Kar, 1995).

Maize varieties (H614, H629 and H6210) showed no significant effect for seed germination percentage and early seedling growth under water stress. This finding disagrees with the trends of Ashagre *et al.* (2014) who observed significant differences in response to water stress on germination and seedlings growth of maize cultivars. The present results could be probably due to the fact that the three varieties of maize (H614, H629 and H6210) belong to the six series family hence the genotypic variation is presumed to be minimum.

Findings from the present study clearly showed that *T. harzianum* played a key role in enhancing maize seed germination and early seedling growth under water stress. Seeds treated with *T. harzianum* showed significant difference in germination from control at water stress. For example, at -0.9MPa, seeds treated with 10^7 spores/ml of *T. harzianum* recorded significant germination (54%) from control (13%). This finding is attributed to the fact that seeds do respond to *T. harzianum* very early in germination, even before the radicle protrudes (Harman *et al.*, 2004). Also, *Trichoderma spp.* have been shown to augment seed germination by enhancing phase III imbibition (cell elongation, followed by radicle protrusion) (Harman *et al.* (2004). The present results are in agreement with those of Mastouri *et al.* (2010). The authors found that tomato seeds that were treated with *T. harzianum* (T22) showed higher seed germination percentage than untreated tomato seeds.

Results further showed maximum seed germination (95%) in seeds germinated at 0MPa in both treated and untreated maize seeds. This could be due to the fact that, seeds require enough water for germination to take place. Water activates the enzymes that coordinate the germination process. Absorbed water also causes the seeds to swell and soften which makes it possible for the plant to break through (Ashagre *et al.*, 2014). Similar results were reported by Gharoobi *et al.* (2012) and Ashagre *et al.* (2014), where maximum maize seed germination was recorded in seeds grown under stress free conditions (0MPa).

Results from the present study also showed significant differences between treated maize seedlings and control for maize seedling growth (seedling length, seedling fresh weight, root and shoot dry weight). For example, seedling length increased from 1.6cm in control to 5.7cm in seedlings treated with 10^7spores/ml of *T. harzianum* at -0.9MPa. The rationale of *T. harzianum* in enhancing early seedling growth under stress could be due to its ability to induce synthesis of hormones mainly indole acetic acids (IAA) that promote growth in plants, probably through the up-regulation of plant genes for hormone biosynthesis or the down-regulation of the genes involved in hormone catabolism (Martínez-Medina *et al.*, 2011). Another reason could be due to the fact *T. harzianum* decreases the synthesis of Abscisic acids (ABA) hormone (Aroca *et al*., 2013).This hormone inhibits plant growth during water stress in its mechanism to enable plants to withstand abiotic stresses (Aroca *et al*., 2013). Moreover, *T. harzianum* has been reported to produce ACC deaminase (ACCD), which reduces the availability of the ACC necessary for ET biosynthesis, which results in inhibited plant root growth (Viterbo *et al.*, 2010). Similar results have been reported by several authors. For instance, Harman

(2000) found that *Trichoderma spp.* conferred tolerance to water stress at least in part through promotion of deeper root penetration into the soil profile. In another report by Bae *et al*. (2009), *T. hamatum* increased tolerance of cocoa plants to water deficit through increasing g root growth that provided greater water resources to treated plants and delayed the onset of water deficit in these plants. Yildirim *et al.* (2006) also showed that squash plants treated with T22 or other showed enhanced root and shoot growth under abiotic stresses. Furthermore, tomato plants were reported to be enhanced by *T. harzianum* in terms of seedling growth under a wide range of abiotic stresses (Mastouri *et al.,* 2010). Previously, Bjorkman *et al.* (1998) revealed that, seed treatment with *T. harzianum* conferred advantages to maize seed with poor vigor caused by genetic manipulations, therefore, increasing vigor of maize seedlings. Experiments on bitter gourd, loofah and cucumber also showed that *Trichoderma* strains significantly increased from 26 to 61 % in seedling height, 85-209 % in root exploration, 27-38% in leaf area and 38 to 62 % in root dry weight after 15 days of sowing Chaur-Tsuen Lo and Chien-Yih Lin (2002). *Trichoderma spp.* was also shown to significantly increase millet seedling height, root length and root dry weight (Hassan *et al.*, 2014).

The results of present study showed that root dry weight was higher than shoot dry weight. This could be probably due to the fact that *Trichoderma spp.* colonizes plant roots directly and establishes symbiotic relationships with a wide range of host plants. As a consequence, plant root growth and performance frequently is enhanced (Harman, 2000). Similar results have been reported by Shoresh and Harman (2008).

It is worth noting that *T. harzianum* promoted growth even at the lowest osmotic potential (-0.9MPa). This could be due to the fact that microbes isolated from stressed

habitats confer habitat-specific stress tolerance to their host plants (Rodriguez *et al*., 2009). This finding corresponds to that of Bano *et al*. (2013) and Mayak *et al*. (2004) where microbial strains isolated from water stressed conditions mitigated severe drought effects in maize. Therefore, results from the present study, along with the frequent observation that the greatest advantage of *Trichoderma* treatments to plants occurs when they are under stress, gives weight to the concept that these beneficial fungi ameliorate abiotic plant stresses.

Findings from our study showed that, early seedling growth was optimum at -0.3MPa. This could be due to the fact that moderate water stress promotes plant growth (Ghajari and Zeinali 2003). Ashagre *et al.* (2014) reported similar results when increase in shoot and radicle lengths was recorded until -0.2 MPa using PEG-6000. Furthermore, Harman *et al*. (2004) showed that *Trichoderma spp.* enhanced plant growth only under suboptimum conditions.

5.3 Minimum and optimum concentrations of *T. harzianum* for maize seed treatment

Results of the present study showed significant differences ($p<0.05$) between maize seeds treated with different concentrations of *T. harzianum* and control. Results also showed that 10^5 spores/ml of *T. harzianum* was the minimum concentration of the fungus applied on maize seeds for significant seed germination and seedling growth. On the other hand, 10^7 spores/ml of *T. harzianum* was shown to be the optimum concentration of the fungus applied on maize seeds for maximum seed germination and seedling growth. Recently, Hassan *et al*. (2014) reported that 10^7 spores/ml of *T. viride* significantly improved

plumule length of millet plant compared to other treatments while 1.2 $\times 10^5$ spores/ml of the fungus was the minimum concentration that enhanced seedling length in millet plant. A previous study by Boyd-Wilson *et al*. (2000) showed that 1.2×10^7 CFU/seed of three fungal isolates was effective against *F. culmorum* and significantly enhanced seedling growth under biotic stress.

5.4 *Trichoderma harzianum* ability in enhancing the antioxidative enzymes of water stressed maize seedlings

Untreated seedlings grown under three levels of osmotic potentials (-0.3, -0.6 and -0.9 MPa) showed an increase in SOD and CAT activity. However, the activities of these enzymes were lower than those of treated maize seedlings. This is probably due to the fact that plants themselves (without microbes' interaction) can alleviate water stress through enhanced SOD and CAT activities. This finding is consistent with that of Sharma and Dubey (2005) where increase in antioxidant enzymes' activities was reported in plants subjected to abiotic stresses. Previously, Pastori and Trippi (1992) had reported an increase in SOD activity that was correlated to induced resistance of plants to drought stress.

In our study we found enhanced SOD and CAT enzyme activities in maize shoots of maize seedlings, yet *T. harzianum* is reported to interact with plant roots. This is credited to the fact that *Trichoderma* spp. induces systemic changes in gene expression through a complex signal transduction network with methyl jasmonate (MeJA) playing the pivotal role (Shoresh *et al.,* 2006). Similar findings were reported by Shoresh and Harman (2008) when proteomics of shoots inoculated with *Trichoderma* showed an increase in levels of

anti-oxidative enzymes mainly Superoxide dismutase as well as increased levels of peroxidase, glutathione-reductase and Glutathione-S-transferase (GST), and other detoxifying enzymes in leaves.

In the present study, *T. harzianum* increased SOD and CAT activity significantly in all the three varieties of maize under -0.3MPa, -0.6MPa and -0.9MPa as compared to control plants. These results are in agreement with that of Navazio *et al.* (2007) where a transient increase in intracellular ROS was detected 5 to 10 min after treating soybean cell culture with culture filtrate of *T. atroviride.* Furthermore, Mastouri *et al.* (2012) also reported that *T. harzianum* enhanced the activity of antioxidant enzymes in tomato plant subjected to water stress.

Results obtained from the present study also showed that the activity of SOD enzyme was higher than that of CAT enzyme. This could be attributed to the fact that SOD enzyme embodies the first line of cell defence against reactive oxygen species (ROS) generated by abiotic stresses like drought in plants, therefore, preventing tissue damage due to oxidative stress (Blokhina *et al.,* 2003) The enzyme converts superoxide radicals to hydrogen peroxide, while CAT enzyme converts hydrogen peroxide to water and oxygen (Blokhina *et al*., 2003). In this respect, increased activity of SOD alone cannot protect plants from toxic effect of oxygen free radicals and therefore, other enzymes like CAT and POD are required to get rid of hydrogen peroxide toxicity (Arora *et al.*, 2002).

This study therefore presents evidence that maize seedling colonization by *T. harzianum* enhances systems of antioxidative enzymes. This consequently indicates that, one of the mechanisms that *T. harzianum* especially those isolated from semi-arid soils employ in

improving plant tolerance to water stress is through the reduction of oxidative stress via increased SOD and CAT activity.

5.5 Effect of *T. harzianum* is modulated by the plant genotype

The response of three different varieties of maize to the growth-promoting fungi *T. harzianum* under different osmotic potential levels was assessed. Seed germination and both seedling length and weight were minimally enhanced by 10^5 spores/ml of *T. harzianum* under water stress in all the three maize varieties. Optimum seed germination and seedling growth was observed in seedlings treated with 10^7 spores/ml of the fungus. Seed germination and seedling growth parameters that were evaluated in this study showed that, maize variety by *T. harzianum*-concentration interaction was not significant. Also, maize variety by *T. harzianum* concentration by osmotic potential interaction was shown to be insignificant. Therefore, our findings demonstrated that the extent of maize seed germination and early seedling growth stimulation is not largely dependent on the maize variety, suggesting that the response to *Trichoderma spp.* is not under genetic control. However, from the few studies that have been carried out, plant genotype affects the activity of most plant growth promoting fungi. For example, Harman *et al*. (2004) clearly showed the importance of the plant genetic background for the interaction between maize and *T. harzianum* (T22). The authors showed that, commercial trials on several T22-treated hybrids and inbred lines revealed that the expected yield increases in most genotypes, with a few actually showing a yield reduction. Furthermore, genetic analysis has demonstrated that maize response is largely conditioned by dominant genes (Harman, 2000).

Another study by Tucci *et al*. (2011) clearly showed the importance of plant variety. Using tomato plant, they assessed the beneficial plant–*Trichoderma* interaction for root growth promotion. They reported a significant increase in root dry weight obtained by treating tomato seedlings with *Trichoderma spp*. However, plant growth promotion differed significantly from different varieties of tomato plant. Results obtained from the present study could be attributed to the fact that, the three varieties that were used in the study belong to a common series (6 series) and therefore, the degree of variation is minimal. Also, the difference could be due to the fact that present study was carried out under water stress conditions while the existing related researches were carried out under water stress- free conditions.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Taken together, the present study concluded that;

1. Treatment of maize seeds with *T. harzianum* isolated from semi-arid soils has beneficial effect on seed germination and seedling growth under water stress. Seedling growth at severe water stress was most probably through enhanced root development which resulted from enhanced phyto-hormones production. The study also showed that optimum activity of *T. harzianum* was maximum -0.3MPa, concluding that *Trichoderma spp.* promotes plant growth mainly under stressed conditions.
2. Approximately 10^5 and 10^7 spores/ml of *T. harzianum* are the minimum and optimum concentration of the fungus respectively that significantly promoted maize seed germination and seedling growth under water stress. However, 10^7 spores/ml of *T. harzianum* was not significantly different from 10^{10} spores/ml of the fungus in terms of maize seed germination and seedling growth.
3. *Trichoderma harzianum* significantly enhanced activities of antioxidant enzymes (SOD and CAT). Maximum activity of these enzymes was recorded under severe water stress (0.9MPa) mainly in seedlings treated with 10^7 spores/ml of *T. harzianum*.
4. Maize variety had no significant influence on the beneficial activity of *T. harzianum*. Significant ($p<0.05$) seed germination and seedling growth were

shown in all the three varieties of maize treated with different concentrations of *T. harzianum.*

6.2 Recommendations

Based on the findings obtained from this study, the following recommendations were made;

1. For maximum maize seed germination and early seedling growth under water stress, 10^7 spores/ml of *T. harzianum* should be used to treat maize seeds irrespective of the plant variety.
2. Since the present investigation was a laboratory study, studies are recommended to evaluate the effect *T.harzianum* on seed germination and seedling growth under stress in the greenhouse and field.
3. The study used macro and micro features to isolate *T. harzianum* from the soil; therefore, it is recommended that molecular work should be done to identify specific strains of *T. harzianum* that can enhance maize seed germination and seedling growth under water stress.

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APPENDICES

Appendix I: Effect of main factors and their interactions on maize seed germination (GP)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A: Conc. of *T. harzianum*	10502.1	3	3500.69	100.82	0.000
B: Variety	29.1667	3	14.5833	0.42	0.658
C:Osmotic potential	89052.1	2	29684	854.9	0.000
INTERACTIONS					
AB	7900.69	9	877.855	25.28	0.000
AC	504.167	6	84.0278	2.42	0.032
BC	254.167	6	42.3611	1.22	0.702
ABC	368.056	18	20.4475	0.59	0.899
RESIDUAL	3333.33	96	34.7222		
TOTAL (CORRECTED)	111944	143			

Appendix II: Effect of main factors and their interactions on maize seedling length

Analysis of Variance for SL - Type III Sums of Squares					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:Conc	153.398	3	51.1327	3055.23	0.000
B:Var	0.160972	2	0.080486	4.81	0.102
C:OP	3257.92	3	1085.97	64888.08	0.000
INTERACTIONS					
AB	0.093472	6	0.015579	0.93	0.576
AC	23.4873	9	2.6097	155.93	0.000
BC	4.42403	6	0.737338	44.06	0.000
ABC	0.790417	18	0.043912	2.62	0.130
RESIDUAL	1.60667	96	0.016736		
TOTAL (CORRECTED)	3441.88	143			

Appendix III: Effect of main factors and their interactions on maize fresh seedling weight (SFW)

Analysis of Variance for SFW - Type III Sums of Squares					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:Conc. *T. harzianum*	0.53251	3	0.177503	2206.72	0.000
B:Variety	0.003564	2	0.001782	22.15	0.051
C:Osmotic Potential	3.20719	3	1.06906	13290.6	0.000
INTERACTIONS					
AB	0.005011	6	0.000835	111.38	0.062
AC	0.085267	9	0.009474	117.78	0.000
BC	0.005718	6	0.000953	11.85	0.000
ABC	0.01059	18	0.000588	7.31	0.071
RESIDUAL	0.007722	96	8.04E-05		
TOTAL (CORRECTED)	3.85757	143			

Appendix IX: Effect of main factors and their interactions on maize shoot dry weight (SDW)

Analysis of Variance for SDW - Type III Sums of Squares					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:Conc. *T. harzianum*	0.005398	3	0.001799	1378.27	0
B:Var	1.85E-06	2	9.24E-07	0.71	0.4955
C:OP	0.02923	3	0.009743	7462.91	0
INTERACTIONS					
AB	1.57E-05	6	2.61E-06	2	0.0733
AC	0.001452	9	0.000161	123.53	0
BC	3.87E-05	6	6.44E-06	4.93	0.0002
ABC	3.52E-05	18	1.95E-06	1.5	0.1079
RESIDUAL	0.000125	96	1.31E-06		
TOTAL (CORRECTED)	0.036296	143			

Appendix V: Effect of main factors and their interactions on maize root dry weight (RDW)

Analysis of Variance for RDW - Type III Sums of Squares					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:Conc	0.03503	3	0.011677	121.32	0.000
B:Var	0.001479	2	0.00074	7.68	0.001
C:OP	0.167683	3	0.055894	580.76	0.000
INTERACTIONS					
AB	0.000647	6	0.000108	1.12	0.055
AC	0.004076	9	0.000453	4.71	0.000
BC	0.004871	6	0.000812	8.43	0.000
ABC	0.002152	18	0.00012	1.24	0.245
RESIDUAL	0.009239	96	9.62E-05		
TOTAL (CORRECTED)	0.225177	143			

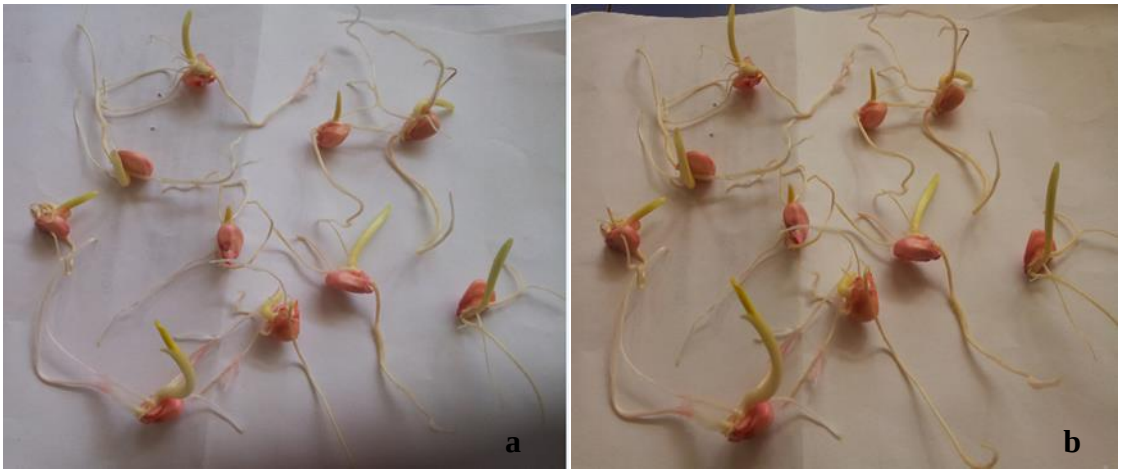
Appendix VI: Effect of main factors and their interactions on superoxide dismutase activity (SOD)

Analysis of Variance for SOD - Type III Sums of Squares					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:Conc	1.66E+06	3	552663	13798004	0.000
B:Var	84.0881	2	42.0441	149.69	0.067
C:OP	6.52E+06	3	2.17E+06	54226483	0.000
INTERACTIONS					
AB	9.15598	6	1.526	38.1	0.054
AC	2.19E+06	9	243168	6071030	0.000
BC	192.197	6	32.0328	799.74	0.000
ABC	128.822	18	7.1568	178.68	0.061
RESIDUAL	3.80512	95	0.040054		
TOTAL (CORRECTED)	1.02E+07	142			

Appendix VII: Effect of main factors and their interactions on catalase activity (CAT)

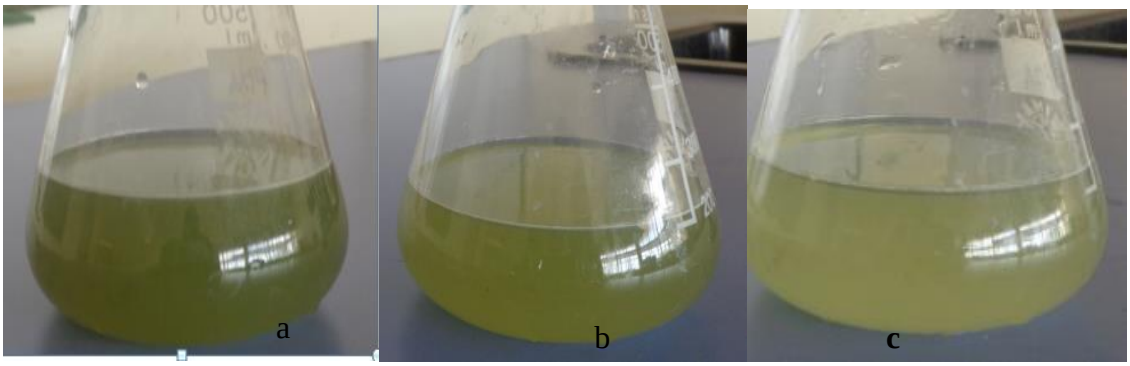
Analysis of Variance for CAT - Type III Sums of Squares

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:Conc. *T. harzianum*	79.2417	3	26.4139	13440.28	0.023
B:Variety	0.039339	2	0.019669	10.01	0.051
C:Osmotic potential	196.494	3	65.4979	33327.53	0.000
INTERACTIONS					
AB	0.011467	6	0.001911	0.97	0.056
AC	59.331	9	6.59233	3354.4	0.011
BC	0.063856	6	0.010643	5.42	0.000
ABC	0.084139	18	0.004674	2.38	0.059
RESIDUAL	0.188667	96	0.001965		
TOTAL (CORRECTED)	335.454	143			



Appendix VIII: Germination of maize treated with 0 spores/ml of *T. harzianum* at 0MPa (a) and with 10^7 spores/ml of *T. harzianum* at 0MPa (b) (Source: Author, 2015)

Appendix IX: Germination of maize seeds treated with 0 spores/ml of *T. harzianum* at -0.9MPa (a) and those treated with 10^7 spores/ml of *T. harzianum* at -0.9MPa (b) (Source: Author, 2015)



Appendix X: Approximately 10^{10} (a) 10^7 (b) and 10^5 (c) spores/ml of *T. harzianum* from semi-arid soils (Source: Author, 2015)