RESPONSE OF BREAD WHEAT (*Triticum aestivum* L.) TO MICRONUTRIENT APPLICATION UNDER GREENHOUSE CONDITIONS AT KULUMSA, SOUTH EAST ETHIOPIA

M.Sc. Thesis

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JIMMA, ETHIOPIA

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In Partial Fulfillment of the Requirements for the Degree of Master of Science in Agronomy

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APPROVAL SHEET

SCHOOL OF GRADUATE STUDIES

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by **Fitsum Desta Mathewos**, entitled **Response of Bread Wheat** (*Triticum Aestivum* **l**.) **To micronutrient Application under Greenhouse Conditions at Kulumsa, Southeastern Ethiopia**

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DEDICATION

To God almighty and Jesus Christ his only begotten son, for nothing is impossible with God and To My Father Desta Mathewos and My mother, Demekeche Soworo, for their allrounded and unconditional support in my life.

STATEMENT OF AUTHOR

First, I declare that this thesis is my own work and that all sources of materials used for thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at Jimma University, College of agriculture and veterinary medicine and is deposited at the University library to be made available to users under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author, Fitsum Desta, was born in 1987 in Wonji Town, East Shewa, Oromiya Regional State of Ethiopia. He attended elementary education (Grade 1-8) from 1993 - 2001 at Wonji No.2 Elementary School. After completing elementary education, he was enrolled at Wonji Compressive and Gelawediwos Comprehensive Secondary School and attended Grade 9-12 from 2002 – 2005 at Wonji and Adama towns, where he pursued and completed his Secondary and Preparatory Educations. He then joined Jimma University in September 2006 and graduated with a BSc Degree in Crop Sciences in July 2008. After graduation, he was employed by Gombora Woreda Agricultural and rural Development Office in Hadiya Zone, Southern Ethiopia and was assigned to work as an expert of Crop Production and Protection. After serving the Gombora Woreda Agricultural Development Office for four years, he then joined the School of Graduate Studies of Jimma University in October 2012 to pursue a study leading to the Degree of Master of Science in Agronomy.

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LIST OF ACRONYMS

| ANOVA | Analysis of variance |
|--------|---|
| CIMMYT | International Center for Wheat and Maize Improvement |
| CSA | Central Statistical Agency |
| FAO | Food and Agriculture Organization of the united Nations |
| HI | Harvest Index |
| KARC | Kulumsa Agricultural research center |
| NSPS | No seed per spike |
| PH | Plant Height |
| RCBD | Rondomize Complete Block Disgen |
| SPAD | Soil Plant Analysis Development |
| TKW | Thousand Kernel Weight |

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RESPONSE OF BREAD WHEAT (Triticum aestivum L.) TO MICRONUTRIENT APPLICATION UNDER GREENHOUSE CONDITIONS AT KULUMSA, SOUTH EAST ETHIOPIA

ABSTRACT

Wheat (Triticum aestivum L.) is one of the leading cereals, which rank first, both in acreage and production among the grain crops of the world. However, its productivity is constrained by a number of problems, among others- there is no use of micronutrients. A pot experiment was therefore conducted at Kulumsa Agricultural Research Center under greenhouse conditions during the 2013/14 growing season, with the objective of investigating the response of bread wheat to micronutrient applications. The trial was laid out in a randomized complete block design. The treatments consisted of sole (Fe, Cu, Zn and Mn along with the recommended NP) and combined applications (Cu+Zn, Cu+Mn, Cu+Fe, Zn+Mn, Zn+Fe, Fe+Mn, Cu+Zn+Mn, Cu+Zn+Fe, Zn+Mn+Fe, Cu+Mn+Fe and Cu+Zn+Mn+Fe with recommended NP) and NP only. Foliar applications were followed at the maximum recommended rates (1.12 kg/ha in 350 litter for Zn, Cu, Mn and F; two times spray during tillering and before flowering). Results revealed that micronutrient application had significant effect (P < 0.05) on plant height, no of seed per spike, thousand kernel weight, grain yield and harvest index. Better values were recorded for all yield and yield components at the combined application of NP+Cu+Zn+Mn+Fe. Grain micronutrient concentrations of Zn, Cu and Mn were high at the combined application of NP+Cu+Zn+Mn+Fecompared to their concentration in the straw. Straw Fe concentrations were higher compared to grain Fe concentrations. It could therefore be concluded that micronutrient application had positive effect on wheat growth and yield under the greenhouse conditions. However, verifying the positive effects of micronutrient in the field condition is suggested to come up with conclusive recommendations.

1. INTRODUCTIONS

Bread wheat (*Triticum aestivum* L.) cultivation reaches far back into history. It belongs to the tribe *Triticeae* which is one of the largest and most important tribes in the grass *Poaceae* family (Dewey, 1984). Bread wheat is the most widely grown type of wheat, occupying about 92% of the world's .Wheat is one of the oldest domesticated grain crops and has been the basic staple food of different civilizations of Europe, West Asia and North Africa. Today wheat is grown almost everywhere with larger land area than any other food grain for humans. Wheat covers an area of about 225.56 million hectares followed by corn 158.19 million hectares and rice (157.83 million hectares). In 2008-09 world production of wheat was 683.13 million metric tons which was second most-produced cereal after maize (784.2 million metric tons) (Anonymous, 2010).

Wheat is most successfully grown between the latitudes of 30° and 60° Nand 27^{0} and 40° S (Nuttonson, 1955). It can be grown beyond these latitudes limits, from within the Arctic Circle to higher elevations near the equator. The optimum temperature for wheat growth is 25° C with minimum and maximum growth temperatures of 3° C to 4° C and 30° C to 32° C, respectively. It is adapted to a wide range of moisture conditions from xerophytic to lithoral. Cultivars of widely differing pedigree are grown under varied conditions of soil and climate and show wide trait variation (Briggle, 1980).

Globally, wheat is the leading source of vegetable protein in human food, having higher protein content than either maize (corn) or rice, the other major cereals .Wheat provides 20% dietary calories of the world. Dough produced from bread wheat flour differs from those made from other cereals in their unique viscoelastic properties (Orth and Shellenberger, 1988). Wheat is the most important source of carbohydrate in majority of countries. Wheat contains minerals, vitamins and fats and with a small amount of animal or legume protein added is highly nutritious. It contains 70% carbohydrates, 22% crude fibers, 12% protein, 12% water2% fat, and 1.8% minerals. A predominately wheat-based diet is higher in fiber than a meat-based diet (Johnson *et al.*, 1978)

Ethiopia is the largest wheat producer in sub-Saharan Africa with about 0.75 million ha of durum and bread wheat. Wheat is one of the major cereal crops in the Ethiopian highlands, which range between 6 and 16°N, 35 and 42°E, and from 1500 to 2800 m. Also at present, wheat is produced solely under rain fed conditions. About 60% of the wheat area is covered by durum and 40% by bread wheat. Of the current total wheat production area, 75.5% is located in Arsi, Bale and Shewa regions. Forty-six percent of the 13 million ha classified as highly suitable for wheat production is located in Arsi and Shewa (Haliu , 2003)

Soil plays a major role in determining the sustainable productivity of an agro-ecosystem. Soil is to be used as medium for plant growth or as a structural material or any other functions (Brady and Weil, 2002). Soil fertility is an important factor, which determines the growth of plant. The growth and development of plants is dependent upon not only an adequate supply of moisture and light, but on a supply of numerous mineral nutrients (Marchers 1995). The terms macronutrient and micronutrient are often used to refer to those elements with essential and specific physiological functions in plant metabolism .Plants require specific amount of certain nutrients in specific format appropriate time, for their growth and development (Sajid *et al*, 2008). The growth and development of plants is dependent upon not only an adequate supply of moisture and light, but on a supply of numerous mineral nutrients (Marchers 1995).

Six micronutrients (Mn, Fe, Cu, Zn, B and Mo) are known to be required for all higher plants (Welch, 1995). Micronutrients play a pivotal role in the yield improvement (Rehm, 2006). Their adequate supply improves nutrients availability and positively affects the cell physiology that is reflected in yield as well (Taiwo *et.al*, 2001). They improves general condition of plants and are known to act as catalysts in promoting organic reactions taking place in plant. Large area of agricultural land has been found to be deficient in one or more micronutrients. Deficiency of essential mineral nutrients especially micronutrients is of general occurrence during the past few decades, due to intensive cropping, with introduction of high yielding varieties, greater use of chemical fertilizers ,loss of micronutrients by leaching, and decreased use of farmyard manure(Bose, 1996).

The deficiency of micronutrients has become major constraint to productivity, stability and sustainability of soils (Bell and Dell, 2008). Low quality seed, salinity, water logging, inadequate use of fertilizers, lack of irrigation water, high input prices, low farmers' education and no use of micronutrients and organic fertilizers are attributed to the low yield and quality of wheat (Khan *et al.*, 1999). The effect of micronutrients Fe, Cu Mn and Zn alone or in combination with each other has not been well studied on bread wheat in Ethiopia. Thus, this study was carried out with the following objective:

□ To evaluate the responses of bread wheat to micronutrient (Zn, Cu, Mn and Fe) and their combinations under greenhouse condition at Kulumsa Agricultural Research Center.

2. LITERATURE REVIEW

2.1 Evolutionary Processes of Bread Wheat

Wheat evolved from wild grasses, probably somewhere in the Near East. A very likely place of origin is the area known in early historical times as the Fertile Crescent - a region with rich soils in the upper reaches of the Tigris-Euphrates drainage basin" (Briggle and Curtis 1987). Naked wheat was cultivated between the late fifth and early fourth millennium B.C in the southern Caucasus in Neolithic settlements. Evidence also shows that naked wheat was found at several sites in the Crimea (1000-900 B.C.) which matches archaeological findings of wheat in Israel from the same period (Korber-Grohne,1988). The process which began some ten thousand years ago involved the following major steps. Wild einkorn *T. urartuc* rossed spontaneously with *Aegilops speltoides* (Goat grass 1) to produce Wild Emmer *T. dicoccoides*; further hybridizations with another *Aegilops (A. taushi)* gave rise to Spelt (*T. spelta)* and early forms of Durum Wheat (cultivated emmer). Bread Wheat finally evolved through years of cultivation in the southern Caspian plains. This evolution was accelerated by an expanding geographical range of cultivation and by human selection, and had produced bread wheat as early as the sixth millennium BC (Feldman, 2001).

2.2 Cultivation History

The development of agriculture and cultivation of wheat occurred approximately 9,000 to 10,000 years ago (Katz, 2003). Wild einkorn, the most common variety of wheat prior to the Neolithic revolution, led to the evolution of wild emmer by crossing with wild goat weed. Emmer is the oldest cultivated variety of wheat, grown as early as 8700 B.C.E. in ancient Turkey and quickly spreading to Mesopotamia, Egypt, Rome and Greece (Kipel and Kriemhild, 2000). During these prehistoric times the cultivation of wheat increased rapidly extending to North Africa and the Indus valley of northern India by 4000 B.C.E. northern China by 3000 B.C.E. and western Europe by 2000 B.C.E. Wheat was introduced the New World by the Spanish and eventually reached America by the early 17th century though English settlers in the North American Colonies (Smith ,2004).

Currently wheat is globally produced and traded amongst all nations. Currently, about 95% of the wheat grown worldwide is hexaploid bread wheat, with most of the remaining 5% being tetraploid durum wheat (Fossati and Ingold, 2001).

2.3 Importance of bread wheat

Bread wheat is the widely grown food crop in the world. World wheat production doubled during the 25 year period to 1984-85. The consumption of wheat in developing countries increased by 73% in the 10 year period 1972-82 (Pomeranz ,1987). The primary use of bread wheat is for bread manufacture. Estimated that national average (per capita per year) bread consumption ranges from about 40 to 300 kg'. Wheat flour is also used to produce biscuits, confectionary products, noodles and vital wheat gluten. Bread wheat is the source of flour for breads, rotis, chapattis, semolina, biscuits and other confectionary products. Wheat grain is also used to manufacture alcoholic beverages. Bran from flour milling is used in livestock feed and the germ is a valuable addition to feed concentrate. Grains are fed to livestock whole or coarsely ground. The wheat plant is also used as a pasture feed before stem elongation and this practice permits plant regeneration and grain harvest. Wheat straw is also used as a source of fibre. The comparatively high protein content of wheat grain makes it a most important source of human nutrition (Briggle and Curtis , 1987).

2.4 Botanical Description

Wheat is a tall, annual plant with a height ranging from two to six feet in early varieties. The plant is made up of leaves surrounding a slender stalk that terminates in spikes, or ears, of grain at the top of wheat (International Starch Institute). Each spike, ear, of grain is made up of spikelet's, which encloses the wheat grain in between the lemma and the pale. The wheat grain is in the shape of an oval and is what gives wheat its nutritional value. The grain may also vary in its length of brush hairs, either long or short. Cultivated wheat is most commonly grown with physical characteristics of fusiform spikes, are awned (bearded) and are easily threshed (McKevith, 2004). Domestic wheat are also bread for strong seed heads which will not shatter during processing (Katz ,2003).

2.5 Growth requirement of wheat

Although the crop is most successful between the latitudes of 30° and 60°N and 27° and40°S (Nuttonson, 1955). Wheat can be grown beyond these limits from within the Arctic Circle to higher elevations near the equator. Development research by the International Maize and Wheat Improvement Center (CIMMYT) during the past two decades (Saunders and Hettel, 1994) has shown that wheat production in much warmer areas is technologically feasible. In altitude, the crop is grown from sea level to more than 3 000 m.a.s.l, and it has been reported at 4570 m.a.s.l. The optimum growing temperature is about 25°C with minimum. Wheat is adapted to abroad range of moisture conditions from xerophytic to littoral. Although about three-fourths of the land area where wheat is grown receives an average of between 375 and 875 mm of annual precipitation. it can be grown in most locations where precipitation ranges from 250 to 1 750 mm (Leonard and Martin, 1963). Optimal production requires an adequate source of moisture Availability during the growing season; however, too much precipitation can lead to yield losses from disease and root problems. Cultivars of widely differing pedigree are grown under varied conditions of soil and climate and show wide trait variations. Although wheat is being harvested somewhere in the world in any given month, harvest in the temperate zones occurs between April and September in the Northern Hemisphere and between October and January in the Southern Hemisphere (Percival, 1927).

2.6 Concept of Soil fertility

Agriculture systems have a crucial role in the provision of food improved livelihoods and income for many being the main occupation of about 80% of poor people in rural areas including women (Pinstrup-Andersen, 2011). Soil one of the most important natural resources the medium in which our food, clothing and shelter are produced. Soil is a layer of organic and mineral substances covering most of the Earth's surfaces. It is created by a slow, and constant physical and chemical breakdown of rock and the action and turnover of living organisms. It takes approximately 500

years to replace 1 inch of soil by these processes, making it critical to keep the soil fertile and productive as well as to prevent its erosion (Poincelot, 2004).

Soil fertility is the status of a soil with respect to its ability to supply elements essential for plant growth without a toxic concentration of any element. Thus soil fertility focuses on an adequate and balanced supply of elements or nutrients to satisfy the needs of plants. (Foth, 1988). According to Martin (1993) the study of soil fertility involves examining the forms in which plant nutrients occur in the soil how these become available to the plant and factors that influence their uptake. This in turn leads to a study of the measures that can be taken to improve soil fertility and crop yields by supplying nutrients to the soil-plant system. This is usually done by adding fertilizers manures and amendments to the soil but sometimes by supplying nutrients directly to the plant parts by means of sprays such productive capacity requires the provision of adequate and balanced amounts of nutrients to ensure proper growth of the plants. Some of these are soil moisture and temperature, aeration, water-holding capacity, a pH that should be near neutral an absence of hardpans that otherwise would inhibit root growth adequate organic matter and other conditions that promote the growth of soil micro-organisms. Other necessary soil factors must be favorable to promote proper nutrient uptake and therefore adequate growth production and yield. Soil fertility is an important factor which determines the growth of plant. Soil fertility is determined by the presence or absence of nutrients i.e. macro and micronutrients. (Wajahat, 2006). Soil fertility decline is considered as an important cause for low productivity of many soil (Sanchez et.al, 2000).

A decline in soil fertility implies a decline in the quality of the soil and soil fertility decline is defined as the decline in chemical soil fertility or a decrease in the levels of soil organic matter, pH, cation exchange capacity (CEC), and plant nutrients (Madison, 1997). One of the major a problem constraining the development of an economically successful agriculture is nutrient deficiency (Fageria and Baligar, 2005). Declining soil fertility has continued to be a major constraint to food production in many parts of the tropical region. The low soil fertility in the tropics has been attributed to the low inherent soil fertility, loss of nutrients through erosion and

crop harvests and little or no addition of external inputs in the form of organic or inorganic fertilizers (Mureithi *et al.*, 2000). Deficiency of essential mineral nutrients especially micronutrients is of general occurrence during the past few decades, due to intensive cropping, with introduction of high yielding varieties, greater use of chemical fertilizers, loss of micronutrients by leaching, and decreased use of farm yard manure. Large area of agricultural land has been found to be deficient in one or other micronutrients. It is realized that productivity of crops is being adversely affected due to deficiencies of micronutrients (Bose and Tripathi, 1996)

2.7 Essential element

The role of macro and micro nutrients is crucial in crop nutrition for achieving higher yields (Raun & Johnson, 1999). Because human nutrition is directly linked to that of plants the production of nutritious foods requires a balanced content of essential macro-, meso- and micronutrients. Macronutrients are required in large amounts and include nitrogen (N), phosphorous (P) and potassium (K). The meso- or secondary nutrients include calcium (Ca), magnesium (Mg) and sulfur (S). Micronutrients or trace elements are required in smaller amounts and include iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), boron (B), chloride (Cl), molybdenum (Mo) and nickel (Ni). Soils are the main source of Micronutrients s for plants, and the soil bioavailability of MNs is influenced by many soil and other environmental factors (Panuccio *et al.*, 2009).

2.8 Micronutrient

Micronutrients are essential for plant growth, but plants require relatively small amounts of them, hence, the term "micro". They include boron (B), chlorine (CI),copper (Cu), iron (Fe), manganese (Mn), Molybdenum (Mo), and zinc (Zn). These elements may be referred to as minor or trace elements, but micronutrients is the preferred term. (Regis Voss, 1998).

2.8.1 Importance of micronutrients in crops

The micronutrient is sometimes referred to as a catalyst. All the micronutrients, Cu, Fe, Mn, Mo, and Zn, except chlorine (CI) are activators of specific enzymes. Micronutrients are generally considered to be enzyme activators in plants.

Copper is involved in the activation of several enzyme systems and apparently cannot be replaced by other metalions. Iron is essential for the synthesis of chlorophyll, the green color of plants which functions in photosynthesis but it is not part of the chlorophyll molecule. Manganese functions as an activator of an enzyme that is involved in the evolution of oxygen in photosynthesis. Zinc is part of auxin, one of the well-known enzymes regulating plant growth. It is necessary for chlorophyll synthesis and carbohydrate formation. (Regis Voss, 1998). Appreciable crop yield increases under local conditions have also been observed in a number of crops at the farmers' fields. Average yield increases with Zn application are 22% in potato and sunflower, 18% in maize, 13% in wheat, 12% in rice and 8% in sugarcane (Anon., 1998). The addition of each of Fe, Zn, Cu and B increased grain yield of various crops from 8% to 30% or from 4% to 11% when the four micronutrients were added together to a soil planted with wheat (Malakouti, 2000). There are numerous examples of the positive effects of Micronutrient addition to crops growing in micronutrient deficient soils. Information on the effects of Micronutrient fertilizers on crop yield and quality as well as their interaction with macronutrients is available (Malakouti, 2007).

Micronutrients play a vital role in gene expression, biosynthesis of protein, nucleic acids, growth substances metabolism of carbohydrates and lipids through their involvement in various plant enzyme systems and other physiologically active molecules (Rangel, 1991). Play key roles in the release of carbon dioxide, and in optimizing the function of vitamin A and the immune system (Marschner, 1995). Micronutrients play a significant role in plant growth and metabolic processes associated with photosynthesis, chlorophyll formation, cell wall development and respiration, water absorption, xylem permeability, resistance to plant diseases, enzyme activities involved in the synthesis of primary and secondary metabolites, and nitrogen fixation and reduction (Adhikary *et. al.*, 2010). Accordingly, Zn, Fe, Mn and Cu are involved in many processes controlling plant growth, and their content in grains and leaves determine the quality of food consumed by humans and animals. Micronutrients Zn deficiency is the most detrimental to crop yield especially in calcareous soils. In general, rice, maize, sorghum bean and fruit trees are among the most sensitive crops to inadequate supply of the four Micronutrients s. Barley, cotton, lettuce, potatoes

and soybean have a medium level of sensitivity to micronutrient deficiency and grasses, asparagus, wheat and oat are among the least sensitive (Martens and Westerman, 1991).

2.8.2 Role of micronutrients on wheat

Extensive research on the effects of micronutrient fertilizers on crop yield and quality has been conducted during the past decade (Malakouti *et al.*, 2005). Micronutrient deficiency has become a major constraint for crop productivity that may either be primary, due to their low total contents or secondary, caused by soil factors that reduce their availability to plants (Sharma and Chaudhary, 2007). Application of Fe, Mn and Zn separately and combined significantly increased number of spikes per plant, number of grain per spike and 1000 grain weight (Masoud *et.al*, 2012). Results of a broad-based study conducted in 815 irrigated wheat growing regions of Iran to evaluate the effect of micronutrients showed an increase of 4 to 11% in wheat grain yield by the addition of each micronutrient (Fe, Zn, Cu, and B) or a combination of Fe + Zn + Cu + B to NPK fertilizer increased grain yield (Malakouti, 2000).

Sarkar *et al.*, (2007) showed that a small amount of nutrients, particularly Zn, Fe and Mn applied by foliar spraying increases significantly the yield of crops. Khan *et al.* (2006) reported that Cu, Fe, Mn and Zn contents of leaf, straw and grain of wheat increased with the application of mineral fertilizers. Ziaeian and Malakouti (2001) reported that total uptake of Zn, Cu, Fe and Mn, in grain and flag leaves was significantly increased. Soylu *et al.* (2005) and Kenbaev and Sade (2002) reported significant increase in number of spikes m^{-2} in wheat with foliar application of different micronutrients individually or in combination. Micronutrients such as Fe, Zn and Mn have a structural role in chlorophyll. These elements can be easily sprayed on leaf, thus leaves chlorophyll concentration increased by micronutrient foliar application, which in turn, lead to an increase in plant height and yield. Also zinc, increased plant height via increasing internodes distances (Kaya and Heggs, 2002). The soil and foliar application of Mn significantly increased the yield (Manal *et al.*, 2010).

2.8.3 Sources of micronutrient

Inorganic micronutrients occur naturally in soil minerals. The parent material from which the soil developed and soil forming processes determine what the micronutrient content of the soil will be. As minerals break down during soil formation, micronutrients are gradually released in a form that is available to plants (Tripathi, 1996). Two sources of readily available micronutrients exist in soil: nutrients that are adsorbed onto soil colloids (very small soil particles) and nutrients that are in the form of salts dissolved in the soil solution (McKenzie, 1992).Organic matter is an important secondary source of some micronutrients. Most micronutrients are held tightly in complex organic compounds and may not be readily available to plants. However, they can be an important source of micronutrients when they are slowly released into a plant available form as organic matter decomposes. The three main classes of commercial fertilizer-Micronutrients are inorganic, synthetic chelates and organic. The inorganic sources include water-soluble sulfate salts of Fe, Zn, Cu and Mn. Other inorganic products comprise of commercial ox sulfates (i.e., mixture of ZnO and ZnSO₄) and oxides of micronutrients that are less readily available to plants relative to sulfate salts. Synthetic chelates of ethylene diamine tetraacetic acid (EDTA) consist of a ring-type structure that binds Fe, Zn, Mn and Cu or other (Rosse, 1992).

2.8.5. Micronutrient deficiencies

Deficiency of essential mineral nutrients especially micronutrients is of general occurrence during the past few decades, due to intensive cropping, with introduction of high yielding varieties, greater use of chemical fertilizers, loss of micronutrients by leaching, and decreased use of farm yard manure. Large area of agricultural land has been found to be deficient in one or other micronutrients. It is realized that productivity of crops is being adversely affected due to deficiencies of micronutrients (Bose and Tripathi 1996). Deficiency of micronutrient in soil and plants is a global nutritional problem and is prevalent in many countries with different magnitude of severity. Induced stress in plants including low crop yield and quality, imperfect plant morphological structure (such as fewer xylem vessels of small size), widespread infestation of various diseases and pests and lower fertilizer use efficiency are some of the adverse effects of micronutrient deficiency(Malakouti, 2008). Their lack greatly influences both the quantity and the quality of plant products (Ahmadikhah *et.al.* 2010)

| Micronutrient | Deficient | Medium | Adequate |
|-----------------------------------|-----------|----------|----------|
| Boron(hot water extractable -ppm) | 0.0-0.4 | 0.5-1.2 | >1.2 |
| Chlorine(water excstactble -ppm | 0.0-0.8a | - | - |
| Copper(DTPA extractable – ppm | 0.0-0.2b | 0.3-1.00 | >1 |
| | 00-0.5c | 0.6-1.0 | >1 |
| | 0.0-2.5d | - | >2.5 |
| Iron(DTPA extractable - pop) | 0.2-2.0 | 2.0-4.5 | >4.5 |
| Manganese(DTPA extractable - ppm) | 0.0-1.0 | - | >1.0 |
| Zinc(DTPA extractable – ppm | 0.0-1.0 | 0.5-1.0 | >1.0 |

Table 1.Range level of micronutrient in the soil

a This level is used by some labs as a critical level for recommending Cl for disease suppression in cereals. b Brown and Dark Brown soil areas, c Black and Grey Wooded soil areas, d Organic soils.

Source. Ross, 1992

2.8.6 Diagnosing symptoms of Micronutrient in plants

As the micronutrient deficient plants may exhibit characteristic symptoms, plant symptoms can be useful indicator of micronutrient deficiencies. Micronutrient usually occurs in older, lower leaves, but may also show symptoms in recently matured tissue. Other factors may cause similar symptoms and it is necessary to do the proper tests to diagnose the problem (www.greenbeam.com). Physical deficiency symptoms for Zn, Fe, Mn and Cu. When the amount of micronutrients removed by crops exceeds the amount supplied by soils and fertilizers, the result is Micronutrient deficiency in food products. Micronutrient deficiency in crops is reflected by different symptoms (Adhikary *et al.*, 2010) expressed in different forms, depending on the specific element (Marschner, 2012)

2.8.6.1 Zinc deficiency

In terms of its potential deficiency, symptoms such as "little leaf" and "rosette" of fruit trees have been identified when zinc levels are low (Salisbury, 1992). These symptoms appear as a reduction in the growth of both young leaves and stem internodes, resulting from zinc's requirement in growth hormones such as indoleacetic acid. In other crops, such as corn or beans, interveinal chlorosis is evident, as is puckering and distortion of the leaf margins. If zinc deficiencies do occur, however, they typically occur under cool and wet conditions encountered in spring (McKenzie 1992).

2.8.6.2 Manganese deficiencies

Manganese deficiency is not common, and is often referred to as "gray speck" in oats (the crop in which deficiencies are most susceptible, McKenzie 1992), "marsh-spot" in peas or "speckled yellows" in sugar beets (Salisbury and Ross 1992). As with most micronutrient deficiencies, it is characterized by interveinal chlorosis of both young and old plant tissues, although this symptom logy is somewhat species-dependant. Following chlorosis, and depending on the severity of the deficiency in soil, necrotic lesions may develop throughout the plant tissue. The manganese content of plants ranges widely from 31 to 100 mg/kg (Knezek and Ellis 1980), with average concentrations of approximately 50 mg/kg (Jones 1991).

2.8.6.3 Iron deficiencies

Symptom logy of iron deficiency in plants includes interveinal chlorosis, beginning first on younger leaves, followed by the eventual chlorosis of the entire leaf (Salisbury and Ross 1992). In severe cases, the entire leaf may become white and develop necrotic lesions. Deficiency of this micronutrient is exacerbated under conditions of high pH and by the presence of bicarbonates in the soil. (McKenzie 1992).

2.8.6.4 Copper deficiencies

When deficient in plants, young leaves often become dark green, misshapen, and develop necrotic spotting (Salisbury and Ross 1992). Wheat, barley and oat crops are considered to be the most

sensitive to deficiencies of copper, and as such, deficiencies have been observed on crops grown in mineral soils in both the Grey-Black and Black soil zones of Alberta (McKenzie 1992). Normal copper concentrations in plant tissues range from 5 to 20 mg/kg, with an average concentration of approximately 6 mg/kg (Jones 1991).

2.8.7 Factor affecting availability of micronutrient

Important factors controlling partitioning of micronutrient between soil and soil solution include pH, inorganic ligands (e.g., HCO3 and Cl), organic ligands (e.g., labile soil organic matter, plant or microbially produced ligands) and competing cations such as Ca+2 and excluded ligands. Other soil factors involve element concentration, organic matter content, redox conditions, soil moisture status, microbial activity, concentrations of other trace elements, the levels of macronutrients and also climate (Guala *et al.*, 2010).Soil pH affects exchangeable Fe, Mn, Cu and Zn similarly (Imtiaz *et al.*, 2010). Several soil properties influence the bioavailability of iron, including high soil pH, high bicarbonate content, plant species and abiotic stresses (Takáč *et al.*, 2009). The availability of Microniutrents to plants is also influenced by both parent material and soil development, but soil properties can operate differently for different trace elements. Organic complexes can be important sources of slow Micronutrients release for plant, uptake especially during soil organic matter decomposition (McKenzie, 2001)

2.8.8 Plant uptake and transport of micronutrients

Most of the Micronutrients are taken up mainly as ion-organic chelates from the soil solution but also as ions. For example, Zn^{+2} , Fe^{+2} , Fe^{+3} , Mn^{+2} and Cu^{+2} are the predominant ionic forms taken up by plants. Micronutrients are acquired from the soil solution by plant roots via diffusion or active transport (Briat *et al.*, 2007). Micronutrients of Fe, Zn, Mn and Cu are transferred from the soil solution through the root or leaf cell membranes into the xylem for transport, utilization, internal recycling and storage in the plant (Marschner, 2012).

| Table. 2 Form of Tr | ansport microi | nutrient in | Plant |
|---------------------|----------------|-------------|-------|
|---------------------|----------------|-------------|-------|

| Element | Ionic form take up by plant |
|------------|-----------------------------|
| Boron | B(OH)3 |
| Chlorine | Cl |
| Copper | Cu ²⁺ |
| Iron | Fe^{2+}, Fe^{3+} |
| Manganese | Mn^{2+} |
| Molybdenum | MoO4 ²⁻ |
| Zinc | $Zn2+, Zn(OH)_2$ |

Source: Regis Voss, (1998)

3. MATERIALS AND METHOD

3.1 Description of the study area

The experiment was conducted in the greenhouse at Kulumsa Agricultural research center (KARC, fig 1). KARC is located 168 km southeast of Addis Ababa at 8°2' N & 39°10'E and at an altitude of 2200 masl. The mean annual rainfall of KARC is 832 mm and maximum and the minimum temperatures are 22° C and 10° C, respectively (www.eiar.g.et.)



Figure.1 Location of study Area

3.2 Experimental treatments

 Table 3 Experimental treatment

| | Treatments combinations |
|-----|--|
| T1 | Recommended NP fertilizer rate |
| T2 | 100% recommended NP + Cu chelate foliar fertilizer 14% Cu |
| T3 | 100% recommended NP + Zn chelate foliar fertilizer 14% Zn |
| T4 | 100% recommended NP + Mn chelate foliar fertilizer 13% Mn |
| T5 | 100% recommended NP + Fe chelate foliar fertilizer 13.2% Fe |
| T6 | 100% recommended NP +Chelate of 14% Cu and 14% Zn |
| T7 | 100% recommended NP + Chelate of 14% Cu and 13% Mn, |
| T8 | 100% recommended NP + Chelate of 14% Cu and 13.2%Fe |
| T9 | 100% recommended NP + Chelate of 14% Zn and 13% Mn, |
| T10 | 100% recommended NP + Chelate of 14% Zn and 13% Fe |
| T11 | 100% recommended NP + Chelate of 14% Fe and 13% Mn, |
| T12 | 100% recommended NP + Chelate of 14% Cu, Zn14% and 13% Mn, |
| T13 | 100% recommended NP + Chelate of 14% Cu, Zn14 % and 13.2%Fe |
| T14 | 100% recommended NP + Chelate of Zn14 %, Mn 13% and 13.2% Fe |
| T15 | 100% recommended NP + Chelate of 14% Cu, Mn 13% and 13.2% Fe |
| T16 | 100% recommended NP + Chelate of $~14\%$ Cu, Zn14 $\%$, 13% Mn and 13.2% Fe |

3.4. Experimental Procedures

3.4.1 Pot preparations

Forty eight pots were prepared. The diameter of each pot was 26.5cm; radius of pot was 13.25cm. The net pot area was 0.055 meter square. The distance between adjacent pots and blocks were 10cm and 15cm apart, respectively.

3.4.1 Field activities

About 11.5 kg of soil was filled in each pot. The soil was collected from a 20cm depth after separating any wanted material. Sowing was done in august 2013/14 at the rate of sixty seeds/pot. Weeding was done manually by hand. Watering was done two times per day, morning and evening.

3.4.2 Fertilizer application

The micronutrient were mixed in water as per the recommendation (Yara, 2005) and Foliar applications were followed at the maximum recommended rates (1.12 kg/ha in 350 litter for Zn, Cu, Mn and F; two times spray during tillering and before flowering). Seeds were broadcasted at the rate of 125 kg/ha. Recommended Nitrogen fertilizer rate was applied to all treatment at the rate of 50kg/ha in the form of Urea, and Phosphorus at 150kg/ha in the form of Di-ammonium Phosphate (DAP). Half dose of Urea and full of DAP were applied at the time of sowing while remaining half UREA was applied at tillering or after 21 days. Weeds were managed by hand. Finally harvesting was done on December, 2014.

3.5. Laboratory analysis

3.5.1 Soil Micronutrient analysis

Soil samples were collected, finely ground and then passed through a 20 mesh sieve to obtain very fine particles. Ten gram of an air-dried, ground and sieved sample was placed in an Erlenmeyer flask and 15 mililiter of the extracting solution (0.05 NHCl + 0.025 N H2SO4) was added to it. Then it was placed in a magnetic stirrer and the mixture was stirred for 20 minutes. The resulting solution was filtered through a Whatman® No42 filter paper into a 50 mL polypropylene vial and diluted to 50 milliliter with the extracting solution. The analytical reagent blanks were also prepared and these contained only the acids. (Perkin Elmererkin, 2009).

3.5.2 Grain and straw analysis

Plant samples were analysed after harvesting. The plants were harvested and separated into grain and straw. Grain and straw samples were separated and prepared for analysis. 0.5gram of grain/straw sample was weighed in 50ml of crucible and Placed into furnace and ashed at a temperature of 550 0 c for 2hrs. After ashing is completed, the sample was dissolved first in 1ml of d.H₂O then in 1ml concentrations of HCl. The procedures outlined in Cottonie *et al* (1982) were followed to determine the micronutrients in the grain and straw samples.

3.6. Data collected

Data on different morphological, yield and yield component characters were recorded as follows:

Plant height: It was measured as the height from the soil surface to the base of the spike of five randomly taken plants from the net pot area at physiological maturity.

Number of grains per spike: Numbers of grains per spike were counted for each pot. Total number of grains in the main spike counted at the time of harvest and recorded.

1000 grain weight (g): It was determined from 1000 randomly selected kernel seeds from each pot and weighed using sensitive balance.

Grain yield (g/ha): Total dry weight of grains harvested from all was taken as grain yield and expressed as grams per pot.

Harvest index (%) It was calculated as the ratio of grain yield to total aboveground biomass

Yield as follows:

HI= <u>GY (kg/ha</u> *100 TBY (Kg/ha)

Where, HI= harvest index GY=Grain yield (at 12.5% moisture base)

TBY=Total biomass yield (Stover +grain yield

3.5. Statistical Data Analysis

Analyses of variances for the data recorded were carried out using the SAS version 9.2 (SAS Institute, 2002). Means of significant treatment effects were separated using the Least Significant Difference (LSD) test.

4. RESULT AND DISCUTIONS

4.2. Yield and Yield component

4.2.1 Plant height

The analysis of variance (ANOVA) showed that the application of each (Zn, Fe, Cu, Mn) and their combinations had a significant effect on plant height (P<0.01). In single applications, the highest plant height was recorded from NP+Zn (54.7cm) and NP+Mn (54.33 cm), followed by Cu (53.667 cm) and Fe (52.66cm) .The lowest plant height (51cm) was obtained from the control plot (Table 4). In a multiple micronutrient application, maximum plant height (61cm) was recorded from NP+Cu+Zn+Mn+Fe, followed by NP+Zn+Mn+Fe, NP+Cu+Mn+Fe, with mean value of 58cm and 57.33cm, respectively.

The results of the experiment indicated that application of both single and multiple micronutrient increases plant height significantly over the control. These results are in agreement with khatoon yousefi (2012), indicated that plant height was significantly affected by foliar application of Fe, Mn and Zn individually and combined. Alloway (2008) showed competitive inhibition between Zn and Fe during unloading in the xylem. Arif *et al.*, (2006) reported significant increase in plant height of wheat crop with foliar application of different micronutrient individually or in combination. Similarly, Fe, Zn and Mn and Cu have a structural role in chlorophyll. These elements can be easily sprayed on leaf; thus, leave chlorophyll concentration increased by micronutrient foliar application, which in turn, lead to an increase in plant height and yield. Also zinc, increased plant height via increasing internodes distances (Kaya and Heggs, 2002). Similarly, Abbas *et al* (2009) reported that soil application and foliar spray of Fe alone or in combination with other micronutrients increase plant height of wheat. Commercial macro and micronutrient formulation "HiGrow" increased plant height and number of branches per plant in chili (Baloch *et al.*, 2008).

4.2.2 Number of Grains per Spike

Result of analysis of variance (ANOVA) (Appendix Table.1) showed that number of seed per spike was significantly affected by micronutrient application. In single application, highest value was recorded on manganese and zinc with mean 27 and 26.7, followed by copper and iron, with mean values of 25 and 24, respectively. In a combine applications the three highest numbers of grains/ spike recored on NP+Cu+Zn+Mn+Fe, NP+Zn+Mn+Fe, and NP+Cu+Mn+Fe with mean values 34.67, 34, 34(Table .4). The lowest mean value was recorded from the control plot. Our results are in line with that of Guenis *et al.* (2003) who also reported that marked increase in number of grains spike⁻¹ of wheat as a result of micronutrient applications. Similar report was also made by Khatoon yousefi (2012), indicating that single or combined application of Fe, Mn and Zn significantly increased number of spikes per plant, number of grain per spike and 1000 grain weight. Yilmaz *et al.* (1997) reported the antagonistic effects between elements of Zn-Cu and Zn-Fe on bread wheat. Similarly , Soleimani (2006) who also reported that foliar application of zinc increased in number of grains spike-1. Zn is known to have an important role either as a metal component of enzymes or as a functional, structural or regulatory cofactor of a large number of enzymes (Grotz and Guerinot, 2006).

4.2.3Thousand Grain Weight

Thousand grain weight (TKW) was significantly affected by micronutrient applications (Table 4). In a single application maximum value was recorded from plots that received recommended NP+Zn and NP+Mn, with mean values of 37.13g and 36.59g. From multiple applications, maximum mean grain weight (42.947g) was recorded from plots that received NP+Cu+Zn+Mn+Fe, followed by NP+Zn+Mn+Fe and NP+Cu+Mn+Fe, with mean TKW of 42.053g and 41.910g, respectively. The lowest value was recorded on the control plot. The result is in agreement with khatoon (2012), who reported that foliar application of single or multiple micronutrients significantly increased TKW. Yassen (2010) reported that the highest increment in grain weight (16%) was obtained when plants sprayed with micronutrients mixtures of Fe + Zn + Mn. Kenbaev and Sade (2002) and Hosseini (2006) reported increase in yield components

from application of zinc. Similarly, Zeidan *et al* (2006) reported that yield components in lentil are enhanced by foliar application of micronutrients.

4.2.4 Harvest index

The analysis of variance results show that application of micronutrient either separately or in mixture significantly affected harvesting index (p<0.01) (appendix Table.1). Mean value of treatment shows, application of micronutrient in single application maximum harvest index recorded on Zn and Mn (mean value 32.67and 32.33) (Table 4). In a multiple combination maximum harvest index recorded on NP+Cu+Zn+Mn+Fe with mean value of 38.33, followed by NP+Zn+Mn+Fe, NP+Cu+Zn+Mn with mean value of 37.33and 37. The increase in the studied characters due to micronutrients may be attributed to its influences in enhancing the photosynthesis process and translocation of photosynthetic products to the seed as a result of increase enzymatic activity and other biological activities. The result accorded with khatoon (2012) reported that foliar application with micronutrient either separately or in mixture significantly increased harvest index. Zayed *et al*, (2011) reported that the three micronutrients Zn+2, Fe+2 and Mn+2 and their combinations significantly affected harvest index.

4.2.5 Grain Yield

The analysis of variance showed that application of micronutrients significantly affected grain yield (p<0.0) (appendix Table.1). The control treatment, which received recommended NP without micronutrients, recorded the lowest yield (9.1 gm/pot). Application of Cu and Mn treatment gave grain yield of 13.06 and 13.50 g/pot, respectively. But application of Fe alone provided similar responses to Cu. As compared to the plots that received a single micronutrient the response of the test crop to Zn application was the highest. In the multiple combinations highest yields in descending order (16.37, 16.07, 15.80, and 14.55g/pot) were obtained from the application of treatments Cu+Zn+Mn+Fe, Zn+Mn+Fe, Cu+Mn+Fe, and Cu+Zn+Mn, respectively. The four lowest yields (13.76, 13.66, 13.57, and 13.38 gm/pot) from multiple micronutrient applications were obtained from treatments of Zn+Fe, Cu+Fe, Zn+Mn and Cu+Zn

respectively. The results of treatments with the highest and lowest yields indicated that both antagonistic and complementary effects from the interaction of the nutrients supplied existed. Antagonistic effects among the four micronutrient interaction is well documented by Samuel L, *et al* (2002), and uptake and translocation of any one micronutrient cations can be affected by the presence or absence of any other micronutrient cation or cations. Iron is critical for chlorophyll formation and photosynthesis and is important in the enzyme systems and respiration of plants, also Manganese is involved in the enzyme systems related to carbohydrate and nitrogen fixation (Babaeian *et al* 2011). Decrease of grain yield in of Zn+Fe, Cu+Fe , Zn+Mn and Cu+Zn can be attributed to antagonistic effect among Fe and Zn, also Mn and Zn. Alloway (2008) showed competitive inhibition between Zn and Fe during loading in the xylem .Similarly, Mn had antagonistic effect with Zn, contrasted to the complementary effect with Fe, indicating that Zn absorption from the soil or its translocation within the plant system is hindered by the concentration of Mn. supply from the soil and treatments.

These result agreed with Sarkar *et al.*, (2007) showed that a small amount of nutrients, particularly Zn, Fe and Mn applied by foliar spraying increases significantly the yield of crops. Similarly with Zayed (2011) reported the triple combination of Zn+2 +Fe+2 + Mn+2 as soil application gave the highest values of grain yield and Concurrence with Radulovic (1996) who applied N, P, K, Ca, Mg and Fe, B, Zn, Mn and Cu as foliar spray and observed increase in growth and yield contributing parameters in chili. The addition of each micronutrient (Fe, Zn, Cu, and B) or a combination of Fe + Zn + Cu + B to NPK fertilizer increased grain yield. The highest yield was obtained by adding all the micronutrients to NPK fertilizer (Malakouti, 2000). Similarly foliar application of micronutrient Fe and Mn individually and combine significantly increase proteins content , Fe, Zn, and Zn content in grain of wheat (Zeidan,2010)

| Treatments | PH | NSPS | TKW | HI | GY |
|----------------|-----------------------|-----------------------|------------------------|-----------------------|----------------------|
| | (cm) | | (g/pot) | (%) | (g) |
| | | | | | |
| RR NP | 51^{i} | 23.6 ^e | 34.79 ^f | $27.0^{\rm f}$ | 9.10 ^g |
| NP+ Cu | 53.66 ^{gh} | 25 ^e | 36.18 ^{fe} | 31.67 ^e | 13.06 ^f |
| NP+Zn | 54.7 ^{dce} | 26.67 ^{ed} | 37.13 ^{de} | 32.67d ^e | 13.54 ^{fe} |
| NP+Mn | 54.33 ^{dceb} | 27 ^{ced} | 36.26 ^{fe} | 32.33 ^{de} | 13.50 ^f |
| NP+Fe | 52.66 ^h | 24.33 ^e | 36.26 ^{cd} | 31.67 ^e | 13.18 ^f |
| NP+Cu+Zn | 56.3 ^{dec} | 31.67 ^{cabd} | 40.67^{cab} | 35.0 ^{cadbe} | 14.38 ^{ed} |
| NP+Cu+Mn | 56.66 ^{dbc} | 32.33 ^{cab} | 41.27 ^a | 35.0 ^{cadbe} | 14.51 ^{cd} |
| NP+Cu+Fe | 55.3 ^{def} | 27.33 ^{ced} | 39.13 ^{cd} | 33.33 ^{cde} | 13.66 ^{fe} |
| NP+Zn+Mn | 55 ^{gef} | 27 ^{ced} | 39.24 ^{cd} | 33.00 ^{cde} | 13.57 ^{fe} |
| NP+Zn+Fe | 56dec | 29 ^{cedb} | 40.07^{cb} | 33.67 ^{cde} | 13.76 ^{fed} |
| NP+Fe+Mn | 56.66^{dbc} | 33 ^{ab} | 41.21 ^{cab} | 35.33 ^{cadb} | 14.54 ^{cd} |
| NP+Cu+Zn+Mn | 56.66 ^{dbc} | 33.33 ^{ab} | 41.82^{ab} | 35.67 ^{cadb} | 14.55 ^{cd} |
| NP+Cu+Zn+Fe | 56.66 ^{dbc} | 33.67 ^{ab} | 41.84^{ab} | 36.33 ^{cab} | 15.33 ^{cb} |
| NP+Zn+Mn+Fe | 58b | 34.33 ^{ab} | 42.05^{ab} | 37.33 ^a | 16.07^{ab} |
| NP+Cu+Mn+Fe | 57.33 ^{bc} | 34 ^{ab} | 41.91 ^{ab} | 37.00^{ab} | 15.80^{ab} |
| NP+Cu+Zn+Mn+Fe | 61 ^a | 34.67 ^a | 42.95 ^a | 38.33 ^a | 16.37 ^a |
| CV (%) | 1.74 | 10.9 | 3.47 | 5.906 | 3.58 |
| Lsd (0.01) | 1.63 | 5.34 | 2.29 | 3.356 | 0.84 |

Table.4 Mean values of Plant heights, No. seed per spike, thousand kernel weight, Grain yield and Harvest index as influenced by application micronutrients, Kulumsa 2014

Whereas RRNP= Recommended Nitrogen and Phosphorus Cu=Copper, Fe= Iron, Mn= Manganese, Zn= Zinc, PH=Plant height, NSPS=No seed per spike, TKW=Thousand kernel weight, HI= Harvest index, GY= Grain yield, NS = non-significant, Variable means followed by the same letters are not significantly different ($P \le 0.01$) according to LSD Tests, CV=coefficient of variations .LSD=List significant difference

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4.3 Micronutrient Concentration of Grains

4.3.1 Copper

Concentration of copper significantly affected grain yield (p<0.01) (appendix Table.2). The mean value of copper content varies from 0.56 to 0.8 mg kg-1. Comparing single micronutrient applied treatments the minimum copper concentration mean value 0.59 mgkg-1, was recorded from the treatment consisting of recommended NP+Fe. Among plot the lowest copper grain concentration recorded on control plots. Comparing combine applications, the maximum concentration (0.8 mg kg-1) was recorded from treatments consisting of NP+Cu+Zn+Mn+Fe. copper grain Concentrations increasing orders of 0.76, 0.76, 0.77, 0.77mgkg-1 were obtained from treatments of NP+Cu+Zn+Mn, NP+Cu+Zn+Fe, NP+Zn+Mn+Fe, NP+Cu+Zn, and NP+Cu+Mn+Fe. The results are in agreement with Kochian (1991) and Kumar et al. (2009) investigated that Cu concentrations in leaves, grain and straw increased significantly with an increase in the level of applied Cu and the applied Cu had significantly reduced the Fe content in wheat leaves. Brar and Sekhon (1978) observed that excess Cu antagonistically affect the translocation of Fe from stem to the leaves. However, no such effect was observed in Zn concentrations of grain and straw of wheat plants. In Fe deficient leaves, Cu concentrations generally increased as Zn supply increased. Yilmaz et al(1997) reported antagonistic effects between elements of Zn-Cu and Zn-Fe in the above mentioned treatments.

4.3.2 Iron

The analysis of variance shows the treatments receiving with recommended dose of NP was significant at the probability level of 1% (p<0.01) to each other with regard to Fe content of wheat grain (Appendix Table.2) Iron content of wheat grains varied from 0.1066 to 0.7733 mg kg-1. In a single application maximum concentration Recorded (mean value 0.333mg kg-1) was observed in the pot receiving Fe + Recommended dose of NP, whereas, followed by concentration recorded (0.3033 0.3066, 0.31 mg kg-1) was recorded in NP+Cu , NP+Mn, NP+Zn. The lowest value recorded on control treatments.

In combined applications the highest iron grain concentration were obtained from NP+Cu+Zn+Mn, NP+Cu+Zn+Fe, NP+Zn+Mn+Fe, NP+Cu+Zn, and NP+Cu+Mn+Fe with mean value of 0.56,0.573,0.583,0.576 mg kg-1, However, the data revealed that Cu+Zn+Mn+Fe applied with NP fertilizer, increased the Fe content of wheat grains. The grain is food of human being and sufficient quantity of iron content in grain surely improves the nutritional values of wheat flour.

4.3.3 Manganese

The statistical analysis showed that grain manganese concentration significant to each other (p<0.01) (Appendix Table 2).The Manganese contents of wheat grain ranged from 0.046 to 0.170mg kg-. In a single application maximum manganese grain concentration obtained from NP+Mn with mean value 0.076 mg kg-1. Maximum Mn content (0.170mg kg-) was recorded in treatment where Cu+Zn+Mn+Fe with recommended dose of NP, whereas, the minimum concentration (0.046 mg kg-1) was found in control plot. The result agreement with those of Mastoi (1998), who reported that Mn content in grain increased with the application of inorganic fertilizer. Cu, Fe, Mn and Zn contents of leaf, straw and grain of wheat increased with the application of various levels of Hal-Tonic and mineral fertilizers (khan *et al*, 2006)

4.6.4 Zinc

The analysis showed that all the treatments are statistically significant (p<0.01) (Appendix table 2). The Zn content varied from 0.383 to 1.087 mgkg-1. The data further showed that all the treatments improved Zn content of wheat grain over control. In combine applications maximum zinc concentration was recorded 1.087 mg kg-1 Cu+Zn+Mn+Fe where was applied with recommended dose of NP. This indicated that Cu+Zn+Mn+Fe, due to its micronutrient contents, improved the Zn nutrition of wheat. This result line with Increase in Zn content in grain and straw due to zinc fertilization was reported earlier (Sakal *et al.*, 1987). Zeidan (2007) indicated that Zn applications significantly increased grain protein and enhanced grain Zn concentration, while simultaneously reduced grain P concentration.

Shaheen *et al* (2007) find out that the number of tillers per hill, grain and straw yield of wheat, Zn concentrations and Zn uptake both in grain and straw and Zn concentrations of pre-sowing and post-harvest soils were significantly increased with the application of Zn. Sharma and Bapat (2000) reported that the content of zinc in various plant parts increased with increased levels of zinc in soil and the content of Cu and Mn was not influenced with applied Zn.

| Treatements | | | | |
|----------------|-----------------------|---------------------|-----------------------|-----------------------|
| RR NP | 0.56 ^g | 0.226 ^f | 0.140 ^j | 0.383 ^h |
| NP+ Cu | 0.63 ^{gedf} | 0.286 ^{ef} | 0.153 ^{hij} | 0.600 ^g |
| NP+Zn | 0.61gef | 0.306 ^{ed} | $0.160^{ m ghij}$ | 0.663 ^{efg} |
| NP+Mn | 0.60^{gef} | 0.293 ^{ef} | $0.170^{ m ghi}$ | 0.630 ^{fg} |
| NP+Fe | 0.59g | 0.306 ^{ed} | 0.150^{ij} | 0.627^{fg} |
| NP+Cu+Zn | 0.72^{cadb} | 0.396 ^c | 0.183 ^{gdef} | 0.733 ^{ecd} |
| NP+Cu+Mn | 0.74 cab | 0.423 ^c | 0.183 ^{gdef} | 0.753 ^{cd} |
| NP+Cu+Fe | 0.66^{cedf} | 0.313 ^{ed} | 0.173 ^{ghif} | 0.717 ^{ecd} |
| NP+Zn+Mn | 0.643^{gedf} | 0.310 ^{ed} | 0.170^{ghi} | 0.697 ^{efd} |
| NP+Zn+Fe | 0.69^{cedb} | 0.3766 ^c | 0.180^{ghef} | 0.720^{ecd} |
| NP+Fe+Mn | 0.75^{cab} | 0.496 ^b | 0.200 ^{cdef} | 0.757 ^{cd} |
| NP+Cu+Zn+Mn | 0.76^{ab} | 0.563 ^a | 0.206^{cdeb} | 0.790 ^c |
| NP+Cu+Zn+Fe | 0.76^{ab} | 0.573 ^a | 0.210 ^{cdb} | 0.790 ^c |
| NP+Zn+Mn+Fe | 0.77^{ab} | 0.583 ^a | 0.233 ^{ab} | 0.993 ^b |
| NP+Cu+Mn+Fe | 0.77^{ab} | 0.576^{a} | 0.220^{cab} | 0.980^{b} |
| NP+Cu+Zn+Mn+Fe | 0.8 ^a | 0.630 ^a | 0.243 ^a | 1.087^{a} |
| Cv (%) | 7.89 | 10.39 | 9.419 | 6.508 |
| Lsd 0.01 | 0.091 | 0.072 | 9.029 | 0.081 |

Table. 5. Mean values of micronutrient concentrations (ppm) in grain

Whereas, RRNP= Recommended Nitrogen and Phosphorus Cu=Copper, Fe= Iron, Mn= Manganese, Zn= Zinc, NS = non-significant, Variable means followed by the same letters are not significantly different (\mathbb{R} 0.01) according to LSD Tests , CV=coefficient of variations .LSD=List significant difference.

4.4 Micronutrients Content of straw

4.7.1 Copper

The analysis of variance (appendix Table 3) showed that copper content of wheat straw significant at probability level of 1% (p<0.01). Mean value (Table.6) shows in a sole application Minimum copper content (0.037) was recorded in control plots, while the maximum copper (0.057) was observed where Cu was applied with recommended dose of NP fertilizer and followed by zinc and manganese (mean value 0.050 and 0.050). Micronutrient increased copper content of wheat straw over control plots. In a combine application maximum copper recorded on NP+Cu+Zn+Mn+Fe (mean value 0.106) and followed by NP+Zn+Mn+Fe, NP+Cu+Mn+Fe (0.096 and 0.083). The effect was more pronounced when Cu+Zn+Mn+Fe was applied with NP fertilizer. The result agreement with Kumar *et al.* (2009) investigated that the Cu concentrations in leaves, grain and straw increased significantly with an increase in the level of applied Cu and the applied Cu had significantly reduced the Fe content in wheat leaves.

4.7.2 Iron

The analysis of variance (appendix table 3) show that iron content of wheat straw significant at the probability level of 1% (p< 0.01). The mean value (Table.6) ranged from (0.11to 0.773). In a single application maximum value recorded on recommended dose of NP with Fe (mean value 0.306). Maximum Iron content of straw was maximum in treatment containing Cu+Zn+Mn+Fe Recommended dose of NP (0.773), whereas minimum copper concentration (0.11) was recorded in control. Statistically all the treatments significant to each other (appendix table 3). The result is agreement with approximately 80% of the total iron found in plants can be found in the plant chloroplast (Mengel and Kirkby 1987), which may explain the higher concentration of this element found in the straw material. Iron can be stored in the stroma of plastids, but can also be found in the xylem and phloem (Marschner, 1995).

4.7.3. Manganese

The analysis of variance (ANOVA) depicted that manganese content of wheat straw significant probability level 1 %(p<0.01). Mean value show (Table. 6) that manganese content of wheat straw ranged from 0.046 to 0.170. The maximum Mn content (0.170) was recorded in combination NP+Cu+Zn+Mn+Fe while, minimum (50.046) was recorded in control. The result agreement with (Kochian1991) Manganese is considered to be an intermediately mobile micronutrient, which due to its phloem mobility, may account for its presence within the grain component.

4.7.4 Zinc

The analysis of variance (appendix Table 3) showed that zinc content of wheat straw ranged from 0.251 to 0.860. The maximum Zinc content (0.860) was found in the treatment received Cu+Zn+Mn+Fe and recommended dose of NP, while minimum value (0.251)(Table.6).The zinc content was higher in grain than in straw. The result line with similar result was reported by Naik and Das (2007). Similarly, Dvorak *et al.* (2003) in studying a wheat crop found that zinc was transported primarily to and accumulated in, the grain produced by the plant, whereas straw concentrations were always lower. In contrast, Mengel and Kirkby (1987) describe zinc as having low mobility within plants and have found that it accumulated in root tissues.

| — | | | | |
|---------------------|-----------------------|---------------------|-----------------------|------------------------|
| Treatments | Cu | Fe | Mn | Zn |
| RR NP | 0.037 ^h | 0.11 ^h | 0.046 ^j | 0.256 ^g |
| NP+ Cu | 0.057^{fge} | 0.30 ^g | 0.073^{hi} | 0.466^{f} |
| NP+Zn | 0.050 ^{fg} | 0.31 ^g | 0.073 ^{hi} | 0.553 ^{edf} |
| NP+Mn | 0.050^{fg} | 0.303 ^g | 0.076^{hgi} | 0.540^{edf} |
| NP+Fe | 0.046 ^{gh} | 0.33 ^g | 0.070^{i} | 0.510 ^{ef} |
| NP+Cu+Zn | 0.066^{de} | 0.49 ^{fe} | 0.120 ^{de} | 0.613 ^{dc} |
| NP+Cu+Mn | 0.070 | 0.513 ^{de} | 0.123 ^{dc} | 0.623 ^{dc} |
| NP+Cu+Fe | 0.060 | 0.436 ^f | 0.100 ^{dc} | 0.590 ^{edc} |
| NP+Zn+Mn | 0.056 ^{fge} | 0.363 ^g | 0.093^{hgf} | 0.563 ^{edf} |
| NP+Zn+Fe | 0.063 ^{de} | 0.460^{fe} | 0.096^{gf} | 0.610 ^{edc} |
| NP+Fe+Mn | 0.070^{cd} | 0.526 ^{de} | 0.140^{dcb} | 0.630 ^{dc} |
| NP+Cu+Zn+Mn | 0.080 ^{cb} | 0.573 ^{dc} | 0.123 ^{dc} | 0.673 ^{cb} |
| NP+Cu+Zn+Fe | 0.080 ^{cb} | 0.603 ^{bc} | 0.143 ^{cb} | 0.673 ^{cb} |
| NP+Zn+Mn+Fe | 0.096 ^a | 0.657^{b} | 0.150 ^b | 0.843 ^a |
| NP+Cu+Mn+Fe | 0.083 ^b | 0.606^{bc} | 0.146^{ab} | 0.836 ^b |
| NP+Cu+Zn+Mn+Fe | 0.106 ^a | 0.773 ^a | 0.170^{a} | 0.860^{a} |
| Cv(%) | 10.613 | 9.355 | 11.82 | 10.012 |
| Lsd _{0.01} | 0.012 | 0.078 | 0.022 | 0.102 |

Table.6 Mean values of micronutrient on straw concentrations

Whereas, RRNP= Recommended Nitrogen and Phosphorus Cu=Copper, Fe= Iron, Mn= Manganese, Zn= Zinc, NS = non-significant, Variable means followed by the same letters are not significantly different (\ltimes 0.01) according to LSD Tests , CV=coefficient of variations .LSD=List significant difference.

5. SUMMURY AND CONCLUSION

Bread Wheat (*Triticum aestivum* L.) cultivation reaches far back into history. Today wheat is grown almost everywhere with larger land area than any other food grain for humans. Soil fertility is an important factor, which determines the growth of plant. Plants require specific amount of certain nutrients in specific format appropriate time, for their growth and development. Six micronutrients that is, Mn, Fe, Cu, Zn, B and Mo are known to be required for all higher plants. Micronutrients play a pivotal role in the yield improvement. Their adequate supply improves nutrients availability and positively affects the cell physiology that is reflected in yield as well

This study was therefore, conducted in the year 2013/14 under green house Conditions at Kulumsa Agricultural Center (KARC), in Oromiya region the in village of Kulumsa, northeast of Asela, in Arsi Zone with objectives of evaluate the response of bread wheat to micronutrient applications under green house conditions. The treatments consisted laid out as a randomized complete block design (RCBD) treatment combinations and replicated three times. The treatments consisted sixteen sole (Fe, Cu, Zn and Mn with recommended NP) conjoined application(Cu+Zn, Cu+Mn, Cu+Fe, Zn+Mn, Zn+Fe, Fe+Mn, Cu+Zn+Mn, Cu+Zn+Fe, Zn+Mn+Fe, Cu+Mn+Fe and Cu+Zn+Mn+Fe with recommended NP) and NP only.

Results revealed micronutrient application showed significant effect on plant height, number of seed per spike, thousand kernel weights, harvest index and grain yield. Higher value of all parameter was obtained in response recommended NP with Cu+Zn+Mn+Fe. Combine application of Cu+Zn+Mn+Fe produce higher plant height (59.6cm),number of seed per spike(34.66),thousand kernel weight (42.6g) , harvest index (38.33) and grain yield (16.03g) to all yield and yield component. Also encouraging result recorded on recommended NP with Zn+Mn+Fe. Treatments NP without micronutrients application showed the least performance in terms of growth parameters, number seed per spike, thousand grain weight, grain yield, harvest index. In grain micronutrient Zn, Cu and Fe was high grain concentration recorded on NP+Cu+Zn+Mn+Fe than strow concentration, where as manganese straw

concentration was higher than grain concentrations. In both grain and strow micronutrient concentration maximum value recorded on NP+Cu+Zn+Mn+Fe. It could be concluded that micronutrient application had positive effect on wheat growth and yield. Thus application of micronutrients is suggested to be applied for better crop nutrition and increased crop growth, which will ensure higher yields. However, further studies are warranted at field condition to provide conclusive recommendation.

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7. APPENDIX

Appendix Table 1. A analysis of variance showing mean squares for thousand kernel weight biological yield, and grain yield and harvest index of maize supplied with different N rate and time of applications

| Source of variance | | Mean square | | | | | |
|----------------------|---------------|----------------------------|-------------------------|-----------------------------|----------------------------|-------------------------|--|
| | Df | РН | NSPS | TKW | HI | GY | |
| REP TRET ERROR | 2 15 30 | 2.083 15.7208** 0.95 | 1.75 46.44** 1.84 | 1.620058 20.55** 1.88 | 0.896 23.356** 4.051 | 0.17 8.44** 0.253 | |
| CV | - | 1.75 | 4.55 | 3.471 | 5.906 | 3.58 | |

Where,; df = degrees of freedom; PH=plant height ;NSPS=No. Seed per spike ;GY= Grain Yield; TKW=Thousand Kernel Weight;, and Harvest index, *, ** and *** = non-significant, significantly different at 5%, 1%,

| Sourc | Source of Variance | | | Mean square | | |
|-------|--------------------|----------|----------|--------------|--------------|--|
| | Df | Cu | Zn | Mn | Fe | |
| REP | 2 | 0.0014 | 0.00317 | 0.00022708 | 0.00525208 | |
| TRET | 15 | 0.0181** | 0.0855** | 0.00275208** | 0.05357097** | |
| ERROR | 30 | 0.00299 | 0.0024 | 0.00030708 | 0.00187431 | |
| CV | - | 7.896 | 6.508 | 9.42 | 10.39558 | |

Appendix Table 2. Analysis of variance showing mean squares for grain concentration of micronutrient

Where; Df = degrees of freedom; Cu=Copper; Zn=Zinc; Mn=Manganese;Fe=iron; *, ** and *** = non-significant, significantly different at 5%, 1%,

| Source of variance | ce Mean square | | | | |
|--------------------|----------------|--------------|--------------|-----------|-----------|
| | Df | Cu | Zn | Mn | Fe |
| REP | 2 | 0.0001187 | 0.01308958 | 0.000133 | 0.00126 |
| TRET | 15 | 0.00131542** | 0.06147333** | 0.00379** | 0.08446** |
| ERROR | 30 | 0.00005208 | 0.00372292 | 0.000167 | 0.00185 |
| CV | - | 10.59358 | 10.01626 | 11.82 | 9.3549 |

Appendix Table .3 Analysis of variance showing mean squares for grain concentration of micronutrient

Where, Df = degrees of freedom; Cu=copper, Zn=zinc, Mn=Manganese, Fe=iron; *, ** and *** = non-significant, significantly different at 5%, 1%.7