BIOFORTIFICATION OF FINGER MILLET GROWN IN BIOCHAR AMENDED SOILS USING ZINC AND IRON

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A DISSERTATION SUBMITTED TO THE DIROCTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN FOOD SECURITY AND COMMUNITY NUTRITION OF GULU UNIVERSITY

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DECLARATION

I, **ALINAFE MANJAWIRA** declare that the content of this research report is my own original work and has never been presented to any institution of higher learning here or elsewhere in pursuit of an academic award

Sign ....AW.....

Date.....30-11-2023.....

APPROVAL

This is to certify that this dissertation has been developed with our supervision and guidance and therefore approve it for submission

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DEDICATION

This work is dedicated to my parents and siblings for their support, patience and encouragement throughout my academic journey.

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LIST OF ACRONYMS

AAS: Atomic Absorption Spectrophotometer

Al: Aluminum

BC: Biochar

CEC: Cation Exchange Capacity

Ca: Calcium

DNA: Deoxyribonucleic Acid

HCL: Hydrochloric acid

H_2SO_4: Sulphuric acid

FAO: Food and Agriculture Organization

Fe: Iron

FYM: Farm yard manure

Mg: Magnesium

Na: Sodium

NH_4: Ammonia

NO_3: Nitrate

N: Nitrogen

NPK: Nitrogen, phosphorous and potassium

SOC: Soil organic Carbon

RDF: Recommended dose of fertilizer

S1: Season 1

S2: Season 2

UNDP: United Nations Development Programme

Zn: Zinc

ABSTRACT

Recurrent dearth of micronutrients from soils and crops aggravated by the impacts of climate change has augmented an increase of hidden hunger with over 2 billion people affected across the globe. This problem is more rampant in developing countries with devastating economic setbacks, which deprive them from diet diversification. This calls for more interventions in enriching the cereal crops which are naturally low in micronutrients despite being the source of staple food for millions of people in African countries. One of the key approaches towards the drive to reducing malnutrition is biofortification. A two-season pot experiment was carried out at Gulu University's Faculty of Agriculture and Environment experimental site. The aim of the experiment was to explore the effects of soil and foliar application of mineral based fertilizers (iron and zinc) in biochar amended soils on yield and grain quality of finger millet grown in allisols and ferralsols. The experiment had eight treatments with four replicates which were arranged in RCBD in both soil types. The results from the experiment revealed that biofortification in biochar amended soils improved yield and quality of finger millet in both soil types. Maximum yield of 50 – 53 g/pot were recorded in both soils with biochar treatments, sprayed with both iron and zinc compared to the yield of 23.8 g/pot -25.5 g/pot in control. Similarly, an increase in protein content from 4.67 % to 11 % was also mirrored in treatments receiving foliar application of both iron and zinc in biochar amended soils. Zinc and iron content were higher in grains (41.5 - 44.6 mg/100g and 11.3 - 13.3 mg/100g, respectively) from treatments which had biochar and were foliarly applied with iron and zinc. Soil organic carbon and moisture content significantly ($P < 0.05$) influenced Zn and Fe uptake from soil. Overall, both soil and foliar application methods of mineral fertilizers in biochar amended soils are ideal in enhancing nutrient availability for crop growth and yield coupled with improved grain quality.

Key words: *Biofortification; hidden hunger; biochar; finger millet; yield, quality parameters; climate change.*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Food security and nutrition well-being are sustainable forces for health and development. They are considered as important indicators for national development. Despite this being the case, increased demand for more food and prevalence of malnutrition are the major issues affecting most developing countries. In this regard, efforts towards mitigating food insecurity and malnutrition should put into consideration dietary quality. Thus, growing of traditional food crops like finger millets which are best suited to different agro-ecologies poses a potential successful approach to achieving food and nutrition security (Pragya Singh, 2012).

Finger millet (*Eleusine Caracana* L) is rich in nutraceuticals as compared to other regularly consumed major cereals (Ajeeshi *et al*., 2021). It plays a significant role for millions of people in sub–Saharan Africa and other developing countries (Bora, 2013). Its wide adaptation to a eclectic environments, less input requirement and resistance to pests and diseases makes it vital for present and future human use (Chandra *et al*., 2016). Finger millet is also drought tolerant as compared to other major cereals like rice, wheat, barley and maize. This is attributed to its C4 photosynthesis system and its ability to grow in marginal agro-ecologies (Gebreyohannes *et al.*, 2021a). In addition, to the aforementioned qualities, finger millets are further associated with numerous health benefits which include; reducing diabetes, obesity, diarrhea and osteoporosis (Anitha *et al.*, 2021).These traits are associated with high amounts of calcium and dietary fiber contained in finger millet.

Millets are produced at an estimated total land area of 33.49 million ha globally with total yield of 31.74 million tones. Finger millets accounts for 12% (4,018,800) of the total world

production area (Bennetzen *et al.*, 2003). In Uganda, finger millet is grown on about 400000 ha and produces 700000 metric tones (FAO, 2005). It widely grown in northern, eastern and south-western regions of Uganda.

Finger millet is one of the most important cereal crops in Uganda. It is ranked second after maize and provides energy to over 80% of Ugandan population (Diego Camps, 2008). Despite this being the case, its production is constrained by various abiotic stresses leading to decreased yield and quality over the years. Declining soil fertility is one of the examples of abiotic stress plummeting its productivity. One of technologies highly regarded as a measure of soil fertility restoration is use of biochar which is a heterogenous combination of pyrogenic compounds with varying physical and chemical properties that interact with soil. Biochar helps in soil fertility restoration through direct addition of nutrients it contains, especially base cations and phosphorous in addition to improved soil nutrient retention amongst others (Obia *et al.*, 2019). Because of this, biochar has been reported repeatedly to increase crop yields (Kätterer et al., 2019).

Low micronutrient content of finger millet, which include zinc and iron is another challenge, which compromises the quality of finger millet grains. It is reported that finger millet contains 2.3 and 3.9 mg/100g mean values of zinc and iron, respectively (Shobana et al., 2013), which are inadequate to meet daily dietary allowance. According to WHO (2007a), daily dietary allowance for zinc in children above six months and adults ranges from 3-12 mg/day and for iron ranges from 11-30 mg/day from infants to adults. Throughout the world, dietary deficiency of iron and zinc are considered as two of the big five silent killers in humans along with vitamin A, iodine and selenium deficiencies. Zinc and iron are vital in central, physiological and structural roles in humans (Connorton & Balk, 2019). Low intake of iron and zinc results in various health problems which include: Pallor (Anemia), skeletal abnormalities, impaired

physical and mental development, poor immune functions and diarrhea (Subbaiah *et al.*, 2016). The deficiencies are common among the growing children, pregnant and lactating mothers, sports men and manual labour workers. Zinc and iron deficiency have affected over 2 billion people across the globe (Chandra et al., 2020). In Uganda, three quarters of under five children and half of women of reproductive ages are iron deficient while prevalence of zinc deficiency ranges from 20-70% in young children and 20-30 % in adults (The Republic of Uganda, 2016).

Additionally, finger millet contains low protein content of approximately 7.3 %, which is meagre to meet the body demands of 0.66 g/kg of body weight (Bruins et al., 2019). Protein is essentially required for catalyzing chemical reactions, regulation of gene expression and immune systems. Its deficiency contributes to protein-energy malnutrition which is currently on the rise with more than14 million cases across the globe (Zhang *et al.,* 2022). In Uganda protein-energy malnutrition is one of the greatest contributors to 60 % of child mortality expressed in form of edematous malnutrition (kwashiorkor), wasting and stunting (Akugizibwe *et al.,* 2013).

Considering the risks associated with zinc and iron deficiency coupled with protein-energy malnutrition to the health of individuals, research to generate novel strategies to enhance Zn, Fe and protein supplementation remains a crucial nutrition-sensitive matter. One such novel strategy which has received growing attention is biofortification which is aimed at improving nutritional value of edible crops (Garg *et al*., 2018). Biofortified crops increases micronutrient intake and have a significant positive impact on human health (Praharaj *et al*., 2021). The idea of biofortification originated around green revolution period which was 1966-1985. After green revolution, food systems in most countries improved tremendously but undernourishment still remained a big challenge. This resulted in high micronutrient shortages in food crops produced as the emphasis was on quantity and not quality, thus augmenting the malnutrition problem

among consumers. Having this awareness, agricultural systems are shifting to develop nutrient dense crops in addition to having copious yields as one way of alleviating challenges of hidden hunger. Thus, micronutrient biofortification has increased exponentially (Szerement *et al.*, 2022).

1.2 Statement of problem

Finger millet is an important crop to most African communities. Approximately 78 % of finger millets are consumed as staple food, 20 % as drinks and other uses, and 2 % as livestock feed (Obilana, 2002). Despite the great value associated with finger millet, its productivity has remained low and poor soil fertility together with its low micronutrient contents is the major reason attributed to this (Gebreyohannes *et al.*, 2021b). It is also low in micro nutrients more specifically zinc and iron with mean values of 2.3 and 3.9 mg/100g, respectively (Shobana *et al.*, 2013). Additionally, it contains low protein content of approximately 7.3 %. It is in this regard therefore that soil amendments interpositions are thus vital to improve these prevailing conditions. This research aimed to expound on the above through biochar and mineral fertilizer amendment to the soils as there is no clear research done currently on how micronutrient biofortification can be influenced by biochar application to increase yield and grain quality parameters of finger millet. The micronutrient biofortification attempts that have been carried out have focused on other crops other than finger millet. In addition to the above, the behavior of micro elements in soils in the presence of biochar remains unclear for weathered tropical soils, which are widespread in Uganda.

1.3 Study objectives

1.3.1 Main objective

The main objective of the study was to determine the effect of biochar and micro-nutrient-based fertilizer applications on the yields and grain quality parameters of finger millet grown in two soil types.

1.3.2 Specific objectives

- To determine effect of biochar and micro nutrient-based fertilizers on growth and yield of finger millet.
- To determine the effects of foliar and soil application of zinc and iron fertilizers on finger millet quality parameters.
- To determine soil factors influencing effectiveness of biofortification using Zinc and iron under biochar amended soils.

1.4 Research hypotheses

This research was steered by the following hypotheses;

- Application of biochar would result to increased yield due to improved soil chemical properties and water availability for the two soil types.
- Both soil and foliar application of zinc and iron with or without biochar would result in improved finger millet grain quality parameters for the two soil types.
- Improved soil chemical properties and water availability by biochar would result in higher Zn and Fe contents in millet grains, independent of soil types.

1.5 Justification of the study

Finger millet has potential to boost food and nutrition security, improve people's livelihoods and contribute to industrialization. However, many countries have not done enough to increase its yield and micro-nutrient content. A lot of research done on biofortification have focused on other crops like maize but a few studies have looked into finger millet, which plays a major role in providing dietary needs of rural people who constitute 80% of Ugandan population (Diego Camps, 2008). Accordingly, there is need to investigate alternative interventions which could enhance its productivity in terms of both quantity and quality.

In addition to promoting food security, it's also essential to curb micronutrient and protein energy malnutrition by promoting production of cereals, which are rich in micro and macro nutrients. Biofortification mostly concentrates on staple food for the people living in rural areas where it is difficult to have access to commercially fortified food. This process may result in increased availability of biofortified crop products within their locality hence lack of nutrients in the food they consume daily can at least be minimized or eliminated entirely. Finger millet is one the mostly consumed staples and its germplasm is naturally good for accumulation of iron and zinc through fe and zn homeostasis pathways which facilitate in the acquisition of iron and zinc in environmentally limiting conditions (Chandra *et al*., 2020).

CHAPTER TWO

LITERATURE REVIEW

2.1 Finger millet production across the regions

Finger millets are enormously vital in the African Semi-Arid tropics with East Africa being the largest producer having a total production of 773,500 tones followed by southern Africa 121,000 tones, Central Africa 58,110 tones and North Arica 11,080 tones (Obilana, 2002).

2.2 Advantages of finger millet and its potential to support food and nutrition security

Finger millet is known to save poor farmers from starvation when other crops have failed due to abiotic factors aggravated by climate change. This is due to its wide adaptation and ability to grow in diverse weather and soil conditions, marginal, arid and mountainous terrains where major cereals have low growth ability. According to research carried out in two regions of Kenya, it was noted that with increasing impacts of climate change, certain crops were more severely affected than others. In the same season and under the same environmental conditions, finger millets were able to produce yield while other cereals like maize experienced total failure (Shibairo *et al*., 2016). This is attributed to the indigenous nature of the crop to the continent hence being better adapted to harsh environmental conditions such as irregular rainfall, drought and increased temperatures.

Availability of ready markets is another advantage associated with finger millet production. Local markets serve as the main selling points in most rural communities, which have weekly rotations. This trend enables farmers to sell the crop more frequently and at a double price compared to other cereal grains. This is majorly attributed to the small size of the grain and high nutritional content and enables the farmers to make more profits.

Finger millets can be stored for years without being destroyed by weevils. This is because of its small grain size, which makes it difficult for pests to move within it (Gebreyohannes *et al.*, 2021a). It is therefore ideal for households who can't afford to buy insecticides. This quality makes it a unique crop that can be reserved for famine.

Health benefits of finger millet is another reason attributed to its wide cultivation and preference. Some of the health benefits associated with finger millet include; lowering blood glucose through phenolic extracts, which are reported to inhibit alpha glycosidase and alpha amylase. This is essential in management of postprandial hyperglycemia (Sbobona *et al.*, 2009). Finger millet has low glycemic value hence being able to last long in the digestive system. This is a desirable trait for diabetic patients. It is further associated with an increase in inbuilt resistance against bacterial and fungal infection diseases due to high content of potassium. The presence of antioxidants reduces risk of heart, kidney problems and cancer. It is in this regard therefore that this research aimed at increasing iron content for enhanced hemoglobin levels and zinc content for physical and mental development coupled with improved immune systems in both children and adults.

2.3 Constraints in finger millet production

Common reported challenges associated with finger millet production include blast diseases, weed infestation, impacts of climate change, declining soil fertility and lack of improved seeds.

Finger millets are highly susceptible to blast disease infection. It is more prevalent in long rain season causing great yield losses ranging from 7.32 - 54.07 % depending on cultivar's susceptibility (Sasmal, 2016).

High weed infestation increases production labour due to its similarity to certain weeds such as Eleusine indica, which are closely related (Jayne *et al*., 2016). Weeding becomes a difficult

and expensive task because of its high resemblance with the weeds as such the crop is weeded more frequently compared to others.

Lack of high yielding and well adapted varieties also limits its production (Gebreyohannes *et al.*, 2021a). Most farmers rely on local seeds from their farms or from their neighbors and relatives usually reserved from previous seasons. This is due to lack of access to improved seeds and mostly emanating from neglects of finger millet by research community and extension service providers. Extension workers mostly focus on popular crops like maize and beans as opposed to traditional crops like finger millet, which are better adapted to the tropical regions. This concurs with research by Handschuch *et al.* (2016) who reported that millets in Africa have been greatly under researched resulting in farmers' limited exposure to improved varieties.

Despite the fact that this crop is able to adapt to poor growing conditions, the declining levels of soil fertility has resulted into reduced productivity (Diego Camps, 2008).This research therefore, aimed at improving soil fertility through biochar application as one way of augmenting finger millet production.

2.4 Approaches to increasing finger millet yield and quality

A number of interventions are being implemented in regards to improving finger millet productivity which include breeding programs, mixed cropping, crop rotation, use of organic and inorganic fertilizers. In regards to improving the grain quality, biofortification has been an applauded approach and widely adopted by many in addition to breeding programs.

A lot of studies have been done to assess the effect of organic fertilizer alongside inorganic fertilizer on finger millet growth and yield. Notable improvements in finger millet yields and soil fertility were observed after application of farm yard manure coupled with NPK (Kumara

et al., 2014). Similar trend was observed by Govindappa *et al*. (2009) in which there was an increase in grain yield, weight and straw yield of finger millet after applying FYM at a rate of 7.5 t/ha alongside NPK. Singh et *al*. (2013) observed an overall increase in plant height, grain weight, and yields when biochar was applied at the rates of 0, 50 and 100 %/ha coupled with NPK.

Intercropping of finger millet with legumes such as groundnuts, pigeon peas, soy beans, and common beans among others is reported to have a positive impact on yields. This is because legumes have the ability to fix atmospheric nitrogen through nitrogen fixation. Consequently, finger millet is able to utilize fixed nitrogen as it unable to fix nitrogen on its own (Thilakarathna & Raizada, 2015). An experiment by Maitra *et al*. (2000) reported an increase in finger millet yield under intercropping with groundnuts and pigeon peas compared to the yields obtained under sole cropping.

Crop rotations helps in improving crop production as residual fertility from the preceding crop contributes to the growth of the crop that follows. It has been reported that finger millet benefits more from the residual nourishment stemming from the previous crop more especially when organic fertilizers are used (Thilakarathna & Raizada, 2015). A study by Ebanyat *et al*. (2010) in eastern Uganda observed an increase in the yield of finger millet following legume crops.

Breeding program has enabled cultivar development, crop improvement and seed improvement of various important crops conventionally through selective mating or hybridization. Currently a number of breeding approaches have been involved. For instance, pure line selection, hybridization, mutation and molecular breeding which has led to development of different finger millet varieties across the world. Examples of varieties developed are PLR-1. K-22, Ro-786 in India, while in Uganda, varieties developed include Gulu-E and Serere-1(Al-Khayri *et al*., 2019).

Biofortification which is aimed at improving the nutritional value of edible crops through selective conventional plant bleeding, mineral fertilization (Agronomic) and plant breeding using genetic engineering (Garg *et al*., 2018) has been adopted as a measure of increasing nutrient content in finger millet grains. This study focused on mineral fertilization and how it can influence grain quality parameters of finger millet when applied either foliarly or through the soil in a local setting.

2.5 Approaches to fertilizer application and agronomic biofortification

Agronomic biofortification is the application of mineral micronutrient fertilizers to soils or plant leaves to enhance the concentration of target minerals in edible portions of crops with the aim of increasing dietary intake of the targeted minerals (Valenca *et al.*, 2017). It is widely believed that the application of mineral nutrients from external sources increases their concentration in developing grains as well as improving soluble and mobilizable mineral elements in the soil. It is an important method especially in Africa and Asia as it is the fastest and easiest method to supply food grains with essential micronutrients (Aciksoz *et al*., 2011). This approach has been widely adopted because it does not require a lot of time and the method of application is straight forward. Agronomic biofortification by applying Zn and Fe containing fertilizers represents a useful complementary approach to breeding as breeding programs are time consuming and expensive.

Other approaches to agronomic biofortification include seed coating, microbial inoculation and cropping systems. Microbial interventions such as application of plant-growth promoting rhizobacteria are known to improve micronutrient intake and content in grains. Cropping systems approaches include intercropping, for example, maize + beans, millet + chickpea coupled with manure. This approach has been proved to be one of the effective measures in improving micronutrient availability and uptake in grains (Nayak *et al*., 2022).

Among fertilizer management approaches, foliar application is deemed as the most effective way of enriching mineral content of cereal grains as compared to soil application. Foliar application requires less fertilizers than soil application. According to a study by Prasad *et al*. (2014) on agronomic biofortification of finger millet using Zn and Ca grown in lateritic soil, the results showed that foliar application at 1% and 2 % of Fe and Ca, respectively resulted in increased Ca content of 343 mg/100g, 15.08 mg/100g for iron and 7.32 % for protein in finger millet grains compared to soil application. Another study by Ebanyat *et al*. (2010) observed that soil application of Zn had very little effect on zinc concentration of brown rice as compared to foliar Zn application which showed a significant increase. Hassan *et al*. (2012) also observed an increase in Zn concentration following increased application rates of 5, 10, 15 and 20 mg Zn/kg of soil and the zinc content in finger millet grains were 43.5, 71.5, 79.5 and 80.4%, respectively higher compared to control treatments. An upsurge was observed under foliar application and distinct increase was observed when the application was done at flowering stage. Another study by Singh *et al*. (2013) observed an average increase in zinc concentration by 47.0 mg/kg grain when 0.5% $ZnSO_4$ solution was applied compared to control which had 33.9 mg/kg soil.

Dhaliwal et al. (2010) observed a significant maximum Fe concentration in PR 113 116 (289.2 mg/kg) and PR 113 (234.4 mg/kg) finger millet cultivars when sprayed with 0.5 % and 1 % levels of $FeSO_4$. $7H_2O$ at tillering stage. Agronomic fertilization works best in a specific situation or in combination with other strategies like use of organic manures, synthetic fertilizers and biofertilizers. According to research done on pearl millet, nitrogen fertilization combined with zinc increased the yield as well as zinc content in pearl millet grains (Prasad *et al*., 2014). Grain zinc content significantly varied with zinc and nitrogen rates and a maximum zinc content was recorded at N application rate of 60 kg/ha. This research therefore expounded on the above by using biochar fertilizers.

2.6 Biochar production and composition

Biochar is a carbon rich material produced from pyrolysis of biomass in oxygen limited environment under temperatures between 300 and 1000 °C (Joseph &Lehmann 2009). Biochar production is a novel approach that tends to have both agronomic and environmental benefits.

Elements found in biochar include carbon with concentrations ranging between 172 - 905 g/kg, phosphorous ranging from 2.7 - 480 g/kg, nitrogen from 1.8 - 56.4 g/kg and potassium from 1.0 - 59 g/kg (Lehmann *et al.*, 2009). It also contains other elements like sulphur, hydrogen, oxygen, base cations and heavy metals in varying amounts (Jacobs & Sovacool, 2012).Variations in amounts of heavy metals contained is attributed to feedstock type and conditions subjected to during pyrolysis (Joseph *et al*., 2012). Biochar pH values ranges from 4 to 12 and it's also dependent on pyrolysis temperatures (Neem *et al*., 2016). Higher pH values of biochar are associated with concentration of alkaline elements emanating from pyrolysis under high temperatures (Mukherjee *et al*., 2011).

2.7 Impact of biochar on soil properties

Biochar influences plant growth by improving soil physical properties such as bulk density, water holding capacity and porosity, soil chemical properties which include nutrient retention and availability, improved CEC, surface area and pH (Abel *et al*., 2013). It also influences soil biological properties by increasing diversity and provision of a conducive environment of soil microbial community (Abujahal *et al*., 2016).

2.7.1 Soil chemical properties

Biochar application brings about changes in soil chemical properties which include an increase in soil pH, CEC, organic carbon and nutrient content (Biederman & Harpole, 2013) coupled with reduction effect on the content of exchangeable Al^{3+} in the soil (Jacobs & Sovacool, 2012).

Soil amendment with biochar has been reported to reduce mobility of toxic elements in acid soils coupled with increased availability of potassium and phosphorous (Major *et al*., 2010). Nutrient availability reduces the costs incurred in buying fertilizers.

Cation exchange capacity (CEC) is useful indicator of soil fertility as it facilitates adsorption of mineral nutrients and ions such as Na^+, Ca^{2+}, Mg^{2+}, K^+ and NH_4^+. It is natural inherent property of any soil as such it is not easily influenced by management (Alkharabsheh *et al*., 2021). Despite this being the case, a number of studies have indicated that biochar amendment can improve the CEC of soil. This trait has been associated with the presence of strong phenolic and carboxylic functional groups coupled with negative charges on the surface of biochar particles (Gao *et al*., 2017). A study by Zhang *et al*. (2012) reported 21 % upsurge in soil's CEC in treatments that had biochar when it was compared to unamended soils. Ndor *et al*. (2015) reported a 21 % increase in the CEC of soils from the treatments which had biochar applied at a rate of 5 mg/kg of soil derived from rice husks and saw dust followed by an upsurge by 44 and 57%, respectively when the application rate was increased to 10 mg/kg of soil.

Biochar application raises soil pH even though the changes depend on soil type, feed stock and liming value of biochar (Tian *et al*., 2017). A study by Martinsen *et al*. (2015) that used biochar derived from oil palm and cacao shell reported an increase in soil pH from 4.7 to 5. Castaldi *et al*. (2011) also observed an increase in soil pH from 5.1 to 6.39 when biochar was added at a rate of 60 t/ha. Soil pH influences mobility and availability of nutrients in the soil.

Melo *et al*. (2016) reported a reduction of organic pollutants like PAHs, dioxins, pesticides and availability of heavy metals under biochar amended soils, which was attributed to biochar's large surface area and high adsorption capacity.

A lot of studies have observed an increase in organic carbon content following soil amendments with biochar. Soil organic carbon is crucial to soil health, fertility and ecosystem services. El-Naggar *et al*.(2018) observed an increase in the organic carbon content in sandy soils by a range of 42 % -72 % in biochar amended soils following biochar application rate of 30 t/ha. Another research by Jeffery *et al*. (2011) reported an increase in soil organic carbon content after application of biochar derived from maize at an application rate of 50, 100 & 200 mg/kg of soil.

2.7.2 Soil physical properties

Biochar has relatively high surface area as compared to soils. This trait influences soil biochar interaction and increases total soil-specific surface area in biochar amended soils (Lehmann *et al*., 2009). It is assumed that biochar application reduces soil bulk density through; dilution effect as biochar's bulk density ranges from 0.08 g/cm^3 to 0.43 g/cm^3 which is lower than soils bulk density which ranges from 1.16 to 2.00 g/cm^3 (Chaudhari *et al.*, 2013). A study by Omondi *et al*. (2016) concurred with above statement as they observed a decline in soils bulk density with values between 3 & 31 % in 19 biochar amended soils out of 22 soils. Another study by Adekiya *et al*. (2020) registered a reduction in soil's bulk density by 75 % following the addition of biochar at 30t/ha when it was compared with untreated soils. Bulk density is an indicator of soil compaction, which influences water retention, rooting depth, aeration and movement of nutrients.

Another influence of biochar application on soil physical property is on porosity which describes the amount of open spaces or pore space that can be found between soil particles.

Biochar helps to increase soil porosity by decreasing soil compaction and improving soil aggregation (Blanco-Canqui, 2017). A study by Zhang *et al*. (2012) found out that biochar application increased soil porosity and capillarity by 23 and 24 % respectively when it was compared to soils without amendment. This concurred with the results by Adekiya *et al*. (2020) who observed an increase in porosity by 65 % after biochar application rate of 30 t/ha as compared to unamended soils.

Application of biochar increases soil water retention as a result of increased soil porosity. More water spaces are available emanating from biochar spaces. A study conducted by Adekiya *et al*. (2020) reported an increase in soil and water infiltration rate after biochar application at a rate of 30 mg/kg of soil. Concurring with the results, Kätterer *et al*. (2019) observed a substantial increase in water holding capacity after applying biochar at a rate of 100 Mg/ha compared to untreated soils. Soil water holding capacity indicates the amount of water that a given soil can retain for crop use.

2.8 Effect of biochar on crop growth and yield

Amendment of soil with biochar influences bioavailability of various nutrients. For example, potassium is present in the forms which are readily available for the plant uptake. An experiment by Borchard *et al*. (2019) observed an increase in maize yields coupled with an increase in N and Ca in the maize leaves after applying biochar at an application rate of 15 g/kg of soil. Agegnehu *et al*. (2015) reported a yield increase ranging between 22 % and 24 % in pod yield of ground nuts which was grown in wood biochar modified soils applied at a rate of 25 t/ha compared to the yield where inorganic fertilizers were utilized.

Furthermore, biochar helps in altering soil pH which in turn influences nutrient solubility hence availability. Ferrosols are acidic in nature and biochar addition may help to neutralize the

acidity. An increase in soil pH due to biochar addition is associated with increased availability of macro and micro nutrients such as K, P, Ca, Mg (Major *et al*., 2010). There is also reduction of aluminium toxicity in acidic soils emanating from an increase in soil pH.

According to a study which was conducted on acidic ferrosol at an application rate of 10 t/ha resulted in a reduction of ammonium acetate extractable aluminium from 1.93 cmol (p^+)/kg of soil to a very small amount which couldn't be detected (Van Zwieten *et al*., 2010). As such the liming effect of biochar described above helps in alleviating aluminiun stress for better crop production (Dai *et al*., 2017).

Improvement in plant growth and yield after biochar application are further associated with modification of physical and chemical properties of soil emanating from improved soil structure and water holding capacity. A study by Jeffery *et al*.(2011) observed an increase of 28% to 39% changes in productivity in esteem to both crop yield and above ground biomass when it was planted in biochar amended soils. This was attributed to biochar's liming effect which enables nutrient and water availability. This study therefore explored more on the impacts of biochar and micro nutrient fertilizers on finger millet growth and yield on common soil types of northern Uganda as biochar's influence on yields tend to vary between the soil types.

2.9 Soil factors influencing the efficiency of agronomic biofortification

2.9.1 Soil pH

Soil pH influences the accessibility of micro nutrients in the soil. Zn solubility decreases with an increase in soil pH due to high adsorptive capacity of the soil solid surfaces emanating from increased pH dependent negative charges, formation of hydrolyzed forms of zinc and co-precipitation in iron oxides (S. Singh *et al.*, 2023). When PH is > 8, calcites bound the Zn more

strongly tumbling its availability to plants. Similarly, Fe solubility increases with a decrease in soil pH. It is in this regard therefore that this research aimed at determining the pH levels which are ideal for enhanced uptake of iron and zinc and subsequent accumulation in the grains.

2.9.2 Moisture content

Movement of zinc and iron through diffusion is mired by low soil moisture which reduces their accumulation in grains (Rengel, 2015). Soil amendments technologies like organic manures could help to improve availability of micronutrients like zinc and iron hence this study explored more on how organic amendments like biochar could influence nutrient availability and support biofortification as most of the researchers have dwelled much on biofortification in soils supplied with inorganic fertilizers.

2.9.3 Organic matter

Soil organic matter contains humic substances which contributes more than 50% organically bound carbon in the soil. Humic substances influence soil solution concentration of several plant nutrients and enhances P, Fe, Zn and Cu solubility thus increasing their availability and subsequent uptake by plants (Gerke, 2022).

2.9.4 Soil aeration

Aeration is required by micro-organisms that work on decomposition of organic matter to release plant nutrients, thus enabling its uptake and subsequent accumulation in grains.

2.10 Significance of iron and zinc in human diets

Iron is a key micro nutrient element as it is a component of hemoglobin through which it is involved in transport and storage of oxygen hence being responsible for the survival of mankind (Prasad *et al.*, 2014). Clinical symptoms of iron deficiency include fatigue, pallor

(Anemia), dizziness, reduced intellectual performance and work capacity (Samson & Fischer, 2022).

Zinc is also essential part of human diet as it plays a key role in physical growth and neurobehavioral development. It also forms integral component of zinc finger proteins which help in regulating DNA transcription (Levenson & Morris, 2011). Zinc deficiency symptoms in children include neurobehavioral disturbances, diarrhea, stunting, anorexia (loss of appetite), poor immune infections and blepharoconjuctivis among others. Common symptoms in adults include recurrent infections, chronic non-healing leg ulcers, and hypogeusia (impaired taste sensitivity) (Brown *et al*., 2004).

2.11 Nutrition profile of fingermillet

Finger millet is one of the most nutritious cereals crop. It contains carbohydrates, proteins, dietery fibre and minerals, which include iron, calcium, manganese among others as compared to maize, polished rice, cassava and plantains. However, its chemical composition is influenced by environmental and genetic factors.

Proximate composition of finger millet is as follows: Fat - 2.95%, crude protein - 10.50%, moisture content - 9.33%, fibre content - 18.45%, ash content - 2.52%, carbohydrate - 74.72% (Okolo *et al*., 2015). Its mineral composition (mg/100g) is as follows: Calcium - 70.89 mg/100g, Sodium- 19.95 mg/100g, Magnesium - 16.45 mg/100g, Potassium - 9.95 mg/100g, Copper - 4.73 mg/100g, Chromiun - 9.83 mg/100g, Manganese - 9.95 mg/100g and Phosphorous - 60.8 mg/100g (Auda *et al*., 2018), Zinc 2.3 mg/100g and iron 3.9 mg/100g (Shobana et al., 2013).

CHAPTER THREE

METHODS AND MATERIALS

3.1 Study area

The experiment was carried out at Gulu University campus at the Faculty of Agriculture and Environment experimental site. Gulu University lies approximately 3 kilometers from Gulu city, northern part of Uganda. The coordinate for the study site is 46′29″ N, 32 17′ 56″E. The study site has two seasons, wet season which starts from March to November peaking in the months of May and October with an average rainfall amount of 154 mm and 161 mm, respectively. This is followed by a dry season which starts in December to February. The district has moderate variations in temperature with highs fluctuating from 26.8 ˚C - 34.2 ˚C and lows ranging from 16.7 ˚C - 21 ˚C. The climate is tropical, wet and dry coupled with relative humidity range of 38 % to 85 %.

3.2 Experimental crop

Naromil 2 (FMS 376) finger millet variety was used for this experiment. This variety is widely grown in eastern and northern Uganda. It was released by National Agricultural Research Organization (NARO) of Uganda in 2017 and has since gained popularity for its ability to withstand the prevailing erratic rainfall conditions. Finger millet is ranked second in terms of importance in Uganda after maize (Diego, 2008). It is a reliable source of energy to over 80% of Ugandan population hence the choice of the crop to be used in the experiment so as to improve its yield and quality.

3.3 Experimental design

A complete randomized design (CRD) with eight treatments and four replications was used. The CRD was preferred because of its flexibility with any number of treatments. A factorial experiment consisting of a three-by-three factor levels was used. These factors were: application methods of mineral fertilizers, application rate of mineral fertilizers, and biochar. Application methods used were foliar and soil coupled with application rates of 1.1% for mineral fertilizers and 10 % volume by volume (1 litre biochar to 9 litres of soil) for soil-biochar mixtures. The treatment details and layout are presented in table 1 and 2 respectively.

Table 1: The experimental treatments.

TNO.	TREATMENT	TREATMENT DETAILS
T1	Control	Finger millet grown in soils without zinc/iron and biochar application.
T2	S + B	Finger millet grown in biochar amended soils without zinc/iron.
T3	S+B+Zn	Finger millet grown in biochar amended soils with soil application of zinc.
T4	S+B+Zn foliar	Finger millet grown in biochar amended soils with foliar zinc application.
T5	S+B+Fe	Finger millet grown in biochar amended soils with soil application of iron.
T6	S+B+Fe Foliar	Finger millet grown in biochar amended soils with foliar application of iron.
T7	S+B+Fe +Zn	Finger millet grown in biochar amended soils with soil application of both Zn and Fe.
T8	S+B+Fe+Zn foliar	Finger millet grown in biochar amended soils with foliar application of both Zn and Fe.

TNO- Treatment number, B – Biochar, S - soil, Fe - iron and Z - zinc

Table 2: The experimental design and layout.

BLOCK 1	BLOCK 2	BLOCK 3	BLOCK 4
S+B+Fe+Zn	S+B	S+B+Zn foliar	Control
S+B+Fe Foliar	S+B+Fe+Zn	S+B+Zn	S+B+Zn foliar
S+B	S+B+Fe	Control	S+B+Fe+ Zn
S+B+Zn	S+B+Fe+Zn foliar	S+B+Fe	S+B+Fe foliar
S+B+Fe	S+B+Zn	S+B	S+B+Fe+Zn foliar
S+B+Fe+Zn foliar	Control	S+B+Fe Foliar	S+B+Fe
Control	S+B+Zn foliar	S+B+Fe +Zn Foliar	S+B+Zn
S+B+Zn foliar	S+B+Fe Foliar	S+B+Fe+Zn	S+B

B-Biochar, Zn -Zinc, Fe-Iron, S-soil

Spacing of 1 m between columns and rows was used. The experiment was conducted in 2022 and 2023. First experiment was conducted from September to December 2022 which was the second rainfall season of the year. The second experiment was conducted from January to May 2023 corresponding to the first rainfall season of the year 2023.

3.3.1 Soil management

The experiment was conducted in pots measuring 25 cm in diameter and 25 cm height, using two soil types collected at a depth of 0-20 cm from common soil types in northern Uganda (Allisols and ferralsols), classified based on the soil map of Uganda. Potted experiment was preferred for easy management and control. The collected soil was air dried, crushed and passed through 5 mm sieve before filling in the pots. 10 litres of ferralsol and allisol soils were filled in each pot. Biochar made from pigeon pea stems using flame curtain technique (Baronti *et al*., 2010) was crushed to ≤5 mm size before mixing with soil. Crushing increases the surface to volume ratio and also enhances the desirable effects on soils. Volume to volume biochar application rate was utilized whereby 1 litre of biochar was added to 9 litres of soil (Adopted from Obia *et al*., 2016). The soil (control – 10 liters of soil) and soil-biochar mixtures (10 liters) were left for a week to allow them get stabilized before planting finger millet after wetting.

3.3.2 Planting

Plump and healthy finger millet seeds were selected and planted using broadcasting method at a depth of less than 5 cm in the pots. Excess millet seedlings were uprooted from each pot leaving 5 plants at the recommended spacing of millet which ranges from 3-5 cm between the plants at the center of the pot. Thinning was done in phases to ensure successful establishment of the plants in each pot before discarding the excess plants. In other cases, backfilling was done whereby finger millet was uprooted and transplanted in pots having similar treatments as one way of ensuring that all pots maintained the 5 plants having the same growth pattern. Watering of the finger millets was done to supplement the prolonged dry spells.

3.3.3 Weeding

First weeding was done at three weeks following the emergence of the finger millets. The second weeding was carried out at six weeks. Hoe weeding was done to remove the weeds around the pots while hand picking was used to remove the weeds inside the pots.

3.3.4 Fertilizer application

Mineral fertilizers were applied at a rate of 1.1% (equivalent to 1.1 mg of $ZnSO_4$ and $FeSO_4$ /kg of soil) solution of Zinc and iron sulphate for foliar application and 1.1 mg/kg of soil for soil application (Adopted from Habibur Rahman *et al.,* 2020). The application of these fertilizers was conducted at 4 weeks after emergence and at flowering stage.

3.3.5 Harvesting

Naromil finger millet takes approximately 110 days to mature. Harvesting was done when the grains were hard but before they dry so much to prevent high losses of the grain through shattering of the finger millet heads. Harvesting was done by cutting off the heads of the finger millet with a knife and were put in separate envelopes having appropriate labels of each treatment used. The harvested finger millet heads were dried using solar drier machines until

constant weight was achieved. The stems were uprooted together with the roots after harvesting. The roots were thoroughly washed and air dried. Later both roots and above ground biomass without heads were oven-dried at 60 °C to determine the biomass on dry weight basis.

3.4 Data collection

3.4.1 Objective one: Plant growth and yield

Total biomass that is below and above ground from each treatment were oven dried and weighed. Grain yield (weight) was determined after solar drying on threshed grains.

3.4.2 Objective two; Finger millet quality parameters

Finger millet grain mineral contents were analyzed using Atomic Absorption spectrophotometer (AAS) on 2 g of ground millet grains from each treatment. The ground samples were ashed followed by digestion using 3 % nitric acid followed by measurement of Fe and Zn contents using AAS. Zinc was analyzed at a wavelength of 213.9 nm and slit size of 0.7 nm while iron was analyzed at a wavelength of 248.9 nm and slit size number 0.2 nm. Crude protein content was determined by estimating the % nitrogen using micro-kjeldahl method (Adopted from AACC, 2009), whereby 1g of millet flour was digested with sulphuric acid in the presence of kjeldhal tablets as a catalyst and N was converted to NH_3 which was distilled and titrated back to N using 0.2 m HCL. Crude protein % was calculated by multiplying N % by a conversion factor of 6.25 as shown below:

$$\%\,Protein = \frac{A - B\ x\ 1.4007x0.2x\ 6.25}{W} x100$$

Where A = Volume (ml) of HCL used for sample titration

B = Volume (ml) of HCL used for blank titration

W = Sample weight (g)

0.2 = Molarity of HCL

1.4007 = atomic weight of nitrogen

6.25 = was used as a conversion factor based on assumption that food proteins contain about 16 % of nitrogen (Mæhre et al., 2018).

3.4.3. Objective three; Soil properties

The two soil types used in the experiment were characterized for basic properties which included, pH, organic carbon and texture. Soil pH was measured using electronic pH meter at a soil to water ratio of 1:2.5, organic carbon using Black and Walkley wet oxidation procedure which quantifies the amount of oxidizable organic matter (Vegard Martinsen *et al*., 2019). Excess of potassium dichromate in the presence of sulphuric acid was used to oxidize organic carbon in the organic matter. The unutilized excess dichromate was back titrated with iron sulphate from where C content was calculated. Soil texture was determined using hydrometer method.

Water holding capacity was determined by the use of core rings of known volume which were labeled according to the corresponding treatments (Mat- *et al*., 1999). These were driven manually into the soil (one directly on top of the other) until one was completely submerged and the second one half way into the soil vertically. A knife was then used to level off the soil in the bottom ring after scooping it out with a shovel. The uppermost part of the soil was covered with a piece of cloth and fastened with a rubber band. The rings with soil were saturated with water in a basin for 24 hours after which they were taken out weighed, recorded and allowed to drain for 48 hours. The drained samples were then weighed and recorded after which they were dried in an oven at 105 °C for 24 hours and weighed again.

Water holding capacity was then calculated from the formula;

$$\text{WHC } \% = \frac{\text{Mass}_{\text{wet}} - \text{Mass}_{\text{dry}}}{\text{Mass}_{\text{dry}}} \text{x}100$$

All analyses were conducted in the labs at the Faculty of Agriculture and Environment and at Multifunctional laboratory of Gulu University.

3.5 Statistical analysis

The effect of biochar on yield, the effect of application methods as well as soil properties on the grain mineral and protein content were determined using a combination of multiple regression models and ANOVA of R software version 4.2.3.

3.6 Ethical considerations

The ethical approval was sought from Gulu University Research Ethics Committee (No. Gurec - 2022-411(Appendix 11). Permission was obtained from Gulu University, Faculty of Agriculture and Environment to conduct experimental studies at its site and conduct laboratory measurements at the faculty and multi-purpose lab. Permission was also obtained from land owners where the soil samples were collected from.

CHAPTER FOUR

RESULTS

4.1 Effects of biochar and micronutrient-based fertilizers on finger millet yield

Significant differences were observed among treatments with respect to grain yield in allisols. Soils with biochar amendments recorded higher grain yield of 42.6 g/pot alongside their biomass yield compared to unamended soil which had lowest yield of 23.8 g/pot (Appendix 2). This presented a significant increase of 79 %. Application of micro-nutrient based fertilizers in addition to biochar had a significant ($P < 0.001$) effect on yield. The treatments which received foliar application of both Fe and Zn in biochar amended soil recorded highest yield of 52.6 g/pot representing an increase of 121 % compared to control pots (Figure 1).

In ferralsols, grain and biomass yield were significantly ($P < 0.05$) influenced by treatments (Appendix 2). Treatments with biochar application recorded higher yield (44.2g/pot) compared to control which had 25.5 g/pot (Figure 1). Treatments which received foliar application of iron and zinc in addition to biochar resulted in a significant yield increase of 99.61%.

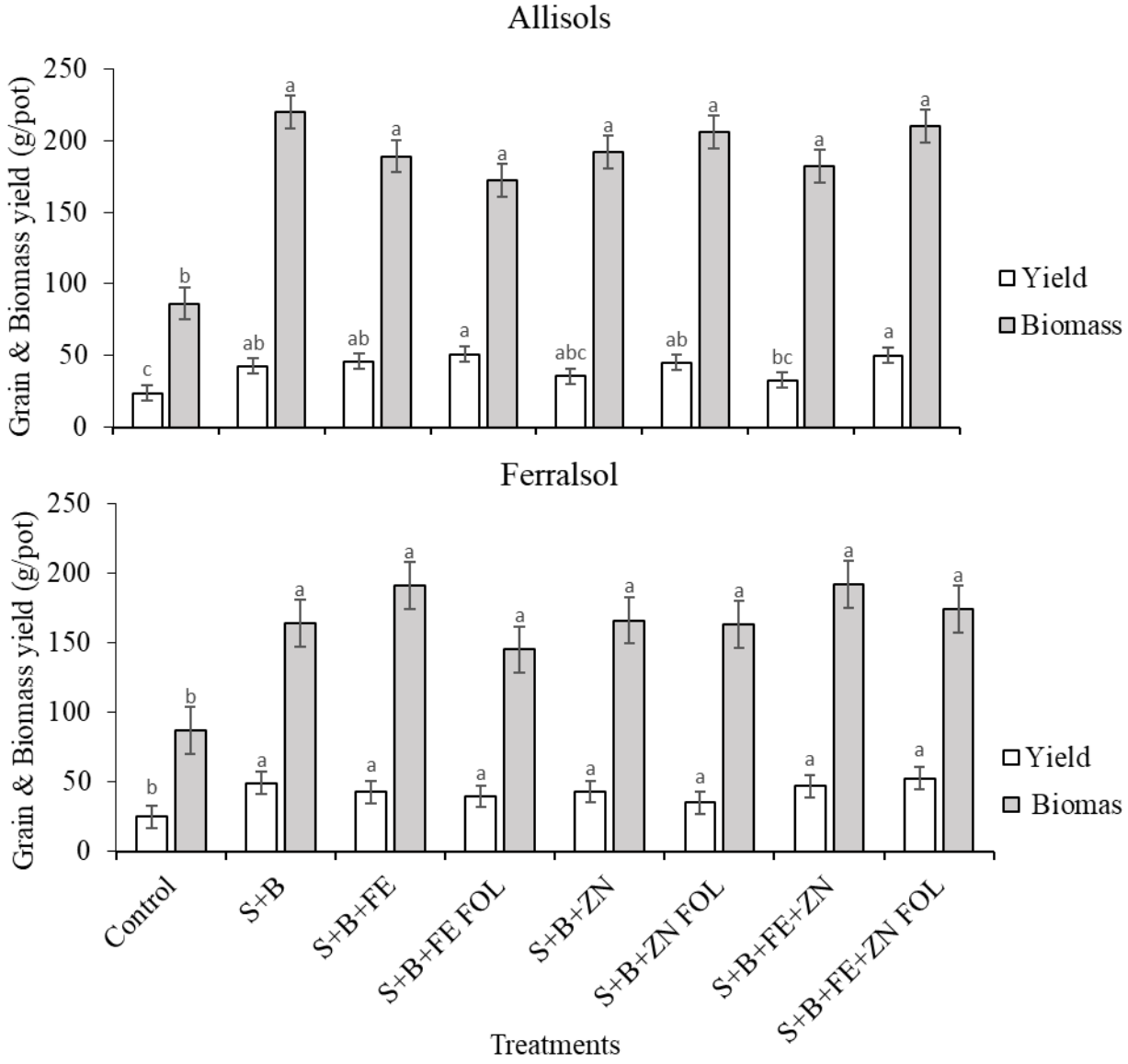

Figure 1: Effect of biochar and micronutrient fertilizers on finger millet grain and biomass yield. S, soil; B, biochar; Fe, iron; Zn, zinc; fol, foliar. D. Error bars represents std error of means. Different letters indicate significant differences (P < 0.05). NB: five plants were maintained per pot before tillering.

4. 2 Effect of soil and foliar application of zinc and iron in biochar amended soils on millet quality

4.2.1 Zinc and iron content in millet grains

Biochar and micro nutrient-based fertilizer application had a significant ($P < 0.05$) effect on zinc and iron content of finger millet grains in both soil types and seasons (appendix 3). In allisols, iron content increased by 6 mg/100g and 4.43 mg/100g in biochar amended soils for season 1 and 2, respectively. Further application of micronutrient-based fertilizers' augmented iron content in finger millet grains by 8.05 mg/100g in the first season and 9 mg/100g for the second season. An increase by 9.1 mg/100g and 14.75 mg/100g in grain zinc content was also registered in season 1 and 2, respectively in biochar treatments over control. Treatments receiving foliar application of zinc in biochar amended soils recorded higher grain zinc content of 41.5 mg/100g and 43.65 mg/100g for season 1 and 2, respectively over the rest of the treatments (Figure 3).

Finger millet grain zinc and iron content was also significantly ($P < 0.05$) influenced by treatments in ferralsols (appendix 3). Treatments which had biochar registered high iron content of 7.6 mg/100g in the first season and 9.7 mg/100g in the second season (Figure 2). A significant increase of 8.13 mg/100g and 9 mg/100g in finger millet grain iron content was also observed in treatments which received foliar application of iron in addition to biochar, in season 1 and 2, respectively. Similar trend increase was also observed in terms of grain zinc content. Biochar treatments recorded higher grain zinc content of 19.25 mg/100g (season 1) and 19.55 mg/100g (season2) over control (Figure 3). Finger millet grain zinc content was further enhanced by 27.6 mg/100g (season 1) & 32.7 mg/100g (season 2) where zinc fertilizers were applied foliarly in biochar amended soils compared to control treatments. Overall, there were significant differences between the soil types and grain mineral content ($P < 0.001$) in both

seasons.

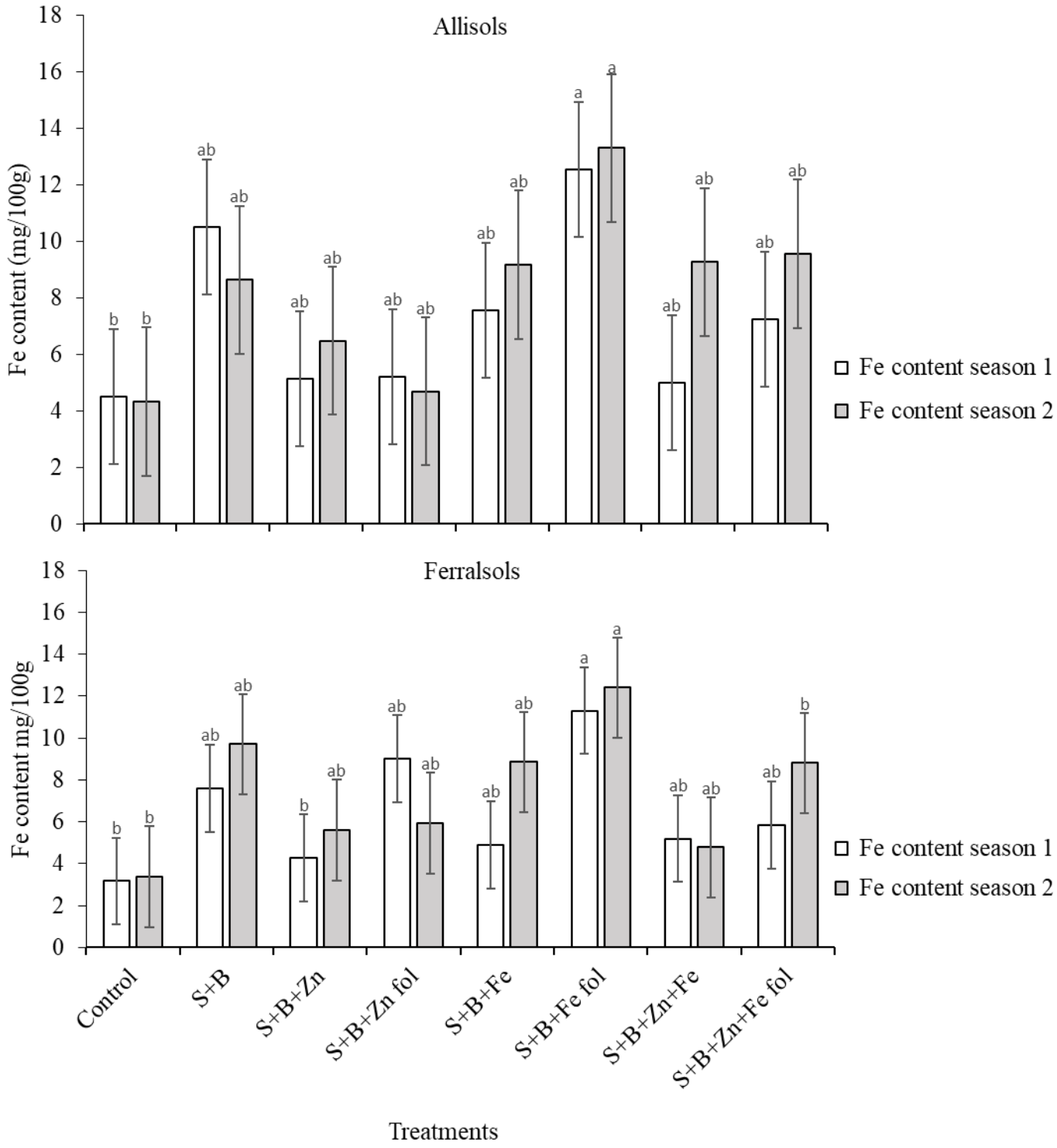

Figure 2: Effect of biochar and micronutrient-based fertilizer on finger millet grain iron content. S, soil; B, biochar; Fe, iron; Zn, zinc; fol; foliar. Error bars represents std error of means. Different letters indicate significant differences (P < 0.05).

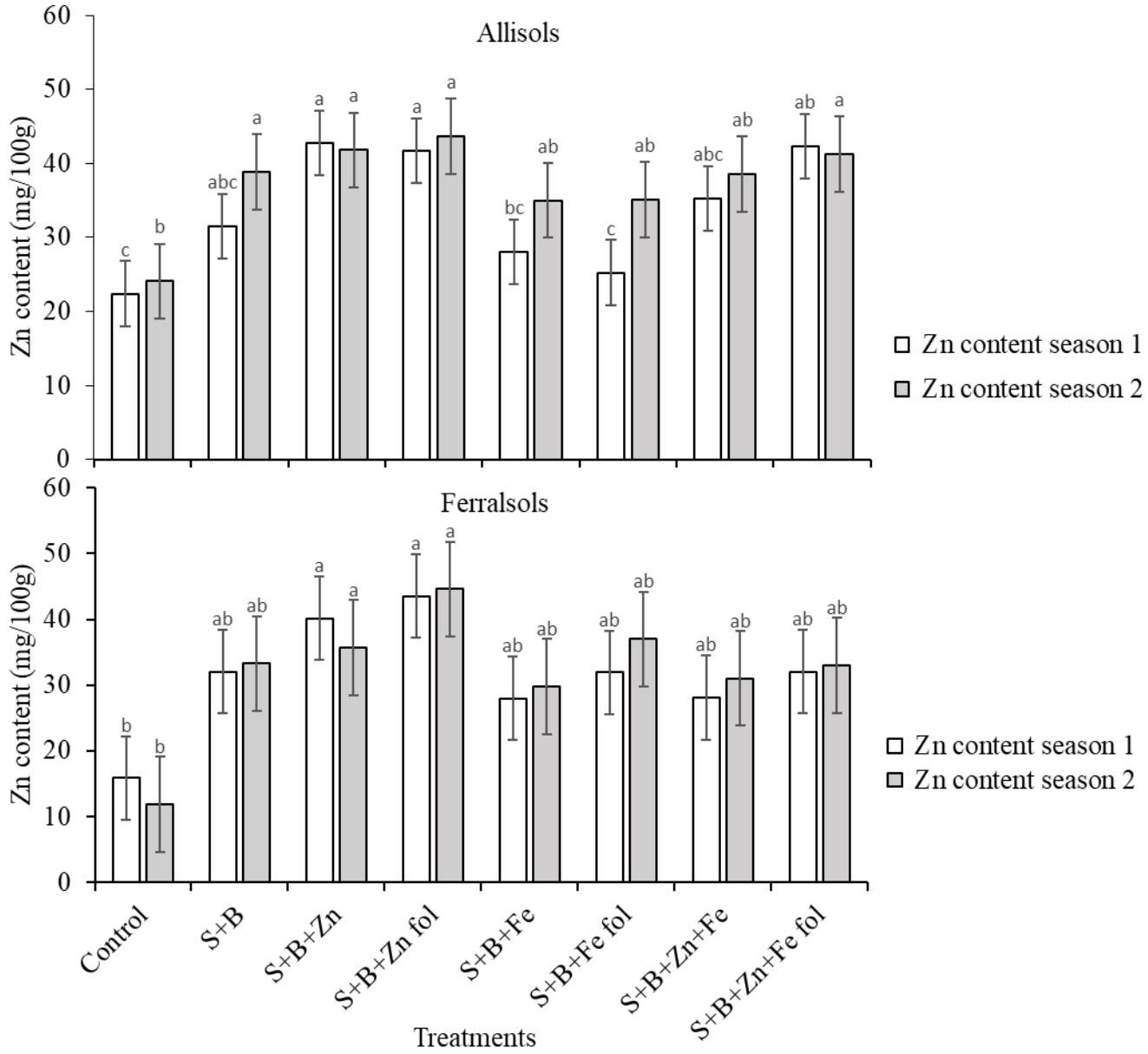

Figure 3: Effect of biochar and micronutrient-based fertilizer application on finger millet grain zinc content. S, soil; B, biochar; Fe, iron; Zn, zinc; fol; foliar. Error bars represent std error of means. Different letters indicate significant differences (P < 0.05).

4.2.2 Crude protein

Significant ($P < 0.05$) differences were observed among treatments in esteem to finger millet protein content (appendix 4) in both soil types in season 1. Treatments which had biochar recorded an increase in protein content by 27.8 % and 11.8 % in allisols and ferralsols, respectively. Further addition of micronutrient fertilizers in biochar amended soil recorded a significant increase in protein content of finger millet grains. Biochar amended soils with foliar application of both iron and zinc registered an upsurge by 109.9 % (allisols) and 64.9 % (ferralsols) over the rest of the treatments. Significant differences ($P < 0.001$) were observed between the soil types*treatments in respect to grain protein content during the first season.

Finger millet grain protein content was significantly ($P < 0.05$) influenced by treatments in the second season in allisols but it was non-significant ($P > 0.05$) in ferralsols (appendix 4). Finger millet grown in biochar amended soils had higher protein content of 9.98 % and 8.59 % in allisol and ferralsols, respectively over control. Foliar application of both iron and zinc in soils that had biochar registered higher grain protein content values of 10.2 % in allisols and 11 % in ferralsols (Figure 4). In general season two recorded an increase in finger millet grain protein content than in the first season. There were no significant ($P = 0.380$) interactions among the soil types *treatments in respect to grain protein content during the second season (appendix 4). Similarly, there were no significant differences ($P=0.333$) in crude protein content between the soil types during the second season.

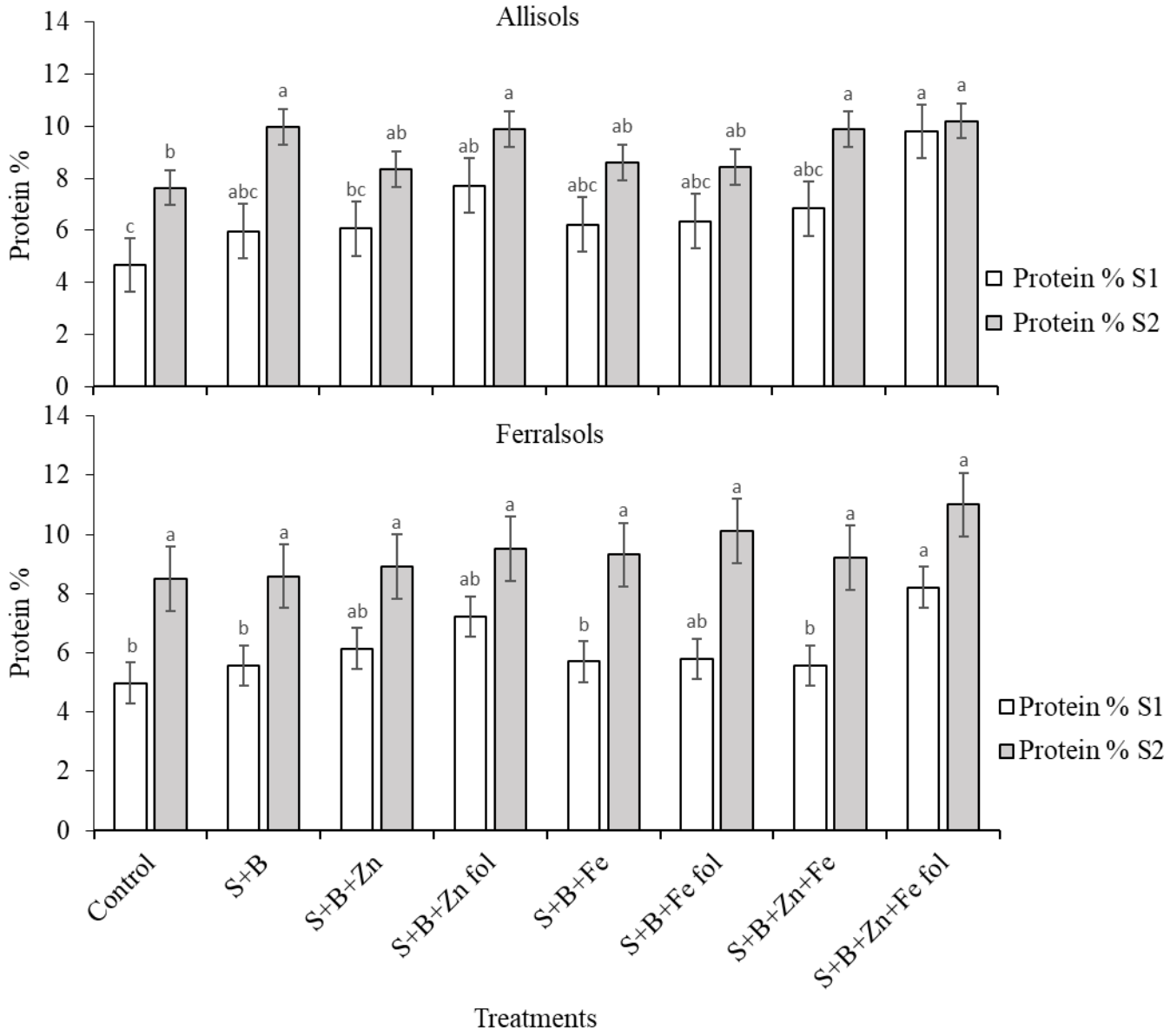

Figure 4: Effects of micronutrient fertilizers and biochar on crude protein content of finger millet grains. S, soil; B, biochar; Fe, iron; Zn, zinc; fol, foliar. Error bars represents std error of means. Different letters indicate significant differences (P < 0.05).

4.3 Soil factors influencing the effectiveness of biofortification of millet grown in biochar amended soil using zinc and iron

4.3.1 Soil pH, organic carbon and water holding capacity

Soil pH was significantly ($P < 0.05$) affected by the treatments in ferralsols but was not significant in allisols (Appendix 5). An increase in soil pH from 5.04 to 5.66 in allisols and 5.03 to 6.18 in ferrosols was registered in biochar treatments representing a change by 12.3 % and 22.86 % respectively. Soil organic carbon was not significantly ($P > 0.05$) affected by the treatments, though an increase by 59.3% and 89.7% was observed in allisols and ferralsols, respectively over control. There was significant increase in water holding capacity in allisols following biochar application while it was non-significant in ferralsols. An increase by 10 % in water holding capacity was registered in treatments that had biochar (Appendix 6 &7). Mineral fertilizers did not have any significant effect on soil pH and organic carbon in both soil types (Appendix 5).

A non-significant relationship was observed between pH and finger millet grain iron and zinc content. As the pH increased from 4.6 to 4.7, grain iron and zinc content increased from 8.51 mg/100g and 21.4 mg/100g, respectively though not significant. Grain iron and zinc content started to decline at the pH beyond 5.8 (Figure 5).

The relationship between grain zinc content and organic carbon was well described by the two parameter Michaelis-Menten equation (Figure 6). The increase in grain zinc content was rapid at low organic carbon of 1 % which reached the modelled half of the maximum (33.61mg/100g) at organic carbon content of 1.41 %. The increase in grain zinc content leveled off at a modelled maximum of 67.22 mg/100g at organic carbon content of 3.5 %.

A linear relationship was observed between organic carbon content and grain iron content whereby an upsurge in organic carbon by 0.5 % resulted in an increase in grain iron content by

2.47 mg/100g. The maximum grain iron content of 15.2 mg/100g was attained at 3 % organic carbon (Figure 6).

A significant linear relationship between water holding capacity and grain zinc and iron content was also mirrored. Grain iron and zinc content increased tremendously following an upsurge in water holding capacity. Maximum zinc and iron content of 26 mg/100g (Fe) and 38.6 mg/100g (Zn) was reached with the water holding capacity of 22 % and 25 %, respectively (Figure 7).

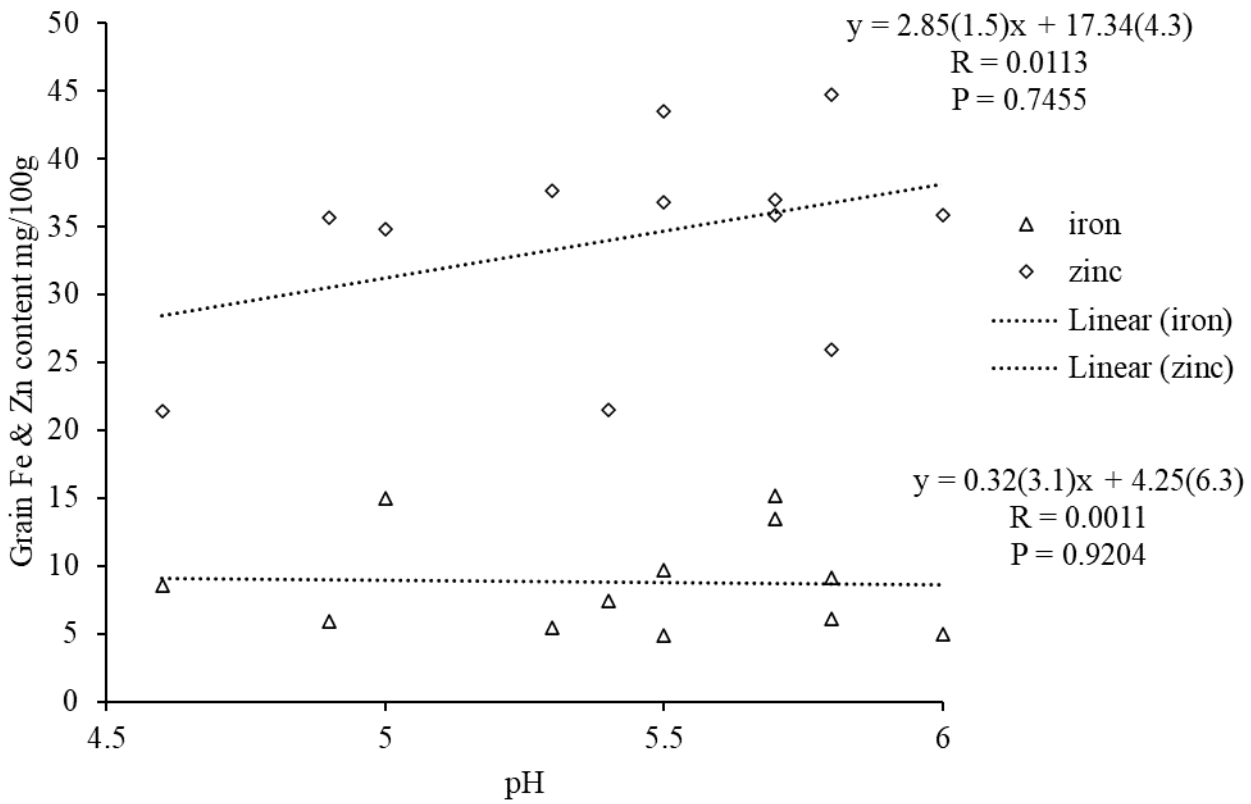

Figure 5: Mean concentration of iron and zinc in finger millet grains with an increase in soil pH. Brackets represent std error.

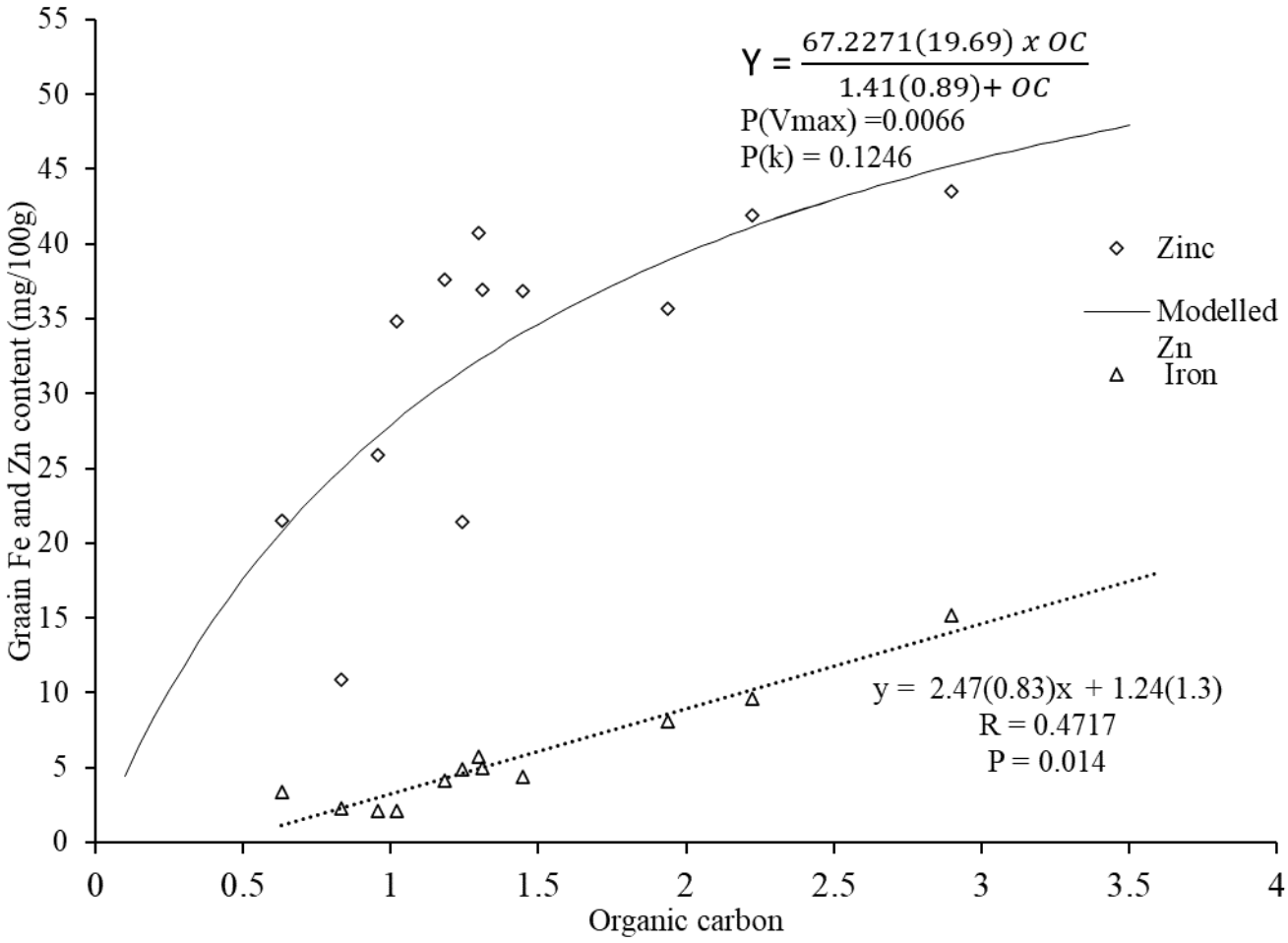

Figure 6: Mean concentration of iron and zinc in finger millet grains with an increase in organic carbon. Brackets represents std error. P(Vmax) and P(K) is the p-value for the maximum accumulation of Zn in the grain and organic carbon content at ½ maximum accumulation of Zn, respectively.

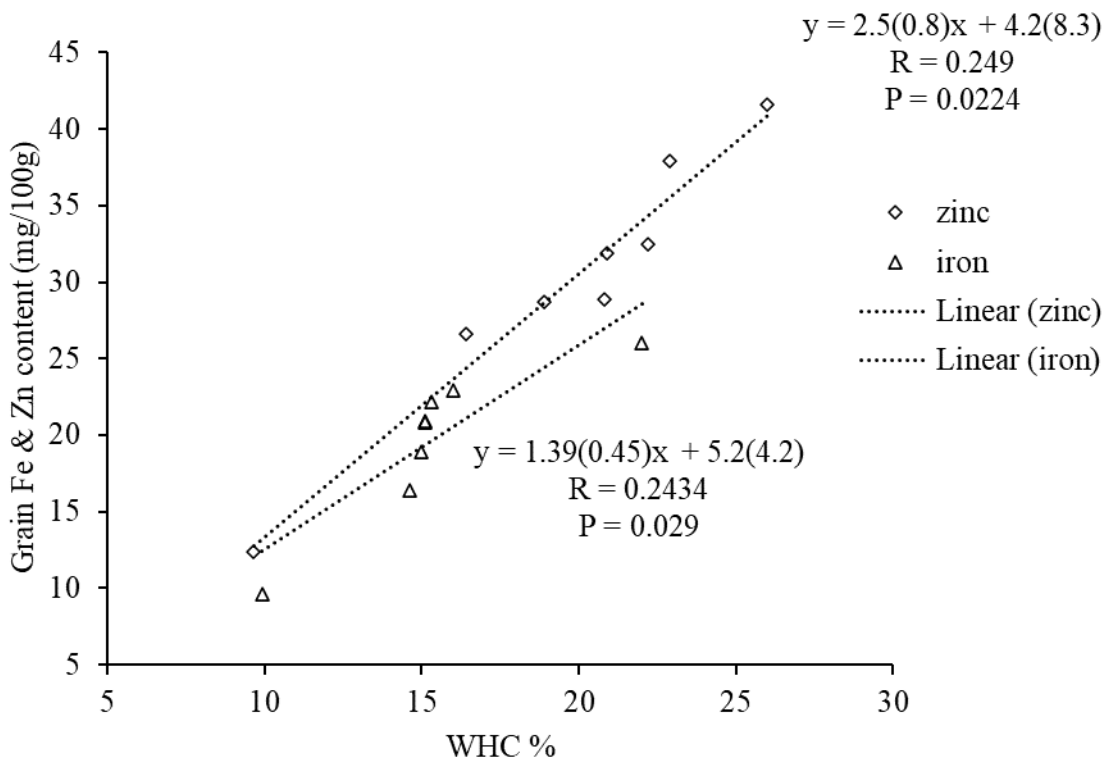

Figure 7: Mean grain iron and zinc content with an increase in water holding capacity.

Brackets represent std error.

CHAPTER FIVE

DISCUSSSIONS

5.1 The effect of biochar and micro nutrient-based fertilizers on finger millet yield

Biochar application resulted in a significant increase in the finger millet yield in both soil types. This could be attributed to biochar's ability to improve physical and chemical soil conditions such as soil water holding capacity and nutrient retention. Additionally, biochar creates a favorable environment for the growth of beneficial soil microorganisms that substantially contribute to nutrient recycling and subsequent increase in crop yield. These results are in agreement with Borchard *et al.* (2019) who also observed an increase in maize yields coupled with an increase in N and Ca in the maize leaves after biochar application at a rate of 15 g/kg of soil. These results were also supported by the findings of Hossain *et al.* (2010) who reported an increase in yield of cherry and tomato by 64 % where 10 t /ha of waste water sludge biochar was applied to chromosol soil due to enhanced availability of nitrogen and phosphorous. Similarly, Taheri *et al.* (2023) reported an increase in millet yield by 58% in biochar amended saline-sodic and calcareous soil as compared to control due to improved moisture content.

Across the soil types a higher yield of 52.6 g was recorded in treatments which were sprayed with iron and zinc in biochar amended soils which could be extrapolated to a yield of 7 t/ha. Iron and zinc play a significant role in chlorophyll formation, starch metabolism and enzyme carbonic anhydrase which accelerates carbohydrate formation in C4 plants (Kryvoruchko, 2017). Results from this research are in consistence with the previous investigators such as Vijayakumar *et al.* (2020) who also recorded an increase in yield from 1613 kg/ha to 2258 kg/ha when sprayed with $ZnSO_4$ (0.5%) +$FeSO_4$(1%) +citric acid (0.1) in NPK treatments twice at vegetative and flowering stage in sandy loam soils. Narimani *et al.* (2010) also reported an increase in wheat yield from 4353 kg/ha to 4640 kg/ha (6.6%) due to balanced fertilization

through foliar application of micro nutrients (iron, zinc & copper). A significant increase was also recorded by Zeidan *et al.* (2010) in all grain attributes when Zn and Fe were sprayed at tillering and booting stage in wheat grown in sandy soils. Foliar spray enhances rapid absorption of zinc and iron through leaves thus aiding in increased cell division, elongation, chlorophyll content and photosynthesis. This method also augments translocation of photosynthates from source to sink. Thus, these results confirmed the hypothesis of the study that application of biochar and micronutrient-based fertilizers would increase finger millet growth and yield.

5.2 Effect of soil and foliar application of zinc and iron in biochar amended soils on finger millet grain quality Parameters

5.2.1 Zinc and iron content

Soil amendment with biochar significantly influenced millet grain iron and zinc content from both soil types and seasons. This could be attributed to increased availability of nitrogen in the soil ascribed to biochar's application which might have increased the abundance of zinc and iron transporters which are required for root uptake and phloem loading of zinc and iron (Mohamed et al., 2017). Additionally, improved soil pH could have also played a role as its slight increase was within the optimum range for enhanced availability of iron and zinc.

Foliar application of Fe and Zn in biochar amended soils increased iron and zinc content of millet grain from both soil types compared to soil application. These results concur with those reported by Saleem *et al.* (2016) who recorded a maximum increase by 55 % and 51.8 % of Zn and Fe, respectively, in treatments which received foliar application rates of 0.1% $ZnSO_4$ & $FeSO_4$ in RDF of NPK compared to soil application. These results are also in line with those reported by Dodake *et al.* (2022) where they recorded high Fe content of 15.08 mg/100g and Ca content of 3.37 mg/100g where $FeSO_4$ was applied at 1 % along with RDF of NPK +basic slag. Anwar *et al.* (2022) also reported an increase by 34 mg/kg and 0.3 mg/kg in maize grain zinc and iron respectively following foliar application rates of 0.1 % $ZnSO_4$ and 0.3 % $FeSO_4$. Many researchers have reported an increase in nutrient accumulation when the fertilizers are foliarly applied due to enhanced absorption of nutrients and other positively charged particles when sprayed onto the leaves. The pores within leaf cuticles are lined up with negatively charged molecules and when the cations are sprayed, it results in increased nutrient uptake (Reddy *et al.*, 2018).

Increased iron and zinc concentration in finger millet grains from this study could be attributed to enhanced absorption of nutrients due to foliar spray but also role of biochar in increasing nitrogen availability. Improved nitrogen availability affects Zn or Fe chelating nitrogenous compounds which are necessary in transporting Zinc and iron (Cakmak, 2010). Additionally, finger millet utilizes a combination of strategies in iron and zinc homeostasis pathways which plays a significant role in acquisition of iron and zinc from the soil (Chandra *et al*., 2020). Overall, there were no significant ($P > 0.05$) differences between soil and foliar application of the mineral fertilizers in biochar amended soils with respect to grain iron and zinc content. This could be associated with improved nitrogen content in the soil emanating from biochar's application, which enhances the availability of zinc and iron transporters. Nevertheless, foliar application registered high grain iron and zinc content in both soil types. These results confirmed the hypothesis of the study that soil and foliar application of micronutrient-based fertilizers in biochar amended soils would increase finger millet grain quality.

5.2.1 Protein content

Soil amendment with biochar enhanced protein content in both soil types and seasons. Improved water holding capacity influenced by biochar application could have played a key role in enhanced moisture content which is essential for nutrient transportation in plants. Results from this study are similar to those of Khan *et al.* (2022) who reported an increase in wheat protein content by 4 %, 6% and 7%, following the application rates of 10, 20 and 30 t BC /ha , respectively in calcareous soil over control. Alli *et al.* (2015) also registered a 20 % increase in wheat grain protein content with an application rate of 25 t BC /ha in saline sodic soil. These increases were attributed to biochar's influence in improving soil nutrient availability and its efficiency in adsorption of NH_4^+ and NO_3^- which repressed their leaching from the soil ensuing in improved absorption by the plants (Hong & Sullivan, 2013). Additionally, Major *et al.* (2010) observed an increase in N uptake in maize at an application

rate of 20 t BC/ha due to adsorption effect. Abukari *et al.* (2018) explained that the binding nature of biochar reduces the N losses. In addition, biochar application augments the availability of urease enzyme which plays a key role in nitrogen assimilation by providing a favorable environment for the growth of soil microorganism followed by enhancement in the concentration of ammonium and nitrate in the solution (Taheri *et al.*, 2023), thus increasing nitrogen concentration in the soil and subsequent uptake and accumulation in grains.

Further, addition of micronutrient-based fertilizers enhanced the protein content in both soil types. Significant increase in protein content were observed in treatments which received foliar application of both iron and zinc over soil application in both soil types and seasons. This could be associated with the role of zinc and iron in activating enzymes which are responsible for protein synthesis and biochar's influence on water holding capacity, thus ensuring in enhanced soil moisture content. Zinc is a key structural component of RNA polymerase, participant in protein synthesis. The results from this study are also in support with the findings of Mohamed *et al.* (2017), who observed an increase in protein content in the alluvial soils amended with 80g of biochar per pot in the presence of micronutrients.

These results were also in agreement with the findings of Kumar (2021) who reported an increase in protein content by 7.5% and 11.25%, respectively following foliar application rates of 0.2% $ZnSO_4$ and 0.5 % $FeSO_4$ in sandy loam soils. Ali *et al.* (2021) also reported an increase in protein content of wheat seeds following an increase in foliar application rate of iron. This was associated with the role of iron in formation of chlorophyll, photosynthesis systems and respiration of plants.

Protein content was significantly affected by the season discrepancies (Figure 4). During the first experimental season, grain filling coincided with onset of dry spell thus a subsequent decrease in available moisture. During the second experimental season, grain filling was at par

with the peak of rains which could have contributed to increased protein content as moisture plays a key role in nutrient movement through diffusion.

Overall, the improvement in yields and grain quality parameters were not statistically different between the soil types.

5.3 Soil factors influencing the effectiveness of biofortification of finger millet grown in biochar amended soil

Soil amendment with biochar improved soil pH in both soil types as compared to control pots. This could be attributed to the biochar's alkaline nature and high pH buffering capacity that can ameliorate soil acidity (Dai *et al.*, 2017). Similar results were also reported by Yuan *et al.* (2011) who registered an increase in soil pH by a range of 0.49 - 0.68 in acidic soils following straw biochar application from mung bean, peanut and faba bean over control applied at the rates of 10 and 20 g/kg of soil. Alkalinity of legume biochar was considered as key factor in influencing their liming potential on acidic soils.

Regression results revealed a non-significant positive relationship between soil pH and finger millet grain content of iron and zinc. Nevertheless, biochar application in this study helped in improving grain mineral content. This is likely due to biochar's influence in increasing the activities of microorganisms, especially mycorrhizal fungi while altering the pH and in the process enabling the accessibility of zinc, iron and phosphorous (Thingujam *et al.*, 2019). These results contradict those reported by Vahedi *et al.* (2022) where they observed a significant decrease in soil zinc with an increase in pH. Higher pH augments zinc adsorption capacity emanating from increased pH dependent negative charges, thus reducing its accessibility in the soil and its subsequent uptake and accumulation in the grains. As observed from this study, it is evident that the grain iron and zinc started to reduce as the pH was getting to 6. However, the slight increase in pH observed from this study was still vital to improving

grain mineral content as the changes were within the optimal range for enhanced availability of iron and zinc.

An increase in soil organic carbon was registered following the soil amendment with biochar in both soil types. This could be ascribed to biochar's stable and recalcitrant structure which provide a protective shield to organic carbon from mineralization, thus resulting in an increase in SOC. The results from this study are in agreement with other researchers such as Ali *et al.* (2021) who also reported an increase in SOC from 8.5 to 8.89 in biochar treatments of saline sodic soil at four application rates: 5, 10, 15 and 20 t BC /ha. Han *et al*. (2016) registered an increase in SOC by 2.67 g/kg, 5.34 g/kg and 10.67 g/kg following biochar application rates of: 4 g/kg, 8 g/kg and 16 g/kg of soil, respectively. These results were also consistent with those reported by Zheng *et al.* (2022) who observed an increase in SOC by 11.02 - 22.13% after applying 6 & 12 t BC /ha, respectively in alluivic primisol soil. This was attributed to the porous nature of biochar which provides a conducive environment for the beneficial soil microorganisms, thus fostering their activities and contributing to the formation of stable soil aggregates (Vetter *et al.*, 2022). These aggregates provide a shield to SOC thereby obstructing its decomposition and enabling its long-term sequestration (Weng et al., 2022). Additionally, soil amendments with biochar improves nutrient availability (N, P, K) which enhances crop growth and productivity. This enhances the input of organic carbon into the soil through root exudates, plant tissue, and rhizo deposition thus resulting in increased accumulation of SOC over time (Pei *et al.*, 2020).

The data revealed that soil organic carbon had a significant influence on grain iron and zinc concentration (Figure 6). This could be ascribed to the role of iron and zinc in formation of soluble complexes with soil organic matter component hence increasing its concentration in the soil solution, enabling its enhanced uptake by plants and subsequent concentration in the

grains (Thingujam *et al.,* 2019). Additionally, grain iron and zinc content was augmented due to improved soil solution concentration of most mineral nutrients necessary for the plant growth and development influenced by soil organic carbon (Gerke, 2022).

Soil amendment with biochar had a significant influence on water holding capacity of the soil. This could be accredited to enhanced pore spaces emanating from biochar's porous nature. This agrees with the findings of Yu *et al.* (2013) who reported a twofold increase in water holding capacity in biochar amended loamy sand at an application rate of 9%.

Grain iron and zinc content were significantly and positively related with water holding capacity of the soils. The amount of water returned in the soil influences soils moisture which is one of the key factors that help in transportation of nutrients through diffusion. Insufficient moisture content result in reduced nutrient transportation and subsequent decline in grain nutrient concentration. Improved grain zinc and iron content from this study could be attributed to biochar's influence in improving water holding capacity of the soils resulting in augmented soil moisture and subsequent transportation of the nutrients, hence the upsurge in concentration of iron and zinc in finger millet grains. These results concurs with Zhao *et al.(* 2009) who reported an increase in the following mineral content of wheat: P, K, Ca, Zn and Mg with increased soil water conditions from 45% to 85 %. The increase in iron and zinc content of finger millet grains is in line with the hypothesis of this study that improved soil properties would enhance grain mineral content due to improved moisture content and soil chemical properties.

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study demonstrated that application of biochar and micro nutrient-based fertilizers could be a viable strategy to enhancing yield, grain iron, zinc and protein content of finger millet. Both foliar and soil application in biochar amended soils are ideal in improving the aforementioned parameters. This could be a promising approach towards the drive to building communities which are food and nutrition secured.

6.2 RECOMMENDATIONS

6.2.1 Recommendation for practice

Iron and zinc to be applied when the soils have enough moisture content for enhanced uptake and subsequent accumulation in the grains.

6.2.2 Recommendation for policy

There is need for inclusion of use of biochar in extension hand books to increase its wide adoption across the farming communities.

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Appendices

Appendix 1: Characteristics of the soils used in the experiment.

(a) Allisols

pH	OM	sand	clay	silt	Textural class
	%	%	%	%	
5.7	0.6	63.2	14	22.8	sandy loam

(b) Ferralsols

PH	OM	sand	clay	silt	Textural class
	%	%	%	%	
5.6	1	38	38	24	Clay loam

Appendix 2: Effect of biochar and micronutrient based fertilizers on grain and biomass yield of finger millet in Allisols and ferralsols.

a) Allisols

Soil type	Treatments	mean grain yield (g/pot) season 2	mean biomass yield (g/pot) season2
Allisols	Control	23.8[c]	86.3[b]
	S+B	42.6[ab]	220[a]
	S+B+Zn	45.9[ab]	189[a]
	S+B+Zn fol	51[a]	172[a]
	S+B+Fe	35.7[abc]	192[a]
	S+B+Fe Fol	45.1[ab]	206[a]
	S+B+Zn Fe	32.8[ab]	186[a]
	S+B+Zn+Fe Fol	50.1[a]	210[a]
P value		P < 0.001	P = 9.026e-09

Different letters indicate significance differences at P < 0.05

a) Ferralsols

Soil type	Treatments	mean grain yield (g/pot)	mean biomass yield (g/pot)
Ferralsols	Control	25.2[b]	87[b]
	S+B	49.1[a]	164[a]
	S+B+Zn	42.7[a]	191[a]
	S+B+Zn fol	39.6[a]	145[a]
	S+B+Fe	43[a]	166[a]
	S+B+Fe Fol	35.2[ab]	163[a]
	S+B+Zn+Fe	47.2[ab]	192[a]
	S+B+Zn+Fe Fol	52.6[a]	174[a]
P value		P < 0.001	P = 0.0129

NB: Yield for the first season was not captured as the experiment was invaded by cows

Appendix 3: Effect of soil and foliar application of iron in biochar amended soil on finger millet grain iron content.

a) Allisols

Soil type	Treatments	mean iron content Mg/100g season 1	mean iron content (mg/100g) season 2
Allisols	Control	4.5^{b}	4.33^{b}
	S+B	10.5^{ab}	8.64^{ab}
	S+B+Zn	5.13^{a}	6.48^{ab}
	S+B+Zn fol	5.2^{a}	4.69^{ab}
	S+B+Fe	7.55^{ab}	9.17^{ab}
	S+B+Fe Fol	12.55^{ab}	13.3^{ab}
	S+B+Zn+Fe	5^{ab}	9.27^{ab}
	S+B+Zn+Fe Fol	7.25^{ab}	9.56^{ab}
P value		P = 0.0209	P = 0.042

b) Ferralsols

Soil type	Treatments	mean iron content (mg/100g) season 1	mean iron content (mg/100g) season 2
Ferralsols	Control	3.175^{b}	3.38^{b}
	S+B	7.6^{ab}	9.7^{ab}
	S+B+Zn	4.27^{b}	5.6^{ab}
	S+B+Zn fol	9^{ab}	5.93^{ab}
	S+B+Fe	4.89^{ab}	8.85^{ab}
	S+B+Fe Fol	11.3^{a}	12.4^{ab}
	S+B+Zn+Fe	5.2^{ab}	4.78^{ab}
	S+B+Zn+Fe Fol	5.85^{ab}	8.8^{ab}
P value		P = 0.01	P = 0.017

Different letters indicate significance differences at $P < 0.05$

Appendix 4: Effect of soil and foliar application of zinc in biochar amended soils on grain zinc content of finger millet.

a) Allisols

Soil type	Treatments	mean zinc content (mg/100g) season 1	mean zinc content (mg/100g) season 2
Allisols	Control	22.4^{c}	24.1^{b}
	S+B	31.5^{abc}	38.85^{a}
	S+B+Zn	42.7^{a}	41.8^{a}
	S+B+Zn fol	41.65^{a}	43.65^{a}
	S+B+Fe	28.1^{bc}	35^{ab}
	S+B+Fe Fol	25.25^{c}	35.1^{ab}
	S+B+Zn+Fe	35.25^{abc}	38.55^{ab}
	S+B+Zn+Fe Fol	42.35^{ab}	41.25^{a}
P value		P = 9.362e-05	P = 0.04

b) Ferralsols

Soil type	Treatments	zinc content (mg/100g) season 1	zinc content (mg/100g) season 2
Ferralsols	Control	15.9^{b}	11.9^{b}
	S+B	32^{ab}	33.3^{ab}
	S+B+Zn	40.1^{a}	35.7^{a}
	S+B+Zn fol	43.5^{a}	44.6^{a}
	S+B+Fe	28^{ab}	29.8^{ab}
	S+B+Fe Fol	31.9^{ab}	37^{ab}
	S+B+Zn+Fe	28.1^{ab}	31^{ab}
	S+B+Zn+Fe Fol	32^{ab}	33^{ab}
P value		P = 0.011	P = 0.015

Different letters indicate significance differences at $P < 0.05$

Appendix 5: Effect of soil and foliar application of mineral based fertilizers in biochar amended soils on crude protein content of finger millet.

a) Allisols

Soil type	Treatments	mean crude protein % season 1	mean crude protein % season 2
Allisols	Control	4.67^{c}	7.6^{ab}
	S+B	5.97^{abc}	9.98^{a}
	S+B+Zn	6.07^{bc}	8.35^{ab}
	S+B+Zn fol	7.72^{ab}	9.89^{a}
	S+B+Fe	6.22^{ab}	8.61^{ab}
	S+B+Fe Fol	6.35^{abc}	8.44^{ab}
	S+B+Zn+Fe	6.83^{abc}	9.88^{a}
	S+B+Zn+Fe Fol	9.8^{a}	10.2^{b}
P value		P = 0.0034	P = 0.0076

b) Ferralsols

Soil type	Treatments	mean crude protein % season 1	mean crude protein % season 2
Ferralsols	Control	4.98^{b}	8.5^{a}
	S+B	5.57^{b}	8.59^{a}
	S+B+Zn	6.14^{ab}	8.92^{a}
	S+B+Zn fol	7.22^{ab}	9.52^{a}
	S+B+Fe	5.7^{ab}	9.31^{a}
	S+B+Fe Fol	5.79^{ab}	10.1^{a}
	S+B+Zn+Fe	5.56^{b}	9.2^{a}
	S+B+Zn+Fe Fol	8.21^{a}	11^{a}
P value		P = 0.0044	P = 0.22

Different letters indicate significance differences at $P < 0.05$, while same letters indicate non-significance, $P > 0.05$

Appendix 6: Effect of biochar and mineral based fertilizers on soil organic carbon and pH.

a) Allisols

Soil type	Treatments	pH	organic carbon
Allisols	Control	5.29[a]	0.387[a]
	S+B	5.66[a]	1.59[a]
	S+B+Zn	5.48[a]	1.437[a]
	S+B+Zn fol	5.38[a]	0.402[a]
	S+B+Fe	5.11[a]	0.442[a]
	S+B+Fe Fol	5.33[a]	0.371[a]
	S+B+Zn+Fe	5.04[a]	0.413[a]
	S+B+Zn+Fe Fol	5.48[a]	0.59[a]
P value		P = 0.3035	P = 0.169

b) Ferralsols

Soil type	Treatments	pH	organic carbon
Ferralsols	Control	5.59[a]	0.883[a]
	S+B	6.18[a]	1.45[a]
	S+B+Zn	5.47[ab]	0.886[a]
	S+B+Zn fol	5.54[ab]	0.738[a]
	S+B+Fe	5.03[b]	1.13[a]
	S+B+Fe Fol	5.68[ab]	1[a]
	S+B+Zn+Fe	5.6[ab]	0.982[a]
	S+B+Zn+Fe Fol	5.67[ab]	1.4a
P value		P = 0.0025	P = 0.163

Different letters indicate significance differences at $P < 0.05$ while same letters indicate non-significance, $P > 0.05$

Appendix 7: Effect of biochar application on water holding capacity.

a) Allisols

Soil type	Treatment	water holding capacity
Allisols	Control	5.93[b]
	S+B	11.5[a]
	S+B+Zn	10.1[a]
	S+B+Zn fol	11.3[a]
	S+B+Fe	10.4[a]
	S+B+Fe fol	10.8[a]
	S+B+Fe+ Zn	10.1[a]
	S+B+Fe+ Zn fol	12.2[a]

Different letters indicate significance differences at $P < 0.05$, while same letters indicate non-significance, $P > 0.05$.

b) Ferralsols

Soil type	Treatment	water holding capacity
Ferralsols	Control	13.7[a]
	S+B	15.1[a]
	S+B+Zn	22.0[a]
	S+B+Zn fol	15.0[a]
	S+B+Fe	14.6[a]
	S+B+Fe fol	16.0[a]
	S+B+Fe+ Zn	15.1[a]

S+B+Fe+ Zn fol	15.3[a]

Same letters indicate non-significance, P > 0.05.

Appendix 8: Application of iron and zinc to finger millets.

Appendix 9: Preparation of finger millet ash for mineral analysis.

Appendix 10: Mineral analysis using AAS.

Appendix 11: GUREC approval

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RESEARCH ETHICS COMMITTEE

17/02/2023

To: Alinafe Manjawira

Gulu University
0726455717

Type: Initial Review

Re: GUREC-2022-411: Biofortification of finger millet grown in biochar amended soils using zinc and iron, Uganda, Gulu, 2.0, 2023-02-09

I am pleased to inform you that at the **94th** convened meeting on **17/11/2022**, the Gulu University REC, committee meeting, etc voted to approve the above referenced application.
Approval of the research is for the period of **17/02/2023** to **17/02/2024.**

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **17/02/2024** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by Gulu University REC:

GULU UNIVERSITY
RESEARCH ETHICS COMMITTEE
APPROVED UNTIL
★ 17 FEB 2024 ★
FACULTY OF MEDICINE

No.	Document Title	Language	Version Number	Version Date
1	Data collection tools	English	2.0	2023-02-09
2	Informed Consent Waiver	English	2.0	2023-02-09
3	COVID-19 & EBOLA risk management plan for the study	English	2.0	2023-02-09
4	Protocol	English	2.0	2023-02-09

Yours Sincerely

Julaina A. Obika

Julaina A. Obika (PhD)
For: Gulu University REC

GULU UNIVERSITY
RESEARCH ETHICS COMMITTEE
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Appendix 12: Plagiarism certificate

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Department:____ FOOD SCIENCE AND POST HARVEST TECHNOLOGY _____

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