UNIVERSITY OF CAPE COAST

AGRONOMIC ZINC BIOFORTIFICATION OF MAIZE (*Zea mays*) AND CARROT (*Daucus carota*) FOR IMPROVED FOOD AND NUTRITIONAL SECURITY

GODFRED OKYERE-PRAH

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BY

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A thesis submitted to the Department of Crop Science of the School of Agriculture, College of Agriculture and Natural Science, University of Cape Coast, in partial fulfilment of the requirements for the award of a Master of Philosophy degree in Crop Science

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my original research, and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: …….. Date: ……..

Name: ……

Supervisor's Declaration

We hereby declare that the preparation and presentation of the thesis were supervised per the guidelines for supervision of the thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: …… Date: ……..

Name: ……

Co-Supervisor's Signature: …… Date: ……..

Name: ……

ABSTRACT

Micronutrient inadequacies, especially those linked to zinc, pose a significant worldwide public health issue, particularly in low-income nations such as Ghana. Improving the zinc content of primary grains and vegetables that form dietary staples through agricultural biofortification provides an economical solution to combat this problem. The study explored the impact of zinc concentration, timing, and fertilisation method on the yield and uptake efficiency of maize and carrots. The study used a 3-Factoral experiment in a Randomized Complete Block Design with fertilisation rate, stage/time, and method being the experimental factors. The current research in maize demonstrated that zinc concentration had a positive impact on physiological parameters, particularly Fv/Fm ratio, with an increase of over 11 % at a dosage of 6 kg/ha compared to the control. However, the timing and method of applying zinc fertiliser did not directly affect the physiology and growth of maize. Despite the evident link between zinc levels and yield parameters, applying it at 8 kg/ha led to a decreased cob weight, grain weight, and overall maize yield. It is noteworthy that a substantial increase of 52 % in cob weight and 28 % in yield was observed with 6 kg/ha zinc fertilisation relative to the control group. The timing of fertilisation had negligible impacts on most measured physiological and yield parameters in maize; however, there was a notable 15 % increase in cob weight when applied before flowering compared to during grain-filling. Additionally, application of zinc at 8 kg/ha had minimal impact on both physiology and yield traits. Nevertheless, a 26 % increase in grain zinc concentration was observed under 8 kg/ha fertilisation relative to the control. The findings revealed that, foliar zinc fertilisation increased grain zinc concentration of grains by 15.8 % compared to soil application. Also, zinc fertilisation at grain filling improved gain zinc concentration by 16 % compared to pre-anthesis. In carrots, method of application had an insignificant effect on yield and growth. However, there was a progressive increase in yield corresponding to higher zinc fertilisation rates. Typically, a double-fold increase in yield was recorded under 6 kg/ha concentration. Also, a 58 % and 14 % increase in root length and yield were recorded at 30 DAS compared to 50 and 70 DAS. Similarly, root zinc concentration exhibited a positive response with increasing zinc concentration with 30.6 mg/kg and 31.6 µg/g root and shoot zinc concentration recorded at 6 kg/ha level. Application at 30 DAS had a pronounced increase in shoot and root zinc concentration compared to 50 and 70 DAS application time. Hence, it is clear, that zinc agro-biofortification could play a significant role in addressing micronutrient inadequacy however, this is contingent on rate of application and time of application. Although application at 8 kg/ha could be lethal or show diminishing marginal returns regarding growth and yield, however, this significantly increased the concentration of zinc in maize. These findings have profound implications for nutritional security and the fight against hidden hunger, particularly in regions where staple crops are the primary sources of essential micronutrients.

KEYWORDS

Maize

Carrots

Zinc deficiency

Hidden hunger

Agronomic biofortification

Zinc

Fertilisation

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DEDICATION

Dedicated to my wife, family, and friends.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Background to the study

Globally, human beings are concurrently confronted with the following challenges from agriculture: ensuring food security, conserving natural wealth, and maintaining environmental health (Tilman et al., 2002). Another concealed and neglected challenge involves increasing the micronutrient concentrations in the edible portions of crop plants to mitigate the prevalent deficiency of essential micronutrients, especially in low-income countries (Bouis & Welch, 2010). Most of the world's population cannot meet their daily micronutrient requirement (Masuda et al., 2020). About 2 million individuals worldwide experience human nutrient deficiencies, or "hidden hunger" (Fongar et al., 2019). In humans, micronutrient deficiencies often go undetected, but they significantly affect immunity and physical and mental growth to a large extent (Ritchie & Roser, 2017). Hidden hunger can adversely affect individuals adhering to dietary limitations due to health, religious, or ethical concerns (Sharma & Verma, 2019). Globally, an estimated 33 % of women of childbearing age suffer from anaemia, which puts them at a greater risk of malnutrition (Mirza et al., 2018).

Zinc is indispensable in human nutrition and the production of crops (Malakouti, 2007). In humans, zinc supports immunity, bone mineralization, tissue growth, sperm production and fertility, as well as protein and synthesis of DNA (Gupta et al., 2019). Additionally, zinc has preventive and therapeutic properties against viruses and bacteria, especially during the COVID-19 pandemic

(Read et al., 2019). Thus, ensuring adequate zinc consumption is essential to preserve optimal human health.

Despite the importance of zinc to human health, its deficiency is a prevalent issue that affects global health and is recognized as the second most widespread nutrient deficiency worldwide (Haider et al., 2021). About 17 % of people worldwide suffer from zinc deficiency, making it one of the most common micronutrient deficits (Chasapis et al., 2020; Hacisalihoglu, 2020). Low and middle incomes (LMICs) countries suffer more from zinc deficiency (Ohly et al., 2019). In developing countries, zinc deficiency ranks fifth in diseases and mortality (Das, et al., 2019). It has been reported that children with insufficient zinc levels experience stunted growth, pneumonia, and diarrhoea. The mortality of infants is notably affected by these last two conditions. (Gupta et al., 2020). It is estimated that around 60-70 % of Asian and Sub-Saharan African populations could suffer from low levels of zinc intake (Das et al., 2019). The amount of zinc consumed by the Ghanaian population is inadequate (Ayensu et al., 2020).

Globally, human zinc deficiency is on the rise, mainly caused by the low intake of bioavailable zinc diets (Gupta et al., 2020). It has been reported that about 50 % of agricultural soils across the globe contain low levels of available zinc (Suganya et al., 2020). Similarly, a review of relevant agronomic literature indicates that Ghanaian soils are deficient in micronutrients (Asiedu-Amoako et al., 2016). Among the micronutrients most deficient in Ghanaian soils are zinc, iron, and manganese (Elias et al., 2017). This invariably results in reduced micronutrient levels in the consumable parts of plants and decreases crop

productivity. Therefore, it is unsurprising that there is a correlation between human zinc deficiency and the geographical distribution of soils with limited zinc supply (De Groote et al., 2021). Also, climate change is projected to exacerbate the issue of human zinc deficiency (Peramaiyan et al., 2022). It has been reported that the emission of carbon dioxide causes a reduction in zinc uptake by plants from soil (Nakandalage et al., 2016) and reduces plant's zinc concentration (Huang et al., 2020).

Conventional approaches to delivering zinc to at-risk individuals have centered on food fortification, supplementation, and programs promoting dietary diversification (Maqbool & Beshir, 2019). However, these approaches have not consistently yielded universal success because they are expensive and not accessible to people living in rural areas. An alternate solution is to elevate the zinc concentration in the edible parts of crops through biofortification. Biofortification is increasing the bioavailable concentration of the target nutrient in the edible portions of crops to address the inadequate dietary consumption of those nutrients in humans (White & Broadley, 2005). This approach employs breeding and agronomic methods to address deficiencies in nutrients. However, developing genotypes with increased nutrient content through breeding is expensive and takes a considerable amount of time (Bouis et al., 2011).

Nonetheless, the agronomic method (agronomic biofortification) provides a rapid remedy for addressing human zinc deficiency. This method entails administering micronutrients via various means, such as soil application, foliar application, and seed treatments (White & Broadley, 2005). Moreover, agronomic

biofortification can potentially optimize the yield of crops cultivated on marginal soils, which could have substantial positive effects (Salehi et al., 2021).

Maize (*Zea mays*) is the third most important cereal behind rice and wheat in global production (Suganya et al., 2020). It serves as a staple diet for over 1.2 billion individuals across 25 developing countries, contributing 15-56 % of total daily calories (Prasanna et al., 2001). In Ghana, maize is the most widely cultivated and consumed cereal crop, accounting for over 50 % of the country's total cereal production (Darfour & Rosentrater, 2016). It can provide food for humans, feed for livestock such as poultry, rabbits, and pigs, and raw materials for various industrial purposes (Ayyar et al., 2019). A yield of 5.1 metric tons per hectare is estimated to be produced across 163.9 million hectares annually, yielding approximately 832.5 million metric tons (Gwirtz & Garcia-Casal, 2014). Besides being consumed fresh, maize can be ground and fermented to be processed into flour or alcoholic beverages (Ekpa et al., 2019).

Hence, increasing the zinc content in maize grain would lead to an increase in the amount of zinc in people whose diets are largely derived from maize. Despite the significant role maize plays in achieving food security (Lopez-Ridaura et al., 2019), its production is constrained by increasing marginal soils, climate change, and infertile soil (St. Clair & Lynch, 2010)**,** especially soils with low levels of available zinc (Zhang et al., 2013). Additionally, most of the zinc in the maize grain is found in the embryo and aleurone layers, which are usually discarded when processing the flour (Suri & Tanumihardjo, 2016). Moreover, zinc is often bound by phytate within maize kernels, and humans do not possess

the enzyme that releases phytate, limiting the amount of zinc available to the body from maize kernels for dietary consumption (Schlemmer et al., 2009).

Similarly, fresh fruits and vegetables have become increasingly popular because healthy eating guidelines are advocated worldwide. Carrot (*Daucus carota* L.) is an important exotic vegetable cultivated across the globe (Norman, 1992). Among the exotic vegetables grown in Ghana, they have a high value and are very popular in urban areas. They are also potential export crops (Asante, 2019). Carrot is cultivated for its fresh root, either cooked or eaten raw (Agbede, 2021). Among the succulent vegetables, it is ranked third in world production in terms of their importance (Bassett et al., 1986). Carrots represent 1.4 % of the world's production of roots, tubers, and other vegetables (Schulzova et al., 2022). Carrot is well-known for its pleasant taste and health benefits, as it contains carotenes, vitamins, minerals, and fibre (Appiah et al., 2017). The leaves are also used to feed livestock (Kahangi, 2004).

Given its significance, various strategies have been applied to successfully grow these crops, including integrated fertilisation, particularly zinc, due to the large proportion of soils deficient in zinc across the globe (Noulas et al., 2018). Since zinc is crucial for all root vegetable crops, its deficiency can adversely impact carrot yield and decrease zinc levels in the tissue, which will pose a widespread health risk to the majority of the population in developing countries (Mehata et al., 2020; Salehi et al., 2021). Thus, increasing the bioavailable concentration of zinc in maize and carrots through targeted fertilisation offers a less expensive and sustainable route to improving dietary zinc consumption and

contributes to the attainment of Sustainable Development Goal 2 (SDG 2), which aims to "end hunger, achieve food security, improved nutrition and promote sustainable agriculture".

Statement of the problem

Micronutrient deficiency, particularly zinc, significantly threatens nutrition and food security throughout the world. Zinc is an essential micronutrient for human health, playing a critical role in various physiological processes and immune functions (Das & Green, 2016). Zinc deficiency has public health ramifications, particularly affecting children, women of reproductive age, pregnant women, and others who rely on it for diverse growth and physiological processes (Gopalan, 1995).

Zinc insufficiency in humans could lead to various health problems, such as impaired brain development, stunted growth, immune system abnormalities, increased vulnerability to infections like diarrhoea and pneumonia, decreased physical capabilities and productivity, as well as adverse birth outcomes in expectant mothers (Black et al., 2008; Terrin et al., 2015). An estimated one-third of the global population has zinc deficiency, with children under five years old being particularly vulnerable due to their heightened need for zinc to support their growth and development (Boonchuay et al., 2013; Wessells & Brown, 2012). Approximately 500,000 children under 5 perish from conditions related to zinc deficiency each year (Stoltenberg, 2006). Zinc insufficiency is caused primarily by malnutrition. It is estimated that a substantial percentage of individuals in many developing countries consume staple crop-based diets that do not contain

adequate amounts of zinc (Liu et al., 2017), contributing to widespread deficiencies and associated health problems. Due to insufficient bioavailable minerals (and vitamins), malnutrition affects many Ghanaians (Boateng et al., 2019), resulting in significant costs in terms of missed opportunities for economic growth, loss of lives, and deteriorated quality of life. Zinc deficiency affects a significant proportion (30 % - 50 %) of children of school-going age in Ghana (Annan et al., 2019). A study has revealed that about 44 % of young children consumed insufficient zinc, with over 30 % identified as zinc deficient (with levels below 70μg/g) based on hair and serum sample analyses (Egbi, 2012). In Northern Ghana, a striking 64.9 % of children were found to have inadequate zinc intake, surpassing the high-risk threshold of 25 % in the population, indicating a pressing need for interventions to enhance dietary zinc consumption (Coomson & Aryeetey, 2022). As a result, increased dietary intake of zinc can mitigate the impact of many diseases in Ghana.

Additionally, zinc is among the 17 essential minerals. It contributes to photosynthesis, pollen development, plant growth, functioning, auxin metabolism, enzyme structure, sugar transformation, membrane permeability, protein synthesis, signal transduction, and gene expression (Alloway, 2009; Hacisalihoglu et al., 2004). Plants absorb zinc from the soil. However, soil zinc deficiency has become a critically significant abiotic stress factor that affects over 49 % of arable lands worldwide (Hacisalihoglu & Blair, 2020). Zinc insufficiency adversely affects the growth of plants, resulting in stunted internodes, small leaves, tissue

death, delayed maturation, and interveinal chlorosis (Hacisalihoglu, 2020). Thus, sufficient zinc is crucial for crop yield and quality.

Significance of the study

Zinc deficiency is a widespread and persistent public health issue that substantially threatens global food and nutrition security, particularly in developing nations (Gupta et al., 2020). Zinc deficiency affects millions worldwide, significantly affecting human health and well-being. Zinc deficiency poses a significant health risk, especially in children, because it adversely impacts their physical growth, immune system, learning abilities, damages DNA, and causes cancer (Black et al., 2008). Therefore, increasing the zinc concentration of staple food crops is imperative to meet humanitarian needs (Lawate et al., 2018).

The problem of zinc deficiency is compounded by the fact that staple crop-based diets in Ghana lack sufficient zinc content to meet daily dietary requirements. These diets predominantly consist of cereals including maize, rice, and sorghum and root and tuber crops like yam, cocoyam, and cassava, which are inherently low in zinc and fail to provide an adequate supply of this essential micronutrient to vulnerable populations. As a result, efforts to combat zinc deficiency have become a critical component of global food and nutrition security initiatives. Agronomic zinc biofortification has emerged as a promising and sustainable approach to address this pressing issue. By enhancing the zinc content of crop plants through targeted zinc fertilisation, the biofortification strategy aims to enhance zinc levels and uptake in edible plant parts, thereby enhancing the nutritional quality of crops.

In addition, the results could lead to reforms in Ghana's current fertiliser subsidy policy to incorporate Zn-based and other micronutrient-enriched fertilisers. This would address the role of plant nutrition for improved system health in the soil-crop-livestock-human continuum.

General Objective

The overall objective was to evaluate the effect of zinc application time, method, and concentration on crop performance, zinc uptake and tissue concentration, in maize and carrots, to establish the efficacy of agronomic biofortification for improving dietary zinc intake.

Specific Objective(s)

Specifically, the study:

1. Examined the effects of application time, method, and zinc concentration on the morphophysiological and yield parameters of maize and carrots.
2. Assessed the effects of application time, method, and zinc concentration on zinc uptake and tissue concentration in maize and carrots.

Research hypothesis

1. Time of zinc application and concertation of fertilisation significantly influence morphophysiological and yield parameters of maize and carrots, whereas the method of application has no significant effect.
2. Method of application, concentration, and stage of application of zinc influence uptake and tissue concentration of zinc in maize and carrots.

CHAPTER TWO

LITERATURE REVIEW

The extent of soil zinc deficiency worldwide

Globally, the average total zinc concentration in uncontaminated soils is around 64 mg kg^{-1} (Kabata-Pendias, 2000). In contrast, the range of zinc concentration in soil solutions varies from 4 to 270 µg L^{-1} (Alloway, 2009). Despite this, much of the arable land around the world lacks zinc (Alloway, 2009; Broadley et al., 2007). Its deficiency is widespread, particularly in crops like cereals, leading to significant variations in the concentration of zinc in grain crops grown in soils with sufficient or deficient zinc (Erdal, 2003).

The major reason zinc nutrition is overlooked is the greater yield responses that N, P, and K fertilisers have driven since agricultural production intensifying in the 1970s. Nonetheless, extended periods of continuous cultivation have mined a significant proportion of the soil's zinc reserves. When no fertilisers are applied, this mining can lead to a severe depletion of soil zinc reserves. For instance, significant zinc removal rates have been reported in India for cereal production (Takkar, 1996). Additionally, historical zinc inputs have decreased due to a shift from relying on animal manures, a significant zinc source, to synthetic fertilisers that focus on potassium, nitrogen, and phosphorus nutrition (Prasad, 2010).

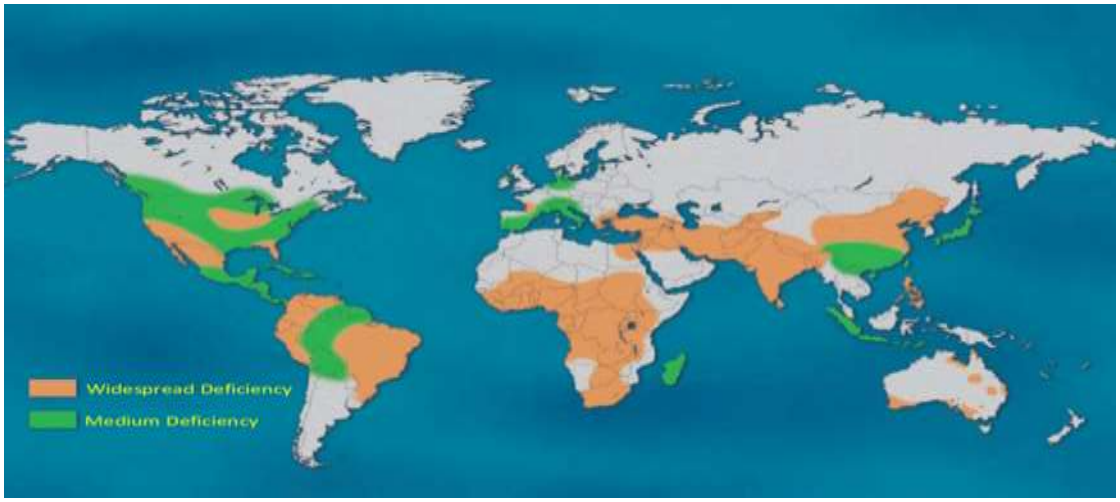

Figure 2. 1: The extent of zinc deficiency in soils worldwide (Alloway, 2009).

The phytoavailability of zinc in soil

In soils, zinc concentrations depend primarily on factors such as atmospheric deposits, parent material, and human activities, including adding fertilisers, farmyard manures, industrial wastes, and sewage sludge (Alloway, 2008). The chemical forms of zinc in soil are diverse, with varying degrees of solubility (Marschner, 2011). These variations encompass zinc associated with organic matter, water-soluble zinc in the soil solution, exchangeable zinc, zinc co-precipitated as secondary minerals, or linked with sesquioxides and as an integral component of primary minerals (Almendros et al., 2022). These distinct forms govern the solubility and accessibility of zinc for plants (Shuman, 2017). Plant roots can easily absorb zinc in the soil solution (Marschner, 2011). Nevertheless, adsorbed zinc maintains an equilibrium with solution zinc, influencing zinc accessibility through adsorption and desorption reactions (Takkar & Sidhu, 1979). The zinc concentration in the soil varies depending on the soil type. Thus, the variation of zinc in the soil ranges between 17 and 125 μg g^{-1}, with a worldwide average of 64 μg g^{-1} for uncontaminated soils (Kabata-Pendias, 2000).

The zinc deficit in plants is not solely influenced by more than just the soil's zinc concentration; its phytoavailability is also crucial (Noulas et al., 2018). Several soil factors influence the availability of zinc to plants. These factors encompass the organic matter content of the soil (Obrador et al., 2003), the pH of the soil (Sadeghzadeh, 2013), the texture of the soil (Sutradhar et al., 2016), the level of carbonate content (Singh et al., 2005), the oxidation state of the soil (Sajwan & Lindsay, 1988), the presence of other elements or compounds capable of forming zinc complexes (Sutradhar et al., 2016), the temperature (Sutradhar et al., 2016), microbial activity and the moisture content of the soil (Alloway, 2009). The combined impact of these factors means that specific types of soils are more prone to expressing zinc deficiency. These include calcareous and alkaline soils, particularly those found in semi-arid and arid conditions (Alloway, 2009), and sandy coastal soils that are highly leached (Alloway, 2008). There is also a likelihood for zinc deficiency in other soil types, such as peats and mucks, as well as soils with high levels of phosphorus, magnesium, silicon, sodium, calcium, and bicarbonate (Hafeez et al., 2013).

Soil type, Parent material, and climate

Soils stem from bedrock materials like granite, quartz or gneiss, naturally possess low zinc levels (Hafeez et al., 2013). As the bedrock disintegrates, they usually form sandy soils with limited cation exchange capacities and larger grain sizes. This situation is worsened by leaching, which further contributes to zinc depletion. The hot and humid climates often found in tropical regions promote weathering and significant zinc leaching from the soil profile. This category of

soils includes “Ferralsols (Oxisols), Acrisols (Ultisols), Plinthosols, and Nitisols” (Deckers et al., 1998).

Mobility of zinc in the soil

Typically, zinc is considered to have limited mobility within soils and tends not to disperse beyond its application site (Hacisalihoglu & Kochian, 2003). Given zinc’s limited diffusion coefficients and constrained movement, depletion zones typically arise near the rhizosphere, especially during periods of low soil moisture (Whiting et al., 2003). The limited diffusion of zinc within the soil structure necessitates that plant roots grow toward soil sources of zinc to absorb sufficient amounts for growth (Hacisalihoglu & Kochian, 2003). However, the diffusion rate of zinc fertilisers to the soil has been demonstrated to be higher when they are applied as chelates instead of sulfate salts, resulting in a greater uptake of zinc by plants (Aiqing et al., 2022).

Soil pH and carbonate content

Soil pH plays a pivotal role in determining the availability of zinc in the soil, as the availability of zinc to crops primarily hinges on variations in the soil pH (Noulas et al., 2018). The absorption of zinc from the soil declines significantly as the pH of the soil rises from 4.6 to 6.8 (Alloway, 2008).

For plants to take up minerals, they must be available in the soil, and the low solubility of zinc in the soil is one of the main contributing factors to widespread zinc deficiency in crops. The ability of zinc to dissolve in the soil solution is influenced by the soil’s pH. As the soil pH rises, zinc's solubility decreases, consequently reducing its availability to plants (Egwu & Agbenin,

2013). This phenomenon occurs due to the reaction between zinc ($Zn^{2+)}$ and hydroxide ions (OH-) to form Zinc hydroxide $Zn(OH)_2$ (Recena et al., 2021), a substance not soluble in water (Clever et al., 1992). An elevated pH increases soil particles' adsorptive capacity due to the co-precipitation of iron oxides, chemisorption on calcite, and increased negative charge dependent on pH (Alloway, 1995).

Treating soils with low pH levels using lime could potentially hinder zinc uptake if excessive quantities are applied (Holland et al., 2018). Liming can adversely affect the substances used with high carbonate (CO_3^-) or bicarbonate (HCO_3^-) levels. In high-pH soils, zinc ions (Zn^{2+}) can form zinc carbonate ($ZnCO_3$) by reacting with carbonate ions (CO_3^-) (Walworth & Heerema, 2015), which is also characterized by its low solubility in water (Clever et al., 1992). The formation of bicarbonate ions (HCO_3^-) may lead to soil alkalinity, resulting in reduced zinc concentrations in plant tissue, especially when soil pH exceeds 8.3 (Tambasco et al., 2000). Conversely, zinc becomes more soluble and bioavailable for plant uptake in soils with lower pH levels (Rutkowska et al., 2015). Therefore, it is necessary to regularly assess soil and plant zinc levels in areas that experience gradual soil acidification as a result of nitrogen fertilisation (Guo et al., 2010) or which are situated in high rainfall areas (White et al., 2000) to prevent zinc toxicity, especially if zinc-based fertilisers are commonly used.

The physiology of zinc uptake and use by plants

Functions of zinc in plants

In plants, zinc plays a direct role in several metabolic processes that include maintaining membranes' function, structure and promoting membrane stability (Tabassum et al., 2014). It contributes to the production of pollen (Marschner, 2011), chlorophyll and cytochrome synthesis (Sutradhar et al., 2016), and the formation of tryptophan, a precursor of the growth hormone auxin (Alloway, 2008). Zinc is a constituent of numerous enzymes like carboxypeptidase, alkaline phosphatase, and phospholipase (Palmer & Guerinot, 2009). It influences the activity of the enzyme "carbonic anhydrase", thereby regulating the pH levels in tissues where photosynthesis occurs (Sadeghzadeh, 2013) and governs root growth (Slaton et al., 2005). Additionally, zinc participates in ionic exchange, respiration, and the closure of stomata (Escudero-Almanza et al., 2012), which consequently impact assimilation of carbon and photosynthesis (Tabassum et al., 2014). It serves to detoxify free radicals and enhance tolerance against oxidative stress (Cakmak, 2000; Sadeghzadeh, 2013) and can even act as a fungicide (Fontes et al., 1999).

Zinc insufficiency in plants

The insufficient zinc supply to plants results in reduced yields, compromised quality of cereal grains, lower biomass production, limited growth, elevated levels of reactive oxygen species, a hindrance to the synthesis of proteins and photosynthesis, and lowered pollen fertility (Cakmak, 2000). Zinc deficiency is characterized by small leaves, bronze in colour, exhibiting wavy edges, and

clustered on stems having short internodes. In severe situations, symptoms of zinc insufficiency entail leaves turning necrotic and chlorotic, greater branching of shoots, poor reproductive fertility, and apical growth points dying off. Plants deficient in zinc are more susceptible to experiencing photo-oxidative damage during prolonged exposure to intense solar radiation (Cakmak, 2000). Since zinc is not easily mobile within plants, the initial signs of zinc deficiency manifest in younger leaves (Alloway, 2008; Goldy, 2013). This is due to plants' limited ability to remobilize zinc from older leaves to younger ones.

The symptoms of zinc deficiency in crops become evident when zinc concentration in the shoot drops to 15-25 mg Zn kg^{-1} DM, which marks a common threshold below which growth is restricted (Sinclair & Krämer, 2012; Singh et al., 2005). Nonetheless, there is substantial information suggesting that these critical values vary with plant parts and growth stages. Notably, during the reproductive phase of the plant, zinc is translocated into grains, making critical tissue concentration values less reliable as indicators of zinc status. The ratios of P: Zn and Fe: Zn in shoots can also indicate zinc insufficiency (Marschner, 2011).

Plants suffering from a zinc deficiency typically exhibit diminished chlorophyll levels in their leaves and lower Chl a:b ratios, indicating that photosystem-II (PSII) units have lost their intrinsic quantum efficiency (Chen et al., 2008). This decline can be attributed to a decrease in the activity of antioxidant enzymes and an elevated oxidative stress damage in the chloroplasts. This damage stems from a disruption in energy transfer from PS-II to photosystem-I (PS-I) (Fu et al., 2015). Ultimately, these negative effects on

photosynthetic centres lead to a reduction in the plant's leaf photosynthetic capacity, resulting from a decreased quantity of PS-II units per unit of leaf area, which will make them more susceptible to photodamage (Chen et al., 2008).

Zinc uptake and translocation in plants

The principle of homeostasis is a biological mechanism for adapting and maintaining equilibrium between molecules, elements, and energy concentrations at constant equilibrium (Sinclair & Krämer, 2012). The process of zinc homeostasis comprises multiple stages, including absorption, transportation, movement, allocation, and storage of zinc (White & Broadley, 2011). These processes collectively maintain optimal zinc levels across different plant components and systems, ensuring the plant's survival (White & Broadley, 2011). This intricate balance is governed by a complex interplay of ligands, proteins, transporters, and finely controlled gene expression (Page & Feller, 2015). The control of this equilibrium hinges on the plant's internal zinc needs and the external zinc supply (Schroeder et al., 2013).

The acquisition route of zinc from the soil to grains involves several stages, encompassing the absorption and uptake by the epidermal cells of the root, apoplastic loading into the xylem or symplastic loading into the phloem pathways for subsequent movement, and unloading to aid seed development (Olsen & Palmgren, 2014; Sinclair & Krämer, 2012). An alternative route that can lead to higher zinc levels in grains is the direct absorption of zinc through the foliage, which is later transferred, primarily from ageing leaves to developing seeds, with the help of the phloem (White & Broadley, 2011). The suitable plants for

biofortification are the ones that efficiently transfer acquired zinc from roots and leaves to grains. However, elevating the zinc content in the edible portions of cereals can be hampered by the transport and distribution of zinc as a result of the barrier between roots and shoots, the limited movement of zinc through the phloem, and the challenges in unloading zinc from the phloem during grain development (White & Broadley, 2011).

The uptake of zinc by roots.

Plants primarily absorb zinc from the soil through their root system through divalent cation (Zn^{2+}) (Widodo et al., 2010). But it is worth noting that under high pH conditions, zinc is taken up as a monovalent cation ($ZnOH^{+}$) (Broadley et al., 2012) or in chelated form when plants secrete certain compounds like phytosiderophores (Widodo et al., 2010). Plants that efficiently utilize zinc have been observed to possess longer root hairs with increased surface area, or they can even alter the chemical and biological soil properties to enhance the availability of zinc for plant uptake (Cakmak et al., 1996). This adaptation is illustrated in plants that can release larger quantities of phytosiderophores to dissolve soil-bound zinc, making it more accessible (Arnold et al., 2010).

After uptake into the root system, zinc can either become fixed within the vacuoles of the root cells or be transported to the vascular bundle made up of xylem and phloem for subsequent movement up the stem (Swamy et al., 2016). At this point, obstacles to effectively moving zinc from roots to shoots, commonly known as the "root-shoot barrier", may arise (Yamaji et al., 2013). Another limitation is the presence of suberin as well as Casparian strips in the roots, which

serve as potential barriers at the junction between roots and shoots, playing a role in restricting root-shoot zinc transfer (Sinclair & Krämer, 2012).

Apoplastic transport of zinc (xylem)

In the apoplastic pathway, substances are passively transported through the cell walls and open spaces between cells with complete permeability (Olsen & Palmgren, 2014). For zinc to move from roots to shoots via the apoplastic pathway, it must first leave the root cells and enter the xylem (Lu et al., 2013). Specific transporters in the plasma membrane regulate zinc transfer into the xylem (Lu et al., 2013). Following its transfer into the xylem, zinc is transported through the stream of the transpiration pull (Hanikenne et al., 2008). Zinc may be "Zn-nicotinamide, Zn^{2+}, Zn-malate, Zn-histidine, and Zn-citrate" within the xylem sap (Lu et al., 2013).

Symplastic transport of zinc (phloem)

In the apoplastic pathway, substances are transported within the interconnected cytoplasmic network of living plant cells via plasmodesmata (Wang et al., 2011). To avert zinc precipitation in the cell cytoplasm or any unintended binding to other molecules, plants utilize chelation to bind zinc with citrate, histidine, 2'-deoxymugineic, and nicotinamide, enabling their transportation through the phloem (Yoneyama et al., 2015). Plants counteract the limited mobility of zinc in the phloem by transporting zinc through the xylem to the desired location then loading it into the phloem (Yamaguchi et al., 2012). Nevertheless, to avoid zinc homeostasis, the transport between the xylem and phloem is meticulously modulated, ensuring no excess movement occurs (van Bel

et al., 2011). Instead, any surplus zinc present in the xylem sap is directed toward the stems for storage (Wang et al., 2011). The zinc movement from the xylem to the phloem has mainly been investigated for rice and wheat, while other crops have received little information.

Remobilizing zinc from the vegetative tissues to grains through the phloem is a significant route for grain zinc buildup, particularly when the zinc available for plant uptake after anthesis is insufficient (Nishiyama et al., 2013). In wheat, for instance, more than 70 % of the zinc stored in the vegetative tissues is mobilized as the grains are filled (Haslett et al., 2001). This becomes even more crucial when foliar application of zinc is employed, as the success of this approach depends on efficiently transporting the absorbed zinc through the phloem to reach the grains (Haslett et al., 2001). Consequently, any hindrance to the movement of substances in the phloem restricts the potential for zinc accumulated before the filling stage from being effectively transferred to the grains.

Zinc transport from xylem and phloem into grains

The final stage in the accumulation of zinc within grains is the transfer of zinc from the phloem and xylem into the grains. In certain grains like wheat, the phloem is the sole vascular tissue connected to the grains (Yokosho et al., 2009). There is a limited understanding of the mechanisms underlying this process (Ren XueLiang et al., 2006), given that the protein transporters accountable for transferring zinc into grains from vascular tissues are undiscovered (Patrick & Offler, 2001). Possible genes involved in this process include “AtYSL2 and

ZmYS1" (Yin et al., 2016), as well as transporters from the family of cation exchangers (Schaaf et al., 2005). Nevertheless, studies have demonstrated that an increase in zinc absorption from roots and enhanced root-shoot zinc transport does not necessarily lead to a corresponding rise in the zinc concentration of the grain (Yin et al., 2016). As the zinc supply rises, this barrier at the point where the shoot connects with the grain becomes more effective (Wang et al., 2011).

The role of phytate in dietary zinc bioavailability

Considering micronutrient distribution within crops is crucial in biofortification endeavours, as it dictates how much the accrued nutrient remains readily available during digestion and absorption in a consumer's gastrointestinal tract (Nestel et al., 2006). It is important to note that substances known as antinutrients, such as phytates, oxalates, antivitamins, and tannins, can significantly reduce the ability of the human body to absorb zinc (Welch & Graham, 2004). Among these, phytate has presented a distinct hurdle to enhancing zinc's bioavailability, especially in the cereal grains.

Characteristics of phytate

Phytate (also known as myo-inositol-1,2,3,4,5,6-hexakis-dihydrogen phosphate) possesses a strong negative charge and a high propensity to form insoluble metal-phytate salts by chelating monovalent, divalent, and trivalent cation metals (Liang et al., 2023). Studies related to human nutrition have confirmed that phytate hinders the uptake of various minerals, including iron, zinc, calcium, potassium, and copper (Egli et al., 2004).

It is worth nothing that Zn^{2+} exhibits a strong affinity for phytate, and its solubility decreases with increasing pH levels (Liang et al., 2023). There are two barriers to zinc absorption in the small intestine. Firstly, the small intestine's alkaline environment reduces zinc-phytate's solubility (Maares & Haase, 2020). Since the human digestive system lacks phytase enzymes to break down phytate, zinc remains bound to phytate, hindering bioavailability and absorption (Brouns, 2021). The balance between phytate and zinc in the diet directly impacts the bioavailability of zinc (Hunt et al., 2008). As many as six zinc cations can be bound to one phytate molecule (Hotz & McClafferty, 2007).

The phytate content of cereal grains

Phytate is the primary compound for storing phosphorus in cereal grains, constituting a significant proportion (60 - 95 %) of the overall phosphorous content in whole-grain seeds (LOEWUS, 2001). In general, cereal grains contain a percentage of between 1 and 7 % phytate, whereas rice contains as much as 8.7 % (Schlemmer et al., 2009). The amount of phytate accumulated depends upon the amount of phosphorus available to the plant and the grain.

The phytate content of grain seeds is stored in globoid crystals rich in proteins found in the aleurone layer and embryo during maturation (Gupta et al., 2015). The primary sites of phytate storage in compact-grained cereals including wheat, maize, barley, and rice are globoid crystals in the aleurone layer and pericarp (Bohn et al., 2008). Once the seed starts to germinate, an enzyme called phytase disintegrates phytate into its constituent inositols, minerals, and

phosphates, allowing them to be utilized by the plants during their growth (Raboy, 2003).

Nutrient requirement of plants, animals, and humans.

Plants and animals need minerals to complete their life cycles, which are divided into major and trace nutrients. Plant nutrients include nitrogen, phosphorus, potassium, sulfur, calcium, and magnesium. These nutrients play vital roles in many biological processes. A wide range of essential molecules require nitrogen, phosphorus, and sulfur. Nitrogen and sulfur are components of amino acids that form proteins, and nitrogen and phosphorus are components of nucleotides that form DNA (Smith, 2007). Phosphorus is also a vital component of phospholipids, essential for forming cellular membranes (Gaude et al., 2008). Potassium plays an active role in activating enzymes and regulating water balance in plants (Britto & Kronzucker, 2008). Calcium is secondary in stress responses, maintaining membrane structural integrity (Maathuis, 2009). Magnesium plays a vital role in the production of chlorophyll (Sirijovski et al., 2008). Plants, animals, and humans require different levels of trace nutrients. Plants require eight essential trace nutrients for optimal growth: “iron, zinc, copper, manganese, chlorine, boron, molybdenum, and nickel” (Bhatla et al., 2018). In addition, silicon, cobalt, vanadium, and sodium have been suggested to enhance plant growth, but their status as micronutrients is yet to be confirmed (Singh et al., 2013).

Humans and animals, like plants, require the same eight essential elements. However, they also need selenium, iodine, silicon, fluorine, lithium,

cobalt, tin, chromium, and arsenic for healthy growth and development (Prasad, 2010). It is essential that plants, animals, and humans get these micronutrients in the right proportion to sustain proper health and also support vital physiological processes.

Incidence of micronutrient insufficiencies in human populations

The major staple foods of many human diets are cereal grains and root tubers. However, these crops have low micronutrient levels and do not meet the daily requirements of humans (Hakim et al., 2010). In developing countries, it is typical for many communities to rely on single-staple food, which can result in severe micronutrient deficiency symptoms (Aphane et al., 2011). The situation is further aggravated by low-income communities residing in areas with severe soil mineral deficiencies, which challenge fertilisation and agricultural development, a common scenario in developing countries (White & Broadley, 2005).

Globally, 33 % of the population is impacted by a lack of essential micronutrients, often called malnutrition or hidden hunger (White & Broadley, 2009). Most people affected by micronutrient malnutrition are in developing countries across Asia, Africa, Latin America, and the Caribbean and Oceania island nations (Tulchinsky, 2010). There are also micronutrient deficiencies in several developed countries (Hakim et al., 2010), primarily due to poor dietary choices rather than limited accessibility of these nutrients.

Zinc deficiency and its health consequences

A zinc deficiency is a common micronutrient deficiency that plagues global populations, and it holds significant importance as an essential mineral for

human health, engaging in a multitude of vital bodily functions (Andreini et al., 2006). Zinc is second to iron (Fe) in terms of humans' most abundant trace minerals and is often found in high concentrations within cells (Deshpande et al., 2013). It is indispensable due to its involvement in diverse cellular processes. Zinc plays an important role in the growth and development of cells (Darnton-Hill & Ahmed, 2010), and it is involved in gene regulation and expression (King, 2011). Further, zinc is crucial for wound healing (Lin et al., 2017). It also serves as a signalling molecule in neurons, sensory cells, and immune cells (Eide, 2006).

The recommended daily zinc intake varies according to the growth stage and gender. It is recommended that adults consume 7 - 11 mg of zinc every day, and 40 mg is the upper limit (UL) for adults (Gibson et al., 2016). However, pregnant and lactating women may need up to 14 mg of zinc daily (Moran et al., 2012). Unfortunately, in developing nations, these recommended intake levels are often unmet because people rely primarily on zinc-deficient cereal grains as their main source of calories for daily nutrition (Bouis & Welch, 2010). The human body cannot store zinc for an extended period. Therefore, continuous intake is necessary to avoid experiencing deficiencies (Wessells et al., 2010). To maintain optimal health and prevent potential health issues associated with zinc deficiency, zinc-rich foods and supplements must be consumed in adequate amounts (Roohani et al., 2013).

In developing nations, over half of all children, elderly, and pregnant women are estimated to be deficient in zinc (Gupta et al., 2020). In these regions, zinc deficiency ranks fifth among disease risk factors, affecting nearly 70 % of the

Sub-Saharan Africa region (Maret & Sandstead, 2006). Thus, insufficient intake of Zn in humans leads to numerous dire consequences.

Among the consequences are impaired growth and development (Black et al., 2013; Wessells & Brown, 2012), increased susceptibility to diseases, and even death, particularly in high-risk populations like pregnant women, the elderly and young children as well as elevated cancer risk due to compromised immune functions and defence mechanisms (Ho, 2004; Prasad, 2007). Zinc deficiency also affects mental lethargy, cognitive development, neuro-behavioural and behaviour, as well as learning abilities (Black, 2003). Moreover, it increases mortality (Hussain et al., 2022). Furthermore, a lack of zinc is directly associated with more severe and frequent occurrence of diarrheal episodes, which contribute significantly to child mortality (up to 4.4 %) in children under the age of 5 (Fischer Walker et al., 2009). There have been extensive studies on the effects of zinc deficiency on health and nutrition (Roohani et al., 2013).

Strategies for preventing human micronutrient deficiency

In the long run, the most effective and long-lasting strategy to alleviate zinc insufficiency entails enhancing dietary quality via diversification of diets to include a variety of plant-based and animal-based foods (Roohani et al., 2013). However, low-income groups, which are mostly at risk of zinc deficiency, cannot access high-quality foods because they are expensive (Bouis, 2003). Moreover, changing people's eating patterns can be challenging. As a result, various preventive measures have been put in place to guarantee that populations acquire appropriate nutrition. Among these strategies are food enrichment programs and

the use of supplements (Harvey & Dary, 2012; Trentmann et al., 2012). Food enrichment has to do with fortifying food during industrial processing by incorporating micronutrients (Black et al., 2012). Notwithstanding, there are worries regarding the efficacy and safety of these food fortification initiatives.

The addition of different forms of iron (Fe), for example, has been found to harm food quality and stability (Rebellato et al., 2018). Moreover, adding one micronutrient may interfere with the absorption of other nutrients due to potential interactions (Rosado, 2003). In spite of the fact that fortification increases micronutrient uptake, there is limited evidence that they have a positive effect on functional health indicators, such as anemia prevalence in women and zinc deficiencies (Hurrell, 2018).

Another approach is to give nutraceuticals to the population at higher risk of deficiencies in the form of dietary supplements. Unfortunately, implementing these programs in poor rural and urban communities has not been universally successful due to high costs, poor health infrastructure, and inadequate logistics (Bouis et al., 2003; Stein et al., 2007). Consequently, only a limited number of governments, institutions, and organizations can afford to sponsor such programmes continuously or long-term (Mkambula et al., 2020).

Biofortification of Crop Plants

The concept of biofortification is a novel approach to providing consumers with a diet rich in essential micronutrients. Biofortification entails elevating nutrient levels in edible portions of crops using agronomic, genetic, or a blend of both techniques (Dhaliwal et al., 2022). Thus, the definition encompasses

agronomic approaches, genetic modification, and traditional breeding techniques to enhance crop elements (Cakmak, 2008).

Primarily, research efforts in biofortification have prioritized staple crops such as maize, rice, and wheat, among others, because these crops form a significant part of the dietary intake of impoverished households (Kutman et al., 2010). The "HarvestPlus Challenge Program" by CGIAR in 2004 was introduced to decrease micronutrient deficiency using biofortified food sources across the globe. The overall target of the research was to increase the Zn, Fe, and Vitamin A content of rice, maize, wheat, pearl millet, cassava, sweet potato, and beans. On the other hand, secondary priority was given to other metabolites, including antioxidants, folate, amino acids, vitamin E, and long-chain fatty acids (Carvalho & Vasconcelos, 2013).

The key to an effective and successful biofortification program is addressing eight fundamentals (Welch & Graham, 2004). Below are these fundamentals:

i. There should be a clearly defined micronutrient target in the crop's edible parts which the breeding programmes aim to achieve.
ii. There should be a clear understanding of the impact of consuming food staples containing micronutrients and human nutritional status.
iii. A mechanism should be in place to ensure sufficient availability of bioavailable micronutrients within the soil in order to sustain crop micronutrient mining rates.

iv. The micronutrient concentrations should be stable in crops across various environmental conditions.

v. The biofortified varieties should have a spin-off effect on yield to encourage farmers to adopt them.

vi. There should be widespread adoption and consumption of biofortified foods among consumers.

vii. The target micronutrients must have a high degree of bioavailability and bio absorption.

viii. The biofortification program should be cost-effective.

Although biofortified crops cannot replace pharmaceutical supplements and processed food for micronutrients, however, they can, enhance the daily mineral and vitamin intake (Bouis et al., 2011). Therefore, to achieve maximum impact, a successful biofortification program should be implemented in conjunction with dietary diversification and disease reduction programs (Zhang et al., 2012).

Agronomic biofortification of crop plants

Agronomic biofortification involves the application of fertilisers containing the target micronutrient to crops as a short-term intervention and complementary strategy to breeding (Ullah et al., 2019). Thus, agronomic biofortification presents a time-saving and simple strategy to increase the bioavailable concentration of the target micronutrient in the edible tissues of crop plants via the application of fertiliser to the soil, foliage and seed enhancement (Cakmak, 2010). Targeted fertilisation strategies and enhanced soil management play a significant role in agronomic practices to attain or facilitate the intended aim of biofortification

(Bouis & Welch, 2010). Providing crops with substantial nutrients is most efficiently and effectively achieved through soil fertilisation (Cakmak, 2008), while foliar fertilisation is often carried out to avoid nutrient fixation problems and protect cultivated soil from micronutrient toxicity (Niu et al., 2021). Several factors influence the efficiency and effectiveness of agronomic zinc biofortification, including the environment, species, and genetics. Regarding soil application of zinc, Regmi et al. (2010) enumerate four factors which include:

1. variations in soil zinc phytoavailability across space and time;
2. immediate fixation of zinc in soil that makes it inaccessible to plants;
3. difficulty for soil zinc to move down the soil profile or be transported across it because its mobility is poor, and
4. varying efficiency of different forms of zinc fertilisers for delivering zinc.

Similarly, according to Fageria et al. (2009), several factors contribute to the efficacy of foliar-applied zinc. They include;

1. inadequate penetration and increased runoff rates on leaves having thick waxy surfaces;
2. swift drying of fertiliser solution resulting in scorching and leaf damage;
3. issue of zinc being fixed and retained by the leaf cuticle and
4. zinc that is absorbed by plants and not efficiently relocated and transported to the various parts.

These factors may limit agronomic zinc biofortification. Therefore, soil-applied zinc would likely be required in combination with foliar-applied zinc to

maximize zinc accumulation in edible portions of crop plants. Hence, agronomic strategies must be adjusted accordingly to meet the needs of the new situation so that biofortification can be successful.

A significant downside of agronomic biofortification is the frequent need to apply fertilisers regularly to maintain the high levels of micronutrients in crops consistently. This incurs both financial and potential environmental implications (Winkler, 2011). Farmers may be discouraged from investing in such endeavours if there are no substantial enhancements in productivity and yield or if biofortified crops do not command a premium price (Cakmak & Kutman, 2018). The move towards environmental sustainability within the fertiliser industry, along with the legal restrictions on fertiliser usage, could further hinder the adoption of agronomic biofortification in society (Cakmak & Kutman, 2018). Therefore, to improve the micronutrient concentration in crops sustainably, a combined approach using both genetic and agronomic biofortification is likely needed (Carvalho & Vasconcelos, 2013).

Soil zinc application

Soil application of zinc is a potent technique and a frequently employed strategy for providing significant quantities of zinc to crops (Cakmak & Kutman, 2018). Adding zinc to the soil increases zinc concentration in seed, enhances crop growth, and increases the yield of crops grown on marginal soils (Khan et al., 2008). Various inorganic zinc fertilisers are used in soil cultivation, including zinc oxide, sulfate, nitrate, and chloride (Mortvedt & Gilkes, 1993). However, because of factors that affect the diffusion rates, solubility, bioavailability, and fixation of

zinc fertilisers in soil environments, Zn-chelates are sometimes used instead of these zinc fertilisers (Zhao & McGrath, 2009). Some of these fertilisers are zinc-ethylenediaminetetraacetic acid (Zn-EDTA), zinc lignosulfonate, and zinc phenolate (Martin-Ortiz et al., 2009). Although zinc chelates tend to be pricier compared to industrial salts, their phytoavailability is high, which results in enhanced uptake by plants (Nowack et al., 2008), with a single study being an exception (Behera et al., 2015).

Despite this, soil microbes have a slow biodegradation rate for chelating agents such as EDTA (Bolton et al., 1993), resulting in EDTA persisting considerably within the environment. The concentrations of EDTA in soil over 300 mg kg^{-1} have been found to exhibit toxicity to plants and algae, as a result of the inhibition of chlorophyll production and cellular replication (Oviedo & Rodríguez, 2003) EDTA existing in soils could potentially intensify heavy metals availability (Chen & Cutright, 2001). Therefore, it is imperative to use chelated zinc fertilisers with utmost caution to prevent the accumulation of pollutants in the soil.

Another technique for administering zinc to crops is to incorporate zinc compounds into macronutrient granules or to coat macronutrient fertilisers with zinc (Milani et al., 2012). Due to the granules' structure and the nature of the binding formulation utilized, this approach facilitates the gradual release of zinc in a controlled manner, aligning with the plants' needs and reducing wastage (Irfan et al., 2018). The precise placement of zinc fertiliser is crucial for maize, given that the root zone crucial for zinc uptake was identified within the 0–30 cm

range (Milani et al., 2012). Evidence from studies indicates that zinc-coated fertilisers effectively enhance zinc concentration in grains and shoots of rice and wheat (Martin-Ortiz et al., 2010).

Foliar zinc application

Zinc applied to the plant in the form of a foliar spray can enter the leaf's stomata and subsequently be translocated through the plant's vascular system to its required destination (Marschner, 2011). Zinc foliar application often complements soil zinc application by providing an additional source of zinc since plants can absorb soluble compounds through their leaves (Kannan, 1990). Sometimes, this approach is preferred over soil application to avoid challenges related to nutrient fixation within the soil (Kolota & Osinska, 1999). However, both soil and foliar fertilisation may have similar outcomes in terms of yield (Kutman et al., 2010). Unlike soil zinc applications, research indicates that zinc absorption applied to leaves is more efficient when using zinc salts than chelated forms of zinc (Wei et al., 2012).

The absorption of zinc chelates by plant leaves tends to be less effective primarily because of their larger molecular size, which reduces zinc’s transport rate via watery pores across the leaf's surface (Popp et al., 2005). Nevertheless, various zinc chelated forms exhibit differing absorption rates via leaf surfaces. For instance, Zn-rhamnolipid and Zn-polyethyleneimine demonstrate efficient absorption rates via cuticles as compared to Zn-EDTA. Zn-polyethyleneimine containing cationic amine groups Zn-polyethyleneimine (Holloway, 1993) might be more effectively associated with aqueous anionic pores for uptake, while

lipophilic Zn-rhamnolipid could permeate water-repellent leaf cuticles (von Harpe et al., 2000). Once inside the plant, zinc chelates translocate more effectively than zinc salts (Ferrandon & Chamel, 1988).

The timing of applying zinc to leaves is essential to align the supply with the onset of zinc requirements (Cakmak et al., 2010). Research has shown that foliar zinc fertiliser has varying effects on zinc concentrations in kernels based on the developmental stage at which it is applied (Tariq et al., 2014). The results of foliar zinc applications at the early milk stage on rice were significantly higher (26 mg kg^{-1} DM), exceeding the outcomes of applications at the active tillering stage "(14 mg kg^{-1} DM) or booting stage (16 mg kg^{-1} DM)" (Mabesa et al., 2013). Compared to a single application at the early milk stage, a second foliar application at the early milk stage following an initial application during active tillering or booting did not significantly increase grain zinc concentration in rice (Mabesa et al., 2013). Similarly, for wheat, foliar zinc fertilisation was most effective at the early milk stage in contrast to the booting stage. Although compared with rice, in wheat, a second fertilisation at the early milk stage, along with a first fertilisation during the booting stage, increased grain zinc levels (Ajiboye et al., 2015).

Genetic biofortification of crop plants

Genetic biofortification is directed towards enhancing the micronutrient absorption and increasing accumulation in the edible portions or improving the genotype's capacity to synthesize antioxidant and vitamin pigments efficiently (Mayer et al., 2008). Genetic biofortification can be achieved simultaneously with crop improvement screening, such as selecting lower concentrations of anti-

nutrients, elevated concentrations of nutrient absorption promoters, and suppressed heavy metal uptake (Bouis, 2003).

Genotypes that exhibit enhanced uptake and accumulation traits must be selected for breeding to increase the uptake and accumulation of micronutrients successfully. Hence, the primary focus of initial genetic biofortification studies was to explore and identify natural sources of variation in micronutrient content among related or progenitor species (Mayer et al., 2008). The advancement of these conventional breeding approaches was hindered by several limitations, including protracted development periods and the requirement for the trait of interest's genetic diversity to exist in the species or among sexually compatible plants (Borrill et al., 2014). In addition, there was limited understanding of molecular physiological mechanisms and genetic regulation of traits that contribute to the uptake and accumulation of micronutrients (Zhao & Shewry, 2011).

In the past few years, molecular technology and genomics advancements have created new opportunities for focusing research at the molecular level (White & Broadley, 2005). In the initial stages of molecular breeding, extensive genome size, the polyploid nature of certain crops, and the considerable nucleotide similarity between genomes posed significant challenges (Paterson et al., 2009). Nevertheless, researchers have been able to characterize existing populations more rapidly due to improvements in the efficiency and cost reduction of genome sequencing technology (Borrill et al., 2014). Through marker-assisted selection, comprehensive SNP (single nucleotide polymorphisms) datasets, and purified

individual chromosome arms, researchers could map micronutrient accumulation and homeostasis traits (Winfield et al., 2012). For instance, Quantitative Trait Loci (QTLs) responsible for zinc accumulation in grains have been identified in maize, wheat, barley, and rice (Reuscher et al., 2016; Zhang et al., 2011; Srinivasa et al., 2014). Transgenic technology and genetic modification have led to great success in producing such crops as multivitamin maize, vitamin A-enriched rice, and high-iron rice (Naqvi et al., 2009).

Despite this, genetic biofortification is still associated with many drawbacks. Although intensive research has been conducted, a significant amount remains to be uncovered regarding the physiological and molecular mechanisms to explain how micronutrients are taken up, transported, used, and accumulated (Naqvi et al., 2009). In addition, each crop species has unique micronutrient regulation pathways, so knowledge from one species may not be applicable to another (Borrill et al., 2014). Due to the incomplete understanding of environmental and genetic interactions, cereal crop genotypes that consistently achieve higher micronutrient accumulation across different areas are not yet available (Joshi et al., 2010).

Further, genetic biofortification could entail a trade-off for other desirable agronomic traits in crops, such as yield, resistance to pests and diseases, and adaptation to various environmental conditions (for example, heat stress, drought) or unintentional buildup of toxic heavy metals that pose a significant threat to consumer health (Shahzad et al., 2014). For instance, efforts to breed crops with higher zinc or iron concentrations in grain have proven challenging, given that

these attributes often show negative correlations with grain yield due to the dilution of mineral content caused by the heightened carbohydrate content in the grains of germplasm that are high-yielding (McDonald et al., 2008). Newly introduced crop varieties may face consumer resistance if attributes like texture, taste, or visual appeal do not conform to current standards (Falk et al., 2002). Therefore, genetic biofortification programmes must assess genotypes across diverse environments to ensure the consistent manifestation of elevated micronutrient levels.

The advancement of nutrient biofortification in main cereal crops

Maize

Most progress in maize biofortification has centred on increasing carotenoid levels, particularly provitamin A (β-carotene). In a thorough germplasm screening, certain phenotypes of temperate maize with high provitamin A were identified and subsequently integrated into tropical maize through breeding. Presently, the IITA and CIMMYT are working to develop maize varieties capable of providing 50 % of the Recommended Daily Intake (RDI) for provitamin A to young and adult women (Kanwal et al., 2010). Provitamin A levels have also been elevated using the genes overexpression of associated carotenoid with synthesis (Decourcelle et al., 2015). Similar strategies have been employed to enhance the vitamin content of maize, targeting vitamins C, E, and B (Lian Tong et al., 2015).

HarvestPlus' goal regarding zinc biofortification is to achieve a zinc concentration of 60 mg per kg of maize dry matter, with an interim target of 38 -

40 mg Zn kg^{-1} (Ortiz-Monasterio et al., 2007). Currently, maize varieties exhibit zinc concentrations ranging from 15 to 35 mg Zn kg^{-1}, with one study reporting a maximum average of 50 mg Zn kg^{-1} (Kanwal et al., 2010; Ortiz-Monasterio et al., 2007). A collaborative partnership between CIMMYT and IITA will lead to efforts to enhance maize's zinc content. However, there has been little progress in the zinc biofortification of maize.

Wheat

Wheat has had the greatest success in enhancing zinc levels through biofortifying all prominent cereal grain crops. The zinc concentration in CIMMYT wheat germplasm is estimated to range from 25 – 65 mg Zn kg^{-1} DM with an average value of 35 mg Zn kg^{-1} (Pfeiffer & McClafferty, 2007). The major wheat-producing region in the US, on the other hand, has an average zinc concentration of 31 mg Zn kg^{-1} (Miner et al., 2022). These average figures indicate that a minimum increase of 10 mg Zn kg^{-1} in the zinc concentration of whole wheat grain is required to confer a noticeable nutritional advantage for human health (Miner et al., 2022). Studies have found An inverse correlation between grain zinc concentration and yield (McDonald et al., 2008). This poses a significant threat to biofortification initiatives, which need to navigate the task of enhancing grain zinc levels while safeguarding the yield improvements attained through extensive historical breeding endeavours.

Since wheat plays an important historical and economic role in many high-income countries, enhancing zinc and iron content through biofortification has earned more extensive research attention as compared to other cereals. In

wheat grains, as in other cereal grains, zinc and iron are mainly localized in the bran, and embryo (Aiqing et al., 2022). These vital micronutrients tend to form complexes with phytate and are insoluble, thereby resulting in their limited bioavailability (Eagling et al., 2014; Neal et al., 2013)

The enhancement of zinc levels in the edible parts of crops has been accomplished through a combination of genetic and agronomic strategies. In contrast, efforts to enhance iron levels have centred on genetic methodologies. Notably, traditional breeding techniques by CIMMYT have successfully created wheat varieties with the potential for heightened zinc accumulation within grains, resulting in 20 - 40 % higher grain zinc concentrations (Velu et al., 2018). Nevertheless, a substantial range in grain zinc concentration exists across different geographical locations, environmental conditions, cropping years, and agricultural management practices for wheat genotypes (Gómez-Becerra et al., 2010; Karami et al., 2009). Hence, implementing agronomic strategies becomes imperative to ensure adequate zinc availability for plant uptake, thereby enabling the realization of the genetic potential for elevated zinc accumulation.

In soils with insufficient amounts of zinc, applying zinc fertiliser to the soil proved effective in increasing the zinc concentration in the harvested grains. Even in cases where the soil contains an adequate amount of zinc, applying zinc through the leaves further heightens the zinc content in grains of high-yielding cultivars (Zou et al., 2012). The zinc content in grains was increased by 60 % compared to untreated control groups and those treated with foliar applications (Zhang et al., 2012). Interestingly, nitrogen (N) application to the soil seemed

crucial for achieving these improved levels (Cakmak et al., 2010). A study found that when plants encounter after-anthesis zinc insufficiency, before-anthesis nitrogen fertilisation increases the amount of zinc remobilized from vegetative tissue into grains (Cakmak et al., 2010).

When the zinc content of the soil was sufficient to optimize grain yield and biomass production, a substantial portion of grain zinc originated from zinc uptake after anthesis, a figure that even reached 100 % when accompanied by high nitrogen applications (Ajiboye et al., 2015). The most elevated grain zinc concentrations were attained when foliar zinc applications were carried out during the later phases of crop growth (Cakmak et al., 2010; Ajiboye et al., 2015). Studies have demonstrated that humans absorbed significantly higher amounts of total zinc from biofortified wheat than non-biofortified wheat varieties (Rosado et al., 2009).

Rice

The primary focus of rice biofortification has been geared toward elevating zinc, iron, and vitamin A levels with significant success in countries like Bangladesh, China, Brazil, and India (Bouis et al., 2011). HarvestPlus breeding initiative aims to achieve a zinc concentration of 28 mg Zn kg^{-1} DM for rice grain (Bashir et al., 2013; Bouis et al., 2011). The International Research Institute (IRRI) evaluated in 1992, involving about 7000 rice genotypes, and reported that zinc concentrations ranged from 15 to 58 mg kg^{-1} (Gregorio et al., 2000). The removal of the aleurone layer, where zinc is deposited, during the processing of rice grains reduces zinc content (Saltzman et al., 2013).

In rice cultivation, agronomic biofortification is almost necessary, considering that many rice fields exhibit low levels of readily available zinc (Johnson-Beebout et al., 2009). Rice grain zinc content was influenced more by soil factors, such as soil type, zinc status, salinity, and acidity, than by zinc treatments or genotypes (Wissuwa et al., 2008). This becomes particularly crucial in rice cultivation, given its adaptability to both waterlogged (paddy) and aerobic (upland) conditions (Gao et al., 2012). These systems exhibit different zinc accumulation processes and agronomic reactions to zinc application.

Research involving aerobic rice has shown that the primary source of zinc in rice grains is the absorption of soil zinc from the soil after anthesis, instead of the uptake of zinc from foliar applications or remobilizing stored zinc within the plant. (Jiang et al., 2007). These differing findings suggest that zinc might become less mobile in flooded soils, potentially contributing to reduced zinc uptake through the roots (Johnson-Beebout et al., 2009). Agronomic strategies to improve the zinc content of rice have also been proven to be more challenging than wheat ones since the zinc harvest index varies from 23.0 % to 38.7 % (Cakmak & Kutman, 2018). In many instances, increases in the concentration of zinc in straw and improved grain yields did not correspond to significant increases in the zinc concentration of the grain (Wissuwa et al., 2008). This disparity may be attributed to zinc's lower mobility through rice's phloem than wheat (Alloway, 2009). Consequently, the success of agricultural biofortification in rice hinges on the selection of genotypes that possess a greater capacity for zinc translocation,

the remobilization of zinc, and the effective deposition of zinc from applied fertilisers into the grain (Nakandalage et al., 2016).

Enhanced levels of iron (Fe) in rice grains have been effectively attained through genetic modification (Trijatmiko et al., 2016), as well as the utilization of transgenic methods Masuda et al. (2012). These approaches have resulted in an almost threefold increase in iron concentration (Lee et al., 2009). Improvements in iron bioavailability have also been accomplished by reducing the phytate content within rice (Saltzman et al., 2013).

Successful strides in genetically enhancing the nutritional content of rice have been achieved through the augmentation of phytoene accumulation, a precursor to provitamin A, within the endosperm of rice grain. This advancement has led to the creation of the commercially recognized 'Golden Rice,' enriched with high levels of provitamin A (Zhao & McGrath, 2009). Genetic modification has additionally proven successful in raising folate concentration in rice by as much as 150 times, reaching levels that now adequately meet the Recommended Daily Intake for adults (Storozhenko et al., 2007; Blancquaert et al., 2015).

The advancement of nutrient biofortification in vegetables

The consumption of vegetables around the globe and their mineral content are potential avenues for increasing mineral intake in the human diet through targeted biofortification strategies (Buturi et al., 2022). Given the growing need to increase vegetable consumption for both sustainability and health reasons and as the global population continues to rise, we will require more sustainable food sources to meet this demand (Ruini et al., 2015). A healthy diet must include a

variety of mineral nutrients to maintain good health. The importance of minerals becomes evident given that vitamins cannot be effectively absorbed or function in isolation, relying on specific minerals that play essential roles in numerous physiological processes (Gupta et al., 2015). The success of any biofortification approach hinges on both market acceptance and the consumption of improved food products. Hence, it is crucial to select vegetables that are commonly consumed in human diets.

An excellent example is the carrot (Daucus carota L.), one of the most widely consumed vegetables worldwide, cultivated across approximately 1.13 million hectares and yielding nearly 41 million tons of production (Bhandari et al., 2022). As a vegetable, carrots are recognized for their versatile taproot, which can be enjoyed fresh or cooked in a variety of ways (Ierna et al., 2020). The use of zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) appears to be the most commonly applied inorganic source (White et al., 2012). Studies have shown that applying $ZnSO_4$ and Zn-EDTA via foliar methods resulted in increased fresh weight and length of the roots. However, Zn-EDTA specifically enhanced the dry matter content, indicating that zinc has a stimulating impact on the photosynthetic metabolism of the crops (Buturi et al., 2023). Similarly, a study involving carrots by Awad et al. (2021) observed an increase in both dry matter and root fresh weight after foliar application of 5.7 mM of Zn-EDTA three times.

Also, a substantial rise in the zinc content of the edible portions of cabbage (200 %) and canola (25 %) has been effectively attained through agronomic biofortification (Mao et al., 2014). Zinc biofortification through foliar

spray was effectively carried out in arugula, resulting in a remarkable 94% increase in leaf zinc concentration (Rugeles-Reyes et al., 2019).

Despite significant strides in using agronomic biofortification to improve the zinc content of crop plants, several critical gaps remain in the literature. The majority of studies focus on the type of zinc, rate of zinc fertilisation, method of application, and stage of fertilisation in independent experiments, with little information available on their interactions in combined experiments (Boonchuay et al., 2013; El-Dahshouri, 2017; Esfandiari et al., 2016). To address these gaps, my research will evaluate the effects of zinc application method, rate, and timing on crop performance, zinc uptake, and tissue concentration in maize and carrots, to optimize agronomic biofortification practices for enhanced crop zinc content and human nutrition

CHAPTER THREE

MATERIALS AND METHODS

Introduction

Separate experiments were conducted for carrots (in pots) and maize (in the field) to test the null hypotheses proposed and accomplish the objectives. The comprehensive overarching methodologies used in these experiments are presented in the subsequent sub-sections. However, specific details regarding materials and methods relevant to each experiment are provided in the individual experiments.

Description of the experimental site for objective one

The fieldwork for the maize cultivation was carried out at the Teaching and Research Farm of the School of Agriculture at the University of Cape Coast, Cape Coast. The site coordinates are approximately 5.1155° N latitude and 1.2909° W longitude. The experiment for maize was conducted from 2nd March 2023 to 30th May 2023. This site is situated in a coastal savannah agroecological zone with Haplic Acrisol, which extends along the coast and widens towards the east (Asamoa, 1973). This region experiences yearly rainfall of 750 to 1000 mm. There are two main seasons of rainfall (Asare-Bediako et al., 2014). The major season occurs between May and July, and the minor season occurs from August to October. The experimental location receives solar radiation that fluctuates between 3151 KJ cm^{-2} per day and 3804 KJ cm^{-2} per day, day length varies from 11.30 to 12.40 hours, relative humidity falls within the range of 60 % - 80 %, and temperature ranges from 24 °C - 32 °C (Adu et al., 2017).

Experiment one: Demonstrating the efficacy of increasing the zinc content and yield of maize through agronomic biofortification.

Genetic material

The maize crop employed in the field experiment was a commonly cultivated and utilized variety in Ghana called Abontem. The crop offers a promising avenue for impoverished rural populations in developing nations like Ghana to progressively enhance their income sustainably. Abontem was introduced in Ghana by CSIR in 2014. This open-pollinated variety typically requires approximately 50 days to reach 50 % silking and 75-80 days to mature fully. It has a plant height of 162 cm, an ear height of 82 cm, and a potential yield of 4.7 tons per hectare.

Experimental design, treatments, and fertiliser applications.

This field experiment was conducted from 2nd March 2023 to 30th May 2023 to investigate the impact of foliar and soil zinc fertilisation on the morphophysiological parameters, yield, and tissue zinc concentration of maize. The study employed a three-factorial experiment in a Randomized Complete Block Design (RCBD) with four (4) replications and twenty (20) treatments from a 2*2*5 factorial experiment. The first factor, factor A, was application time or growth phase, which was at two levels – pre anthesis and grain filling. Factor B was method of application which was either soil of foliar. Factor C was zinc concentration at five levels, 0, 2, 4, 6, and 8 kg per hectare of $ZnSO_4 \cdot 7H_2O$. The various zinc fertiliser rates were dissolved in 600 ml of water and sprayed onto the plant canopy for the foliar treatment. Plants in the control plots were sprayed with 600 ml of water using a 2L pump spray bottle. Drift shields were used during

foliar applications to prevent cross-contamination between treatments. Similarly, for the soil application, the various zinc fertiliser rates were dissolved in 600 ml of water and carefully applied at a distance of 12 cm from the base of each plant to ensure even distribution and prevent direct contact with the plant stem. The zinc application was done at two stages of growth. The first and second applications were carried out at pre-anthesis (40 DAG) and during the early grain-filling (70 DAG) stages respectively. All plots received a basal application of Nitrogen, Phosphorous, and Potassium fertilisers at the rate of 217 kg N/ha as urea (46 % N), 133 kg P/ha as triple superphosphate (TSP) (45 % P_2O_5), and 100 kg K/ha as muriate of potash (MoP) (http://mofa.gov.gh/site/?page_id=14167; accessed 03/02/2023). The nitrogen fertiliser was split applied. The first application was carried out two weeks after sowing at a rate of 130 kg urea, 133 kg TSP, and 100 kg MoP, while the second split of urea application also known as top-dressing of 87 kg was administered during the vegetative stage (Norman, 1992).

Field layout of the experiment

The experimental area measured 68 meters in length and 15.5 meters in width, resulting in a total plot area of 1054.5 square meters. There were 72 plots, each measuring 5 meters by 2 meters, equal to 10 square meters per plot. 0.5 m row paths separated the experimental plots, and the blocks were separated by 1 m row paths (Figure 3.1).

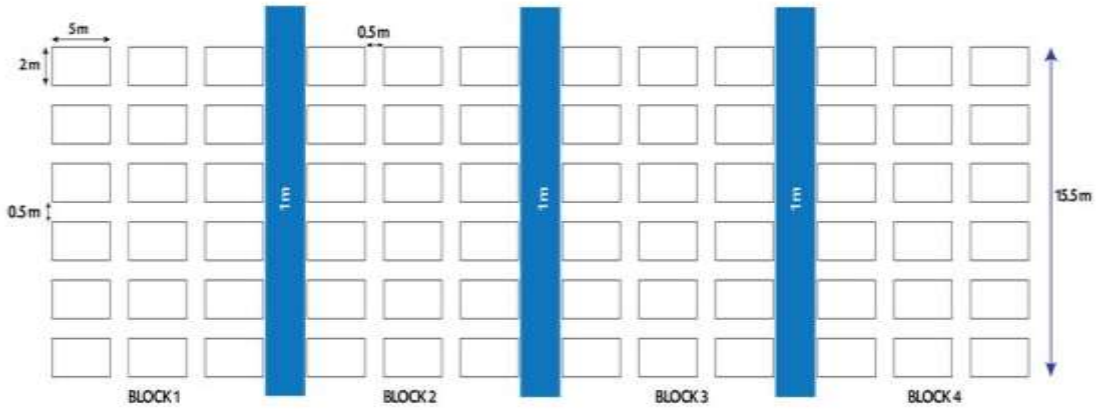

Figure 3. 1: Experimental layout of the field for maize biofortification trial.

Land preparation

The experimental area was weeded, ploughed, and harrowed to approximately 30 cm depth. According to the treatments and design, there were four blocks of 18 plots each, constituting 72 plots in total. Before planting, random composite surface soil samples were collected at 0 - 15 cm depth, air-dried, and then ground to pass through a 2 mm sieve. The physical and chemical properties of the soil were analyzed before the commencement of the experiment. The results are presented in Table 3.1.

Table 3. 1: Basic soil characterization of experimentalfield

Parameter	Unit	Value
pH		6.75
Organic carbon	%	1.64
Organic matter	%	1.6
Electrical conductivity	mS cm^{-1}	0.2
Calcium	cmol kg^{-1}	5.36
Magnesium	cmol kg^{-1}	2.08
Sodium	cmol kg^{-1}	0.15
Potassium	cmol kg^{-1}	0.2
Zinc	µg/g	2.28
Exchangeable acidity		0.44
Sand	%	54
Silt (0.002-0.02 mm)	%	30
Clay (<0.002 mm)	%	16
Textural class		Sandy loam

Planting and Agronomic Practices

The field was irrigated with pipe-borne water using a sprinkler to ensure proper moisture conditions before sowing. The seeds were primed and manually planted at a density of two seeds per hole with a planting distance of 30 cm between rows and 60 cm apart within rows. The seedlings were reduced to one seedling per stand a fortnight after planting. Good Agronomic Practices (GAP) such as weeding, diseases, and pest control were employed to nurture the plants. The plots were weeded two times before reaching total crop cover to smother weeds. Emamectin benzoate pesticide was applied once at the seedling stage and twice during the vegetative stage using a knapsack sprayer, following the manufacturer's instructions for preparation and dosage to control fall armyworm infestations. The pesticide solution was prepared by diluting the product in water within the knapsack sprayer and applied early in the morning when armyworms were actively feeding on the maize plants. The nozzle of the sprayer was directed to ensure uniform application, targeting the whorls and leaves of the maize plants where the armyworms were concentrated. The plots were irrigated with sprinklers as and when needed.

Data collection

Six plants per plot were randomly chosen and labelled to collect growth and physiological data. The data was collected at intervals of two weeks, starting from three weeks after sowing. The yield data was collected for every plot. In each plot, twenty plants were chosen from the middle, covering an area of 3.6 m^2. The cobs were harvested and weighed immediately. The yield data included cob

weight, cob length, 100-seed weight, and grain weight. The following (plant height, chlorophyll index, chlorophyll fluorescence ratio, and performance index) growth and physiological parameters were measured in the experiment.

Plant height

Plant heights were determined using a meter rule, measuring from the shoot's base at ground level to the tip of the plant's apical meristem on each plot. This was carried out from three weeks after planting until the initiation of flowering. When the plants reached harvest maturity, three were randomly picked from the middle row of each plot, and their heights were measured with a tape measure after excavation. The average plant height was expressed in centimetres.

Chlorophyll index

The portable SPAD-502-meter device was used to accurately and non-destructively measure the leaf's chlorophyll content. The readings from SPAD-502-metre provide relative SPAD values, which correlate with the leaf's chlorophyll content. The measurements were taken at a consistent time of the day (between 8:00 am and 10:00 am) to minimize variations due to diurnal changes in leaf hydration and light intensity. The relative SPAD values were assessed on uniform leaves from six plants per plot. Fully expanded mature leaves were selected to reduce variability. Three readings were taken per plant, avoiding the midrib and edges, and the average value was recorded.

Chlorophyll fluorescence ratio and Performance Index (PI)

The Pocket Plant Efficiency Analyzer (PEA) Portable Chlorophyll Fluorimeter device was used to measure the chlorophyll fluorescence ratio

(Fv/Fm) and the Performance Index (PI). The selected leaves were dark-adapted for 30 minutes before Fv/Fm and PI values were recorded (Mishra et al., 2016).

Sample preparation and nutritional analysis

Plant biomass samples were taken for zinc analysis at both physiological and harvest maturity stages. Three plants were randomly selected from the centre of each plot and excavated. Each excavated plant was partitioned into its root and shoot portions. The roots were thoroughly washed with tap water, and both the roots and shoots were then separately placed into envelopes before it was conveyed to the laboratory to maintain the integrity of the samples. The root and shoot (comprising leaves and stem) were washed with deionized water, after which their fresh weight was taken. The cob weight for each plant was recorded and separated into grain, cob, husk, and silk. All the component samples were placed in separate envelopes and oven-dried at 70°C until constant weight. The oven-dried samples were then finely ground with a household stainless-steel grinder, and the resulting powdered sample was stored in Ziploc bags pending tissue analysis.

Experiment two: Demonstrating the efficacy of increasing the zinc content and yield of carrots through agronomic biofortification.

Genetic material

The carrot (*Daucus carota*) variety chosen was "Kuroda king", which matures over 85-95 days. The seeds were acquired from the Sakaata Agro shop in Accra. This variety was chosen because farmers widely use it in the Central region and across Ghana.

Experimental design, treatments, and fertiliser application

A pot experiment was carried out in May 2023 and August 2023 to examine the impacts of both foliar and soil zinc applications on tissue zinc concentration and yield of carrots. The study employed a three-factorial experiment in a Randomized Complete Block Design with three (3) replications and 24 treatments from a 2*4*3 factorial experiment. The first factor, factor A, was method of application which was either soil or foliar. Factor B was zinc concentration at four levels 0, 2, 4, and 6 kg per hectare of $ZnSO_4 \cdot 7H_2O$. Factor C, was application time or growth phase, which was at three levels - 30 DAS, 50 DAS and 70 DAS. The different zinc fertiliser rates were dissolved in 100 ml of water. Each 100 ml portion of the zinc solution was applied to the soil in the pot for soil zinc application, while a 2L Pump Spray Bottle was employed to evenly spray the 100 ml of the zinc solution onto the plant canopy for each foliar treatment. Drift shields were used during foliar applications to prevent cross-contamination between treatments. Recommended basal fertilisation with 222 kg/ha nitrogen (N) from urea (46 % N), 133 kg/ha phosphorous (P2O5) from TSP (triple supper phosphate), and 999 kg/ha potassium (K2O) from MOP (muriate of potash) were applied to all the pots (http://mofa.gov.gh/site/?page_id=14167; accessed 03/02/2023). The basal applications were administered three weeks after planting when the seedlings had become established.

Soil preparation

Zinc-deficient and highly weathered soil used for the study was collected near A. G Carson Technology Village, School of Agriculture, at a depth of 0-15

cm. The soil can be described as a sandy loam, typically found in the coastal savanna region, and exhibits characteristics of Haplic Acrisols (WRB, 2015). The soil samples were collected from different locations at the site and combined into a composite sample after removing all plant debris. The composite samples were allowed to air-dry for three days and then ground to pass through a 2 mm sieve. The resulting fine soil fraction, measuring less than 2 mm, was used for the laboratory analysis and the pot experiment. Standard laboratory procedures were employed to assess the physicochemical properties of the soil. The result of the soil's physicochemical properties is presented in Table 3.2. Before planting, the soil was incubated at ambient temperature for 30 days after it had been watered to 80 % field capacity (determined gravimetrically). In the study, nursery polybags with a radius of 10 cm, a height of 35 cm, and a volume of 10,996 cm^3, each with drainage holes beneath were filled with 18 kg of soil repacked to a bulk density of 1.1 g cm^{-1}.

Table 3. 2: Basic soil characterization of experimental site field

Parameter	Unit	Value
pH		5.77
Organic carbon	cmol kg^{-1}	1.48
Organic matter	cmol kg^{-1}	0.86
CEC	cmol kg^{-1}	3.65
Calcium	cmol kg^{-1}	2.01
Magnesium	cmol kg^{-1}	0.18
Sodium	cmol kg^{-1}	0.27
Potassium	cmol/kg	0.10
Zinc	µg/g	2.19
Exchangeable acidity	cmol kg^{-1}	0.09
Sand (0.02-0.2 mm)	%	68.39
Silt (0.002-0.02 mm)	%	23
Clay (<0.002 mm)	%	8.32
Textural class		Sandy loam

Planting and agronomic practices

The seeds were manually planted directly into the pots at a depth of approximately 1-2 centimetres and then lightly covered with a thin layer of soil. Palm fronds were used to cover pots to minimize excessive heat and prevent small seeds from falling off when watering. The seeds germinated a week after planting, and the palm fronds were removed from the pots. A bamboo and palm frond shelter were erected above the pot to shield the seedling from the heavy rainfall, which could otherwise damage the young seedling at the initial growth stage. The seedlings were thinned to about 3 cm between plants, giving ten plants in each pot. Each pot was watered daily with piped water to 80 % field capacity except on rainy days. Every two weeks, the spaces between the rows of carrot plants were gently stirred using a hand fork to eliminate weeds and loosen the soil to enhance aeration and infiltration. Also, the upper part of the roots was earthed up to prevent them from turning green.

Data collection

Three plants in each pot were randomly selected and tagged for data collection. Data on plant height and chlorophyll content were collected using a wooden meter rule and SPAD meter. Data were collected seven days after the imposition of the treatment and collected every two weeks until the plants reached maturity.

Harvesting, yield data collection, and sample preparation for nutritional analysis

The plants were harvested exactly 90 days after planting. After harvesting, the roots were thoroughly washed with tap water, and the shoot was separated from the root. Three plants were randomly selected after harvesting, washed with deionized water, and separated into aboveground and belowground biomass, after which the fresh weights were recorded and placed in an envelope. The diameter of the three randomly selected roots was measured using veneer calipers at a distance of about 1cm from the shoulder of the root. The length of the root was measured with a 30 cm ruler. The yield was determined by recording the carrot (root) weight from ten plants in each pot. The three aboveground and belowground biomass placed in an envelope were oven-dried at 70°C until they attained a stable weight and documented as shoot dry weight and root dry weight, respectively. They were then ground with a household stainless-steel grinder, and the resulting powder was stored in Ziploc bags for nutritional analysis.

Determination of tissue zinc content

Approximately, 0.4 grams of the milled samples were digested using the Aqua regia digestion method at 360°C over a period of two hours (Allen et al., 1974). The samples, post digestion, were adjusted to their final volume and kept at room temperature until they could be analyzed for their elemental composition. The digested samples were examined for zinc content using an atomic absorption spectrophotometer (AAS; model 210 VGP, Buck Scientific). The samples were aspirated into the AAS and calibrated for zinc (Welz & Sperling, 2008).

Statistical analysis

Analysis of Variance (ANOVA) was conducted with GenStat Twelfth Edition, to assess the significance within the dataset, considering both individual and interactive effects on zinc fertilisation rates, methods, and timings on the measured data. The means were differentiated using the least significant difference (LSD) post-hoc test at a 5% significance level, and the Origin Lab Program was used to represent the data graphically.

CHAPTER FOUR

RESULTS

Experiment one: Demonstrating the efficacy of increasing the zinc content of maize through agronomic biofortification.

Yield Parameters

Grain yield

Increasing the zinc fertilisation rate resulted in a significant ($P<0.001$) improvement in yield to an asymptote at 6 kg ha^{-1} zinc concentration and then declined at 8 kg ha^{-1} concentration (Figure 4.1A). Grain yield in tons per hectare varied from 5.32 t ha^{-1} to 6.82 t ha^{-1}, representing a 28 % increase in grain yield of zinc fertiliser rates of 6 kg ha^{-1} compared to the control treatment (Figure 4.1A). Similarly, grain yield was significantly ($P<0.001$) influenced by the timing of zinc fertilisation (Figure 4.1B). Grain yield in response to time of application ranged from 5.92 t ha^{-1} to 6.52 t ha^{-1}. The Zinc application at pre-anthesis recorded a higher grain yield than the application at grain filling. The interaction of method × rate of zinc fertilisation ($P<0.001$) as well as the interaction of rate × time zinc fertilisation ($P<0.001$) influenced grain yield (Figures 4.1C & 4.1D). However, neither the zinc fertilisation method ($P = 0.221$), the interaction between method and time ($P = 0.185$), nor the three-way interaction of method, application rate, and time ($P = 0.902$) led to a statistically significant increase in grain yield (Appendix 1A - C).

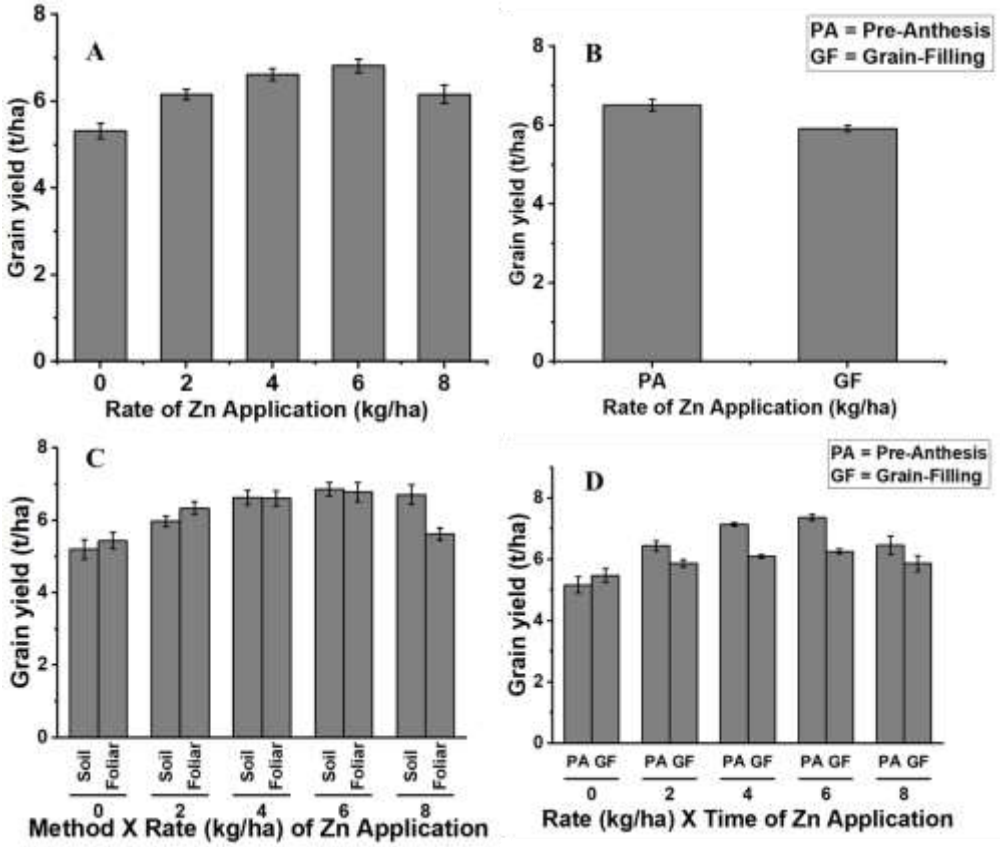

Figure 4. 1: Effect of Zn fertilisation on field-grown maize grain yield. Effect of Zn fertilisation rates on maize grain yield (A); effect of Zn fertilisation timing on maize grain yield (B); interactive effect of Zn fertilisation method and rate on maize grain yield (C); and interactive effect of Zn fertilisation rate and timing on maize grain yield (D). Error bars show s.e.m.

Cob weight harvested at physiological and harvest maturity stages.

The various rates of zinc fertiliser application significantly ($P<0.001$) affected maize's fresh cob weight (FCW) harvested at the physiological maturity stage (Figure 4.2A). A maximum (249.9 g) increase in FCW was observed at zinc fertilisation rate of 6 kg ha^{-1}. In comparison, the control with no zinc fertiliser application recorded the minimum (154.3 g) FCW, showing approximately a 1.6-fold difference between the control with no zinc fertilisation and zinc fertilisation

rate of 6 kg ha^{-1}. Fresh cob weight showed a rising trend with increasing rates of zinc fertilisation up to 6 kg $ha^{-1,}$ levelling off at 8 kg ha^{-1} (Figure 4.2A). Similarly, FCW differed significantly ($P<0.001$) among the various times of zinc fertiliser application (Figure 4.2B). Fresh cob weight ranged from 190.5 g to 219.7 g, indicating a 15 % increase between zinc application at pre-anthesis and grain filling. The interaction of rate × time of application appeared to have a significant ($P<0.001$) impact on the FCW of maize harvested at the physiological maturity stage (Figure 4.2C). However, there was no significant effects on the method of zinc fertilisation ($P = 0.506$), the interaction between method × rate ($P = 0.915$), the interaction between method × time ($P = 0.990$), and the three-way interaction between method × rate × time ($P = 0.957$) (Appendix 1D - G).

At the harvest maturity stage, a comparable trend was detected in the dry cob weight (DCW). There were no significant effects on the method of zinc fertilisation ($P = 0.146$), the interaction of rate × method ($P = 0.286$), and the interaction of method × time ($P = 0.258$) (Appendix 2A - C). However, the different rates of zinc fertilisation resulted in a significant ($P<0.001$) gain in DCW (Figure 4.3A). Dry cob weight varied from 153.7 g to 233.9 g, representing a 52 % increase in DCW between control with no zinc fertiliser application and 6 kg ha^{-1} zinc fertiliser application.

Generally, DCW increased with increasing zinc fertilisation until 6 kg ha^{-1}, which plateaued at 8 kg ha^{-1} (Figure 4.3A). Similarly, times of zinc fertilisation led to a significant ($P<0.001$) improvement in the DCW of maize harvested at the harvest maturity stage (Figure 4.3B). Zinc fertiliser application at the grain filling stage

recorded the minimum (185.0 g). Conversely, zinc fertiliser application at the pre-anthesis stage recorded the maximum (213.2 g), indicating an approximately 15 % increase in DCW over zinc application at the grain-filling stage. The interaction of rate × time of zinc fertilisation ($P<0.001$) and interaction of method × rate × time of zinc fertilisation ($P = 0.007$) had a significant influence on the DCW of maize harvested at the harvest maturity stage (Figures 4.3C & 4.3D).

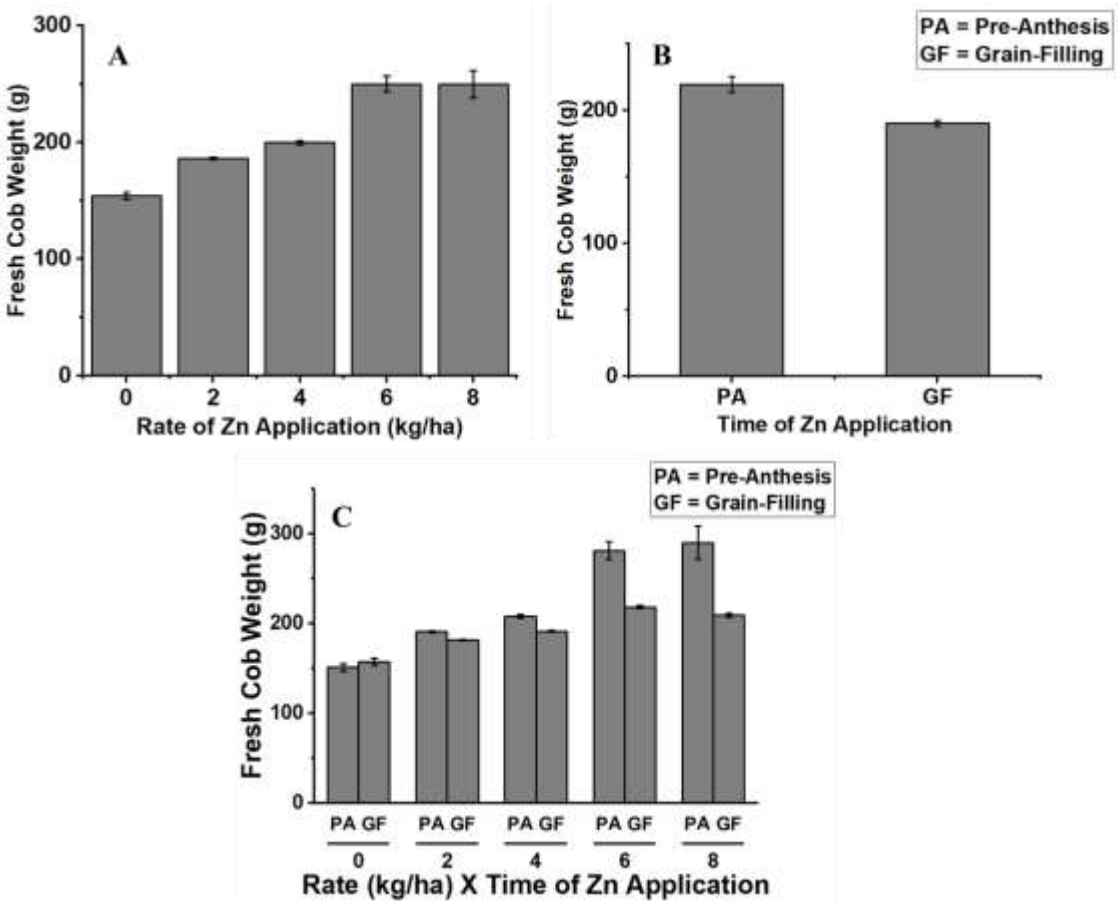

Figure 4. 2: Effect of Zn fertilisation on fresh cob weight (FCW) grown under field conditions. Effect of rate of Zn fertilisation on FCW of maize (A); effect of time of Zn fertilisation on FCW of maize (B); and interactive effect of rate × time of Zn fertilisation on FCW of maize (C). Error bars show s.e.m.

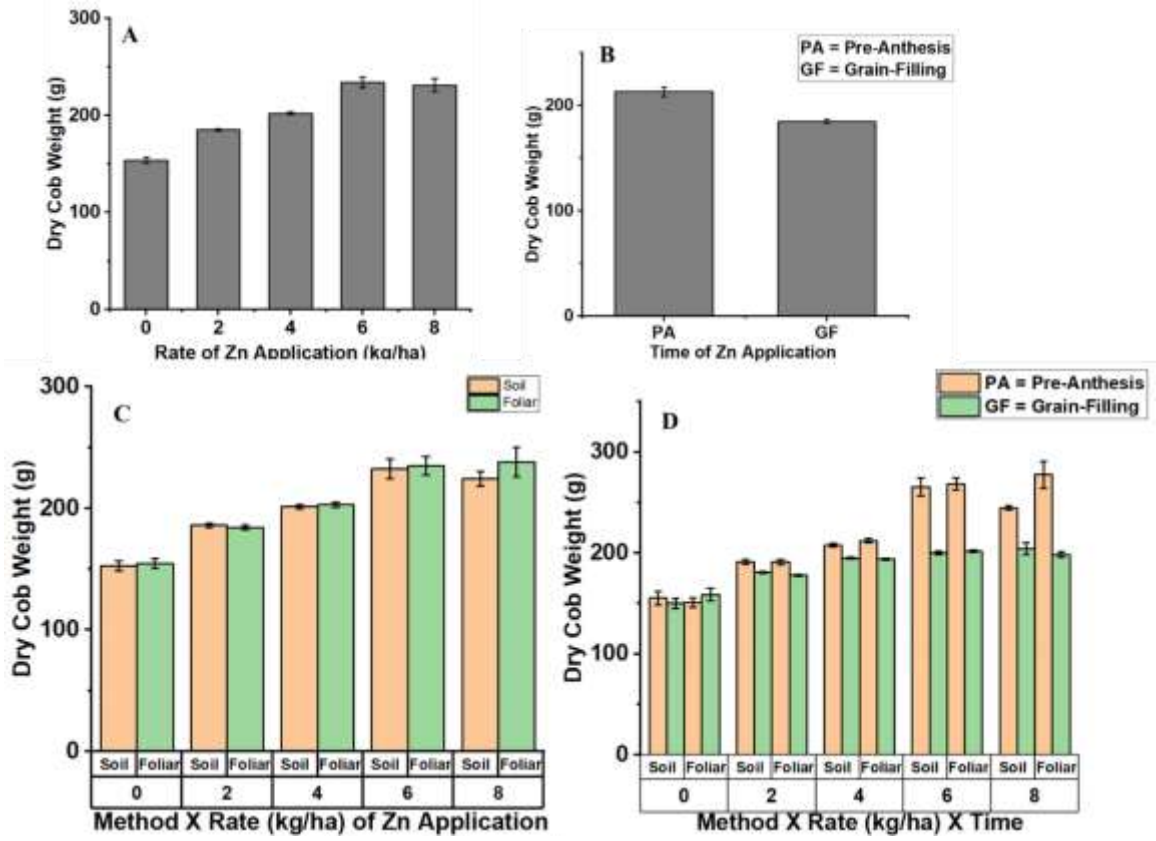

Figure 4. 3: Effect of Zn fertilisation on dry cob weight (DCW) of maize grown under field conditions. Effect of rate of Zn fertilisation on DCW of maize (A); impact Zn fertilisation timing on DCW of maize (B); interactive effect of rate × time of Zn fertilisation on DCW of maize (C); and interactive effect of method × rate × time of Zn fertilisation on dry cob weight of maize (D). Error bars show s.e.m.

Cob weight

The method of zinc fertilisation (P = 0.244), the interaction of method × time of zinc fertilisation (P = 0.546), and the three-way interaction effects for method × rate × time of zinc fertilisation did not significantly lead to increases (P = 0.605) in cob weight of maize (Appendix 2D - F). However, maize cob weight varied significantly (P<0.001) among the different zinc fertilisation rates (Figure 4.4A). The cob weight varied from 3.056 kg to 3.725 kg, suggesting approximately 21 % increase in maize cob weight of zinc fertiliser rates of 6 kg ha^{-1} compared to the

control treatment. Cob weight increased with an increasing rate of zinc application to an asymptote at a rate of 6 kg Zn ha^{-1} and then declined at 8 kg Zn ha^{-1} (Figure 4.4A).

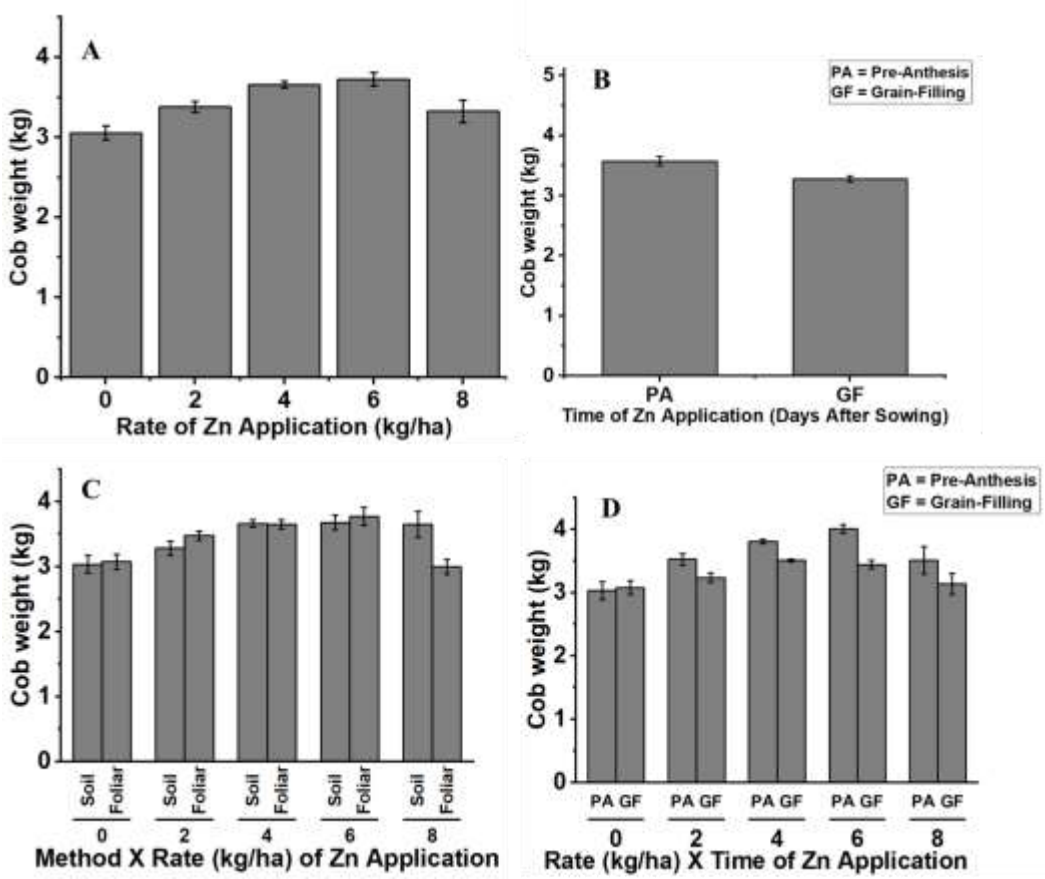

Figure 4. 4: Effect of Zn fertilisation on cob weight (CW) of maize grown under field conditions. Effect of rate of Zn fertilisation on CW of maize (A); effect of Zn fertilisation timing on CW of maize (B); interactive effect of method × rate of Zn fertilisation on CW of maize (C); and interactive effect of rate × time of Zn fertilisation on CW of maize (D). Error bars show s.e.m.

Similarly, the time of zinc fertilisation resulted in a significant ($P<0.001$) influence on cob weight (Figure 4.4B). Cob weight ranged between 3.282 kg/ha and 3.576 kg/ha for zinc application at grain filling and pre-anthesis. The interaction of method × zinc fertilisation rate ($P<0.001$), as well as the interaction

between zinc fertilisation rate × time (P<0.018) significantly affected cob weight (Figures 4.4C & 4.4D).

Grain weight

Grain weight varied significantly (P<0.001) among the different rates of zinc fertilisation (Figure 4.5A). Grain weight ranged from 1.92 kg to 2.46 kg, indicating an approximately 1.2-fold variation between the control group, which had no zinc fertilisation and the zinc fertilisation rate of 6 kg ha-^{1}. Generally, it was clear that grain weight increased with an increasing rate of zinc fertiliser to an asymptote at a rate of 6 kg ha^{-1} and then declined at the rate of 8 kg ha^{-1}. Similarly, the time of zinc application significantly (P<0.001) affected grain weight (Figure 4.5B). Grain weight ranged from 2.13 kg to 2.35 kg. The lowest grain weight was recorded for zinc application at grain filling and the highest grain weight was recorded for zinc application at pre-anthesis. The interaction of method × rate of zinc fertilisation (P<0.001) as well as the interaction between rate × time of zinc fertilisation (P<0.001) influence grain yield (Figures 4.5C & 4.5D). However, there were no significant interaction effects of method × time (P = 0.185), method × rate × time (P = 0.902), and the method of zinc fertilisation (P = 0.221) on grain yield (Appendix 3A - C).

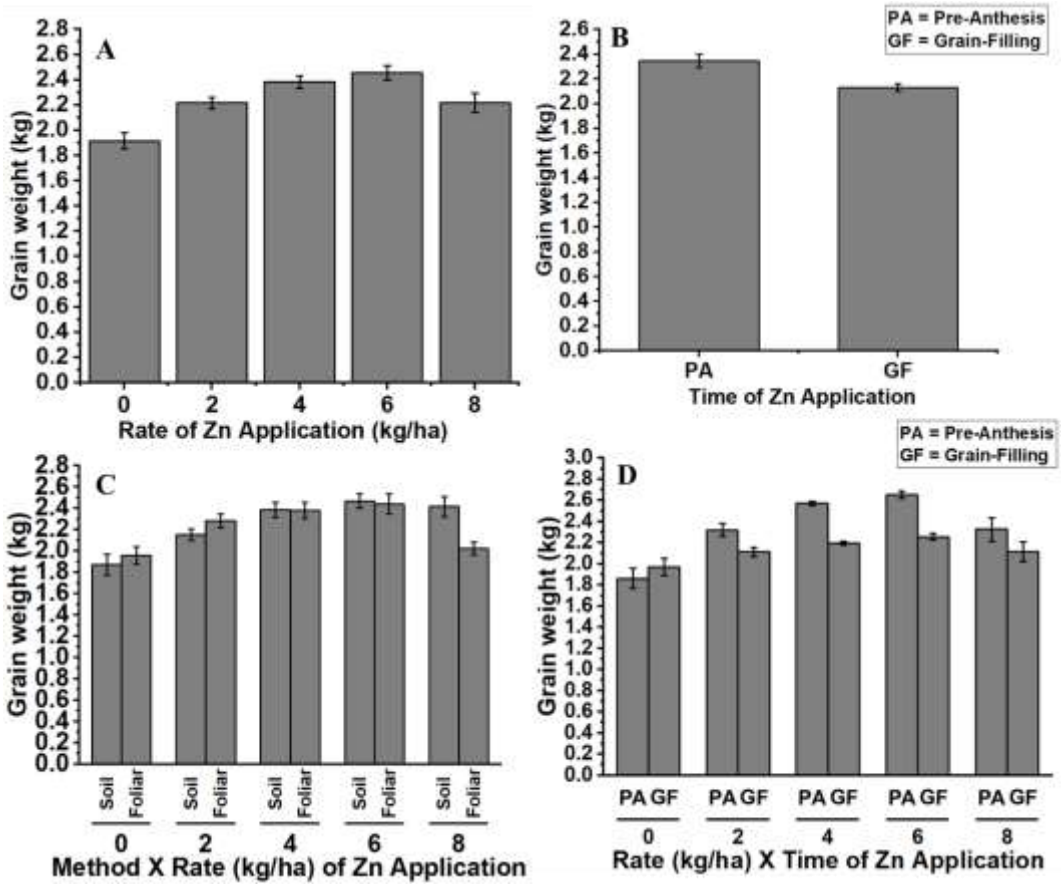

Figure 4. 5: Effect of Zn fertilisation on grain weight (GW) of maize grown under field conditions. Effect of Zn fertilisation rate on GW of maize (A); effect of time of Zn fertilisation on GW of maize (B); interactive effect of method × rate of Zn fertilisation on GW of maize (C); and interactive effect of rate × time of Zn fertilisation on GW of maize (D). Error bars show s.e.m.

Cob length

Effect of zinc fertilisation method (P =0.737), rate (P = 0.877), time (P = 0.737), the interaction of method × rate (0.642) the interaction of method × time (P = 0.339), the interaction of rate × time (P = 0.644), and the three-way interaction of method × rate × time (P = 0.817) did not show a significant impact on cob length of field-grown maize (Appendix 4A - G).

100 seed weight

The main effect of zinc fertilisation method (P =0.817), rate (P = 0.325), time (P = 0.398), the interaction of method × rate (0.636) the interaction of method × time (P = 0.196), the interaction of rate × time (P = 0.478), and the three-way interaction of method × rate × time (P = 0.764) did not show a significant impact on 100 seed weight of field-grown maize (Appendix 5A -G).

Biomass Parameters

Shoot dry weight at Physiological and harvest maturity.

Shoot dry weight (SDW) at physiological maturity varied significantly (P<0.001) among the different rates of zinc fertilisation (Figure 4.6A). Shoot dry weight ranged from 88.42 g to 138.39 g, indicating an approximately 1.5-fold variation between control with no zinc fertilisation and zinc fertilisation rate of 8 kg ha^{-1}. Generally, it was evident that as the rate of application increases, SDW also increases (Figure 4.6A). However, the method of application (P = 0.591), time of zinc fertilisation (P = 0.979), the interaction between method × rate of zinc fertilisation (P = 0.767), the interaction between method × time of zinc fertilisation (P = 0.275), the interaction of rate × time (P = 0. 083) and the three-way interaction of method × rate × time of fertilisation (P = 0.928) did not have a significant effect on SDW (Appendix 6A - F).

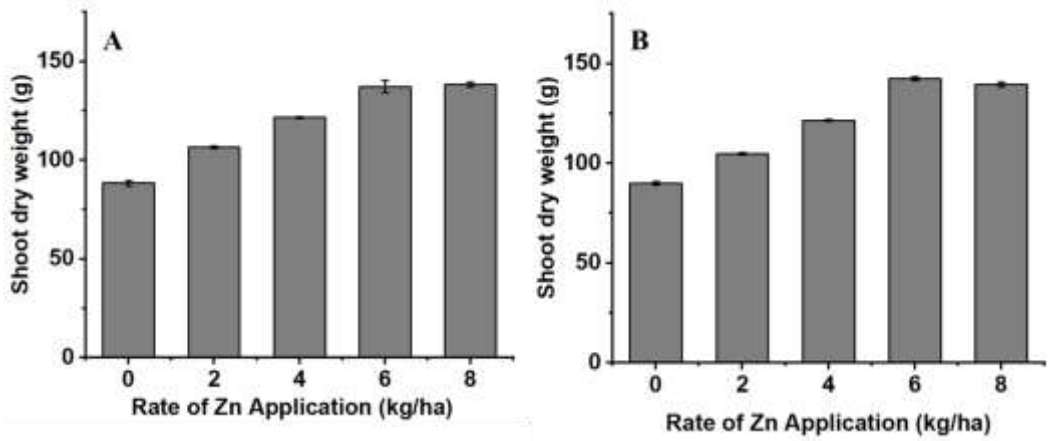

Figure 4. 6: Effect of Zn fertilisation on Shoot dry weight (SDW) of maize plants harvested at the physiological and harvest maturity stages. Effect of different Zn fertilisation rates on SDW of maize plants harvested at the physiological maturity stage (A); and effect of different rates of Zn fertilisation on SDW of maize plants harvested at the harvest maturity stage (B). Error bars show s.e.m.

Similarly, the different rates of zinc fertilisation significantly ($P<0.001$) influenced the shoot dry weight at harvest maturity (Figure 4.6B). Shoot dry weight ranged from 90.05 g to 142.47 g, indicating an approximately 58 % increase in SDW of zinc fertiliser rates of 6 kg ha^{-1} compared to the control treatment. Generally, it was clear that SDW weight increased with an increasing rate of zinc fertilisation to an asymptote at a rate of 6 kg ha^{-1} and then declined at the rate of 8 kg ha^{-1}. However, the method of zinc fertilisation ($P = 0.055$), time of zinc fertilisation ($P = 0.234$), the interaction between method × rate of zinc fertilisation ($P = 0.068$), the interaction between method × time of zinc fertilisation ($P = 0.081$), the interaction between rate × time of zinc fertilisation ($P = 0.062$) and the three-way interaction of method × rate × time (0.812) did not have a significant effect on SDW (Appendix 7A - F).

Root dry weight

Root dry weight (RDW) at physiological maturity varied significantly ($P<0.001$) among the various rates of zinc application (Figure 4.7A). Root dry weight ranged from 8.98 g to 21.41 g, indicating an approximately 2.3-fold variation in RDW of zinc fertiliser rates of 8 kg ha^{-1}compared to the control treatment.

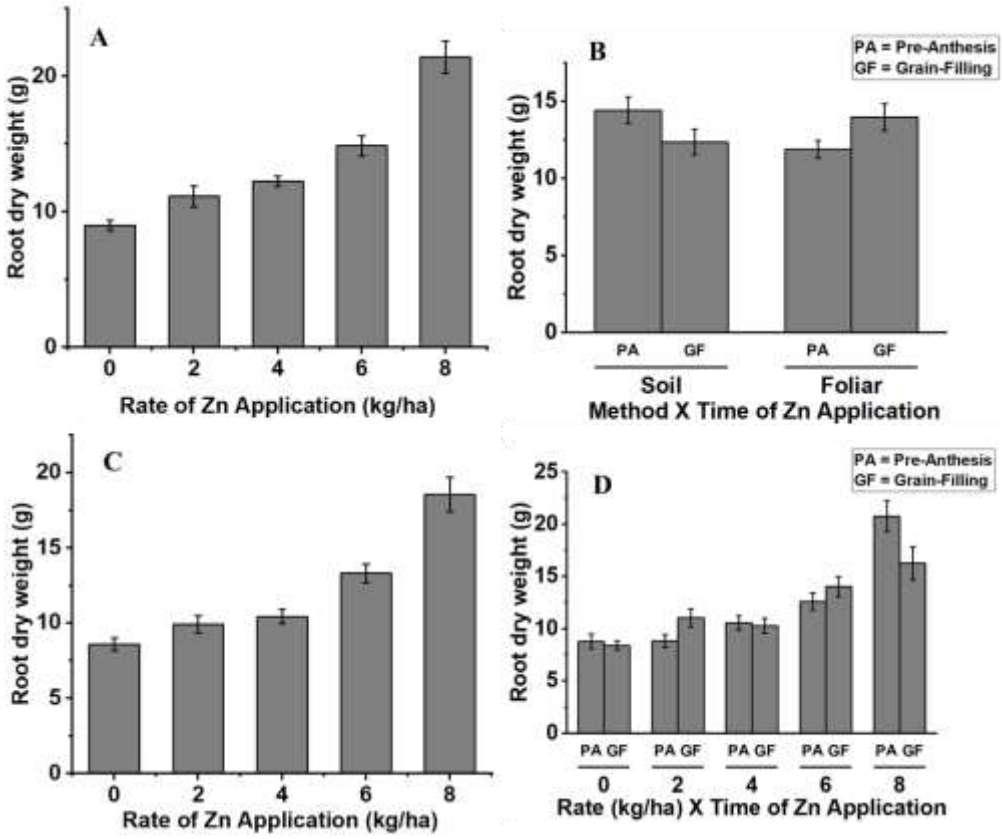

Figure 4. 7: Effects of Zn fertilisation on root dry weight (RDW) of maize plants harvested at the physiological and harvest maturity stages. Effects of different Zn fertilisation rates on RDW of maize plants harvested at the physiological maturity stage (A); effects of method × time of Zn fertilisation on RDW of maize plants harvested at the physiological maturity stage (B); effects of different rates of Zn fertilisation on RDW of maize plants harvested at the harvest maturity stage (C); and, effects of rate × time of Zn fertilisation on RDW of maize plants harvested at the harvest maturity stage (D). Error bars show s.e.m.

The data indicated that RDW increased progressively with increasing rate of zinc fertilisation (Figure 4.7A). The interaction of method and time of zinc application had a significant($P<0.001$) effect on RDW (Figure 4.7B). However, the method of application ($P = 0.434$), the timing of fertilisation ($P = 0.968$), the interaction between method × rate ($P = 0.140$), the interaction of rate × time ($P = 0.250$), and the three-way interaction of method × rate × time ($P = 0.337$) did not have a significant impact on RDW (Appendix 8A - E).

Similarly, the different rates of zinc fertilisation led to a significant ($P<0.001$) increase in root dry weight (RDW) at harvest maturity (Figure 4.7C). Root dry weight ranged from 8.59 g to 18.54 g, indicating an approximately 2.1-fold variation in RDW of zinc fertilser rates of 8 kg ha^{-1} compared to the control treatment. Generally, it was evident that RDW increased with an increasing rate of zinc fertilisation. The interaction of rate and time of zinc application significantly ($P<0.005$) affected RDW (Figure 4.7D). However, the method of fertilisation ($P = 0.924$), the time of fertilisation ($P = 0.993$), the method × rate combination ($P = 0.337$), the method × time combination ($P = 0.210$), and the method × rate × time combination ($P = 0.667$) did not significantly impact RDW (Appendix 9A - E).

Growth and physiological parameters

Plant height

Analysis of maize plant height showed no statistically significant effect of fertilisation methods ($P = 0.987$). Furthermore, there was no significant interactive effects of fertilisation method × rate of fertilisation ($P = 1.000$),

method × time of fertilisation (P = 0.973), rate × time of fertilisation (P = 0.134) and, method × rate × time of fertilisation (P= 0.994) (Appendix 10A - E). However, the rates of zinc application significantly (P<0.001) affected the plant height of maize (Figure 4.8A). The mean plant height of maize varied from 114.1 cm to 129 cm, indicating a notable 13 % increase in plant height of zinc fertiliser rates of 6 kg Zn ha^{-1}compared to the control treatment. As the zinc fertilisation rate increased, the plant height of maize generally increased until reaching a maximum and then decreased when the fertilisation rate reached 8 kg Zn ha^{-1} (Figure 4.8A). Similarly, the plant height of maize varied significantly (P<0.001) with the timing of zinc application (Figure 4.8B). Zinc fertiliser application at the pre-anthesis stage recorded the maximum mean plant height of 128.6 cm and the minimum mean plant height of 118.3 cm for zinc application at the grain-filling stage (Figure 4.8B).

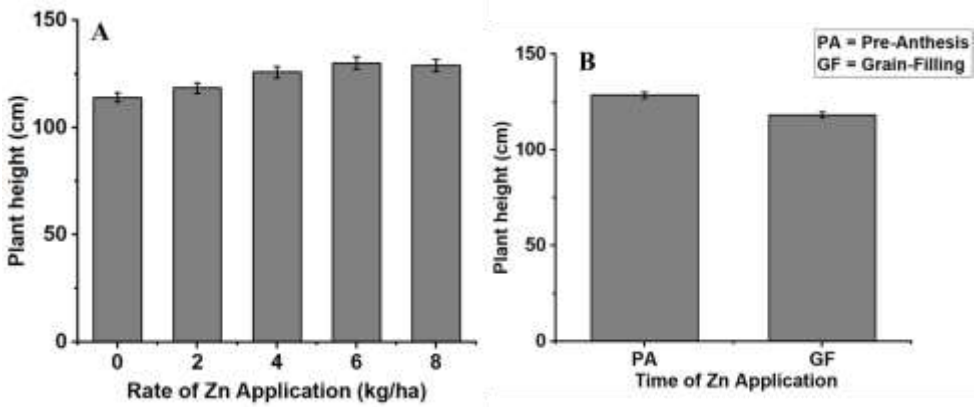

Figure 4. 8: Effect of Zn fertilisation on field-grown maize plant height (PH). Effect of different Zn fertilisation rates on PH of maize plants (A); and effect of time of Zn fertilisation on PH of maize plants (B). Error bars show s.e.m.

Maximum quantum yield of photosystem II (PSII) photochemistry (Fv/Fm)

The Fv/Fm ratio of field-grown maize plants varied significantly ($P<0.001$) with rates of zinc fertilisation (Figure 4.9). The mean Fv/Fm ratio varied between 0.6585 to 0.7589. However, the method of fertilisation ($P = 0.453$), time of fertilisation ($P = 0.170$), the interactive effects of method × rate of fertilisation ($P = 0.914$), method × time of fertilisation ($P = 0.175$), rate × time of fertilisation ($P = 0.861$) and the three-way interaction of method × rate × time ($P = 0.745$) did not significantly impact the Fv/Fm ratio of maize plants (Appendix 11A - F).

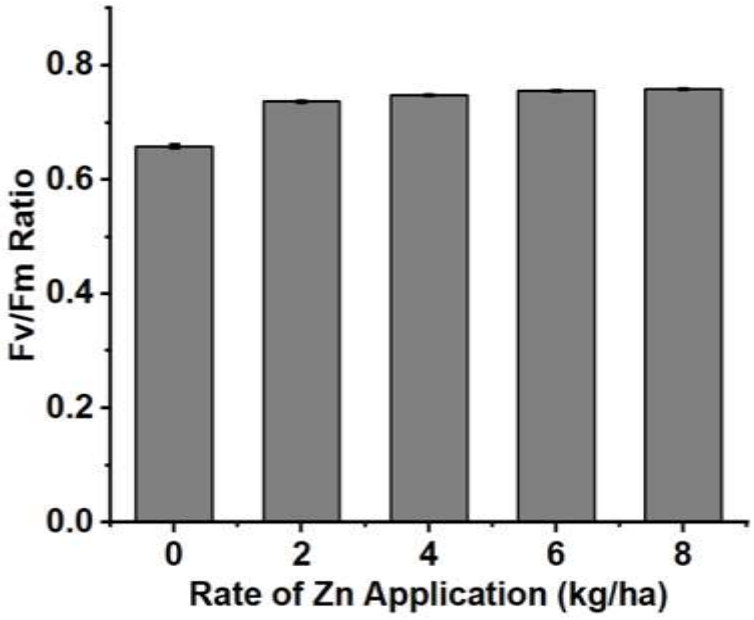

Figure 4. 9: Effect of different Zn fertilisation rates on Fv/Fm ratio of field-grown maize plants. Error bars show s.e.m.

Performance Index (PI)

The PI did not differ significantly among the method of fertilisation ($P = 0.522$), the interaction between method × rate of fertilisation ($P = 0.725$), and the three-way interaction between method × rate × time of application ($P = 0.254$) (Appendix 12A - C). However, among the rates of zinc fertilisation, statistically

significant ($P<0.001$) effects were observed (Figure 4.10A). The performance index varied from 2.208 to 3.825, indicating a 1.7-fold variation in PI of zinc fertiliser rates of 6 kg ha^{-1} compared to the control treatment. Generally, the PI increased with a corresponding increase in zinc fertilisation up to 6 kg ha^{-1} and declined at 8 kg ha^{-1}. The timing of zinc fertilisation significantly ($P<0.001$) impacted the PI of field-grown maize plants (Figure 4.10B). The maximum (3.671) PI was observed when zinc was applied at the pre-anthesis stage, while the minimum (2.983) PI was observed at the grain filling-stage, pointing to a 23 % increase in PI for zinc application at grain filling. Similarly, the interactive effects methods of zinc application × time of fertilisation ($P = 0.034$) and rates of zinc application × time of zinc fertilisation ($P<0.001$) significantly influenced the PI of maize plants (Figures 4.10C & 4.10D).

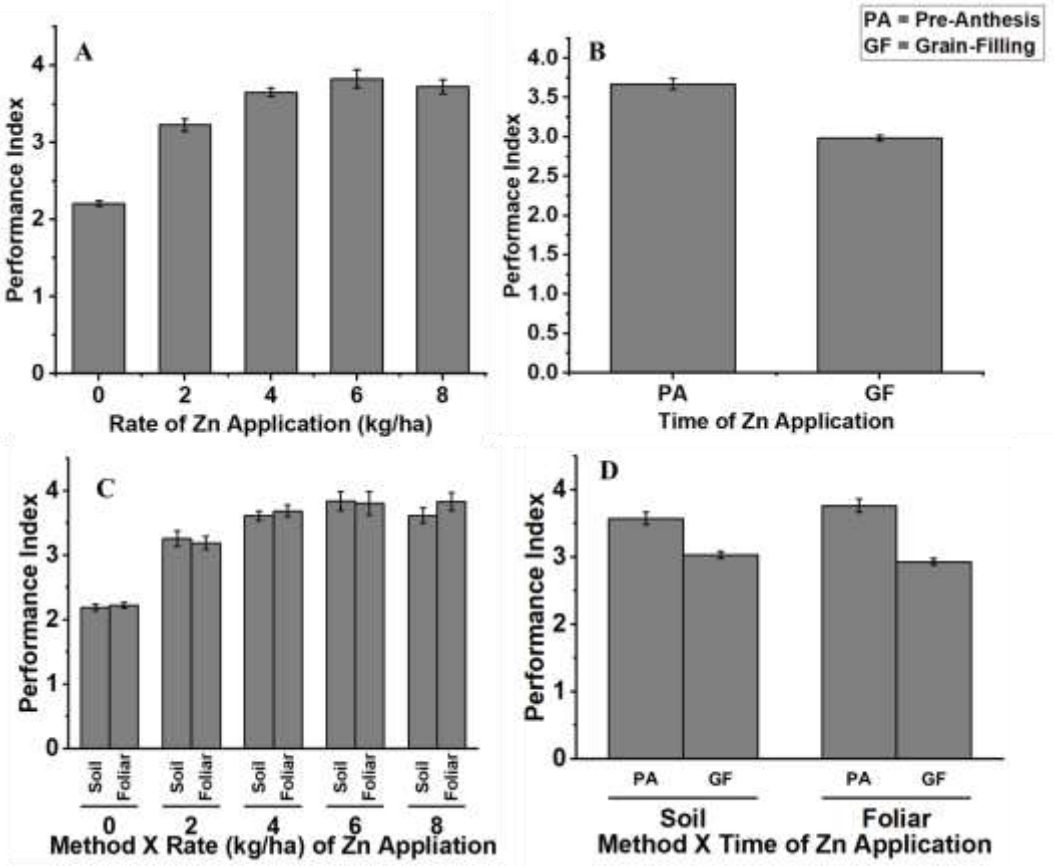

Figure 4. 10: Effect of Zn fertilisation on field-grown maize plants' performance index (PI). Effect of Zn fertilisation rates on PI of field-grown maize plants (A); effects of Zn fertilisation timing on PI of field-grown maize plants (B); effects of methods of Zn application × rate of Zn fertilisation on PI of field-grown maize plants (C); and effects of methods × time of Zn fertilisation on PI of field-grown maize plants (D). Error bars show s.e.m.

Chlorophyll content

Rates of zinc fertilisation had a significant ($P<0.001$) effect on the chlorophyll content in the leaves of field-grown maize (Figure 4.11A). The average chlorophyll content varied from 40.12 mg m^{-2} to 45.54 mg m^{-2}, implying a 13.5 % increase in chlorophyll content of zinc fertiliser rates of 6 kg Zn ha^{-1} compared to the control treatment. Overall, the chlorophyll content in maize generally increased with increasing zinc fertiliser rate, peaking at 6 kg $ZnSO_4$ ha^{-1} before

falling. The timing of zinc fertilisation significantly ($P<0.001$) influenced the chlorophyll content of field-grown maize (Figure 4.11B). The minimum (41.22) mean chlorophyll content was observed for zinc application at the grain-filling stage, while the maximum (44.79) chlorophyll content was observed for zinc application at the pre-anthesis stage. Similarly, there was significant interactive effects of method of zinc application × rate of zinc fertilisation on the chlorophyll content of field-grown maize plants (Figure 4.11C).

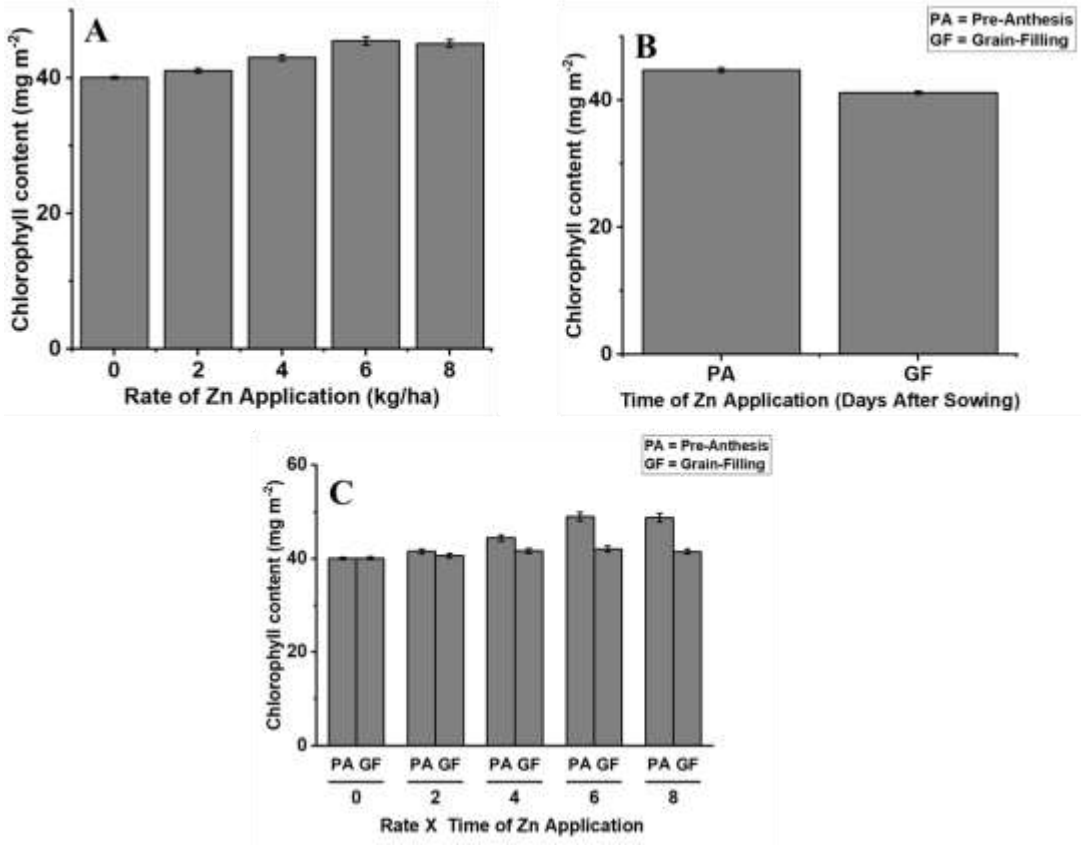

Figure 4. 11: Effects of Zn fertilisation on chlorophyll content of field-grown maize plants. Effects of Zn fertiliser rates on chlorophyll contents of field-grown maize plants (A); effects of Zn fertiliser timing on chlorophyll content of field-grown maize plants (B); and effects of Zn fertiliser rates × time of Zn fertilisation on chlorophyll contents of field-grown maize plants (C). Error bars show s.e.m.

However, zinc fertilisation methods (P = 0.869), and the interactive effects of zinc application methods × rates of zinc fertilisation (P = 0.998), the zinc application methods × time of zinc fertilisation (P = 0.216), and the three-way interactive effects of zinc application methods × rates of zinc fertilisation × time of zinc fertilisation did not significantly influence the chlorophyll contents of maize (Appendix 12D - G).

Zinc concentration in grain harvested at physiological and harvest maturity stages.

Zinc concentration in grain at physiological maturity growth stage

Significant differences were observed in the grain zinc concentration of grains harvested at physiological maturity among the zinc fertilisation methods (P<0.001), rates of zinc fertilisation (P<0.001), and timing of zinc fertiliser application (P<0.001). Generally, the zinc concentration within the grain harvested at the physiological maturity stage varied from 19.76 mg Zn kg^{-1} DW to 51.94 mg Zn kg^{-1} DW with a grand mean of 32.55 mg Zn kg^{-1} DW. Zinc fertilisation method recorded concentration of 30.16 mg Zn kg^{-1} DW to 34.94 mg Zn kg^{-1} DW. The minimum grain zinc concentration of 30.16 mg Zn kg^{-1} DW was observed for soil applied zinc, while the maximum grain zinc concentration of 34.94 mg Zn kg^{-1} DW was observed for foliar applied zinc. The foliar applied zinc increment represents approximately 16 % increases of zinc concentration over soil applied zinc method.

Similarly, the different rates of zinc fertilisation significantly (P<0.001) impacted grain zinc concentration (Figure 4.12C). Rates of Zn application resulted in grain

zinc concentrations of 19.76 mg Zn kg^{-1} DW to 51.94 mg Zn kg^{-1} DW. The highest zinc concentration translates to approximately 2.6-fold increases in zinc concentration of the 8 kg ha^{-1} zinc application rate compared to the control. Generally, it was apparent that grain zinc concentration increases with an increasing rate of zinc fertiliser application (Figure 4.12C). Furthermore, zinc fertiliser application at pre-anthesis recorded the minimum grain zinc concentration of 27.85 mg Zn kg^{-1} with the grain-filling stage recording the maximum grain zinc concentration of 37.25 mg Zn kg^{-1} DW, indicating an approximately 1.3-fold increase in grain zinc concentration at grain-filling stage of fertiliser application.

Zinc concentration in grain at harvest maturity growth stage

The zinc concentration within the grain harvested at the harvest maturity stage followed the same trend as that of physiological maturity growth stage. The overall zinc concentration in the grain ranged from 17.60 mg Zn kg^{-1} DW to 45.31 mg Zn kg^{-1} DW with a grand mean of 29.39 mg Zn kg^{-1} DW. Significant differences were observed in the zinc concentration of grains harvested at the harvest maturity stage among the methods ($P<0.001$), rates ($P<0.001$), and timing ($P<0.001$) of zinc fertilisation. The various methods of zinc application significantly ($P<0.001$) influenced grain zinc concentration (Figure 4.12B). Soil zinc application recorded the lowest grain zinc concentration of 27.93 mg Zn kg^{-1} DW, while foliar zinc application recorded the highest grain zinc concentration of 30.86 mg Zn kg^{-1} DW (Figure 4.12B). In the same vein, the various rates of zinc application had a significant ($P<0.001$) effect on grain zinc concentration (Figure

4.12D). The grain zinc concentration varied between 17.60 mg Zn kg^{-1} DW and 45.31 mg Zn kg^{-1} DW, indicating roughly a 2.5-fold variation in grain zinc concentration of zinc fertiliser rates of 8 kg ha^{-1} compared to the control treatment.

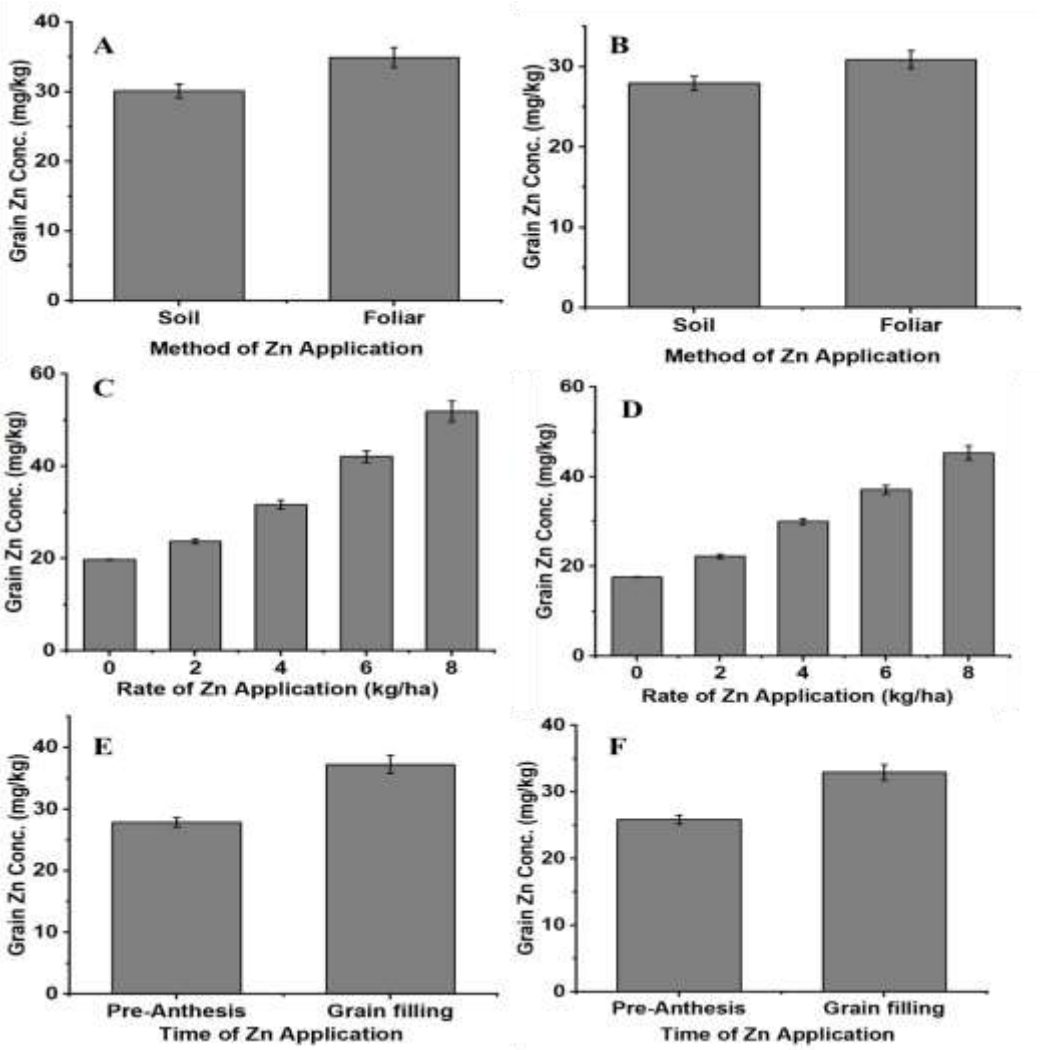

Figure 4. 12: Effects of Zn fertilisation on Zn concentration of grains harvested at physiological and harvest maturity stages. Effects of Zn fertilisation method on zinc concentration of grain harvested at the physiological maturity stage (A); effects of Zn fertilisation method on zinc concentration of grain harvested at the harvest maturity stage (B); effects of different Zn fertilisation rates on zinc concentration of grain harvested at the physiological maturity stage (C); effects of different Zn fertilisation rates on zinc concentration of grain harvested at the harvest maturity stage (D); effects of different timing of Zn fertilisation on zinc concentration of grain harvested at the physiological maturity stage (E); and effect

of different timing of Zn fertilisation on zinc concentration of grain harvested at the harvest maturity stage (F). Error bars show s.e.m.

The timing of zinc fertiliser application had a significant ($P<0.001$) effect on grain zinc concentration (Figure 4.12F). Zinc fertilisation at pre-anthesis yielded the minimum concentration (25.83 mg Zn kg^{-1} DW), in contrast to the maximum concentration (32.96 mg Zn kg^{-1} DW) achieved during the grain-filling stage.

Interaction effects on grain zinc concentration

The combined effect of method of Zn application × rate of zinc fertilisation significantly ($P<0.001$) influenced the zinc concentration of grains harvested at the physiological maturity stage (Figure 4.13A). Grain zinc concentration varied in response to the methods and rates of zinc fertiliser application. Foliar zinc fertilisation resulted in higher grain zinc concentrations than the soil zinc applied methods across all the various zinc fertilisation rates (Figure 4.13A). The interaction of method × timing of zinc fertilisation significantly ($P<0.001$) affected grain zinc concentration (Figure 4.13B). Zinc fertilisation at the grain-filling-stage for soil and foliar applications yielded a greater zinc concentration compared to the pre-anthesis stage (Figure 4.13B). The interaction effect of rate × time of zinc application significantly ($P<0.001$) affected the zinc concentration of grains harvested at the physiological maturity stage (Figure 4.13C). Grain zinc concentration varied in response to the timing and rates of zinc fertiliser application (Figure 4.13C). Zinc fertilisation at the grain-filling stage resulted in a higher zinc concentration compared to zinc fertilisation at the pre-anthesis stage. Similarly, the three-way interaction (method × rate × time) significantly ($P<0.001$) affected the zinc concentration of grains harvested at physiological

maturity (Figure 4.13D). For the same application rate, grains harvested under foliar application treatments recorded a higher zinc concentration than those harvested under soil treatments. Zinc application during grain filling across all zinc application rates and methods appears higher than at the pre-anthesis stage.

Similarly, the interaction effect of method × rate of zinc application significantly ($P<0.001$) affected the grain zinc concentration of grains harvested at the harvest maturity stage (Figure 4.14A). Grain zinc concentration responded differently to the various methods and rates of zinc fertiliser application. The grain zinc concentration seems to be higher with foliar zinc application compared to soil zinc application across all the different rates of zinc application (Figure 4.14A). The interaction of method × time of zinc fertilisation significantly ($P<0.001$) affected grain zinc concentration (Figure 4.14B). Zinc fertilisation at the grain-filling stage for soil and foliar applications produced greater grain zinc concentration compared to fertilisation at pre-anthesis stage (Figure 4.14B). The combined effect of zinc application rate and time significantly ($P<0.001$) impacted the grain zinc concentration of grains harvested at the harvest maturity stage (Figure 4.14C). Grain zinc concentration responded differently to the timing and rates of zinc fertiliser application (Figure 4.14C). Applying zinc during the grain-filling stage led to a greater zinc concentration in the grains compared to application during the pre-anthesis stage. In the same vein, the three-way interaction (method × rate × time) significantly ($P<0.001$) affected the zinc concentration of grains harvested at physiological maturity (Figure 4.14D). Plants subjected to foliar application treatments produced a higher grain zinc

concentration than those harvested under soil treatments at the same application rate. Zinc application during grain filling across all zinc application rates and methods was higher than that of the pre-anthesis stage. Although the grain zinc concentration had a similar trend across all main and interaction effects for grains harvested at both physiological and harvest maturity stages. However, grains harvested at physiological maturity had a higher zinc concentration compared to those harvested at harvest maturity across all treatments

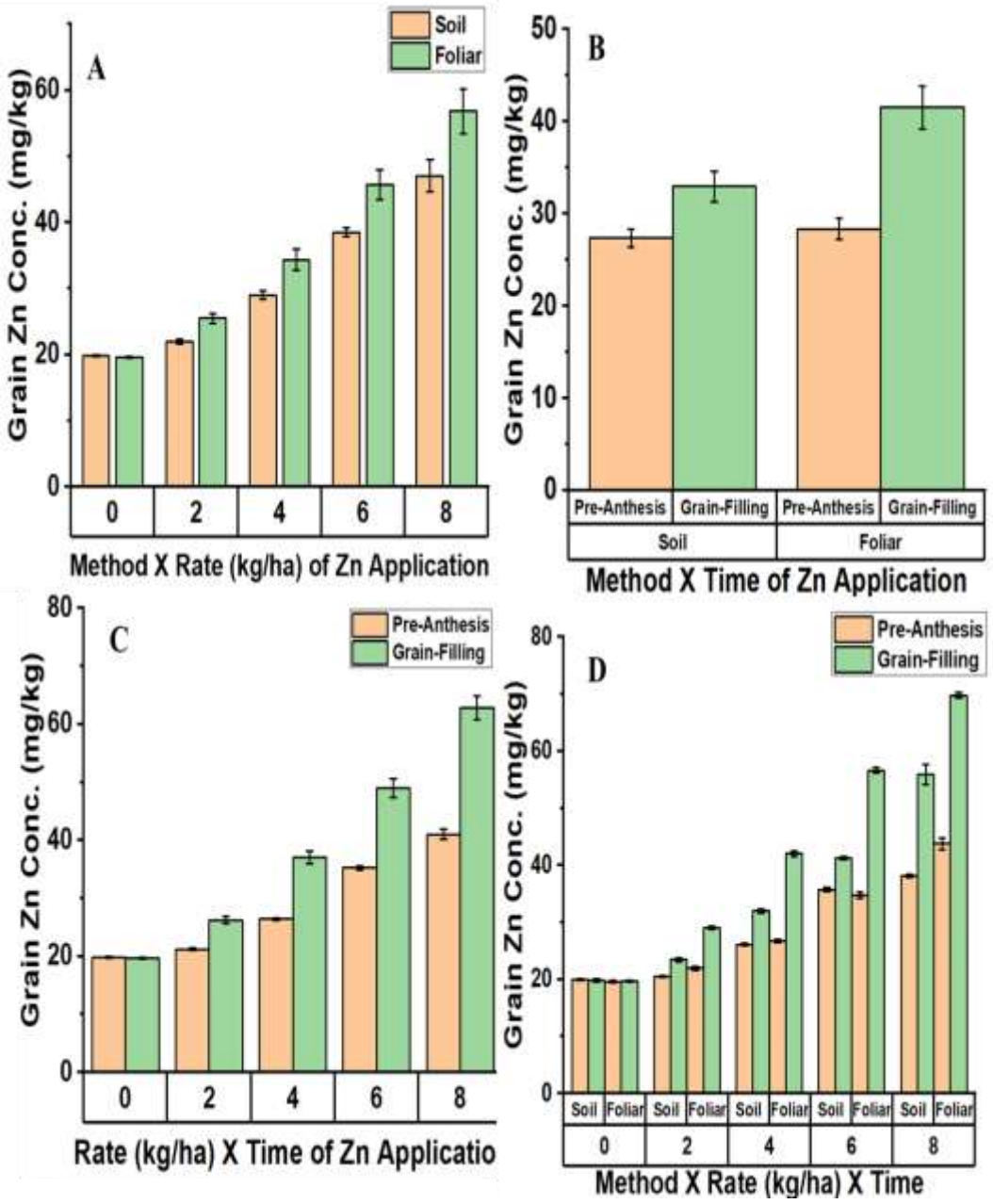

Figure 4. 13: Interaction effects of method × rate of Zn fertilisation on grains harvested at the physiological maturity stage (A); the interaction effects of method × time of Zn fertilisation on grains harvested at the physiological maturity stage (B); interaction effects of rate × time of Zn fertilisation on grains harvested at the physiological maturity stage (C); and the interaction effects of method × rate × time of Zn fertilisation on grains harvested at the physiological maturity stage (D). Error bars show s.e.m.

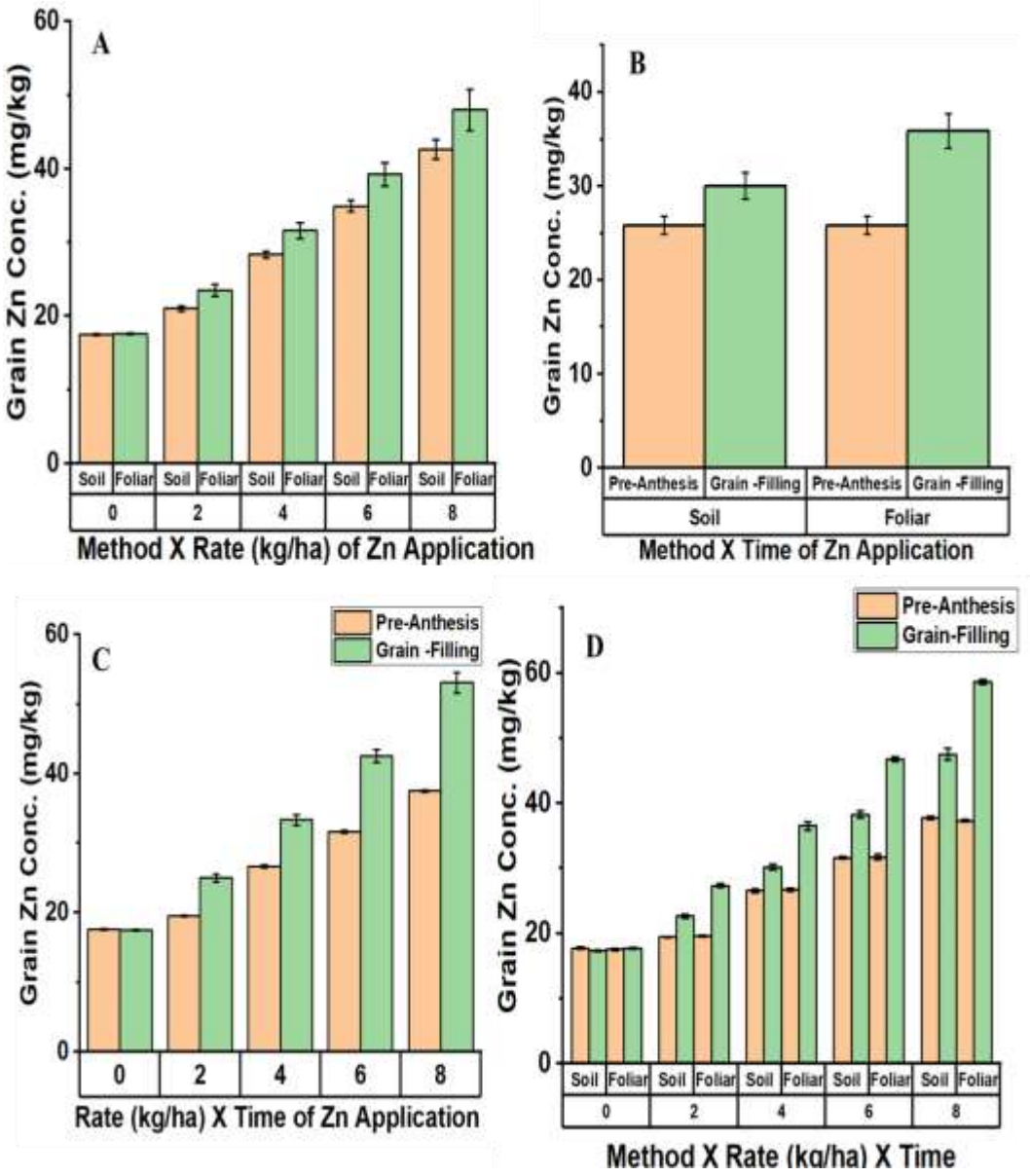

Figure 4. 14: Interaction effects of method × rate of zinc fertilisation on grains harvested at the harvest maturity stage (A); the interaction effects of method × time of zinc fertilisation on grains harvested at the harvest maturity stage (B); interaction effects of rate × time of zinc fertilisation on grains harvested at the harvest maturity stage (C); and the interaction effects of method × rate × time of zinc fertilisation on grains harvested at the harvest maturity stage (D). Error bars show s.e.m.

Experiment two: Demonstrating the efficacy of increasing the zinc content of carrots through agronomic biofortification.

Biomass Parameters

Shoot fresh weight

Analysis of carrots shoot fresh weight (SFW) showed no statistically significant response to the method of zinc fertilisation (P = 0.734), the time of zinc fertilisation (P = 0.891), the interaction between method × rate of fertilisation (P = 0.907), the interaction between method × time of fertilisation (P = 0.609), the interaction between rate × timing of fertilisation(P = 0.187) and the three-way interaction of method × rate × timing of fertilisation (P = 0.136) (Appendix 13A - F). However, higher zinc fertilisation rate led to a significant (P<0.001) improvement in shoot fresh weight with an increasing rate of zinc fertilisation (Figure 4.15). Shoot fresh weight ranged from 59.31 g to 25.49 g, indicating an approximately 2.3-fold variation in SFW of zinc fertiliser rates of 6 kg ha^{-1} compared to the control treatment.

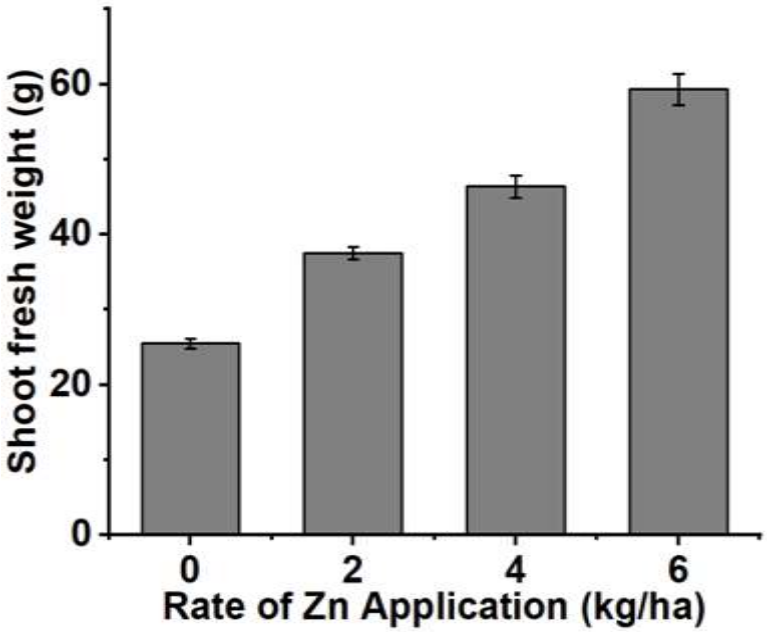

Figure 4. 15: Effects of zinc fertiliser rates on shoot fresh weight of pot-grown carrot. Error bars show s.e.m.

Root fresh weight

Root fresh weight (RFW) varied significantly ($P<0.001$) among the different rates of zinc fertilisation (Figure 4.16A). Root fresh weight ranged from 25.81 g to 61.13 g, indicating more than a double-fold variation in RFW of zinc fertiliser rates of 6 kg ha^{-1} compared to the control treatment. Generally, it was clear that RFW increased with an increasing rate of zinc fertilisation (Figure 4.16A). The time of zinc fertilisation significantly ($P<0.001$) impacted the RFW of pot-grown carrots (Figure 4.16B). Root fresh weight varied from 41.17 g to 47.18 g. The highest RFW was recorded 30 days after sowing, while the lowest was recorded 70 days after sowing, indicating a 14 % increase over plants under 70 days after sowing (Figure 4.16B). The interaction between rate × time of zinc fertilisation significantly influenced ($P<0.001$) the SFW of pot-grown carrots (Figure 4.16C). However, the main effect of method ($P = 0.840$) of fertilisation, the interaction

between method × rate of zinc fertilisation (P = 0.445), the interaction between method × time of zinc fertilisation (P = 0.079) and the three-way interaction of method × rate × time of zinc fertilisation (P = 0.264) did not show a significant effect on root fresh weight of pot-grown carrots (Appendix 14A - D).

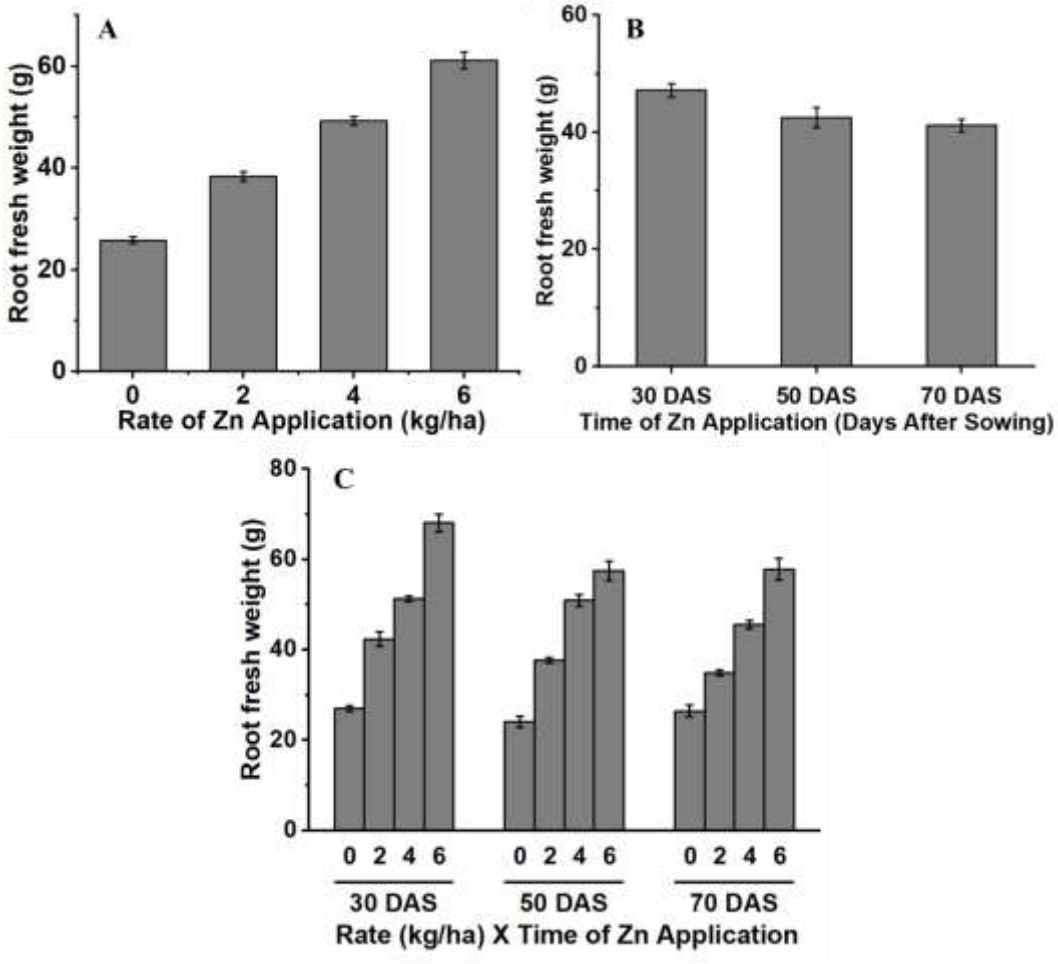

Figure 4. 16: Effects of Zn fertilisation on pot-grown carrots' root fresh weight (RFW). Effects of different zinc fertilisation rates on RFW of pot-grown carrots (A); effects of different timing of zinc fertilisation on RFW of pot-grown carrots (B); interaction effect of rate × time of zinc fertilisation on RFW of pot-grown carrots (C). Error bars show s.e.m.

Shoot dry weight

There were significant variations in shoot dry weight (SDW) ($P<0.001$) among the different zinc fertilisation rates (Figure 4.17). Shoot dry weight ranged from 3.57 g to 8.39 g, indicating a 2.3-fold variation between control and 6 kg ha^{-1} zinc fertilisation rate. Generally, it was evident that increasing the zinc application rates resulted in increases in SDW (Figure 4.17). However, there was no significant difference in the time of zinc application ($P = 0.729$), method of zinc application ($P = 0.571$), interaction of method and rate ($P = 0.440$), interaction of method and time ($P = 0.369$), interaction of rate and time ($P = 0.500$) and interaction of method, rate and time ($P = 0.186$) concerning SDW (Appendix 15A – F).

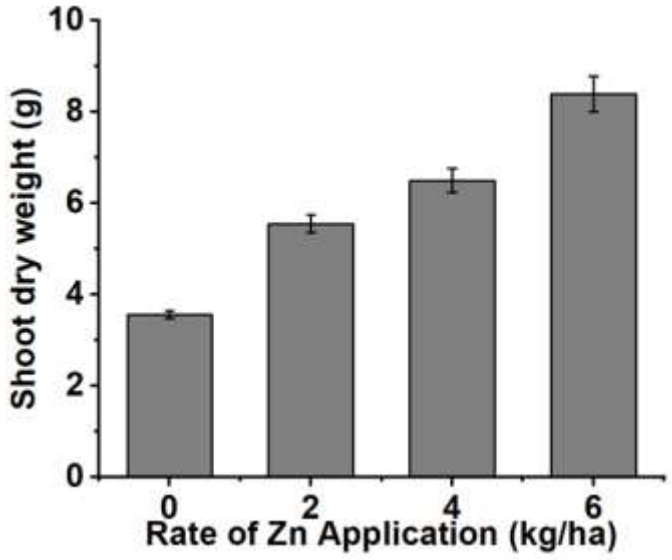

Figure 4. 17: Effects of different zinc fertilisation rates application on shoot dry weight of pot-grown carrots. Error bars show s.e.m.

Root dry weight

There was a significant ($P<0.001$) difference in root dry weight (RDW) among the methods of application (Figure 4.18A). Root dry weight ranged from 4.41 g to

5.55 g, indicating a significant 25 % increase in carrot's RDW under foliar zinc fertilisation over soil zinc fertilisation (Figure 4.18A). Root dry weight varied significantly ($P<0.001$) among the different rates of zinc application (Figure 4.18B). Root dry weight ranged from 3.76 g to 5.78 g, resulting in a 53 % increase in plants under 6 kg ha^{-1} fertilisation rate over control with no zinc fertilisation (Figure 4.18B). Based on the two-way analysis, RDW was influenced by the interaction of method and rate of fertilisation ($P<0.001$) (Figure 4.18C). However, there was no significant difference in the timing ($P = 0.765$) of fertilisation, the interaction between method and timing of fertilisation ($P = 0.764$), the interaction between rate and timing of fertilisation ($P = 0.623$), and the three-way interaction of method, rate and time ($P = 0.867$) did not have a significant impact on RDW (Appendix 16A - D).

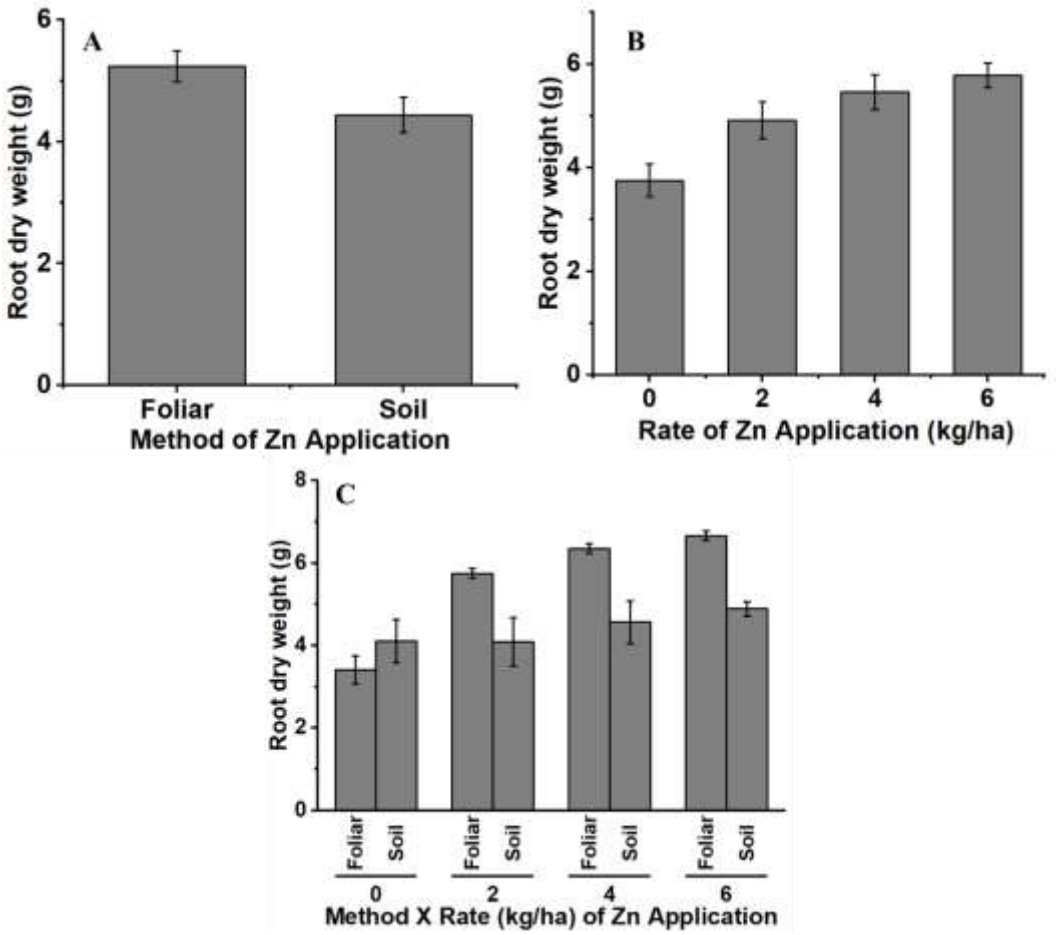

Figure 4. 18: Effects of Zn fertilisation on pot-grown carrots' root dry weight (RDW). Effects of zinc fertilisation method on RDW of pot-grown carrots (A); effects of different zinc fertilisation rates on RDW of pot-grown carrots (B); interaction effect of method × rate of zinc fertilisation on RDW of pot-grown carrots (C). Error bars show s.e.m.

Root length

The rate of application had a significant ($P<0.001$) effect on root length (Figure 4.19A). Root length ranged from 8.62 cm to 13.62 cm, indicating a 58 % increase in root length of zinc fertiliser rates of 6 kg ha-^{1} compared to the control treatment (Figure 4.19A). The time of zinc fertilisation had a significant ($P<0.001$) impact on the root length of carrots (Figure 4.19B). The maximum root length was observed for zinc fertilisation 30 days after sowing (DAS), followed by 50 DAS

and 70 DAS, respectively. The two-way analysis showed that the interaction of rate and time of zinc fertiliser application significantly ($P<0.001$) affected the root length of carrots (Figure 4.19C). However, no statistically significant effects were found for the method of fertilisation ($P = 0.776$), interaction between method and fertilisation rate ($P = 0.583$), interaction between method and timing of fertilisation ($P = 0.352$) and the three-way interaction between method, rate and timing of fertilisation ($P = 0.256$) (Appendix 17A - D).

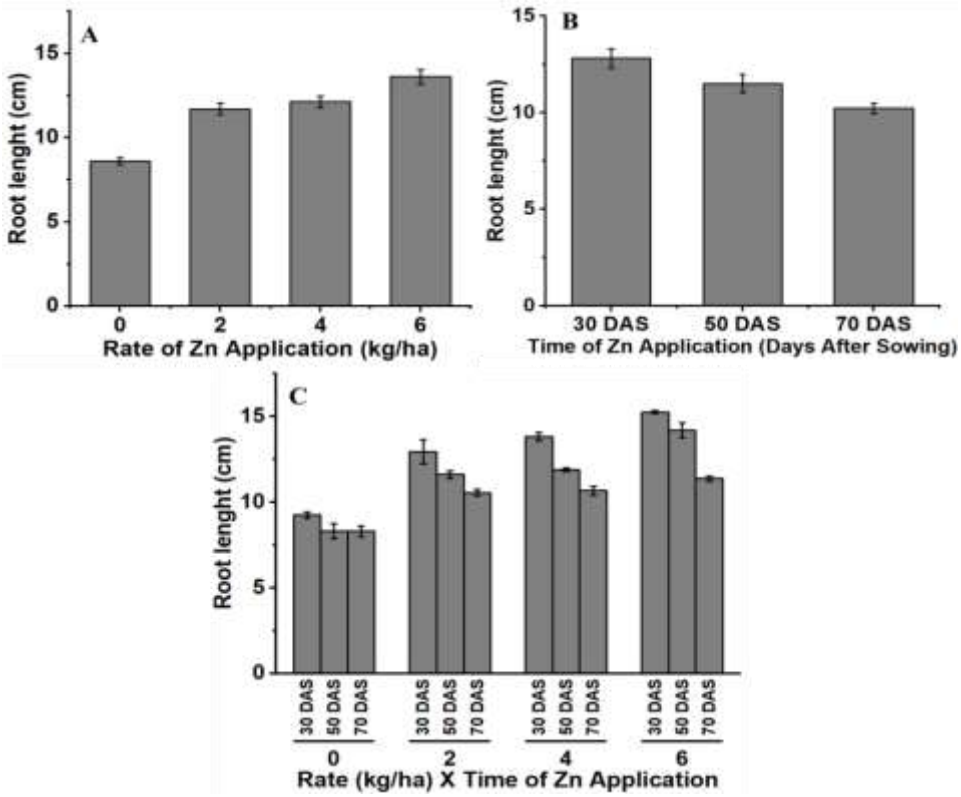

Figure 4. 19: Effects of Zn fertilisation on pot-grown carrots' root length (RL). Effects of different Zn fertilisation rates application on RL of pot-grown carrots (A); effects of Zn fertilisation timing on RL of pot-grown carrots (B); the interaction effects of rate × time of zinc fertilisation on root length of pot-grown carrots (C). Error bars show s.e.m.

Root width

The various rates of zinc fertiliser application had a significant ($P<0.001$) effect on root width (Figure 4.20A). Root width ranged from 11.85 mm to 21.10 mm, indicating a 1.7-fold variation in root width of zinc fertiliser rates of 6 kg ha^{-1} compared to the control treatment (Figure 4.20A). Generally, it was evident that root width increases with an increasing rate of zinc fertilisation (Figure 20A). The timing of zinc application had a significant ($P<0.001$) effect on root diameter (Figure 4.20B). Zinc application at 30 days after sowing (DAS) recorded the maximum root width, followed by zinc application at 50 days after sowing (DAS), and zinc application at 70 days after sowing (DAS) recorded the minimum root width (Figure 4.20B). From the two-way analysis of root width, the interaction between the rate and time of zinc fertilisation significantly ($P<0.001$) improved the root width of carrots (Figure 4.20C). However, no significant effects were found for the method of fertilisation ($P = 0.475$), interaction between method and rate of fertilisation ($P = 0.129$), interaction between method and timing of fertilisation ($P = 0.707$), and the three-way interaction between method, rate and timing of fertilisation ($P = 0.159$) (Appendix 18A - D).

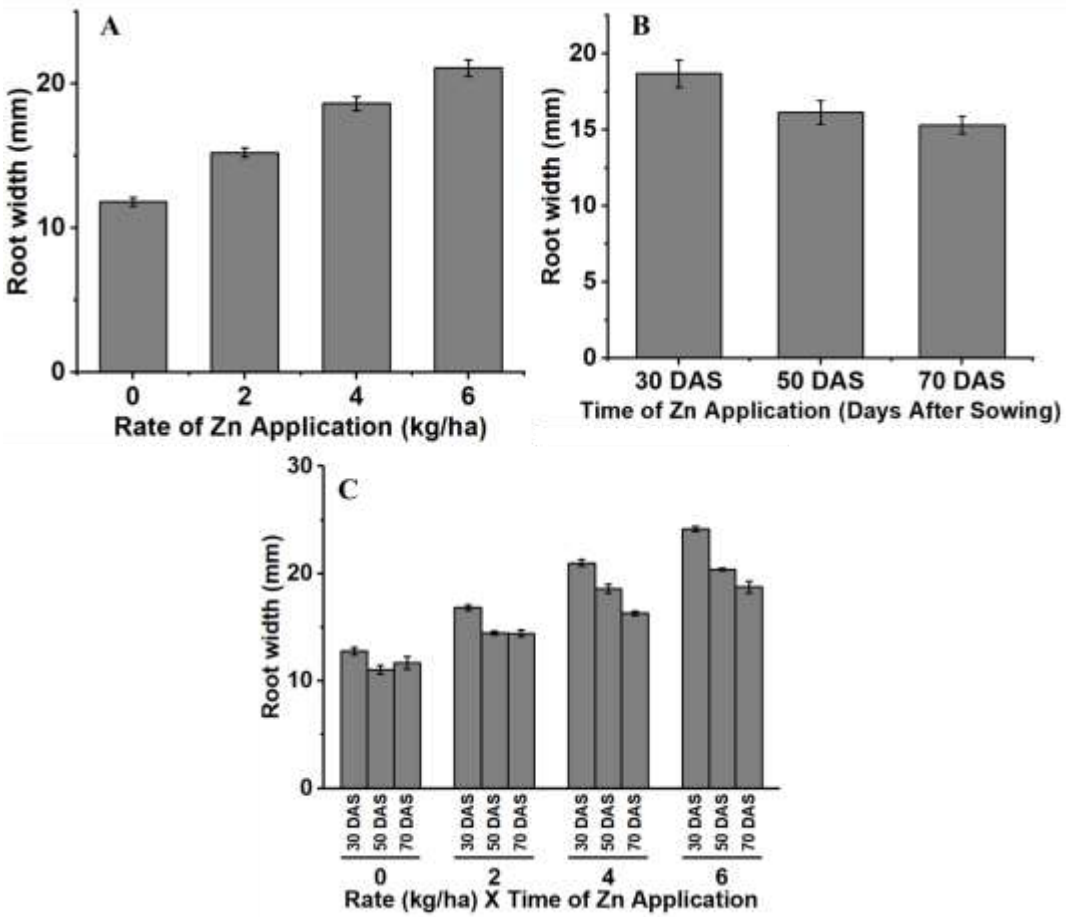

Figure 4. 20: Effects of Zn fertilisation on pot-grown carrots' root width (RW). Effects of Zn fertiliser rates on RW of pot-grown carrots (A); effects of timing of Zn fertilisation on RW of pot-grown carrots (B); interaction effects of rate × time of Zn fertilisation on RW of pot-grown carrots (C). Error bars show s.e.m.

Yield

Yield analysis showed a statistically significant ($P<0.001$) effect among the different rates of zinc application (Figure 4.21). Carrot yield varied between 9.64 t ha^{-1} (control) to 21.12 t ha^{-1} (6 kg Zn ha^{-1}), signifying approximately 2.1-fold increase over control. Generally, there was an upward yield increase with an increased zinc application rate (Figure 4.21). However, no significant effects were found for the method of fertilisation ($P = 0.187$), timing of fertilisation ($P =$

0.749), interaction between method and rate of fertilisation (P = 0.269), interaction between method and timing of fertilisation (P = 0.955), interaction between rate and timing of fertilisation (P = 0.510) and the three-way interaction between method, rate and timing of fertilisation (P = 0.814) (Appendix 19A - F).

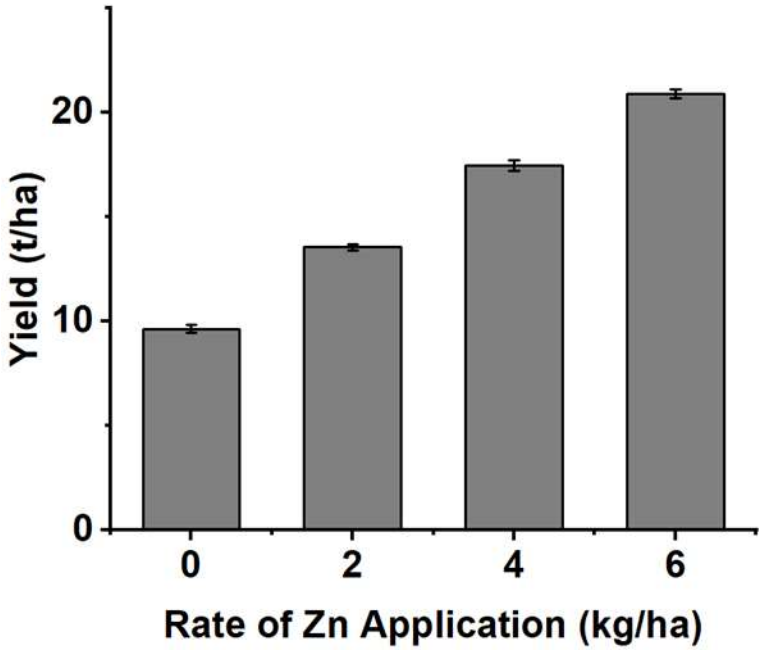

Figure 4. 21: Effects of different zinc fertilisation rates on the yield of pot-grown carrots. Error bars show s.e.m.

Growth and physiological parameters

Plant height

The main effect of the method of zinc fertilisation (P = 0.972), rate of zinc fertilisation (P = 0.874), timing of fertilisation (P = 0.906), as well as the interaction between method × rate of zinc fertilisation (P = 0.983), interaction between method and timing of zinc fertilisation (P = 0.953), interaction between rate × timing of zinc fertilisation (P = 1.000) and the three-way interaction

between method × rate × and time of zinc fertilisation exhibited no significant changes in plant height of pot-grown carrots (Appendix 20A - D).

Chlorophyll content

The rates of zinc fertiliser application had a significant ($P<0.001$) effect on the chlorophyll content in the leaves of pot-grown carrots (Figure 4.22A). The average chlorophyll content ranged from 21.55 µmol m^{-2} to 29.35 µmol m^{-2}, implying an approximately 1.3-fold increase in chlorophyll content of 6 kg ha^{-1} zinc fertiliser rates of compared to the control treatment. Generally, the chlorophyll content in carrots leaves rose as zinc fertilisation rates increased. The time of zinc application significantly ($P = 0.026$) affected the chlorophyll content of pot-grown carrots (Figure 4.22B). Chlorophyll content ranged from 24.55 µmol m^{-2} to 29.35 µmol m^{-2}. Generally, zinc fertilisation at 30 days after sowing recorded the highest chlorophyll content, followed by the application at 50 and 70 days after sowing, respectively (Figure 4.22B). However, no significant effects were found for the method of zinc fertilistion ($P = 0.092$), interaction between method × rate of zinc fertilisation ($P = 0.822$), interaction between method × time of zinc fertilisation ($P = 0.772$), interaction between rate × time of zinc fertilisation ($P = 0.745$) and the interaction between method × rate × time of zinc fertilisation (0.994) (Appendix 21A -E).

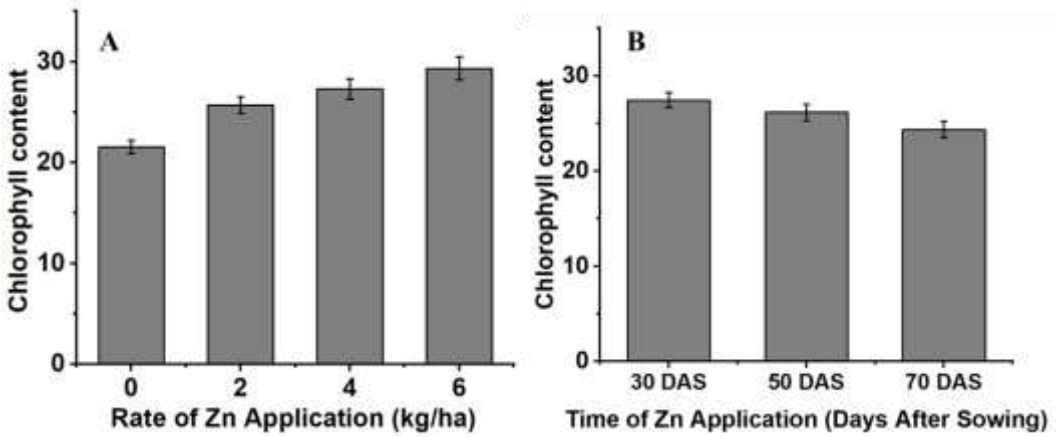

Figure 4. 22: Effects of Zn fertilisation on chlorophyll content of pot-grown carrots. Effects of zinc fertiliser application rates on the chlorophyll content of pot-grown carrots (A); effects of timing of zinc fertiliser application on the chlorophyll content of pot-grown carrots (B). Error bars show s.e.m.

Zinc concentration

Root zinc concentration

The various zinc application rates significantly ($P<0.001$) affected the root zinc concentration of carrots (Figure 4.23A). Maximum elevation in root zinc concentration (30.67 mg Zn kg^{-1} DW) was recorded for 6 kg ha^{-1} zinc application rate, and minimum root zinc concentration (15.73 mg Zn kg^{-1}) occurred in the control treatment, indicating a 1.9-fold variation in root zinc concentration of zinc fertiliser rates of 6 kg ha^{-1} compared to control treatment. Generally, root zinc concentration increased with an increasing rate of zinc fertilisation. Similarly, the time of zinc application markedly ($P<0.001$) influenced root zinc concentration (Figure 4.23B). Zinc fertilisation at 30 days after sowing recorded the highest root zinc (24.13 mg Zn kg^{-1}), followed by zinc fertilisation at 50 days after sowing (22.03 mg Zn kg^{-1}) and fertilisation at 70 days after sowing (20.45 mg Zn kg^{-1}). The interaction between rate × time of zinc fertilisation significantly impacted

(P<0.001) the root zinc concentration (Figure 4.23C). However, no significant effects were found for the method of fertilisation (P = 0.265), the interaction between method × rate of fertilisation (P = 0.435), the interaction between method × time of fertilisation (P = 0.692), and the three-way interaction between method × rate × time (P = 0.098) (Appendix 22A - D).

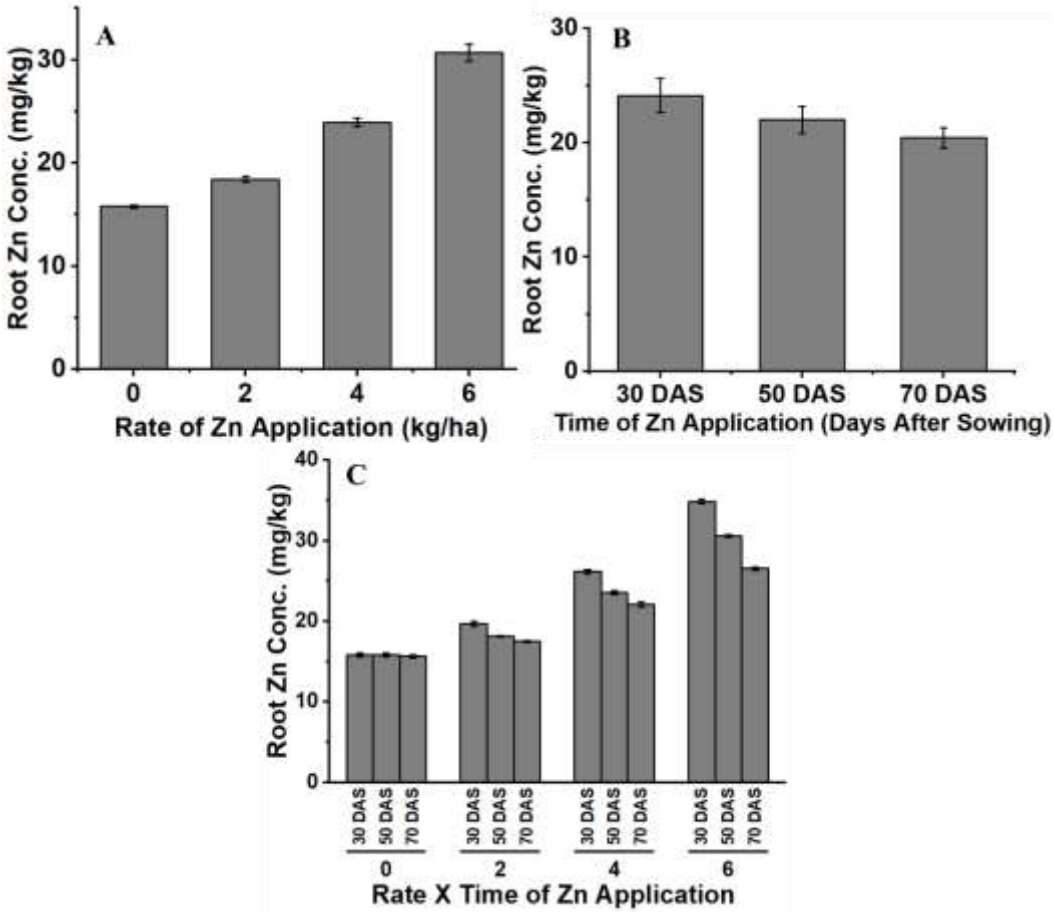

Figure 4. 23: Effects of Zn fertilisation on root zinc concentration of pot-grown carrots. Effects of Zn fertilisation rates on the root Zn concentration of pot-grown carrots (A); effects of timing of Zn fertilisation on the root Zn concentration of pot-grown carrots (B); interaction effects of rate × timing of Zn fertilisation on the root Zn concentration of pot-grown carrots (C). Error bars show s.e.m.

Shoot zinc concentration

Shoot zinc concentration exhibited significant (P<0.001) differences among the methods of application (Figure 4.24A). Shoot zinc concentration ranged from 20.76 ug g^{-1} to 31.65 ug $g^{-1,}$ indicating approximately 1.5-fold variation between soil and foliar zinc fertilisation. Soil zinc fertilisation resulted in the least shoot zinc concentration, while foliar zinc fertilisation produced the highest. Significant differences (P<0.001) in shoot zinc concentration were observed among the various rates of zinc fertilisation (Figure 4.24B). Shoot zinc concentration varied between 7.04 ug g^{-1} to 50.67 ug g^{-1}. The lowest shoot zinc concentration was recorded for control, whereas the highest shoot zinc concentration was recorded for the 6 kg ha^{-1} zinc fertilisation rate. Generally, shoot zinc concentration increased with an increasing rate of zinc fertilisation. Similarly, the interaction of method × rate (P<0.001) (Figure 4.24C), the interaction of method × time (P<0.001) (Figure 4.24D), the interaction of rate × time(P<0.003) (Figure 4.24E), and the interaction of method × rate × time (P<0.001) (Figure 4.24F), significantly affected shoot zinc concentration. However, no statistically significant (P = 0.114) effect found for shoot zinc concentration in terms of time of fertilisation (Appendix 22E).

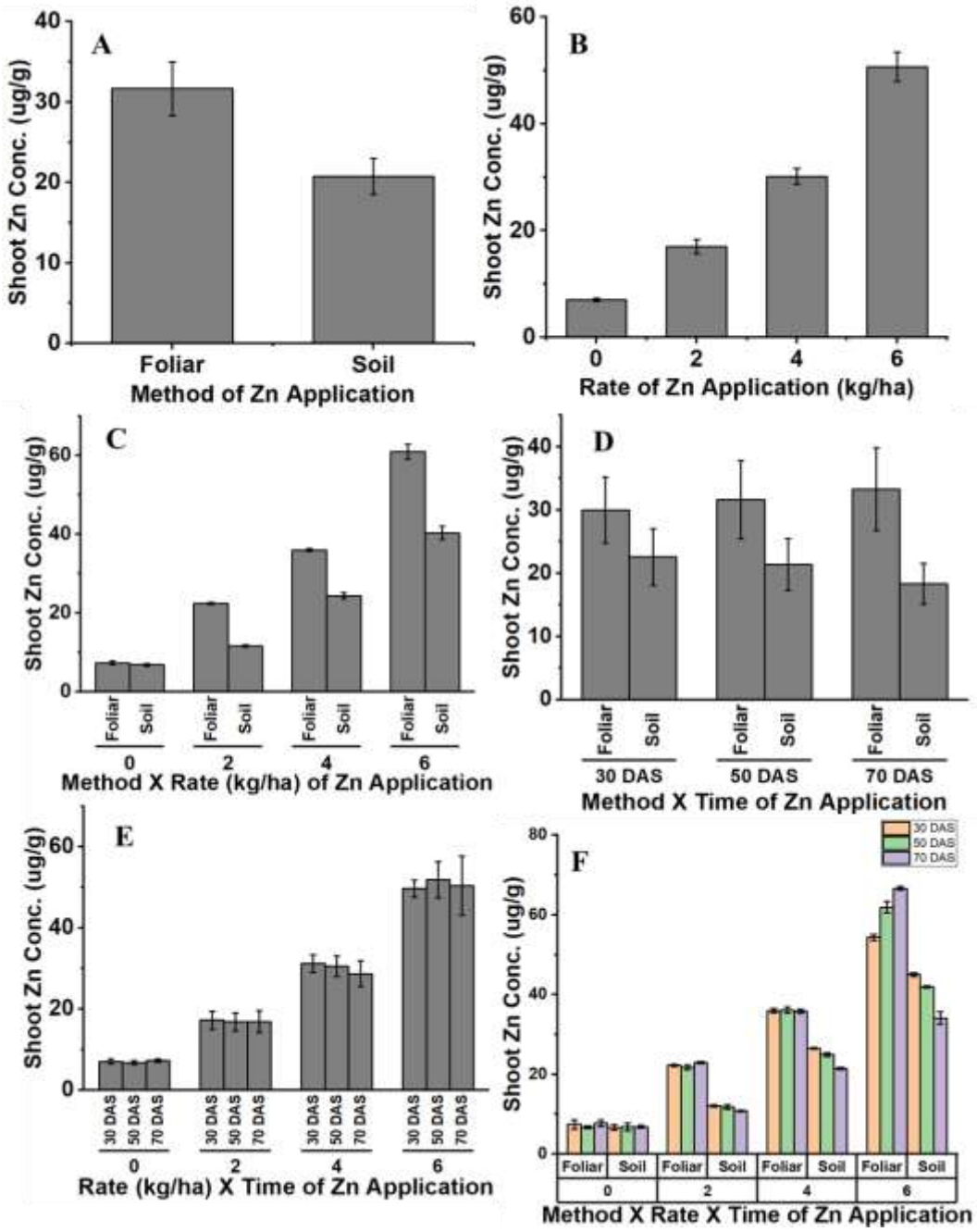

Figure 4. 24: Effects of Zn fertilisation method on the shoot Zn concentration of pot-grown carrots (A); effects of Zn fertilisation rates on the shoot Zn concentration of pot-grown carrots (B); interaction effects of method × rate of Zn fertilisation on shoot Zn concentration of pot-grown carrots (C); interaction effects of method × time of Zn fertilisation on shoot Zn concentrations of pot-grown carrots (D); interaction effects of rate × time of Zn fertilisation on shoot Zn concentration of pot-grown carrots (E) and interaction effects of method × rate × time of Zn fertilisation on shoot Zn concentration of pot-grown carrots (F). Error bars show s.e.m.

Multivariate analysis

Correlation between measured traits of maize and carrot

A diverse association was observed between measured traits in the present study, as illustrated in Figure 4.25. Regarding carrots, yield had a positive and significant association ($r = 0.96 – 0.97$, $p < 0.001$) with biomass trait, including shoot fresh weight, root fresh weight and, shoot dry weight, but had a negative insignificant ($r = -0.34$, $p = 0.23$) with root dry weight (Figure 4.25A). Zinc concentration of root and shoot had a positive and significant association ($r = 0.66 – 0.97$, $p < 0.001, 0.01, 0.05$) with yield and biomass traits such as shoot dry weight and root and shoot fresh weight (Figure 4.25A). Conversely, root dry weight had a significantly negative ($r = -0.74 – 0.86$, $p < 0.001, 0.01$) relationship with root and shoot zinc concentration (Figure 4.25A). Root length was not associated with most biomass and yield traits except for root dry weight ($r = 0.93$, $p < 0.001$). Similarly, root length was positively associated ($r = 0.78$ to 0.87, $p < 0.001$) with chlorophyll content and plant height but had a significant negative ($r = -0.68$, $p < 0.05$) relationship with root zinc concentration (Figure 4.25A).

The relationship between measured morphophysiology, agronomic yield, biomass, and tissue zinc concentration traits of maize is illustrated in Figure 4.25B. Grain yield and grain weight are associated significantly and positively ($r = 0.57$ to 0.95, $p < 0.001, 0.01$) with biomass traits such as shoot fresh and dry weight, physiological traits such as Fv/Fm ratio, chlorophyll content and performance index, and zinc tissue concentration (Figure 4.25B). Tissue zinc concentration at physiological and harvest maturity stages exhibited a significant

and positive (r = 0.68 – 0.99, p <0.001, 0.05) relationship with morphophysiological traits such as plant height, chlorophyll content, Fv/Fm ratio, performance index, yield traits including 100 seed weight, cob length and cob weight and biomass traits shoot fresh and dry weight (Figure 4.25B).

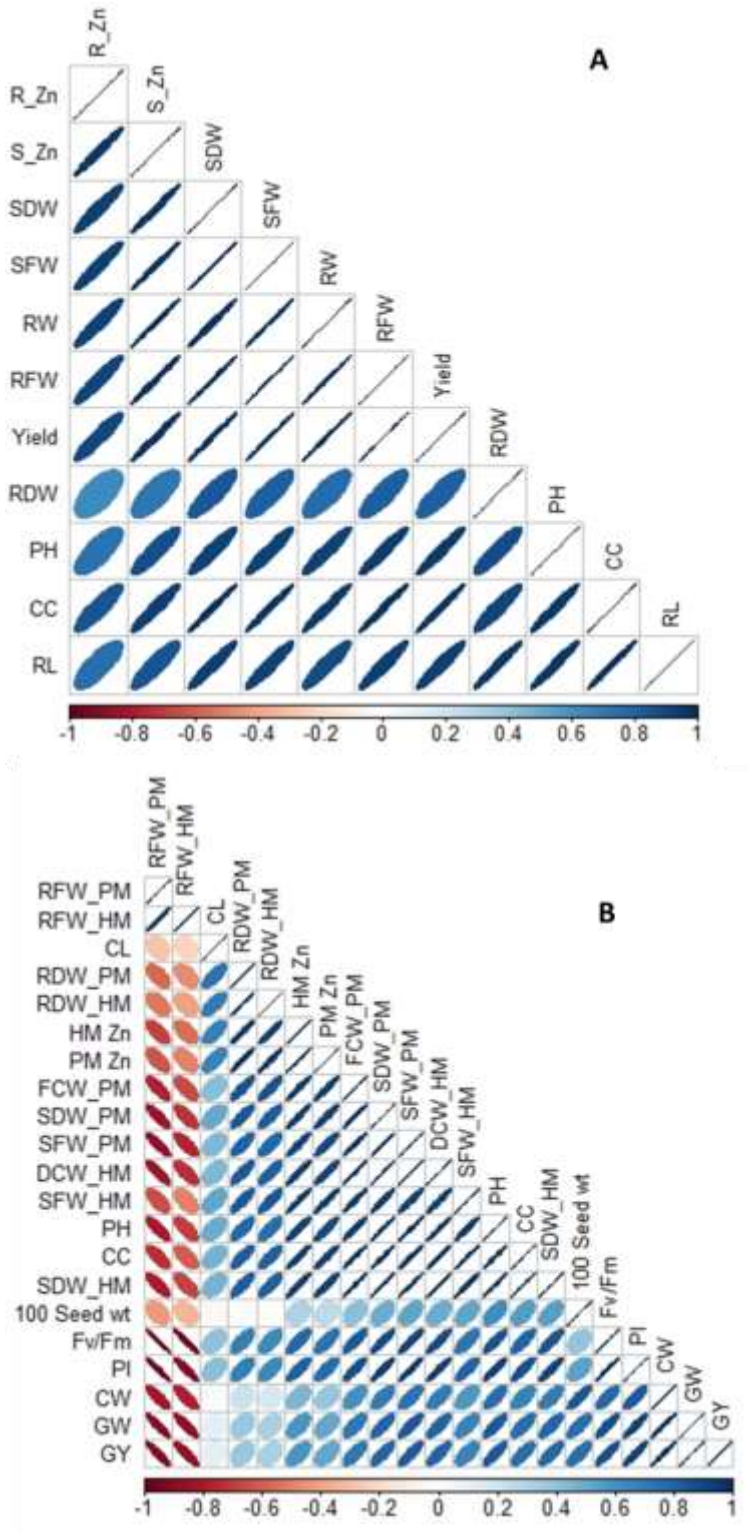

Figure 4. 25: Correlation analysis of measured morphophysiological, biomass, yield, and zinc concentration of (A) Carrot and (B) Maize.

CHAPTER FIVE

DISCUSSION

Zinc fertilisation rates and timing, but not application method, influenced morphophysiological traits.

Maize (*Zea mays* L.) remains an important dietary staple next to rice and wheat for more than 200 million people, especially in Sub-Saharan Africa (Rosales et al., 2023). However, maize is inherently poor in content of protein and minerals, particularly zinc (Suganya et al., 2020). Hence, improving zinc levels in maize grain could enhance zinc intake and level among individuals whose diets predominantly consist of maize-based foods, either directly or indirectly, may benefit from interventions to alleviate zinc deficiency and related health implications (Virk et al., 2021). In this context, agronomic biofortification has been used to improve the zinc content of major cereals such as maize, wheat, and rice. (Botoman et al., 2022; Cakmak et al., 2017). However, the majority of these studies focus on the rate of zinc fertilisation, type of zinc, stage of fertilisation, and method of zinc fertilisation in independent experiments with little information on their interactions in combined experiments (Boonchuay et al., 2013; El-Dahshouri, 2017; Esfandiari et al., 2016).

Similarly, the world population is growing. As a result, our demand for sustainable food sources will increase in response to the growing need to increase vegetable consumption for sustainability and health reasons (Reddy et al., 2021). Given that vegetables are enjoyed globally and serve as a natural source of minerals, they could improve the intake of dietary zinc through the application of

biofortification techniques (Buturi et al., 2022). Therefore, choosing a common vegetable in human diets cannot be overstated. Carrot stands out as Ghana's leading vegetable crop in terms of economic significance for export and domestic markets in recent years, potentially playing a vital role in nutrition and food security (Asante, 2019). The optimal production of root vegetable crops requires zinc; a lack of it severely affects nutritional quality and yield, thereby posing a significant health threat to many people in underdeveloped nations (Salehi et al., 2021). Additionally, little information is available regarding Ghana's agronomic zinc biofortification of maize and carrots. Hence, the present study provides insight into zinc fertilisation at different stages and rates as an agronomic biofortification tool to improve zinc concentration in the edible portions of maize and carrots.

Plant growth, development, and productivity can benefit from Zn treatment (Kandil et al., 2023). It was revealed that zinc fertilisation and application stage significantly influence crops' morphophysiological traits. Results of the present study showed that zinc rate had a direct association with plant height of maize, confirming a major effect of zinc on morphological growth (El-Badawy & Mehasen, 2011). The positive response of plant growth parameters to zinc fertilisation could be attributed to the role of zinc in the synthesis of plant growth hormones such as indoleacetic acid hence, increases in cell division and elongation, thereby contributing toward an increase in the growth and development of plants (Suganya et al., 2020). Several studies support the positive response of plant growth parameters, including number of leaves, stem girth, leaf

area, plant height, etc., to zinc application (Suganya et al., 2020; Sun et al., 2020). However, symptoms of zinc toxicity emerged at an application rate of 8 kg/ha, as evidenced by decreased plant height. Symptoms of zinc toxicity have been characterized by reduced biomass, stunted growth, wilting, inhibition of cell division, and elongation (Rossi et al., 2019).

Furthermore, the results revealed that zinc fertilisation directly effects the photosynthetic machinery of maize, which was clear in increased Fv/Fm ratio, performance index, and chlorophyll content under zinc fertilisation compared to control (Figures 4.9, 4.10 & 4.11). However, the effect was dependent on the rate, and the stage of growth as reported by Palacio-Márquez et al. (2021). Increased photosynthetic efficiency among maize in response to zinc could be attributed to the ability of zinc to improve transpirational rate and water uptake, hence maintaining cellular integrity and protection for photosynthetic enzymes (Iqbal et al., 2022; Kandil et al., 2023). In line with the present observation, zinc application has a greater impact on chlorophyll formation and carbonic anhydrase activity hence, plays a key role in photosynthesis-related enzymatic processes (Bashir et al., 2019; Hernández et al., 2020; Zafar et al., 2023). Zinc toxicity reduces ATP synthesis and chloroplast activity, leading to a decline in photosynthesis (Mousavi et al., 2013). The aforementioned justifies the decrease in physiological traits such as performance index and chlorophyll content observed when plants were cultivated under 8 kg/ha of zinc, indicating a toxicity level which directly impaired crops' overall performance.

In general, the timing of zinc fertiliser application can significantly impact its effectiveness in enhancing crop growth, yield, and quality (Asadpour et al., 2022). The present study revealed that not only did the rate of application influence plant morphophysiology, however, the application of zinc at 6 kg/ha at pre-anthesis and 30 days after sowing for maize and carrots, respectively, had a significant increase in photosynthetic and growth parameters compared to applying at the grain filling stage and at 70 days after sowing. Consistent with the results, applying zinc at flowering or early growth stages improves overall plant growth performance in crops, including rice, wheat, chicken pea, etc. (Pandey et al., 2013; Tuiwong et al., 2022). This could be largely linked to zinc's significant role in chlorophyll synthesis and hormone regulation. Adequate zinc levels during the early growth stage ensure the proper production of chlorophyll and plant hormones, critical for photosynthesis and plant growth regulation (Umair Hassan et al., 2020). Thus, zinc fertilisation at the early growth could boost chlorophyll content and promote shoot and root development, enhancing nutrient capture and overall plant health.

Although previous studies have presented significant variation in the method of zinc application on various morphophysiological parameters of crops such as maize, rice, sorghum, etc. (El-Dahshouri, 2017; Sher et al., 2022), the results revealed a non-significant impact of the method of zinc fertilisation (foliar and soil) on plant growth and physiological performance. Such contrasting trends could be attributed to variations in zinc fertilisers, soil properties, variety of crops,

etc., as zinc's bioavailability and functional properties are contingent on various factors (Rosales et al., 2023; Suganya et al., 2020).

Zinc fertilisation rates, stage of growth but not method of application, affected agronomic yield parameters

In the present study, an over 58 % increase in biomass parameters such as root and shoot dry weight was observed when maize was cultivated under 6 kg/ha zinc fertiliser rates compared to the control treatment (Figures 4.6A & 4.6B). Similarly, carrots cultivated under zinc fertilisation resulted in a 135 % increase in shoot dry weight and a 53 % increase in root dry weight compared to the control (Figures 4.17 & 4.18B). Increased biomass under zinc application could be related to improved physiological traits such as performance index, and chlorophyll content (Figures 4.10A, 4.11A, and 4.22A). A positive association was observed between biomass traits and photosynthetic traits (Figure 4.25A and 4.25B), which further justifies the significant increase in biomass traits observed in the present study. Zinc is a necessary component of several enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids, as well as in the metabolism of other micronutrients, and plays an important role in the production of biomass (Solanki, 2021; Suganya et al., 2020). Correspondingly, previous studies in maize, wheat, carrot, cowpea, etc. have reported an increase in biomass components (Ali Raza et al., 2021; Awad et al., 2021; Datcu et al., 2019; Galindo et al., 2021; Hassanein et al., 2019). Thus, zinc fertilisation is crucial as it supports numerous physiochemical processes, both

directly and indirectly, thereby enhancing the production of dry matter in crops (Hussain et al., 2018).

The study found that carrots grown with foliar zinc amendment had an over 25 % increase in root dry weight relative to those grown with soil zinc amendment (Figure 18A). In this context, it is clear that foliar application could improve plant metabolism and carbon assimilation. However, as shown in the carrot (Figure 4.18A), the effectiveness of zinc fertilisation may be contingent on a variety of factors including the crop's growth stage.

Zinc is an important micronutrient that improves yield because it plays a key role in photosynthesis-related enzymatic processes (Bashir et al., 2019; Umair Hassan et al., 2020; Zafar et al., 2023). Numerous studies have shown that carrots, maize, and wheat exhibit a positive yield response to zinc fertilisation (Palai et al., 2020; Awad et al., 2021). Liu et al. (2020) observed maize yield improvements ranging from 4 % to 17 % with soil zinc fertilisation. Awad et al. (2021) also reported that carrot yields increased by over 85 % with foliar zinc fertilisation. In the present study, applying zinc at 6 kg/ha improved maize grain yield by 28 % compared to the control treatment, whereas over 119 % increase in yield was observed in carrots cultivated under 6 kg/ha zinc amendment compared to the control treatment. Increased yield under zinc application could be related to the direct effect of zinc on yield parameters such as grain weight, cob length cob weight, root length, and root width of plants. Additionally, the direct impact of zinc on photosynthetic efficiency traits such as Fv/Fm ratio, chlorophyll content, and performance index hence, enhancing carbon accumulation and assimilation

could have accounted for increase in yield (Hernández et al., 2020). The positive and significant correlation observed among yield parameters of both carrot and maize and measured morphophysiological traits as indicated in the results further justifies the aforementioned increase (Figure 4.25A and 4.25B).

The findings from the present study indicate that the yield traits of maize (cob weight, grain weight, etc.) (Figure 4.1 – 4.5) to zinc fertiliser is directly dependent on the time of application rather than a method of application for maize. However, in carrots, the influence of zinc on some yield-related traits directly depends on the method and time of application (Figure 4.16B, 4.18A & 4.19B). Zinc fertilisation at the pre-anthesis of maize resulted in about a 21 % increase in yield parameters including grain yield, cob dry and fresh weight, and cob length of maize compared to zinc fertilisation at grain filling. Similarly, in carrots, the application of zinc at 30-DAS increases yield traits such as root weight, root length, and root width. This suggests that applying zinc fertiliser at pre-anthesis and early growth stages should be sufficient to have a measurable influence on productivity hence, crucial for achieving optimal crop growth and yield. The results corroborate previous findings in wheat, where higher yield was recorded when zinc was applied at the stem elongation and tillering stage compared to when applied at the milking stage (El-Dahshouri, 2017). In contrast, Boonchuay et al. (2013) reported that zinc application at different rice growth stages did not significantly impact the grain yield. Nonetheless, grain yield was higher when zinc was applied two weeks after flowering.

Zinc application generally has beneficial effects on overall plant physiology and agronomic performance. However, the effectiveness of zinc fertiliser is dependent on the formulation, the source, the time of application, the method of application, and the particle size (Palacio-Márquez et al., 2021). In this context, several studies have reported that foliar zinc application is more effective in improving crop productivity than soil application (Xue et al., 2023). In the present study, the method of zinc fertilisation had an insignificant effect on all the yield parameters measured on maize. However, foliar zinc fertilisation had a greater magnitude of increase in carrot root dry weight than soil zinc fertilisation. Foliar zinc fertilisation is an effective method and technique for ameliorating plant zinc deficiency compared to soil treatment (Esfandiari et al., 2016). Similar results were found in wheat, rice, and maize (Naeem et al., 2021; Stewart et al., 2021; Zou et al., 2019). The non-significant impacts of zinc fertilisation methods on maize and carrots yields might be due to the responsiveness of the experimental soils to zinc fertilisation and the robust root system of maize and carrot facilitating better nutrient uptake.

Zinc fertilisation rates, application time, and application method affected zinc concentration in maize grains and carrots' shoots and roots.

In both plants, zinc fertilisation significantly influenced the zinc concentration of root and grain. Maize grain zinc concentration increased with an increasing zinc rate of 8 kg/ha, obtaining an over 160 % increase compared to the control (Figures 4.12C & 4.12D). Carrots cultivated under 6 kg/ha exhibited a 95 % rise in root zinc levels and a 619 % rise in shoot zinc levels relative to the

control treatment (Figures 4.23 and 4.24). An increase in zinc concentration under 6 kg/ha zinc fertiliser rates could be attributed to carrots' extensive root system facilitating better nutrient uptake and the role of zinc in increasing the translocation of nutrients from vegetative organs to other parts of the plant (Chen et al., 2016). Similar to this finding, studies in wheat reported a range of 11.2 to 31.8 mg Zn kg^{-1} for control and zinc treatment, respectively (Xue et al., 2023), while Buturi et al. (2023) reported over 94 % rise in root zinc levels of carrot when Zn-EDTA was applied to the foliage. Irrespective of the positive association between zinc fertilisation rate and grain zinc concentration, grains harvested at physiological maturity exhibited significantly higher zinc levels compared to those harvested at harvest maturity. The observed decline in grain zinc concentration could be related to the reduction in the active transport of zinc from the vegetative tissues and soil to the developing grains during the later stage of growth or senescence and the increased carbohydrate content of maize which could dilute a given concentration of zinc (Bänziger & Long, 2000; Brkić et al., 2004). Xue et al. (2019) examined the zinc content in maize grains at various developmental stages after silking. They observed that zinc content was initially high right after silking but began to decline around 15 days later.

The present study revealed that grain zinc concentration is contingent not just on the rate of zinc fertilisation but also on the method and the growth stage. In this study, foliar zinc fertilisation had a 16 % increase in grain zinc concentration compared to soil application, suggesting that foliar zinc fertilisation could be beneficial in increasing zinc concentration in plants. Furthermore, the

positive association observed between grain and tissue zinc concentration, morphophysiological and yield parameters suggests that soil and foliar application of a zinc fertiliser represents an effective strategy to biofortify maize simultaneously without yield and morphophysiological trade-off in maize. Several studies have shown that foliar zinc fertilisation has a positive impact on zinc concentration in cereal grains (Gomez-Coronado et al., 2016; Wang et al., 2015; Palacio-Márquez et al., 2021). Also, several studies have shown that, compared to zinc sulphate fertilisers, foliar zinc fertilisation using ZnO-NPs were more efficient at increasing grain Zn concentration of maize and wheat (Wang et al., 2021; Subbaiah et al., 2016). In crops like maize and wheat, foliar application of zinc resulted in a 26.4 % increase in wheat grain yield and a 51.3 % increase in wheat grain zinc concentration from 31.0 to 46.9 mg Zn kg^{-1} (Kumar et al., 2021).

Similarly, in this study, foliar zinc application had a 61 % increase in carrot shoot zinc concentration compared to soil application, suggesting that foliar zinc fertilisation could be beneficial in increasing zinc concentration in plants. Foliar application offers several benefits, such as preventing zinc fixation by soil and avoiding the impact of antagonistic nutrients on zinc uptake, among others (Prasad et al., 2014). Thus, foliar zinc fertilisation allows for efficient absorption and transportation via the phloem, as evidenced in wheat studies using radio-labeled zinc (^{65}Zn), especially under conditions of low zinc availability (Erenoglu et al., 2002; Haslett et al., 2001). The superiority of foliar zinc fertilisation over soil application highlights its potential as a practical strategy for addressing crop zinc insufficiency.

The findings of this study underscore the importance of timing of zinc fertilisation for achieving the greatest enhancements in grain zinc concentration via foliar application. Thus, the effectiveness of foliar zinc fertilisation may also depend on several factors, including the time at which zinc application is prominent (Boonchuay et al., 2013). Typically, application at the grain filling stage had a 33.9 % increase in grain zinc concentration compared to the pre-anthesis growth stage. Higher zinc concentration obtained at the grain filling stage could be attributed to increased translocation of zinc in various parts of the plant (sources) to grains of maize. Thus, applying zinc at the grain-filling stage could have coincided with the period of maximum nutrient uptake and utilization by the plant. This optimal timing ensures that the applied zinc is efficiently utilized and accumulated in the grains. Consistent with these results, increased grain zinc of wheat was observed when foliar zinc was applied after flowering compared to before flowering (Cakmak et al., 2010; Ozturk et al., 2006). Brown rice zinc concentration showed a remarkable increase of 56 % with zinc fertilisation after flowering or at a later growth stage. Boonchuay et al. (2013) attributed the observed increase to the efficient translocation of zinc at the late growth stage. This finding closely aligns with Phattarakul et al. (2012), who found that foliar zinc fertilisation during later growth stages in rice cultivated under field conditions resulted in a more substantial elevation in grain zinc relative to application before the flowering stage.

Conversely, zinc application in carrot roots at 30 days after sowing resulted in an 18 % increase in root zinc concentration compared to zinc

fertilisation at 70 days after sowing (Figure 4.23B). The higher zinc concentration observed when zinc fertilisation was carried out 30 days after sowing could be explained by the fact that early application coincides with the period when the root system most actively absorbs nutrients, leading to increased zinc accumulation in the roots. In contrast, later applications may be less effective as the root uptake mechanisms become less active as the plant matures (Buturi et al., 2023).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The results obtained in this study demonstrate that the effect of zinc fertilisation on morphophysiology, yield, and biomass parameters is contingent on the rate of application, time of application or growth stage of the crop, and to the least extent, method of fertilisation which could be attributed to the responsiveness of the experimental soil to zinc fertilisation. An increasing trend in measured traits was observed with increasing zinc fertilisation, with the application at 6 kg/ha having a pronounced effect on morphophysiological traits such as plant height, Fv/Fm ratio, performance index, chlorophyll content, etc.; biomass traits such as shoot and root dry weight and yield traits such as cob weight, root weight, 100-seed weight, cob length etc. Therefore, applying zinc at 6 kg/ha could be paramount in improving the plant's physiological, morphological and cellular activities while ensuring higher overall yield. While the application of 8 kg/ha zinc rate in the case of maize resulted in a notable increase in grain zinc concentration, the study identified a trade-off between yield and zinc concentration. Specifically, the 8 kg/ha dosage appeared to fall within a toxicity range detrimental to plant growth and yield.

Furthermore, the magnitude of foliar zinc fertilisation on measured traits was significantly higher than soil application; therefore, foliar zinc fertilisation should be preferentially applied at various growth stages to improve root and grain zinc concentration while maintaining higher growth and yield. It was shown

that, among various application times, foliar application increased zinc concentration in both maize and carrot relative to soil application.

The present study has indicated that optimal timing for zinc application is crucial as it plays both direct and indirect roles in maximizing crop yield and zinc concentration. The research suggests that applying zinc during the grain filling and later growth stage significantly improves zinc concentration within plants' tissues (grain and carrot root). This indicates that this stage is critical for zinc uptake and accumulation in the grain. Unlike zinc concentration, pre-anthesis stage of zinc application significantly enhances yield and biomass production compared to application during the grain filling stage. The findings suggest a trade-off between zinc concentration and morphophysiological and yield traits. While applying zinc during grain filling enhances zinc concentration, it may not be the most effective timing for maximizing yield, morphophysiology and biomass.

In light of these findings, the hypothesis that time of application and concentration of zinc fertilisation significantly influence the morphophysiological and yield parameters of maize and carrots, whereas the method of application has no significant effect, was supported and accepted. Similarly, the hypothesis that method of application, concentration, and stage of application of zinc significantly influence uptake and tissue concentration of zinc in maize and carrots was also supported and accepted. These findings contribute meaningful insights into optimizing zinc fertilisation strategies, reinforcing the importance of considering

application timing, concentration, and method to maximize crop performance while mitigating potential trade-offs between yield and zinc accumulation.

Recommendation(s)

Based on the findings of the present study, the following recommendations were made:

1. Despite the advantages of foliar zinc fertilisation observed in this study, its full adoption may require a comprehensive cost-benefit analysis to guide final decision-making. While the foliar application may offer superior results, it may involve higher application costs or additional labour requirements. Farmers and agricultural practitioners could consider incorporating foliar application methods into their nutrient management practices to optimize zinc uptake and enhance crop performance.
2. The significant difference between foliar and soil application prompts further investigation into these contrasting effects' underlying mechanisms. Future research could explore factors such as nutrient mobility, uptake kinetics, and physiological responses to elucidate why foliar application outperforms soil application in enhancing plant traits.
3. The present study used maize and carrots as test crops to provide insight into zinc biofortification using an agronomic approach. Hence, it is crucial to note that ongoing efforts in breeding to create high-yield genotypes must be combined with agronomic practices, such as foliar application of zinc, to attain high grain yield and optimal grain nutritional quality for human health.

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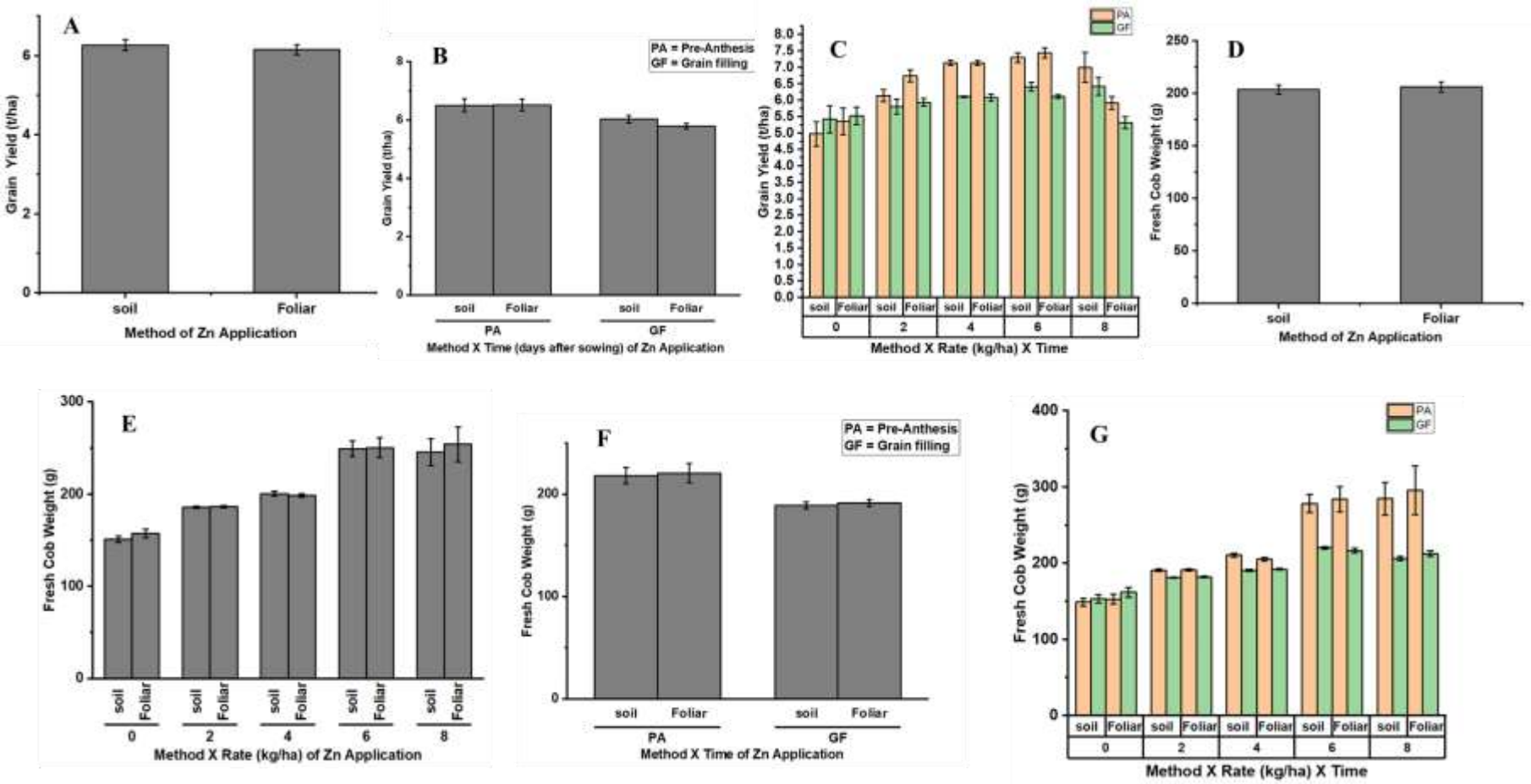
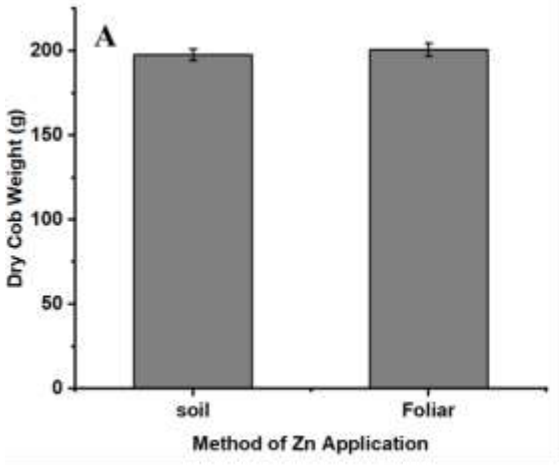
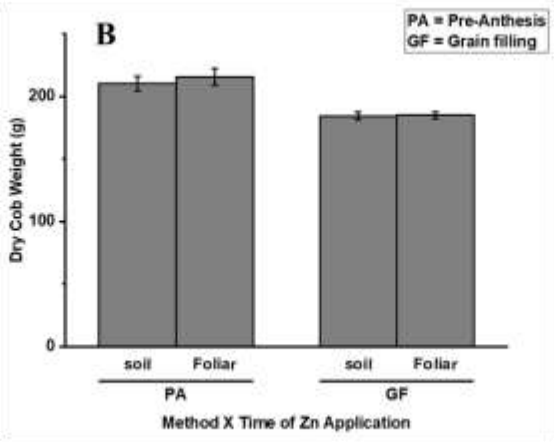
APPENDIX

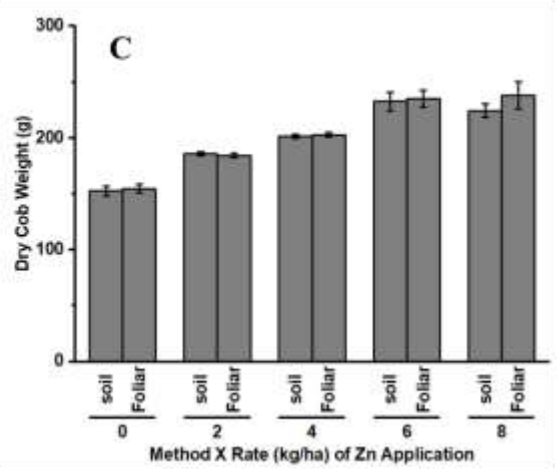
Appendix 1: Impact of Zn fertilisation on grain yield and fresh cob weight of maize.

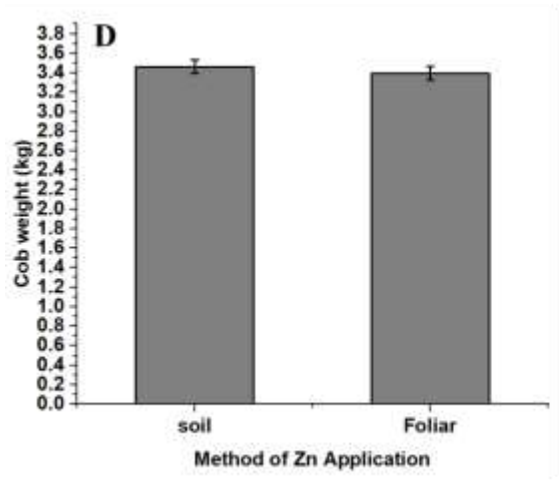


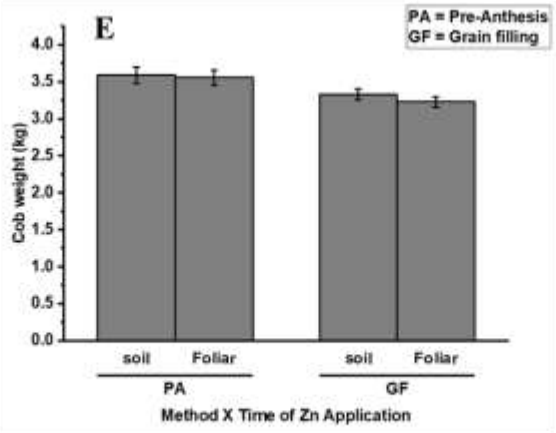
Appendix 2: Impact of Zn fertilisation on dry cob weight and cob weight of maize.

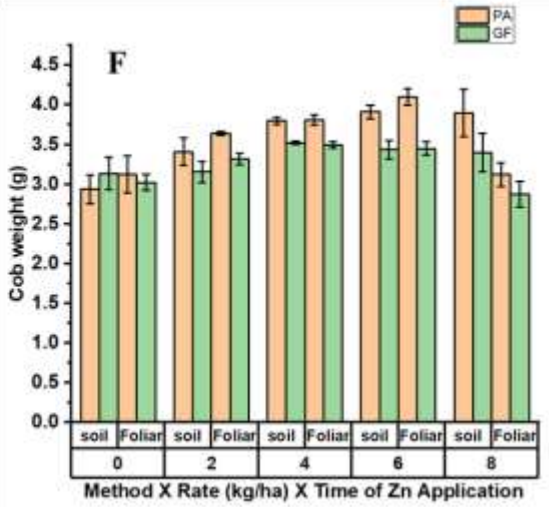




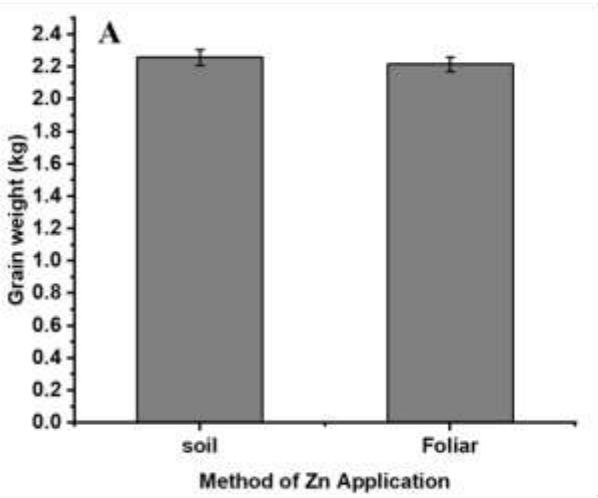


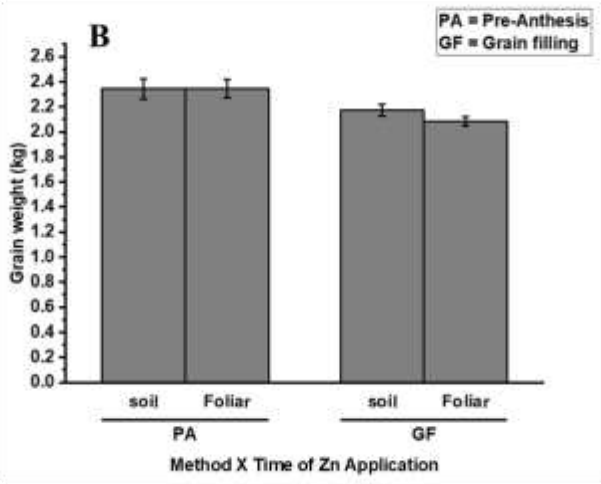


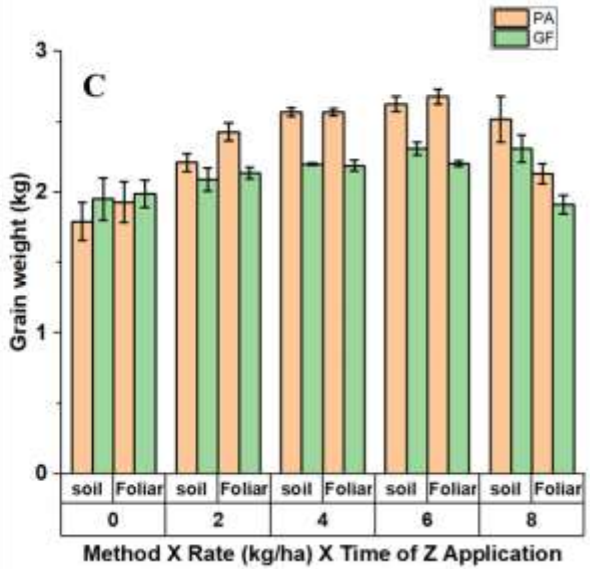




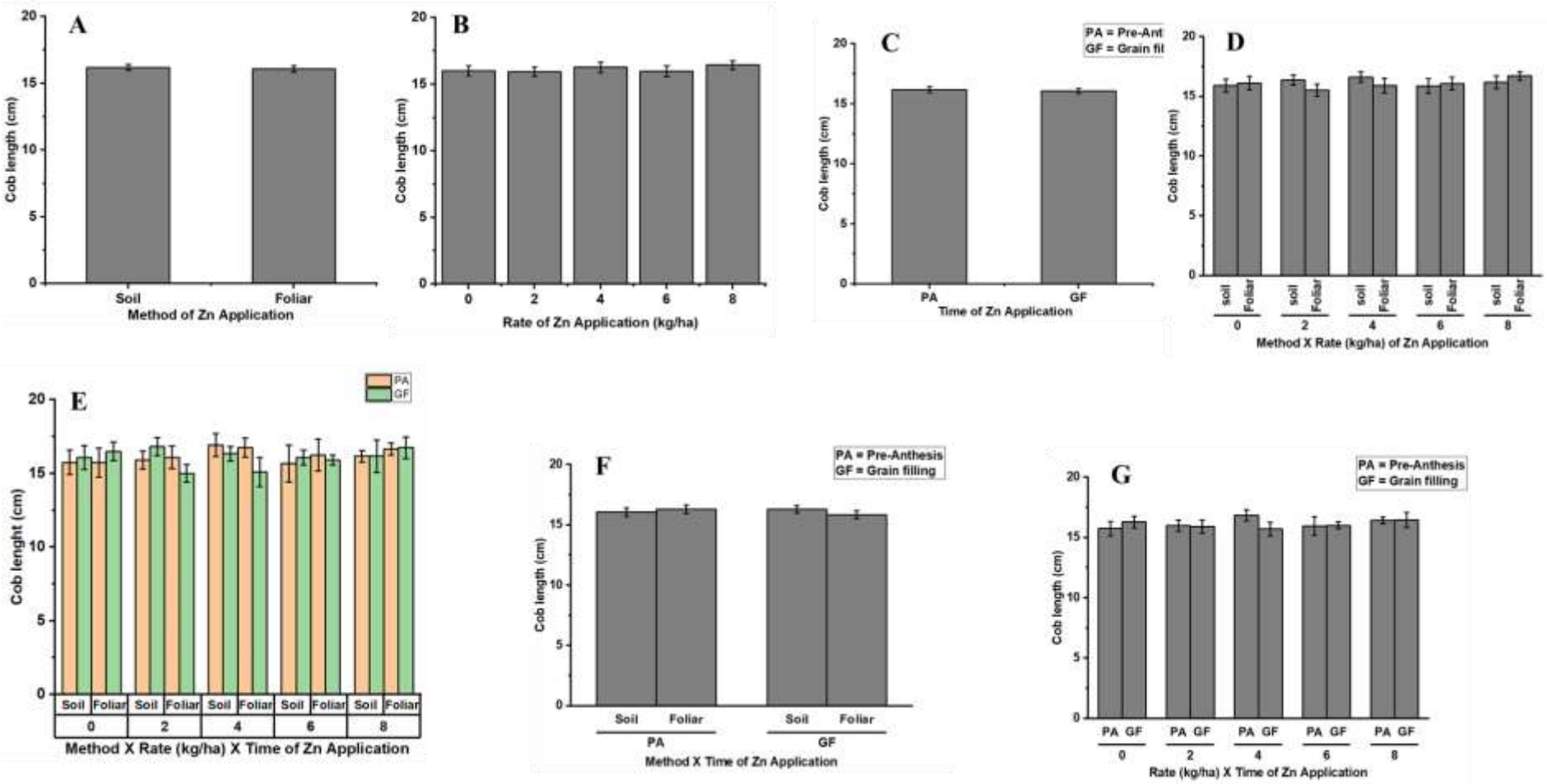
Appendix 3: Impact of Zn fertilisation on grain weight of maize.



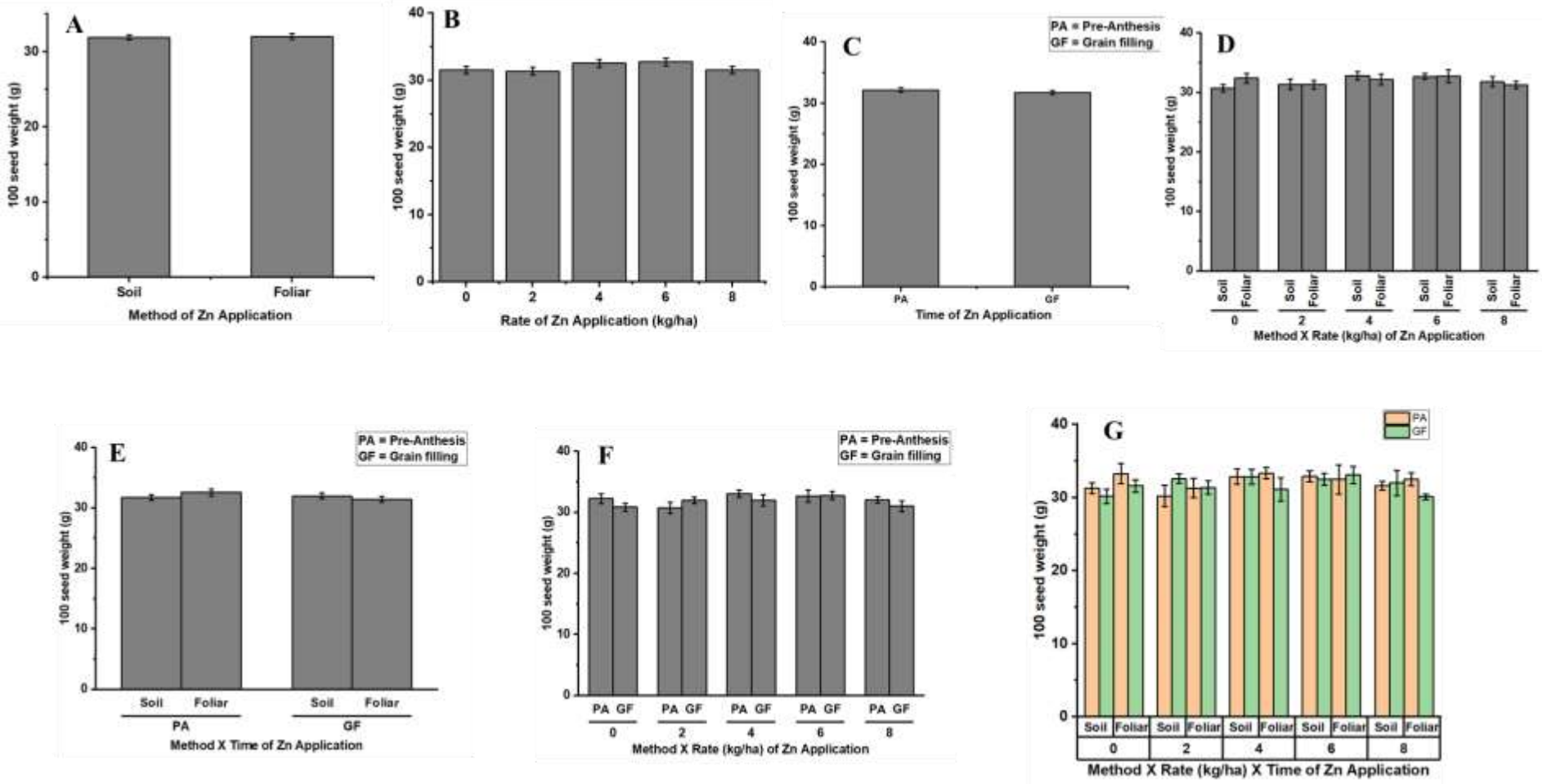




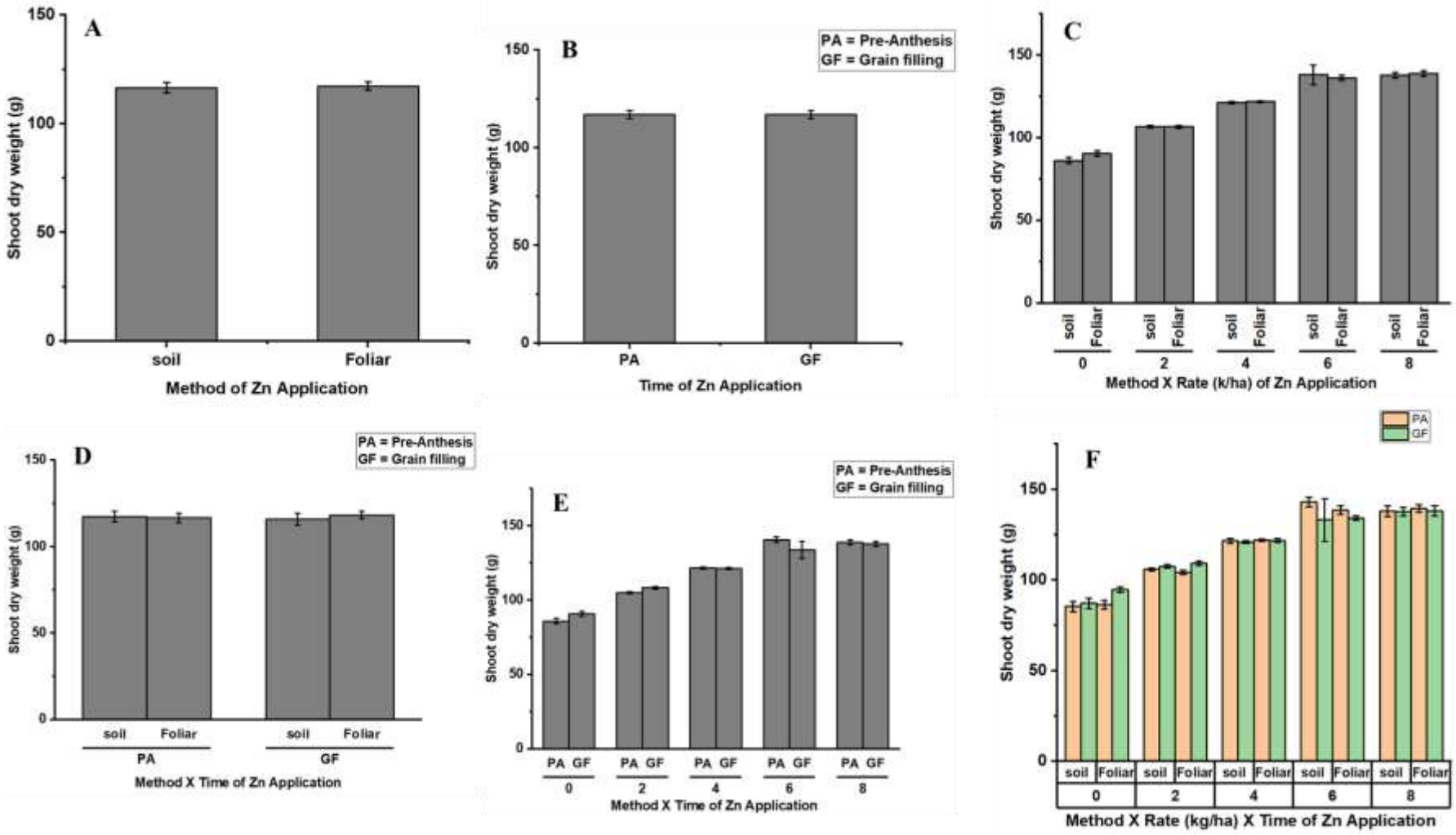
Appendix 4: Impact of Zn fertilisation on cob length of maize.



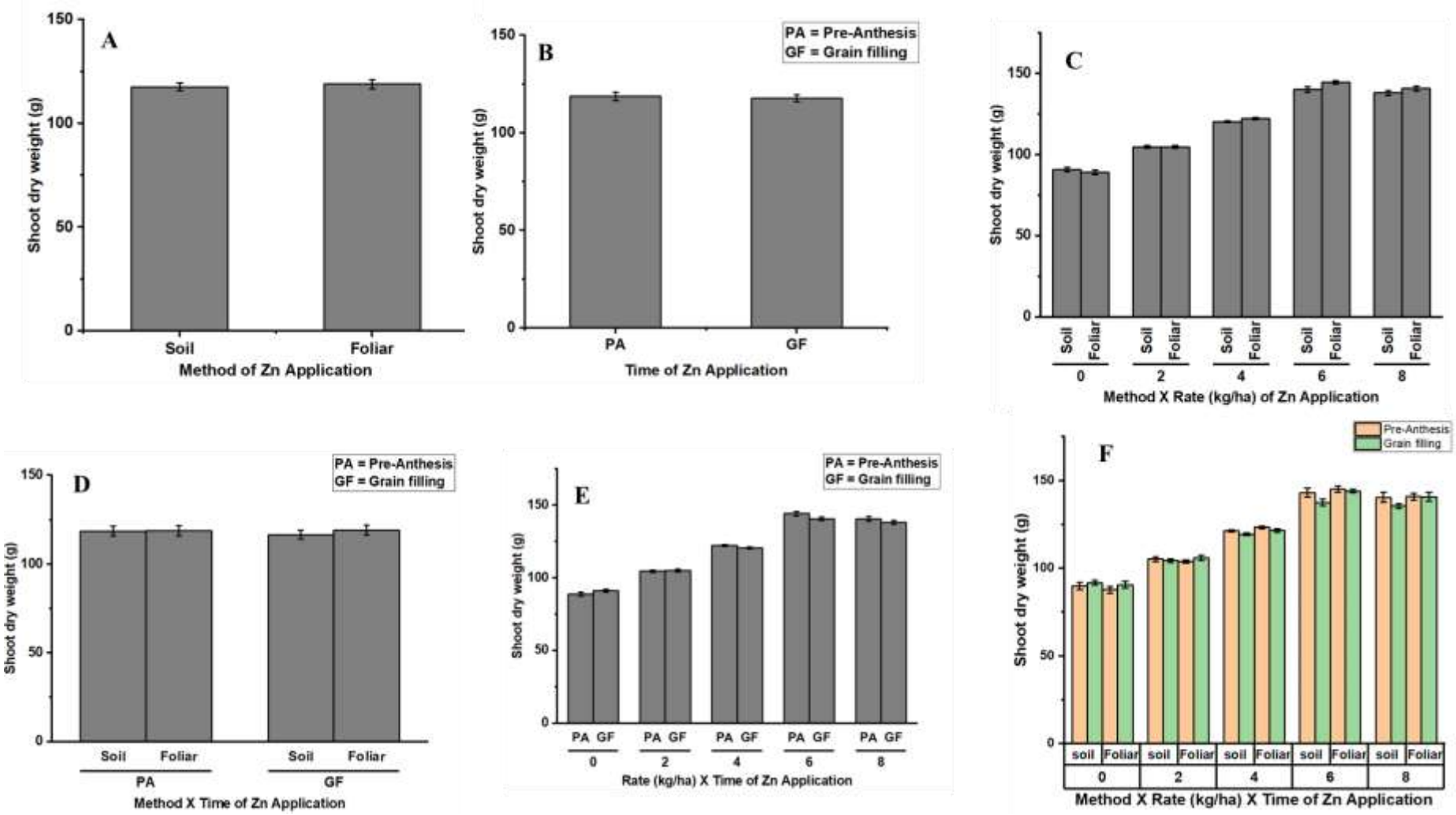
Appendix 5: Impact of Zn fertilisation on 100 seed weight of maize.



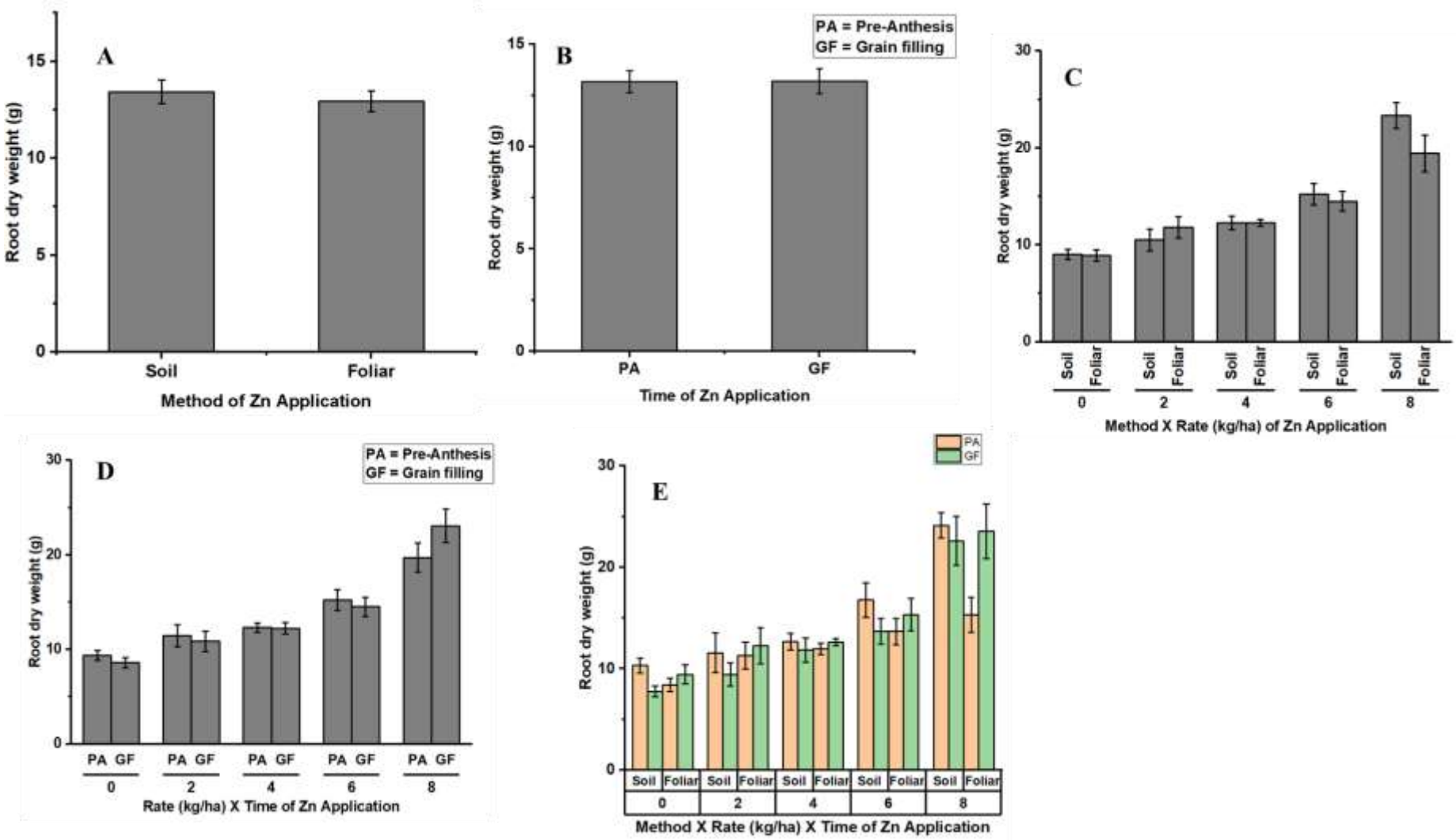
Appendix 6: Impact of Zn fertilisation on shoot dry weight of maize plants harvested at physiological maturity stage.



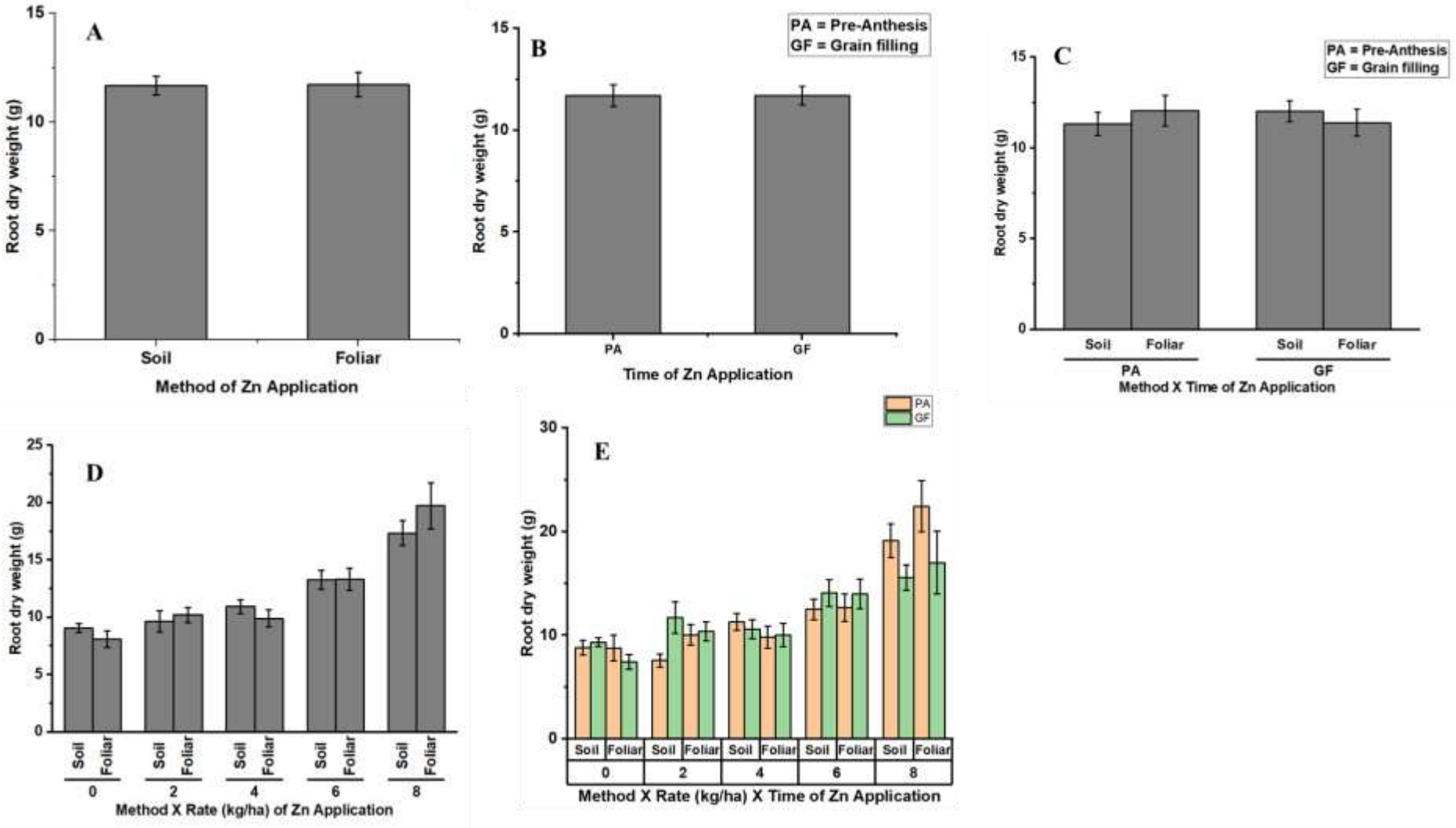
Appendix 7: Impact of Zn fertilisation on shoot dry weight of maize plants harvested at harvest maturity stage.



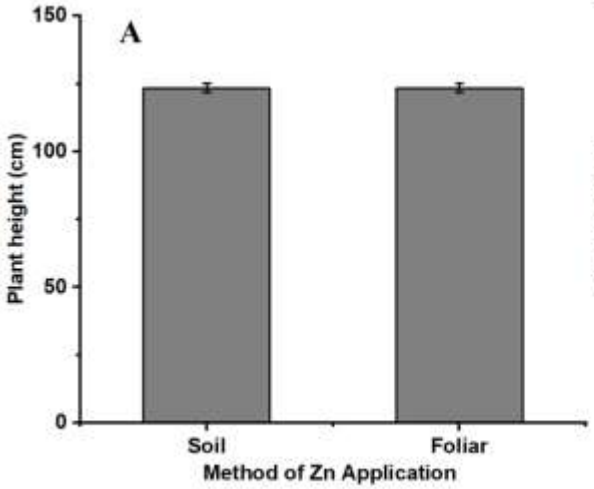
Appendix 8: Impact of Zn fertilisation on root dry weight of maize plants harvested at physiological maturity stage.

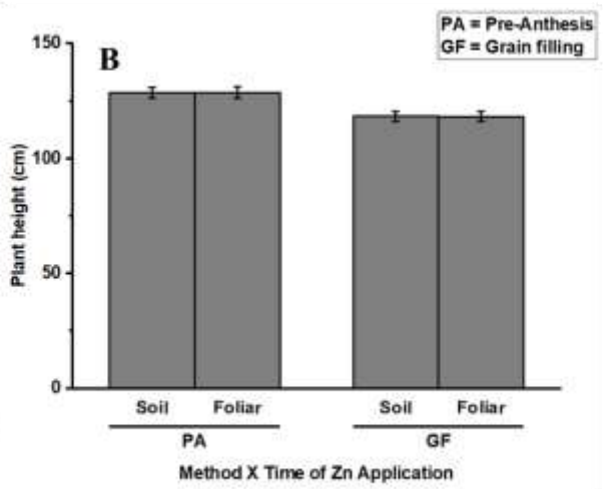


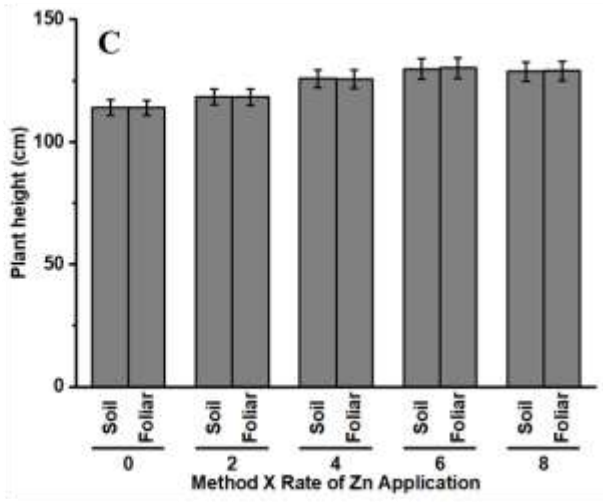
Appendix 9: Impact of Zn fertilisation on root dry weight of maize plants harvested at harvest maturity stage.

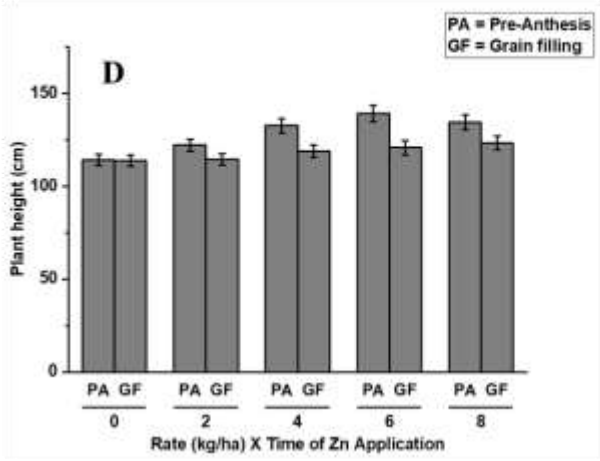


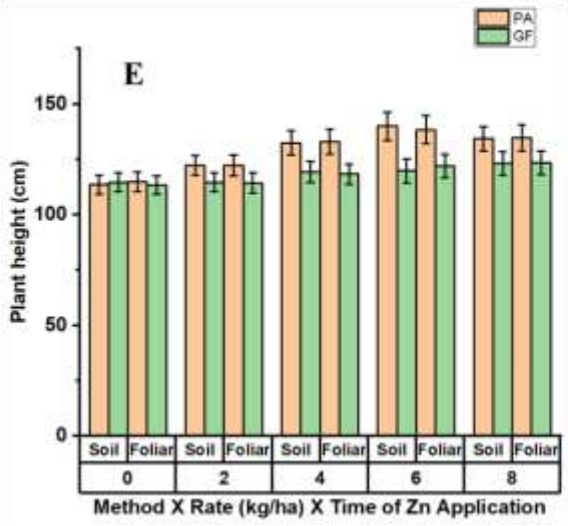
Appendix 10: Impact of Zn fertilisation on plant height of maize plants.



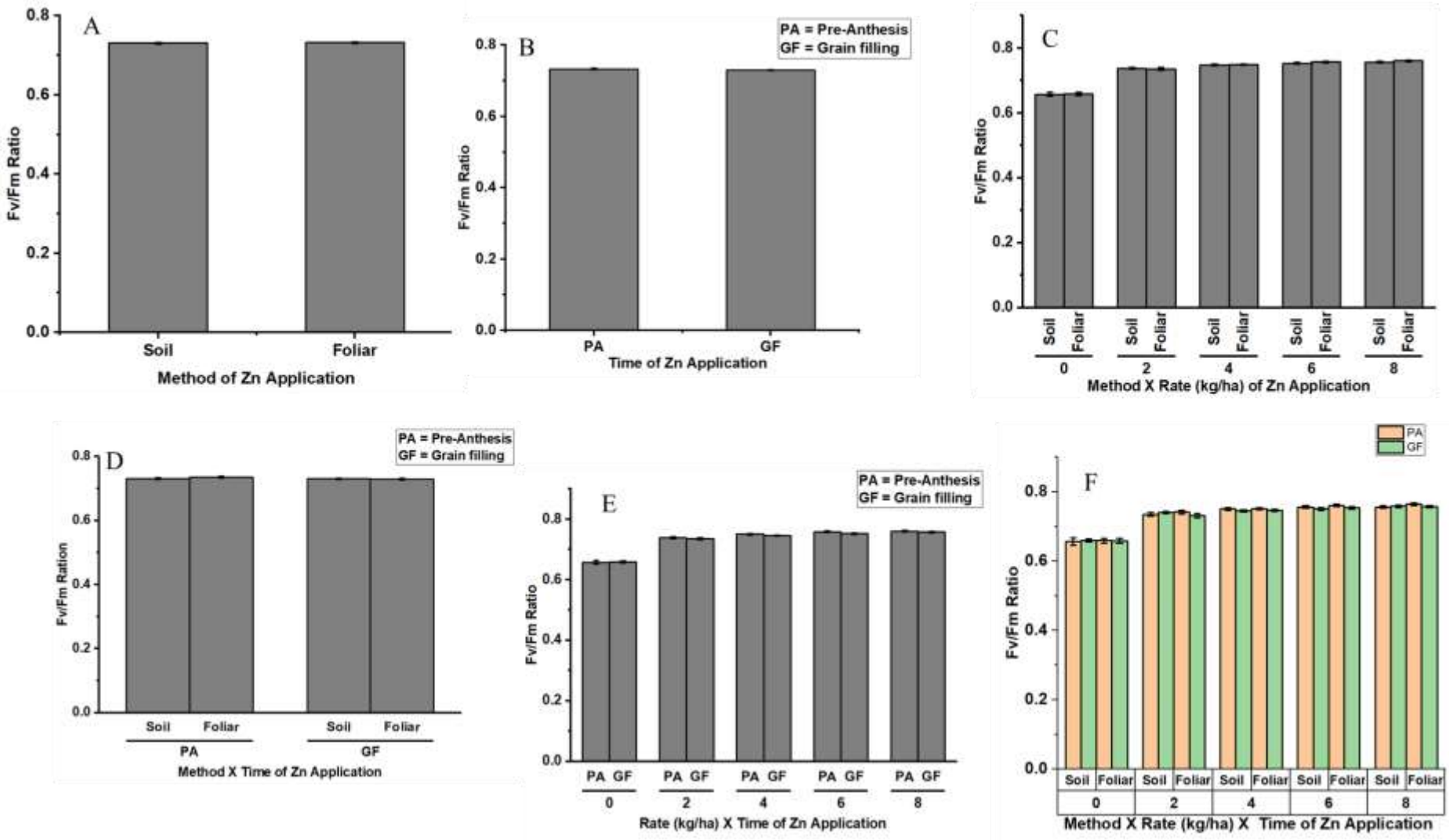




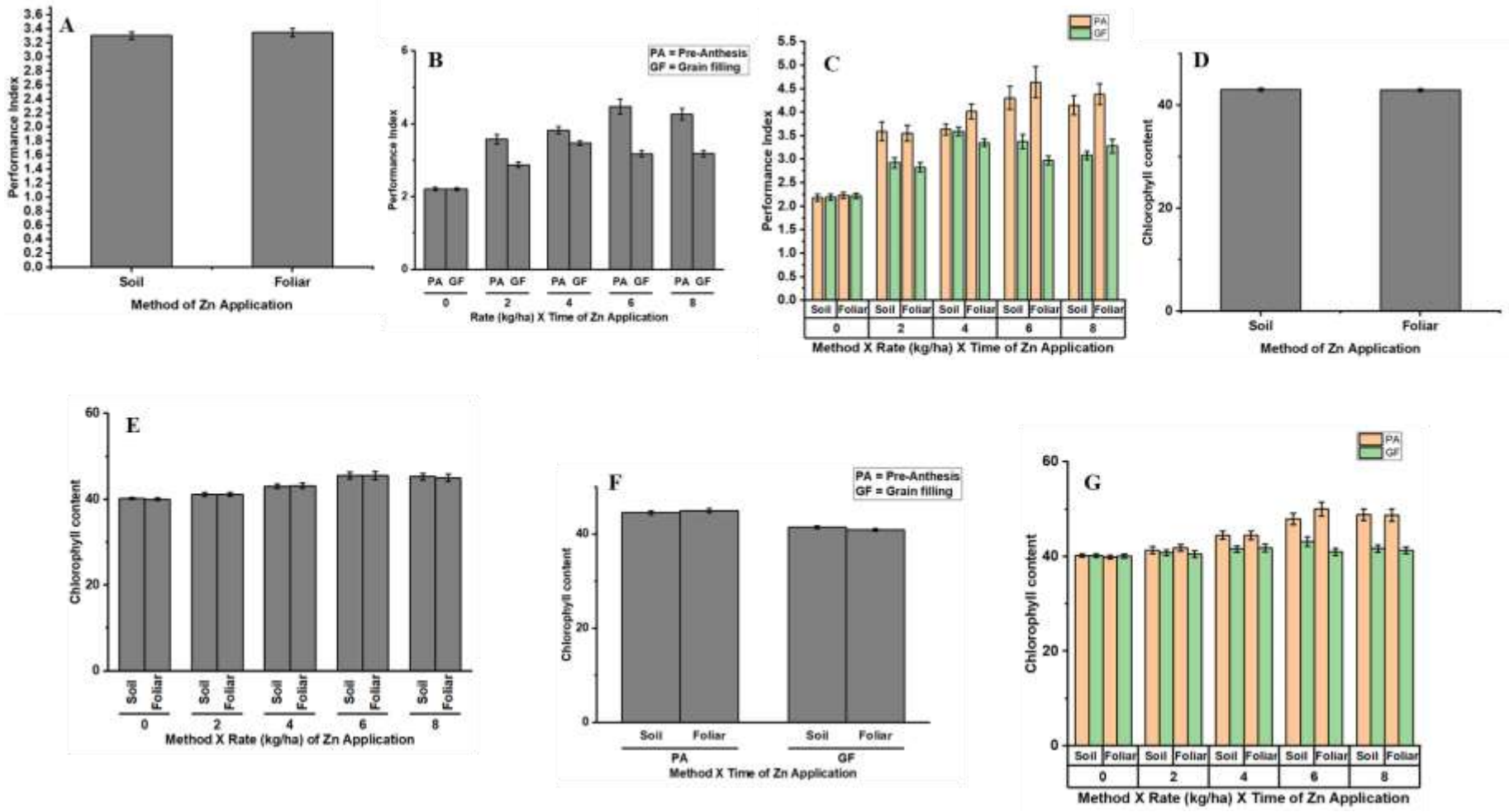




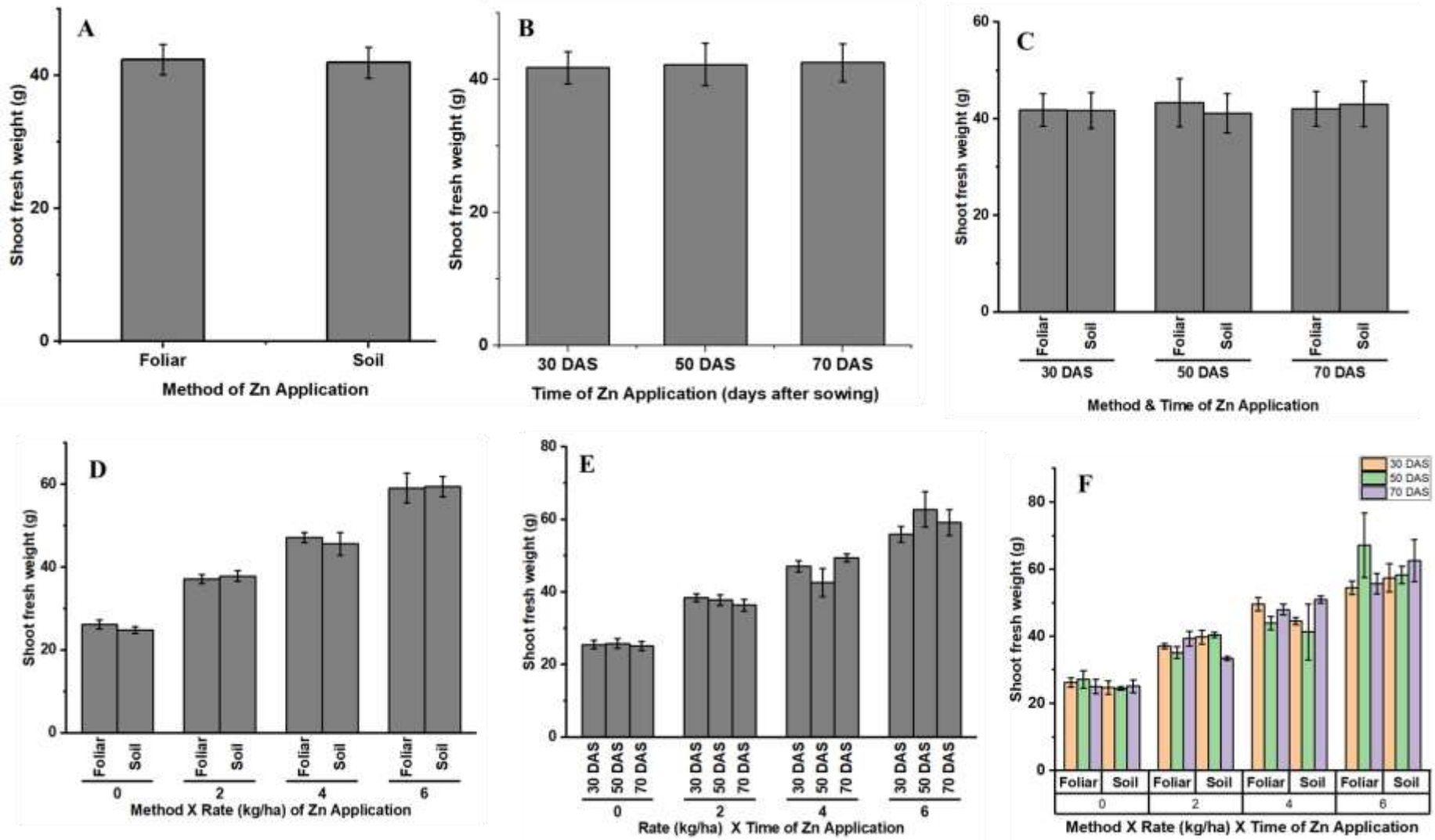
Appendix 11: Impact of Zn fertilisation on Fv/Fm ratio of maize plants.



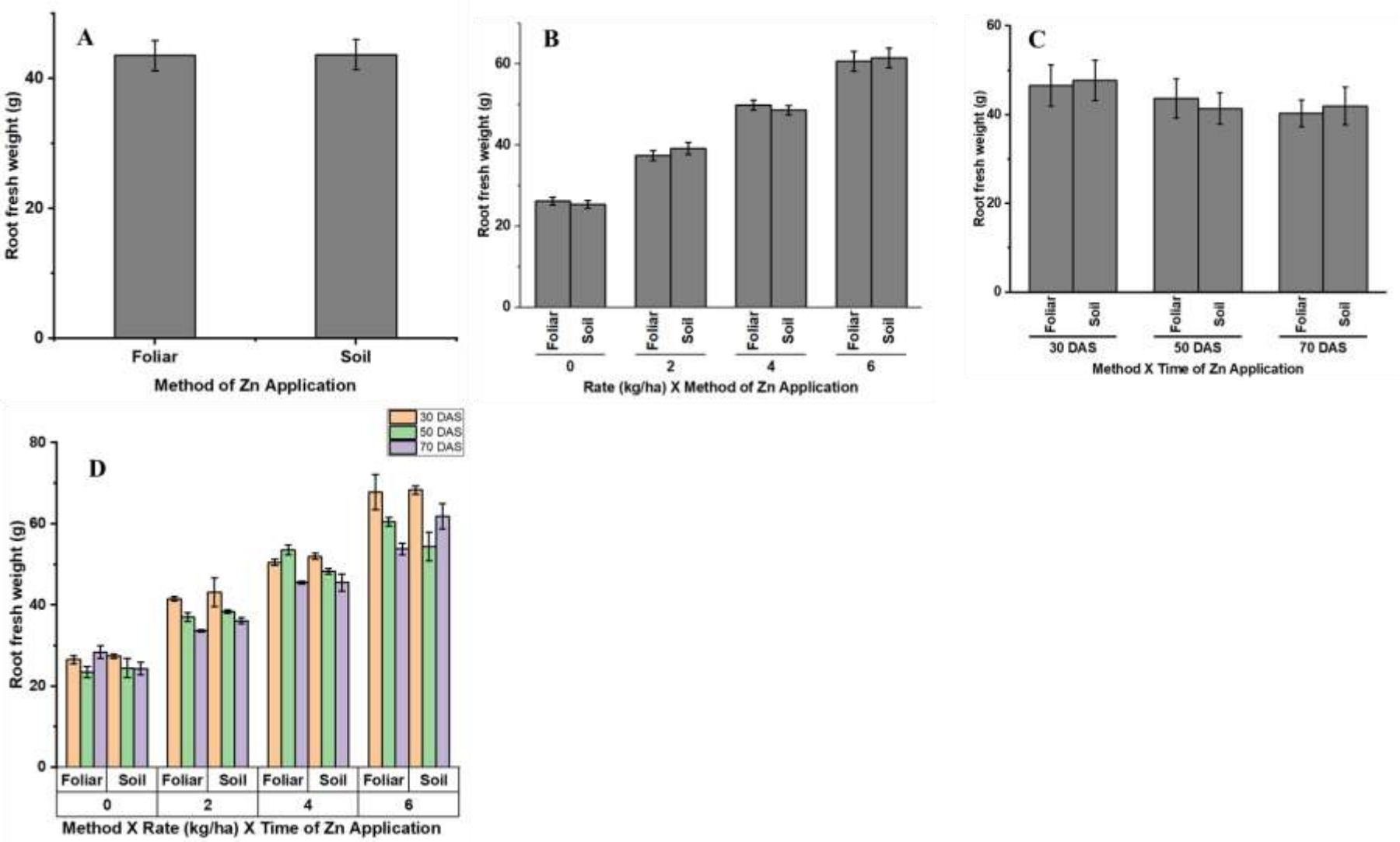
Appendix 12: Impact of Zn fertilisation on performance index and chlorophyll content of maize plants.



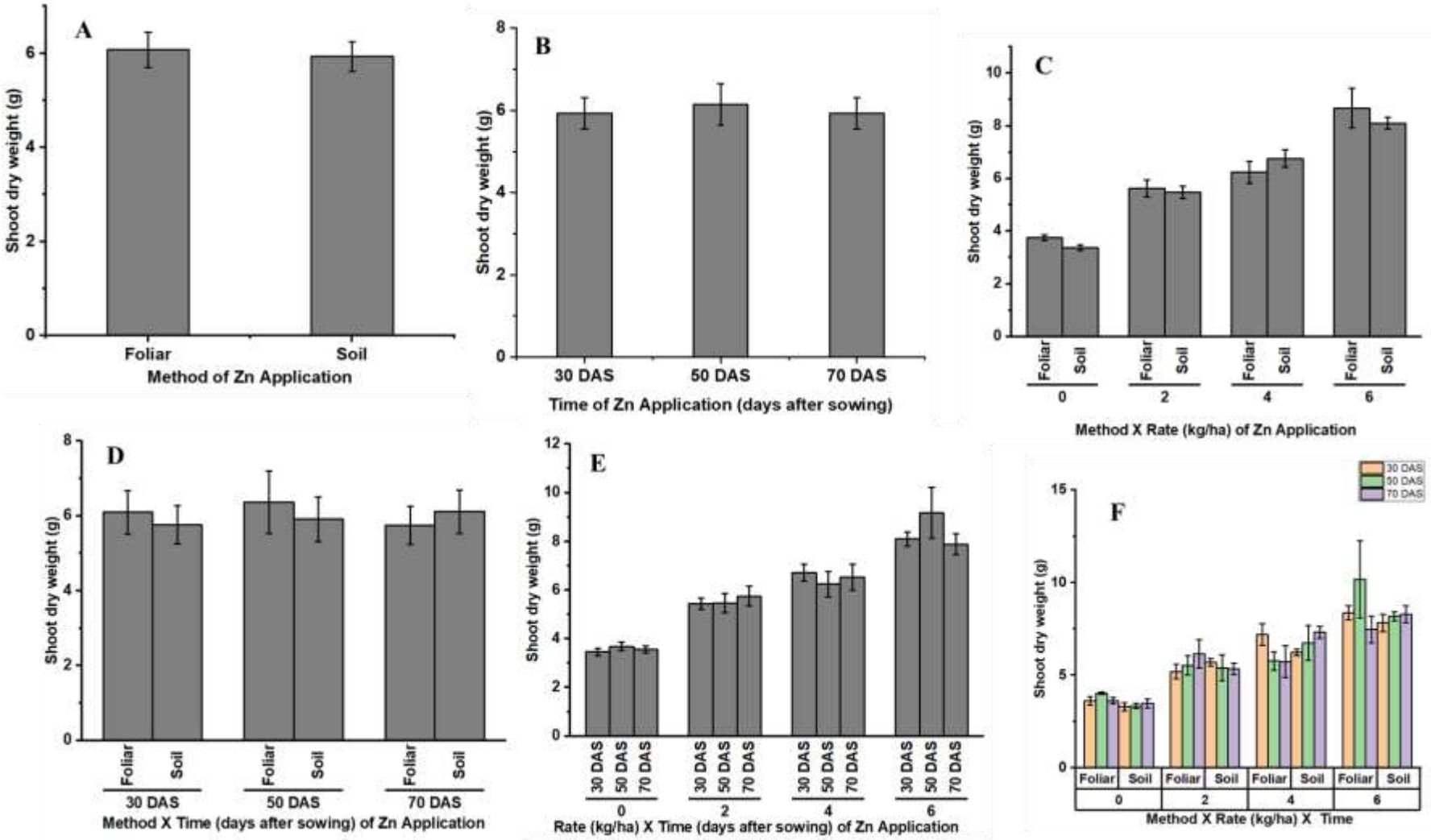
Appendix 13: Impact of Zn fertilisation on shoot fresh weight of pot-grown carrots.



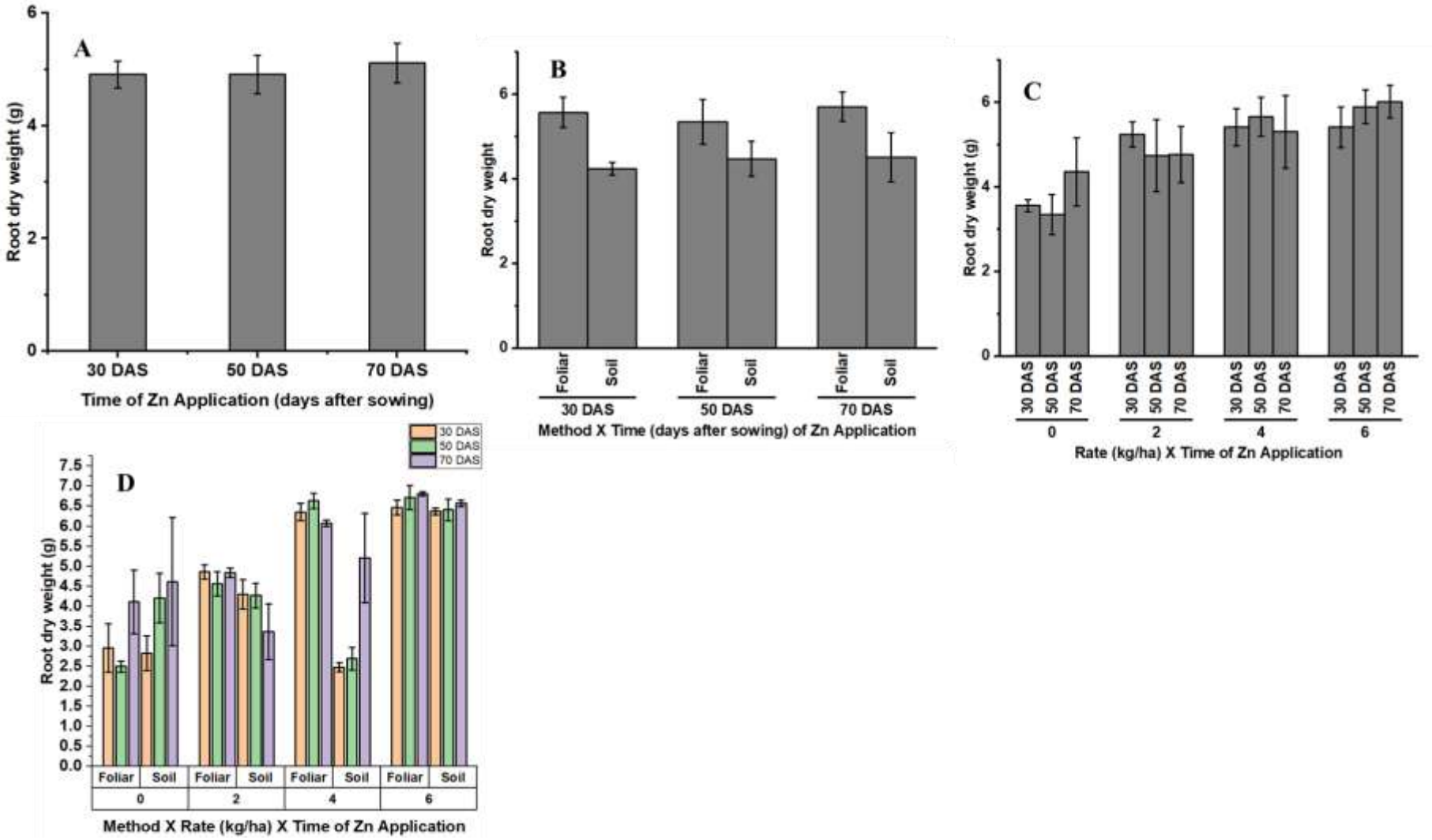
Appendix 14: Impact of Zn fertilisation on root fresh weight of pot-grown carrots.



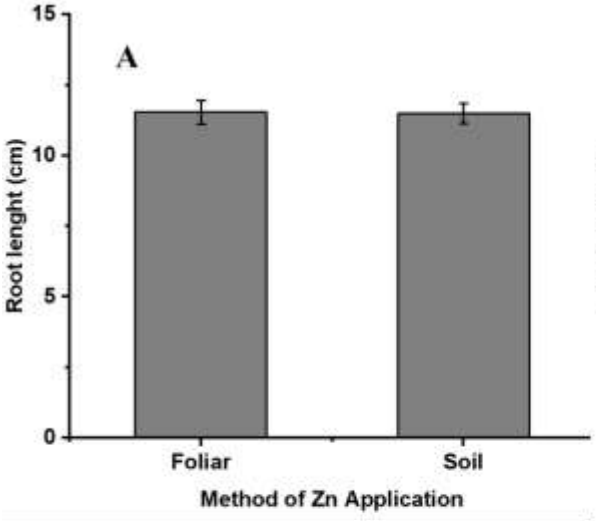
Appendix 15: Impact of Zn fertilisation on shoot dry weight of pot-grown carrots.

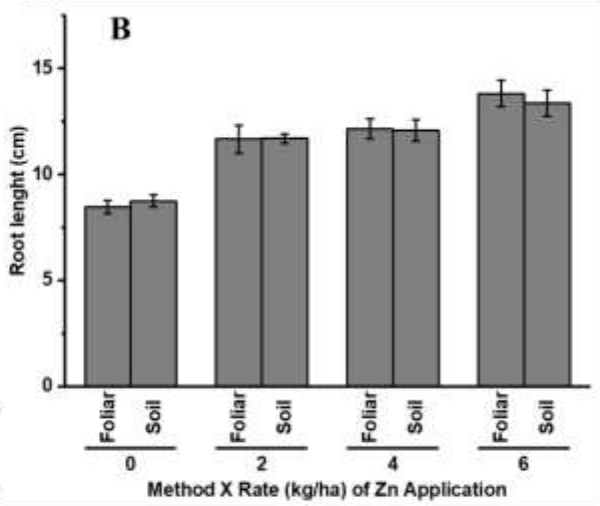


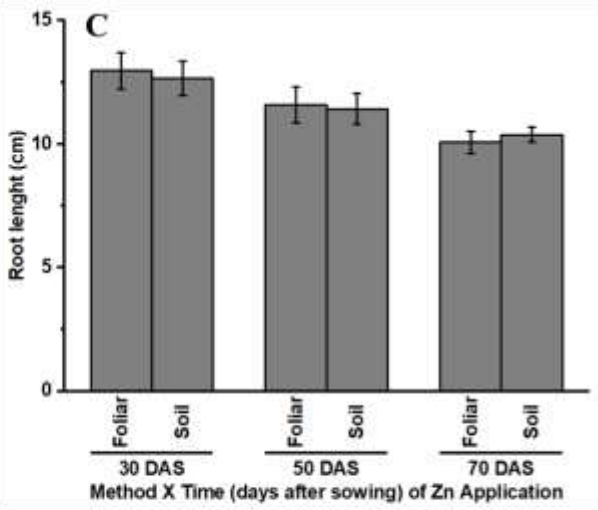
Appendix 16: Impact of Zn fertilisation on root dry weight of pot-grown carrots.

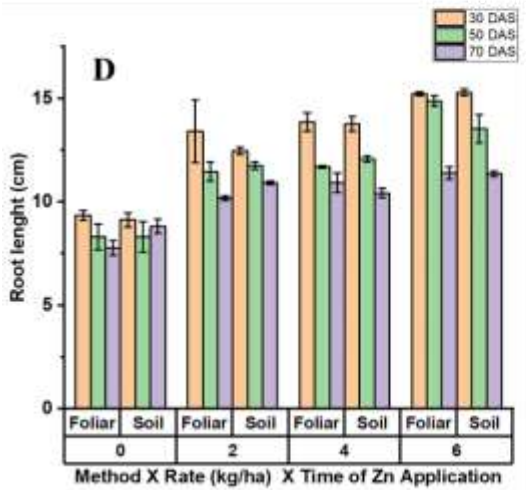


Appendix 17: Impact of Zn fertilisation on root length of pot-grown carrots.

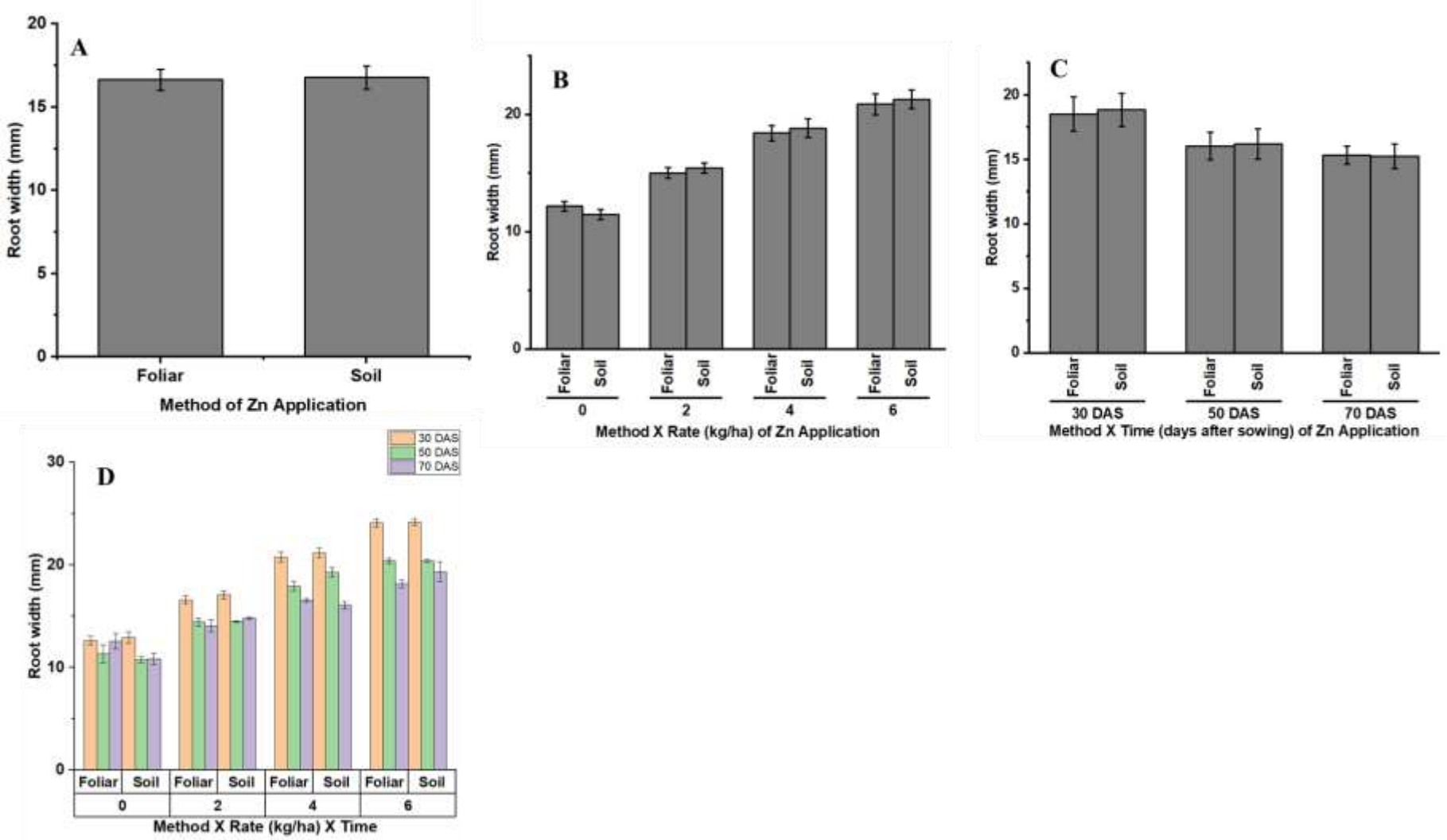




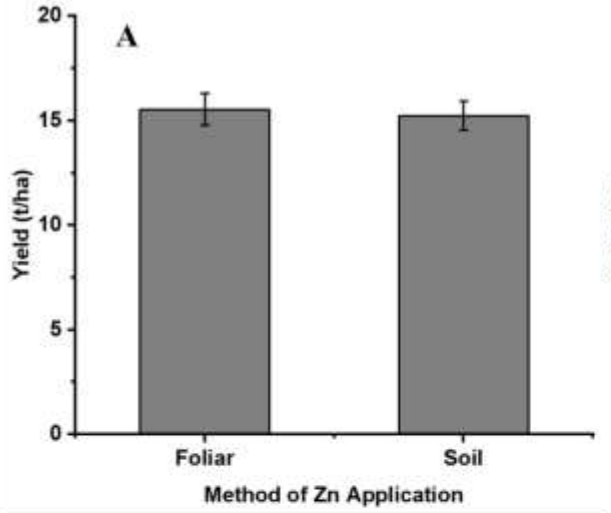


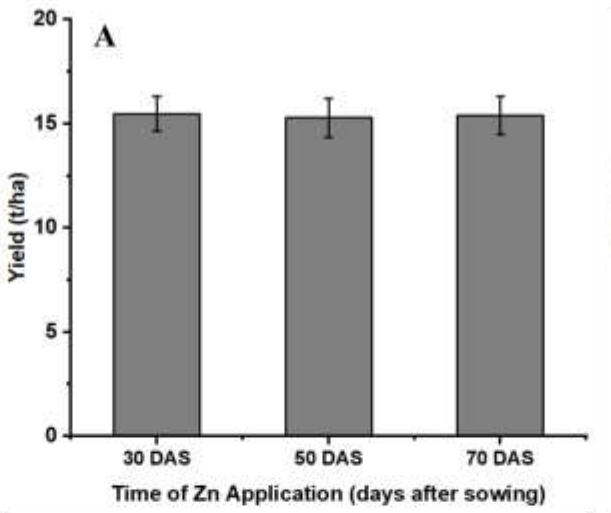


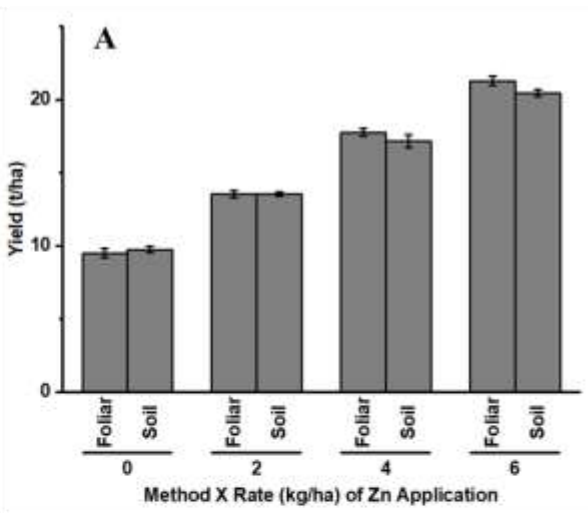
Appendix 18: Impact of Zn fertilisation on root width of pot-grown carrots.

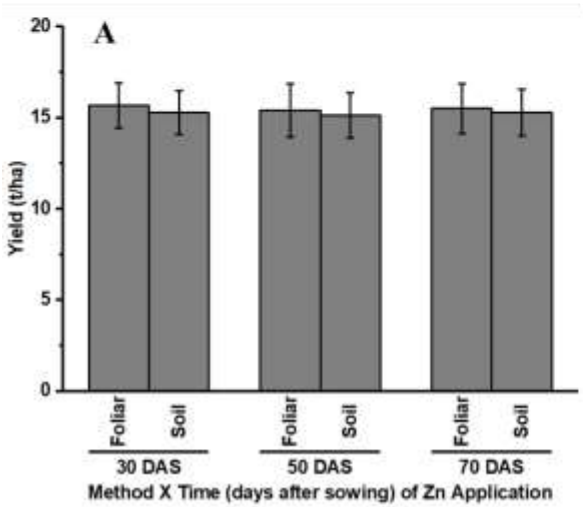


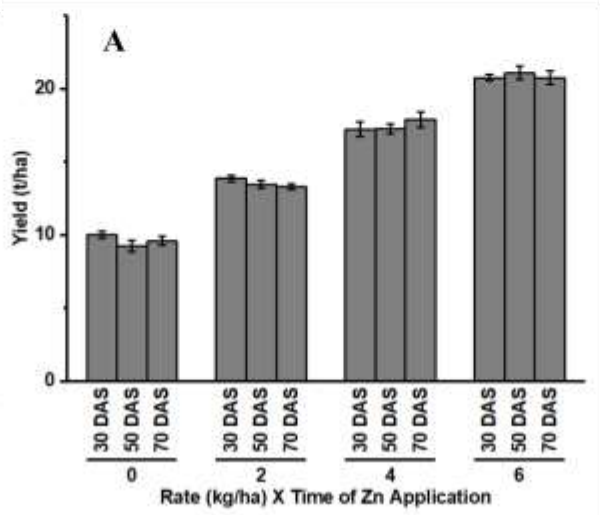
Appendix 19: Impact of Zn fertilisation on yield of pot-grown carrots.

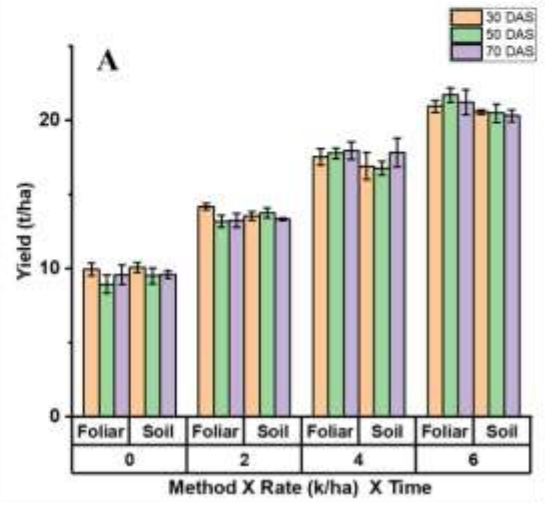




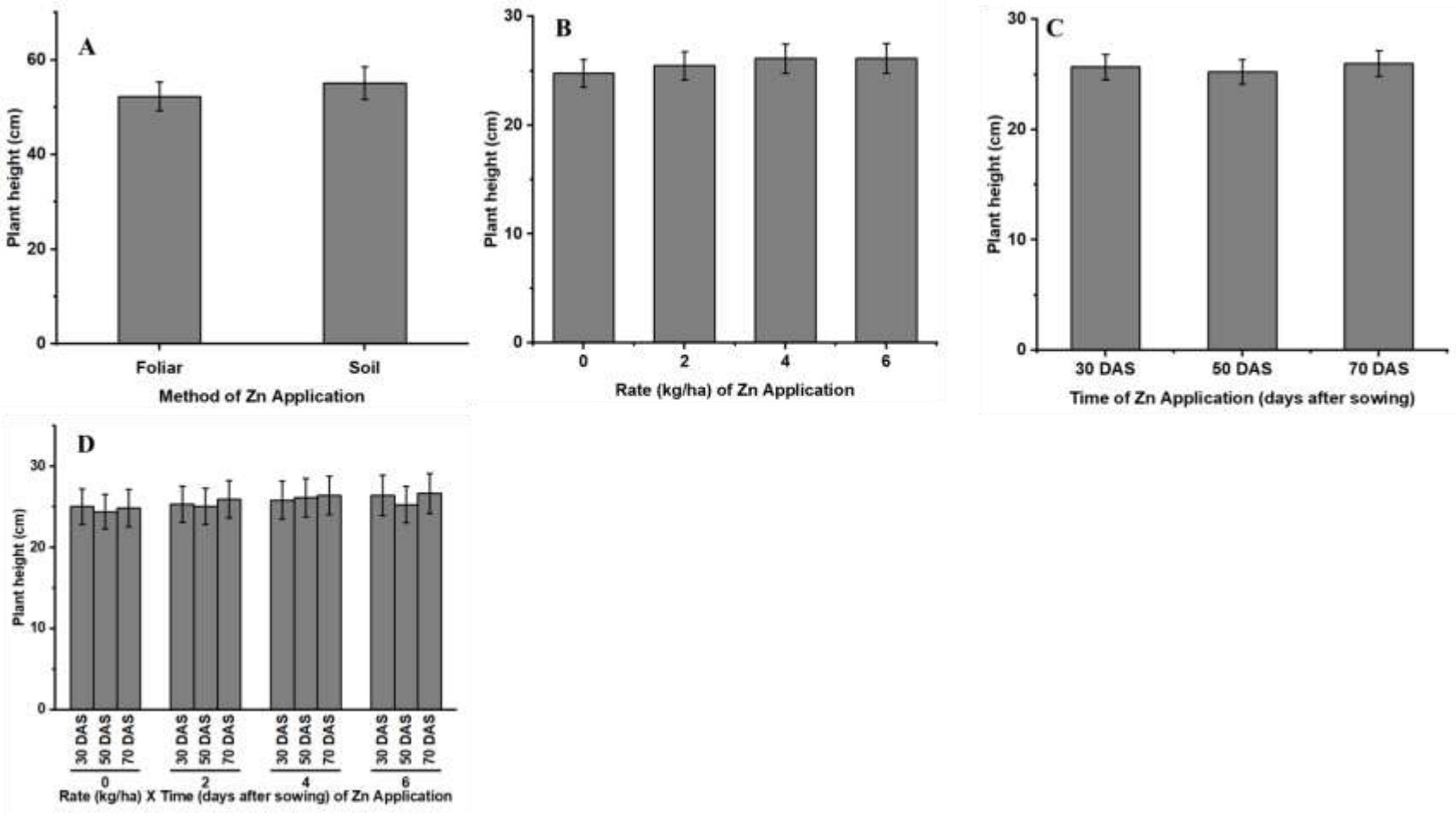




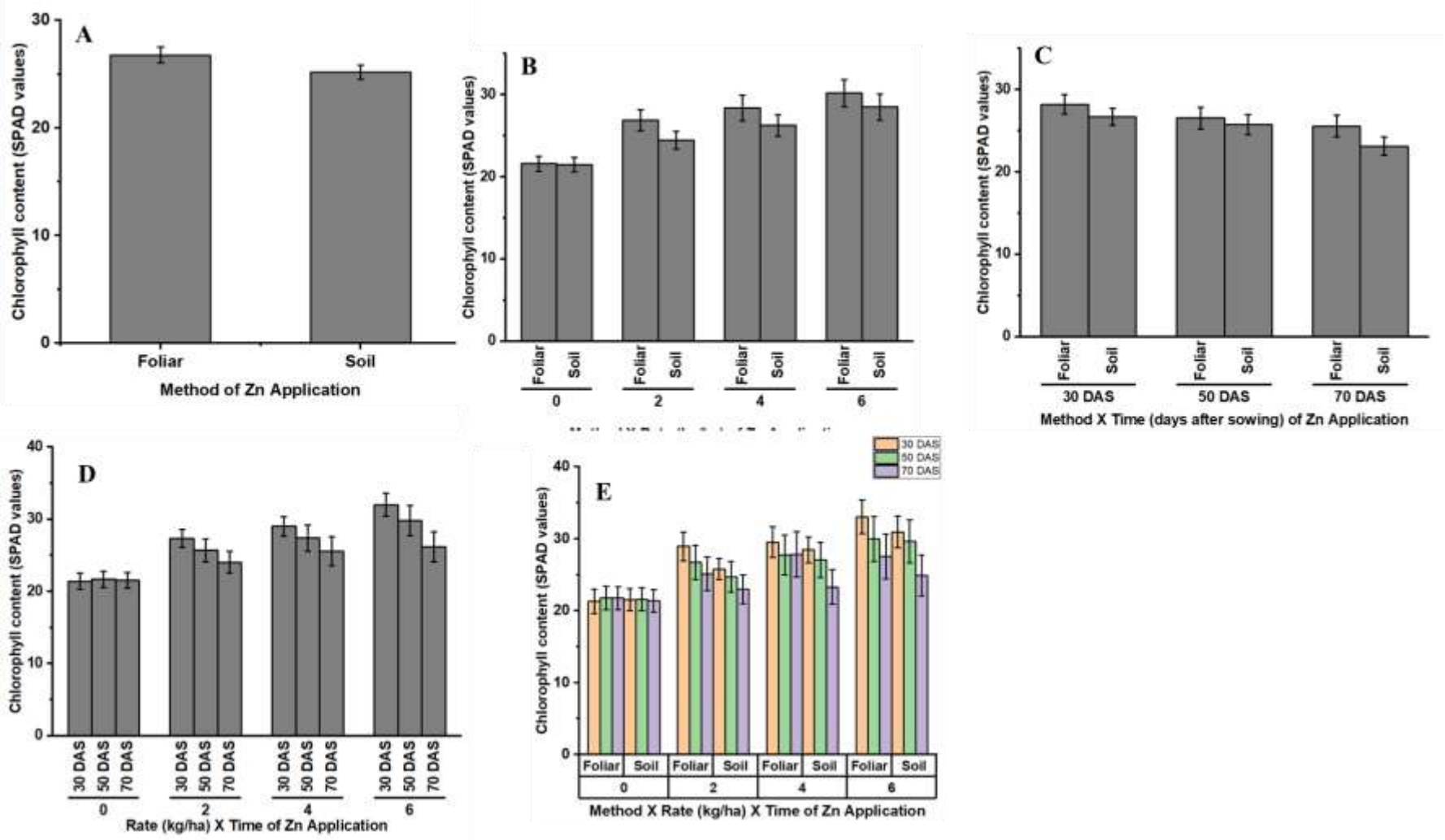




Appendix 20: Impact of Zn fertilisation on plant height of pot-grown carrots.



Appendix 21: Impact of Zn fertilisation on chlorophyll content of pot-grown carrots.



Appendix 22: Impact of Zn fertilisation on root zinc concentration of pot-grown carrots.