# EFFICACY OF MICRONUTRIENT SEED PRIMING ON MAIZE (*Zea mays*) GROWTH AND YIELD IN MICRONUTRIENT DEFICIENT SOILS IN LIMPOPO PROVINCE

**by**

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## DEDICATION

I dedicate my mini-dissertation to my grandfather Mr M.G. Shokane, my parents Mr L.P. and Mrs M.P. Rapetsoa, my brothers Mr M.B. and Mr M.K. Rapetsoa and my younger sister Ms S.T. Rapetsoa.

## DECLARATION

I, **Mokgatla Collen Rapetsoa**, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Agriculture (Soil Science) has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution, while related materials contained herein had been duly acknowledged.

Mr M.C. RAPETSOA

Date: 28-02-2019

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## ABSTRACT

One of the major constraints to crop productivity in South Africa is crop nutrient deficiency especially micronutrients. Laboratory, glasshouse and field studies were carried out during the 2016/2017 growing season to assess the effects of micronutrient (Zn, B and Mo) seed priming on maize growth and yield in micronutrient deficient soils of the Limpopo province. The laboratory experiment was carried out in a completely randomized design (CRD) laid out in a 3 x 5 x 3 factorial treatment structure with three replications. The assessments of the micronutrients were made at 0%, 0.01%, 0.05%, 0.1%, and 0.5% concentrations. The seeds were primed in the solutions for 24 h, 12 h and 8 h. The glasshouse experiment was carried out in a completely randomized design (CRD) laid out in a 3 x 4 x 2 factorial treatment structure with three replications. In the field, a single factor experiment in a randomised complete block design (RCBD) with three replications and a control was used, with micronutrient concentrations as levels. The laboratory study investigated the effect of seed priming with Zn, Mo and B on germination (germination percentage (GP), germination rate (GR), the coefficient velocity of germination (CVG), days to germination (DG) and mean germination time (MGT)). The interaction between seed priming duration and concentration levels of seeds primed with B had significant effect ($P < 0.05$) on germination. The interaction between seed priming duration and concentration levels of seeds primed with Zn had significant effect ($P < 0.05$) on germination. The interaction between seed priming duration and Mo concentration levels on CVG, MGT and DE had no significant different ($P > 0.05$). Meanwhile, the effect on GP and GR had significant ($P < 0.05$) effects. Seed priming with the micronutrients and water resulted in improved GP, MGT and CVG for seeds primed with 0.01, 0.05 and 0.1% for 24 h in laboratory conditions. The glasshouse study was established to investigate the effect micronutrient seed priming on seedling establishment and growth. The effects of the interaction between Mo concentration levels and duration, Zn concentration levels and duration, B concentration levels and duration and control had no significant ($P > 0.05$) effect on days to emergence (DE), seedling wet weight (WW), dry weight (DW), chlorophyll, stem diameter, plant height and final root length (RL). Furthermore, seeds primed at lower concentration levels (0.05, 0.01 and 0.1%) with longer duration priming period (24 hours) for Mo, Zn and

B recorded higher results on the seedling emergence and establishment parameters. The field investigation showed that priming with micronutrients solutions had no significant difference on the final values of dry biomass, prolificacy, harvest index and grain yield. Seeds primed with 0.05, 0.01 and 0.1 % concentration levels with longer duration priming period (24 hours) showed improvement in germination and seedling establishment. The grain yield and grain nutrient content was not improved, meanwhile emergence was improved. This confirms that not only micronutrients limit yields maximization and crop nutrients content retention solely, rather that the complexity of the agricultural crop production environment should be well understood by all farmers to archive their goals.

Keywords: **Boron, Maize, Micronutrients, Molybdenum, Nutrient seed priming, Zinc.**

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# CHAPTER 1: INTRODUCTION

## 1.1. Background

A major constraint to soil and crop productivity in South Africa is soil micronutrient deficiency. This is particularly true in the smallholder maize farms (Mandiringana *et al*., 2005). Hence, there is poor crop establishment and low yield, which poses a threat to food security. Barnard and du Preez (2004) reported that micronutrients are deficient in most South African soils. Most of the deficiencies occur due to ill agricultural practices that degrade the nutrient status while some soils are naturally occurring deficient as a result of soil type in the area. Guan *et al*. (2009) and Matsushima and Sakagami, (2013) indicated that micronutrients are affected by soil conditions such as pH, leaching, salinity, organic matter, clay content, drainage and nutrient interactions. Most compound fertilizers have micronutrients such as Zn added to them, which can be a solution. However, Ali *et al*. (2008) reported that high costs have led to limited access to compound fertilizers by smallholder farmers. Nonetheless, micronutrient deficiency problems in agricultural fields could be corrected by low-cost technologies such as nutrient seed priming (NSP).

Nutrient seed priming is a physiological method used to improve seed performance and provide faster germination and good crop establishment (Yohannes and Abraha, 2013). It is an easy, low cost and low-risk method used to supplement micronutrients and is hence a promising method that can provide an adequate amount of micronutrients and significantly improve maize growth. The importance of micronutrient is highlighted in a study by El-Fouly *et al*. (2012). In the study, the results indicated that the NPK dose based on soil testing plus spraying of micronutrients, improved all growth parameters, ear characteristics and resulted in improving nutrient concentrations in maize leaves and also enhanced nutrients uptake which induced significant increase in grain yield as compared to other treatments". Although in South Africa there is limited information about nutrient seed priming influence on crop yield and quality. A positive influence on growth and germination on cotton and maize was reported in Zimbabwe (Murungu *et al*., 2005). The success of NSP can be attained through the development of optimum

concentration and appropriate priming procedure. Overall, the development of NSP could reduce the cost of nutrients and improve grain nutrient composition and yields.

## 1.2. Problem statement

Low soil fertility status is a major constraint to smallholder farming in South Africa (Barnard and du Preez, 2004). Mavengahama *et al*. (2014) indicated that micronutrients, especially zinc (Zn), molybdenum (Mo) and boron (B) are deficient in South African soils. Micronutrient deficiency problems of the soil, in turn, result in plant nutrient deficiency, consequently resulting in poor maize growth, yield and low nutrient compositions in grains (Ayeni *et al*., 2012). Poor maize growth and low grain nutrient status are exacerbated by farmer‟s lack of appreciation of soil micronutrient deficiency and their corrections (Ayeni *et al*., 2012). Poor agricultural practices in the small-scale farming sector are a huge contributing factor on degradation of the nutrient status of the soil. Practises such as fallowing are not conducted due the financial pressure experienced by most small-scale farmers as most depend on farming as source of income or food. It is therefore imperative that South African smallholder farmers correct the deficiency problems on their farmlands by adopting cheaper and effective technologies to supply micronutrients.

## 1.3. Rationale

There is a need to come up with effective and user-friendly agronomic interventions such as nutrient seed priming (NSP) to improve smallholder farmer productivity. Before farmers can adopt and use NSP technology, researchers need to assess the efficacy of the technology in improving maize growth, yield and grain micronutrient compositions on deficient soils of South Africa. An estimated 8,0 million tons of maize grain are produced annually in South Africa on commercial farmers and more than 12 000 small farms. Over 3.1 million ha of land is used for production of maize with aim of consumptions by producers (small scale farmers) and market purposes (sold commodities) (Du Plessis, 2003). Furthermore the report indicated that maize is highly consumed in rural areas as staple food and most smallholder farmers rely on maize for household income, hence the adoption of the crop as a test crop. Despite significant indications of the effectiveness of NSP in increasing maize yield

in some Asian countries and Zimbabwe (Harris, 2006), there is a lack of information on the technology in South Africa. Little effort has been made to refine this technology to suit marginal soils in many smallholder farms in South Africa. It is therefore vital to conduct studies on the performance of NSP on South African micronutrient deficient soils to improve the availability of the data on the technology in the country.

## 1.4. Purpose of the study

### 1.4.1. Aim

The aim of this study was to assess the effects of micronutrient seed priming on maize growth and yield in micronutrients deficient soils under smallholder resource-poor farmers‟ conditions in Limpopo province.

### 1.4.2. Objectives

The objectives of this study were to:

i. Determine the optimum micronutrient (Zn, Mo and B) concentrations required to improve maize germination and seedling vigour.

ii. Evaluate the effect of micronutrient seed priming (Zn, Mo and B) on maize establishment, grain yield and grain nutrient composition on deficient soils.

## 1.5. Hypotheses

i. Appropriate micronutrient (Zn, Mo and B) concentration levels can result in improved germination rates and seedling vigour.

ii. Nutrient seed priming using Zn, Mo and B can improve establishment, grain yield and nutritional quality of maize

# CHAPTER 2: LITERATURE REVIEW

## 2.1. Introduction

Maize (*Zea mays L*.) is an annual grass from the family *Gramineae* (*Poaceae*) (Verheyen, 2008). Maize is widely planted in subtropical and temperate agro-climatic regions throughout the world (du Plessis, 2003; Aref, 2011). Today maize serves as food for humans, feed for animals and industries derive products such as oil, beverages and flour (Tabrizi *et al*., 2011a; Zhang *et al*., 2013). Du Plessis (2003) indicated that maize is highly consumed in rural areas as staple food with most smallholder farmers relying on maize for household income. Furthermore, Garnett (2010) showed that in rural areas maize forage is used as a livestock feed. Therefore, it is important for maize crop to improve micro and macronutrient content in the grain and forage for human consumption and livestock feed, respectively. Also, the need for the adequate amount of micro and macronutrient content in maize grains is encouraged by a report from NAMC and DAFF (2015), which indicates that maize is highly consumed in developing countries. Failure to satisfy the nutrient content in crops can result in low nutrients status of the grains and leaves. Whereas Johnson *et al*. (2005) further showed that failure to boost the nutrients contained in the food substances can lead to micro and macronutrient malnutrition problems to human beings. For maize to effectively use the macronutrients, micronutrient such as B, Mo and Zn are required in small quantities (Havlin *et al*., 2014; FSSA, 2007). Micronutrients such as B, Zn, and Mo, which are responsible for enzymes that help the plants to best utilize macronutrients (NPK) should be balanced in the soil or provide adequate quantities.

## 2.2. Importance and demand for maize

Maize is ranked the third most important cereal crop after wheat and rice amongst all cereal grain crops in the world (Verheye, 2008). Concerns were raised that as global population increases, the demand for maize will also increase (Sihlobo and Kapuya 2015; NAMC and DAFF, 2015). Locally South Africa‟s population has been estimated by Stats SA to have steadily increased from 50 million in 2010 to 55 million in 2015 (Stats SA, 2010, 2015). Furthermore, the increase in maize demand

is supported by a report by Sihlobo and Kapuya (2015), which indicated that maize demand superseded supply with 5 602 metric tons supplied against a demand of 5 984 metric tons maize yield in 2015. Du Plessis (2003) also indicated that 3.1 million ha of land produces an estimated 8.0 million tons of maize grain in South Africa, with half produced for human food consumption. One important reason causing smallholder South African farmers to fail to meet the demand for maize is low fertility status of smallholder farms lands and their poor soil fertility management strategies (Roberts *et al*. 2003; Fey, 2010). There is, therefore, a need to come up with effective and low-cost techniques, which can adequately supply micronutrients and improve the efficacy of macronutrients (NPK) to maize crops (Havlin *et al*., 2014; FSSA, 2007).

## 2.3. Role of micronutrients (Zn, B and Mo)

### 2.3.1. Micronutrient uptake by plants (Zn, B and Mo)

Zinc is an essential micronutrient for maize and other plants‟ optimal growth and reproduction (Tabrizi *et al*., 2011b). Zinc can be accessed by plant roots in the form of $Zn^{2+}$ and as $ZnOH^{+}$ in soil (Henriques *et al*., 2012). Meanwhile, the primary source of Zn in the soil is physical and chemical weathering of parent rocks. Herselman (2007) indicated that South African top soils contain a mean Zn soil concentration between 0.62 – 6.03 mg/kg (low – sufficient). Furthermore, Gupta *et al*. (2016) stated that only a small fraction of Zn in the soil is available for plant use. Availability of Zn is dependent on factors such as soil physicochemical properties, the activity of plant roots and microflora in the rhizosphere and other non-edaphic factors (Alloway, 2008). A small portion of soil Zn is found as insoluble complexes or exchangeable form. Meanwhile, another fraction exists in a water-soluble form accessible for plant use. Among the soil factors, pH is one major property that enhances the risk of Zn deficiency in agricultural fields as it influences the availability of Zn in the soil for plant use (Alloway, 2008; Gupta *et al*., 2016). Increase in soil pH will cause Zn adsorption to cation exchange sites thus reducing free Zn in solution (Henriques *et al*., 2012). Other factors which influence the availability of Zn in the soil are soil moisture, organic matter and sandy soil (Alloway, 2008).

Boron can be accessed by plants in the form of boric acid ($H_3BO_3$) and/or borate ($H_2BO_3$). Similar to Zn, the availability of B in the soil is affected by soil pH, soil texture, soil moisture and organic matter. Boron is pH sensitive and is soluble in low pH conditions in the form of boric acid (Sarkar *et al*., 2014). Deficiencies of B prevail in alkaline conditions and liming practices results in “B fixation” (Da Rocha Pinho *et al*., 2015). The percentage of clay content in the soil also affects the availability of boron. Sarkar *et al*., (2012) Indicated that B in the soil increases with an increase in the clay content. Organic matter is an important constituent of the soil, which directly affects nutrient availability and uptake. Essentially, mineralisation of organic matter in the soil releases B to the soil (Sarkar *et al*., 2014). Soil moisture control factors such as diffusivity and mobility by which B decreases when both are reduced in the soil (Sarkar *et al*., 2012).

Molybdenum in soil is found in the form of oxyanion molybdate ($MoO_4^{2-}$) (Bittner, 2014). The high abundance of Mo in the lithosphere and Mo availability and/or solubility is highly sensitive to pH and presences of phosphorus in the soil (Liu *et al.*, 2010). Furthermore, Liu *et al.* (2010) indicated that there is a significant reduction of P concentration due to the presence of Mo and vice versa, this is normally attributed to the fact that both phosphates and molybdates are absorbed in anionic forms, and they compete with each other for the adsorption sites. Kaiser *et al*. (2005) showed that Mo availability is favoured at pH over 5 and reduced at pH less than 5. Kaiser *et al*. (2005) and Bittner (2014) indicated that an average 1.2 mg/kg is abundant in the lithosphere and this figure labels Mo as one of the scarcest micronutrients in the soil.

### 2.3.2. Morphological, physiological and biochemical importance of Zn, B and Mo in maize

Maize crops are highly sensitive to Zn deficiencies, thus making Zn an important micronutrient especially to maize. The symptoms of Zn deficiency are first observed in young leaves because of poor translocation of Zn in plant tissue (Camberato and Maloney, 2012). A very common visual Zn deficiency symptom occurs on the leaf midrib where it turns white to yellowish-white on both sides with the leaf edges remaining green (McCauley *et al*., 2011). Internodes are affected by Zn deficiency as they are shortened. Chilian *et al*. (2015) and Camberato and Maloney (2012)

indicated that a Zn tissue concentration of 20-70 (ppm or mg/kg) is considered sufficient for seedling, early growth, and tasselling in maize. Thus, the call for nutrients seed priming techniques to sustain adequate Zn tissue concentration levels in maize is a genuine one. Zinc also has an important role as a metal component of enzymes or as a functional, structural, or regulator cofactor of a large number of enzymes (Salem and El-Gizawy, 2012; De Vasconcelos *et al*., 2011). Zinc plays a key role in many biochemical pathways of crops (De Vasconcelos *et al*., 2011). It is required by cellular membranes for keeping the structural orientation of the macromolecules and ion transport system (Tsonev and Lidon, 2012; Dang *et al*., 2010). Zinc affects the production of auxin and other growth hormones (Tsonev and Lidon, 2012).

Boron deficiency shows different symptoms in crops, firstly they appear in young leaves or terminal shoots (da Rocha Pinho *et al*., 2015). The leaves become discoloured during the reproductive stages with boron causing abortion of flowers and fruits and reduced number or no seeds and reduced seed size (Gupta and Solanki, 2013). In wheat, plant with B deficiency form a normal ear, which later fails to flower and it also restricts development of inflorescence and setting of grains (Sarkar *et al*., 2014). Nonetheless, B toxicity results in leaf burn, chlorotic and necrotic patches at the margins and tips of older leaves (Da Rocha Pinho *et al*., 2015; Sarkar *et al*., 2012). Boron also influences the uptake of NPK by crops and deficiency of B influences the balance of other macronutrients in crops (Singh *et* al., 2014). There are several reports, which indicate that B is required for the maintenance of the cell wall structure and functions of membranes especially, plasma membrane (Camacho-Cristóbal *et al*., 2008; Aref, 2011). Furthermore, Camacho-Cristóbal *et al* 2008 stated that B has an important role in sugar translocation, protein synthesis, sucrose synthesis, cell wall composition, membrane stability and $K^+$ transportation. In cereals and oilseed crops, B promotes pollen tube growth and germination (Gupta and Solanki, 2013).

Haque (1987) indicated that Mo concentration less than 1 ppm in the soil causes the plants leaves to curl and turn pale yellow. Hence, concentration between 1 and 4 ppm results in healthy plants with deep green leaves whilst concentration between 8 and 16 ppm causes stunted growth and dark brownish colour on the roots. The role

of Mo in plant growth is inconsistent with respect to the total quantities required by different plants. Moreover, Mo is directly associated with N activities to such an extent that even the deficiency symptoms are similar. “Since Mo activities are directly associated with the metabolism of nitrogen Singh *et al*. (2014)” nitrogen uptake can thus be inhibited due to low amounts of Mo in the soil. Since Mo is vital for nitrate reductase and nitrogen enzymes, Mo deficiencies in the soil can be diagnosed with nitrogen deficiency symptoms (Kaiser *et al*., 2005). The symptoms include stunted growth, chlorosis pale leaves which may be scorched, cupped, or rolled and leaves which appear thick or brittle (McCauley *et al*., 2011; Singh *et al*., 2014). Toxicity levels of Mo in the soil can cause leaves to turn yellowish, reduce seedling growth and increase anthocyanin concentrations (Kaiser *et al*., 2005; Bittner, 2014). Tabrizi *et al*. (2011b) further indicated that Mo is responsible for oxidation and reduction reactions of enzymes and it is a necessity for most plants. Hence, the authors stated that Mo deficiency can cause a high accumulation of organic amino acids and cause reproductive disorders in a number of crops in the male and female sexual organs. Farmers, in general, use different kinds of methods to supplement the micronutrients to minimize the symptoms and growth restrictions mentioned above.

## 2.4. Common micronutrients application methods

Normally, inorganic micronutrients occur naturally in soil minerals and maize crops obtain them directly from the soil (Lohry, 2007). The parent materials and soil forming processes from which the soils develop from will determine the type and quantity of micronutrient found in the soil (Brady and Weil, 2008). In most cases, the minerals are released in a form accessible by plant roots from mineral break down during soil formation processes such as organic matter decomposition (Jenny, 1994). The most important secondary soil constituent for micronutrient source is organic matter (Brady and Weil, 2008; Havlin *et al*., 2014). Organic matter releases micronutrients to the soil slowly due to the ability of organic matter to hold micronutrients tightly in complex organic compounds (Fageria *et al*., 2011). However, organic matter is still regarded as an important secondary source and reservoir for micronutrients in soil (Brady and Weil, 2008; Havlin *et al*., 2014). Manure (kraal and chicken) and compost are common organic materials used to supplement nutrients by smallholder farmers (Van Averbeke and Yoganathan, 2003). Thirdly, foliar

application and soil fertilization with inorganic industrially produced nutrients are used to supply micronutrients to crops and soil respectively (Salem and El-Gizawy, 2012; Johnson *et al*., 2005). In some cases farmers‟ use drip irrigation systems to supply the micronutrients to the soil (Jat *et al*., 2011). Most common industrially produced fertilizers used to supply micronutrients (Zn, B and Mo) are sodium borate (20% B), boric acid (17% B), zinc sulfate (36% Zn), NPK with Zn, ammonium molybdate (49%) and sodium molybdate (46%) (Lohry, 2007; Brady and Weil, 2008). Some of these mentioned products are expensive or inaccessible for smallholder farmers to supply the micronutrients or even when they are available there are still additional challenges which limit the farmers from using them to full extent.

## 2.5. Micronutrient problems in agriculture

South African smallholder farmers are faced with soil nutrient deficiencies in their agricultural lands (Maqubela *et al.*, 2010; FSSA, 2007). Du Plessis (2003) indicated that over 3.1 million ha of land is used for maize production in South Africa. Moreover, Tariq *et al*. (2015) showed that maize is a high nutrient demanding crop. This calls for proper nutrient management practices to balance the demand and the availability of nutrients in the soil. Soil conditions, yield targets and cultivars are other factors which affect the micro and macronutrients requirement of a crop for sufficient growth (Tariq *et al*., 2015). Smallholder farmers are reported to have difficulties in assessing and correcting the nutrient deficiency in agricultural fields, which in return results in low production of maize (Van Averbeke and Yoganathan, 2003). An assessment in the Vhembe district showed that insufficient funding (48%), transport costs (27%), accessibility (5%), limited knowledge on fertilizer use (5%) and others (15%) affected the use of fertilizers in the area (Odhiambo and Magandini, 2008). An abundant of cations in most agricultural soil also contributes to micronutrients deficiencies (Helias *et al*., 2012). Furthermore, micronutrients such as Mo and iron (Fe) are normally deficient when the soil has high manganese and/or lime content (Malvi, 2011). One of the most important challenges of micronutrient availability is soil pH which affects micronutrient availability and fixation in the soil (Nubé and Voortman, 2006). This is supported by reports which indicated that micronutrients are pH sensitive and their availability is influenced by pH status (Miller, 2016; McCauley *et al*., 2009; Rutkowska *et al*., 2014). Therefore there is a need to

introduce simple and efficient support techniques which will help smallholder farmers to archive optimum yields targets. Camacho-Cristóbal *et al*. (2008) provided evidence that B is very pH sensitive and can be accessed by plants only at a pH less than 8. The high abundance of iron and aluminium in soils leads to the formation of complexes with boron, thus causing a higher deficiency of boron (Singh *et al*., 2014). McCauley (2009) illustrated that nutrients can be fixed in soils due to pH and will not be available for plant uptake. Furthermore, McCauley (2009) indicated that majority of micronutrients (B, Cu, Fe, Mn, Ni, and Zn) are accessible for plant uptake within a pH range of 5 to 7. Furthermore, smallholder farmers in high rainfall areas experience micronutrients deficiencies due to a high level of leaching and these lead to yield losses (Biederman and Harpole, 2013; Yao *et al*., 2012).

Micronutrients are required in small quantities and they are important for sufficient use of macronutrients and crop growth and development. FSSA (2007) indicated that South African soils have micronutrient deficiencies as a result of salinity conditions in the soil which inhibits micronutrient availability. Yohannes and Abraha (2013) showed that salinity conditions have restricted seedling growth of maize. Furthermore, Yohannes and Abraha (2013) indicated that calcareous and low clay content soils are known to have micronutrient deficiencies. Availability of micronutrients also severely decreases under drought conditions and in acidic soils with a large content of reactive iron oxide hydrates (Lohry, 2007). Another limiting factor that is not soil or crop specific to farmers is low capital and unavailability of fertilizers in the market at an affordable price and at critical times (Ali *et al*., 2008). Methods such as seed priming which is used to enhance the seedling performance should be tested to verify its effectiveness in supplying micronutrients.

## 2.6. Seed priming

Seed priming is a technique where seeds are soaked in water for a certain duration before sowing. Seed priming can improve and speed up germination, early seedling growth and decrease physiological germination heterogeneity. Imran *et al.* (2013) indicated that the resulting improvement in maize germination and seedling growth upon sowing primed seeds is due to the seeds rapid imbibition of water to quickly restore the seed metabolism process. The normal germination process and seed

priming process are illustrated in figure 2.1. The process can be divided into three phases (imbibition, activation and growth). Seeds are soaked in water for a given period of time and grown against the non-primed seed to observe the growing pattern from imbibition, activation to the growth process. Normal seed grow at a slow rate while primed seeds after re-imbibition in soil have a rapid growth (Figure 2.1).

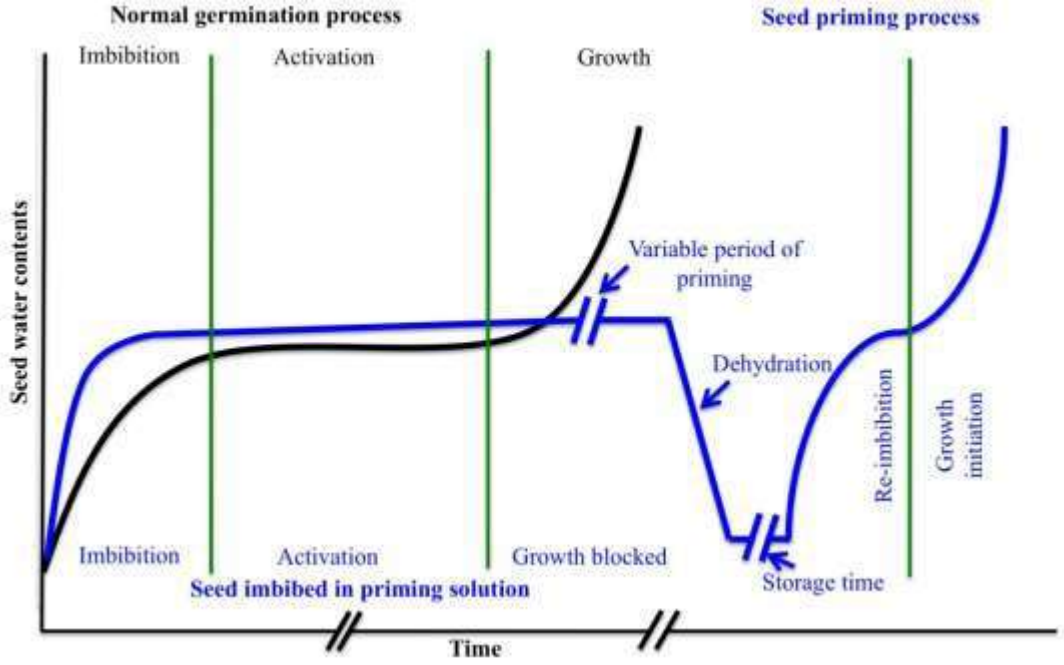


Figure 2.1: Normal germination and primed seed priming process (Imran *et al.,* 2013).

One special key aspect, which smallholder farmers are struggling with is to maximize the use of resources they have to improve yields. Seed priming improves quality of seed vigour and viability when used (Imran *et* al., 2012; Badiri *et al*., 2014). Since micronutrients are required in small quantities, solutions which contain micronutrients can be used during seed priming to incorporate those micronutrients during the process of priming. Nutrient seed priming can then become a solution to micronutrient deficiencies for smallholder farmers.

Nutrient seed priming (NSP) is a process whereby seeds are soaked in solutions with trace amounts of nutrients. During germination and the early stages of seedling seed nutrient content is of great importance. Thus, seedlings acquire their nutrients in the early stages from the seed reserves and from the soil (Imran *et al*., 2013). It is

therefore important to increase the seed nutrient content through a process such as NSP, which can improve seed nutrient content. Therefore, NSP can assist the current nutrients supply systems by submitting adequate level of nutrients to the seed thereby solving the problems farmers encounter in micronutrients deficient soils. Limited studies have been done on nutrient seed priming in South Africa. The work done on NSP technique in South Africa to my best knowledge is either restricted or not published in the public main stream of information pool. The findings of this study can provide the base line for further investigations in South Africa to adequately test the technique in the country deficient soils and small scale farmer‟s conditions. The growth, yield and economic impact, application and adaptability of the NSP technique in South Africa still remain un-answered.

The technique can be effective and it has been reported to have both economic and environmental benefits in some Asian countries and in Zimbabwe (Harris *et al*. 2006; Ali *et al*. 2008). Badiri *et al*. (2014) showed that total biomass and time to emergence was reduced with the priming of iron (Fe), zinc (Zn) and manganese (Mn) for broadleaf plantain. High maize emergence was reported in a study by Rahman *et al.* (2014) after sowing primed seeds compared to non-primed seeds in Bangladesh. Similarly primed cotton and maize seed resulted in higher germination percentages at low water potentials than non-primed seeds in a study conducted in Zimbabwe (Murungu *et al.,* 2005). El-Saifi *et al*. (2010) showed improved results on growth and yield of tomato. Uche *et al*. (2016) indicated seed priming improved germination of green-pepper seeds. Growth parameters such as germination mean germination time and coefficient velocity of germination were improved after seed priming mountain rye (Ansari *et al*., 2013).

# CHAPTER 3: RESEARCH METHODOLOGY

## 3.1. Description of study site

Laboratory, glasshouse and field studies were carried out during the 2016/2017 growing season. The laboratory and glasshouse studies were carried out at the soil science lab and Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). Minimum/maximum ambient temperatures averaged 13/25°C, with maximum temperatures controlled using thermostatically activated fans in the glasshouse. A field trial was conducted during the 2016/2017 growing season at Ofcolaco, 43km south-east of Tzaneen at 24° 6' 0" S, 30° 23' 0" E in the Mopani District of the Limpopo Province of South Africa. The landscape is covered by a broadleaved deciduous forest and is characterised by clay loam soils of Hutton form. The climate is classified as a humid subtropical (dry winter and hot summer). The area receives an annual average rainfall of 700 mm with minimum and maximum average temperatures of 9 °C and 30°C, respectively.

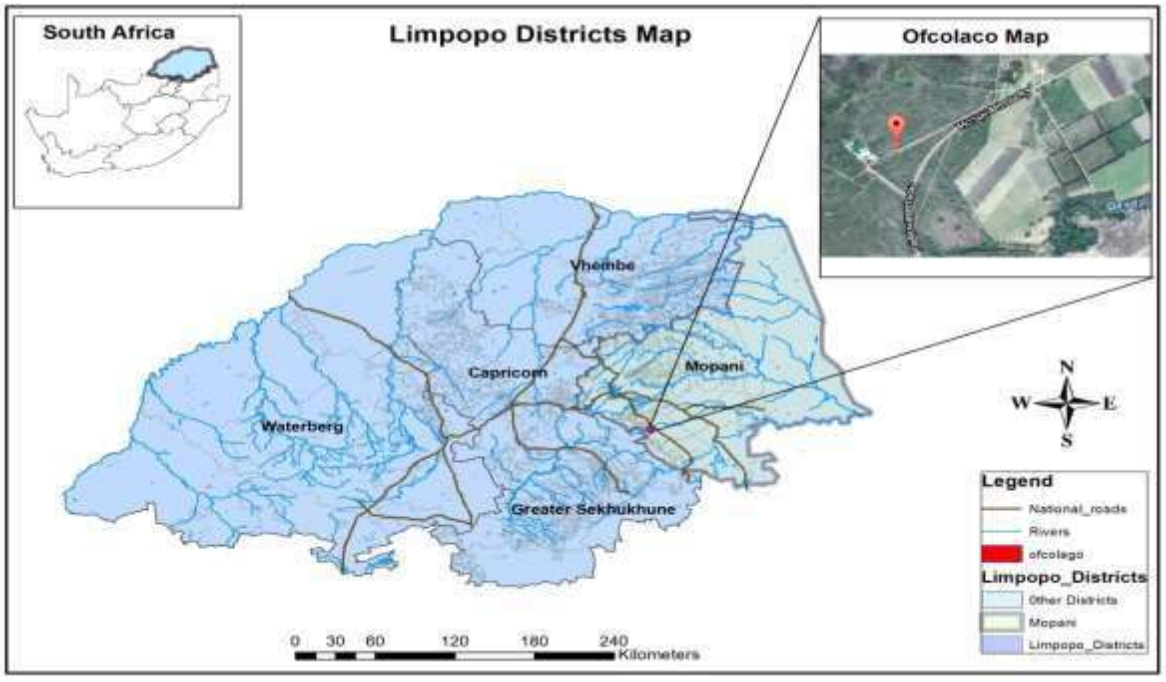


Figure 3.1: Map of Ofcolaco.

## 3.2. Soil characterization

Soil samples were randomly collected using an auger from each plot and the geographical coordinates of the auger points were determined using a geographical positioning system (GPS). Soil samples collected from the surface up to a depth of 0.15 m were mixed to have a composite sample. The soil sample was taken to the laboratory, air-dried at room temperature and sieved through a 2 mm sieve for analysis.

Micronutrients (Zn, Mo and B) were determined using water paste saturation (U.S. Salinity Laboratory Staff, 1954). Total nitrogen (N) was analysed with total N digestion (Kirk, 1950). Phosphorous (P) was analysed using the Bray-1 (Bray and Kurtz, 1945). Exchangeable bases (potassium ($K^+$), calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$) and sodium ($Na^+$)) were determined with ammonium acetate extraction method (The non-affiliated soil analysis work committee, 1990). Particle size was done using the hydrometer method (Bouyoucos, 1962) and organic carbon (Org. C) with the Walkley-Black method (Walkley and Black, 1934). Soil pH ($H_2O$) was measured through 1:2.5 mass of soil to water methods (Blakemore *et al*., 1987).

Table 3.1: Selected chemical and physical properties of the soil in Ofcolaco‟s farmers‟ field.

| Soil properties | | Rating | Source of rating |
|---|---|---|---|
| Clay | 26% | | |
| Silt | 15% | | |
| Sand | 59% | | |
| Soil texture | | Sandy clay loam | Bouyoucos, 1962 |
| Zn | 0.364 mg/kg | Deficient | Sellamuthu *et al.,* 2011 |
| B | 0.362 mg/kg | Deficient | Sellamuthu *et al.,* 2011 |
| Mo | 0.0072 mg/kg | Deficient | Sellamuthu *et al.,* 2011 |
| EC | 43.8 µS/m | Normal | Ravikumar and Somashekar (2013) |
| pH ($H_2O$) | 6.572 | Neutral | Ravikumar and Somashekar (2013) |
| Organic carbon | 1.072% | High | Ravikumar and Somashekar (2013) |
| Total N | 0.055% | Low | Ravikumar and Somashekar (2013) |
| P | 38.718 mg/kg | Medium | Ravikumar and Somashekar (2013) |
| Ca | 764.25 mg/kg | Medium | Ravikumar and Somashekar (2013 |
| Mg | 203.406 mg/kg | Low | Ravikumar and Somashekar (2013 |
| K | 140.176 mg/kg | Medium | Ravikumar and Somashekar (2013) |
| Na | 11.734 mg/kg | Low | Ravikumar and Somashekar (2013 |

## 3.3. Experimental procedure

### 3.3.1. Laboratory study

#### 3.3.1.1. Procedure

Micronutrient seed priming experiments were initially conducted in the laboratory and glasshouse to determine optimum priming concentrations and procedure before NSP technique was evaluated under field conditions. A completely randomized design (CRD) laid out in a 3 x 5 x 3 factorial treatment structure [micronutrients x concentration x duration] with three replications was used for the laboratory. Micronutrients include Zn, Mo B and a control in an incubator (25°C). The concentrations assessed for each micronutrient were 0%, 0.01%, 0.05%, 0.1%, and 0.5%, as described by Imran (2012) where 0% (hydro-priming ($H_2O$)) was used as the control for the experiment. The seeds were primed in the solutions for 24 h, 12 h and 8 h. For priming, seeds were subjected to hydro-priming ($H_2O$) for control and priming with zinc sulphate ($ZnSO_4$), sodium molybdate ($Na_2MoO_4$) and boric acid ($H_3BO_3$) (example in Figure 3.2).

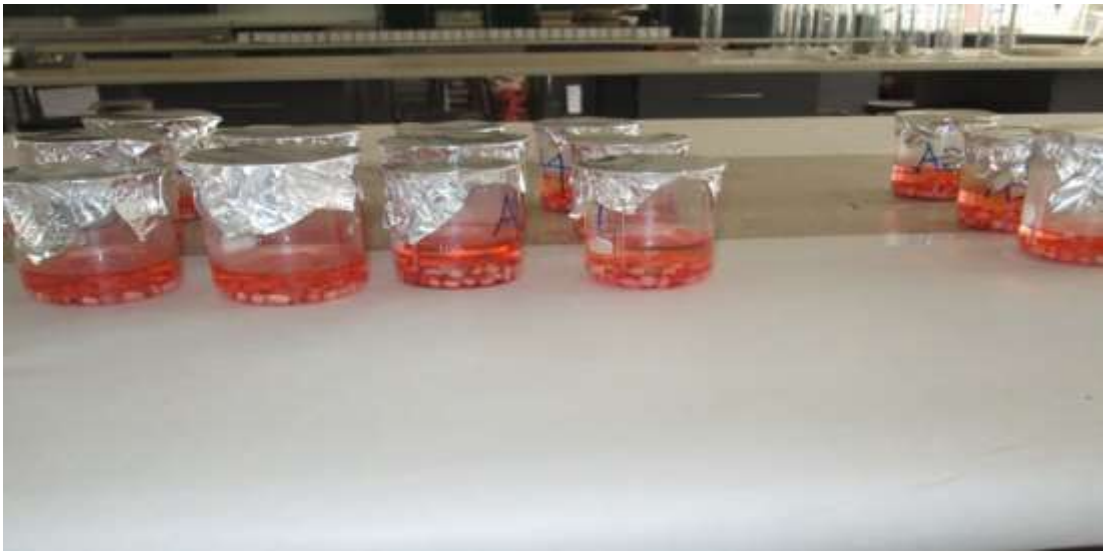

Figure 3.2: Seeds immersed in nutrients solutions.

Salts were washed off the seed coat three times using deionized water. The seeds were air dried under shade for 24 h back to almost their original weight. The seeds

were sown between germination papers and incubated at 25°C for 10 days. The germination paper was irrigated with 10 ml of distilled water using a pipette every other day. The concentrations levels (with the control) that performed well and two best duration periods were further tested in the glasshouse experiment.

3.3.1.2. Data collection

Data were recorded on final germination percentage (GP), germination rate (GR), mean germination time (MGT), days to germination (DG) and coefficient of velocity (CVG). Equations in Table 3.2 were used to determine the characteristics of seedlings for the laboratory experiment.

Table 3.2: Equations used to determine selected seedling characteristics.

| No | Equation | Reference |
|---|---|---|
| 1 | $\left(\frac{\qquad\qquad\qquad\qquad\qquad\qquad}{}\right)$ | (Zahedifar andZohrabi, 2016; ISTA, 1996) |
| 2 | $\sum -$ | (Zahedifar and Zohrabi, 2016; ISTA, 1996) |
| 3 | $\sum$ $\sum$ | (Zahedifar and Zohrabi, 2016; Ellis and Roberts, 1981) |
| 4 | $\frac{\qquad\qquad\qquad\qquad\qquad\qquad}{(\quad)\ (\quad)\qquad\qquad(\quad)}$ | (Zahedifar and Zohrabi, 2016; Scott, *et al*.,1984) |

*Where $n_i$ is the number of seeds emerged on an $i^{th}$ day and $D_i$ is the number of days counted from the beginning of the experiment. J is set to 7 days in this experiment, n is the number of seeds germinated on day and d is the number of days from the beginning of the experiment, $G_1 – G_n$ is the number of germinated seeds from the first to the last day.*

Seeds were considered germinated when at least 2 mm long radicle protruded through the seed coat. Days to germination was recorded when 50% of the seeds had germinated. The final germination percentage was calculated on the $7^{th}$ day of incubating the seeds.

### 3.3.2. Glasshouse study

#### 3.3.2.1. Procedure

A completely randomized design (CRD) laid out in a 3 x 4 x 2 factorial treatment structure with 3 replications and a control (hydro- priming) was used for the glasshouse study (Figure 3.3). The treatments consisted of three micronutrients (Zn, Mo and B) at four different concentrations each with the 0% treatment level as control and primed at two different durations.

Figure 3.3: Experimental set up of nutrient seed priming of maize with zinc, molybdenum and boron for 12 and 24 h in a glasshouse study.

Three best performing micronutrients levels for each nutrient and two durations from the laboratory experiment were used for the greenhouse experiment. A total of 72 pots were used for the experiment. The experiment was run for a period of 4 weeks (i.e. up to the V3 – V5 stage of maize) before termination. Soil used for the experiment was collected from Ofcolaco and filled into a 25 cm diameter pots. The pots were placed on benches in the glasshouse with inter-row and intra-row spacing of 0.25 m each. Three seeds were sown in each pot at depth of 0.03 m. Each plant was irrigated with 500 ml tap-water on a three-day interval.

#### 3.3.2.2. Data collection

Data were recorded on days to emergence (DE), chlorophyll content, stem diameter, seedling height, seedling biomass (wet (WW) and dry (DW)) and final root length (RL). After emergence stem diameter was measured with a digital Vernier calliper, chlorophyll content with chlorophyll meter (MINOLTA SPAD-502) and seedling height was measured using a measuring tape. Stem diameter and plant height were collected at three different stages (VE, V1 and V3) after emergence while chlorophyll was collected on V1 and V3 stages after emergence. On the final day of the experiment, seedlings were uprooted and washed off to remove all the soil from the roots. Root length was measured with a ruler and wet weight was measured with a weighing balance.

### 3.3.3. Field experiment

#### 3.3.3.1. Procedure

The field experiment was carried out to evaluate the effect of NSP on, chlorophyll content, maize plant dry weight, plant height, grain yield and grain nutrient composition of Mo, Zn and B. Two best-performing priming solution concentrations for each and single duration from glasshouse experiment were used for the field experiment. A single factor experiment in a randomised complete block design (RCBD) with three replications and a control was used. Micronutrients treatment levels used were $Zn_1$, $Zn_2$, $Mo_1$, $Mo_2$, $B_1$, $B_2$, non – primed (NP) and $Zn_0 + Mo_0 + B_0$ as the control (Table 3.3). A total of 24 plots of 14 $m^2$ each were grouped into three blocks and the blocks were 1 m apart. Every plot contained 70 plants with a target population of 50 000 plants per ha.

Table 3.3: Treatment details for field experiment.

| Treatment label | Nutrient $_{conc(\%)}$ | Treatment details |
|---|---|---|
| $Zn_1$ | $Zn_{0.05}$ | Seeds primed at 0.05% concentration for 24 h with zinc sulphate heptahydrate |
| $Zn_2$ | $Zn_{0.1}$ | Seeds primed at 0.1% concentration for 24 h with zinc sulphate heptahydrate |
| $Mo_1$ | $Mo_{0.05}$ | Seeds primed at 0.05% concentration for 24 h with sodium molybdate dehydrate |
| $Mo_2$ | $Mo_{0.1}$ | Seeds primed at 0.1% concentration for 24 h with sodium molybdate dehydrate |
| $B_1$ | $B_{0.05}$ | Seeds primed at 0.05% concentration for 24 h with boric acid powder |
| $B_2$ | $B_{0.1}$ | Seeds primed at 0.1% concentration for 24 h with boric acid powder |
| NP | NA | Non-primed seeds |
| $Zn_0 + Mo_0 + B_0$ | $H_2O$ | Seed primed at 0% for 24 h with water |

3.3.3.2. Data collection

Plant height was measured at the V8-V9 stage, V16-VT, tasselling and maximum plant height at grain physiological maturity from five randomly selected plants per plot using a tape measure. Chlorophyll content was determined from the youngest fully extended leaves at three different stages (V8-V9, V16-VT and tasselling) before physiological maturity (Coste *et al*., 2010). Dry shoot biomass was determined at V8 – V9 stage from three random plants per plot which was oven dried at 65$^{\circ}$C for three days. Total dry matter yield, grain yield, harvest index and components of yields where determined following the procedure by Dobermann (2005). The moisture content of the seed was determined at harvest using a Wile 65 grain moisture meter (Farm-crop Agro-electronics). Grain nutrient composition of micronutrients (Zn, Mo and B) and NPK were determined with water paste saturation, total N digestion, Bray-1 and ammonium acetate methods (Bray and Kurtz, 1945; The Non-affiliated Soil Analysis Work Committee. 1990). Grain yield was expressed in kg/ha after accounting for grain moisture content.

#### 3.3.3.3. Cultural practices for a field experiment

A medium early maize cultivar (PAN 5Q-751BR) planted at depth of 0.05 m was used. The experiment was rain fed and weed control was carried out by mechanical removal with hand hoes. Maize was planted at inter and intra row spacing of 0.75 m and 0.3 m respectively. Fertilisers were applied in accordance with the soil analysis results, except for the nutrients in question. The fertilisers were band-placed at planting 0.05 m aside and 0.05 m below the seed and top-dressed at the V8-V9 stage.

## 3.4. Data analysis

Data was subjected to analysis of variance (ANOVA) for effect of treatments on maize development and yield parameters using JMP 12 statistical software. Mean comparison was performed using Tukey's HSD test at α = 0 05

# CHAPTER 4: RESULTS AND DISCUSSION

## 4.1. Results of the laboratory experiment.

### 4.1.1. Effect of boron seed priming on maize seed germination.

Boron seed priming duration and concentration had significant ($P < 0.05$) effects on GP, GR, CVG, DG and MGT. The interaction between these two factors was significant ($P < 0.05$) (Table 4.1).

The lowest GP (87.33%) was observed after seed priming with 0.5% B for 24 h. However, no difference was observed on GP from 0 up to 0.1% B in both durations (Figure 4.1). Within each B concentration level including the control, seeds primed for 24 h had a higher GR than the both the 8 and 12 hour durations except for the 0.5% B concentration where priming for 24 h resulted in the lowest GR (Figure 4.2). Priming seeds with B at 0.5% for 24 h significantly slowed down the overall GR as compared to seeds primed with water (0%). Hence, seed primed with B at 0.01% for 24 h increased GR (47.53% per day), but not significantly different from seed primed with 0, 0.05 and 0.1% B for 24 h (Figure 4.2). The seeds primed with B for 24 h at 0, 0.01, 0.05 and 0.1% levels obtained a faster CVG of 0.85, 0.84, 0.84 and 0.8, respectively, while the slowest was achieved for seeds primed for 8 h at 0.05%, 12 h at 0.01% and 24 h and 0.5% (0.46, 0.44 and 0.38, respectively) (Figure 4.3). The earliest germination was observed for seeds primed with B for 24 h at 0, 0.01, 0.05 and 0.1% (one day), these treatments were significantly different from the rest of the treatments. However, the rest of the treatments were not significantly different from one another and germinated after two days (Figure 4.4). Mean germination time ranged between 1.17 and 2.66 days across all interactions. The longest MGT was for seeds primed with B at 0.5% for 24 h and the shortest was for seed primed for 24 h at 0, 0.01, 0.05 and 0.1% B (Figure 4.5).

Table 4.1: ANOVA for the effect of concentration levels, duration and interaction of concentrations levels and duration period on seed germination.

| Concentration | | | | | |
|---|---|---|---|---|---|
| Source | GP | GR | CVG | DG | MGT |
| Nparm | 4 | 4 | 4 | 4 | 4 |
| DF | 4 | 4 | 4 | 4 | 4 |
| Sum of Squares | 181.87 | 1159.34 | 0.31 | 1.20 | 3.23 |
| F Ratio | 8.75 | 76.97 | 22.27 | 6.51 | 44.96 |
| Prob> F | < 0.0001* | < 0.0001* | < 0.0001* | 0.0008* | < 0.0001* |
| Duration | | | | | |
| Nparm | 2 | 2 | 2 | 2 | 2 |
| DF | 2 | 2 | 2 | 2 | 2 |
| Sum of Squares | 5.51 | 655.74 | 0.23 | 5.38 | 0.56 |
| F Ratio | 0.53 | 87.07 | 32.16 | 58.41 | 15.64 |
| Prob> F | 0,5941 | < 0.0001* | < 0.0001* | < 0.0001* | < 0.0001* |
| Concentrations (g/L)*Duration (h) | | | | | |
| Nparm | 8 | 8 | 8 | 8 | 8 |
| DF | 8 | 8 | 8 | 8 | 8 |
| Sum of Squares | 270.93 | 1606.84 | 0.37 | 1.73 | 3.93 |
| F Ratio | 6.52 | 53.34 | 13.32 | 4.71 | 27.38 |
| Prob> F | < 0.0001* | < 0.0001* | < 0.0001* | 0.0010* | < 0.0001* |

*Pr> F = < 0.0001*. significantly different (*), Germination percentage (GP), Germination Rate (GR), the Coefficient velocity of germination (CVG), Days to germination (DG) and Mean germination time (MGT).*

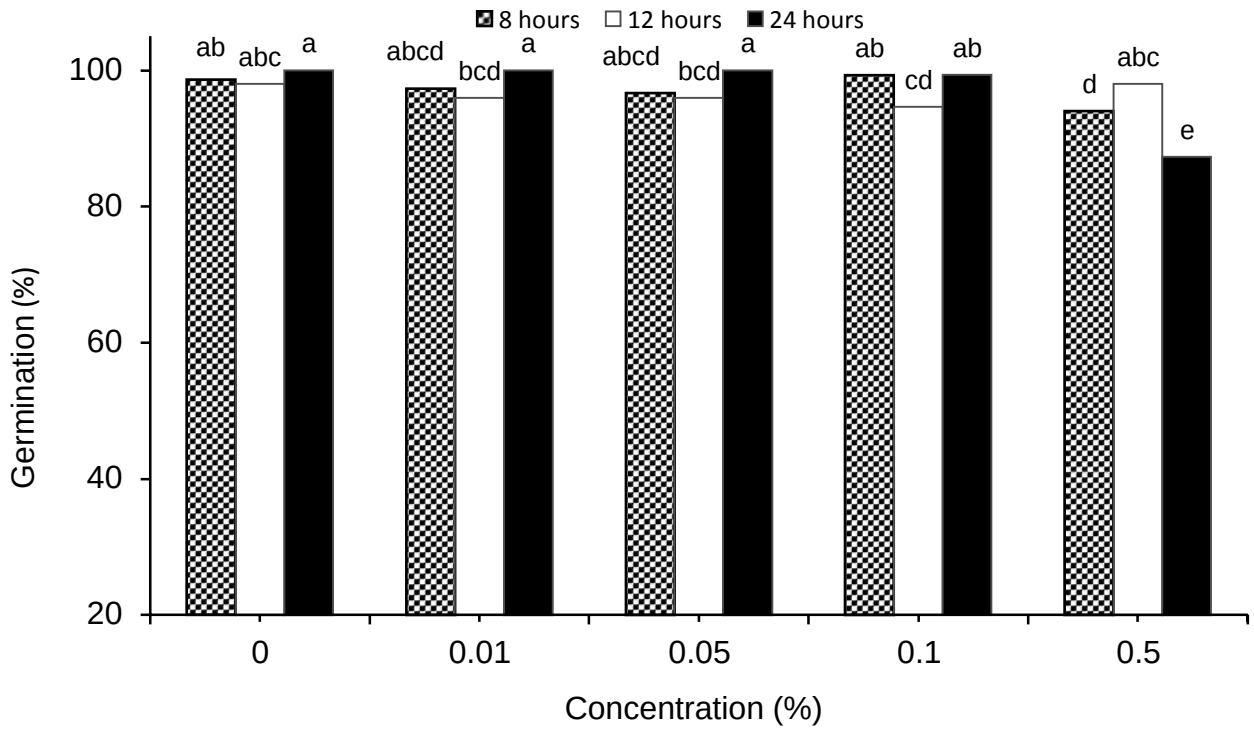


Figure 4.1: Effect of priming duration and boron concentration levels on germination percentage.

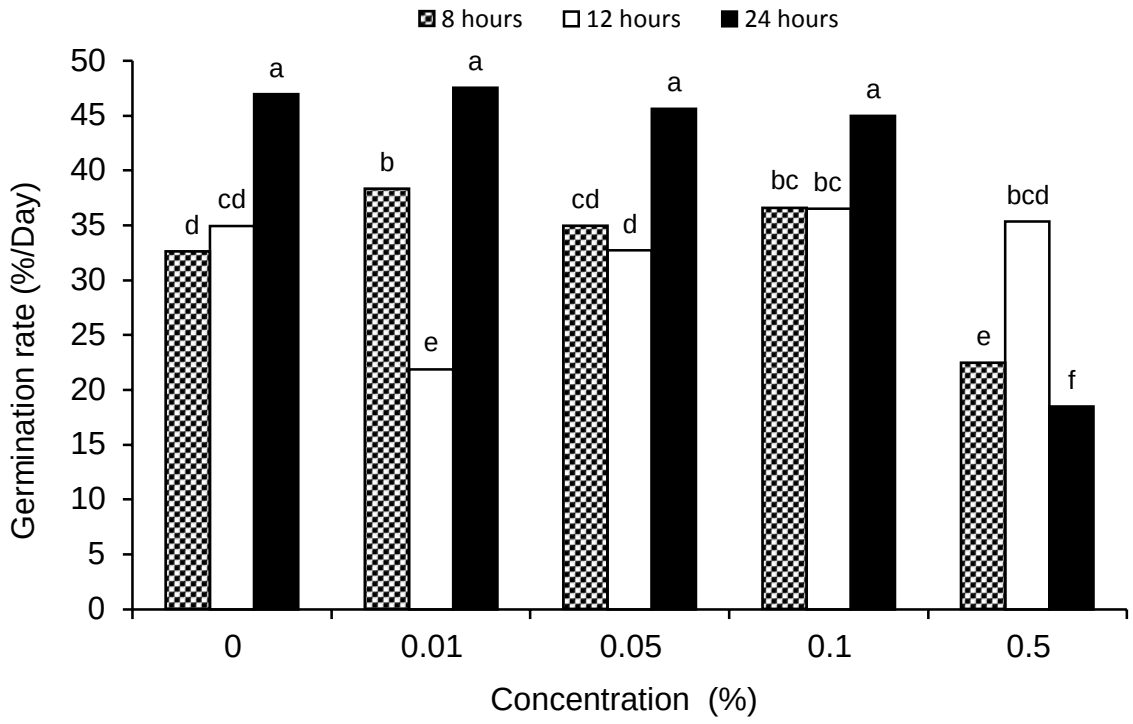


Figure 4.2: Effect of priming duration and boron concentration levels on germination rate.

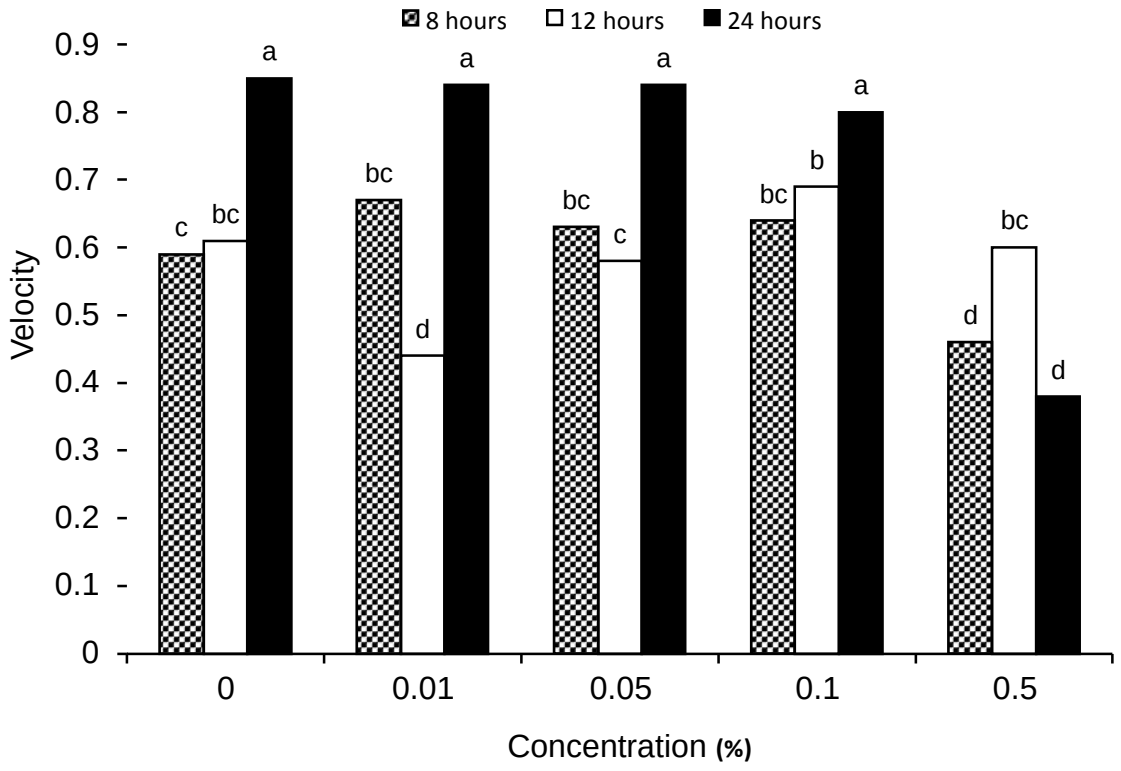


Figure 4.3: Effect of priming duration and boron concentration level on the coefficient velocity of germination.

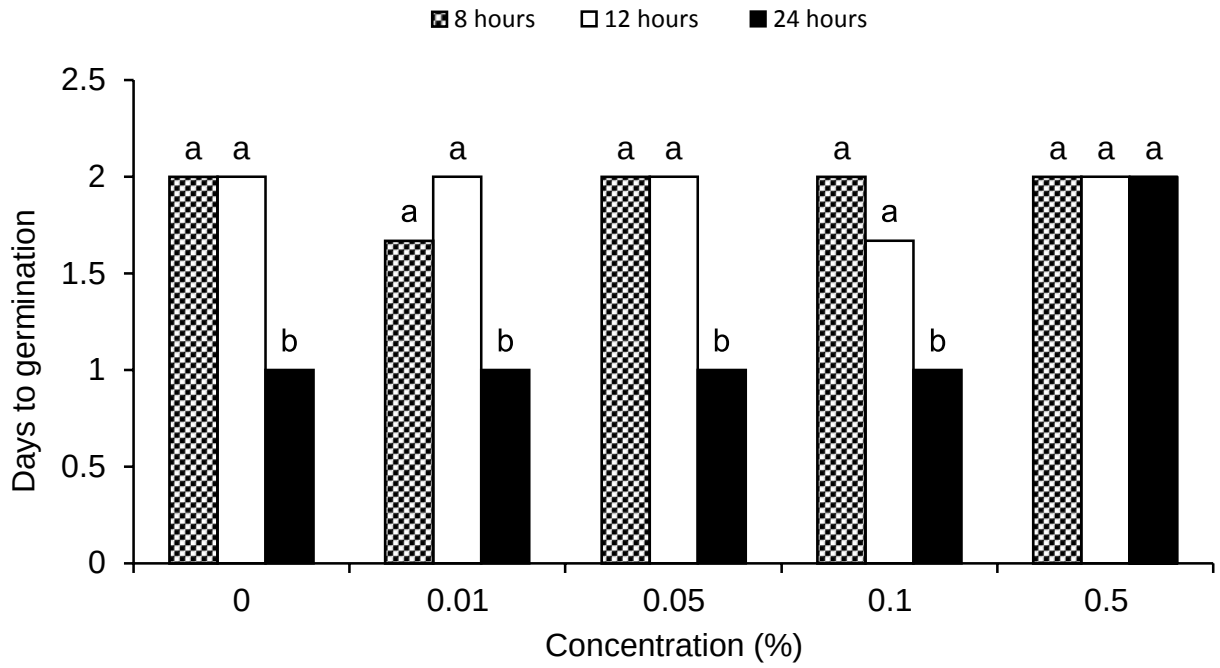


Figure 4.4: Effect of priming duration and boron concentration levels on days to germination.

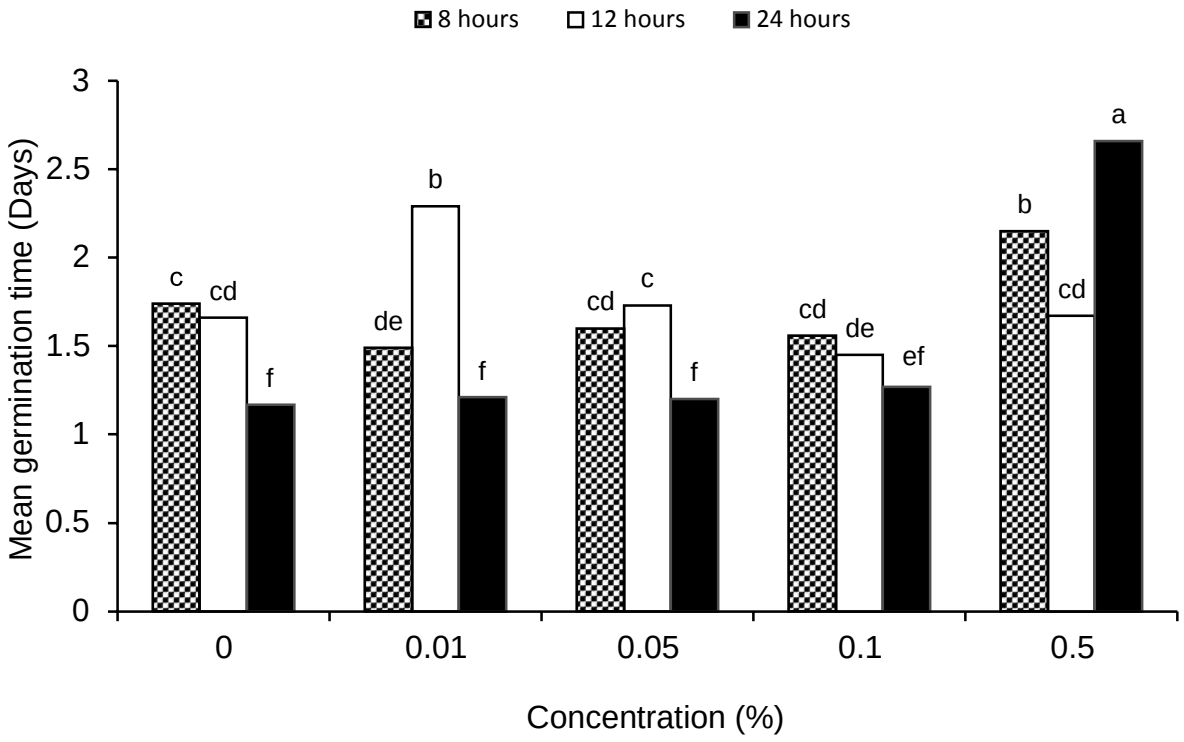


Figure 4.5: Effect of priming duration and boron concentration levels on mean germination time.

### 4.1.2. Effect of zinc seed priming on maize seed germination

Zinc seed priming duration and concentration levels had significant ($P < 0.05$) effects GP, GR, CVG, DG and MGT. The interaction between seed priming duration and Zn concentration levels on GR, CVG, DG and MGT was significant ($P < 0.05$) but not significant for GP (Table 4.2). Seed priming with Zn at 0.01% for 24 h significantly increased the overall GR (48.06% / day) as compared to seeds primed at 0% Zn (21.62 % per day) (Figure 4.7). A faster CVG of 0.87, 0.86 and 0.85 were obtained for seeds primed with Zn for 24 h at 0, 0.01, 0.05 and 0%, respectively. The slowest was observed for seeds primed for 8 h at 0% (Figure 4.8). The earliest germination resulted after seed priming with Zn for 24 h at all concentration levels (one day). Seeds primed with Zn for 12 h and 8 h (except at 0.05% Zn) germinated after an average of 2 or 3 days (Figure 4.9). Mean germination time ranged between 1.17 and 3.21 days across all interactions. The longest MGT was observed for seeds primed with Zn for 8 h at 0% and the shortest was observed for seed primed for 24 h at 0.05% (Figure 4.10).

Table 4.2: ANOVA for the effect of concentration zinc levels, duration and interaction of concentrations levels with duration on germination.

| Concentration | | | | | |
|---|---|---|---|---|---|
| Source | GP | GR | CVG | DG | MGT |
| Nparm | 4 | 4 | 4 | 4 | 4 |
| DF | 4 | 4 | 4 | 4 | 4 |
| Sum of Squares | 22.58 | 383.59 | 0.14 | 3.42 | 2.34 |
| F Ratio | 1.67 | 8.03 | 8.26 | 3.15 | 4.23 |
| Prob> F | 0.1854 | 0.0002* | 0.0002* | 0.0294* | 0.0083* |
| Duration | | | | | |
| Nparm | 2 | 2 | 2 | 2 | 2 |
| DF | 2 | 2 | 2 | 2 | 2 |
| Sum of Squares | 51.38 | 2404.50 | 1.13 | 16.53 | 11.69 |
| F Ratio | 7.59 | 100.68 | 130.79 | 30.46 | 42.29 |
| Prob> F | 0.0023* | < 0.0001* | < 0.0001* | < 0.0001* | < 0.0001* |
| Concentrations (g/L)*Duration (h) | | | | | |
| Nparm | 8 | 8 | 8 | 8 | 8 |
| DF | 8 | 8 | 8 | 8 | 8 |
| Sum of Squares | 52.62 | 397.35 | 0.098 | 7.91 | 3.15 |
| F Ratio | 1.94 | 4.16 | 2.85 | 3.64 | 2.8469 |
| Prob> F | 0.0926 | 0.0022* | 0.0190* | 0.0050* | 0.0190* |

*Pr> F = < 0.0001*. significantly different (*), Germination percentage (GP), Germination Rate (GR), the Coefficient velocity of germination (CVG), Days to germination (DG) and Mean germination time (MGT).*

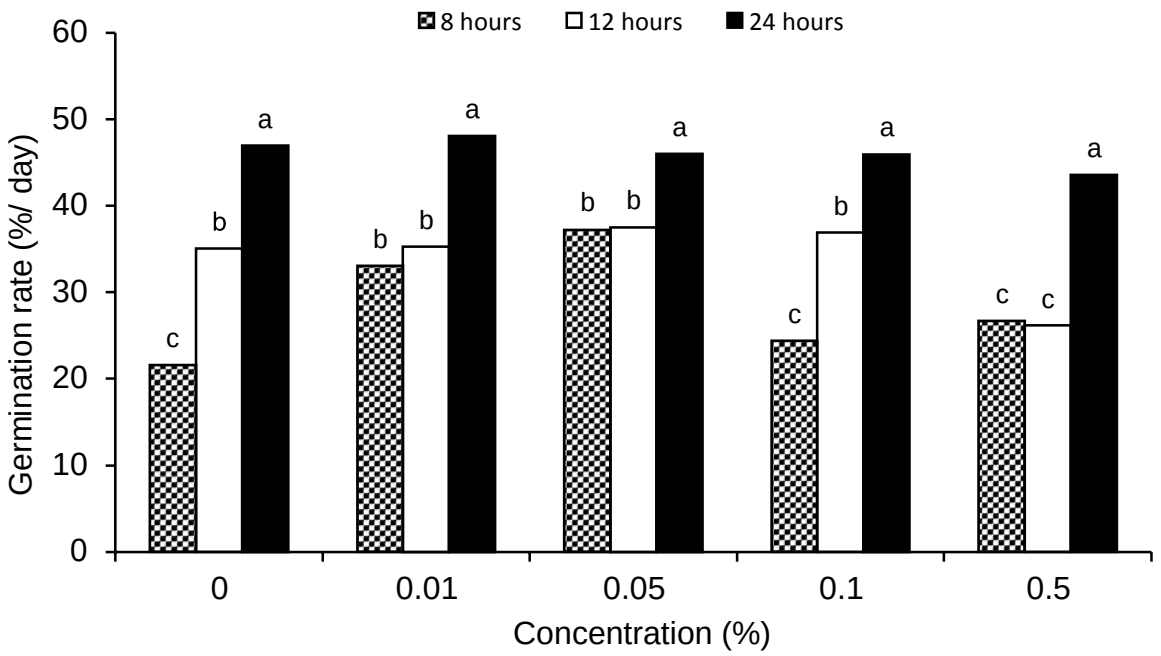


Figure 4.6: Effect of priming duration and zinc concentration levels on germination rate.

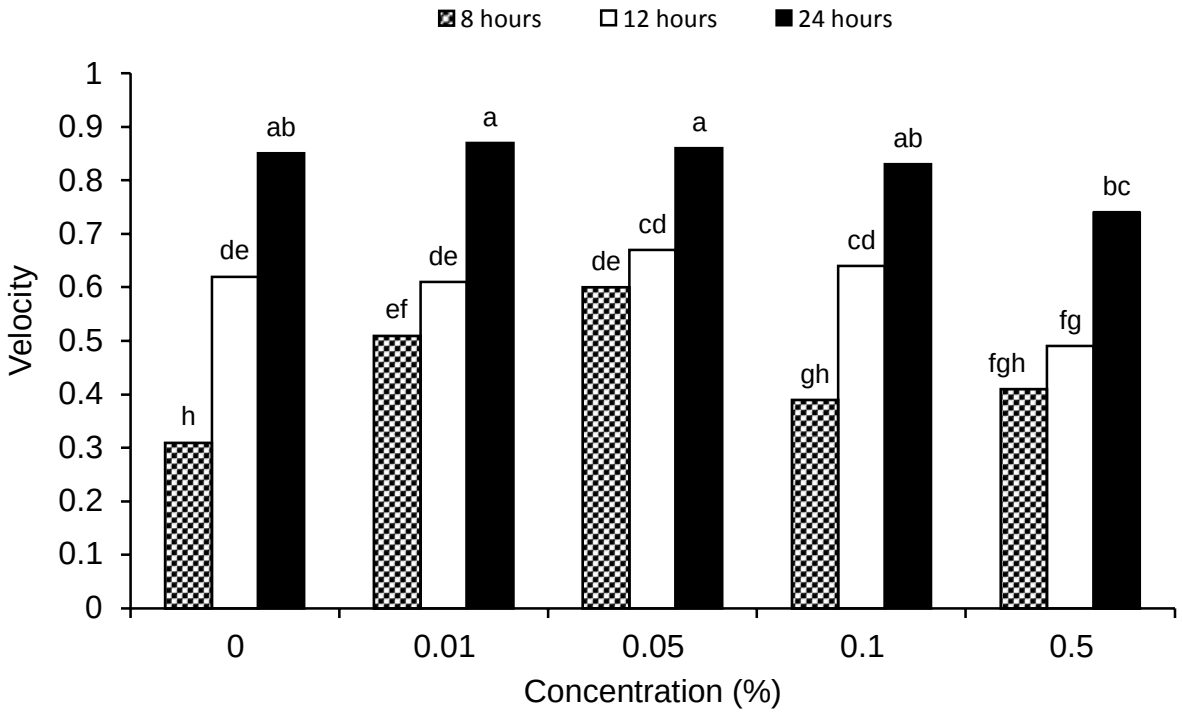


Figure 4.7: Effect of priming duration and zinc concentration levels on the coefficient velocity of germination.

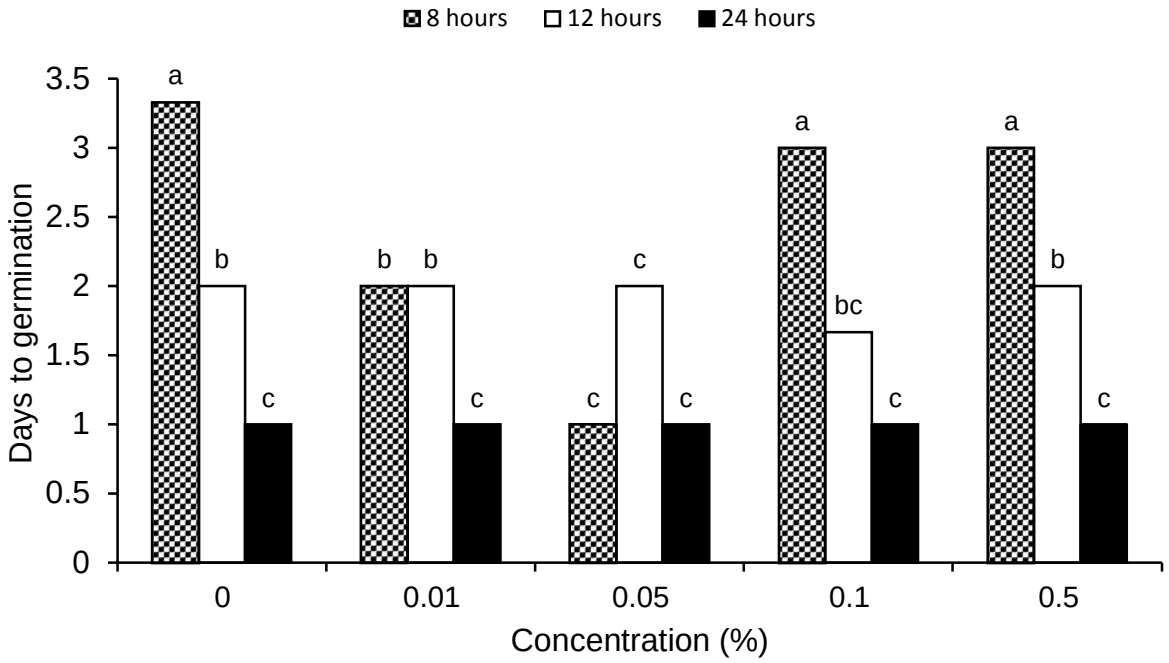


Figure 4.8: Effect of priming duration and zinc concentration levels on days to germination.

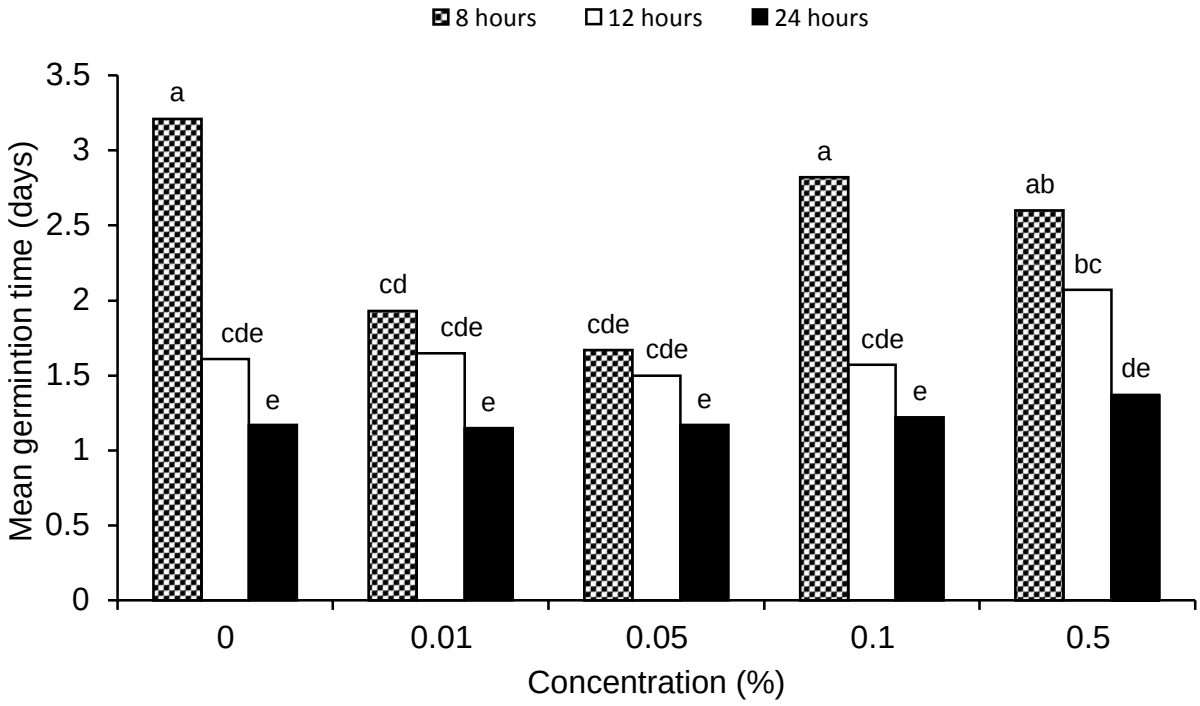


Figure 4.9: Effect of priming duration and zinc concentration levels on mean germination time.

### 4.1.3. Effect of molybdenum seed priming on seed germination

Molybdenum seed priming duration and concentration levels had significant effects ($P < 0.05$) on GP, GR, CVG, DG and MGT (Table 4.3). The interaction between seed priming duration with concentration levels on CVG, MGT and DE was not significant ($P > 0.05$) while the effect on GP and GR was significant ($P < 0.05$) (Table 4.3). The highest GP (99.33%) was observed for seeds primed with Mo for 24 h at all concentration levels and the lowest GP was 80% for seed primed with Mo for 12 h at 0.5% (Figure 4.11). Seeds primed with Mo had a similar GR for the duration of 24 h priming (Figure 4.11). However, seed priming with Mo for 12 h at 0.5% and for 8 h at 0.5% resulted in the slowest GR of 18.62 and 24.36% per day, respectively. Mean germination time, the coefficient of velocity and days to emergence were statistically not significant, seeds primed with 0.01% Mo for 24 h resulted in the short germination time (1 day) and seed primed with 0.5% Mo for 12 h resulted in the longest germination time (2.47 days). All the seeds primed for 24 h with Mo germinated after 1 day (the earliest) at all concentration levels (Figure 4.13). Meanwhile, seeds primed with 0.5% Mo for 12 h were the latest (2.67 days) to germinate and resulted in the lowest germination velocity (Figure 4.12 and 4.13).

Table 4.3: ANOVA for the effect of concentration molybdenum levels, duration and interaction of concentrations levels with duration period on seed germination.

| Concentration | | | | | |
|---|---|---|---|---|---|
| Source | GP | GR | CVG | DG | MGT |
| Nparm | 4 | 4 | 4 | 4 | 4 |
| DF | 4 | 4 | 4 | 4 | 4 |
| Sum of Squares | 282.67 | 822.55 | 0.33 | 0.98 | 2.52 |
| F Ratio | 11.39 | 29.57 | 14.71 | 2.00 | 11.61 |
| Prob> F | < 0.0001* | < 0.0001* | < 0.0001* | 0.1219 | < 0.0001* |
| Duration | | | | | |
| Nparm | 2 | 2 | 2 | 2 | 2 |
| DF | 2 | 2 | 2 | 2 | 2 |
| Sum of Squares | 364.98 | 2188.58 | 0.74 | 9.38 | 3.49 |
| F Ratio | 29.42 | 157.38 | 65.68 | 38.36 | 32.17 |
| Prob> F | < 0.0001* | < 0.0001* | < 0.0001* | < 0.0001* | < 0.0001* |
| Concentrations (g/L)*Duration (h) | | | | | |
| Nparm | 8 | 8 | 8 | 8 | 8 |
| DF | 8 | 8 | 8 | 8 | 8 |
| Sum of Squares | 394.13 | 689.31 | 0,07 | 1.96 | 0.95 |
| F Ratio | 7.94 | 12.39 | 1.47 | 2.00 | 2.19 |
| Prob> F | <0.0001* | <0.0001* | 0.2131 | 0.0838 | 0.0590 |

*Pr> F = < 0.0001* . significantly different (*), Germination percentage (GP), Germination Rate (GR), the Coefficient velocity of germination (CVG), Days to germination (DG) and Mean germination time (MGT)*

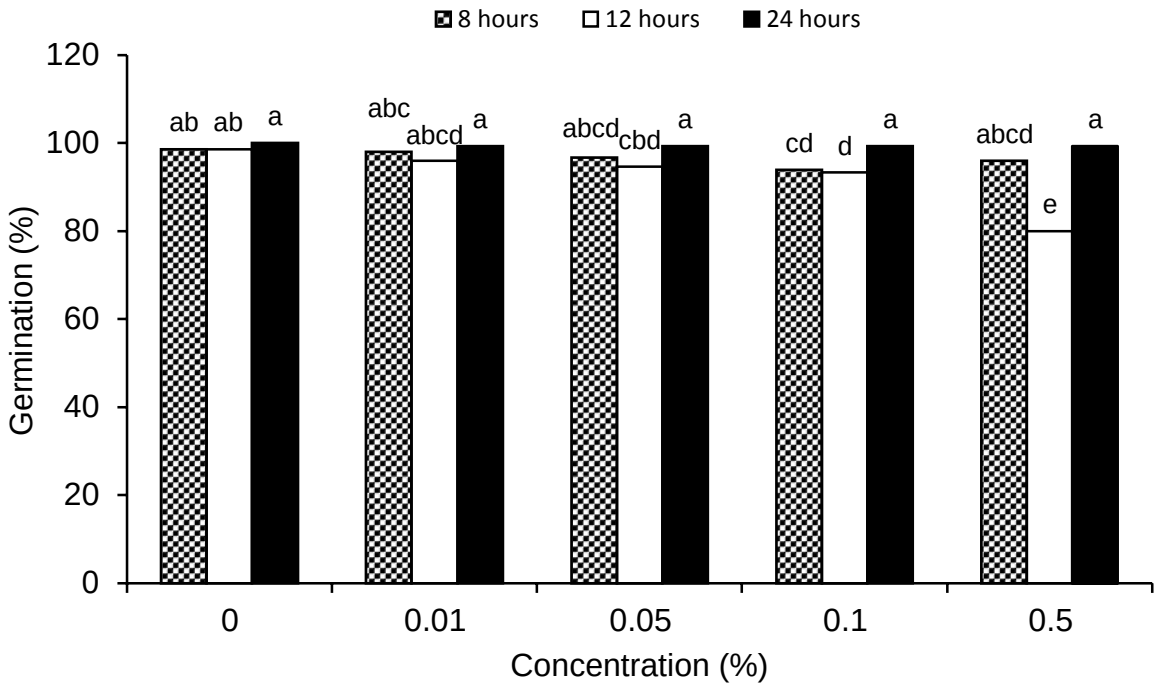


Figure 4.10: Effect of duration and molybdenum concentrations on final germination percentage.

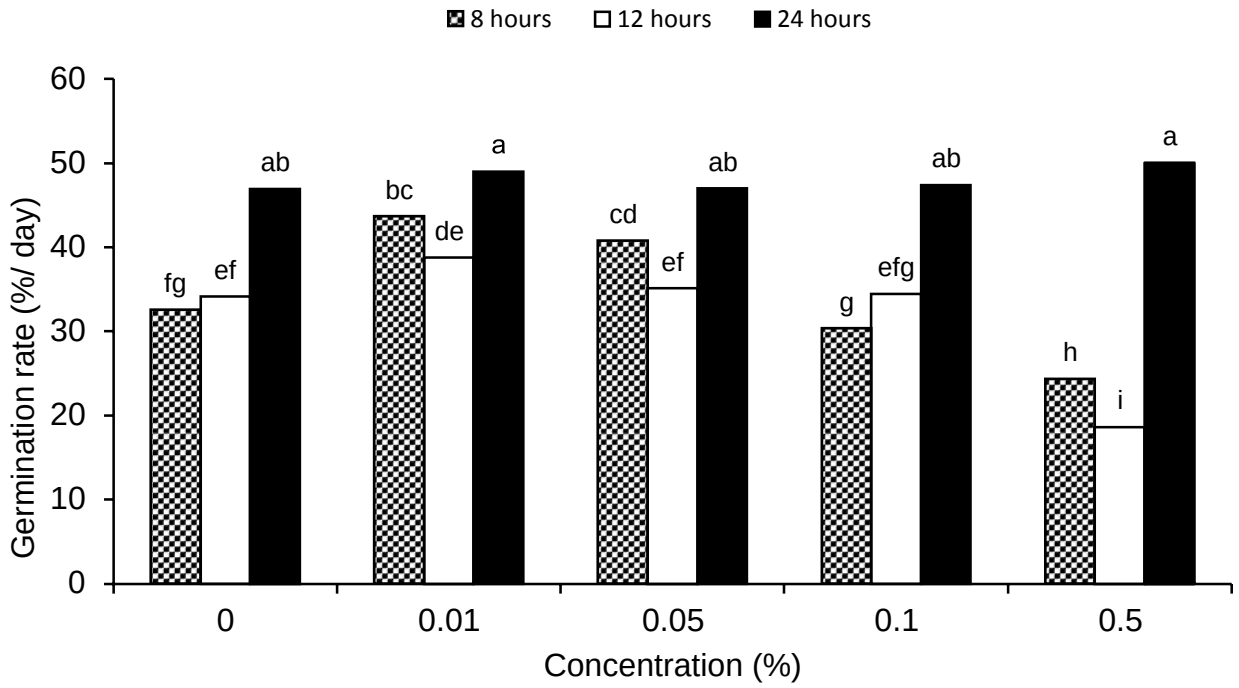


Figure 4.11: Effect of duration and molybdenum concentrations on germination rate.

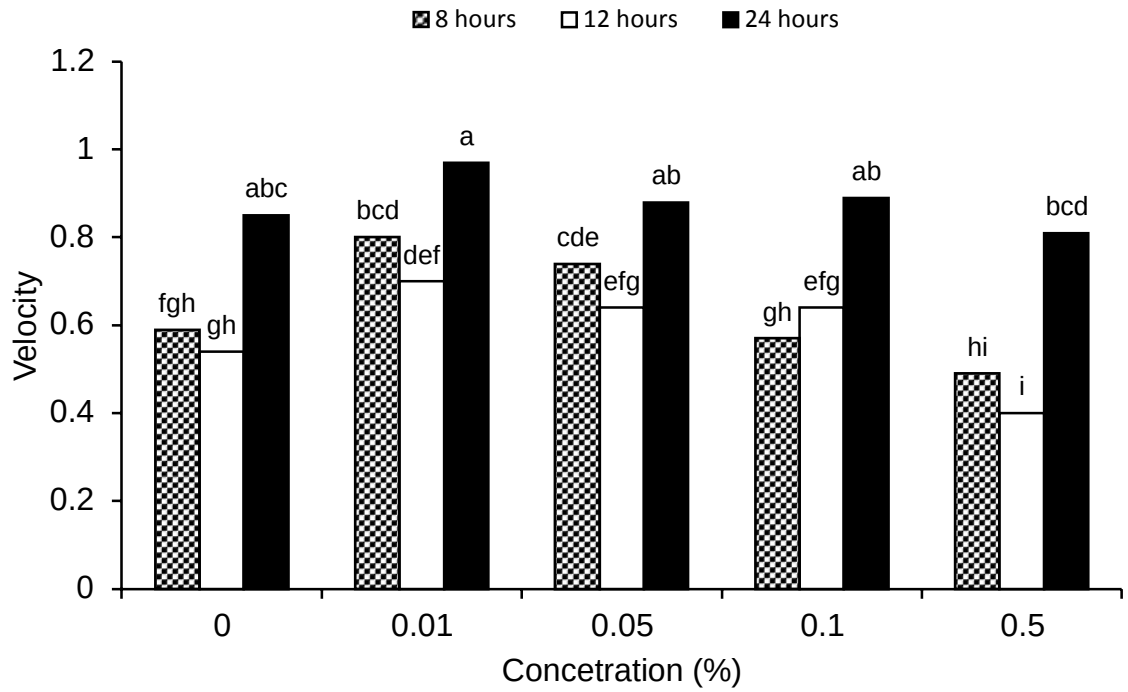


Figure 4.12: Effect of duration and molybdenum concentration levels on the coefficient velocity of germination.

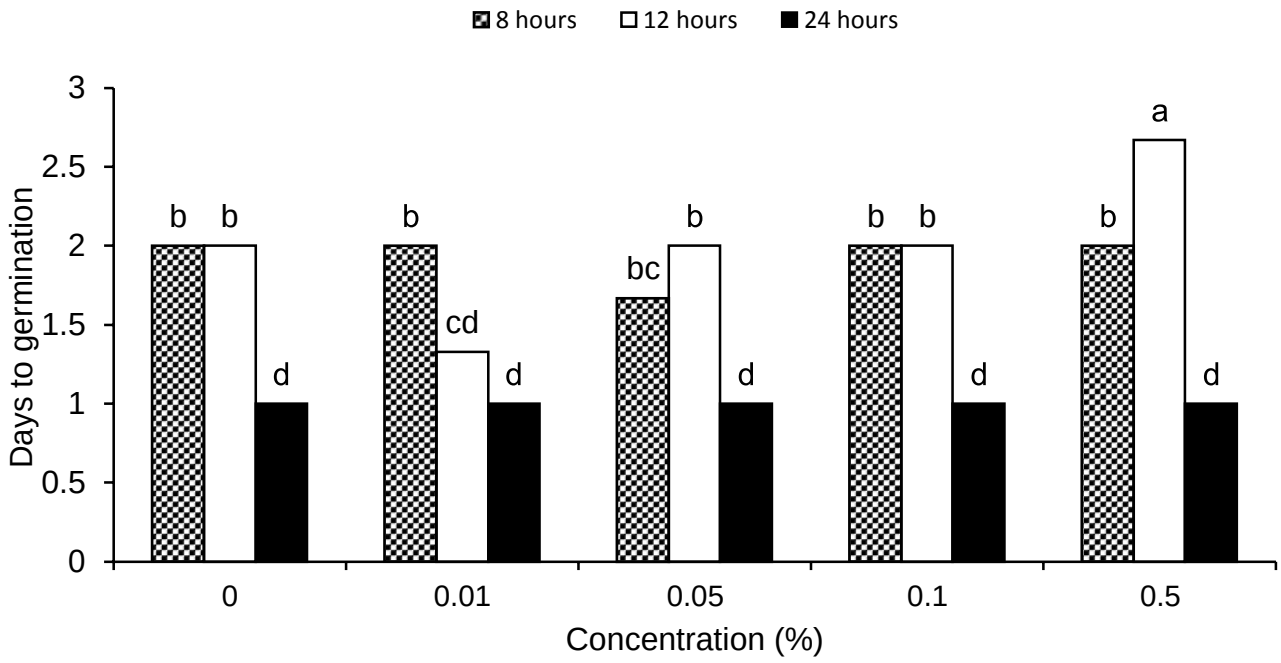


Figure 4.13: Effect of duration and molybdenum concentration levels on days to germination.

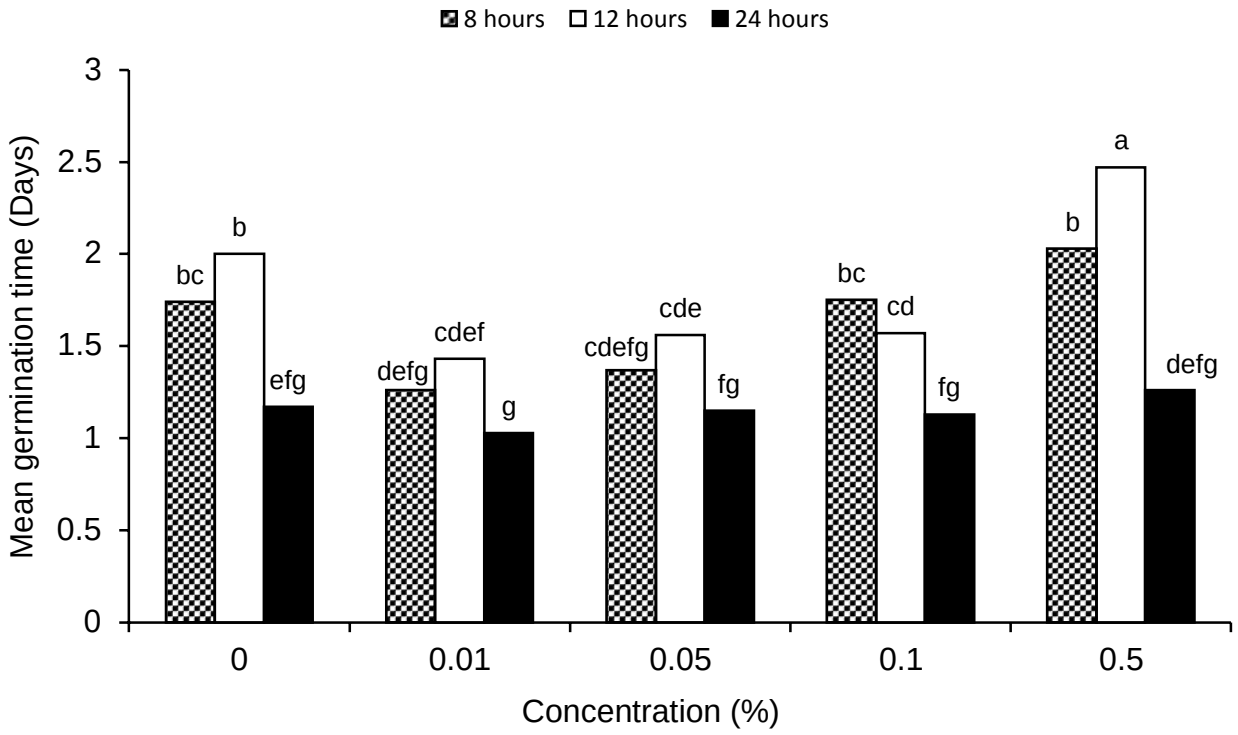


Figure 4.14 Effect of duration and molybdenum concentration levels on mean germination time.

## 4.2. Results of the glasshouse experiment

### 4.2.1. Micronutrient seed priming on maize

The main effects i.e. duration and concentration had significant ($P < 0.05$) effects on days to emergence (DE), seedling wet weight (WW), dry weight (DY), chlorophyll and final plant height. While the effect of interaction between duration and concentration levels on seedling weight, seedling height and roots length (RL) was statistically significant ($P < 0.05$). Meanwhile, the interaction between concentration, nutrients and duration on DE, WW, DW, chlorophyll, stem diameter, plant height and RL was not statistically significant ($P > 0.05$) (Table 4.4). The seeds primed with 0.01% Zn for 24 h emerged the earliest (after 3 days) while seeds primed with water and non-primed seed were the last to emerge, after 9.22 and 10.33 days respectively. The effects of interaction of concentration levels and duration are shown in Figure 15. The seeds primed at 0.01% for 24 h had the heaviest wet and dry weight, highest height and longest root length (Figure 15). The seeds primed with

0.1% at 12 h had the lowest wet and dry weight while the seeds primed with water (0%) for 24 h had the shortest height and root length (Figure 15). The non-primed seeds and those primed with water only resulted with short final roots lengths as compared to primed seeds (Figure 4.16). The seeds primed with 0.1% Zn for 24 h resulted in the longest roots length (49.82 cm) and the shortest was for non-primed seeds at 16.18 cm. Nevertheless, seeds primed with 0.01% Mo and Zn for 24 h recorded the second and third longest RL of 48.49 and 48.06 cm respectively. No significant difference between the RL of the seeds primed with Zn, Mo and B were found (Figure 4.17).

Table 4.4: ANOVA for the effect of concentration levels, duration and interaction of concentrations levels and duration period for seed priming on maize in glasshouse experiment.

| Source | DF | DE | Seedling weight | | Chlorophyll | | Shoot diameter | | | Seedling height | | | RL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | WW | DW | VI | V3 | VE | VI | V3 | VE | VI | V3 | |
| Rep | 2 | 0.3107 | 0.4911 | 0.2920 | 0.1681 | 0.1201 | 0.8591 | 0.5207 | 0.3518 | 0.2646 | 0.2505 | 0.1844 | 0.1216 |
| N | 2 | 0.9492 | 0.3802 | 0.7954 | 0.0184* | 0.2637 | 0.9375 | 0.1845 | 0.5583 | 0.1925 | 0.1682 | 0.2830 | 0.6336 |
| C | 3 | <.0001* | 0.0037* | 0.0002* | 0.0335* | 0.4320 | 0.0183* | 0.0003* | 0.2006 | 0.0045* | <.0001* | <.0001* | 0.0032* |
| D | 1 | <.0001* | <.0001* | <.0001* | 0.6788 | 0.3995 | 0.8370 | 0.0003* | 0.0120* | 0.1409 | <.0001* | <.0001* | 0.4701 |
| N x C | 6 | 0.5398 | 0.6707 | 0.5078 | 0.1172 | 0.3471 | 0.7491 | 0.6912 | 0.8282 | 0.7466 | 0.9499 | 0.5112 | 0.6989 |
| N x D | 2 | 0.6585 | 0.3428 | 0.2560 | 0.0763 | 0.4042 | 0.2586 | 0.0751 | 0.4318 | 0.2966 | 0.1742 | 0.1973 | 0.1558 |
| C x D | 3 | 0.0612 | 0.0489* | 0.0004* | 0.0698 | 0.1817 | 0.1121 | 0.0002* | 0.3119 | 0.0251* | 0.0004* | <.0001* | 0.0005* |
| C x D x N | 6 | 0.6303 | 0.8375 | 0.3449 | 0.4165 | 0.9554 | 0.5375 | 0.5017 | 0.9874 | 0.5417 | 0.5012 | 0.8496 | 0.3455 |

*Pr> F = < 0.0001 *. significantly different (*), Nutrients(N), concentarions (C), duration (D), days to emergence (DE), wet weight (WW), dry weight (DW), root length (RL), boron (B), Zinc (Zn), Molybdenum (Mo) concentration (Conc) and hours (h) maize stages (VE, VI and V3) .*

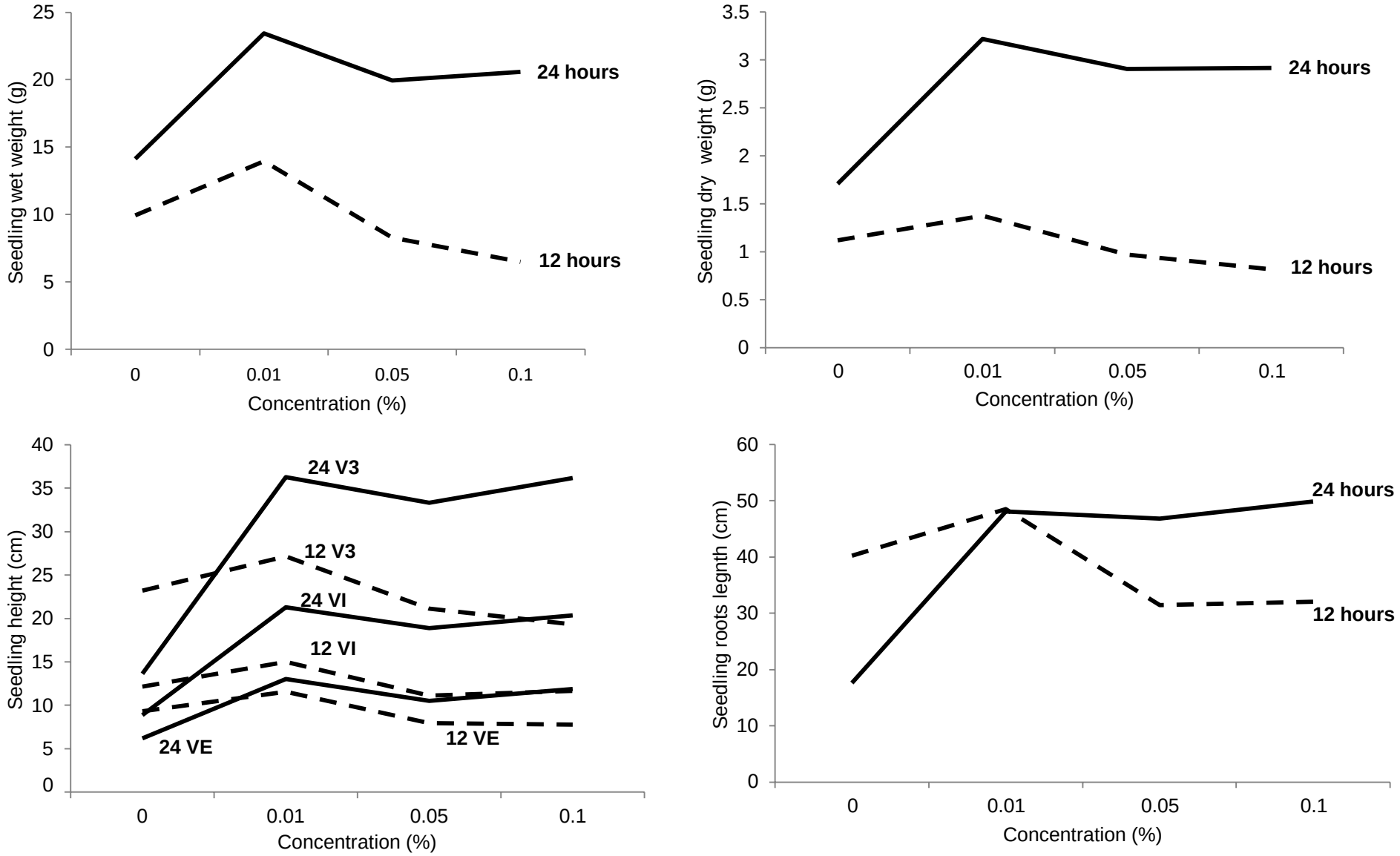


Figure 15: Effect of interaction of concentration and duration on seedlings wet and dry weight, height and roots length.

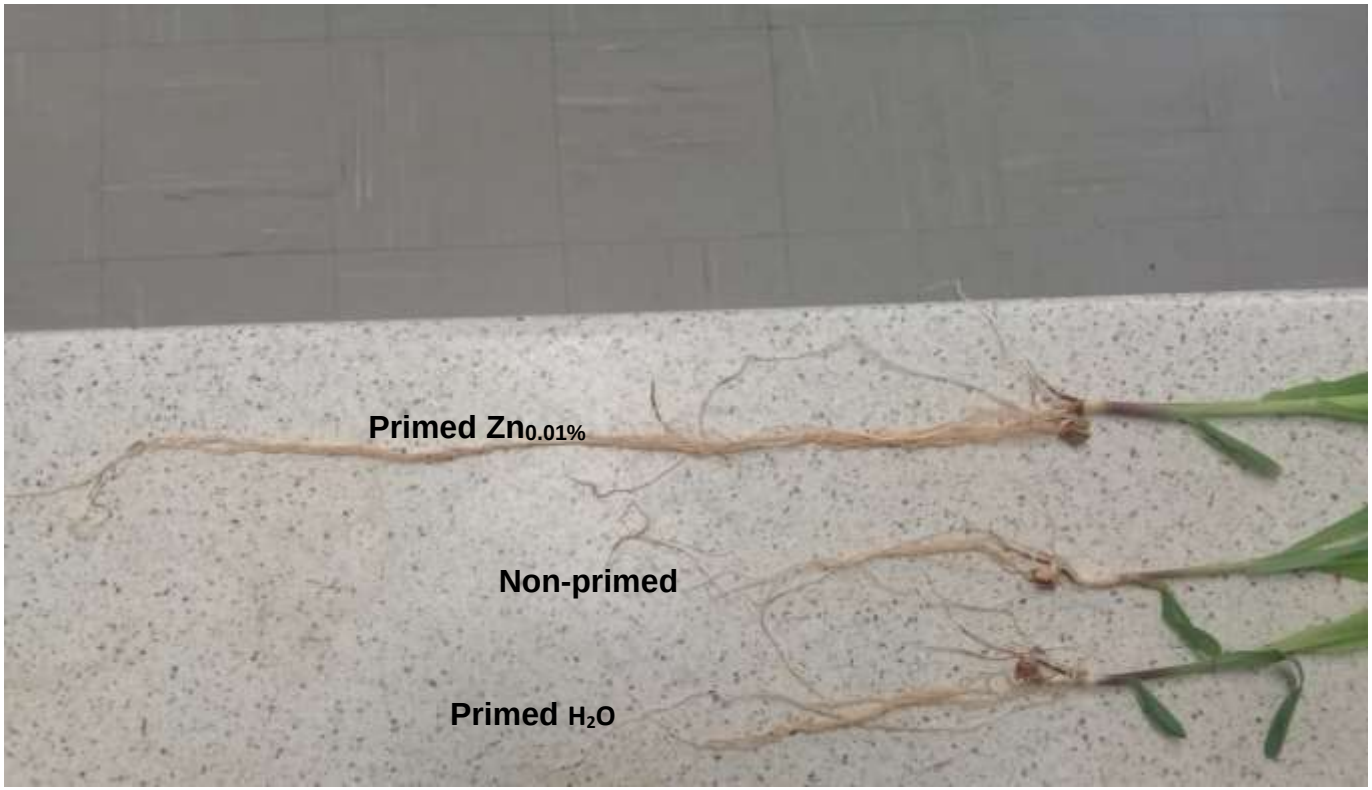


Figure 4.16: Root length of maize seeds primed with water, non-primed and primed with nutrient ($Zn_{0.01}$).


Figure 4.17: Root length of maize seeds primed with 0.01% of zinc, molybdenum and boron.

## 4.3. Results of the field experiment

### 4.3.1. Effect of micronutrient seed priming on maize growth and yield

Nutrient seed priming had statistically significant effects ($P < 0.05$) on only two of all the measured variables i.e. a number of rows per cob and days to emergence (Table 4.5). However, seeds primed with 0.05% of B, Mo and Zn recorded overall better results for all maize growth parameters. Seeds primed with 0.05% Zn emerged earliest (after 7.33 days) and recorded the highest overall plant height throughout the stages of growth (Table 4.5). Nonetheless non – primed seeds and seeds primed with water emerged latest and recorded the shortest heights throughout the growth period. Seeds primed with 0.05% B tended to have a higher GP than the rest. Seeds primed with water recorded the highest dry shoot biomass of 5.53 g at the V8-V9 stage.

Seeds primed with 0.05% (B) emerged after 10 days had the highest final plant height (2.06 m), chlorophyll content of 16.89, 38.01 and 29.67 µmol. $m^2$ (V8 – V9, V16 – VT and T – F respectively) and heaviest dry shoot biomass of 5.43 g. While seeds primed with 0.05% Mo emerged, after 9.33 days and had 66.67% GP, final plant height of 1.76 m, chlorophyll content of 21.60, 39.59 and 28.0 µmol. $m^2$ (V8 – V9, V16 – VT and T – F respectively) and dry shoot biomass of 3.43 g. Nonetheless seed primed with 0.05% Zn emerged earliest after 7.33 days and observed 79.67% GP, final plant height of 1.98m, chlorophyll content of 17.84, 37.45 and 24.87 µmol. $m^2$ (V8 – V9, V16 – VT and T – F respectively) and dry shoot biomass of 4.30 g. Seeds, which were not primed emerged the latest after 16.33 days followed by seeds primed with water (Table 4.5). However, seeds primed with B, Mo and Zn with 0.05% concentration level recorded overall better results for maize growth parameters (Table 4.5).

The effect of NSP treatment on all maize yield parameters was not significant ($P > 0.05$) (Table 4.6). Even though there was no significant effect for seeds primed with 0.05% Mo the highest prolificacy (74.07%) and yield (6438 kg/ha) was achieved for seeds primed with 0.05% Mo. Meanwhile seeds not primed had a prolificacy of 70.37

and the lowest yield of 4417.80 kg/ha. The highest dry biomass was for seeds primed with 0.1% Zn and the lowest was for seeds primed with water ($B_0$) (Table 4.6). The seeds primed with 0.1% B had the highest number of rows per cob, grain per row and 100 seed weight and those primed with 0.05% B had the highest harvest index and cob length. Lastly, the seeds which were not primed (NA) had the lowest harvest index, cob length, number of rows per cob and grain per row.

### 4.3.2. The effect of micronutrients seed priming on NPK content of maize grains and leaves

The effect of nutrient, concentrations and interaction between nutrient and concentrations were not significantly different ($P > 0.05$) for the final values nitrogen (N), phosphorus (P) and potassium (K) in grains and leaves (Table 4.7). Nonetheless, seeds primed with micronutrients resulted in higher levels of NPK in grains and leaves than seeds primed water and non-primed. Seeds primed with 0.05% B had the highest N content of 1.69% in grains and the lowest was 1.11% for seeds that were not primed. The non-primed seeds also resulted in the lowest final value of P and K for grains (0.13 and 0.09% respectively) and leaves (0.16 and 1.47% respectively) also. The seeds primed with water resulted in the lowest leaf N content of 2.64%. Meanwhile, nitrogen content in the leaves was the highest (3.39%) for seeds primed with B at 0.1%, Mo at 0.05 and 0.1%. Seeds primed with 0.05% Zn and 0.05% B resulted in the highest P and K content in leaves respectively. Finally grain content for P and K was highest at 0.33 and 0.47% for seeds primed with 0.1% B.

Table 4.5: Effect of nutrient seed priming with zinc, boron and molybdenum on selected maize growth parameters.

| Treatment | DE | GP | Plant height (m) | | | | Chlorophyll | | | Dry shoot biomass (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | V8-V9 | V16-VT | T-F | F-P | V8-V9 | V16-VT | T-F | V8-V9 |
| $B_0$ | $13.67^{b}$ | $68.00^{a}$ | $0.28^{a}$ | $0.85^{ab}$ | $1.78^{a}$ | $1.79^{a}$ | $17.90^{abc}$ | $32.87^{ab}$ | $26.86^{a}$ | $3.60^{a}$ |
| $B_{0.05}$ | $10.00^{cd}$ | $80.00^{a}$ | $0.28^{a}$ | $0.94^{a}$ | $1.90^{a}$ | $2.06^{a}$ | $16.89^{abc}$ | $38.01^{ab}$ | $29.67^{a}$ | $5.43^{a}$ |
| $B_{0.1}$ | $10.33^{cd}$ | $68.00^{a}$ | $0.28^{a}$ | $0.84^{ab}$ | $1.74^{a}$ | $1.83^{a}$ | $19.39^{abc}$ | $31.99^{ab}$ | $25.67^{a}$ | $5.00^{a}$ |
| $Mo_0$ | $14.00^{b}$ | $73.67^{a}$ | $0.26^{a}$ | $0.80^{ab}$ | $1.95^{a}$ | $1.86^{a}$ | $19.99^{ab}$ | $33.71^{ab}$ | $25.98^{a}$ | $3.76^{a}$ |
| $Mo_{0.05}$ | $9.33^{d}$ | $66.67^{a}$ | $0.27^{a}$ | $0.98^{a}$ | $1.76^{a}$ | $1.76^{a}$ | $21.60^{a}$ | $39.59^{a}$ | $28.80^{a}$ | $3.43^{a}$ |
| $Mo_{0.1}$ | $11.00^{c}$ | $69.33^{a}$ | $0.27^{a}$ | $0.92^{a}$ | $1.84^{a}$ | $1.87^{a}$ | $17.38^{abc}$ | $35.60^{ab}$ | $26.94^{a}$ | $3.67^{a}$ |
| NA | $16.33^{a}$ | $74.33^{a}$ | $0.26^{a}$ | $0.70^{b}$ | $1.76^{a}$ | $1.93^{a}$ | $14.03^{c}$ | $29.32^{b}$ | $33.19^{a}$ | $4.27^{a}$ |
| $Zn_0$ | $13.33^{b}$ | $76.00^{a}$ | $0.24^{a}$ | $0.87^{ab}$ | $1.84^{a}$ | $1.86^{a}$ | $15.89^{bc}$ | $33.61^{ab}$ | $27.09^{a}$ | $5.53^{a}$ |
| $Zn_{0.05}$ | $7.33^{e}$ | $79.67^{a}$ | $0.29^{a}$ | $0.95^{a}$ | $1.96^{a}$ | $1.98^{a}$ | $17.84^{abc}$ | $37.45^{ab}$ | $24.87^{a}$ | $4.30^{a}$ |
| $Zn_{0.1}$ | $10.00^{cd}$ | $70.00^{a}$ | $0.25^{a}$ | $0.89^{ab}$ | $1.71^{a}$ | $1.87^{a}$ | $16.23^{abc}$ | $32.85^{ab}$ | $28.56^{a}$ | $3.07^{a}$ |
| Pr> F | <0.0001 * | 0.5480 | 0.7381 | 0.2212 | 0.4924 | 0.7591 | 0.2606 | 0.4180 | 0.8171 | 0.6908 |

*Pr> F = < 0.0001* Means not connected by same letter are significantly different (α = 0.05, t = 2.10092). NA = Not primed, DE = Days to emergence, GP = Germination percentage, V8 – V9 = four weeks after planting, V16 – VT = 10 weeks after planting, T – F = Tasseling and flowering stages.*

Table 4.6: Effect of nutrient seed priming with zinc, boron and molybdenum on maize yield parameters.

| Treatment | Prolificacy (%) | Cob length (m) | Rows per cob | Grains per row | 100 Seed weight (kg) | Seed Moisture (%) | Grain Yield (kg/ha) | Dry biomass (kg/ha) | Harvest index |
|---|---|---|---|---|---|---|---|---|---|
| $B_0$ | 55.56$^{a}$ | 0.19$^{ab}$ | 12.33$^{a}$ | 33.67$^{ab}$ | 0.037$^{a}$ | 12.67$^{abc}$ | 4283.21$^{a}$ | 6656.01$^{a}$ | 0.64$^{ab}$ |
| $B_{0.05}$ | 66.67$^{a}$ | 0.19$^{ab}$ | 12.67$^{a}$ | 32.67$^{ab}$ | 0.037$^{a}$ | 13.17$^{ab}$ | 4959.60$^{a}$ | 7349.68$^{a}$ | 0.67$^{ab}$ |
| $B_{0.1}$ | 62.96$^{a}$ | 0.19$^{ab}$ | 13.00$^{a}$ | 34.00$^{ab}$ | 0.039$^{a}$ | 12.97$^{abc}$ | 4609.20$^{a}$ | 6877.01$^{a}$ | 0.67$^{ab}$ |
| $Mo_0$ | 70.37$^{a}$ | 0.18$^{b}$ | 13.00$^{a}$ | 33.00$^{ab}$ | 0.039$^{a}$ | 12.33$^{bc}$ | 5322.53$^{a}$ | 7993.95$^{a}$ | 0.66$^{ab}$ |
| $Mo_{0.05}$ | 74.07$^{a}$ | 0.19$^{ab}$ | 12.67$^{a}$ | 36.00$^{a}$ | 0.038$^{a}$ | 13.13$^{ab}$ | 5682.44$^{a}$ | 8282.99$^{a}$ | 0.69$^{a}$ |
| $Mo_{0.1}$ | 66.67$^{a}$ | 0.19$^{ab}$ | 12.67$^{a}$ | 36.67$^{a}$ | 0.039$^{a}$ | 12.03$^{c}$ | 5020.31$^{a}$ | 7572.33$^{a}$ | 0.65$^{ab}$ |
| NA | 70.37$^{a}$ | 0.18$^{b}$ | 10.00$^{b}$ | 29.33$^{b}$ | 0.040$^{a}$ | 12.80$^{abc}$ | 4417.80$^{a}$ | 7298.98$^{a}$ | 0.61$^{b}$ |
| $Zn_0$ | 66.67$^{a}$ | 0.19$^{ab}$ | 12.06$^{a}$ | 35.00$^{ab}$ | 0.037$^{a}$ | 12.37$^{bc}$ | 4801.90$^{a}$ | 7170.95$^{a}$ | 0.66$^{ab}$ |
| $Zn_{0.05}$ | 66.67$^{a}$ | 0.19$^{ab}$ | 12.67$^{a}$ | 37.00$^{a}$ | 0.038$^{a}$ | 12.07$^{c}$ | 5559.73$^{a}$ | 8363.51$^{a}$ | 0.66$^{ab}$ |
| $Zn_{0.1}$ | 70.37$^{a}$ | 0.20$^{a}$ | 13.00$^{a}$ | 37.67$^{a}$ | 0.039$^{a}$ | 13.60$^{a}$ | 6438.97$^{a}$ | 9544.97$^{a}$ | 0.67$^{ab}$ |
| Pr> F | 0.8514 | 0.2558 | 0.0003 | 0.2129 | 0.6507 | 0.0593 | 0.7611 | 0.7377 | 0.6932 |

*Pr> F = < 0.0001* Means not connected by same letter are significantly different (α = 0.05, t = 2.10092). NA = Not primed, DE = Days to emergence, GP = Germination percentage, V8 – V9 = four weeks after planting, V16 – VT = 10 weeks after planting, T – F = Tasseling and flowering stages.*

Table 4.7: The effect of nutrients seed priming with Mo, B and Zn on maize grains and leaves NPK content.

| Treatments levels | Grains | | | Leaves | | |
|---|---|---|---|---|---|---|
| | N % | P % | K % | N % | P % | K % |
| Non-primed | 1.11$^{c}$ | 0.13$^{b}$ | 0.09$^{b}$ | 2.68$^{ab}$ | 0.16$^{b}$ | 1.47$^{b}$ |
| Water | 1.28$^{c}$ | 0.19$^{ab}$ | 0.10$^{b}$ | 2.64$^{ab}$ | 0.21$^{ab}$ | 1.55$^{b}$ |
| Zn (0.05%) | 1.49$^{b}$ | 0.26$^{a}$ | 0.35$^{a}$ | 2.99$^{ab}$ | 0.35$^{a}$ | 2.50$^{a}$ |
| Zn (0.1%) | 1.46$^{b}$ | 0.27$^{a}$ | 0.34$^{a}$ | 3.03$^{a}$ | 0.33$^{a}$ | 2.34$^{a}$ |
| B (0.05%) | 1.69$^{a}$ | 0.28$^{a}$ | 0.32$^{a}$ | 3.14$^{a}$ | 0.32$^{a}$ | 2.56$^{a}$ |
| B (0.1%) | 1.46$^{b}$ | 0.33$^{a}$ | 0.47$^{a}$ | 3.09$^{a}$ | 0.32$^{a}$ | 2.19$^{ab}$ |
| Mo (0.05%) | 1.57$^{ab}$ | 0.27$^{a}$ | 0.34$^{a}$ | 3.39$^{a}$ | 0.33$^{a}$ | 2.42$^{a}$ |
| Mo (0.1%) | 1.62$^{a}$ | 0.27$^{a}$ | 0.36$^{a}$ | 3.09$^{a}$ | 0.31$^{a}$ | 2.32$^{a}$ |
| | Prob > F | | | | | |
| Nutrient | 0.2194 | 0.6027 | 0.5036 | 0.9433 | 0.9291 | 0.9893 |
| Concentration (%) | 0.4373 | 0.4235 | 0.3305 | 0.9859 | 0.3953 | 0.9915 |
| Nutrient*Concentration (%) | 0.1164 | 0.3549 | 0.3367 | 0.9962 | 0.7902 | 0.9999 |

*Pr> F = < 0.0001* Means not connected by same letter are significantly different (α = 0.05, t = 2.10092). Zinc (Zn), boron (B), molybdenum (Mo), nitrogen (N), phosphorus (P) and potassium (K).*

## 4.4. Discussion of the laboratory, glasshouse and field experiment

### 4.4.1. Effect of micronutrient seeds priming on seed germination

Faster seed germination and emergence is very important for maize growth because it improves its competitive ability against weeds for water and nutrients. Seed priming with micronutrients (Zn, B and Mo) improved mean germination time (MGT), germination percentage (GP), the coefficient velocity of germination (CVG), germination rate (GR) and reduced days to germination (DG). Similarly, Rahman *et al*. (2014) also indicated that higher germination percentage, decreased MGT and increased CVG were obtained for seeds primed with nutrients. Meanwhile soaking seeds in solutions for a longer period at low concentration levels resulted in higher GP, improved CVG, low MGT and high GR. These could be due to the synthesis of DNA, RNA and proteins during NSP (Afzal *et al*., 2008). This finding was further supported by Dezfuli *et al*. (2008) and Yohannes and Abraha (2013) who reported that seeds primed for a longer period at low concentration levels performed better than other treatments. The decrease in germination and germination rate for seeds primed for a longer period at higher concentration levels could be due to increases in nutrient toxicity in the seed coat (Figures 4.1 and 4.2). Toxicity alters the enzymes of the nucleus and metabolism causes protein metabolism to interfere with hormonal balance and cuts the utilization of seed food reserves during germination (Yohnnes and Abraha 2013). Priming duration is a critical factor in NSP technique, thus the longer priming period has improved CVG, DG and MGT. The sensitivity of priming period and improvements on GP, GR, CVG, DG and MGT was highlighted by Murungu *et al* (2005) who noted that “final germination percentage of cotton and maize seed decreased as the water potential was lowered, but the non-primed seed was much more sensitive to moisture stress than primed seed. Whereas Johnson *et al*. (2005) and Guan *et al*. (2009) stated that at higher concentration levels, the maize germination is decreased while at lower concentration they are elevated.

The improvement in GP, GR, CVG, DG and MGT of NSP treated treatments as compared to the control (only water primed), could be due to the increased nutrient content in the seeds. During priming proteins like the beta subunit of the globulin is

increased, lipid peroxidation is reduced and antioxidative is enhanced as they are responsible for germination in the seeds (Elouaer and Hannachi, 2012). After drying the primed seed upon re-absorption of water rapid growth is observed and radicle and plumule appear earlier. Nutrient seed priming technique is applied on a wide range of crops and other beneficial effects were reported in tomato (El-Saifi *et al*., 2010), green-paper (Uche *et al*., 2016), mountain rye (Ansari *et al*., 2013) and fenugreek plant (Soughir *et al*., 2012). Seed germination is a critical stage in maize growth and yield and the application of NSP to improve germination and crop establishment can provide a solution to poor crop establishment and germination in Limpopo province.

### 4.4.2. Effect of micronutrient seed priming on maize seedling growth

Seedling emergence and establishment are the key processes in the survival and growth of plants. Seedling establishment was improved for seeds primed with micro-nutrients as compared to seeds primed with water. Seedlings primed with micro-nutrient solutions resulted in higher, longer, thicker and heavier seedlings. Lizárraga-Paulín *et al*. (2013) and Zeng *et al*. (2012), support these findings with similar conclusions that NSP improved seedling height, length, thickness and weight of maize and soybean respectively. The availability of micronutrients in the seeds is vital for protein synthesis and enzymes responsible for seedlings to effectively utilize the other nutrients in the soil, resulting in improved seed germination and seedling establishments. These can be traced in various reports which indicate the vitality of micronutrients such as Mo and B to effectively use the NPK nutrients by varies crops (Kaiser *et al*., 2005; Singh *et al*., 2014). In addition, these improvements in growth and development of seedlings primed with solutions could adequately be due to the earlier uptake of micronutrients which activated the germination process. Seeds which are soaked in water for a particular duration and dried before seminal root protrusion can develop and grow faster (Sozharajan and Natarajan, 2014). Also, seed priming sometimes decreases the basic water potential towards more negative values, increasing the ability of the seed to germinate under lower water availability (Zahedifar and Zohrabi, 2016). There is an increase in the root length of seeds primed with micronutrients and this could be due to activation of cell respiration and cycling during priming. Activation of cell respiration and cycling, repair of

macromolecules, assimilated materials translocation and weakening of seed coat structure for faster root emergence (Vasquez-Ramos and Sanchez, 2004; Cantliffe *et al*., 1984).

### 4.4.3. Effect of micronutrient seed priming on maize growth and yield

The difference which, occurs in the final values of yield and growth parameters between the primed and non-primed seeds is accounted for by access to the micronutrients by seeds at early stages of development. This is consistent with the conclusions by Al-Baldawi and Hamza (2017) and Singh *et al*. (2015), who indicated that priming seeds with micronutrient solutions improved germination and yields better than non-primed seeds. The final values of dry biomass, prolificacy, harvest index and grain yield were all statistically not significant although the seeds primed with solutions had slightly higher values than those of seeds primed with water and non-primed seeds (Table 4.6 and 4.7). Similar trends differences between seeds treated with micronutrients solution and water were observed by Murungu *et al*. (2005), El-Saifi *et al*. (2010) and Uche *et al*. (2016). Micronutrients such as Zn and B are responsible for the production of auxin and other growth hormones, which influence germination and other growth parameters. Tsonev and Lidon (2012) indicated that lack of Zn in crops has a physiological impediment in the growth of the seedling. Meanwhile, Guptas and Solonki (2013) showed that seeds which access B at planting have increased chances of faster germination. Whereas access to adequate amounts of Mo by seeds at early stages promotes seedling growth (Bittner, 2014). Therefore, the wellbeing of a crop seedling is the primary building block of much-improved yields and growth.

There was no significant difference in yield parameters of seeds treated with micronutrients, water and non-primed seeds. The crop performance is reliant on factors such as nutrients status (NPK) in the soil, moisture content, management of diseases and pest and on farm conditions. During the 2016/17 growing season there was a national outbreak of army worm (DAFF, 2017). The army worm was well controlled within the plots, nonetheless, it appeared that seeds primed with micronutrients were much more resistant. The tolerance is evident in the data as its shows that seeds primed had slightly higher values of dry biomass, prolificacy,

harvest index and grain yield than those of seeds primed with water and non-primed seed. Dordas (2008) indicated that nutrients availability in plants can assist reduce susceptibility to pest and disease attack to an acceptable level at which further control by other cultural practices or conventional organic biocides can be performed. Nutrient seed priming technique has shown that solely it will not improve crop performance in general. Perhaps the soil macronutrient status and other components such as soil pH and moisture, which affects the ability of a crop to grow, should be optimized as well for NSP to succeed micronutrient deficient soils.

Maize crops are very sensitive to Zn deficiency and Zn plays an important role as a metal component of functional, structural and regulator cofactor of a large number of enzymes (Salem and El-Gizawy, 2012; de Vasconcelos *et al*., 2011). The success of NSP to deliver Zn to the maize seeds for growth period is vital, especially in micronutrient deficient soils. Although the emergence and seedling height were statistically not significant, the seed primed with nutrient and water recorded better results than non-primed seeds. These could be due to the speed at which the primed seeds re-absorb water to regenerate its metabolic activities resulting in faster development of the plumble and radicle of the maize seed (Imran, 2013). Badiri *et al*. (2014) also discussed this effect of priming on germination and emergence. They drew a conclusion that time to emergence was shortened and seed yield was improved for seeds primed with micronutrients solutions of Zn, Fe and Mn. Healthy and fast-growing seedlings are very critical for agricultural yields for maize and lots of factors can limit seedling emergence and growth. The major limiting factors are drought, low soil nutrient content, salinity and high temperature (Mabhaudhi and Modi, 2010; Molatudi and Mariga, 2009).

Overall, seeds which were not primed (NA) recorded the lowest harvest index, cob length, number of rows per cob and grain per row. One important factor to consider is that a micronutrient deficiency especially of B, Zn and Mo affects the efficacy of plants to use NPK (Liu *et al*., 2010; Sarkar *et al*., 2012). Lack of micronutrients in non-primed and water primed seeds promoted low absorption of macronutrients (NPK). This is shown in Table 4.7, where seeds primed had slightly higher values of nutrient residual content even though the treatments effects were not statistically significant. Days to emergence were statistically significant (Table 4.5). The seed

primed with micronutrient emerged earlier, implying that the length of the crop growing period up to maturity is reduced hence cutting the cost that can be incurred by a farmer with prolonged days for the crop to reach maturity. A key factor to farmers in agricultural fields is to maximize yields and growth at the minimum cost of operation and nutrient seed priming could lower the cost of operation.

# CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

## 5.1. Conclusion

The study focused mainly on the recommendation of optimum nutrient seed priming concentration levels necessary to improve the performance of maize crop on micronutrients deficient soil of Limpopo province. The laboratory investigation showed that priming at 0.05, 0.01 and 0.1% concentration levels for 24 and 12 h improved germination parameters (MGT, GP, DG, CVG and GR) compared to seeds primed with water. The concentrations where taken further for glasshouse testing. The glasshouse showed the effect of 24 h of priming duration at 0.05 and 0.01% concentration level improved the seedling growth. The field investigation showed that priming with micronutrients solutions for 24 h improved number of rows per cob and days to emergence. The effect of nutrients seed priming on maize growth and yield parameters were not significant. Meanwhile, the quantity of micronutrients (Zn, Mo and B) in the grain and leaves at harvest was low for seeds not primed and primed with water.

Seed priming with micronutrients (Zn, Mo and B) improved the germination and seedling growth. This implies that with optimum micronutrients concentration levels nutrient seed priming can improve germination and seedling growth. The overall improvement of maize seedlings primed with micronutrients confirms that NSP technique can provide a solution to the problems encountered by farmers. It is important to note that seeds primed at lower concentration levels (0.05, 0.01 and 0.1) with longer duration priming period (24 h) had satisfactory seedling growth and development in both the laboratory and glasshouse experiment. Meanwhile, grain yield, crop establishment and grain nutrient content were not improved by nutrient seed priming using Zn, B and Mo. The evaluation of the effect of nutrients seed priming on maize establishment, grain yield and grain nutrient composition on deficient soils showed that not only micronutrients limit the maximization of the yield parameters; rather there are other external factors which must be improved owing to the poor status of the soil. Nutrient seed priming can provide a strong foundation to farmers under micronutrients deficient soils to maximize maize germination, reduce days to the emergence and lowering financial losses from sowing to seedling

emergence. The farmer would be expected to provide an additional operation to maintain and maximize the yields after seedling emergence.

## 5.2. Recommendations

The data provides the basis for assisting farmers to select optimum concentration level for nutrient seed priming to improve yield in micronutrient deficient soils in and around Limpopo province. The laboratory and glasshouse data also provide useful information to investigate and develop the nutrient seed priming technique for different nutrients, crops and field conditions. One will have to question the financial and return implication of seed priming. Therefore, NSP technique needs further investigation to cover the economic and flexibility of the technique to smallholder farmers in Limpopo and other parts of the country. Nutrient seed priming with appropriate micronutrient (Zn, Mo and B) concentration levels improved germination and seedling vigour. There is also a need to study on whether the technique can increase tolerance/resistance to pests such as army warm. Lastly, the NSP using Zn, Mo and B did not improve grain yield and crop establishment, therefore a farmer will have to provide additional operations such as proper pest control, sufficient irrigation, and adequate macro fertilizer application to maximize the yield.

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## APPENDICES

Appendix 1: Selected pictures taken during the laboratory experiment.

Plate 1: Nutrient primed maize seeds during germination count.

Plate 2: Maize seed drying after priming with micronutrient solutions and water.

Appendix 2: The ANOVA(s) of the effect of nutrient seed priming on seedlings under glasshouse condition.

Table 1: The ANOVA of the effect of nutrient seed priming on days to emergence.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Rep | 2 | 2 | 3.17 | 1.19 | 0.3107 |
| Nutrient | 2 | 2 | 0.13 | 0.05 | 0.9492 |
| Concentration | 3 | 3 | 282.22 | 71.25 | <.0001* |
| Duration | 1 | 1 | 82.49 | 62.48 | <.0001* |
| Concentration *Duration *Nutrient | 6 | 6 | 5.75 | 0.72 | 0.6303 |

*Pr> F = < 0.0001* significantly different (*).*

Table 2: The ANOVA of the effect of nutrient seed priming on seedling wet weight.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Rep | 2 | 2 | 39.92 | 0.72 | 0.4911 |
| Nutrient | 2 | 2 | 54.62 | 0.98 | 0.3802 |
| Concentration | 3 | 3 | 430.12 | 5.18 | 0.0037* |
| Duration | 1 | 1 | 1694.42 | 61.31 | <.0001* |
| Concentration *Duration *Nutrient | 6 | 6 | 75.46 | 0.45 | 0.8375 |

*Pr> F = < 0.0001* significantly different (*).*

Table 3: The ANOVA of the effect of nutrient seed priming on seedling dry weight.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Rep | 2 | 2 | 0.75 | 1.26 | 0.2920 |
| Nutrient | 2 | 2 | 0.14 | 0.23 | 0.7954 |
| Concentration | 3 | 3 | 6.97 | 7.88 | 0.0002* |
| Duration | 1 | 1 | 45.83 | 155.39 | <.0001* |
| Concentration *Duration *Nutrient | 6 | 6 | 2.05 | 1.15 | 0.3449 |

***Pr> F = < 0.0001**** *significantly different (*).*

Table 4: The ANOVA of the effect of nutrient seed priming on chlorophyll.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Chlorophyll at day 22 | | | | | |
| Rep | 2 | 2 | 96.23 | 1.85 | 0.1681 |
| Nutrient | 2 | 2 | 226.87 | 4.37 | 0.0184* |
| Concentration | 3 | 3 | 245.94 | 3.16 | 0.0335* |
| Duration | 1 | 1 | 4.50 | 0.17 | 0.6788 |
| Concentration *Duration *Nutrient | 6 | 6 | 160.75 | 1.03 | 0.4165 |
| Chlorophyll at day 28 | | | | | |
| Rep | 2 | 2 | 217.72 | 2.22 | 0.1201 |
| Nutrient | 2 | 2 | 134.53 | 1.37 | 0.2637 |
| Concentration | 3 | 3 | 137.311 | 0.93 | 0.4320 |
| Duration | 1 | 1 | 35.44 | 0.72 | 0.3995 |
| Concentration *Duration *Nutrient | 6 | 6 | 74.43 | 0.25 | 0.9554 |

*Pr> F = < 0.0001* significantly different (*).*

Table 5: The ANOVA of the effect of nutrient seed priming on the steam diameter.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Steam diameter at day 16 | | | | | |
| Rep | 2 | 2 | 0.0028 | 0.15 | 0.8591 |
| Nutrient | 2 | 2 | 0.0012 | 0.06 | 0.9375 |
| Concentration | 3 | 3 | 0.10 | 3.70 | 0.0183* |
| Duration | 1 | 1 | 0.0004 | 0.04 | 0.8370 |
| Concentration *Duration *Nutrient | 6 | 6 | 0.04 | 0.85 | 0.5375 |
| Steam diameter at day 22 | | | | | |
| Rep | 2 | 2 | 0.007 | 0.66 | 0.5207 |
| Nutrient | 2 | 2 | 0.02 | 1.76 | 0.1845 |
| Concentration | 3 | 3 | 0.12 | 7.56 | 0.0003* |
| Duration | 1 | 1 | 0.08 | 15.19 | 0.0003* |
| Concentration *Duration *Nutrient | 6 | 6 | 0.03 | 0.90 | 0.5017 |
| Steam diameter at day 28 | | | | | |
| Rep | 2 | 2 | 0.03 | 1.07 | 0.3518 |
| Nutrient | 2 | 2 | 0.02 | 0.59 | 0.5583 |
| Concentration | 3 | 3 | 0.08 | 1.61 | 0.2006 |
| Duration | 1 | 1 | 0.11 | 6.85 | 0.0120* |
| Concentration *Duration *Nutrient | 6 | 6 | 0.014 | 0.15 | 0.9874 |

*Pr> F = < 0.0001* significantly different (*).*

Table 6: The ANOVA of the effect of nutrient seed priming on plant height.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| | Plant height at day 16 | | | | |
| Rep | 2 | 2 | 34.29 | 1.37 | 0.2646 |
| Nutrient | 2 | 2 | 42.79 | 1.71 | 0.1925 |
| Concentration | 3 | 3 | 187.55 | 4.99 | 0.0045* |
| Duration | 1 | 1 | 28.12 | 2.25 | 0.1409 |
| Concentration *Duration *Nutrient | 6 | 6 | 63.52 | 0.85 | 0.5417 |
| | Plant height at day 22 | | | | |
| Rep | 2 | 2 | 52.50 | 1.43 | 0.2505 |
| Nutrient | 2 | 2 | 68.21 | 1.86 | 0.1682 |
| Concentration | 3 | 3 | 548.06 | 9.94 | <.0001* |
| Duration | 1 | 1 | 411.66 | 22.39 | <.0001* |
| Concentration *Duration *Nutrient | 6 | 6 | 99.63 | 0.90 | 0.5012 |
| | Plant height at day 28 | | | | |
| Rep | 2 | 2 | 138.32 | 1.76 | 0.1844 |
| Nutrient | 2 | 2 | 102.30 | 1.30 | 0.2830 |
| Concentration | 3 | 3 | 1670.07 | 14.13 | <.0001* |
| Duration | 1 | 1 | 879.24 | 22.32 | <.0001* |
| Concentration *Duration *Nutrient | 6 | 6 | 103.49 | 0.44 | 0.8496 |

*Pr> F = < 0.0001* significantly different (*).*

Table 7: The ANOVA of the effect of nutrient seed priming on final root length.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Rep | 2 | 2 | 937.37 | 2.21 | 0.1216 |
| Nutrient | 2 | 2 | 195.67 | 0.46 | 0.6336 |
| Concentration | 3 | 3 | 3387.62 | 5.32 | 0.0032* |
| Duration | 1 | 1 | 112.62 | 0.53 | 0.4701 |
| Concentration *Duration *Nutrient | 6 | 6 | 1474.36 | 1.16 | 0.3455 |

***Pr> F = < 0.0001**** *significantly different (*).*

Appendix 3: Selected pictures taken during the glasshouse study.

Plate 3: Maize plant at the three leaf (V3) stage.

Plate 4: Roots evaluation and measuring after unrooting.

Appendix 4: Field layout of the experiment in Ofcolaco for season 2016/17.

**Field map: OFCOLACO**

| $R_1$ | $R_2$ | $R_3$ |
|---|---|---|
| $Mo_{0.1}$ 1 | $Mo_0$ 11 | $B_{0.05}$ 21 |
| $Zn_{0.05}$ 2 | NA 12 | $Zn_0$ 22 |
| $Zn_{0.1}$ 3 | $B_0$ 13 | $Zn_{0.05}$ 23 |
| $Mo_{0.05}$ 4 | $B_{0.05}$ 14 | $Mo_{0.1}$ 24 |
| NA 5 | $Zn_{0.05}$ 15 | NA 25 |
| $Zn_0$ 6 | $Mo_{0.05}$ 16 | $Mo_{0.05}$ 26 |
| $Mo_0$ 7 | $Zn_{0.1}$ 17 | $B_{0.1}$ 27 |
| $B_{0.05}$ 8 | $B_{0.1}$ 18 | $Mo_0$ 28 |
| $B_0$ 9 | $Zn_0$ 19 | $Zn_{0.1}$ 29 |
| $B_{0.1}$ 10 | $Mo_{0.1}$ 20 | $B_0$ 30 |

*N.B. Plot size = 3 m x 4 m. Number of plots = 30. Space between the plots = 1 m. Borders = 2 m from the plots. Total area = 20 m x 53 m. Boron = B, Zinc = Zn, Molybdenum = Mo and NA= normal aPplication. Concentrations = 0, 0.01, 0.05, 0.1 and 0.5. Replication = R. Plot number = 1, 2, 3, ……., 30.*

Appendix 5: Selected pictures taken during the field study.

Plate 5: Maize plant at V8 – V9 stage.

Plate 6: Maize plant at tasseling stage.