STATUS OF COPPER LEVEL AND EFFECTS OF RHIZATECH®TM VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI ON WHEAT GROWTH, YIELD AND COPPER ION UPTAKE IN NAROK NORTH SUB-COUNTY, KENYA

BY

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REGISTRATION NUMBER: I56/36141/2019

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY OF THE UNIVERSITY OF NAIROBI

DEPARTMENT OF BIOLOGY

FACULTY OF SCIENCE AND TECHNOLOGY

UNIVERSITY OF NAIROBI

JUNE 2023

DECLARATION

I DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination or award of degree. Where other people's work has been used, this has been properly acknowledged and referenced in accordance with the University of Nairobi's requirements.

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DEDICATION

I dedicate this thesis to my parents, donors from Kenya Climate Smart Agriculture, my supervisors and KALRO for their support and guidance.

ACKNOWLEDGEMENT

I sincerely wish to acknowledge the support of my supervisors Dr. Catherine Kitonde, Dr. Virginia Wang'ondu, and Dr. Esther Gikonyo, whom this research was carried out under their supervision. I am grateful to them for their guidance and kind assistance throughout the research process. My sincere thanks also go to my donors from Kenya Climate Smart Agriculture for their financial and moral support throughout my studies. I also acknowledge Kenya Agricultural and Livestock Research Organization (KALRO) for their immense support especially for allowing me to use their greenhouse and farms. My special thanks also go to my parents, siblings and friends for always believing in me.

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LIST OF ABBREVIATIONS AND ACRONYMS

°C	Degrees Celsius
AAS	Atomic Absorption Spectrometer
VAMF	Vesicular arbuscular mycorrhiza Fungi
SOD	Superoxidase dismutase
VAM	Vesicular Arbuscular Mycorrhiza
TE	Trace Elements
SSA	Sub-Saharan Africa
KALRO	Kenya Agricultural and Livestock Research Organization
RCBD	Randomized Complete Block Design
CRD	Completely randomized design
pH	Potential Hydrogen
DAP	Di-ammonium phosphate

UNITS OF MEASUREMENTS

µg	Microgram
µL	Microliter
g	Grams
Kg	Kilograms
L	Litre
mL	Milliliter
ng	Nanogram
nm	Nanometer
ppb	Parts per billion
ppm	Parts per million
Meq%	Mill equivalent

ABSTRACT

Soil related abiotic factors hindering wheat production in wheat growing regions in East Africa include poor soil fertility, pests, diseases, drought and salinity. Copper (Cu) deficiency has been identified in soils in major wheat growing areas in Kenya. Majority of the farmers in the region use chemical fertilizers to alleviate this deficiency, which may be harmful to human health, animals and environment. Bio fertilizers can serve as safe natural alternative to reduce reliance on chemical fertilizers. Thus, the overall objective of this study was to determine the effect of RHIZATECH ® TM (a mixture of strains of *Glomus* species; including *Glomus intraradices, Glomus etunicatum and Glomus mosseae*) vesicular arbuscular mycorrhiza fungi, on wheat growth, grain yield and copper ion uptake under copper deficient soils in wheat growing areas in Narok North Sub-County. Fifty soil samples were collected from 50 wheat growing farms in five wards: Narok Town, Olokurto, Nkareta, Oloropil and Melili. The levels of copper in the soil samples were determined using Atomic Absorption Spectrophotometer. Greenhouse and field experiments were set up in Completely Randomized Design (CRD) and Randomized Complete Block Design (RCBD), respectively. Wheat seeds from four wheat varieties including King bird, Kenya Eagle 10, Njoro BW2 and Kenya Tai were planted in pots and furrows after treatment with or without the RHIZATECH ® TM. Roots of the germinated wheat seedlings were sampled 8 weeks after sowing to assess wheat root colonization by RHIZATECH®TM mycorrhiza. Plant height, total dry weight and grain yield were measured as response variables, 120 days after inoculation with RHIZATECH ® TM. The dry roots, shoots and grains were ground into small particles for determination of Cu ion concentration using microwave-assisted acid extraction and measured using atomic absorption spectrophotometer (AAS). Copper concentration levels varied between and within the farms of which 29 soil samples (58%) of the total 50 soil samples were below the critical value of 1.0 mg/kg and was deemed as low concentration whereas 21 soil samples (42%) of the total 50 soil samples were above the critical concentration value of 1.0 mg/kg hence were adequately supplied with copper for wheat growth. The total average mean of

copper concentration in the soil samples was 0.98±0.11 (SE) mg/kg, which according to soil classification was below the critical value of copper hence, these findings are an indication of copper deficiency in soils in wheat growing areas in Narok North Sub-County. In both greenhouse and field trials, inoculation of the wheat varieties with RHIZATECH ® TM inoculant resulted in a positive impact on the wheat varieties over the non- inoculated wheat varieties (controls). In the greenhouse study, this reflected in significant mean increase at p=0.00 in RHIZATECH ® TM VAMF colonization rate from 4% to 46.5%. Biomass also significantly increased at p=0.001 from12.16 g to 16.12 g. Wheat grain number significantly increased at p=0.005 from 204 to 273. Whereas grain weight significantly increased at p=0.021 (5.10 g to 6.36 g). Copper ion uptake significantly increased at p=0.001 (8.29 ppm to 9.93 ppm). However, there was no significant increase at p=0.96 (68.7 cm to 68.83 cm) in wheat stem height. In Narok and Kabete field trials, there was significant mean increase at p=0.001 in RHIZATECH ® TM VAMF root colonization rate. It also led to significant increase in means of wheat grain number (p=0.003) and grain weight (p=0.019) at Nkareita site. Wheat height significantly increased at Oloropil site at p= 0.001 (60.8 cm to 63.5 cm) and at Kabete sites at p=0.005 (47.9 cm to 54.4 cm). However, wheat height had no significant increase at p=0.114 (59.2 cm to 62.8 cm) at Nkareita site. Copper uptake significantly increased at p=0.05 (8.4 ppm to 9.8 ppm) and at P =0.007 (8.8 ppm to 9.8 ppm) at Nkareita and Oloropill sites respectively. Wheat biomass significantly increased at p=0.005 (982 g to 1062 g) at Nkareita site. However, effects of RHIZATECH®TM application did not lead to significant biomass increase at p=0.98 (1047 g to 1128 g) and at p=0.11 (433 g to 438 g) at Oloropill and Kabete sites respectively. This study found that there is need of bio fertilizer use such as RHIZATECH ® TM fungi as economical and environmentally sustainable method of bridging wheat yield gap in Cu-deficient soils.

Key Words: Vesicular arbuscular mycorrhiza, wheat variety, Copper deficiency, Narok County.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Globally, most of the arable soils are deficient in micronutrients including Copper (Cu), which affect human nutrition (Cakmak *et al*., 2017). Yet, most staple cereals in Africa like maize, rice and wheat are grown in these soils that are deficient of micronutrients (Hengl *et al*., 2017). Micronutrient deficiency is common in soils of Sub-Saharan Africa (SSA) due to poor soil fertility caused by deforestation, soil erosion, over cultivation of land and excessive use of nitrogen based chemical fertilizers (Kamoni and Makhoha 2010; Berkhout *et al*., 2017).

Wheat is the second most imported cereal crop in Kenya after maize and consumption is expected to increase by 5 percent to 2.2 million metric tons as from year 2022 as Kenya's tourism and hospitality sectors partially recover from COVID-19 restrictions (USDA, 2022). However, majority of Kenya's wheat-growing regions have experienced wheat yields decline over time and output has been inconsistent, ranging from 264,457 tons in 1991 to almost 126,000 tons in 2000 (Tadesse *et al.,* 2018). Production is estimated to fall from 300,000 to 250,000 metric tons during year 2021 and 2022 in key growing counties including Meru, Uasin Gishu, Laikipia, and parts of Narok (Tadesse *et al.,* 2018). This is mainly due to poor soil fertility, pest, diseases, drought, salinity and deficiency in micronutrients including Copper (Cu), which is very important in wheat growth and development (Pilon *et al*., 2019). According to Gicheru, *et al.,* (2012), several areas in Narok such as Suswa is characterized by loss of land cover, soil erosion, reduced water catchment areas and reduced soil nutrient availability such as copper. Many studies have shown Cu deficiency in remote non-wheat growing areas of the country such as Shimba Hills (Sutton *et al*., 2002). This may be indicative of widespread Cu deficiency in Kenyan soil including wheat-growing areas.

This study targeted copper because, while a deficiency of any other micronutrient may reduce wheat yields, the crop is particularly sensitive to copper (Karamanos *et al*., 2004). Pilon, *et al*. (2019) further

identified that copper is essential and is required for a number of plant processes, including electron transfers, chlorophyll formation, protein synthesis, and respiration. Wheat production can be severely impacted by copper shortage, which leads to delay in heading, limpness or wilting, aborted spikelet and heads are all indications of copper deficiency in wheat (Lal, 2016).

To solve the micronutrient deficiency and improve productivity, majority of the farmers in the region have resorted to use of synthetic chemical fertilizers. In Kenya, copper oxychloride has been used as foliar feed and seed dressing in crop production such as wheat (Owuoche *et al*., 2002). Moreover, copper-based plant protection products and fertilizers are explicitly allowed according to the European Commission implementing Regulation (EU) No 2021/1165 (European Commission, 2021a).

These chemical fertilizers affect the entire plant by increasing wheat growth, wheat grain yield and vigor (Karamanos *et al.,*2004). Almost 2–3 billion people globally, especially from developing nations suffer from micronutrient deficiencies (Goudia and Hash, 2015). Consumption of micronutrient deficient foods, because of micronutrient deficiency in soils, causes micronutrient deficiency in humans, a phenomenon now recognized as 'hidden hunger' (Joy *et al*, 2015; Manzek *et al.*, 2019).

These micronutrients such as copper, manganese, iron and zinc are biologically important in plants, acting as cofactors of several proteins (Zeng *et al.,* 2019). These elements could be deficient in both plants and animals. In excessive concentrations, trace elements such as copper negatively affect crop yield (Saleem *et al.,* 2020a). Vesicular arbuscular mycorrhizal fungi (VAMF) can increase nutrients uptake by plants, (Zhou *et al.,* 2020), and they provide the host plant with water and mineral nutrients and in exchange for photosynthetic products (Zhou *et al.*, 2020).

However, plant hosts show varied responses to AM inoculation, showing increase, decrease or no response, thereby limiting their application in crop production (Smith and Smith, 2011). Thus, the main objective of this research was to determine the effect of RHIZATECH ® TM fungi on wheat growth, grain yield and copper uptake from copper deficient soils of Narok North Sub-County. RHIZATECH ®

TM is a mixed culture of *Glomus intraradices, Glomus etunicatum* and *Glomus mosseae* and it is in form of spores and mycelia fragments of vesicular arbuscular mycorrhizal at a concentration of 50 propagules per cubic centimeter ($50/cm^3$). Vesicular Arbuscular mycorrhizal fungi especially of *Glomus* species have proven to be adaptable to a variety of soil types and climatic circumstances. It colonizes swiftly and aggressively, provides water and nutrients to plants extremely well, and has good field performance on a range of crops .

1.2 Problem statement

Narok North Sub-County is one of the major wheat growing areas in Kenya, however farmers have experienced a decline in wheat production over the years. Kenya's wheat production decreased by 23.1 per cent from 214.7 thousand tons recorded in 2016 to 165.2 thousand tons in 2017 (KNBS, 2018). This has been due to factors such as poor soil fertility, pests, diseases, drought and salinity (Asseng *et al*., 2015). Deficiency in micronutrients including copper (Cu) which is very important in wheat growth and development, has been identified in wheat growing counties such as Nakuru and Narok.

Copper was chosen as the target of this study because, while a deficiency of any other micronutrient may reduce wheat yields, the crop is particularly sensitive to copper. Copper is a crucial nutrient for wheat plant because it aids in the production of chlorophyll and proteins from amino acids, and gives plants stiffness (Pilon *et al*., 2019). More than 30 enzymes in tall plants, including nitrate reductase, cytochrome oxidase, and hemocynin, operate as redox catalysts or as dioxygen carriers (Zeng *et al*., 2019). Insufficient amounts of copper promote spikelet sterility in many empty grains and have an impact on a variety of plant metabolic activities, including photosynthesis and pollen respiration (Marques *et al*., 2018).

Copper deficient plants display symptoms such as chlorosis in younger leaves (A), stunted growth and the wheat's leaves become shriveled and twisted, trapping an ear(C), lodging, melanosis and delayed maturity (D) (Reuter *et al.*, 1981). Grain fill and yield are low in cereals, and grain heads may not develop

at all after severe deficiency (McCauley *et al.*, 2009) as shown in Table 2.2. Wheat may be grown with a Cu content of 1 mg/kg of soil (Farnandez and Hoeft, 2012). In tiny levels, copper is necessary for numerous metabolic processes in soils, but it becomes poisonous at excessive doses (Nazir *et al.*, 2019).

1.3 Justification of the study

Wheat (*Triticum aestivum L.*) is one of the major crops in East Africa (EA). There has been a change in dietary pattern toward quick-to-cook meals in many EA nations, especially wheat-based diets, as a result of rising population growth and urbanization as well as rising family incomes (Negassa *et al*. 2013). Wheat consumption has increased in Kenya and is expected to increase by 5 percent to 2.2 million metric tons as from year 2022 (USDA, 2022). This trend is alarming for a Country that depends mainly on agriculture that contributes about 63 percent of Gross Domestic Product (KIPRA, 2013). The inability to satisfy the local demand could be attributed to limited production due to soil infertility (Berkhout *et al*., 2017).

Soil fertility could be replenished either through biological means or by use of organic or chemical fertilizers but since chemical fertilizers are costly, unsafe and out of reach for smallholder farmers, they may opt to use more affordable biological methods like vascular arbuscular mycorrhiza fungi. However, sufficient information on the efficacy of VAMF fungi especially of *Glomus species* is limited by inadequate knowledge of screening under greenhouse and field conditions. It is therefore necessary to conduct greenhouse and field trials of bio fertilizers such as RHIZATECH ® TM fungi for better management of soil fertility, wheat growth and production.

Bio fertilizers are cost effective, safe, environmentally friendly, and can be produced on the farm itself when needed in large quantities (Wadhwa *et al*., 2017). Bio fertilizers comprised 2% to 2.5% phosphorus, 1.5% potassium, and 3.5% to 4% nitrogen and it was discovered to have higher N, P, and K values (Mukhopadhyay, 2006). Thus, this study was aimed to determine the levels of copper concentration in wheat growing soils in Narok North Sub-County and to establish the effectiveness of RHIZATECH ®

TM vesicular arbuscular mycorrhiza fungi in nutrient acquisition by the wheat plant, following greenhouse experiments and field trials.

1.4 Research questions

1. What is the status of copper ion concentration in wheat growing soils in the five wards of Narok-North Sub-County; including Narok Town, Olokurto, Nkareta, Oloropil and Melili?
2. How effective is RHIZATECH ® TM vesicular arbuscular mycorrhizal fungi association in increasing growth, yield and copper ion uptake in wheat varieties?

1.5 Objectives of the study

1.5.1 Broad objective

To increase wheat production through sustainable soil fertility management in the five wheat growing wards of Narok North Sub-County.

1.5.2 Specific objectives

1. To determine the concentration of copper ion in wheat growing soils in the five wards of Narok North Sub-County.
2. To evaluate the effect of RHIZATECH®TM vesicular arbuscular mycorrhizal fungi on wheat growth, grain yield and copper ion uptake by selected wheat varieties.

1.6 Hypotheses

1. Soils in wheat growing areas in the five wards of Narok North Sub-County, have low level of copper ion.
2. RHIZATECH®TM Vesicular arbuscular mycorrhizal association with wheat varieties has no significant effect on wheat growth, grain yield and copper ion uptake.

CHAPTER TWO: LITERATURE REVIEW

2.1 History of wheat

Wheat *(Triticum aestivum*) is an old native grain of Gramineae family and is amongst the cereal crops mostly used in the food industry (Wrigley 2009). Wheat was used by humans before agriculture commenced (Mac Key, 2005). Nonetheless, from that point forward, the process of selecting the largest wheat kernel for sowing gradually modified those ordinary grasses for human utilization, kicking off an approach to a plant ideotype with improved agronomic traits including higher yields (Hillman and Davies, 1990).

The first "green revolution" for wheat began around 10,000 Before Present (B.P.) with the gradual domestication of some wheat species, most notably the eikorn (*Triticum monococum*) and emmer wheats (*Triticum turgidum ssp. Dicoccum*), which were largely utilized in old times and from which modern bread and durum wheat descended (Mac Key, 2005). The cultivated emmer has a lower total known weight than durum (Faris *et al*., 2014) and bread wheat because it is hulled and lacks the Q "domestication" gene linked to the pleiotropic effects of free threshing ability, rachis stiffness, and glumes tenacity (Konvalina *et al*., 2008).

2.2 Wheat production and consumption in Kenya

Kenya is the fourth most important wheat producer in Africa after Ethiopia, South Africa and Sudan with some of the prominent wheat growing areas being Laikipia, Nakuru, Nyandarua, Keiyo and Narok (Kamwaga, 2016). In the 1990s, yearly wheat output averaged 258,207 tons in Kenya. However, output has been inconsistent, ranging from 264,457 tons in 1991 to almost 126,000 tons in 2000 (Tadesse *et al.*, 2018). Production is estimated to fall from 300,000 to 250,000 metric tons during the year of 2021 and 2022, due to drought and soil infertility in all of Kenya's key growing counties including Meru, Uasin Gishu, Laikipia, and parts of Narok (USDA, 2022). To solve this soil infertility/micronutrient deficiency and to improve productivity, majority of the farmers in the region have resorted to use of synthetic

chemical fertilizers especially nitrogen based, which are unsafe for human health, animals and environment if not applied in the correct amount (Kamoni and Makhoha 2010, Berkhout *et al.*, 2017).

Excessive use of chemical fertilizers over a long period of time has become a major source of soil contamination (Pahalvi *et a*l., 2021). Excessive chemical fertilizer application decreases the soil pH and soil fertility, which enhances heavy metals availability (Pahalvi *et a*l., 2021). Excessive heavy metal buildup degrades soil's physical and biological features, as well as plant development, which can lead to plant mortality (Nazir *et al.*, 2019). Moreover, farmers are presently increasing wheat yield horizontally by planting in locations that were previously deemed unsuitable for farming (FAO, 2002).

As early as 1990 in Kenya, Alary *et al*. (2007) and Tanner and Mwangi (1992) sought to build a sustainable wheat production system for the altitude zones below 1700m above sea level that are historically used by pastoralists. Additionally, Kenya Agriculture and Livestock Research Organization (KALRO) has created wheat cultivars for Kenya's arid regions (Kamwaga *et al*., 2016). The bread varieties have been developed for various qualities in a period spanning over 85 years. Over 180 wheat varieties have been made available to farmers throughout this time. The objective has been to contribute to increased food security as well as farm, community, and national economic growth (Kamwaga *et al*., 2016).

Contrary to production, wheat consumption has increased in Kenya (FAO, 2002). Although there have been some years where there has been a reduction, there has been a rise, especially in the years 1992, 1995, and 1997 (FAO, 2002). Kenya’s population continues to rise at roughly 2 percent per year, providing a steady increase in wheat demand (USDA, 2022). For the year 2022 and 2023, feed consumption is estimated to remain flat on stalled production of animal feed due to high ingredient costs whereas food, seed, and industrial consumption is expected to increase by 5 percent to 2.2 million metric tons as Kenya’s tourism and hospitality sectors partially recover from COVID-19 restrictions (USDA,2022).

Table 2.1: Four wheat varieties with desirable traits that are commonly cultivated in Narok.

Wheat Varieties	Altitude (masl)	Yield potential (tons)	Maturity period (Days)	Special attributes
Eagle10 (*Triticum aestivum* L)	1800 – 2100	6.5	100-110	Resistant to stem rust Early Maturing Good industrial and home baking
Kenya Kingbird (*Triticum aestivum* L)	1800-2400	6.0	90-110	High resistance to both stem and yellow rusts Early maturing Good for industrial and home baking
Njoro BW 2 (*Triticum aestivum* L)	2100-2400	8.0	140-160	High yielding red hard wheat Tolerant to acid soils, lodging and pre-harvest sprouting Excellent baking qualities
Kenya Tai (*Triticum aestivum* L)	1800-2100	6.5	100-110	Moderately resistant to stem rust Red hard grain Heavy biomass hence an excellent source of straw for livestock feed

Source: Kenya Wheat Production Handbook

2.3 Nutritional value and economic importance of wheat

Wheat is an old native grain of *Gramineae* family and is amongst the cereal crops mostly used in the food industry (Wrigley, 2009). The most significant are the *Triticum aestivum* mainly common in bread production, *Triticum durum* is used to manufacture several pastes such as macaroni and spaghetti (Wrigley 2009), while *Triticum compactum,* a softer type is mainly for pastries, cookies and baking cakes (Huebner *et al.,* 1999). Wheat grain nutritional contents are not the same and it varies depending with the overall climatic conditions and soil types (Tadess *et al.,* 2018). Notably, the kernel contains water, carbohydrates, proteins, fat, minerals and crude fibers. In addition, riboflavin, thiamin, and vitamin A are also present in the kernel (Tadess *et al.,* 2018).

However, during the milling process a lot of nutrients are removed together with the barn and germ (Tadess *et al.,* 2018). The largest portion of processed wheat is used for bread baking (Wrigley 2009). Wheat plants that thrive well in dry conditions are hard type and containing protein best for bread baking, whereas softer wheat is from areas with high humidity and has relatively weak gluten (Braun *et al.,* 2010). Wheat provides proteins and calories needed by humans globally on a daily basis, in form of bread, chapatti, biscuits and cookies as well as commercial products of starch such as gluten (Braun *et al.,*2010). Thus, wheat is one of the contributor to food security globally, with wheat occupying 10 million hectares in Africa.

The main market for wheat products manufactured in Kenya is the country itself. Supermarkets, establishments including schools and hotels/restaurants, retail stores, and aid organizations like UNHCR, UNICEF, and CARE Kenya that purchase high-energy biscuits for 37 refugees are some examples of outlets (UNCTAD and Cyclope, 1999). Consequently, an indirect export market exists. Uganda, Tanzania, Zaire, and Rwanda account for the majority of the companies' exports to the East African market. However, only a small fraction of the products is exported, average 6.7 percent per business and ranging from 2 to 11.8 percent overall (UNCTAD and Cyclope, 1999).

2.4 Wheat production constraints in Kenya

2.4.1 Abiotic constraints

A third of arable soils worldwide are low in micronutrients such as zinc (Zn) and copper (Cu) (Cakmak *et al*., 2017). Major key constraint of wheat production in Kenya is poor soil fertility and other effects such as climate change, which include rising temperatures and irregular rainfall (Asseng *et al*., 2015).There exists diversity in the fertilities of soils in the region due to difference in soil forming and environmental factors (Eswaran *et al*., 1992) but majority of the soils are weathered, and infertile thus not able to supply minimum quantities of essential micro and macronutrients like Zn, Cu, Fe (Cakmak *et al.,* 2017).

This can also be attributed to extended periods of soil degradation, low and unbalanced use of fertilizers containing Nitrogen, Phosphorus and Potassium (Goudia and Hash, 2015). Moreover, numerous processes including adsorption-desorption or precipitation-dissolution influence the behavior of metals including copper in soil. Post addition of a mineral to soil, both adsorption and precipitation processes reduce the available mineral concentration by conversion into less available forms (McLaughlin *et al.,* 2010).

Copper deficiency in soils in wheat growing areas has been identified in Nakuru and Narok (Pinkerton, 1967) but many studies have shown Cu deficiency in remote non-wheat growing areas of the country such as Shimba Hills (Sutton *et al*., 2002). This may be indicative of widespread Cu deficiency in Kenyan soil including wheat-growing areas. It can then be concluded that the general trend in poor production of wheat in East African countries are likely due to poor soil fertility (Chand *et al.,* 2014). Due to decreased growth phases, early leaf senescence, lower biomass, and detrimental biochemical and physiological changes, high temperatures cause considerable grain quality and production loss in wheat (Chand *et al.,* 2014).

2.4.2 Biotic constraints

Biotic issues that impact the production of wheat in SSA are pests and diseases that farmers are not able to control effectively (Solh *et al.,* 2012). Brown leaf rust caused by fungus *Puccinia triticina* is one of the common diseases in wheat and it is presented as slightly elliptical or circular pustules that are smaller contrasted to stem rust (Kamwaga *et al.,* 2016).

Yellow rust disease caused by *Puccinia striiformis* mainly affects the leaves and is characteristically presented in form of yellow spores. Initially, they appear as small and circular and advance into parallel yellow stripes on the upper leaf surface and as yellow powder within the glumes whereas black stem rust infects the glumes, stems, leaves and or leaf sheaths (Kamwaga *et al.,* 2016). The infection appears as dark-brown pustules on the spikes and stems of the plant and the farmer could lose the entire produce if left untreated (Kamwaga *et al.,* 2016).

Barley Yellow Dwarf is the viral disease caused by yellow dwarf virus shown by yellow discoloration from the leaf's tip and dwarfing of the plant. At the head of the plant also becomes discolored mostly during the ripening stage. The pests also have significant impact on wheat crop in Kenya (Kamwaga *et al.,* 2016). The field pests include aphids, termites, grasshoppers, beetles, nematodes (of roots and grain), larvae and birds among others (Kamwaga *et al.,* 2016). All of these pests that attack different parts of the crop at different growth phases have detrimental losses to the farmers. Some of these pests include; African armyworms, Cereal aphids and the Russian Wheat Aphid (*Diuraphisnoxia*) (Kamwaga *et al.,* 2016).

2.5 Vesicular arbuscular mycorrhizal fungi

Vesicular arbuscular mycorrhizal fungi belong to the fungi kingdom, and it is composed of several genera including *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* (Smith and Read, 2011). The genus *Glomus* involved in the constitution of RHIZATECH®TM vesicular arbuscular mycorrhizal fungi in this study is classified under phylum Glomeromycota, class Glomeromycetes, order

Glomerales, and family Glomeraceae. There are different species in the *Glomus* genus including *Glomus aggregatum, Glomus etunicatum, Glomus fasciculatum, Glomus intraradices, Glomus microaggregatum* and *Glomus mosseae* (Smith and Read, 2011*).* From these mentioned species, only three *Glomus* species are used to constitute RHIZATECH®TM vesicular arbuscular mycorrhizal fungi, including *Glomus etunicatum*, *Glomus intraradices*, and *Glomus mosseae* according to Dudutech Finlay Horticulture Limited (DFH, 2014).

Vesicular arbuscular mycorrhizal fungi forms mutualistic relationships with over 95% of all vascular plants (Brundrett and Tedersoo, 2018) enabling plants to obtain soil mineral nutrients including those unreachable to the plant roots via their extra-radial mycelium (Luginbuehl *et al*., 2017). The fungus enters roots parenchyma and create their hyphae on cortex cells of the root. The hyphae rapidly colonize the root cortical cells and branch extensively forming arbuscules and vesicles that last for 6-11 days that act as primary site for plant and fungus nutrient exchange (Montero *et al.,* 2019). The obligate symbiotic relationship occurs in the form of the VAM fungus obtaining carbon from the plant while providing nutrients to the plant, in the process the fungus obtains 10-40% of carbohydrates manufactured through photosynthesis. Even after the death of the plant shoot, the fungus is capable of obtaining nutrients from the dead plant for up to 5 months (Pepe *et al.*, 2018).

Plant growth limits caused by insufficient nutrient input can be alleviated by AMF (Nouri *et al.,* 2014). Besides nutritional benefits, AM is also important in improving tolerance to abiotic stressors like salinity (Porcell *et al.,* 2011; Auge *et al*., 2015) and disease resistance (Pozo and Azcón- Aguilar, 2007). AMF also plays an important part in metal homeostasis. Metals including Cu, Fe and Zn play an important part in cellular processes but they are toxic at high concentrations (Tamayo *et al.,* 2014). Vesicular arbuscular mycorrhizae fungi alleviate heavy metal toxicity in plants, including Cu toxicity (Göhre and Paszkowski, 2006; Cornejo *et al.*, 2013). Vesicular arbuscular mycorrhiza fungus can also improve plant mineral nutrient uptake, especially in low-nutrient environments (Smith and Smith, 2011).

The fungi are capable of increasing the plant acquisition of major macronutrients such as Phosphorus, Nitrogen (Luginbuehl *et al.,* 2017). Wheat is a crop with a high N fertilizer demand, and nitrogen deficit is a significant limiting factor for growth, grain production, and grain quality (Liu, 2020b). Chronic nitrogen deprivation had a negative impact on durum wheat plant height, tillering, flag leaf area, spike and seed characteristics, and grain total N content (Curci, 2017).

Different processes have been proposed to explain why mycorrhizal plants are better able to absorb these nutrients. These include examining a broader area of soil, hastening the flow of P and N into mycorrhizal hyphae and solubilizing the phosphorus in the soil (Zhou *et al.,* 2020). AM fungi have also been shown to enhance the uptake of and phyto-availability of plant essential trace elements such as Cu, Fe and Zn (Lehman and Rillig, 2015). Therefore, VAMF could be a vital alternative way to improve micronutrient concentration in crops and improve the crop quality attributed to its enrichment to crops of both micro and macronutrients (Pellegrino and Bedini, 2014).

2.6 Role of copper in plant physiology and implications of its deficiency

Copper is a vital micronutrient in a variety of metabolic activities. Plastocyanin is the most abundant copper protein in higher plants, and it is involved in photosynthetic electron transport in the chloroplasts (Pilon *et al.,* 2019). Plants can adjust uptake by roots (Sancenon *et al*., 2003), tighten Cu transport via P-type ATPase and copper chaperones (Chu *et al.,* 2005). Copper deficient plants display symptoms shown in Figure 2, such as chlorosis in younger leaves**,** stunted growth and the wheat's leaves become shriveled and twisted, trapping an ear, lodging, melanosis and delayed maturity (Reuter *et al.,* 1981). Grain yield are low in cereals, and grain heads may not develop at all after severe deficiency (B) (McCauley *et al.,* 2009). Wheat may be grown with a Cu content of 1 mg/kg of soil (Farnandez and Hoeft, 2012). In tiny levels, copper is necessary for numerous metabolic processes in soils, but it becomes poisonous at excessive doses (Scheiber *et al.,* 2013).

The concentration of Cu ions varies greatly, depending on interactions between parent rocks, physical

and chemical characteristics of soil and external inputs (Steffan *et al.,* 2018). The behavior of metals including copper in soil is influenced by numerous processes including adsorption-desorption or precipitation-dissolution (Steffan *et al.,* 2018). In few moments post addition of a mineral to soil, both adsorption and precipitation processes reduces the available mineral concentration by conversion into less available forms (McLaughlin *et al.,* 2010).

While the levels of micronutrients in soil solutions and their availability are curbed by adsorption reactions at the surfaces of soil colloidal materials (Steffan *et al.,* 2018), desorption process controls the amount and rate of release of micronutrients into solution for plant uptake (Kuo and Mikkelsen, 1980). Desorption of nutrients is a vital process since uptake by plants depletes nutrients in the root zone. However, desorption process remains poorly understood, thus a difficulty persists in predicting the potential for deficiency and/or toxicity (Covelo *et al.,* 2004). The rate of desorption occurs at a slower rate compared to adsorption reactions attributed to transformation of adsorbed ions from one state to the other and also reaction with other soil minerals (Singh *et al.,* 2006).

Table 2.2: Copper deficiency symptoms in wheat

Symptom	**Degree of Deficiency**		
	Slight	Moderate	Severe
Wilting or limpness at mid-tillering.			X
Chlorosis in younger leaves			X
Whip tailing- the tip of the leaf dies and can roll and turn white.		X	X
Wheat has unusually high amounts of take-all or "take-all-like" effects.	X	X	X
Poor stem growth		X	X
Delay in heading causes non-uniform heading, especially on light loamy soils with uniform crop emergence and early growth.		X	X
Spikes and heads are nearly normal, but contain many empty spikelet- grain production and fill are often poor		X	X
Aborted spikelet and heads.			X

Source: (Graham and Nambiar, 1981)

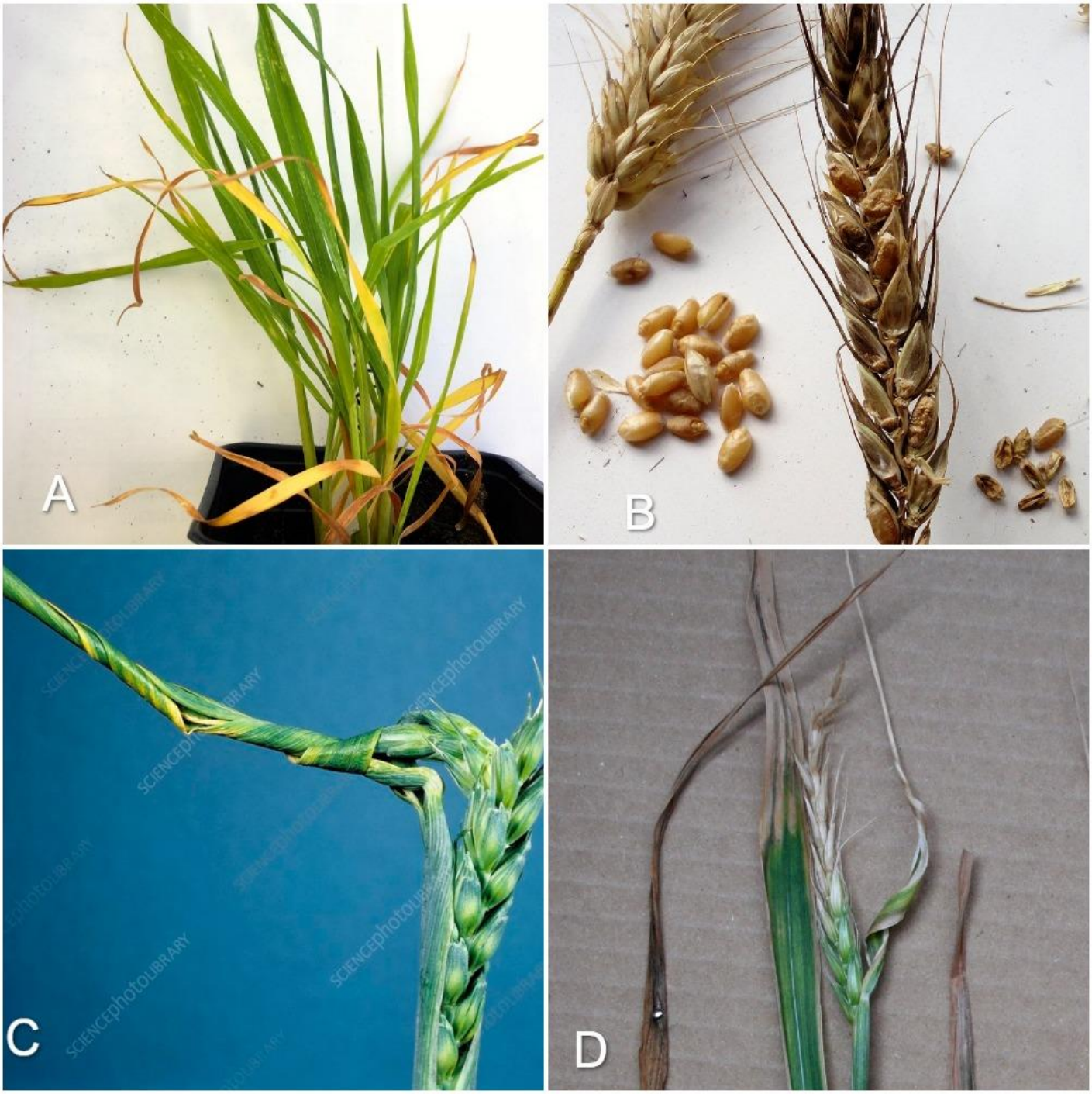

Figure 2.1: Copper deficiency symptoms in wheat plant, Source: (Graham and Nambiar, 1981)

Key: Chlorosis in younger leaves (A), poor grain fill (B) Wheat leaves become shriveled and twisted and trapping of an ear (C), melanosis (brown discoloration) (D).

2.7 Use of bio fertilizers in wheat farming

Bio fertilizers are latent or live cells of efficient strains of mineralizing and cellulolytic microbes and the application of such fertilizers has emerged as a potential solution to promote plant fitness, boost yield, and tolerance to environmental constraints (Raklami *et al.,* 2019). Because of the aforementioned benefits provided by these microorganisms, one of the techniques for sustainable farming includes the use of bio fertilizers (Wadhwa *et al.,* 2017). Farmers can reduce their dependency on chemical fertilizers by using bio fertilizers, which is a win-win situation (Withers *et al*., 2017).

Bio fertilizers are better options to inorganic fertilizers, which are applied repeatedly and indiscriminately, which is harmful to the soil ecosystem, animals and people (Shen *et al*. 2013)). Furthermore, these chemical fertilizers are not fully used by the plants, with little quantity used by the crops (Kumar, 2019). However, bio fertilizers contain live organisms and their effectiveness is influenced by the environment (Chen, 2008). As a result, the consequences are guaranteed to be unpredictable (Rahim, 2002). Short shelf life, a lack of adequate carrier materials, sensitivity to high temperatures, and transportation and storage issues are of the constraints that must be addressed before effective inoculation can be achieved (Chen, 2008).

It is widely recognized that preserving the soil's nutrient levels, which are crucial for plant growth, is necessary for the wheat crops' performance improvement (NIIR, 2004). According to Kader *et al*. (2002), the addition of *Azotobacter* and VAMF inoculants increased wheat grain production above the control by 18%. Furthermore, Suri and Choudhary (2010) noted that inoculating *Glomus mosseae* and *Glomus intraradices* with phosphorus levels that ranged from 50% to 75% of the recommended dosage improved wheat growth, yield, and nutrient absorption over time. In addition, Khan and Zaidi (2007) discovered that inoculating *Azotobacter chroococcum* with *Bacillus* and *Glomus fasciculatum* significantly increased wheat biomass and nutrients concentrations as well as improved wheat grain quality compared to non-inoculated plants.

Use of bio fertilizers like compost and AMF in agriculture is on the rise due to the high cost of mineral fertilizers particularly in the country's arid and semi-arid regions (Hazra, 2014). According to Tilman *et al*. (2002), the usage of fertilizers containing nitrogen (N) and phosphorus (P) has expanded globally by 7 and 3.5 folds, respectively, during the previous six decades. By 2050 use of both fertilizers is predicted to increase by another three fold.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of the study area

Narok North Sub-County is located at Narok County, southern part of Kenya bordering Tanzania and geographically located at latitude 1° 5’ 0” S and longitude 35° 52’ 0” E, and altitude of about 1750 m to 1827 m above sea level (Jaetzold *et al*., 2010).

It lies across two agro-ecological zones (AEZ) as shown in Figure 3.1, namely, Lower Highland (LH) and Upper Highland (UH), which support wheat production in Kenya (Jaetzold *et al*.,2010). The average annual rainfall varies between 500 and 1800 mm (Jaetzold*et al*., 2010).

The areas feature two rainy seasons: short rains from October to December for both zones. Upper highland zone experience long rains from late February to May each year, whereas lower highland zones experience long rains from early February to May, with temperatures ranging from 17 to 29°C (Jaetzold *et al*., 2010). In both zones, the soils are deep, well drained and loamy (Jaetzold *et al*., 2010). The soil samples were collected from the five wards in the Sub-County, which are Narok Town, Olokurto, Nkareta, Oloropil and Melili as shown in Figure 3.1.

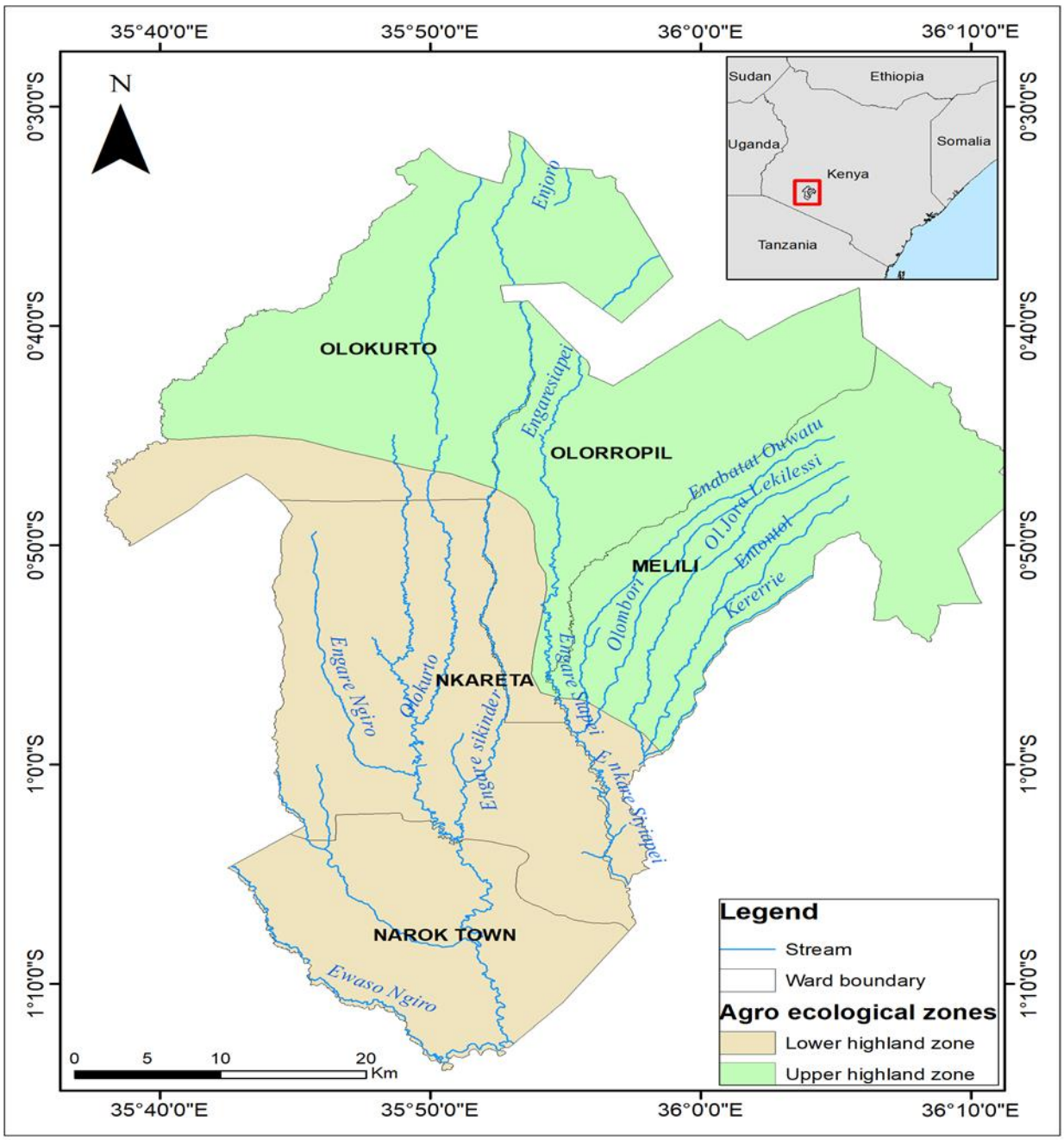

Figure 3.1: Map of Kenya showing location of the five wards in Narok North Sub-County with the two Agro-ecological zones, Lower highland and Upper highland zones (source; Jaetzold *et al*., 2010)

3.2 Description of the green house and field study sites

Greenhouse experiments and field trials were conducted at Kenya Agricultural and Livestock Research Organization (KALRO) and in Narok North Sub-County. Two field trials were conducted in Narok North Sub-County, whereas two greenhouse experiments and a field trial were conducted at (KALRO)-Kabete. Kabete is in agro-ecological zone III and is located at latitude 01° 15' S and longitude 36° 41' E, with altitude of 1800 meters above sea level (Sombreak *et. al.*, 1982).

The climate is typically sub-humid, with mean annual temperatures ranging from 12 to 23 °C and total annual rainfall ranging from 1200 to 1800 millimeters (Jaetzold *et al*., 2010). There are extensive rains from March to May and brief showers from October to November throughout the rainy season (Jaetzold *et al.,* 2010). The soils are categorized as humic nitsols according to the FAO-UNESCO classification system (FAO, 1990). They are well-drained, very deep, dark reddish brown, and friable clay when wet (FAO, 1990).

3.3 Source of wheat varieties and vesicular arbuscular mycorrhiza fungi

Four wheat varieties namely, Kenya Eagle10, Njoro BW2, Kenya Kingbird and Kenya Tai which do well in Narok North Sub-County (Kenya Wheat Production Handbook, 2016) were purchased from Crops Research Centre at KALRO – Njoro.

The standard strains of vesicular arbuscular mycorrhiza fungi used were RHIZATECH ® TM (A mixed strains of three *Glomus* species, including; *Glomus intraradices, Glomus etunicatum and Glomus mosseae)* which were purchased from Dudutech Division of Finlay's Horticulture in Naivasha. The strains are vesicular arbuscular mycorrhizae fungi which belong to phylum Glomeromycota and form mutualistic relationships with over 95% of all vascular plants (Simon *et al*., 1993). According to the manufacturer, Dudutech Finlay Horticulture Limited (DFH, 2014), RHIZATECH ® TM formulation has spores and mycelia fragments of the three *Glomus* species of VAM Fungi at a concentration of 50 propagules per cubic centimeter product. It is available in granular form. The inoculant may be kept for

up to 24 months in a cool dry place away from sunlight.

3.4 Collection of soil samples

A total of 50 soil samples were collected from 50 randomly selected wheat growing farms in the five wards of Narok North Sub-County, including Narok Town, Olokurto, Nkareta, Olorropil and Melili according to Okalebo *et al*. (2002)**.** Ten soil samples were collected from ten wheat farms which were 15 meters a part in each ward. At the farm site, 15 soil sub-samples were randomly collected in a zigzag manner at a depth of 0-30cm using soil auger (Mahler &Tindall, 1990).

The collected soils were placed in a plastic bucket and thoroughly mixed and about 1kg composite sample was put in a clean plastic bag. The bag was then labeled with details such as name of the farmer or farm name, date of sample collection, depth of the soil and name of the sampler. Finally, the soil samples were transported at room temperature (27°C) to Kenya Agricultural and Livestock Research Organization (KALRO) - Kabete Center soil laboratory within 24 hours. It was stored in the soil preparation room under room temperature ready for soil test analysis.

3.5 Soil analysis for plant nutrients

The stored soil samples were each air-dried for 3 days and sieved through a 2 mm sieve. Plant essential macronutrients, micronutrients and pH were all determined according to Okalebo *et al*. (2002)

3.5.1 Determination of Soil pH

Soil pH water was measured using a pH meter (model AD 10) and a glass electrode in a soil water ratio of 1:1 (Mehlich *et al*., 1962). Twenty-five mL of distilled water were added to a 50 mL beaker containing 10g of soil. The solution was mixed or stirred using a clean glass rod for ten minutes and allowed to stand for up to 30 minutes. After the soil settled, a pH electrode was immersed and readings were taken after the pH readings had stabilized.

3.5.2 Determination of Macronutrients

Calorimetric method described by Anderson (1993) was used to determine Total organic carbon. In the presence of sulphuric acid, total organic carbon was oxidized with potassium dichromate to yield soil organic matter. To facilitate uniformity and oxidation, 10 g of soil sample that had been passed through a 2 mm sieve was pulverized to pass a 0.5 mm sieve. Two grams of soil were put in a digestion tube and 2 mL of distilled water was added to wet the soil and 10 mL of 1 N potassium dichromate was pipette-added and spun to oxidize the carbon in the soil. Then 5 mL of concentrated sulphuric acid was poured in a constant stream into the soil dichromate mixture. The heat of dilution created by adding sulphuric acid aided the oxidation process. The mixture was then let to cool for 20 minutes. After adding 50 mL of 0.4% barium chloride it was vortexed using a vortex mixer and allowed to stand overnight. It was then analyzed using Ultra Violet-Visible Spectrophotometer at 600 nm.

Kjeldahl method as outlined by Bremner *et al*. (1982) was used to determine Total Nitrogen whereby 0.3 g of each soil sample was digested in a digestion tube with copper (II) sulfate **(**CuSO4), Nitric acid (HNO3) and selenium (Se) in a digestion mixture. The temperature of the heating block was kept at 360°C for 2 hours, following which the sample was allowed to cool before being transferred to a 50 mL volumetric flask and the volume was calculated. After allowing it to settle, 10 mL of the aliquot was transferred to the distillation container, along with 10 mL of 46 percent sodium hydroxide. As soon as the indicator became green, it was steam distilled for 2 minutes into 5 mL of 1 percent boric acid together with 4 drops of mixed indicator. Sulfuric acid (H_2SO_4) was used to titrate the distillate, and the end point was achieved when the indicator changed from green to grey to definite pink. The formula below was used to calculate total nitrogen.

% N= (Vs-Vb) xNx14x (100x100/axb)

Where,

V_s = volume of Sulfuric Acid used for the titration of soil sample in mL

V_b= volume of Sulfuric Acid used for the titration of the blank in mL

N= normality of Sulfuric Acid

14=weight of N in grams

a=volume of digest taken for distillation in mL

b=weight of the sample taken for analysis in mg.

100=conversion factor

3.5.3 Determination of Micronutrients

The Mehlich 1 method (1962) was used to determine the amount of soil Phosphorous, exchangeable Potassium, Sodium, Calcium and Magnesium (Mehlich *et al*., 1962). Five grams of air dried soils were extracted with 25 mL of a 0.1M hydrochloric acid (HCl) and 0.025M Sulfuric Acid **(**H2SO4) combination solution. The suspension was centrifuged at 240 rpm for 1 hour and then filtered using Whatman filter paper No. 42 after being shaken for 60 minutes in an automated shaker. After adding 1 mL of ammonium vanadate and ammonium molybdate mixture to 5 mL of the extraction for color development, after standing for 1 hour, the extractable phosphorous was measured calorimetrically using a spectrophotometer at 430 nm wavelength (Mehlich *et al*., 1962).

Flame photometry was used to measure the amounts of calcium (Ca), sodium (Na), and potassium (K). After combining 1 mL of the extract with 5 mL of Mg compensatory solution, plus 2 mL of both titan yellow and 8 percent sodium hydroxide (NaOH) solutions for 1 hour, magnesium (Mg) was determined by atomic absorption spectrophotometry (AAS) manufactured by Perkin-Elmer Corporation, USA (Mehlich *et al*., 1962).

For trace elements (Cu, Fe and Zn) soil samples were air-dried at room temperature (22±3°C) for three days according to Okalebo *et al*. (2002)**.** Five grams of each of the dried soil were weighed using the precision balance (Phillips Harris, Shemstone England) and placed in a 100 mL glass bottle and 50 mL of 0.1N hydrochloric acid (extracting solution) was added (Hinga *et al*., 1980)**.** The extracting solution

facilitates freeing of elements to be analyzed. It was then mixed well and shaken mechanically with a table universal shaker (Vortex shaker, Jack A. Kraft and Harold D. Kraft, City, U.S.) for an hour. Qualitative Filter Paper, Whatman Grade 1 was used with V-shaped funnel to filter the suspension. Blank with 10 mL of 0.1N hydrochloric acid and an internal reference sample with 10 g of standard soil were included in each series (Crop Nutrition Laboratory Services, 2013). Cu, Fe and Zn concentrations were read over an Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer Corporation, USA) (Mehlich *et al.,* 1962). A wavelength of 324.7 nm was used to quantify the absorption of Fe, Zn and Cu in the soil samples by use of Atomic Absorption Spectrophotometer (Shimadzu Corporation, 1968).

The standard was prepared from a stock solution of 1000 ppm Cu (3.929 g), 1000 ppm Fe (3.929 g), 1000 ppm Zn (3.929 g) from Copper Sulphate Pentahydrate, ammonium ferrous Sulphate and Zinc Sulphate Pentahydrate respectively. From each stock solution of Cu, Fe and Zn, 2 mL were pippeted into a 100 mL volumetric glass flask and filled up with 0.1 N HCL as stock solutions (20 ppm Cu, 20ppm Fe, 20ppm Zn). From the 20 ppm Cu, 0,2,4,6,8,10, 20ppm Fe, 0,2,4,6,8,10, 20ppm Zn, 0,2,4,6,8,10 were pippeted into each 100 mL volumetric flask and filled up with 0.1 N HCL to make the standard series. These standard series had a concentration of 0-0.4- 0.8-1.2- 1.6-2.0 ppm Cu, 0-0.4 -0.8-1.2 -1.6-2.0 ppm Fe, 0-0.4 -0.8-1.2 -1.6-2.0 ppm Zn. The instrument was calibrated using the standard series. The blank extract (10 mL of 0.1N hydrochloric acid) was nebulized into a blue flame and Cu, Fe, Zn concentration measured (Okalebo *et al.,* 2002). After measurement, the trace elements concentration levels in the soil samples was calculated using the following formula by Okalebo *et al*. (2002): Cu (ppm) = (a-b) x 10 x D, Where,

a = the concentration of Cu, Fe, Zn in the soil sample extract (ppm)

b =concentration of Cu, Fe, Zn in the blank extract (ppm)

D = dilution factor, if any.

A table of soil classifications of available nutrients in soils by Mehlich method (1962) was used to establish the concentration of the nutrients.

3.6 Treatments and experimental designs

3.6.1 Greenhouse experimental design

Two greenhouses trials were conducted at KALRO-Kabete, Nairobi. Soils used in the experiment were collected from one of the farms in Oloropil ward, Narok (Resiato's farm). Before setting up the experiments, characteristics of the soils were determined following routine soil analysis procedures (Okalebo *et al*., 2002). The treatments included inoculation with the RHIZATECH ® TM inoculum and controls that were not treated with RHIZATECH ® TM inoculation. Treatments consisted of

i. Kenya Eagle10 + Control (without RHIZATECH ® TM)
ii. Kenya Eagle10 + RHIZATECH ® TM
iii. Njoro BW2 + Control (without RHIZATECH ® TM)
iv. Njoro BW2 + RHIZATECH ® TM
v. Kenya Tai + Control (without RHIZATECH ® TM)
vi. Kenya Tai + RHIZATECH ® TM
vii. Kingbird + Control (without RHIZATECH ® TM)
viii. Kingbird + RHIZATECH ® TM

A pot experiment was set up according to the method of Steel and Torrie (1990). For soil preparation, large soil clods and debris were removed by passing the soil through a 5 mm mesh sieve. Approximately 3 kg of soils were used per pot and total of 24 pots were used. Each pot was applied with 15 g of granules of RHIZATECH ® TM strains by mixing with soil before planting 3 seeds in each of the pots per wheat variety. Wheat plants, which were untreated with RHIZATECH ® TM inoculum were used as controls for each wheat variety. Completely Randomized Block Design (CRD) was used with three replications. Four wheat varieties were used.

Nitrogen, Phosphorous, and Potassium Fertilizer (N: P: K 23:23:0) was used at a rate of 50 kg per acre following recommendation by National Agricultural Research Laboratory (NARL-Kabete) since total nitrogen content in the soil was inadequate for wheat growth. Constant irrigation was provided for seed germination and growing of the wheat plants for the entire growth period of 120 days when the wheat was ready for harvesting. Wheat plants infested with insect pests during the growth period were regularly sprayed with insecticidal soaps (DECIS OPTIONS) at the recommended rate of 2 mL/L using Smith 190285 1-Gallon Bleach and Chemical Sprayer (D.B. Smith, United States).

3.6.2 Field experimental design

One field trial was conducted at KALRO- Kabete center, Nairobi whereas two field trials were conducted) in Narok North Sub-county at Nkareita and Oloropil wards to cater for the two agro-ecological zones. They were conducted according to the method of Steel and Torrie (1990). Before planting, characteristics of the soils were established in all the field trials following routine procedures (Okalebo *et al.,* 2002). Narok fields had been planted with wheat for three years and none of the experimental locations had previously been infected with VAMF. Kabete field had not been previously infected with VAMF and even though the field had adequate copper in their soils, the experiment was carried out since copper in the soils can be adequate but unavailable to the wheat plants which can be facilitated or made available by treating the soils with arbuscular mycorrhiza fungi.

Wheat grows best at soil pH of 6.0 to 7.0, and to raise the low soil pH in Kabete field which was acidic (4.85 ppm), agricultural lime was applied through broadcasting at rate of 500 kg per acre. To achieve homogeneity in the field blocks, the research locations were done over moderate slopes in all trials. They were manually ploughed using a hoe and stacked into mounds of 2 m wide and 1.2 m length yielding a total of 24 mounds. Total plot size used was 11.8 m by 8.5 m as shown in Table 3.1.

The test crops were four wheat varieties, Kenya Eagle10, Njoro BW2, Kenya Tai, and Kingbird. The treatments included inoculation with the RHIZATECH ® TM and controls (Without RHIZATECH ®

TM). It consisted of 8 treatments arranged in a completely randomized block design as shown in table 3.3, with three replicates. The inoculum was applied per mound (2.4 m^2) at a rate of 50 g based on the manufacturers' recommendations. The inoculum was spread below the wheat seeds for the treated blocks and covered with soil lightly during planting. The plots which acted as controls were untreated with RHIZATECH ® TM inoculums. The same procedures were repeated for the other replications.

Nitrogen, Phosphorous, and Potassium Fertilizer (N: P: K 23:23:0) was used at a rate of 50 kg per acre following recommendation by National Agricultural Research Laboratory (NARL-Kabete) since some of the sites had low total nitrogen and available soil phosphorus level for wheat growth. Wheat plants infested with insect pests during the growth period were regularly sprayed with insecticidal soaps (DECIS) at the recommended rate of 20 g/L using Smith 190285 1Gallon Bleach and Chemical Spray (D.B. Smith, United States), which reduces most of the pests such as aphids, mites and whiteflies.

Table 3.1: Field experimental design using Completely Randomized Block Design.

0.25M↕															
REP 1 2M↕	1 V3+C		2 V4+M		3 V3+M		4 V2+C		5 V1+C		6 V2+M		7 V1+M		8 V4+C	
1M↕																
REP 2 2↕	9 V2+C		10 V3+M		11 V4+C		12 V2+M		13 V3+C		14 V1+C		15 V4+M		16 V1+M	
1M↕																
REP 3 2↕	17 V4+C		18 V2+M		19 V1+M		20 V4+M		21 V3+M		22 V3+C		23 V2+C		24 V1+C	
0.25M↕																

Treatments:

V1 + C = Kenya Eagle10+ Control (without RHIZATECH ® TM)

V1 + M = Kenya Eagle10 + RHIZATECH ® TM

V2 + C-= Njoro BW2 + Control (without RHIZATECH ® TM)

V2 + M = Njoro BW2+ RHIZATECH ® TM

V3 + C = Kenya Tai + Control (without RHIZATECH ® TM)

V3 + M= Kenya Tai + RHIZATECH ® TM

V4 + C=Kingbird + Control (untreated without RHIZATECH ® TM)

V4+ M= Kingbird + RHIZATECH ® TM

3.7 Assessment of mycorrhizal root colonization

Wheat plants were collected from each experimental sites after eight weeks from sowing for assessment of root mycorrhizal development following the method of Koske and Gemma (2005). Five wheat samples were randomly collected from each plot, while two wheat sample of the wheat plants were randomly collected from each pot by pulling out from the soil by hand. They were then placed in plastic bags, labelled, and transported to KALRO-Kabete laboratory for the analysis. The roots of the wheat plants grown in the green house and field trials were cleaned with clean water, cut into small fragments of one-centimeter-long separately using a scalpel blade and thoroughly mixed separately.

Sub-samples of one gram of roots were used to determine root colonization with the fungi. The root samples were then cleaned with 10% potassium hydroxide (KOH) and bleached with 30% (v/v) hydrogen peroxide solution. The root samples were then neutralized in 1% hydrochloric acid (HCL) and stained with 0.03% (v/v) trypan blue in glycerol. Mycorrhizal colonization was examined by assessing five glass slides with ten segments of the root samples. It was examined under a microscope (Olympus CH2, Tokyo, Japan) at magnification power of 40X x 10X. The presence of blue-stained mycorrhizal structures such as vesicles and arbuscules on the root portions were used to determine if they were positive for AMF. The percentage of root colonization, was calculated following formula by Giovannetti and Mosse (1980).

Colonization root percentage (%) = Number of segments colonized with vesicular arbuscular mycorrhizal / Number of segments observed × 100.

3.8 Determination of copper content in wheat tissues

Wheat plants were harvested at 120 days after planting (Crop Nutrition Laboratory Services, 2013). Those that received mycorrhizal treatments and the non-inoculated controls, were both harvested for measuring copper content. Five rows were harvested in all the plots leaving behind the three guard rows on each side of the plot, whereas two wheat plant was harvested per pot in the greenhouse trial. The

wheat plants were uprooted and soils in the roots were removed and washed off with clean water.

The dry roots and shoots of wheat plants were ground into small particles using a stainless mill (IKA, Wittman Battenfeld, Hamburg, Germany) for copper ion determination using microwave-assisted acid extraction or dissolution of plant material (Navozamsky *et al*., 2013). Copper ion was measured over atomic absorption spectrometer (AAS) (Perkin-Elmer Corporation, USA) (Mehlich *et al*., 1962).

The procedure involved weighing of 0.3 g of dried wheat plant materials. It was then transferred into digestion tubes and 2.5 mL of the digestion mixture (sulfuric acid) was added in each tube and mixed slowly and carefully by swirling (Navozamsky *et al*., 2013). The tubes were then heated for about two hours in a heating block at 100 ºC. It was then removed and cooled at 33ºC. Successive three 1-mL aliquots of hydrogen peroxide were added and mixed carefully after each addition. The tubes were then transferred to a preheated block of 33ºC and the digestion was deemed complete when the mixture turned into yellow or colorless. The tubes were then removed and cooled to 27ºC before adding 48.3 mL of distilled water to each tube, mixed and left to stay overnight (Navozamsky *et al*., 2013)

A wavelength of 324.7 nm was used to quantify the absorption of copper ion in the wheat plant digest by use of Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer Corporation, USA) (Mehlich *et al*., 1962). The standard was prepared from a stock solution of 1000 ppm Cu (3.929 g) from Copper Sulphate Pentahydrate. From the stock solution 2 mL were pipetted into a 100 mL volumetric flask and filled up with distilled water as stock solution (20 ppm Cu). From the 20 mL Cu, 0 mL, 2 mL, 4 mL, 6 mL, 8 mL and 10 mL were pipetted into 100 mL volumetric flask and filled up with 0.1 N HCL to make the standard series. These standard series had a concentration of 0-0.4 -0.8 -1.2 -1.6-2.0 ppm Cu. The instrument was calibrated using the standard series. The blank digest of (10 mL of 0.1N hydrochloric acid) was nebulized into a blue flame and Cu concentration measured ((Mehlich *et al*., 1962). After measurement, the Cu concentration in the dried wheat plant material was calculated using the following formula by Okalebo *et al*, (2002): Cu (ppm) = (a-b) x V/W

Where,

a = concentration of Cu in the wheat sample digest (ppm)

b=concentration of Cu in the blank digest (ppm)

V=total volume of digest at the end of the digestion procedure (mL)

W=weight of wheat plant material sample digested.

3.9 Wheat harvesting for determination of height, biomass, and grain yield

Wheat plants were harvested at 120 days after planting (Crop Nutrition Laboratory Services, 2013). The plants were harvested for determining grain yield and biomass. Five rows which had over 500 wheat plants were randomly chosen and plants harvested in all the plots leaving behind the three guard rows on each side of the plot, whereas two wheat plants were harvested per pot in the greenhouse trial. Soil in the uprooted roots of wheat plants was removed and washed off with clean water.

3.9.1 Determination of plant height and biomass

Plant height was measured twice during heading stage and at harvesting stage (Crop Nutrition Laboratory Services, 2013). The height was measured from the base of the stem to the tip of the plant with a meter ruler. All the harvested wheat plants from each pot /plot were cut into small pieces and put carefully in labeled polythene bags for measuring biomass. Samples were dried for 3 days using an oven at 90°C (Okalebo *et al*., 2002). The dried wheat samples were measured using the precision balance (Phillips Harris, Shemstone England). The weight of the bags was accounted by weighing each bag before placing the biomass sample in it, and ensuring that the bag was dry (Crop Nutrition Laboratory Services, 2013).

3.9.2 Determination of grain yield

Five rows which had over 500 wheat plants were randomly chosen and plants harvested in all the plots leaving behind the three guard rows on each side of the plot, whereas two wheat plants were harvested per pot in the greenhouse trial. The wheat plants were uprooted and soils in the roots were removed and washed off with clean water. The yield components of wheat plants grown in the green house and field

trials were determined as the number of grains and weight of grains. Before weighing, harvested wheat plants were threshed and the resulting grains sun-dried. The grains were counted using automatic seed counter (V-Tech Automatic Seed Counter, Germany) and the total weight of the grains obtained using a precision balance (Phillips Harris, Shemstone England) to determine yields per pot /plot (Crop Nutrition Laboratory Services, 2013).

3.10 Statistical analysis

Data was analyzed using two-way analysis of variance (ANOVA) at Confidence Interval of 95 % using GenStat 15th edition statistical software, to determine whether the VAMF inocula caused any significant changes in the wheat growth, yield and copper uptake. The treatment effects were compared using the Fisher's least significant difference (LSD) tests at 0.05 significance level. Descriptive statistical evaluation for soil samples comprised of ranges, means and Standard Deviation (SD; which were utilized to explore collected data through graphical plotting on MS excel 2010 and STATA program.

CHAPTER FOUR: RESULTS

4.1 Characteristics of the soils used in greenhouse and field trials in Kabete- Nairobi and Nkareita and Oloropil wards in Narok North Sub-County

Soil pH in water (1:1) in all the trial sites before planting ranged from 4.84 to 6.13 as shown in Table 4.1 and Table 4.2. It was categorized as highly acidic or slightly acidic. Kabete soils were highly acidic with a pH of 4.84 as shown in Table 4.1 and Table 4.2. Narok soils and soil used in Kabete greenhouse were slightly acidic with a pH of 6.13, 6.03 and 6.03 respectively. Total Soil Organic Carbon was adequate for wheat growth in all the trial sites and it ranged from 1.76 ppm to 3.56 ppm as shown in Table 4.1 and Table 4.2. The soil's total nitrogen content was adequate in two trial sites of Nkareita and Oloropil wards in Narok North, from 0.2 to 0.33%)except for Kabete trial site in Nairobi, which was low at 0.16% as shown in in Table 4.1 and Table 4.2.

All the macronutrients were adequate in Kabete and Nkareita field sites based on National Agricultural Research Laboratories (NARL. It ranged from 1.6 ppm to 4 ppm for calcium, 1.08 ppm to 1.7 ppm for potassium, 2.3 ppm to 3.3 ppm for magnesium and 0.73 ppm to 0.82 ppm for manganese as shown in in Table 4.1 and Table 4.2. However, the available soil phosphorus level in the soil at Oloropil site was low at 26.0 ppm.

The soils had adequate contents of Fe and Zn in all the trial sites based on the ratings presented in FURP (1988) and it ranged from 8.49 ppm to 16.7 ppm for zinc and 12.6 ppm to 37.6 ppm for iron as shown in Table 4.1 and Table 4.2. Soils used in Kabete greenhouse and in the study sites in Narok Nkareita and Oloropil wards were found deficient in copper, at 0.33 ppm, 0.72 ppm and 0.33 ppm respectively as shown in Table 4.1. Soils in Kabete trial field were adequate in copper at 2.25 ppm as shown in Table 4.2.

Table 4.1: Characteristics of the soil used in the Kabete greenhouse experiment collected in one of the farm in Oloropil ward, Narok North -Sub-County

Soil depth (cm)	0-30	
Fertility results	Value	Class
Soil Ph	6.03	Slightly acidic
Total Nitrogen %	0.2	Adequate
Total Organic Carbon	2.23	Adequate
Phosphorus ppm	26	Low
Potassium meq%	1.7	High
Calcium meq%	4	Adequate
Magnesium meq%	3.3	High
Manganese meq%	0.82	Adequate
Copper ppm	0.33	Low
Iron ppm	12.6	Adequate
Zinc ppm	8.49	Adequate

Key: ppm=Parts per million, % =Percentage, Meq %=Millequivalents per 100 gram of soil

Table 4.2: Characteristics of the soils used in three trial sites at KALRO-Kabete in Nairobi, Nkareita and Oloropil wards in Narok North Sub-County

SOIL DEPTH 0-30CM						
Farm	**KALRO-KABETE NAIROBI**		**NKAREITA WARD NAROK**		**OLOROPIL WARD NAROK**	
Fertility results	Value	Class	Value	Class	Value	Class
Soil Ph	4.84	Highly acidic	6.13	Slightly acidic	6.03	Slightly acidic
Total Nitrogen %	0.16	Low	0.33	Adequate	0.2	Adequate
Total Organic Carbon %	1.76	Adequate	3.56	Adequate	2.23	Adequate
Phosphorus ppm	44	Adequate	35	Adequate	26	Low
Potassium meq%	1.26	Adequate	1.08	Adequate	1.7	High
Calcium meq%	1.6	Adequate	1.6	Adequate	4	Adequate
Magnesium meq%	2.3	High	2.3	High	3.3	High
Manganese meq%	0.73	Adequate	0.73	Adequate	0.82	Adequate
Copper ppm	2.25	Adequate	0.72	Low	0.33	Low
Iron ppm	28.6	Adequate	37.6	Adequate	12.6	Adequate
Zinc ppm	15.9	Adequate	16.7	Adequate	8.49	Adequate
Sodium meq%	0.12	Adequate	0.2	Adequate	0.22	Adequate

Key: ppm=Parts per million, % =Percentage, Meq %=Millequivalents per 100 gram of soils

4.2 Status of copper ion concentration in wheat growing soils in the five wards of Narok Town, Olokurto, Nkareta, Oloropil and Melili in Narok North Sub-County

The concentration of Cu was analyzed in the 50 soil samples from wheat growing areas in Narok North Sub- County. The results showed the concentration levels varied between the soil samples. The concentration was found to range from 0.1 mg/Kg to 2.63 mg/kg in the analyzed soil samples in the 5 wards as shown in Table 4.3. It was classified as low or adequate according to critical value of Cu which is 1.0 mg/kg. Twenty-nine soil samples (Table 4.3), which makes up to 58% (Table 4.4) contained Cu below the critical value of 1.0 mg/kg and was deemed as low, whereas 21 soil samples (Table 4.3) which makes up to 42% (Table 4.4) were above the critical value of 1.0 mg/kg hence were adequate in copper. The total mean of the copper concentration in the soil samples was 0.98±0.11 (SE) mg/kg (Table 4.4.) which is below the critical value of Cu (1.0 mg/kg).

The farms in the five wards had different concentration levels of copper in their soils. Most of the soil samples from the farms in Melili, Narok town, and Nkerita wards had inadequate levels of copper compared to soil samples from the farms in Oloropil and Olokurto wards. Three out of 10 soil samples from Oloropil ward farms had low copper levels ranging from 0.1 to 2.63 mg/kg (Table 4.3). These results were almost similar to soil samples from Olokurto ward farms where 4 out of 10 soil samples had low copper levels ranging from 0.37 to 2.62 mg/kg (Table 4.3). Seven out of 10 soil samples from Melili ward farms had low copper levels ranging from 0.33 to 1.99 mg/kg. Narok town ward farms had 7 out of 10 soil samples that had low copper levels ranging from 0.33 to 1.84 mg/kg (Table 4.3). For soil samples from Nkareita ward farms, 8 out of 10 were low in copper levels ranging from 0.1 to 1.02 mg/kg (Table 4.3).

Table 4.3: Status of Copper (Cu) ion concentrations (mg /kg) of the 50 soils samples collected from the fifty farms in Narok North Sub-County

Soil Depth 0-30 cm			
Soil samples	Copper value (mg/kg)	Class	
Sample 1 (Oloropil)	2.32	Adequate	-
Sample 2 (Oloropil)	2.35	Adequate	-
Sample 3 (Oloropil)	0.1	-	Low
Sample 4 (Oloropil)	1	Adequate	-
Sample 5 (Oloropil)	0.24	-	Low
Sample 6 (Oloropil)	2.63	Adequate	-
Sample 7 (Oloropil)	1.06	Adequate	-
Sample 8 (Oloropil)	1	Adequate	-
Sample 9 (Oloropil)	2.22	Adequate	-
Sample 10 (Oloropil)	0.67	-	Low
Sample 11 (Olokurto)	0.9	-	Low
Sample 12 (Olokurto)	2.1	Adequate	-
Sample 13 (Olokurto)	2.62	Adequate	-
Sample 14 (Olokurto)	1.11	Adequate	-
Sample 15 (Olokurto)	0.37	-	Low
Sample 16 (Olokurto)	1	Adequate	-
Sample 17 (Olokurto)	1.6	-	Low
Sample 18 (Olokurto)	1	Adequate	-
Sample 19 (Olokurto)	1.6	Adequate	-
Sample 20 (Olokurto)	0.72	-	Low
Sample 21 (Melili)	0.33	-	Low
Sample 22 (Melili)	0.45	-	Low
Sample 23 (Melili)	0.84	-	Low
Sample 24 (Melili)	0.97	-	Low
Sample 25 (Melili)	0.79		Low
Sample 26 (Melili)	1.89	Adequate	-
Sample 27 (Melili)	0.77	-	Low
Sample 28 (Melili)	1.28	Adequate	-
Sample 29 (Melili)	0.62	-	Low
Sample 30 (Melili)	1.99	Adequate	-
Sample 31(Narok Town)	1.84	Adequate	-
Sample 32 (Narok Town)	0.67	-	Low
Sample 33 (Narok Town)	0.33	-	Low
Sample 34 (Narok Town)	0.48	-	Low
Sample 35 (Narok Town)	0.68	-	Low
Sample 36 (Narok Town)	0.59	-	Low
Sample 37 (Narok Town)	0.97	-	Low
Sample 38 (Narok Town)	0.72	-	Low
Sample 39 (Narok Town)	1	Adequate	-
Sample 40 (Narok Town)	1.21	Adequate	-
Sample 41 (Nkareita)	0.36	-	Low
Sample 42 (Nkareita)	0.12	-	Low
Sample 43 (Nkareita)	0.1	-	Low
Sample 44 (Nkareita)	0.3	-	Low
Sample 45 (Nkareita)	0.42	-	Low
Sample 46 (Nkareita)	0.65	-	Low
Sample 47 (Nkareita)	1	Adequate	-
Sample 48 (Nkareita)	1.02	Adequate	-
Sample 49 (Nkareita)	0.12	-	Low
Sample 50 (Nkareita)	0.15	-	Low
Total =50		21	29

Table 4.4: Summary statistics (Mean, SD, and Range) and percent of copper concentration in the fifty soils samples from five wards of Narok Town, Olokurto, Nkareta, Oloropil and Melili in Narok North Sub- County.

Descriptive Statistics	Value (mg/kg)	Percentage (%) No of soils with adequate and low copper ion levels
Mean	0.98	
Range	0.1 -2.63	
Standard deviation	0.72	
Standard error	0.11	
Number of sample	< 1.0	58%
Number of sample	>1.0	42%

4.3 Effect of RHIZATECH®TM vesicular arbuscular mycorrhizal fungus on wheat growth and copper ion uptake under greenhouse conditions at KALRO-Kabete

4.3.1 Assessment of RHIZATECH®TM fungal colonization of four wheat varieties under greenhouse conditions at KALRO-Kabete

ANOVA data in Table 4.5 show that, RHIZATECH®TM treatment is a significant variable (factor), positively affecting root colonization of the four wheat varieties at p=0.001. The other factors such as wheat variety and number of varieties treated were not significant in affecting the colonization of wheat roots at P value 0.05 confidence level (Table 4.5).

According to data on Table 4.6, Njoro BW2 variety had the highest mean increase in root colonization from 2.67±0.67% (SE)% to 45±3.46 (SE)% (2150.1%). K Eagle 10 variety followed with an increase from 4.67±1.45 (SE)% to 49.33±0.33 (SE)% (1300. 4%). Kingbird had an increase from 3.67±1.16 (SE)% to 42.67±1 (SE)% (1125.2%). Lastly Kenya Tai had an increase in root colonization from 5±1.20 (SE)% to 49±1.76 (SE)% (880.7%).

Table 4.5: ANOVA analysis showing factors affecting root colonization of four wheat varieties under greenhouse conditions at KALRO-Kabete

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Wheat Variety	3	74.833	24.944	3.09	0.057	
Treatment	1	10837.5	10837.5	1340.72	<.001	*
Variety. Treatment	7	28.833	9.611	1.19	0.345	
Residual	16	129.333	8.083			
Total	23	11070.5				

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.6: Root colonization rate of four wheat varieties as influenced by RHIZATECH ® TM inoculant under greenhouse conditions at KALRO-Kabete

Wheat Variety	Treatment		Percentage (%) increase in root colonization
	Control	Treatment	
K Eagle 10	4.67a	49.33a	1300.4
Njoro BW2	2.67a	45a	2150.1
Kenya Tai	5a	49a	880.7
Kingbird	3.67a	42.67b	1125.1
Average Mean	4a	46.5b	1062.5
P (Treatment)	0.001		
LSD (Treatment)	2.461		
LSD (Variety.Treatment)	4.9		
CV (%)	11.3		

Key: LSD- Least significant difference, CV-Coefficient of variability, Treatment-inoculated with RHIZATECH®TM inoculant, Control-non-inoculated with RHIZATECH ® TM inoculant.

Means accompanied by the same letter (s) are not significantly different from each other according to Fisher's least significant difference test at p= 0.05.

4.3.2 Effects of RHIZATECH®TM inoculation on plant height and biomass in the four wheat varieties under greenhouse conditions at KALRO-Kabete

Analysis of variance data on Table 4.7 shows that no factor or variable under greenhouse conditions affected wheat height during the growth period at p=0.096. The analysis of variance data on Table 4.8 shows that RHIZATEC®TM treatment was the only significant factor/variable that caused increase in wheat biomass at p=0.001. However, Njoro BW2 variety and Kenya Tai variety had slight increase in stem height from 67.67±0.88 (SE) cm to 69.33±2.85 (SE) cm (2.9%) and an increase from 65.33±2.01 (SE) cm to 67±2.52 (SE) cm (3.1%) respectively over the controls.

K Eagle 10 variety had a slight decrease in stem height from 71±2.01 (SE) cm to 70 ±2.31 (SE) cm (-1.4%). Kingbird variety had decrease in stem height from 71±2.52 (SE) cm to 69±2.65 (SE) cm (- 2.8%) as shown in Table 4.9. Kingbird variety had the highest mean increase in total dry weight (biomass) from 10.87±1.05 (SE)g to 17.03±1.64 (SE) g (70.1%) followed by K Eagle variety 10 with an increase from 11.91±1.72 (SE) g to 16.79±1.89 (SE) g (16.7%). Njoro BW12 variety had an increase in biomass from 12.42±0.50 (SE) g to 14.96±1.03 (SE) g (15.4%. Lastly, Kenya Tai variety had an increase in biomass from 13.45±1.41 (SE) g to 15.72±1.17 (SE) g (4.5%).

Table 4.7: ANOVA analysis showing factors that affected height of four wheat varieties during growth under greenhouse condition

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.
Wheat Variety	3	68.12	22.71	1.43	0.271
Treatment	1	0.04	0.04	0	0.96
Variety. Treatment	7	15.79	5.26	0.33	0.803
Residual	16	254	15.88		
Total	23	337.96			

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.8: ANOVA data showing factors affecting biomass during growth of four wheat varieties under greenhouse condition at KALRO-Kabete

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Wheat Variety	3	2.909	0.97	0.17	0.913	
Treatment	1	94.169	94.169	16.77	<.001	***
Variety .Treatment	7	15.817	5.272	0.94	0.445	
Residual	16	89.868	5.617			
Total	23	202.764				

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.9: Means of height and biomass of four wheat varieties as influenced by RHIZATECH ® TM inoculations under greenhouse conditions at KALRO-Kabete

	Treatment					
	Wheat height			Wheat biomass		
Variety	Control	Treatment	Percentage (%) increase in biomass	Control	Treatment	Percentage (%) increase in height
K Eagle 10	71a	70a	-1.4	11.91a	16.79a	16.7
Njoro BW2	67.7a	69.3a	2.9	12.42a	14.96a	15.4
Kenya Tai	65.3a	67a	3.1	13.45a	15.72a	4.5
Kingbird	71a	69a	-2.8	10.87a	17.03a	70.1
Mean	68.7a	68.83a		12.16a	16.12b	32.6
P (Treatment)	0.96			P (Treatment)		0.001
LSD(Treatment)	3.448			LSD(Treatment)		2.05
LSD (Variety. Treatment)	6.9			LSD (Variety. Treatment)		4.1
CV (%)	5.8			CV (%)		16.8

Key: LSD- Least significant difference, CV-Coefficient of variability, Treatment-inoculated with RHIZATECH ® TM inoculant, Control-non-inoculated with RHIZATECH ® TM inoculant.

Means accompanied by the same letter (s) are not significantly different from each other according to Fisher's least significant difference test at p= 0.05.

4.3.3 Effects of RHIZATECH®TM inoculation on grain number and grain weight in wheat varieties under greenhouse conditions at KALRO-Kabete

Data on Table 4.10 indicates that RHIZATECH®TM treatment was a significant factor/variable affecting the increase in wheat grain weight at p=0.021 and grain number at p=0.005 (Table 4.11). According to means difference data on Table 4.12, Kenya Eagle 10 variety had the highest mean increase in grain number from 177±37.69 (SE) to 259 ±45.47 (SE) (46.3%) followed by Kingbird variety with an increase from 218±12.48 (SE) to 282±18.00 (SE) (29.4%). Njoro BW2 variety had an increase in grain number from 210±13.75 (SE) to 25±39.74 (SE) 2 (20.9%) and lastly Kenya Tai variety had an increase from 210±3.18 (SE) to 218±35.5 (SE) (3.8%).

Data on grain weight on Table 4.12, shows that wheat variety K Eagle 10 had the highest mean increase in grain weight from 4.42±0.86 (SE) g to 5.76±1.15 (SE) g (32.5%) followed by Njoro BW2 variety with an increase from 4.93±0.45 (SE) g to 6.19±0.95 (SE) g (31. 6%). Kenya Tai variety had an increase from 5.38±0.44 (SE) g to 6.86±0.57 (SE) g (29.2%) and lastly King bird variety had an increase from5.65±0.01 (SE) g to 6.61±0.37 (SE) g (20.7%).

Table 4.10: ANOVA data showing factors affecting grain weight of four wheat varieties under greenhouse condition at KLRO-Kabete

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Wheat Variety	3	4.483	1.494	1.04	0.403	
Treatment	1	9.507	9.507	6.6	0.021	*
Variety. Treatment	7	0.213	0.071	0.05	0.985	
Residual	16	23.057	1.441			

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.11: ANOVA data on the effect of RHIZATECH®TM inoculation on grain number of four wheat varieties under greenhouse condition

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Variety	3	4919	1640	0.62	0.61	
Treatment	1	27983	27983	10.65	0.005	**
Variety. Treatment	7	1759	586	0.22	0.879	
Residual	16	42038	2627			
Total	23	76699				

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.12: Mean grain weight and grain number of four wheat varieties as influenced by RHIZATECH ® TM inoculations under greenhouse conditions at KALRO-Kabete

	Treatment					
	Grain Weight			Grain Number		
Variety	Control	Treatment	Percentage (%)increase in grain weight	Control	Treatment	Percentage (%)increase in grain no
K Eagle 10	4.42a	5.76a	46.3	178a	259a	32.5
Njoro BW2	4.93a	6.19a	20.9	210a	252a	31.6
Kenya Tai	5.38a	6.86a	3.8	210a	296a	29.2
Kingbird	5.65a	6.61a	29.4	219a	283a	70.1
Mean	5.10a	6.36b		204a	273b	20.7
P (Treatment)	0.021			P (Treatment		0.005
LSD (Treatment)	1.03			LSD (Treatment)		44.4
LSD (Variety. Treatment)	2.1			LSD (Variety. Treatment)		88.7
CV (%)	21			CV (%)		21.5

Key: LSD- Least significant difference, CV-Coefficient of variability, Treatment-inoculated with RHIZATECH ® TM inoculant, Control-non-inoculated with RHIZATECH ® TM inoculant.

Means accompanied by the same letter (s) are not significantly different from each other according to Fisher's least significant difference test at p= 0.05.

4.3.4 Effects of RHIZATECH®TM inoculation on copper ion uptake in four wheat varieties under greenhouse conditions at KALRO-Kabete

Data on Table 4.13 shows RHIZATECH®TM treatment was a significant factor affecting copper ion uptake at p=0.001 during growth of four wheat varieties under greenhouse conditions. According to data on Table 4.14, Kingbird wheat variety had the highest mean increase in copper uptake from 8.11±0.12 (SE) ppm to 9.9±0.56 (SE) ppm (23.7%) followed by Kenya Tai variety with an increase from 8.55±0.48 (SE) ppm to 9.81±0.29 (SE) ppm (22.8%). Njoro BW2 variety had an increase from 8.06±0.48 (SE) ppm to 9.9±0.28 (SE) ppm (22.3%). Lastly variety K Eagle 10 an increase from 8.41±0.56 (SE) ppm to 10.1 ±0.35 (SE) ppm (20.1%).

Table 4.13: ANOVA data showing factors affecting copper ion uptake during growth of four wheat varieties under greenhouse condition, KALRO-Kabete

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Variety	3	0.3225	0.1075	0.22	0.884	
Treatment	1	16.1704	16.1704	32.52	<.001	***
Variety. Treatment	7	0.2988	0.0996	0.2	0.895	

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.14: Means on copper ion uptake by four wheat varieties as influenced by RHIZATECH ® TM inoculations under greenhouse conditions at KALRO-Kabete

Variety	Treatment		Percentage (%) increase copper ion uptake
	Control	Treatment	
K Eagle 10	8.41a	10.1a	20.1
Njoro BW2	8.06a	9.9a	22.3
Kenya Tai	8.55a	9.81a	22.8
Kingbird	8.11a	9.9a	23.7
Average Mean	8.29a	9.93b	19.7
P (Treatment	0.001		
LSD (Treatment)	0.61		
LSD(Variety.Treatment)	1.22		
CV (%)	7.7		

Key: LSD- Least significant difference, CV-Coefficient of variability, Treatment-inoculated with RHIZATECH ® TM inoculant, Control-non-inoculated with RHIZATECH ® TM inoculant.

Means accompanied by the same letter (s) are not significantly different from each other according to Fisher's least significant difference test at p= 0.05.

4.4 Effects of RHIZATECH®TM vesicular arbuscular mycorrhizal fungi on wheat growth and copper ion uptake on four wheat varieties at field trials in KALRO-Kabete and Narok North Sub-county (Nkareita and Oloropil sites)

4.4.1 Assessment of RHIZATECH®TM inoculant colonization rate on the four wheat varieties at the KALRO-Kabete in Nairobi and Nkareita and Oloropil wards in Narok North Sub-County

According to data on Table 4.15, RHIZATECH®TM treatment was a significant factor that affected root colonization at p=0.001 of the four wheat varieties in all the three field sites. Data on Table 4.16 shows that, Kenya Tai variety had the highest mean increase in root colonization at Kabete and Nkareita trial sites which was from 1.3 to 49.3±0.42 (SE)% (3692%) and from 2.7 ppm to 50.7±0.36 (SE)% (1778%) respectively.

Kingbird variety had the lowest mean increase in copper uptake at Kabete and Nkareita trial sites which was an increase from 4.7±0.31 (SE)% to 46±0.22 (SE)% (688%) and an increase from 8.7±0.32 (SE)% to 48±0.32 (SE)% (455%) respectively as shown in Table4.15. Njoro BW2 variety had the lowest mean increase in root colonization in all the three sites, Oloropil trial site with an increase from 7.3±0.21 (SE)% ppm to 46±0.12 (SE)% (530%), and Nkareita site with an increase from 11.3±0.22 (SE)% to 48.7±0.11 (SE)% (327%), Kabete site Njoro BW2 had an increase from 8.7±0.33 (SE)% to 46±0.25 (SE)% (621%).

Table 4.15: ANOVA data showing factors affecting mycorhizal development during growth of four wheat varieties under field conditions at KALRO-Kabete and Narok North Sub-County

Kabete
Mycorhizal Development

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.
Variety	3	18	6	0.33	0.806
Treatment	1	10250.67	10250.67	559.13	<.001
Variety. Treatment	7	101.33	33.78	1.84	0.18
Residual	16	293.33	18.33		

Nkareita (Narok)
Mycorhizal Development

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.
Variety	3	57.833	19.278	2.89	0.068
Treatment	1	9841.5	9841.5	1476.22	<.001
Variety. Treatment	7	116.5	38.833	5.82	0.007
Residual	16	106.667	6.667		

Oloropil (Narok)
Mycorhizal Development

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.
Variety	3	205.83	68.61	3.58	0.037
Treatment	1	9841.5	9841.5	513.47	<.001
Variety. Treatment	3	29.83	9.94	0.52	0.675
Residual	16	306.67	19.17		

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table4.16: Means of root colonization rate of the four wheat varieties as influenced by RHIZATECH®TM inoculations under field conditions at KALRO-Kabete and Narok North Sub-County

Mycorhizal Colonization

Site	Kabete			Nkareita (Narok)			Oloropil (Narok)		
Variety	C	T	Percentage (%)increase (colonization)	C	T	Percentage (%) increase (colonization)	C	T	Percentage (%) increase (colonization)
Kenya Eagle 10	6 a	44.6b	643	7.3a	44.6 b	633	13.3b	51.3b	286
Njoro BW2	8.7a	46b	621	11.3a	48.7b	327	7.3b	46b	530
Kenya Tai	1.3a	49.3b	3692	2.7a	50.7b	1778	6b	48b	600
Kingbird	4.7a	46b	688	8.7a 48b		451 46b	2.7b	1 1603	
Mean	5.6a	46.5b	681	7.5a	48b	540	7.3b	47.8b	555
P (Treatment)	0.001			0.001		0.001			
LSD Treatment	3.7			2.2		3.8			
LSD(Variety.Treatment)	2.5			4.5		7.6			
CV (%)	16.6			9.3		15.8			

Key: LSD- Least significant difference, CV-Coefficient of variability, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant.

Means accompanied by the same letter (s) are not significantly different from each other according to Fisher's least significant difference test at p= 0.05.

4.4.2 Effects of RHIZATECH®TM inoculation on wheat height and biomass of the four wheat varieties at KALRO-Kabete Nairobi, Nkareita and Oloropil wards in Narok North Sub-County

RHIZATECH®TM mycorrhizal inoculation resulted in a significant increase in height of the four wheat varieties at p= 0.001 by 6% at Oloropil site and at p=0.005 by 13% at Kabete sites as compared to non-inoculated controls (Table 4.17). However, wheat height had no significant increase at p=0.114 (5%) Nkareita site. Wheat variety Kingbird had the highest mean increase in stem height which was from 37±4.27 (SE) cm to 49±2.96 (SE) cm (32%) at Kabete field site. Njoro BW2 variety had the lowest mean increase in stem height from 62.3±1.58 (SE) cm to 63.3±1.14 (SE) cm (1.4%) at Oloropil field site as shown in Table 4.18.

Wheat biomass resulted in no significant increase at Kabete site at p=0.98 and at Oloropil site at p=0.11 as compared to non-inoculated controls (Table 4.18). However, there was a significant increase at Nkareita site at p=0.005. Wheat variety Kenya Eagle 10 had the highest mean increase in biomass in all the three sites, Oloropil trial site with an increase from 876±31.1(SE) g to 1080±412(SE) g (23%), Nkareita site with an increase from 852±52 (SE) g to 1111±130.1 (SE) g (30%) and Kabete site with an increase from 118±20.3 (SE) g to 327±25.3 (SE) g (177%) as shown in Table 4.19. Kingbird variety had the lowest mean increase in wheat biomass in all the three sites, Oloropil site with an increase from 967±34.2 (SE) g to 978±22.1(SE) g (1%), Nkareita site with an increase from 903±10.2 (SE) g to 961±19.1 (SE) g (6%) and Kabete site with a decrease from 348±33.1 (SE) g to 46±25 (SE) g (-63%).

Table 4.17: ANOVA data showing factors affecting wheat height during growth of four wheat varieties under field conditions at KALRO-Kabete and Narok North Sub-County

Kabete
Wheat Height

Source of variation	D.F.	S.S.	M.S.	V.R.	F	Pr.	
Variety	3	804.33	268.11	11.21		<.001	***
Treatment	1	253.5	253.5	10.6		0.005	**
Variety. Treatment	7	84.83	28.28	1.18		0.348	
Residual	16	382.67	23.92				

Nkareita (Narok)
Wheat Height

Source of variation	D.F.	S.S.	M.S.	V.R.	F	Pr.	
Variety	3	36.46	12.15	0.44		0.728	
Treatment	1	77.04	77.04	2.79		0.114	***
Variety. Treatment	3	5.46	1.82	0.07		0.977	**
Residual	16	442	27.63				

Oloropil (Narok)
Wheat Height

Source of variation	D.F.	S.S.	M.S.	V.R.	F	Pr.
Variety	3	205.83	68.61	3.58		0.037
Treatment	1	9841.5	9841.5	513.47		<.001
Variety. Treatment	7	29.83	9.94	0.52		0.675
Residual	16	306.67	19.17			

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.18: ANOVA data showing factors affecting wheat biomass during growth of four wheat varieties under field conditions at KALRO-Kabete and Narok North Sub-County

Kabete Wheat Biomass

Source of variation	D.F	S.S.	M.S.	V.R.	F Pr.	
Variety	3	1012462	337487	2.02	0.151	
Treatment	1	148	148	0	0.977	*
Variety. Treatment	7	202438	67479	0.4	0.752	
Residual	16	2671310	166957			

Nkareita (Narok) Wheat Biomass

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Variety	3	4301.2	1433.7	2.17	0.132	
Treatment	1	3479.6	3479.6	5.26	0.005	*
Variety. Treatment	7	3048	1016	1.53	0.244	
Residual	16	10591.6	662			

Oloropil (Narok) Wheat Biomass

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Variety	3	303048	101016	7.27	0.003	**
Treatment	1	39692	39692	2.86	0.11	
Variety. Treatment	7	32399	10800	0.78	0.523	
Residual	16	222182	13886			

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.19: Means of height and biomass of the four wheat varieties as influenced by RHIZATECH ® TM treatment under field conditions at KALRO-Kabete in Nairobi, Nkareita and Oloropil wards in Narok North Sub-County

Wheat Height

Site	Kabete			Nkareita (Narok)			Oloropil (Narok)		
Variety	C	T	Percentage (%) increase (Height)	C	T	Percentage (%) increase (Height)	C	T	Percentage (%) increase (Height)
K Eagle 10	45.3a	53b	16	60a	62.3a	3	60a	61.7a	2
Njoro BW2	57.7a	59.6b	3	59.3a	64.3a	8	62.3a	63.3a	1.4
Kenya Tai	51.6a	56b	8	57.3a	60.7a	5	58.7a	63.3a	8
Kingbird	37a	49b	32	60.3a	64a	6	62a	65.7a	6
Mean	47.9a	54.4b	13	59.2a	62.8a	5	60.8a	63.5a	6
P (Treatment)	0.005			0.114			0.19		
LSD Treatment	4.2			4.6			4.25		
LSD (Variety. Treatment)	8.5			9.1			8.5		
CV (%)	9.6			8.6			7.9		

Wheat Biomass

Site	Kabete			Nkareita (Narok)			Oloropil (Narok)		
Variety	C	T	Percentage (%)increase (Biomass)	C	T	Percentage (%) increase (Biomass)	C	T	Percentage (%) increase (Biomass)
K Eagle 10	118a	327a	177	852a	1111a	30	876a	1080a	23
Njoro BW2	582a	743a	27	1126a	1153a	2	1185a	1215a	3
Kenya Tai	682a	553a	-23	1049a	1024a	-2	1158a	1239a	7
Kingbird	348a	128a	-63	903a	961a	6	967a	978a	1
Mean	433a	438a	1	982a	1062b	8	1047a	1128b	7
P (Treatment)	0.98			0.05			0.11		
LSD Treatment	353.6			89.9			102		
LSD (Variety. Treatment)	707.3			179.8			204		
CV (%)	93.9			10.2			10.8		

Key: LSD- Least significant difference, CV-Coefficient of variability, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant.

Means accompanied by the same letter (s) are not significantly different from each other according to Fisher's least significant difference test at p= 0.05.

4.4.3 Effects of RHIZATECH®TM inoculation on grain number and grain weight of the four wheat varieties at KALRO-Kabete Nairobi, Nkareita and Oloropil wards in Narok North Sub-County

Application of RHIZATECH ® TM inoculant on the four wheat varieties led to a significant increase in means of wheat grain number at p=0.019 and grain weight at p=0.03 at Nkareita field site as compared to non-inoculated controls (Table 4.20 and Table 21). However, there was no significant increase in wheat grain number and wheat grain weight in Oloropil and Kabete sites. Kenya Eagle 10 variety had the highest mean increase in grain number and grain weight at Kabete field site which was an increase from 642±26.7 (SE) to 1220±123 (SE) (90%) and an increase from 48±14.7 (SE) to 91±8.3 (SE) (89%) respectively as shown in Table 4.21 and Table 22. King bird variety had the lowest mean increase in grain number and grain weight at Kabete field site which was an increase from 1162±112.37 (SE) to 684±31.6 (SE) (-41%) and an increase from 87±1.4 (SE) g to 52±2.19 (SE) (-67%) respectively.

Table 4.20: ANOVA data showing factors affecting grain number during growth of four wheat varieties under field conditions at KALRO-Kabete and Narok North Sub-County

Kabete Grain Number

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Variety	3	4243549	1414516	2.17	0.131	
Treatment	1	543305	543305	0.83	0.134	
Variety. Treatment	7	1236358	412119	0.63	0.604	
Residual	16	10412766	650798			

Nkareita (Narok) Grain Number

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Variety	3	4301.2	1433.7	2.17	0.132	
Treatment	1	3479.6	3479.6	5.26	0.03	*
Variety. Treatment	7	3048	1016	1.53	0.244	
Residual	16	10591.6	662			

Oloropil (Narok) Grain Number

Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Variety	3	303048	101016	7.27	0.003	**
Treatment	1	39692	39692	2.86	0.241	
Variety. Treatment	7	32399	10800	0.78	0.523	
Residual	16	222182	13886			

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.21: ANOVA data showing factors affecting grain weight during growth of four wheat varieties under field conditions at KALRO-Kabete and Narok North Sub-County

	Kabete Grain Weight				
Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.
Variety	3	23558	7853	2.15	0.134
Treatment	1	3133	3133	0.86	0.368
Variety. Treatment	7	6754	2251	0.62	0.614
Residual	16	58451	3653		

	Nkareita (Narok) Grain Weight				
Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.
Variety	3	4301.2	1433.7	2.17	0.132
Treatment	1	3479.6	3479.6	5.26	0.019 *
Variety. Treatment	7	3048	1016	1.53	0.244
Residual	16	10591.6	662		

	Oloropil (Narok) Grain Weight				
Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.
Variety	3	24776	8259	5.57	0.008 **
Treatment	1	2639	2639	1.78	0.201
Variety. Treatment	7	1002	334	0.23	0.878
Residual	16	23728	1483		

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.22: Means of grain weight and grain number of four wheat varieties after treatment with RHIZATECH ® TM inoculum under field conditions at KALRO-Kabete in Nairobi, Nkareita and Oloropil in Narok North Sub-County

Grain Number

Site	Kabete			Nkareita (Narok)			Oloropil (Narok)		
Variety	C	T	Percentage (%)increase (Grain No)	C	T	Percentage (%) increase (Grain No)	C	T	Percentage (%) increase (Grain No)
K Eagle 10	642a	1220a	90	2172a	2870a	32	1977a	2551a	29
Njoro BW2	1622a	2258a	39	2799a	3066a	10	3204a	3350a	5
Kenya Tai	1208a	1675a	38	2637a	2779a	5	2174a	2324a	7
Kingbird	1162a	684a	-41	2300a	2645a	15	2235	2493a	12
Mean	1158a	1459a	26	2477a	2840b	15	2397a	2680a	12
P (Treatment)	0.37			0.019			0.19		
LSD Treatment	698.2			295.6			442.5		
LSD(Variety.Treatment)	1396.3			591.1			885		
CV (%)	61.6			12.8			20.1		

Grain weight

Site	Kabete			Nkareita (Narok)			Oloropil (Narok)		
Variety	C	T	Percentage (%)increase (Grain weight)	C	T	Percentage (%) increase (Grain weight)	C	T	Percentage (%) increase (Grain weight)
K Eagle 10	48a	91a	89	158a	214a	16.7	148a	190b	28
Njoro BW2	122a	169a	38	207a	230a	5.4	240a	250b	4
Kenya Tai	90a	126a	40	198a	190a	4.5	163a	173b	6
Kingbird	87a	52a	-67	172a	198a	15	167a	187b	12
Mean	87a	110a	26	184a	208b	13	179a	200a	12
P (Treatment)	0.37			0.03			0.20		
LSD Treatment	52.3			22.3			33.3		
LSD(Variety.Treatment)	104.6			44.5			66.7		
CV (%)	61.5			13.1			20.3		

Key: LSD- Least significant difference, CV-Coefficient of variability, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant.

Means accompanied by the same letter (s) are not significantly different from each other according to Fisher's least significant difference test at p= 0.05.

4.4.4 Effects of RHIZATECH®TM inoculant on copper ion uptake of the four wheat varieties at KALRO-Kabete Nairobi, Nkareita and Oloropil wards in Narok North Sub-County

Inoculation with RHIZATECH ® TM resulted in a significant increase in copper ion uptake by four what varieties at p=0.05 (11%) and p =0.007 (8%) at Nkareita and Oloropil sites in Narok North (Table 4.23). There was no significant increase in copper uptake at p=0.163 in the wheat varieties at Kabete field site. Wheat variety K Eagle 10 had the highest mean increase in copper ion level in Kabete and Oloropil sites, which was an increase from 7.5 ppm to 8.9 ppm (19%) and an increase from 8. Ppm 7 o 10.3 ppm (18%) respectively as shown in Table 4.24.

Kingbird variety had the lowest mean increase in copper ion uptake at Kabete and Nkareita sites which had an increase from 7.4±0.31 (SE) ppm to 7.5±0.22 (SE) ppm (1%) and a decrease from 9.0±0.32 (SE) ppm to 8.7±0.32 (SE) ppm (-3%) respectively. Njoro BW2 variety had the lowest mean increase at Olorropil site which was an increase from 9.9±0.12 (SE) ppm to 10.1±0.3 (SE) ppm (2%) as compared to the performance of the other 3 wheat varieties.

Table 4.23: ANOVA data showing factors affecting copper leves during growth of four wheat varieties under field conditions at KALRO-Kabete and Narok North Sub-County

	Kabete Copper	**Levels**			
Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.
Variety	3	5.417	1.806	1.79	0.19
Treatment	1	2.16	2.16	2.14	0.163
Variety. Treatment	7	1.35	0.45	0.45	0.724
Residual	16	16.147	1.009		

	Nkareita Copper	**(Narok) Levels**			
Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.
Variety	3	3.6979	1.2326	2.11	0.139
Treatment	1	2.4704	2.4704	4.23	0.05
Variety. Treatment	7	5.0179	1.6726	2.87	0.069
Residual	16	9.34	0.5837		

	Oloropil Copper	**(Narok) Levels**				
Source of variation	D.F.	S.S.	M.S.	V.R.	F Pr.	
Variety	3	9.2183	3.0728	6.18	0.005	**
Treatment	1	4.86	4.86	9.77	0.007	**
Variety. Treatment	7	2.06	0.6867	1.38	0.285	
Residual	16	7.96	0.4975			

Key: ANOVA- analysis of variance D.F- Degree of freedom, S.S-Sum of squares, M.S-Mean squares, V.R-Variance ratio, F- mean squares treatment / mean squares error, Pr-Probability (confidence interval of 95 %)

Table 4.24: Copper ion uptake of wheat variety influenced by VAM inoculations under field condition under field conditions at KALRO-Kabete and Narok North Sub-County

Copper ion Uptake

Site	Kabete			Nkareita (Narok)			Oloropil (Narok)		
Variety	C	T	Percentage (%) increase in Copper uptake	C	T	Percentage (%) increase (Copper uptake)	C	T	Percentage (%) increase (Copper uptake)
K Eagle 10	7.5a	8.9a	19	8.4a	9.8a	17	8.7a	10.3b	18
Njoro BW2	8.6a	9.0a	5	9.6a	9.9a	3	9.9a	10.1b	2
Kenya Tai	7.8a	8.3a	6	7.9a	9.6a	22	8.9a	10.3b	16
Kingbird	7.4a	7.5a	1	9.0a	8.7a	-3	8.1a	8.6b	6
Mean	7.8a	8.4a	7	8.4a	9.8b	11	8.9a	9.8b	8
P (Treatment)	0.163			0.05			0.07		
LSD Treatment	0.9			0.7			0.6		
LSD(VarietyTreatment)	1.7			1.3			1.2		
CV (%)	12.4			8.4			7.5		

Key: LSD- Least significant difference, CV-Coefficient of variability, T-inoculated with RHIZATECH ® TM inoculant, Control-non-inoculated with RHIZATECH ® TM inoculant.

Means accompanied by the same letter (s) are not significantly different from each other according to Fisher's least significant difference test at p= 0.05.

4.4.5 Comparison on performance of the three field trial sites of Kabete, Nkareita, and Oloropil in the parameters of the four wheat varieties

The three sites had different rate of root colonization. Oloropil ward site (Narok) had the highest mean of 32.33 followed by Nkareita ward site (Narok) with a mean of 30. KALRO-Kabete field had the lowest mean of 27 (Table 4.25). Generally, wheat varieties in Narok sites had a significant increase in root colonization rates compared to those of KALRO-Kabete site as a result of RHIZATECH®TM application.

Wheat growth performance was also different in the 3 sites. Oloropil ward site (Narok North) had the highest mean of wheat height and wheat biomass of 62.12 cm and 1004.4 g respectively (Table 4.25). It was followed by Nkareita ward site (Narok North) with a mean of 61.04 cm stem height and 946.7g biomass respectively. Generally, wheat varieties in Narok sites had a significant increase in wheat growth as compared to those of KALRO-Kabete site as a result of RHIZATECH®TM application.

Nkareita ward site (Narok North) had the highest wheat grain number and wheat weight mean of 2651 and 260 g respectively (Table 4.25). It was followed by Oloropil ward site (Narok North) with a mean of 2538.04 grain number and 189.7g grain weight respectively. KALRO-Kabete field had the lowest mean of wheat grain and wheat weight of 1330 and 99.8g respectively. For copper ion uptake by the four wheat varieties, Oloropil ward site (Narok North) had the highest mean of 9.35ppm copper ion uptake (Table 4.25). It was followed by Nkareita ward site (Narok North) with a mean of 9.11ppm. KALRO-Kabete site had the lowest mean of 8.11ppm.

Table 4.25: Grand means of wheat parameters due to effect of RHIZATECH ® TM inoculation on the wheat varieties in the field sites at Kalro Kabete, Nkareita and Oloropil ward

Least significant difference of means (5% level)						
Site	Root colonization	Wheat Height	Biomass	Grain number	Grain weight	Copper ion
1. Oloropil Site (Narok North)	32.33a	62.12a	1004.5a	2538a	189.7a	9.35a
2. Nkareita Site (Narok North)	30a	61.04a	946.7a	2651a	260.6a	9.11a
3.KALRO-Kabete	27b	51.17b	435.1b	1330b	99.8b	8.11b

Least significant difference of means (5% level) of the effect of RHIZATECH ® TM inoculation on wheat parameters of the four wheat varieties of Narok fields (Olloropil ward and Nkareita ward) and Kalro-kabete field. Means accompanied by the same letter (s) are not significantly different from each other according to Fisher's least significant difference test at p= 0.05

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECCOMMENDATIONS

5.1 Discussion

5.1.1 Soil characteristics of the sites including KALRO-Kabete Nairobi, Nkareita and Oloropil wards in Narok North Sub-County

5.1.2 Soil pH

Soil pH in water (1:1) in all the three trial sites before planting ranged from 4.85 to 6.5. Narok soils and soils used in KALRO-Kabete greenhouse were slightly acidic, which is suitable for most crops including wheat (Kamwaga, 2016). KALRO-Kabete soil, was highly acidic which is unsuitable for wheat growth hence lime was applied at a rate of 50 kg per acre to the plots 2 weeks before planting as recommended by National Agricultural Research Laboratories. This supports the finding of Mwendwa *et al*. (2020) whereby he found most soils in the humid areas such as Kabete to be acidic. The widespread soil acidity in KALRO-Kabete soils is due to prolonged removal of crop residues and decreased organic matter as compared to Narok North fields which had been left fallow for up to two years (Mangale *et al.,* 2015).

5.1.3 Soil macronutrients

Total soil organic carbon was adequate for plant growth in all the trial sites and it ranged from 1.76 ppm to 3.56 ppm except for Kabete field trial site which was moderate. Humid nitisols have been described to be rich in most plant nutrients such as exchangeable cations and macronutrients except nitrogen and phosphorous (mwendwa *et al.,* 2020). Adequate crop residual and adequate use of fertilizers might have contributed to increased harvest and therefore high levels of residues reverted back to the soil.

Because of this, the organic matter levels were significantly high in Narok soils. This conforms to the research reported by Ali *et al.* (2022) whereby he found mineral application of fertilizers increases crop growth and yield leading to increase in soil organic matter. Narok North Sub-County is warmer and drier as compared to Kabete (Jaetzold *et al*., 2010). The differences in climate may possibly have influenced the frequency of carbon decomposition and accumulation within soil organic matter (Smith *et al*., 1998).

The soil's total nitrogen and available soil phosphorus content was adequate in the two sites except for KALRO-Kabete site which was low in total nitrogen and Olorropil site which was low in available phosphorous. This could be due to continuous cropping and prolonged removal of crop residues which led to low organic carbon (Esilaba *et al*., 2021). Moreover, continuous cultivation without nutrient replenishment, particularly manure, leads to inadequate nitrogen levels in the soils. This is in line with research by Willy *et al*. (2019), who linked ongoing farming to decreased soil nitrogen.

5.1.4 Soil micronutrients

Narok soils, specifically, may have inherent characteristics that contribute to low copper levels. Factors such as soil pH, organic matter content and the specific mineralogy of the soil can affect copper availability (Njoroge, 2017). Moreover, copper is classified as a relatively immobile element in soil and it form various complexes with organic matter and soil particles, which can affect its movement within the soil profile (Alloway, 2013).

High zinc and iron levels in all trial sites might be due to high organic content in the sites . Refaey e*t al*. (2017) have found that iron and zinc content increases with the raise in organic matter levels in the soils.

5.2 Status of copper ion concentration in wheat growing soils in the five wards of Narok Town, Olokurto, Nkareita, Oloropil and Melili in Narok North Sub-County

Farms in the five wards had different Cu ion concentration in their soils. Most of the soils from the farms in Melili, Narok town and Nkareita wards had inadequate levels of copper compared to soils from the farms in Oloropil and Olokurto wards. The inadequate levels of Cu could have resulted from the relatively low organic matter status of the soils especially in the farms of Melili, Narok town and Nkareita wards. This is in agreement with the studies of Refaey *et al*. (2017) who have found that iron, zinc and copper content increases with the raise in organic matter levels in the soils and retain low levels of these soil micronutrients.

Relatively low copper content in the soils from 3 wards; Melili, Nkareita and Narok town compared to the soils from the other 2 wards; Oloropil and Olokurto might also be due to difference in original rock material from which soil is formed, difference in modes of deposition, transport and variations in weathering regimes (Bruthans *et al*., 2017). Generally, more than a half of the total soil samples analyzed in the five wards were low in copper ion concentration considering total Cu in average arable soils is estimated to vary from 1 to 50 ppm (Gilbert, 1952). This observation is consistent with the findings of P Gicheru *et al*. (2013) whereb, Suswa area is characterized by loss of land cover, soil erosion, reduced water catchment areas and reduced soil nutrient availability such as copper.

This finding is also in accordance to studies of Sutton *et al*. (2002), who have shown Cu deficiency in remote non-wheat growing areas of the country, such as Shimba Hills and Solio Wildlife Conservancy. This might be indicative of widespread Cu deficiency in Kenyan soils including wheat-growing areas yet copper is a vital micronutrient in wheat growth and development. It promotes metabolic activities such as protein synthesis and photosynthetic electron transport in the chloroplasts (Pilon *et al.,* 2019).

This copper deficiency in some of these wheat growing areas in the sub-county can be attributed to the genesis of the soil from volcanic activity that lack micronutrients (Njoroge, 2017). Moreover, copper is classified as a relatively immobile element in soil and it form various complexes with organic matter and soil particles, which can affect its movement within the soil profile as reported by Alloway (2013) in his findings.

Farming practices have also potentially contributed to copper deficiency in Narok soils. Intensive agricultural practices often involve the use of chemical fertilizers (Alloway, 2013). If farmers excessively use fertilizers without considering copper supplementation, it can deplete the available copper in the soil (Alloway, 2013). Improper land management techniques can lead to soil erosion, which can result in the loss of topsoil containing essential nutrients like copper (Lal, 2015). When erosion occurs, the copper-rich topsoil can be carried away, leaving behind soils with lower copper content (Lal, (2015). Moreover,

Continuous farming of crops that have high copper demands can deplete the available copper in the soil, leading to deficiency over time (Goudia and Hash, 2015).

5.3 Effect of RHIZATECH®TM fungi on wheat growth and copper ion uptake under greenhouse conditions at KALRO-Kabete

The presence of significant RHIZATECH®TM colonization in all inoculated treatments demonstrates mycorrhiza's compatibility with the wheat varieties. This was observed in the microscope study due to few identifiable VAM structures (arbuscules and vesicles) in wheat varieties which were not inoculated with RHIZATECH®TM compared to inoculated varieties which had more presence of VAM structures. This is similar to Cruz-Paredes *et al*. (2017) findings who also reported an increased root colonization rates on wheat infected with *Glomus mosseae*. This further support the findings of Damin *et al*. (2020), whereby he established that the ability of VAMF to form a colonization with the plant roots and form a mutual relationship with the host plant is critical to its benefits.

Previous metaanalyses of several inoculation trials (Koide, 2005) observed that inoculation with several VAMF strains, increased root colonization of the wheat plant. Furthermore, Ortas (2012) who was researching on different crops including wheat inoculated with *Glomus mosseae* for a period of three years observe an increase in root colonization rate compared to non-inoculated treatments which concurs with the findings of this research.

This study also found that rates of mycorrhizal colonization differed among the wheat varieties. This might be due to differences in the genetic make-up of the varieties which influence host specificity of the VAMF to wheat (Baum *et al*., 2015). This further supports previous findings of Mutairi *et al*., 2020 whereby differences in root colonization rates among VAMF strains of *Glomus species* shows that plants exhibit some form of specificity or preference for individual AMF resulting to different levels of mycorrhizal root colonization. Moreover, Han *et al*., (2020) found that the effect of vesicular arbuscular

mycorrhizal root colonization rate on their hosts has been shown to vary, and this variation has been observed even at the variety level.

Wheat varieties on plots previously inoculated with RHIZATECH ® TM fungi in the greenhouse conditions showed significant increase in biomass whereas there was no significant increase in plant height. These findings suggest that seeds inoculated with RHIZATECH ® TM stimulate early emergence and vigor significantly improving biomass of the plant. The observations are in consistence with those of Mastouri *et al*. (2010) who found that wheat seed treatment with *Glomus eutinicatum* improved the plant biomass, height and seed quality.

Farmers are increasingly turning to beneficial microorganisms' particularly vesicular arbuscular mycorrhizal fungus because of their ability to boost agricultural production (Singh *et al.,* 2011). Moreover, the improved growth of the wheat varieties inoculated with the consortium of the three *Glomus* species could be due to synergism effect and this is in line with several studies conducted in the past. Colla *et al*. (2015) discovered that inoculating lettuce, tomato, and zucchini squash seedlings with both *Glomus intraradices* BEG72 boosted biomass output by a factor of two when compared to inoculating *Glomus intraradices* or *Trichoderma atroviride* alone which further supports the findings of this study. Furthermore, wheat height and biomass varied among wheat varieties which could be due to genotypic differences and the VAMF's host specificity to wheat cultivars as reported by De Vita *et al*., (2018).

The findings are contrary to the observation of Ortas *et al*. (2011). He found that the connection of wheat roots with the RHIZATECH ® TM fungus resulted to an increase in the amount of plant nutrients such as copper accessible to the plants hence increase in the wheat height.

Al-Karaki and Al-Raddad (1997) further found that wheat plants can generate greater height with vesicular arbuscular mycorrhizal inoculation due to the possible increase in nutrient absorption which do not agree with this study since the inoculum had no significant effect on the wheat height.

Due to several environmental factors that may impact the fungus or the plant, particularly in a short period of time like a year or two, the same findings would not be easily obtained in field experiments with the same inoculation. This is due to the fact that effectiveness is fully dependent on favorable environmental factors, soil characteristics, the requirement for a symbiotic connection with the host plant, AMF species diversity, and the size of the fungal population (Melo *et al*., 2019).

The highest seed number and seed weight per pot/plot was obtained by treating the soil with the RHIZATECH ® TM fungus which was significant, while the lowest seed number and seed weight per pot and plot was obtained when the RHIZATECH ® TM inoculant were not applied. These findings support the work of Tarafdar *et al.* (1995) who studied the effect of the vesicular arbuscular mycorrhizal fungus (VAMF); the *Glomus mosseae* on durum wheat variety 'Sifnos' growth in 10 different soils. They found that, when compared to non-inoculated wheat plants, inoculation improved tillering, increased plant growth by 11.6 times, and boosted grain output by 5.4 times. Moreover, Colla *et al.* (2015) found that transplanting *Glomus intraradices* BEG72 considerably increased the chlorophyll content and biomass output of five vegetable transplants at early stages of development.

The improved yield of the wheat varieties inoculated with the RHIZATECH ® TM could also have been due to synergism effect which is similar to Chandanie *et al.* (2009) findings, that co-inoculation of *Glomus mosseae* and *T. harzianum* had a synergetic effect on plant development. Moreover, the grain number and grain weight varied among wheat verities. This might be due to the fact that the inoculant strains may have had varying impacts on the wheat plant growth metrics and these effects may not have been the same for each plant species as reported by Ciftci *et al*. (2010) which agrees with the finding of this study.

Li *et al*. (2019), on the other hand stated that there is growing evidence of host-specific distinctions in plant response to AM fungus and fungal response to plants which is vital in plant growth and yield.

Ellouze *et al*. (2016) also found that, one of the key variables that affects how AMF affects plant yield and nutrition is plant identification (species and genotype). Different plant genotypes' responses to the colonization of arbuscular mycorrhizal fungi vary widely, exemplified by a number of crucial crops for agriculture such as wheat (Ellouze *et al*., 2016).

Copper ion uptake by the four wheat varieties was significant due to application of RHIZATECH ® TM compared to non-inoculated varieties. This is in line with several previous studies' findings about VAMF *Glomus* species' high performance in improving plant Cu uptake (Kayama and Yamanaka, 2014; Yaseen *et al.,* 2011) whereby, vesicular arbuscular mycorrhizal fungi especially of *Glomus mosseae* enhances nutrient uptake such as copper especially in areas with nutrient deficiency.

Ercoli *et al*. (2017) state that application of VAMF fungus may be of considerable importance specifically the development of bio fortified foods given the significant rise in Cu levels seen in the durum wheat biomass which further support the finding of this study. This is made even more relevant by the fact that more than 60% and 30% of the global population, respectively, have diets that are low in Copper (White and Broadley, 2009). Moreover, the findings are consistent with those of Cardarelli *et al.* (2010) and Rouphael *et al.* (2010), who found that using *Glomus intraradices* BEG72 increased nutrient uptake.

The enhanced nutritional status of Cu is seen in wheat plants arising from coated seeds in open field studies which could be regarded an indirect consequence of the beneficial fungus (Cardarelli *et al.,* 2010). Similarly, Minaxi *et al.,* (2013) found that an inoculum of VAMF of *Glomus etunicatum* increased Cu levels in wheat shoots. Increased soil exploration and supply to host roots are likely responsible for this. The additional rise in Cu levels in mycorrhizal plants might be ascribed to the VAM fungi's ability to attach the roots to the soils, which is advantageous for nutrient absorption (Imran, 2022c).

Wang *et al.* (2016), discovered that AMF can change root morphology, resulting in more surface area for water and nutrient absorption. Moreover, vesicular arbuscular mycorrhizal symbiosis can modify a

plant's physiology and environment thereby enhancing nutrient uptake (Zhang *et al*., 2018). Additionally, AM fungus aided plant nutrient uptake by enhancing the availability and transport of several minerals, including copper (Wu, 2017). In the current investigation, all mycorrhizal species raised the copper accumulation in the wheat varieties which may be attributed to mycorrhizal-treated plants having a stronger and more effective root system (Wang *et al.*, 2020)

5.4 Effect of RHIZATECH®TM fungi on wheat growth and copper ion uptake at field trials in KALRO-Kabete Nairobi, Nkareita, and Oloropil wards in Narok North Sub-County

Significant mycorrhizal development at p=0.001 was observed in all RHIZATECH®TM treatments due to presence of vesicles and arbuscules in the infected roots while there were few or no root colonization in the controls (non-inoculated treatments). This supports the observations of Field *et al. (*2019) who found entophytic fungi particularly the *Glomus mosseae* are found in close proximity to AMF and are linked to the majority of terrestrial plants. The significant differences in the effect of wheat varieties due to RHIZATECH®TM application might have been as a result of thorough hand ploughing which was followed by harrowing at a depth of 7 cm a few days before planting.

The unique aggregation and mycorrhizal competitive interactions were undoubtedly influenced by this management practice. According to Verbruggen *et al.* (2013), intensive tillage before inoculation reduces the aggregation of indigenous fungi which will have a significant impact on the success of the introduced allochthonous one. In reality, the new imported RHIZATECH®TM fungus was significantly more prevalent locally than the existing fungi due to 'dilution' of the new introduced fungi if they are applied patchily following plowing (Verbruggen *et al*., 2013).

Rate of root colonization by the RHIZATECH®TM inoculants differed within the sites. There was high rate of colonization in the RHIZATECH®TM inoculated wheat varieties in Narok North sites than in Kabete sites which was significant. These might be due to differences in soil properties between Narok

and Kabete sites. According to soil analysis, Narok soils were slightly acidic which is suitable for most crops including wheat (Kamwaga, 2016), whereas soils at KALRO-Kabete were highly acidic which is unsuitable for wheat growth hence might have contributed to low rate of RHIZATECH®TH mycorrhizal colonization. The leaching of basic cations under the humid conditions is the cause of the soil's acidic pH, as seen by higher pH values in the top 0 to 30 cm of the soil. In a similar study in Nepal, Panday *et al.* (2018) attributed moderately acidic soil reaction to leaching of major cations.

Stem height and wheat biomass in some field sites resulted in significant increase compared to non-inoculated wheat varieties. The significant effect of the mycorrhizal fungus in boosting nutrient uptake through increased surface area for absorption might be linked to the considerable wheat plant height obtained in the mycorrhizal plants as reported by Wang and Feng (2021).

Greater nutrient absorption must have resulted in a higher photosynthetic rate, hence the increase in wheat height (Pilon *et al.,* 2019). According to Dumlao *et al*. (2012), plant photosynthetic activity is intimately linked to wheat biomass buildup and chlorophyll fluorescence indices which have been proven to be effective indicators of photosynthetic ability and energy conversion efficiency and does not support the non-significant increase of wheat biomass in this study.

Nutrient deficit typically reduces photosynthesis (Turnbull *et al*., 2007). Through improved photosynthesis, the AMF symbiosis can reverse the poor growth condition of host plants under nutritional stress. *Glomus mosseae* boost soybean plant growth and nutrition by improving photosynthesis in leaves according to Pilon *et al.* (2019) especially when soil nutrients levels are low. Plant roots interacting with mycorrhizal lead to increase in the quantity of copper accessible to the plants, resulting in a rise in wheat growth (Abdel-Fattah *et al.,* 2014).

The significant difference in height between Narok North and KALRO-Kabete was due to difference in total nitrogen content of the sites. The soil's total nitrogen content was adequate for plant growth in all the trial sites except for Kabete. These results agree with those of Mwenda *et al*. (2020) who found

Kabete to be deficient in nitrogen. Moreover, organic matter is essential for preserving the structure of the soil and binding nitrogen, which enhances infiltration and plant uptake. This observation is in line with research by Lelago and Buraka (2019) and Cheng *et al.* (2016) that indicated organic carbon to be crucial for nitrogen availability. Additionally, it agrees with Ye *et al*. (2021) findings, which showed a favorable link between soil nitrogen and soil carbon.

Grain number and grain weight of the four wheat varieties in some field sites resulted in significant increase in mean compared to non-inoculated controls. The RHIZATECH ® TM enhanced the performance of the experimental wheat varieties in terms of both wheat grain number and grain weight. Wheat varieties colonized by the inoculant demonstrated considerable benefits in terms of both nutrient deficit mitigation and root growth colonization led to increase in the wheat grain number and weight. However, in terms of wheat yield, the performance of mycorrhizal strains differed depending on the wheat varieties. According to Amirnia *et al.* (2019), these could be due to the combined actions, such as nutrients uptake and more efficient photosynthesis.

Mycorrhiza have been shown to assist plants increase their resistance to salt stressors through a variety of processes, including enhanced water and nutrient absorption as reported by Maitra *et al*. (2021) As a result, and as compared to non-mycorrhizal controls, the overall impacts boost the wheat yield (Zhou *et al.,* 2020). A meta-analysis study of wheat field trials (Pellegrino *et al*., 2015) also shows that AMF inoculation can boost wheat grain number and weight in field circumstances which further agrees with the finding of this study.

The significant increase in Cu uptake in the field trials was as a result of application of RHIZATECH®TM inoculant. The key dynamic qualities that assist increase the favorable elements of AMF colonization on overall plant performance include improvements in plant nutrition Wang *et al*. (2020)). These findings further agree with that of Pellegrino (2014) who found that mycorrhizal chickpeas grew faster and had higher amounts of protein and Cu. Furthermore, it further supports the two meta-analysis papers

published a few years ago which demonstrated the importance of mycorrhizal symbiosis in the absorption of several micronutrients in crops including copper (Cu) (Lehmann and Rillig, 2015).

More findings have also shown that, under drought, the fungal relationship increased the uptake of micronutrients such as copper according to Jansa *et al.* (2019). Some studies have also shown that arbuscular mycorrhizal fungi such as *Glomus mosseae* have enhanced nutrient uptake in the shoot and nutrients like Cu which are immobile in the soils and are absorbed by plants with the help of vesicular arbuscular mycorrhizal fungi (Ali *et al*., 2015).

Enhanced copper uptake by the inoculated wheat varieties might be due to the VAMF's extra radical mycelium's which increases root surface area, increasing the root volume for nutrient absorption (Yang Dou, *et al.* (2022). The immobilization of Cu by rhizospheric could explain this significant increase in Cu uptake. In fact, the microbial communities that surround a plant's rhizosphere is vital in nutrient cycling as found by Bowles *et al.* (2018); which further compliment this study.

In soils with high mineral content, these fungi have also been demonstrated to control certain nutrient intake, reducing plant toxicity (Gupta, Thokchom, and Kapoor, 2021). The plant benefits from this symbiosis by having improved access to and absorption of soil nutrients and water. It has been demonstrated that symbiotic association aids plants in distributing mineral nutrients from the soil, particularly immobile elements like phosphorus, zinc, and copper (He *et al*., 2021).

Therefore, RHIZATECH®TM fungi such as *Glomu* mosseae is vital in enhancing yield and micronutrient concentration in wheat crops which could be attributed to its enrichment to crops of both micro and macronutrients (Bedini, 2014). These findings also suggest that different wheat varieties respond to RHIZATECH®TM inoculants differently and are able to affect the soil microbial population through secreting root exudates hence different rates of nutrients uptake as reported by Wang *et al. (*021b)

5.5 Conclusion

Soil samples from the farms especially in Melili, Narok town and Nkerita wards had inadequate levels of copper and this might indicate copper deficiency in Narok County. The study also showed that the RHIZATECH ® TM inoculant improved the growth and yield of the experimental four wheat varieties. Root colonization was higher in pots/plots with wheat varieties inoculated with VAMF, resulting in higher Cu ion uptake, biomass, and grain yields compared to the control (non-inoculated pots and plots). Therefore, RHIZATECH ® TM inoculant could be a sustainable tool to improve micronutrients in the soils and yield in wheat varieties, attributed to its enrichment of both micro and macronutrients.

5.6 Recommendations

1. Farmers should be educated about the benefits of soil testing and the use of environmentally friendly bio fertilizers such as RHIZATECH ® TM to enhance soil fertility, nutrient availability, and crop yield through training programs.
2. This study recommends the application of 50 g/acre of RHIZATECH ® TM inoculum to soils in wheat growing areas of Narok County, for increased wheat growth, biomass and grain yield.
3. Further research should be conducted to compare the performance of RHIZATECH ® TM inoculant and other commercial inocula with respect to growth of wheat varieties.

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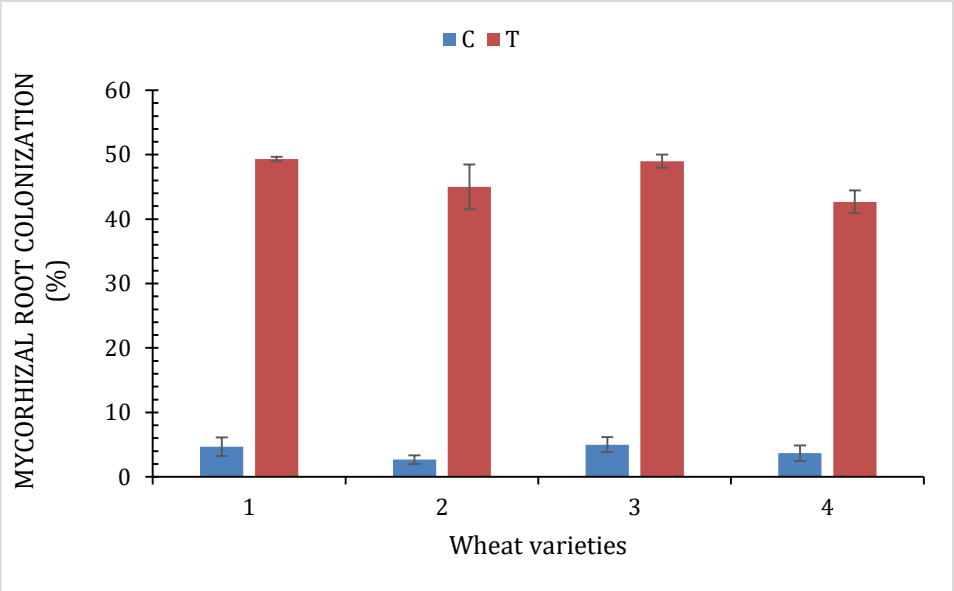
APPENDICES

Appendix 1: Soils Nutrients Classification Table

Nutrient	Deficiency level	Adequate level	Excessive level	Remarks
Sodium, me%	Seldom applies	0-2.0	> 2.0	Excessive
Potassium, me%	< 0.24	0.24-1.5	> 1.5	
Calcium, me%	< 2.0	2.0-15.0	> 15.0	
Magnesium, me%	< 1.0	1.0-3.0	> 3.0	
Phosphorus, mg kg^{-1}	< 30	30-80	> 80	
Manganese, me%	< 0.11	0.11-2.0	> 2.0	Excessive
Extraction with 0.1 M HCl				
Copper, mg kg^{-1}	< 1.0			
Iron, mg kg^{-1}	< 10			
Zinc, mg kg^{-1}	< 5.0			

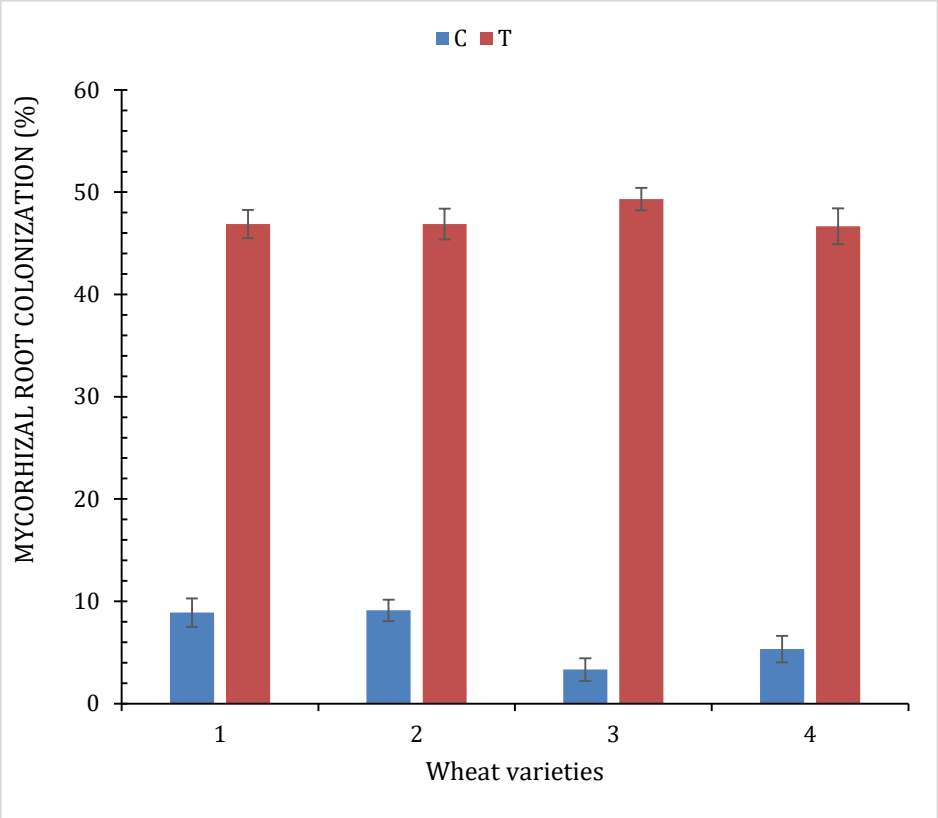
Key: % =Percentage, Meg %=Mill equivalents per 100 gram of soils. Source: (Melich Method, 1962)

Appendix 2: Wheat roots colonization influenced by RHIZATECH ® TM **inoculation under greenhouse condition at Kabete-Nairobi**



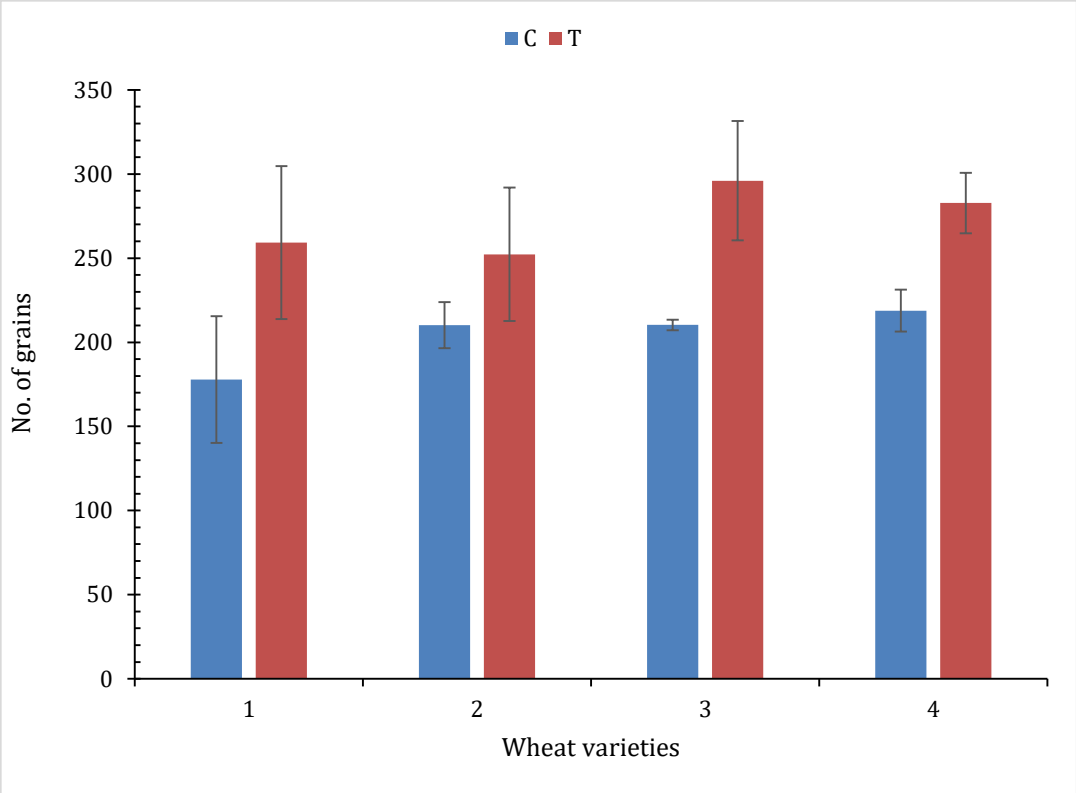
Bars indicate standard error bars, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant

Appendix 3: Wheat roots colonization influenced by RHIZATECH ® TM inoculation under field condition at Kabete-Nairobi



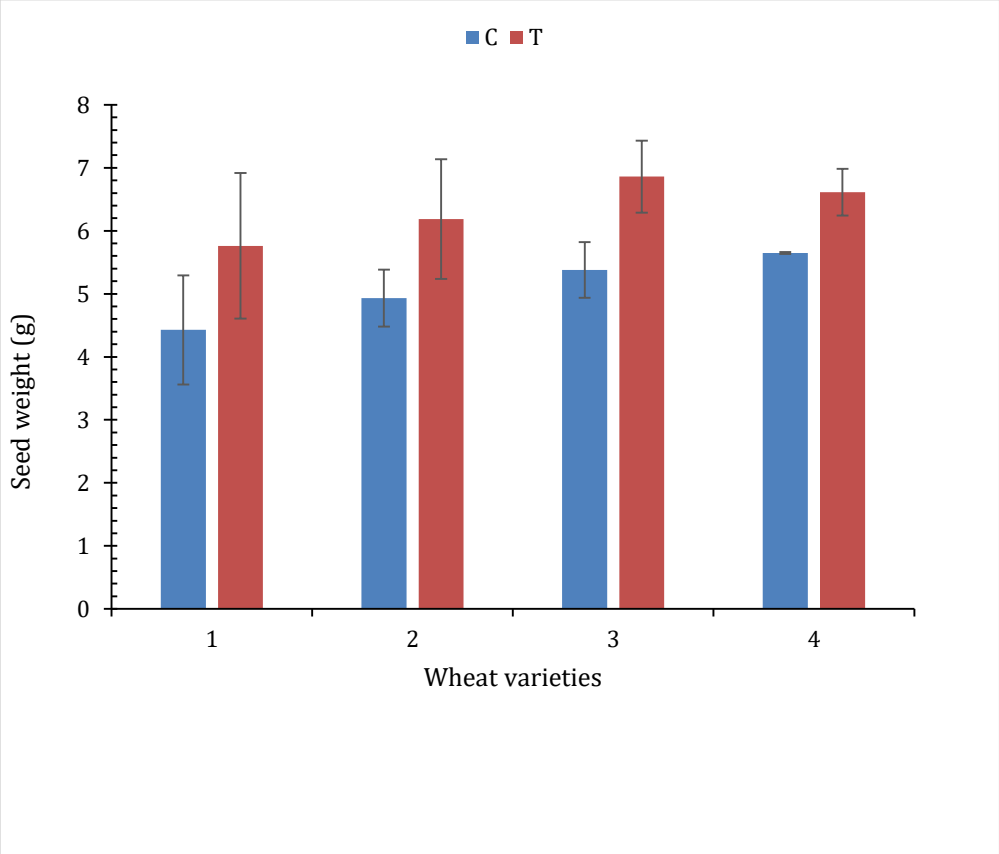
Bars indicate standard error bars, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant

Appendix 4: Wheat number of grains, influenced by RHIZATECH ® TM inoculation under greenhouse conditions at Kabete-Nairobi



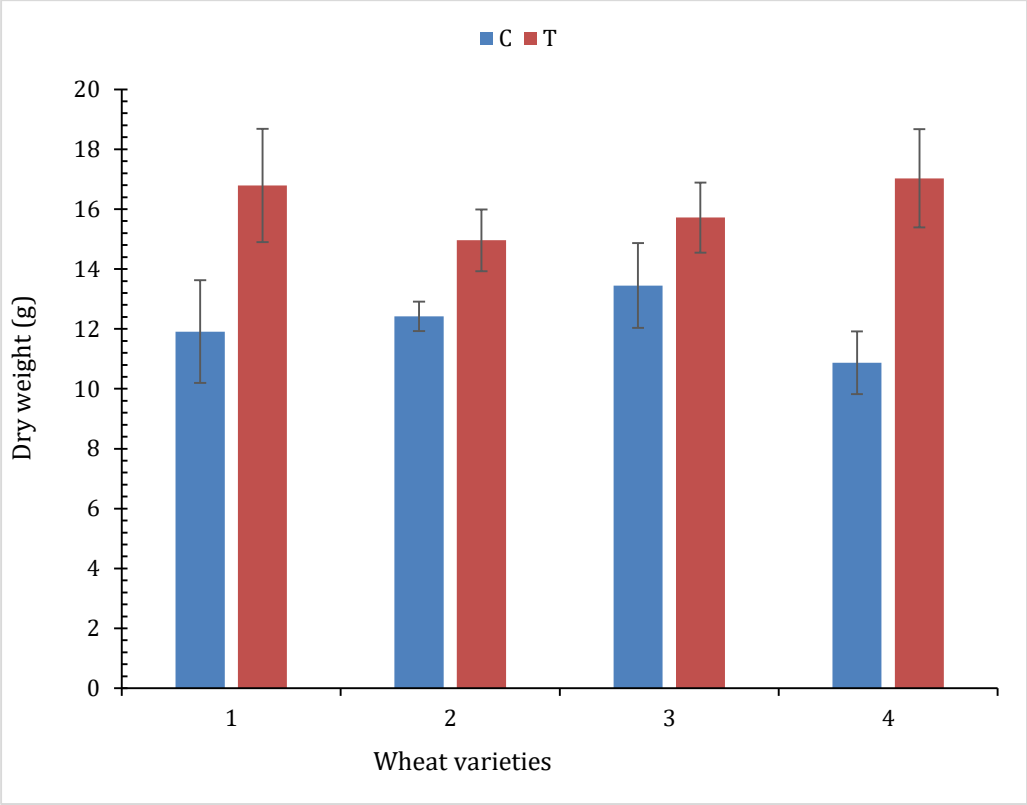
Bars indicate standard error bars, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant

Appendix 5: Wheat grain weight influenced by RHIZATECH ® TM inoculation under field conditions at Kabete-Nairobi



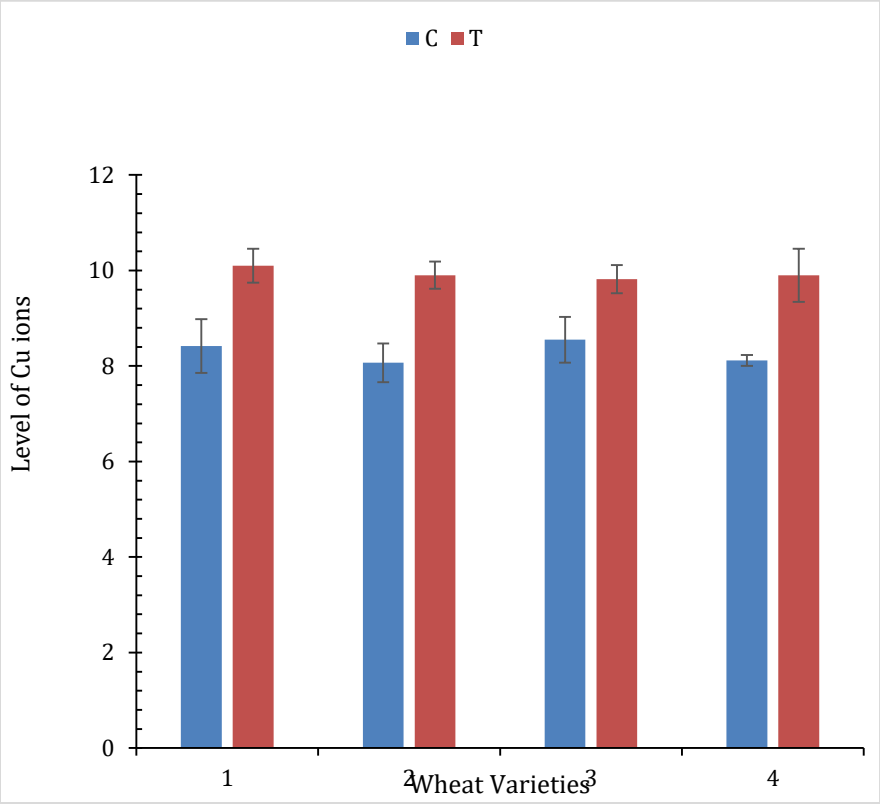
Bars indicate standard error bars, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant.

Appendix 6: Wheat total dry weight of wheat varieties influenced by RHIZATECH ® TM inoculation under greenhouse conditions at Kabete-Nairobi



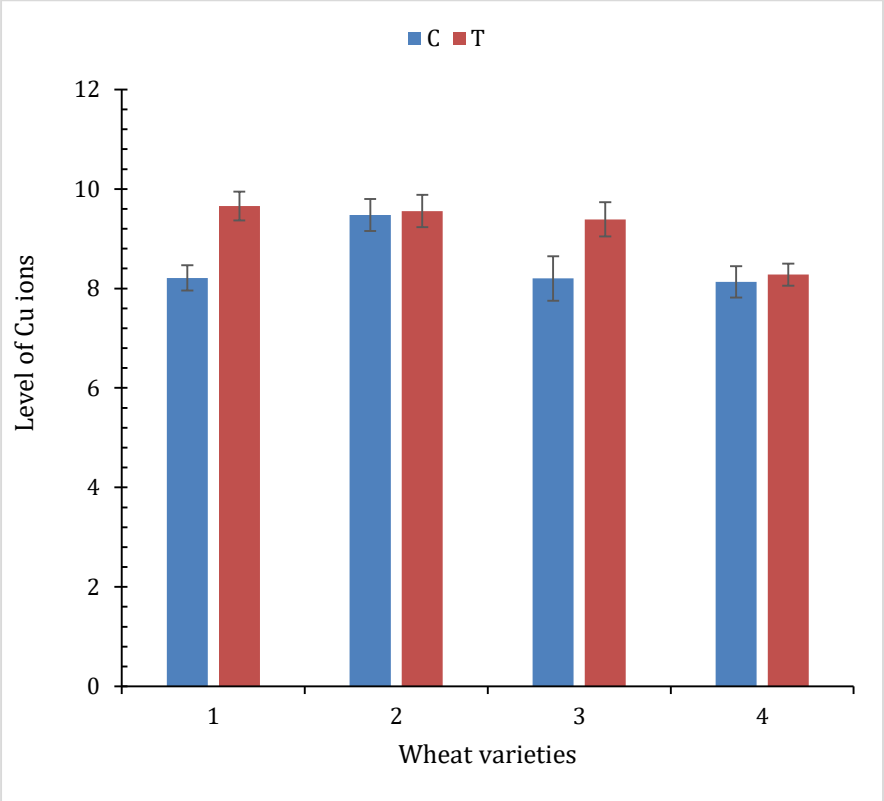
Bars indicate standard error bars, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant

Appendix 7: Copper ion uptake influenced by RHIZATECH ® TM inoculation on four wheat varieties under greenhouse conditions at Kabete-Nairobi



Bars indicate standard error bars, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant

Appendix 8: Copper ion uptake influenced by RHIZATECH ® TM inoculation on four wheat varieties under field conditions at Kabete-Nairobi.



Bars indicate standard error bars, T-inoculated with RHIZATECH ® TM inoculant, C-non-inoculated with RHIZATECH ® TM inoculant