

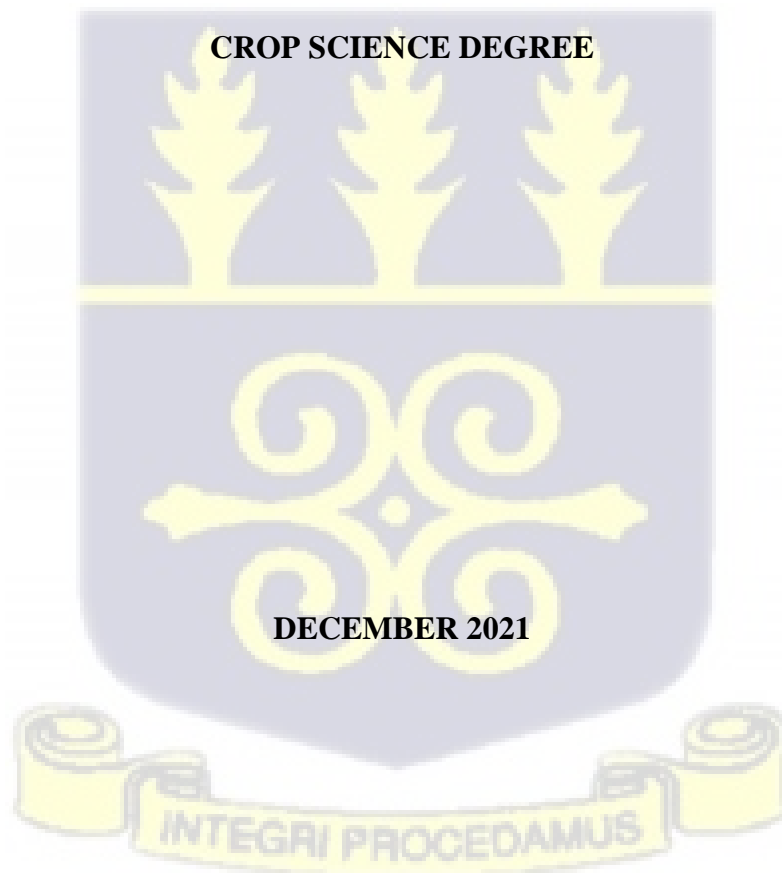
**RESPONSE OF RICE TO NITROGEN SOURCE AND ZINC FERTILIZATION IN
AN IRRIGATED ECOLOGY IN GHANA.**

BY

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10536996

**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF
MPHIL**



DECLARATION

I, Baidu Elijah declare that this work was carried out by me and has never been presented in whole or part anywhere for another degree. All assistances have been duly acknowledged. This work was summited as a thesis for the degree in MPhil Crop Science (Agronomy).

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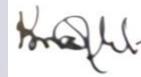


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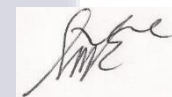


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DEDICATION

I dedicate this to the Lord Almighty and my parents, Mr. & Mrs. Baidu for the moral and financial support they gave to me throughout the past two years in the University.



ACKNOWLEDGEMENT

I would like to express gratitude to Prof. Ofosu-Budu and Dr. Stephen Narh who supervised this work, for their dedication and technical skills they offered to me throughout this work.

I also express my profound gratitude to Mr. Kwabena Adjei of the Soil and Irrigation Research Centre, Kpong and other individuals who contributed in diverse ways to make this work successful.



ABSTRACT

Nutrient management is important to achieve optimum growth and yield of rice. Nitrogen and zinc deficiencies are widespread in most rice cultivating soils and is very important to address these deficiencies to promote high paddy yield. Nitrogen and zinc fertilizers are used to address these deficiencies in rice growth and yield, and the nitrogen source is very important to achieving yield targets. The source of nitrogen fertilizer may influence its zinc uptake due to its unique chemical changes on the soil. Pot and field experiments were conducted at the University of Ghana Soil and Irrigation Research Centre, Kpong (SIREC) under irrigated conditions to examine the effect of nitrogen sources and zinc application rate on the growth, yield and grain zinc content of rice. For the pot experiment a 2 x 6 factorial experiment was laid out in a completely randomised design, with 3 replicates. Two N sources, area and sulphate of ammonia (SoA) at 120 kgN/ha served as the main plots and Zinc rates at, 0 kg ha⁻¹, 5 kg ha⁻¹, 10 kg ha⁻¹, 15 kg ha⁻¹, 20 kg ha⁻¹, 25 kg ha⁻¹, served as subplots with three replicates. The pots were flooded to represent irrigated conditions. Results from the pot experiment showed that interaction between the rate of zinc application and N source did not influence growth and yield parameters of rice. Secondly SoA was a better N fertilizer source than urea in promoting growth and grain yield. SoA application produced higher number of tillers, productive tillers, above ground biomass and percentage filled grains than urea. The grain yield induced by SoA was 13 % higher than urea. Growth and yield components like above ground biomass, plant height, tiller number at maturity, effective tillers, panicle length, 100-grain weight and grain yield did not respond to Zinc application. For the field experiment, it was a randomised complete block design, laid out in a split plot design with 3 replicated. it was made of 4 sources of N as main plot and 4 Zinc rates as subplots. The level of N sources included 100 % N from chemical

fertilizer (CF₁₀₀), 75 % N from chemical fertilizer + 25 % N from Poultry manure (CF₇₅PM₂₅), 50 % N from chemical fertilizer + 50 % N from poultry manure (CF₅₀PM₅₀) and a control, No nitrogen application (No). The chemical fertilizer was applied in the form of urea. The levels of zinc were 0 kg Zn/ha.(Zn₀), 5 kg Zn/ha (Zn₅), 10 kg Zn/ha(Zn₁₀) and 15 kg Zn/ha(Zn₁₅). Results obtained from the pot experiment showed there was a significant interaction between N source and zinc rate for number of tillers/m² and number of panicles/m². Besides those two parameters interaction between N source and zinc rate did not show any effect on the other growth parameters, grain yield and zinc content in the rice. The relative contribution of N from urea and PM influenced the growth and yield of rice. The grain yield of CF₇₅ PM₂₅ treatment that replaced 25% of the recommended rate of inorganic fertilizers (urea) with PM was at par with the grain yield of urea 100% (CF₁₀₀). However, (CF₁₀₀) urea, performed better than (CF₅₀ PM₅₀), when the urea and PM contributed equal parts of N in a ratio of 50: 50. The CF₇₅ PM₂₅ treatment produced the highest grain yield and was not statistically different from Urea 100% (CF₁₀₀). The results showed that grain yield and number of productive tillers of rice responded to zinc application. Zinc application at 5 kg Zn /ha recorded the highest grain yield. Zinc fertilizer application did not influence zinc concentration in the grains. Application of 5 kg Zn/ha increased zinc concentration in the straw by 13%. Increasing the rate of application (>5kg Zn/ha) did not result in any further increase in the zinc concentration in the straw.



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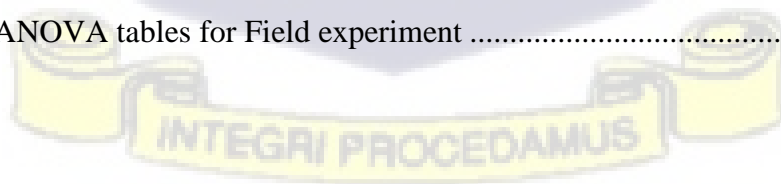
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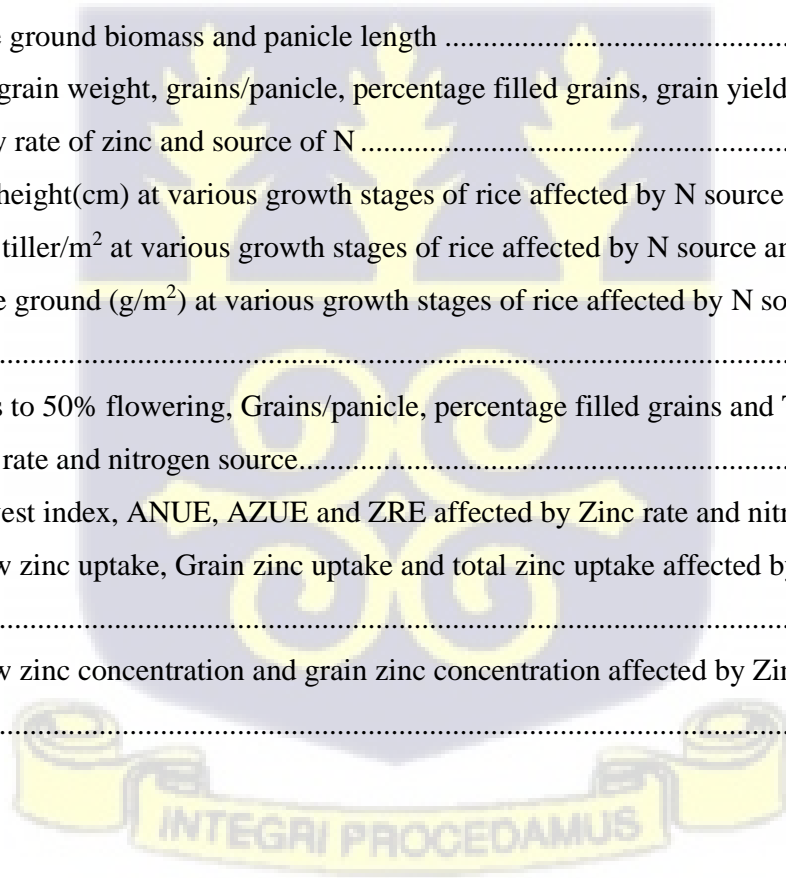
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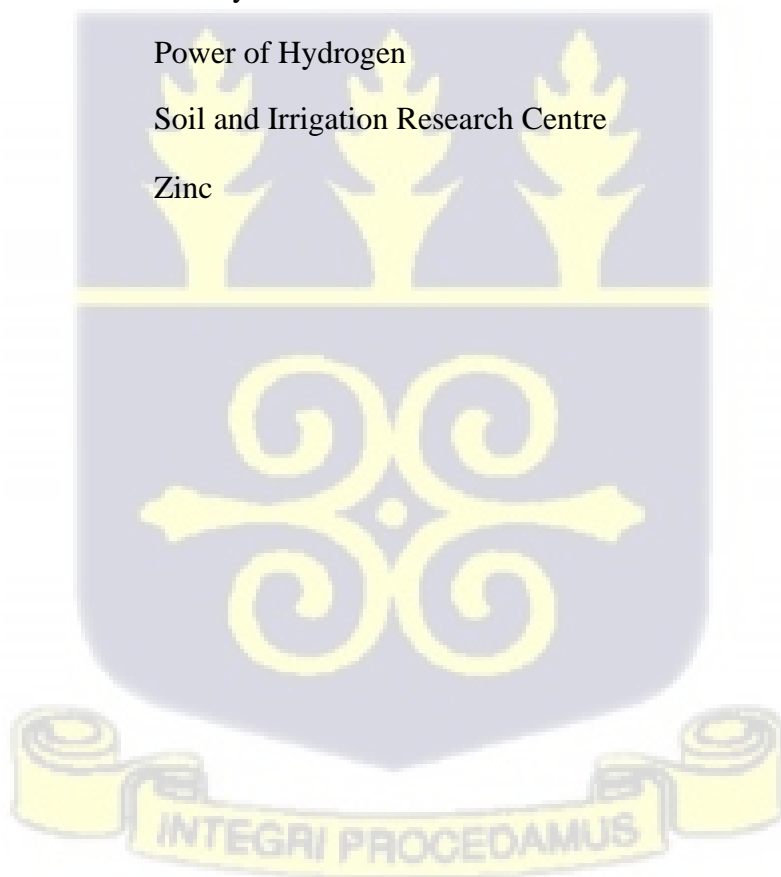
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LIST OF ABBREVIATIONS

ANUE	Agronomic Nitrogen Use Efficiency
AZUE	Agronomic Zinc Use Efficiency
SoA	Sulphate of Ammonia
CF	Chemical fertilizer
DAT	Days after transplanting
IF	Inorganic fertilizer
INF	Integrated nutrient management
MoFA	Ministry of Food and Agriculture
N	Nitrogen
PM	Poultry manure
pH	Power of Hydrogen
SIREC	Soil and Irrigation Research Centre
Zn	Zinc



CHAPTER ONE

1.0 INTRODUCTION

Rice (*Oryza sativa* L) serves as a staple food for over 50 % of the world population, which makes it a key player in ensuring global food security (Chauhan *et al.*, 2017). It supplies more than 20 % of the daily calories consumed by the human population and is consumed by more than 3.5 billion people over the globe every day, from which Asia accounts for 90 % of the consumption (Maclean *et al.*, 2013). Rice accounts for 21 % and 15 % of global per capita energy and protein intake, respectively (Hasan *et al.*, 2020).

Rice production is expected to increase by 40 % in response to global demand for rice by 2050 (Khush, 2005). As a result of rapid increase in population growth and increasing demand for food there has been a significant increase in global rice production, however the demand for rice still exceeds the supply. In 2020 over 497.69 million metric tonnes of milled rice was produced by more than hundred countries (Shahbandeh, 2021). Asian countries are the world's leading producers of rice. India has the largest area of land for rice cultivation at 43200 ha whereas China is the world's largest rice producer (Statista, 2021). The African continent produces only 4.8 % of the world's rice, and despite its potential, only 8 % of the continent's arable land is currently under cultivation (IRRI, 2020). The average rice yield in Africa (2 metric tonnes/ha) is significantly lower than that in Asia (7 tonnes/ha) (MINAG, n.a).

Rice is a staple food consumed by many households in Ghana. The African rice (*Oryza glaberrima*) and Asian rice (*Oryza sativa*) are cultivated in Ghana. The African rice use to be the most cultivated species, however more farmers have shifted toward the cultivation of the

Asian rice in recent years (Ragasa *et al.*, 2014). The main rice producing ecologies in Ghana are rainfed uplands, rainfed lowlands and the irrigated ecology. Domestic rice production is estimated to be 450,000 MT (Ashitey, 2018), which is less than half of the country's rice demand. Ghana spent \$375 million in 2019 on rice imports (Tradeconomy, 2021). In 2020, Ghana imported 950,000 MT of rice (Knoema, 2021). Recent interventions by the Government such as the introduction of subsidies on rice seeds and fertilizers has increased the domestic production of rice.

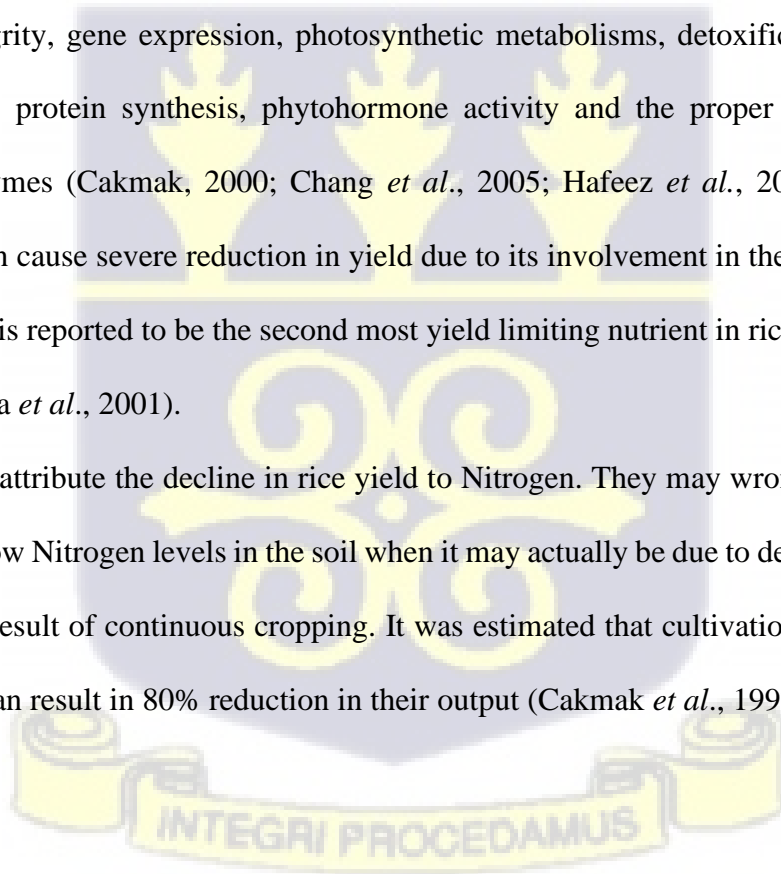
Some of the rice production constraints in Ghana include low soil fertility status, inadequate human resource capacity and post-harvest management technology, erratic rainfall pattern, relatively low usage of fertilizers due to high cost, diseases and pests' problems (CRI & MoFA, 2005; Tetteh, 2009). Among these constraints, poor soil fertility management has been identified as a major factor to the low rice productivity. The recommended fertilizer rate to rice farmers is 95 kg/ha N, 60 kg/ha P, 60 kg/ha K, irrespective of the rice production ecology (Ragasa *et al.*, 2013). Furthermore, there is no recommendation on micronutrient fertilizer application. Most farmers are unable to apply the recommended fertilizer type and rate. This results in nutrient imbalances that could account for low rice yields.

Proper and adequate nutrient management is important in order to achieve optimum rice yields. The inherent soil fertility cannot sustain intensive rice production as nutrients are removed after each harvest, depleting the already low soil nutrient status. About 1023.74 kg of nutrients (micro and macro-nutrients) was reported to be removed from the soil every rice growing season (Datta, 1981). It was estimated that 16-17 kg of nitrogen is required to produce each Metric tonne of rice, including the straw (Sahrawat, 2000). This phenomenon makes fertilization for each rice growing season a necessity for any farmer to attain a high yield.

Rice cultivated under the irrigated ecology is known to be the most productive among the three ecologies of rice production in Ghana (Ragasa *et al.*, 2013). Under this ecology rice is flooded for most of the growing season. In order to develop an efficient fertilization strategy, it is imperative to understand rice nutrition under flooded conditions which differs from non-flooded conditions. When the soil is flooded, it undergoes physical, biochemical, and physiochemical changes, resulting in a unique micro-environment for rice growth and nutrition. Some of the changes include reduction of the redox potential of the soil, Reduction of SO_4^{2-} to S^{2-} , changes in pH of soil, increase in specific conductivity and the increase in availability of nitrogen and decrease in the amount of soluble zinc in the soil (Datta, 1981).

Zinc (Zn) is involved in various important aspects of the metabolism in plants such as cell membrane integrity, gene expression, photosynthetic metabolisms, detoxification of reactive oxygen species, protein synthesis, phytohormone activity and the proper functioning of a number of enzymes (Cakmak, 2000; Chang *et al.*, 2005; Hafeez *et al.*, 2013). The lack of sufficient Zn can cause severe reduction in yield due to its involvement in the above metabolic activities. Zinc is reported to be the second most yield limiting nutrient in rice cultivation after Nitrogen (Guerta *et al.*, 2001).

Farmers mostly attribute the decline in rice yield to Nitrogen. They may wrongly diagnose the situation to be low Nitrogen levels in the soil when it may actually be due to decline of Zn levels in the soil as a result of continuous cropping. It was estimated that cultivation of cereal on Zn deficient soils can result in 80% reduction in their output (Cakmak *et al.*, 1997).



Rice yield is significantly improved when Zn fertilizers are applied on Zn deficient soils (Cakmak *et al.*, 2017). The effect of Zn fertilization is likely to be observed when Zn is applied on soils which are Zn deficient. Out of 10 soil types evaluated, Zn application increases the yield and dry matter production in only three. The soil types that responded to Zn fertilization were the only ones with zinc levels below the critical Zn level of 1.4 mg kg⁻¹ (Kalala *et al.*, 2016).

Not all the Zn in the soil is made available to the plant. Zinc uptake depends on soil characteristics such as pH levels and mineral composition of the soil. Zinc availability in the soil decreases with increasing pH. Due to this Zn uptake is low on alkaline soil. Grain yield and yield attributes of rice were significantly increased by Zn fertilization on alkaline calcareous soil (Hakoomat *et al.*, 2014). Different Zn rates did not significantly affect the growth of rice, however, increase in Zn fertilizer rates increased the nutrients status in the plant (Hosseini & Maftoun, 2008). Different responses of rice to Zn fertilizations from previous studies suggests the need to study the effect of Zn fertilization on nutrient uptake and yield for different types of soils.

Apart from the crucial role Zn fertilization plays in healthy growth of the plant, it also improves the quality of rice grains which is of primary importance to human nutrition. Zinc deficiency in staple crops such as rice and maize are major causes of malnutritional related health problems in many Developing countries (Impa *et al.*, 2013). It is estimated that around two billion people are affected by Zn deficiency globally (IZiNCG, 2004). One major reason for this predicament is because of the high consumption of cereals low in Zn concentration. This has raised concerns about ways through which agronomists can improve the Zn content in cereals. Zinc fertilization has the potential of addressing such problems. Zinc fertilization has been reported to improve

the Zn content in rice grains in the past (Guo *et al.*, 2015; Yin *et al.*, 2016). Yin *et al.* (2016) reported that Zn fertilizer application increased the brown rice Zn concentration only by 20%, however there was 100% increase in Zn concentration in vegetative parts. In order to improve on the Zn content in the rice grains, there is need to find new Zn fertilization strategies to overcome the barrier that prevent the translocation of Zn from the straw to the grains.

Nitrogen application influences the uptake of Zn from the soil through the regulation of the pH of the soil. N and Zn interaction in alkaline soils improves grain yield and yield components of Basmati rice (Hakoomat *et al.*, 2014). It was also observed that increasing the N supply could improve grain Zn and Fe content, and that Zn and N applications have a synergistic effect on the grain Zn content of durum wheat (Kutman *et al.*, 2010; Shi *et al.*, 2010).

Nitrogen (N) is the major yield-limiting factor in flooded rice cultivation systems. It promotes vegetative development, increases the number of effective tillers, increases the number of grains per panicle, and enhances grain protein content during grain filling. Nitrogen use efficiency (NUE) is generally low in rice due to N-loss through runoff, ammonia volatilization, leaching, and denitrification (Hosseini & Maftoun, 2008). To increase NUE and ensure optimal supply of N, there is an urgent need to decrease N-losses by adopting strategies that would reduce these losses.

Selection of the right N source can effectively reduce these losses. Nitrogen uptake and bioavailability of N in the soil is heavily dependent on the source of N fertilizer applied (Jagtap *et al.*, 2018). The sources of N fertilizers can be divided into organic and inorganic (chemical). chemical fertilizers are synthetically generated from minerals, atmospheric gases, and inorganic waste products while organic fertilizers acquire their nutrients from natural sources such as

microbes, organic waste, and other similar sources. Some of the popular sources of organic fertilizers for rice farmers include cow dung, rice straw, green manure, poultry manure and compost.

The Application of inorganic N fertilizers provides crops with large quantities of N within a short period of time. However, the continuous application of these fertilizers gradually decreases the fertility of the soil over time (Yang *et al.*, 2015). Its continues application could result in 38% decrease in grain yield (Singh *et al.*, 2001). Rice growers may achieve and maintain higher yields over longer periods of time by using organic fertilizers. The application of pig manure compost to cropping fields for four years resulted in higher organic-C and nitrogen concentrations in the soil (Wang *et al.*, 2012). Organic N fertilizers enhances soil quality by increasing water holding capacity and microbial activities (Chandra *et al.*, 2004). One major advantage of organic N fertilizers over inorganic N fertilizers is the other micro-nutrients such as Zn that accompany N in the organic source. The major setback with inorganic N fertilizers is because of its high cost many farmers can't afford it. Although it has been established that organic fertilizers are critical for long-term rice cultivation, they are not produced in sufficient amounts (Iqbal *et al.*, 2019). Organic N alone fertilizer cannot meet crop N demands, but it needs to be supplemented with inorganic N fertilizer to improve N availability and synchronization with the crops nutrient demands at various growth stages (Bharti *et al.*, 2016).

The combined usage of organic and inorganic fertilizers in rice production could be a better option for sustainable production of rice. The application of organic fertilizers in addition to inorganic fertilizers has been used by rice farmers to increase their yield (Hope, 2005). There is

a need to assess the best possible combination of different types of nitrogen fertilizers and their effect on yield and profitability to the farmer. The grain yield of rice was significantly increased by replacing 60% of the recommended urea application with 30 % poultry litter and 30% cattle manure when compared to 100% urea (Ismael *et al.*, 2021).

1.1 PROBLEM STATEMENT AND JUSTIFICATION

Soils used in rice production are low in soil fertility, and accounts for the low productivity and quality of crops (Stewart *et al.*, 2020). Intensive crop cultivation has resulted in soils low in organic matter, and deficient in macronutrients, notably nitrogen and phosphorus and Zn (Gemenet *et al.*, 2016; Giday, 2015). In an attempt to remedy the situation farmers are recommended to apply large quantities of inorganic fertilizers such as Urea, NPK, MoP, and SoA for sustainable production. However, most of the small-scale farmers cannot afford the recommended amounts needed to attain optimum yields. In addition, excess application of inorganic fertilizers for long periods deteriorates the productivity of the soils (Yang *et al.*, 2015). The continues application of these fertilizers results in nutrient imbalance in the soil and deficiency in micro nutrients such as Zn and copper because most of them are straight fertilizers and do not contain micro nutrient.

As a result, most soils in west Africa are classified as Zn deficient and this has affected the productivity and quality of crops in the region (Cakmak *et al.*, 2017). The extent of Zn deficiency across the region is depicted in Fig 1.0. Cultivation of rice on Zn deficient soils results in significant reduction in grain yield, impairment of growth and reduction of Zn in both

vegetative and reproductive parts (Cakmak, 2008; Dampare, 2012). The number of effective tillers, dry matter accumulation, grain yield, number of grains per panicle are all examples of yield and growth characteristics of rice that have been reported in the past to be significantly reduced as a result of cultivation of rice on zinc deficient soils (Rao *et al.*, 2019; Rehman *et al.*, 2012; Veer *et al.*, 2020).

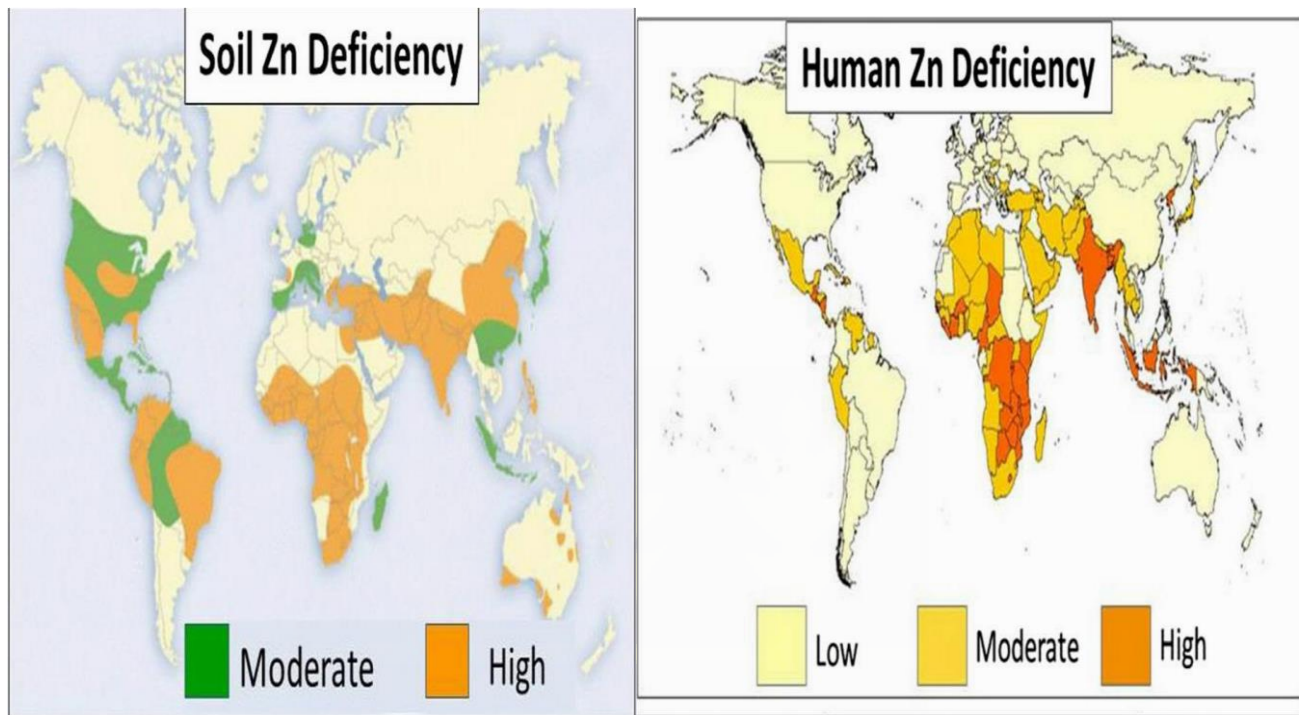


Fig 1.1: Global distribution of human Zn deficiency and soil Zn deficiency (Map adapted from (Cakmak *et al.*, 2017)).

Zinc fertilization on rice farms has been linked with human health and nutrition in recent years. In latest development, claims have been made that the consumption of zinc biofortified food can be used in the fight against the COVID 19 pandemic (Celik *et al.*, 2021). The consumption of rice grains low in zinc resulting from the zinc deficient soils they are cultivated could result in increase in zinc deficiency cases across the globe (Cakmak *et al.*, 2017). Zinc deficiency was responsible for 14.4% of diarrheal deaths, 10.4% of malaria deaths, and 6.7 percent of

pneumonia deaths in African children 6 months to 5 years (Berhe *et al.*, 2019). Consequently, zinc bioavailability in rice grains is a vital global challenge. It was mentioned during the 4th International Zinc Symposium held in 2015, that the only sustainable and feasible solution to Zn deficiency in humans is through agriculture. Zinc concentration in cereal are significantly improved when cultivated on soils with adequate quantities of phytoavailable zinc (Cakmak *et al.*, 2017). Field experiments have shown that zinc fertilization on zinc deficient soils improves bioavailability of zinc in the soil (Liu *et al.*, 2019). The efficacy of zinc fertilization could be improved by nitrogen fertilization. Results obtained from a field experiment shows that increased N fertilization improved zinc uptake and grain Zn concentration only in cultivars with naturally low grain Zn concentrations, but not in cultivars with essentially high grain Zn concentrations (Jaksomsak *et al.*, 2017).

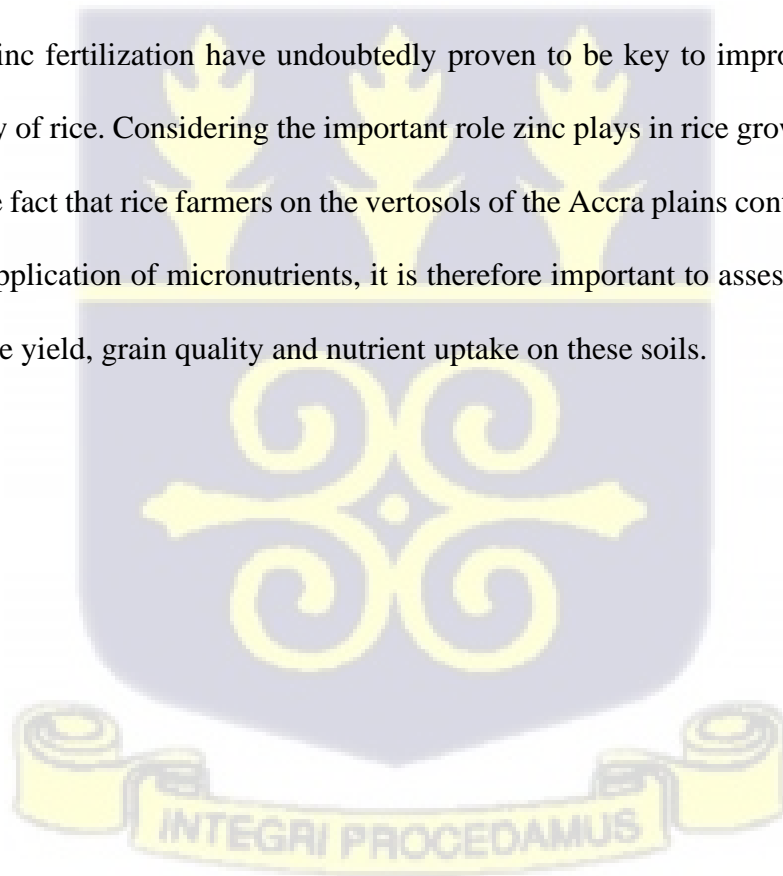
The reduction in zinc in the soils below critical levels in the soils could be averted by the application of organic fertilizers which supply the soil with the needed micronutrient including zinc. The recommendation to rice farmers is to apply these organic nitrogen fertilizer after every four years on rice fields to improve the health and avoid any problems that may arise from zinc deficiency (Khalofah *et al.*, 2021). Most of the locally available poultry manure could be a good option to improving the yield and grain quality on the zinc deficient soils. The litter from the poultry farms are rich in zinc because the feed stock regularly used in the poultry farms are rich in zinc (Chastain *et al.*, 2021). This makes poultry manure a very strong competitor, when picking an organic Nitrogen fertilizer that can improve zinc availability to rice.

A number of studies have looked at the potential of the application of organic nitrogen fertilizers on improving the productivity of rice on zinc deficient soils (Ismael *et al.*, 2021). Significant

variations in nutrient uptake, growth and yield of rice was found among 10 different types of soils in Mozambique (Ismael *et al.*, 2021). Results from similar studies suggest that the response of rice to N source and zinc fertilization is based on the underlining characteristics of the soil (Oinam *et al.*, 2017; Shahane *et al.*, 2018).

In Ghana SoA and urea are the two most popular choice of N source for top dressing (Ragasa *et al.*, 2013). Zinc nutrition under these two N sources may differ due to the difference effects they have on soil pH and nitrogen availability in the soil which regulates uptake of zinc from the soil. Ferrante *et al.* (1986) observed difference response of growth and yield of rice to SoA and urea under lowland condition. It has been well documented that the nutrition of rice under irrigated ecology is different form upland conditions.

Nitrogen and zinc fertilization have undoubtedly proven to be key to improving the growth, yield and quality of rice. Considering the important role zinc plays in rice growth, yield, human nutrition and the fact that rice farmers on the vertosols of the Accra plains continually crop their fields without application of micronutrients, it is therefore important to assess how zinc and N source affect rice yield, grain quality and nutrient uptake on these soils.



1.2 OBJECTIVE:

The objective of the study was to examine the effect of different nitrogen sources and rate of application of zinc on the growth, yield and grain zinc content of rice under an irrigated environment

1.2.1 Specific objectives:

1. Examine the growth and yield response of Legon rice 1 to Sulphate of ammonia and Urea
2. Examine the growth and yield response of Legon 1 rice to zinc fertilization.
3. Examine the growth and yield response of rice to poultry manure and inorganic N fertilizer (Urea and ammonium sulphate).
4. Examine the effect of rice Zn uptake to combined application of Zinc and Nitrogen application.
5. Determine the effect of N source and zinc fertilization on the zinc content of Legon rice 1.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTORY OF RICE: ORIGIN DISTRIBUTION AND DOMESTICATION

The rice plant is an annual crop belonging to the family *Poaceae*, order *Poales* and genus *Oryza* L. The genus *Oryza* consist of two cultivated species, *Oryza sativa* L. (Asian rice), and *Oryza glaberrima* (African rice). Apart from the two cultivated species, a number of wild species of the genus *Oryza* have been discovered around the world namely *O. rufipogon*, *O. alta*, *O. punctata*, *O. minuta*, *O. minuta*, *O. punctata*, *O. granulata*, *O. schlechteri* and *P. coarctata* (N. M. Nayar, 2014).

The Asian rice is believed to have originated 10000 years ago from Asia. However, there is a lot of controversy surrounding the exact origin of the species. Molina *et al.* (2011) pointed out Yangtze of China as exact origin of the Asian rice. Their claims were supported by archeological evidence which points to Yangtze as the earliest place of rice cropping. According to genetic evidence domestication of rice started 8,200–13,500 years ago, in Pearl River valley region of China (Huang *et al.*, 2012; Molina *et al.*, 2011). On the contrary, others believe Asian rice originated from southern India, before spreading to China (Pallavi, 2011). After its establishment in China, it was spread to Japan, Korea and Indonesia around 1000BC. The Asian rice was later spread by the Arab traveler from Asia to European countries such as Portugal and Spain (Hassan *et al.*, 2018).

As the name suggest the African rice (*Oryza glaberrima*) is indigenous to Africa. Earlier reports proposed *Oryza glaberrima* originated from west Africa (Tateoka, 1965). The popular believe is that the African rice originated from the wild rice, *O. barthii* Cheval about 3500 years ago.

On the contrary Nayar, (2010) argued the African rice originated from the Asian rice. The data he collated suggests the Asian rice was introduced to West Africa during the common era by the Europeans, and then the African rice emerged from it years later. Currently, the Asian rice is the universally cultivated *Oryza* species whereas the African rice is grown only in Africa typically on a small scale.

Oryza sativa exhibits a wide range of morphological and physiological variations which allows it to be grown under a wide range of environmental conditions (Ahmadi *et al.*, 2021). The phenotypic diversity is distinguished into two sub-species namely, *japonica* and *indica*. Recent evaluation of the genetic variation across different rice varieties in the world suggests *Oryza rufipogon* as the common ancestor for the two main sub species of rice. It is believed that humans first started the gathering and consumption of *Oryza rufipogon*, a wild species of grass found growing in the swampy areas of Subtropical Asia more than 10000 - 14,000 years ago (Kovach *et al.*, 2007). These ancient farmers slowly transformed the wild rice (*Oryza rufipogon*) to the modern rice (*Oryza sativa*), through the selection of types with desirable traits for human consumption. The domestication of rice resulted from selection from wild species that led to genetic changes which made it more cultivatable and consumable for the human population (Kovach *et al.*, 2007).

2.2 RICE PRODUCTION: GLOBAL, AFRICA

Rice is a major food for many households around the globe. Rice farming serves as the main source of income for over hundreds of millions of people across the world. Rice contributes 20% of the world's dietary and serves as a staple meal for over 3.5 billion people (Alexandratos &

Bruinsma, 2012; Maclean *et al.*, 2013). All the evidences suggest Rice will unquestionably always play a role in global food security, both now and in the future.

Rice is produced in over a hundred countries, with an annual output of about 700 million tons and 470 million tons of milled rice (CGIAR, 2020). Asian countries produce (64 million tons) and consumes over 90% of the world's rice (Bandumula, 2018). China is the world's leading rice producer and India has the largest area of land for rice cultivation at 43,200 ha and second largest rice producer. Aside from China and India, the remaining top 10 rice producing countries in the world as of December 2020 include Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil, and Japan (Statista, 2021) .

Rice is the fastest-growing food staple in Africa. Important rice producing countries in Africa includes Guinea, Guinea-Bissau, Sierra Leon and Nigeria. Nigeria is the biggest rice producer in West Africa, accounting for around 5% of all rice produced globally (CGIAR, 2020). Rice production has increased significantly over the years throughout Africa, however there is still a large dependency on imported rice due to rapid population growth and increase in demand and obsolete production methods by rice farmers.

2.3 RICE PRODUCTION IN GHANA

Both species of the genus *oryza*, *o. stivava* and *o. glaberrima* are cultivated in Ghana. In previous years, local rice variety was popularly cultivated in Ghana, however in recent years more farmers in Ghana are changing from the local varieties to foreign varieties. Ragasa *et al.* (2014) reported that 45% of rice farmers within the country are now cultivating aromatic rice varieties

and more farmers are expected to shift toward the production of aromatic rice varieties. Aromatic rice varieties have gross margins that are twice as high as non-aromatic varieties (Ragasa *et al.*, 2014).

Rice is a staple crop in Ghana which is cultivated across all the major ecological zones (such as semi-deciduous rain forest, coastal savanna and high rain forest zones) within the country (MoFA/IFPRI, 2020). Rice is produced across almost all the regions in the country. The Volta, Upper East and Northern Regions are the highest rice producing region accounting for 45,000 to 160,000 tons of rice produced annually in the country (MoFA, 2011). Regional rice yields vary greatly due to differences in production conditions and practices (Ragasa *et al.* 2014).

Due to rising demand, rice has become the second most important staple in Ghana after maize. In 2019 rice production in Ghana was 963,000 tons, which corresponded to 665,000 tons of milled rice (MoFA-IFPRI, 2020). A steady increase of 11.1% per annum in rice production within the country between 2008 and 2019 was reported (MoFA-IFPRI, 2020). One major factor contributing to the increase in rice production is the increase in the area of land rice is cultivated under in the country. Although the records indicate a significant improvement in rice production in Ghana over the years, more than 50% of rice consumed within the country is imported (Ashitey, 2018).

Some of the challenges contributing to low rice productivity in Ghana includes poor soil fertility, inadequate harvesting and post-harvest management technology, erratic rainfall pattern, relatively low usage of chemical fertilizers due to high cost and, diseases and pests' problems. The inability of rice farmers to meet demands of the population has led to over dependency of the Ghanaian population on imported rice which has been detrimental to the economy.

Improvement of rice production has remained a priority for the government of Ghana since the 1970's. In recent year the government of Ghana has established a number of agricultural policies to support and help increase rice production. Major policies that support rice production by the government includes the National Rice Development Strategy of 2009 and the Planting for Food and Jobs (PFJ) campaign launched in 2017. As part of the aims of these policies, is to help in improving yield and quality of locally produced rice in order to meet the demands of the population. Henceforth effectively reducing the overdependency on imported rice (MoFA, 2019). Some of the main component of the rice support programmes includes intensive rice extension services, and subsidizing 50% fertilizers cost and provision of rice seeds for rice farmers.

2.3.1 RICE PRODUCING ECOLOGIES IN GHANA: IRRIGATED, LOWLAND, UPLAND

Rice production in Ghana can be divided into three types based on the ecology the crop is cultivated under. This includes upland rain fed, the valley bottom (lowland rainfed) and controlled flooding (irrigated). About 78% of rice cultivated in Ghana is under the lowland rain fed ecology, while the upland and irrigated ecologies occupies 6% and 16% respectively (MoFA, 2009). The valley bottom and upland rice production system is mostly for the cultivation of the local varieties. Rice production under the lowland and upland ecologies are regularly cultivated on a lands less than one hector in size (Asante-Poku & Angelucci, 2013).

Under the upland rain fed production method rice cropping is dependent on the type of season thus if it is the raining season or the dry season, because of this rice can only be cultivated once a year (Kranjac-Berisavljevic *et al.*, 2003). The African rice variety is mostly grown under upland conditions because they are more tolerant to harsh biotic and abiotic conditions such as

low soil fertility, weed competition, diseases and pest infestation (Agbanyo, 2012; MoFA, 2009). Upland New Rice for Africa (NERICA) varieties which were developed by crossing the Asian rice and the African rice are able to tolerate the harsh upland condition and still produce higher yield than the African rice (Somado *et al.*, 2008).

Under lowland conditions the rice field is mostly water-logged which increases water availability to the rice plant, suppress the weed growth and effectively reduces weed competition for resources with the crop. Lowland rice fields are regularly waterlogged due to the high-water holding capacity of the soil and high underground water table level. Rice yields are mostly better under lowland rain fed systems than the upland ecology. The soils are able to effectively retain water for a long for the rice roots uptake even during reduced rainfall (Agbanyo, 2012).

Unlike the upland production systems, rice cultivation can be cultivated all year round under the irrigated rice cropping systems. Improved land preparation, cultivars, fertilizer application, and water management are all employed under the irrigated ecology. This results in relatively higher yields when compare with the other ecologies (MoFA, 2009). Weed competition is low in this ecology because the rice field is constantly submerged to control weeds. Rice cultivation under irrigation systems is the most expensive method of rice production primarily due to the cost of irrigation. However, it is the most profitable among all the methods of rice production Ghana. In a survey conducted with 3550 rice farmers across Ghana (John,2017), it was reported that farmers producing rice under irrigated ecology are more economic and technically efficient in rice production than those producing under rain fed. The cultivation of lowland rice during the dry season without adequate irrigation can lead a 45% decrease in grain yield (Oort & Zwart, 2018).

2.4 RICE NUTRITION UNDER IRRIGATED ECOLOGY

An in-depth knowledge on nutrient requirement of rice at all the growth stages and the soil ability to provide the crop with these nutrients is vital to successful and profitable production.

All 16 plant nutrients are known to be important for rice. N, P and K are recognized as primary macronutrients and fertilizers containing them are the most commonly applied by the rice farmers. Mg, Ca, and S are considered secondary micronutrients whereas Zn, Fe, Mn, Cu, B, Mo, and Cl are micronutrients (Shrestha *et al.*, 2020a). Rice requires all of the mentioned nutrients in the proper proportions for optimal growth and development.

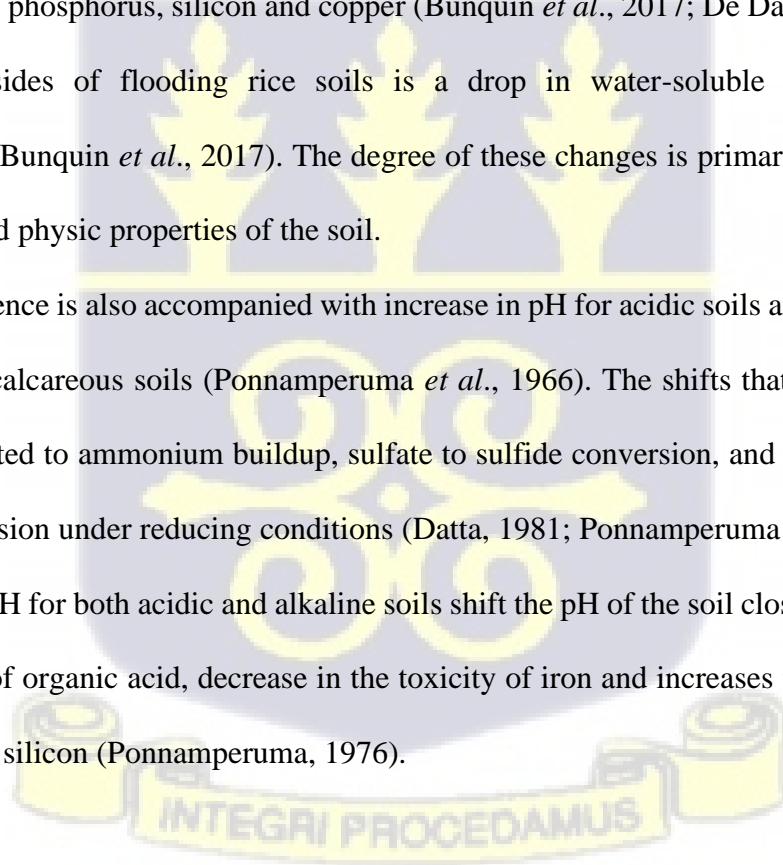
Every nutrient has its own characteristics and plays different roles in metabolic and biological processes of the plant's life. Phosphorous(P) is required for root development during early stages of the rice plant and the supply of energy for all biochemical processes (Agritech, 2021; De Datta, 1981). Maathuis (2009) observed that P was required in cell formation and is also required by rice in large amounts of nitrogen during the vegetative stages. Nitrogen encourages vegetative growth, maximize the number of effective tillers, increases number of grains per panicle and increases protein content of the grains during grain filling (Dobermann, 2000; Maathuis, 2009). Potassium increases the resistance of the rice plant to stress, diseases and unfavorable conditions (Nieves-Cordones *et al.*, 2019). Potassium also serves as a cofactor for more than 40 enzymes in biochemical processes in the rice plant (Wang & Wu, 2013). Sulphur is involved in lipids synthesis, chlorophyll production and protein synthesis (Agrinfobank, 2019). The adequate supply the essential nutrients increase the productivity of rice, however

higher levels of minerals such as iron, aluminum and boron have been reported to be toxic to the plant (Shrestha *et al.*, 2020a).

The growth and yield of rice is better when grown under submerged soil conditions than when grown under non flooded conditions. The main advantage of rice grown under flooded condition is adequate supply of water for the plant. Submergence of the soil is accompanied with physical, biochemical and physiochemical changes which creates a unique and different environment for the growth and nutrition of rice when compared to non-flooded condition (Ismail *et al.*, 2012).

Some of the important chemical changes that occur after flooding includes reduction of the redox potential of the soil, reduction of SO_4^{2-} to S^{2-} , decrease in concentration of plant available zinc, changes in soil PH, increase in specific conductivity and the increase in availability of minerals such as nitrogen phosphorus, silicon and copper (Bunquin *et al.*, 2017; De Datta, 1981). One of the few downsides of flooding rice soils is a drop in water-soluble zinc and copper concentrations (Bunquin *et al.*, 2017). The degree of these changes is primarily determined by the chemical and physic properties of the soil.

Soil Sub-emergence is also accompanied with increase in pH for acidic soils and decrease in pH for alkaline or calcareous soils (Ponnamperuma *et al.*, 1966). The shifts that occur in soil pH could be attributed to ammonium buildup, sulfate to sulfide conversion, and carbon dioxide to methane conversion under reducing conditions (Datta, 1981; Ponnamperuma *et al.*, 1966). The changes in the pH for both acidic and alkaline soils shift the pH of the soil close 7, which favors mineralization of organic acid, decrease in the toxicity of iron and increases the availability of phosphorus and silicon (Ponnamperuma, 1976).



The supply and availability of nitrogen to rice is increased after flooding. Flooding also results in the reduction of NO_3^- and NO_2^- to N_2 and N_2O . Although nitrate is the main form of nitrogen for the rice plant from the soil under aerobic conditions, under submerged conditions the rice prefers the absorption of ammonium to nitrate (Nurulhuda *et al.*, 2018). Low quantities of ammonia are hazardous to many upland crops; however, rice tolerates and uses very high doses with ease under anaerobic or flooded conditions.

2.5 RICE FERTILIZATION IN GHANA UNDER IRRIGATED ECOLOGY

The understanding of the dynamics involved in the nutrition of rice would help both researchers and farmers to develop efficient fertilizer management strategies for the crop. The rate of nutrient removal for every rice growing season is high. Due to this application of large quantities of fertilizers is the only sustainable way of meeting the nutritional requirement of rice. In Ghana the application of inorganic fertilizers by farmers to rice under controlled flooding has been found to be more than under lowland and upland rain fed. According to survey by Ragasa *et al.* (2013), NP-K 15: 15: 15 was the most commonly used type of fertilizers by rice farmers and is applied to 90% of the rice plots in Ghana. Sulphate of ammonia was found to be the second most commonly applied fertilizer, followed by urea (Ragasa *et al.*, 2013).

The recommended application rates of N, P and K inorganic fertilizers for irrigated rice by MoFA and CSIR in Ghana is 84kg/ha, 43kg/ha, 43kg, respectively. These rates are generally higher than the recommended rates under lowland rain fed and upland rain fed. Rice yield is markedly higher across all irrigated ecologies even for fields where the farmers use uncertified seeds. Nevertheless, irrigated field where certified seeds are used in combination with the

recommended rates of fertilizers application by MoFA and CSIR records the highest yields (Ragasa *et al.*, 2013).

In addition to the rates of application the timing of application is also important to increasing fertilizer use efficiencies. A split application of the endorsed rates by MoFA is recommended to the rice farmers. The first known as basal application is to be applied within few weeks after transplanting. The second application known as topdressing is recommended to be applied just before booting or five to six weeks after planting. Surveys done by MoFA suggest most farmers do not adopt the recommended timing for the fertilizer application. Most farmer prefer applying it the full recommended rates during the first application. Only 43% of transplanted fields within the country apply the fertilizers twice (Ragasa *et al.*, 2013).

The application of micronutrients-based fertilizers is not common among the rice growers in Ghana. Ragasa *et al.* (2013) reported that Ghanaian rice farmers hardly apply fertilizers that contains micronutrients. This mostly result in nutrient imbalances and deficiencies in micronutrients such as zinc and boron on rice fields in the long run. A number of studies have proven that most of the soils used for cereal cultivation is deficient in zinc (Cakmak *et al.*, 2017; Dampare, 2012; Liu *et al.*, 2020). The lack of application of fertilizers that would replenish the loss of micro-nutrient in the soil could be a major reason why most farmers experience low yields in the country. Nevertheless, it is recommended to farmers to apply organic fertilizers such as poultry manure and compost after a number of successive cropping seasons in order to avoid any micronutrient deficiencies and nutrient imbalances on rice fields. Currently the application of organic fertilizers is not common among the rice growers.

2.6 IMPORTANCE OF NITROGEN FERTILIZATION IN RICE PRODUCTION

Nitrogen is an essential nutrient needed for the growth and development of all plants. It plays an important role in physiological and biochemical activities of the plant to promote the healthy growth and development of crops. Nitrogen is the most important nutrient for the growth and development of rice. It is the main yield contributing nutrient in every rice production system (Djaman *et al.*, 2018). According to Sahrawat (2000) 16 kg to 17 kg of nitrogen is removed for every one tone of rice grains produced. The rate of nitrogen removal is dependent on several factors such as variety, soil type, PH, water, source of N, N rate and the timing of application (Djaman *et al.*, 2018; Jahan *et al.*, 2020; Z. Liang *et al.*, 2015). Nitrogen fertilizers is the only means through which rice growers can replenish the amount of nitrogen removed after every cropping season.

Rice plants require nitrogen in order to enhance tillering, panicle initiation, grain filling and number of grains per panicle (Datta, 1981). Nitrogen is able to influence rice yield due to the important role it plays in photosynthesis, biomass accumulation, effective tillering, and spikelets formation (Yoshida *et al.*, 2006). Most of the developed high yielding rice varieties is heavily dependent on adequate nitrogen supply in order to attain its yield potential (Barker & Dawe, 2001). Several studies have shown that Nitrogen fertilization plays a significant role in the growth, yield and grain quality of rice.

2.6.1 Importance of nitrogen fertilization to growth and yield.

The role of N fertilization at the various growth stages of rice has been well documented. All most all growth attributes of rice, such as height, number of tillers, leaf area index, and

chlorophyll concentration, are influenced by nitrogen fertilizer application (El-Refae *et al.*, 2007; Malik *et al.*, 2014). In field experiments increasing nitrogen fertilizer rate from 45kg/ha to 80kg/ha significantly increase the plant height of rice. However, increasing the rate above 80 Kg/ha did not result in further increase (Chaturvedi, 2005).

The rate and source of nitrogen fertilization has a significant effect on tillering in rice. Nitrogen increases the cytokinin content within the tiller nodes which enhances germination of the tiller primordium (Y. Liu *et al.*, 2011). This phenomenon makes nitrogen fertilization the most effective agronomical means of increasing tiller population. In addition, nitrogen plays a significant role in tiller development (Sakakibara *et al.*, 2006). Increasing nitrogen supply to rice especially at the early growth stages enhances tillering (El-Refae *et al.*, 2007; Mannan *et al.*, 2012).

Increasing nitrogen levels improves the contribution of late maturing tillers to yield. However, the number of grains per panicle and number of filled grains of the late maturing tillers were lower compared to that of the early maturing tillers (Wang *et al.*, 2017).

Leaf area and photosynthesis in rice are enhanced by nitrogen fertilization. Chaturvedi (2005) reported an increase in the total leaf area of rice in response to nitrogen fertilization. Haque & Haque (2016) obtained a LAI of 4.17 at 45DAT with 100kg N/ha while the control (0kgN/ha) recorded the lowest LAI (1.90).

Dry matter accumulation in rice is heavily dependent on nitrogen supply. Chaturvedi (2005) reported a positive correlation between dry matter accumulation and nitrogen levels. Application of urea at 95 DAT recorded the greatest increase in dry matter accumulation (Ye *et al.*, 2013). The influence of nitrogen fertilization on dry matter production is due to the

important role nitrogen plays in chlorophyll formation, photosynthesis and protein synthesis (Agrinfobank, 2019).

Nitrogen is the most important nutrient when it comes to the determination of yield in rice. Several studies have confirmed the significant effect nitrogen application has on yield, and yield determining attributes such as number of total number of panicles, panicles per grain, test weight and total grain yield. In a field Experiment, application of 120 kg/ha of N recorded the highest number of grains per panicles while application of rates lower than 100 kg/ha decrease the number (Malik *et al.*, 2014). Panicles per m², panicle length and grains/panicle of rice were significantly improved by the application of nitrogen at 60kg N/ha (Singh *et al.*, 2001). The yield response of rice to nitrogen differs for different varieties of rice genotypes (Djaman *et al.*, 2018).

El-Refaee *et al.* (2007) argued that nitrogen fertilization does not have any effect on the test weight of rice. According to them the test weight is a genetic trait of the rice variety with minimal influence from the environment. Jamil & Hussai, (2000) confirmed that nitrogen rate had no significant influence on the test weight. On the contrary, Islam *et al.* (1990) reported a significant effect of nitrogen fertilization on test weight at a rate of 80 kg/ha. He observed an increase in test weight from 22.7 to 26.3 when N rate was increased from 69 kg/ha N to 115 kg/ha N.

2.6.2 Importance of nitrogen to grain quality

Apart from the impact of nitrogen fertilization in yield and growth of the rice, a number of studies have examined the impact it has on grain quality. The application of nitrogen fertilizer generally improves the protein content, amino acid composition, kernel integrity and milling

characteristics in cereals. Blumenthal *et al.* (2008) reported that the application of 75 Kg/ha of ammonia nitrate increased the grain protein concentration by 12 g/kg in winter wheat. Several field studies have provided evidence that the adequate supply of nitrogen to the rice plant throughout its life cycles ensures high grain quality. The nitrogen status and nutrient supply determines the protein content in both white and brown rice (Blumenthal *et al.*, 2008). In an experiment nitrogen fertilizer application during top dressing resulted in increase in rough rice yield by 25% and grain protein by 25% compare to plots that only received nitrogen fertilizers during panicle initiation (Alcantara *et al.*, 1996).

The increase in grain protein by nitrogen fertilization improves resistance of grains to cracking and breakage during milling. The Author provides evidence that the protein content and translucency of rice grains can be significantly be improved through proper nitrogen management while maintain the whiteness of the grains within the accepted limits of the market. Alcantara *et al.* (1996) reported 76.4 % to 85.5 % increase in translucency as a result of nitrogen fertilizer application. Nitrogen fertilization also affects the grain Nitrogen content during grain filling (Wang *et al.*, 2017).

2.7 RESPONSE OF RICE TO SOURCE OF NITROGEN

The selection of a type Nitrogen fertilizer is a major yield contributing factor in rice cultivation. The source may be either from organic or inorganic (chemical or synthetic) sources. Organic fertilizers acquire their nutrients from natural sources such as microbes, organic waste, and other similar source while inorganic fertilizers are synthetically generated from minerals, atmospheric gases, and inorganic waste products. In rice farming a number of studies have proven that the source of Nitrogen fertilizers on the field results in remarkable difference in

growth, grain quality and yield (Hosseini & Maftoun, 2008; Ismael *et al.*, 2021; Jagtap *et al.*, 2018). The difference between the various types of Nitrogen fertilizers is primarily due to their distinctive characteristics such solubility, rate of nutrient release and impact on the soil.

Since the green revolution the use of chemical fertilizers has become the most widely used and accepted source of nitrogen for sustainable rice production. The dramatic use of the inorganic fertilizers such NPK, urea and sulphate of ammonium has been abused in rice cultivation, resulting in not just pollution but also an increase in production costs, nutrient imbalance in soil and lower nitrogen use efficiency and recovery in rice (Conant *et al.*, 2013; Mueller *et al.*, 2014; Yan *et al.*, 2014). Chemical fertilizers are getting increasingly expensive, making them unaffordable for smallholder farmers in Africa (Ismael *et al.*, 2021)

The application nitrogen from chemical sources initially improves the productivity of the soil which result in significant increase in both yield and growth attributes of rice such plant height, dry matter production, chlorophyll content, number of tillers, grain weight and grains per panicle (Rosenzweig *et al.*, 2014). on the other hand, the positive influence of the chemical fertilizers on rice productivity gradually decreases with time due to decrease in soil fertility, reduction in soil organic carbon and deterioration of physical, biological and chemical properties of the soil (Ladha *et al.*, 2003; Pathak *et al.*, 2003). The continuous application of these fertilizers resulted in 38% decrease in rice grain yield (Singh *et al.*, 2001).

The application of organic fertilizers has gained much attention among farmers and scientist in recent years. This surge resulted from the negative impact of the usage of synthetic fertilizers on the soil, crops and the environment. Rice farmers are able to attain and sustain larger yield over long period of time by the application of various types of organic fertilizers. Some of the most

widely applied organic nitrogen sources by the farmers include compost, poultry manure and cow manure.

Manure application enhances soil quality by increasing water holding capacity and microbial activity (Chandra *et al.*, 2004). Wang *et al.* (2012) discovered that applying pig manure and compost to crop farms for four years resulted in higher organic-C and nitrogen concentrations in the soil which reflected on yield. In soils with minimal organic matter, nitrogen losses are higher. Farmyard manure is applied to lower these losses and improve soil health or tilth (Khalofah *et al.*, 2021).

The application of organic manure is accompanied with the increase in the production of greenhouse gases such as Methane and Nitrous oxide. The emission of the gases could be reduced when the manures are added at recommended amounts (Win *et al.*, 2021). However, there is no clear recommendation for the usage of organic manure. Farmers frequently apply either too much or too little organic fertilizer, causing soil conditions to be altered and the amount of nutrients delivered to plants to be insufficient or overdosed (Mbatha, 2008). Excessive application of organic manure has the potential of increasing the toxic effects from metabolic intermediates, and should be avoided (Liang *et al.*, 2003; Moe *et al.*, 2019). Organic fertilizers with reduced nutrient release ability, limits nutrient uptake and fail to meet short-term crop requirements (Iqbal *et al.*, 2019).

Although it has been clearly established organic fertilizer application is vital to for sustainable rice production, they are not produced in sufficient quantities to meet production demands. Organic N fertilizer alone cannot meet crop N demands, however combining it with chemical N

fertilizer increases N availability (Bharti *et al.*, 2016). As a result, integrated nutrient management appears to be a promising strategy for improving soil health and yields of rice.

2.7.1 Combined application of organic and chemical N sources

Synchronization of the nutrient release from fertilizers with crop demands has been a major challenge in rice farming. Each of the stages in rice growth cycle has its own nitrogen requirement. However, most of the chemical fertilizers applied at the early stages are rendered unavailable to the rice root during the latter growth stages. This results in secondary application of the chemical fertilizer in a bid to curb the problem. With organic fertilizers the nutrients are made available to the roots only in small quantities over a long period which may not be able to meet the immediate demand of the crop in the required quantities (Iqbal *et al.*, 2019). One main way of solving the problem of the inadequacies of both sources of nitrogen fertilizers is by combining them. The fertilizer management strategy of combining different sources of fertilizers falls under the Integrated Nutrient Management (INM). Previous studies have shown that combining organic and inorganic source at the right proportion has positive implications on growth, yield, grain quality of rice and cost of production to farmers (Selim, 2020). INM also help avoid or can effectively corrects nutrient deficiencies and nutrient imbalance in the soil. The superiority of combined application to sole application in terms of growth and yield of rice is well documented. Ismael *et al.* (2021) evaluated the response of rice to sole application of Urea, poultry litter and beef cattle manure and their different combination. They reported that 50% urea + 50% beef cattle manure, 50% urea + 50% poultry litter and 40% urea + 30% beef cattle manure + 30% poultry litter recorded the highest grain yield (425 g m^{-2}), plant height (115

cm), number of tillers (18), and thousand-grain weight (34 g). In a similar study Combined fertilizers had a positive impact on height, spikelet's per square, tillering, and grain production when compared to sole application and unfertilized plots (Moe *et al.*, 2019). The combined application of 50 percent inorganic fertilizer with 50 percent organic fertilizer can potentially increase the weight of grain in the upper and middle sections of a panicle (Tang *et al.*, 2015). The combined application of manure and urea N increased grain yield and yield components better than a single organic source (Amanullah *et al.*, 2016). The increase in yield may have resulted from the combined effect of urea and manure on physiochemical properties of the soil such as soil porosity and water holding capacity, and nutrient availability (Duan *et al.*, 2016). Beside nitrogen, integrated fertilization also enhances availability of P, K and Ca in the soil (Ismael *et al.*, 2021).

The grain yield of rice can be markedly increased by replacing 10%-20% of chemical nitrogen with organic nitrogen, which would lead to improved N consumption efficiency (Meng *et al.*, 2009). The combination of urea and farmyard manure in ratio of 75% to 25% recorded the highest number of grain yield and number of effective tillers than 50% to 50% combination and sole application of urea or farmyard manure (Karki *et al.*, 2018). Iqbal *et al.* (2019) argued that combining 30 percent N from poultry manure or cow manure with 70 percent N from a chemical fertilizer was the most promising way of increasing soil quality and rice productivity at the same time in the long term.

The positive response of rice growth to INM is as a result of the synchronization of the nutrient release with the crop, leading to increase in nutrient uptake and dry matter production (Banik *et al.*, 2009). In support of this claim Moe *et al.* (2017), observed an increase in N uptake and

nitrogen use efficiency in rice as a result of the integrated fertilizer application. In comparison to the inorganic fertilizer (IF)-only treatments, the integrated manure and IF treatments improved post-anthesis Dry matter accumulation and soil characteristics such as bulk density, organic carbon, total N and microbial biomass carbon (Iqbal *et al.*, 2021).

Apart from yield and growth of rice, combining organic and inorganic fertilizers has an impact on grain quality (Dou *et al.*, 2017). Plots treated with a combination of organic and inorganic fertilizers increased the protein content of rice grains by 2.61% and 22.66% when compared to plots treated with only chemical fertilizers and control plots (Liu *et al.*, 2019). The combined use of organic and chemical fertilizers improved rice consumption and nutritional quality (Wang *et al.*, 2004). The amylose content in milled rice is used as a measure of rice eating quality. The combined application of organic and chemical fertilizer resulted in a 2.86% reduction in the amylose content of milled rice (Liu *et al.*, 2019).

Studies have shown the combined application of N sources is more economically beneficial than sole application. Chemical fertilizers are getting increasingly expensive, making them unaffordable for smallholder farmers in Africa. Replacement of portions of the chemical fertilizers with organic sources which are less expensive leads to significant reduction in cost of production for these farmers. Integrated N management resulted in a significant increase in net benefit (US\$ 779, 960) and crop yield (7,1686,405 kg/ha) for maize and wheat (Sarwar *et al.*, 2021).

2.7.2 Application sulphate of ammonia and urea

Chemical fertilizers are the most popular choice when it comes to selecting a nitrogen fertilizer for rice. Examples of frequently applied nitrogen chemical fertilizers include ammonium nitrate,

Urea, NPK, sulfate of ammonium and anhydrous ammonia. These fertilizers are mostly used because large quantities of N are made available to meet the immediate N requirement of the rice plant. Globally urea is the most popularly used source of N fertilizer applied to rice. For the second application (which is also termed as Topdressing) of Sulfate of ammonia is more extensively used than urea by rice the rice farmers (Ragasa *et al.*, 2013). The response of rice to Urea and SoA containing the same amount of N could differ from each other based on their unique characteristics such solubility, rate of nutrient release and impact on the soil pH.

SoA releases nitrogen to the soil as ammonium NH_4^+ . After which it is then transformed to nitrate nitrogen by bacteria. The process of this transformation depends on factors regulating microbial activities such as warm temperatures, moisture, and organic materials.

The breakdown of urea starts immediately after it is applied to the soil whenever there is sufficient soil moisture available with the help of the enzyme urease. After two to four days the urea hydrolyzes and is transformed into ammonium and carbon dioxide. This process could happen faster in more alkaline soils.

The ammonium released from Urea is more susceptible to volatilization losses to the environment after application to the soil. When urea is applied to wet soils, ammonia volatilization is enhanced. However, Flooding the soil immediately after urea application helps reduce these losses. Ammonium sulfate, unlike urea, is more resistant to volatilization loss, which is a major concern when urea is applied to muddy soils. The nitrogen loss was only approximately 5% when ammonium sulfate was employed instead of urea, and the yield loss was insignificant when the flood was postponed (Wilson, 2003). This finding suggests rice can

greatly benefit from ammonium sulfate as a nitrogen source. This is mainly true for fields that must be flooded over a long of time.

Soil acidity is a major soil attribute that influences the uptake of nutrients from the soil and productivity in rice farming. The type of chemical N fertilizer the farmers use over a period of time has a significant impact of the PH of the soil. Application of SoA and Urea has an acidifying effect on the soil. From A field experiment Lungu & Dynoodt (2008) observed a significant increase in soil acidity over controls plots after four years application of urea. Among all the commonly used N fertilizers on rice fields the acidifying effect of SoA on the soil is the greatest. Continuous use of SoA could significantly reduce the soils pH, thereby reducing uptake of certain nutrients in the long run.

One Major advantage SoA has over Urea is the sulfur it supplies to the soil. Sulfur is essential to produce chlorophyll, amino acids and lipids. Sufficient sulfur levels are required for maximum test weights, protein, and yield levels in wheat, according to studies. According to Dillon *et al.* (2012), because of the S when SoA is applied tillering is enhance and canopy closure is hastened about four days earlier than when Urea is applied.

The application time of SoA appears to be more sensitive than that of urea. In a rice field experiment, it was reported that SoA enhanced growth and yield *Calrose* Rice better than urea when the fertilizer application is than before permanent flooding. However after permanent flooding, urea was found to be superior to SoA until panicle initiation where the performances of both fertilizers were identical (Heenan & Bacon, 1987). They suggested SoA is a better option

for N fertilizer choice when the application is going to be done before permanent flooding.

While after permanent flooding Urea is a better choice.

After SoA dissolves the ammonium cations binds bonds quickly and strongly to the nearest clay particles. Unlike SoA, for urea after dissolution the cations are not strongly bonded to it and may be transported deep into the soil profile beyond the reach of the roots of the developing rice seedling, while the remaining left on the soil surface is prone to volatilization. However at the latter growth stages of the rice the rice root becomes more dense and developed and can reach the ammonium cations that moved down the soil profile (Heenan & Bacon, 1987). Due to this phenomenon N uptake from the SoA is higher than Urea only for the early stages of rice development

2.8 ORGANIC SOURCES OF NITROGEN

The use of organic sources of nitrogen in rice cultivation has been around for a long time. In 1995 the intensive use of organic sources to supplement chemical fertilizers reached its peak in Japan. However, there was a steady decline in the use of organic fertilizers due to the low cost and abundance of chemical fertilizers. An average of 6.5 t/ha and 4.5 t/ha of compost was reported to be used on in rice cultivation in 1955 and 1970 respectively (Datta, 1981).

In recent years the use of organic sources in rice has received a lot of attention. This is mostly due to the fear that the continuous use of chemical fertilizers may result in buildup of soil acidity and deteriorate the soil productivity. For example, the continuous use of ammonium sulfate for a long time on the same soil increases soil acidity (Datta, 1981).

Some of the popular organic sources of nitrogen used in rice farming are composts, farmyard manure and green manure. Javier *et al.* (2002) reported a steady increase in rice yield after 6 continuous cropping seasons with Organic fertilizer application, however the yield of all fertilized plots were significantly greater than unfertilized plots. The increase in yields of organic chicken manure over inorganic fertilizers after the third year advocates the need to integrate the organic fertilizers in order to maintain a sustainable yield.

Compost refers to a mixture of decomposed organic materials. The mixture could consist of organic substance ranging from crop residues, kitchen waste and animal dropping or dung. In rice production the kind of compost mostly used by farmers contains some amount of rice residues because they are readily available to them. Rice residues-based compost is made of a mixture of rice straw, rice husk, animal droppings and leguminous residues.

The application of compost has a positive influence on grain yield through improving on the fertility of the soil (Kadoglidou *et al.*, 2019). Hope (2005) recorded an increase in grain yield and nutrient uptake in NERICA 1, as a result application of compost. The plant height, tiller number and straw weights were also affected by the compost application. Kadoglidou *et al.* (2019) observed an increase in rice height (8%–64%) and biomass (32%–113%) after they applied 80, 160, 320 kg ha⁻¹ of compost. The 160 kg ha⁻¹ rates gave significantly greater yields than the 80kg/ha, however it was not significant difference from 320 kg ha⁻¹.

Green manure is another important and popular organic source of nitrogen in rice production. In this system of rice cultivation leguminous crops are grown as cover crops and slashed to decomposed in order to improve the nitrogen content and organic constituent of the soil weeks before the rice is transplanted (Efretuei, 2016). Azolla is one of the most common green manure

used in rice production. Azolla, also referred to a duckweed fern, is an aquatic fern that forms a symbiotic relationship with blue – green algae (called *Anabaena azollae*) which capture atmospheric nitrogen and convert it to ammonia which is absorbed by the Azolla. The atmospheric nitrogen taken by the azolla becomes accessible to the rice plant after the decomposition of the Azolla. The use of green manuring in rice production is a popular nitrogen fertilization practice in China and many other countries (Efretuei, 2016).

Farmyard manure has also been used as alternative source of organic nitrogen by farmers. It is made up of animal dung, urine, straw and litter used as bedding material. The effectiveness of the manure on improvement of rice yield depends on the nutrition content of the dung or litter present in the manure. The use of farmyard manures generally increases the concentration of the soil organic carbon which may reflect in higher yields and consequently increases nitrogen content in the soil (Zhe *et al.*, 2018).

2.8.1 The effect of poultry litter on growth and yield of rice

The term poultry litter refers to the waste produced from poultry farming. It consists mainly of chicken or turkey manure mixed with, urine, spilled feed, straw and sawdust used as bedding material. Poultry litter has a variable moisture content ranging from 40-45% and rich in nutrients such as nitrogen, potassium, phosphorous, magnesium and chlorine (Belefant-Miller, 2007; Chastain *et al.*, 2021). 70% of the N and 90-100% of P and K in poultry litter are made available for crop uptake during the first year of application (Hossain *et al.*, 2010). The nutritional composition of the various nutrients and the moisture content varies greatly based the feed.

The application of zinc rich poultry manure helps to correct and avoid zinc deficiencies on rice fields. It is suggested to rice farmers to apply their locally available poultry manure in order to

improve the yield and grain quality on the zinc deficient soils. The litter from the poultry farms are rich in zinc because the feed stock used by the poultry farmers are rich in zinc (Chastain *et al.*, 2021). This makes poultry manure a very important source, when picking an organic Nitrogen fertilizer that can improve zinc availability to rice.

Poultry litter is used by farmers as soil amendment to increase soil organic matter and nutrient. The application rates of the litter are based on the N, P and K percentages after analysis (Sistani *et al.*, 2004). In places where poultry litter is readily available rice, farmers prefer using it because it is a relatively cheaper source of both micro and micronutrients such as nitrogen, potassium, phosphorous, zinc etc. A number of studies have shown on the benefit application of poultry litter on the growth and yield of rice (Wiatrak *et al.*, 2004).

Poultry litter was proven to be more effective in rice production than swine and cattle manure in enhancing yield and biomass of rice (Eneji *et al.*, 2001). Javier *et al.* (2002) reported chicken manure to be as good as chemical fertilizer when it comes to providing nutrients for rice uptake and yield. Belefant-Miller (2007) reported that poultry litter induces and increases tillering in low and high yielding rice varieties, resulting in high yields. poultry litter result in a significant increase in tillering when used to fertilize rice, however the main obstacle in use of poultry litter is its slow releasing rate (Eneji *et al.*, 2001).

Apart from the mineral composition of poultry litter the age of the litter also influences its efficiency. In an experiment that evaluated the effect of the age of poultry litter on yield of Boro rice, 30 days old litter produced significantly higher grain yield and nutrient uptake than 60 days, 90 days, 120 days and 135 days old poultry litter (Hossain *et al.*, 2010). They also reported

a significant decrease in the nitrogen content from 2.25 at day 0 to 0.55% at day 135 due to the decomposition of the litter.

The addition of poultry manure increases the soil pH, N ammonification, base saturation, and phosphorus, potassium, calcium, and zinc content more when compared to the use of inorganic fertilizers (McGrath *et al.*, 2010). Schmidt & Knoblauch (2020) reported that Soil fertility and chemical properties of the soil were altered on rice fields after 5 years of continues poultry manure application. Although the poultry manure improved the soil fertility it did not favor Mineral nitrogen use efficiency. This resulted to a relatively lower grain yield compared to synthetic fertilizers.

2.9 RESPONSE OF RICE TO ZINC FERTILIZER

Zinc is a key nutrient required for numerous metabolic and biochemical activities in rice plants. When the available zinc in the soil for crops is low, the productivity of crops is impaired. Zinc deficient soils may be caused by a number of factors such as high soil pH, High HCO₃, high soil organic matter content, high magnesium to calcium ratio, Excessive liming and application of excessive P fertilizer which results in the formation of zinc phosphate (Wissuwa *et al.*, 2006; yara, 2018). Rice can recover from zinc deficiency when the right zinc fertilization strategies are employed immediately after zinc deficiency symptoms are noticed on the field. On all soils except alkaline soils, the effect of Zn application to soil can persist up to 2 to 5 cropping seasons. For alkaline soils, Zinc fertilizers needs to be administered to each crop to ensure high efficacy (Balasubramanian *et al.*, n.d.).

Symptoms of zinc deficiency starts to manifest about two weeks after transplanting. Some of the reported symptoms for rice include, delay in maturity, decrease in number of filled grains,

appearance of dusty brown to reddish spots on chlorotic young leaves, stunted growth, uneven growth among the plant population and reduction in yield (Impa & Johnson-Beebout, 2012; Wissuwa *et al.*, 2006). Tillering diminishes or even stops when there is a significant Zn deficit (yara, 2018).

These deficiencies are due to the limiting amounts or the absence of zinc for certain metabolic processes in the rice plant. Zinc is involved in several important physiological and biochemical processes in the rice plant, including chlorophyll formation, protein synthesis, enzyme activation, lipids, gene expression, pollen formation and carbohydrate production (Chang *et al.*, 2005; Mengel & Kirkby, 2012; Rehman *et al.*, 2012). The role zinc plays in these processes make zinc fertilization a key contributing factor to the growth, yield and quality of rice.

2.9.1 Effect of zinc application on growth of rice

Zinc is involved in metabolic and biochemical activities which are responsible for the determination of the growth and yield of rice. A number of studies have shown the impact that zinc fertilization can have on growth and yield attributing characteristics such the number of effective tillers, plant height, leaf area index, growth rate, biomass accumulation, grains per panicle and grain yield.

The growth rate, grain milling quality and net assimilation varied for different zinc fertilization treatments for rice under irrigated ecology (Ghasal *et al.*, 2016). Applying zinc at 3 kg ha⁻¹ resulted in a substantial increase in the production of IAA, an essential growth hormone responsible for plant development, which eventually enhanced growth (Jat *et al.*, 2018). The chlorophyll content in rice leaves was markedly increase by zinc fertilization (Kandoliya *et al.*, 2018).

Zinc fertilizer application on zinc deficient soil is most likely to result in the increase in total dry matter production. Kalala *et al.* (2016) observed that three (Kisawasawa, Mbaso-2, and Mang'ula-1) out of 10 soils gave a significant increase in dry matter yield of rice, when 5 and 10 mg kg⁻¹ of zinc was applied. Two out of the three soils that responded to zinc fertilization had a zinc level below 1.5 mg kg⁻¹, however zinc fertilization was not expected to affect the third soil which was taken from Kisawasawa because it had zinc level of 2.6 mg kg⁻¹ which was far above the determined soil critical zinc level of 1.4 mg kg⁻¹. In a similar study when 5 and 10 mg Zn kg⁻¹ were applied to soils by (Msolla *et al.*, 1994), nine out of ten soils with Zn levels below 1.1 mg kg⁻¹ DTPA- Zn showed substantial dry matter yield responses. Zinc fertilization did not affect the dry matter yield of the tenth soil because the zinc concentration in the soil was above the critical zinc concentration of the soil (Zare *et al.*, 2009). In contrast, Fageria *et al.* (2011), did not observe any significant effect on dry matter production after the application of 5 to 120 mg Zn kg⁻¹ on acidic soil with 1.4 mg Zn kg⁻¹ level. The lack of response to zinc fertilization could be as a result of decrease in the availability of soluble zinc due to continuous flooding.

A number of researchers have reported responses of rice straw to zinc fertilization on soils with low levels of zinc. Straw yield was significantly increased, after the application 4.2 mg Zn ha⁻¹ on a soil with zinc level of 0.55 mg Zn kg⁻¹ (Kandali *et al.*, 2015). Straw yield was significantly increased in an Inceptisol, by the application of 5 mg Znkg⁻¹ (Fageria *et al.*, 2011). An increase in both total weight and zinc concentration in rice straw was observed in response to soil plus foliar application of zinc (Ghasal *et al.*, 2016).

The involvement of zinc in cell growth and protein synthesis suggest that zinc fertilization may influence the plant height of rice. Rice plant height was increased by soil and foliar fertilizer

application of zinc when compared to unfertilized plots (Rao *et al.*, 2019). They suggested the response of plant height to zinc fertilization might have been because of the role zinc plays in root development and internode elongation. Similarly, Kadam *et al.* (2018) and Kandoliya *et al.* (2018) reported increase in rice plant height in response to the combined application of zinc sulphate and ferrous sulphate.

Studies have shown that zinc fertilization has an impact on the leaf area index and chlorophyll content in the leaves. The highest leaf areas of 13.74 and 10.92 at 60 DAT and 120DAT respectively, were recorded for plots with soil application of zinc sulphate over the foliar application of zinc and the control. Similarly, Veer *et al.* (2020) reported a significant effect of zinc fertilization on the leaf area index of rice.

Application of zinc on low zinc soils can increase tillering in rice. Zinc treated plots statistically gave a higher number of tillers per plant compared to the control group (Khan *et al.*, 2007). The treatment receiving 10 kg Zn ha⁻¹, recorded the highest number of tillers per plant at 17.41. Zinc may have enhanced enzymatic activity and auxin metabolism in plants, which explains the increase in tillering observed. Kadam *et al.* (2018) reported that combined application of zinc and iron to the soil enhances tillering in rice. In a field experiment, plots treated with a combined application of zinc sulphate and foliar application of zinc sulphate recorded the greatest number of productive tillers (11.33) per hill (Rao *et al.*, 2019). The increase in the number of effective tillers could be justified by increases in the rate of photosynthesis, excessive buildup of sucrose and glucose, and enhancement of fructose content in leaves which consequently resulted in increase in zinc uptake and availability to the plant.

2.9.2 Effect of zinc fertilization on rice yield

The yield of cereal crops can be reduced by up to 80% when grown on zinc-deficient soils

(Cakmak et al., 1997). In rice the response of zinc application is primarily based on the level of zinc in the soil, soil pH and water management strategy employed on the field. For instance, zinc deficiency is more common in calcareous soils than on acidic soils. In a field experiment conducted on a calcareous soil, soil application of Zn at 4 mg kg⁻¹ and 6 kg ha⁻¹ yielded the highest rice grain yield (Kausar et al., 2001).

Zinc application is needed to optimize yield especially on zinc deficient soils. Out of 10 soils studied, zinc treatment statistically improved rice yield in four (Mbasa-1, Mbasa-2,

Mang'ula-1, and Magombera) which had Zn levels below 1.3 mg kg⁻¹ and (Kalala *et al.*, 2016).

The lack of response in yield to zinc fertilization in the remaining soils was primarily due to their relatively higher levels of zinc within them. In the same study the application zinc at 10 mg Zn kg⁻¹ resulted to decrease in yield when the fertilizer was applied on soil with zinc above 3.0 mg kg⁻¹. This suggests that high concentrations of zinc may be toxic to the soil. In support of this (Sakal *et al.*, 1982) observed decline in rice yield after the application of 5 and 10 mg Zn kg⁻¹ calcareous soil with high levels of Zinc .

Several researchers have proven that zinc fertilization can play a significant role in rice yield determination. In a study by (Rao *et al.*, 2019), a combined application of zinc sulphate, foliar zinc EDTA and iron EDTA recorded the highest yield at 5.52 tones/ha. Cakmak (2008) found that soil, soil + foliar, and seed + foliar treatments with zinc all resulted in the significant increases in grain yield relative to plots that did not receive any zinc treatment. The application of zinc had a significant impact on grain yield aromatic rice, however, harvest index was not significantly influenced by zinc fertilization (Veer *et al.*, 2020). In a field study, rice yield was reported to be 4.75 t ha⁻¹ without Zn application, however the grain yield increased to 5.75 t ha⁻¹

¹ after application of 25 kg ZnSO₄ ha⁻¹ (Salam & Subramanian, 1993). It was reported from earlier studies that folia application of zinc increases the grain yield and quality of rice (Beutler *et al.*, 2014; Phattarakul *et al.*, 2012; Rehman *et al.*, 2012)

A number of researchers have tried to explain the mechanisms through which zinc application influences grain yield. The most obvious explanation is that zinc fertilization increases tillering in rice which result in the increase in the number of panicles/m². According to Fageria *et al.* (2011) the responses of rice to zinc application, is because zinc is involved with enzymes related to reproduction. Zinc fertilization has been observed to increase viability of pollen grains (Jat *et al.*, 2011; Karim *et al.*, 2012). Zinc is actively involved in seed production and pollen formation in rice, which consequently results in the increase in the grains per panicle. The beneficial impact of zinc on yield could also be attributed to its catalytic or stimulatory action on the majority of plant physiological and metabolic processes (Rao *et al.*, 2019).

2.9.3 The effect of zinc application on the grain quality

Zinc concentration in rice grains is affected by both genetic and environmental factors. Zinc content in rice gains ranging from 10 to 22 mg/kg has been reported for rice produced under aerobic condition on zinc deficient soils (Jiang *et al.*, 2008). In an experiment that evaluated the concentration of zinc in 99 genotypes, the zinc concentration ranged from 7.3 to 52.7 mg/kg (Sanjeeva Rao *et al.*, 2020). Zinc concentration in the polished grains was also observed to be ≥ 28 mg/kg. In a related study Gregorio *et al.* (2000) reported the concentration of zinc within 1138 accession of rice to be 14–58 mg/kg.

A significant amount of the zinc in the gains is loss through milling and polishing of brown rice. Although the zinc concentration in rice is around 20-25 Zn mg/kg its concentration in polished rice is 16-17 mg/kg (Duffner *et al.*, 2014; Martínez *et al.*, 2010). An average of 24% of the zinc is loss during milling (Palmgren *et al.*, 2008). Polishing of the rice also result in further zinc losses before human consumption.

A number of studies in the past have looked into the potential of improving zinc content in rice through zinc fertilizer application mostly on zinc deficient soils. Zinc fertilization is reported to result in highly significant increase zinc concentration in wheat (Cakmak, 2008). For rice, zinc fertilizer application has not been as successful in enhancing the grain quality as has been reported for wheat and other cereals. For example, Zinc applied in the form sulfate ($ZnSO_4$) at 23 kg/ha had no significant effect on grain zinc content either for upland or lowland rice (Gao *et al.*, 2006). similar result where zinc fertilization only marginally improved grain zinc content under anaerobic conditions (Srivastava *et al.*, 1999).

The lack of response to the fertilizers may be due to physiological barriers which prevent the movement of the zinc to the grains during filling. Radiolabeling with ^{65}Zn in rice showed that a large percentage of zinc in grains originates from zinc root uptake after flowering and not remobilization in the plant (Jiang *et al.*, 2007). The results obtained suggest applying zinc fertilizers the plant after flowing to be the most effective means of increasing zinc content in rice grains. A study by Wu *et al.* (2010) associated zinc concentration in rice to the plant ability to retranslate zinc into the grains instead of improved root uptake.

In contrast to the previous studies zinc fertilizer application was reported to have increase the grain zinc concentration, however the rate of increase is usually insignificant in terms of the nutrition requirements of humans. Combing soil and folia application is known to be the most effective method of agronomically improving grain zinc content in brown rice. Yin *et al.* (2016) reported that Zinc fertilizer application increased the brown rice zinc concentration only by 20%, however there was 100% increase in zinc concentration in vegetative parts. The wide gap between zinc concentration in the vegetative parts and the grains means that internal translocation of Zn from shoot to panicle or from rachis to grain is the primary cause of low concentration of zinc in the grains, rather than root uptake of Zn from the soil. Zhu, (2019) in a pot experiment, observed a significant increase of zinc in brown rice at 10 and 15 mg Zn when compared with zinc at rate 0, 5 mg Zn.

2.10 IMPORTANCE OF ZINC BIOFORTIFICATION OF RICE

Zinc deficiency is very prevalent in crops as well as well as humans. The extent of zinc deficiency across the world is depicted in Fig 1.0. 20 million people are estimated to be affected by zinc deficiency, among which children and pregnant women are the most vulnerable (Brown *et al.*, 2004). Zinc deficiency was responsible with 14.4% of diarrheal deaths, 10.4% of malaria deaths, and 6.7 percent of pneumonia deaths in African children 6 months to 5 years (Berhe *et al.*, 2019). One of the main causes of zinc deficiency in the human population is as a result of the high daily consumption of cereals which are low in zinc concentration (Hotz, 2009). In latest development, claims have been made that the consumption of zinc biofortified food can be used in the fight against the COVID 19 pandemic (Celik *et al.*, 2021). Clinical investigations for the

prophylaxis / treatment of COVID-19 using zinc alone or in combination with other medications are now underway (Doboszewska *et al.*, 2020).

Rice biofortification with zinc offers a way for enrichment of the zinc concentration in rice for the benefit of the human population, through agronomic and genetic intervention (Graham *et al.*, 2001). After evaluating the zinc content of almost 100 genotypes the zinc concentration ranged from 15.9 to 58.4 mg/kg (Graham *et al.*, 1999). Gao *et al.* (2006) reported that the average zinc content in rice grain ranges from 10 to 30 mg/kg (Rerkasem *et al.*, 2010). The reported average zinc content is relatively on a low in respect to human nutritional required.

Two approaches are widely acknowledged as a feasible means of increasing Zn content in grain and other edible plant components. The first strategy is through genetic biofortification. This involves breeding of rice varieties that can accumulate high zinc content in their grains. There have also been several attempts to genetically develop zinc biofortified rice varieties with sufficient concentration of zinc which would contribute to meeting the daily zinc requirements of the population. The aim of the genetic improvement is to increase grain zinc content to around 40–50 mg/kg (Graham *et al.*, 2007). The second strategy for zinc biofortification in rice is through agronomic methods. Agronomical biofortification involves employing proper timing, methods, sources and rates of zinc fertilizer in rice farming in order to improve the uptake of zinc from the soil and translocation into the grains. Physical fortification can also be achieved by parboiling with Zn.

2.11 THE DYNAMICS OF ZINC AVAILABILITY AND UPTAKE IN RICE

The amount of zinc across different types of soils depends on factors such as parent material, human activities(fertilization) and atmospheric decompositions (Alloway, 2008). Zinc in the

soil exist in different chemical forms which regulates the availability of zinc to the plant. Zinc is readily available to the plant in its soluble form. According to Barber (1995), the average concentration of water-soluble zinc in the soil ranges from 4×10^{-10} to 4×10^{-6} M. On rice fields soil pH, phosphorous status, redox potential, organic matter content and water management practices are very key element that influences the solubility and availability of zinc to crops.

The soil available zinc and pH are inversely related. The availability of zinc is reduced as pH increases, due to this zinc deficiency is very common in alkaline and calcareous soils with pH greater than 8 (Srinivasa Rao *et al.*, 2008). Zinc availability is also affected by the soils redox potential and zinc status. At low redox potential there is a high probability of precipitation of Zn to ZnS which results in the decrease in the quantity of available zinc on calcareous soils (JohnsonBeebout *et al.*, 2009)

The soil undergoes physical, chemical and biochemical changes after flooding which has serious implications on the availability of zinc for the rice plant (Rehman *et al.*, 2012). For instance, when rice fields are flooded with water in acidic soils there is a decrease in redox potential and decrease in PH, while on Alkaline soils there is a decrease in pH and chemical reduction of both micro and macro nutrients (Xu *et al.*, 2003). The rate of the chemical changes that occurs depends on the soils physical properties, the rhizosphere temperature and water management practices on the rice field (Rehman *et al.*, 2012). As a result of these physical, chemical changes and biochemical changes after flooding the concentration of soluble zinc decreases, although it temporarily increases immediately after flooding (Mikkelsen & Kuo, 1977). This decrease is associated with precipitation of $Zn(OH)_2$ with an increase in pH, High concentration, formation of ZnS in acidic soils and $ZnCO_3$ in alkaline soils (Bostick *et al.*, 2001).

Another factor that influences the availability of zinc in the soil is the P status of the soil. At high soil Phosphorus levels zinc availability in the soil is reduced. The phosphorous interacts with the zinc which leads to decrease in zinc translocation from the roots to the shoot (Rehman *et al.*, 2012). In a greenhouse experiment conducted by Mandal & Mandal (1990), increase in P application reduced water soluble zinc and exchangeable zinc under both flooded and non-flooded condition. They also observed that P fertilization had a more detrimental effect on rice in non-flooded condition as compared to flooded conditions (Almendros *et al.*, 2008).

2.12 INTERACTION OF ZINC WITH NITROGEN

Several studies have emphasized on the role nitrogen fertilization plays in zinc uptake by the roots and accumulation of Zinc in edible parts of a crop. This has resulted in nitrogen receiving special considerations in yield and quality improvements when it comes to zinc nutrition. Zinc and nitrogen have been reported to have a synergetic effect towards the yield of aromatic rice varieties. (Shahane *et al.*, 2018). Arora & Singh (2004) observed a significant interaction effect on yield for Zinc and nitrogen on the grain yield of Barley (*Hordeum vulgare L*)

In a two years field experiment Hossain *et al.* (2010) compared the effects of organic fertilization (48 m³ ha⁻¹ of compost) to a combined application of different Nitrogen levels (0, 72 and 144 kg N ha⁻¹) and zinc rates (0, 20 and 40 kg Zn ha⁻¹) on yield and nutrient uptake of rice. Increasing the rates of zinc and nitrogen fertilization resulted in the increase of grain yield, plant height, straw yield and dry matter content. On the other-hand compost application recorded a higher value than the mixed application of zinc and nitrogen in the previous parameters. They also confirmed nitrogen zinc interaction has positive effects on grain yield and yield parameters.

From a study by Hakoomat *et al.* (2014) a combination of 120 kg N ha⁻¹ and 14 kg Zn recorded the maximum grain yield.

The source of N applied on rice fields affect the availability and uptake of zinc from the soil, mostly through the regulation of the soil pH. For instance, urea and ammonium-based fertilizer influenced anion-cation uptake ratio and lowered the PH in the rhizosphere (Broadbent & Mikkelsen, 1968). According to Kirk & Bajita (1995) zinc availability to rice under flooded conditions is improved due to increase in H⁺ of extrusion by the roots. Under the same conditions the uptake of ammonium is accompanied with the release of H⁺ ions which leads to the reduction of pH (Rehman *et al.*, 2012). The reduction in the PH of the rhizosphere is very imported in alkaline soils which reduces the availability of zinc. On the other hand, in lowland rice cropping nitrogen is taken in a form of nitrate (NO₃) which leads to the release of OH⁻ ions. The release of OH⁻ ions increase the pH of the rhizosphere which results in decrease in zinc uptake of the roots (Gao *et al.*, 2006).

Lindsay, (1972) suggested the formation of Zn-NH₃ at high pH may increase the solubility thereby increasing zinc availability. The results from the previous studies implies the need to select the most appropriate source of nitrogen fertilizer based on the prevailing soil conditions and water management practices in order improve zinc availability (Gao *et al.*, 2012). For example, the use of Ammonium sulfate under on Acauline calcareous helps decrease the soil PH thereby increasing the availability of zinc.

In addition to N source, appropriate timing and rate of N application in combination with the right source and rate of zinc fertilizer improves zinc availability in the soil for rice and increases accumulation of zinc during grain filling. Nitrogen can influence zinc status in plant is through enhancement of root growth. The increase in N rates increases root growth which may improve

zinc uptake (Giordano, 1979). In a field experiment that evaluated effects of ZnSO₄ and Zn-EDTA through folia and soil application in combination with four rates of N (0, 30, 60, 90 kg ha⁻¹) on lowland rice cultivation, the highest nitrogen level at in , 90 kg ha⁻¹ combination with zinc recorded the greatest N and Zn uptake and grain yield (Khanda & Dixit, 1996). They also revealed that soil application was superior over folia application. The source of N fertilizer did not show any significant impact on zinc bioavailability in wheat however combining it with 0.25% folia applied zinc significantly increased zinc bioavailability (Chattha *et al.*, 2017).

It is important to note than some of the mechanisms involved in Nitrogen zinc interaction in the soil remains unclear and more studies should be done on the topic. Furthermore, nitrogen and zinc have been declared as the two most yield limiting nutrients in rice production and also 50% of the rice-growing soils are zinc deficiency (Rehman *et al.*, 2012).

2.13 ZINC TOXICITY IN RICE

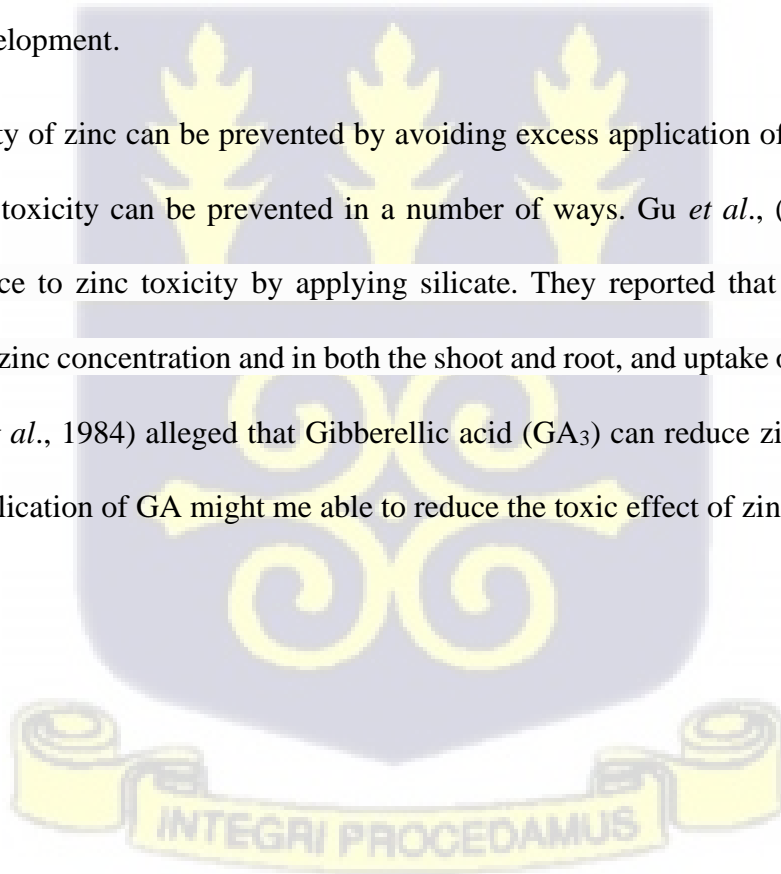
Zinc is a micro-nutrient needed in small quantities for healthy development and productivity of the crop. However larger quantities of zinc can be toxic to the plant by interfering with uptake, transport, and equilibrium of important ions and other metallic activities (Borkert *et al.*, 1998; Chaney, 2010)

Zinc is regarded as a phytotoxic element for a number of cereals and legumes such as maize, soyabean, rice and peanut. (Benton Jones Jr, 1991) claimed that cereals are extremely sensitive to Zinc and higher concentrations ranging from 100 to 400 mg kg is likely to have a toxic effect of them. The minimum amount of any element in the plant that can reduce yield is regarded as

the critical toxic level (CTL). In an experiment conducted in the green house (Borkert *et al.*, 1998) concluded the zinc CTL in the soil to be between 158 and 318 mg/dm³ rice. He reported the plant Zn CTL for soybean and peanut to be 230 mg kg⁻¹ and 140 mg kg¹.

Phytotoxicity in rice is manifested in a variety of ways in rice. Major symptoms of zinc toxicity in rice include drop in flowering, leaf necrosis and decline of growth rate (Debruyne & McIllrath, 1996). According to Patterson (1971) the cultivation of cereal susceptible to zinc toxicity such as maize and rice on soils with zinc concentration beyond 150 mg/kg might result in yield reduction. (Nag *et al.*, 1984) also emphasized that application of zinc fertilizers can results in yield reduction in rice. They also reported that excess soil zinc levels can significantly reduce water uptake capacity of the soil and impair root growth in and chlorophyll development.

The phytotoxicity of zinc can be prevented by avoiding excess application of zinc fertilizers on the fields. Zinc toxicity can be prevented in a number of ways. Gu *et al.*, (2012) was able to increase tolerance to zinc toxicity by applying silicate. They reported that the application of silicate reduced zinc concentration and in both the shoot and root, and uptake of excess zinc from the soil. (Nag *et al.*, 1984) alleged that Gibberellic acid (GA₃) can reduce zinc inhibition. This suggest that application of GA might me able to reduce the toxic effect of zinc.



CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION OF EXPERIMENTAL SITE

3.1.1 Study area

A field and a pot experiment were conducted at the University of Ghana, Soil and Irrigation Research Centre (SIREC) from September 2020 to February 2021. The research center is located 8 km away from the township of Kpong in the Eastern Region of Ghana. The center lies within the lower volta basin of the coastal savanna agroecological zone on latitude $6^{\circ} 09' N$, longitude $00^{\circ} 04' E$, and at an altitude of 22 m above mean sea level (Figure 3.1).

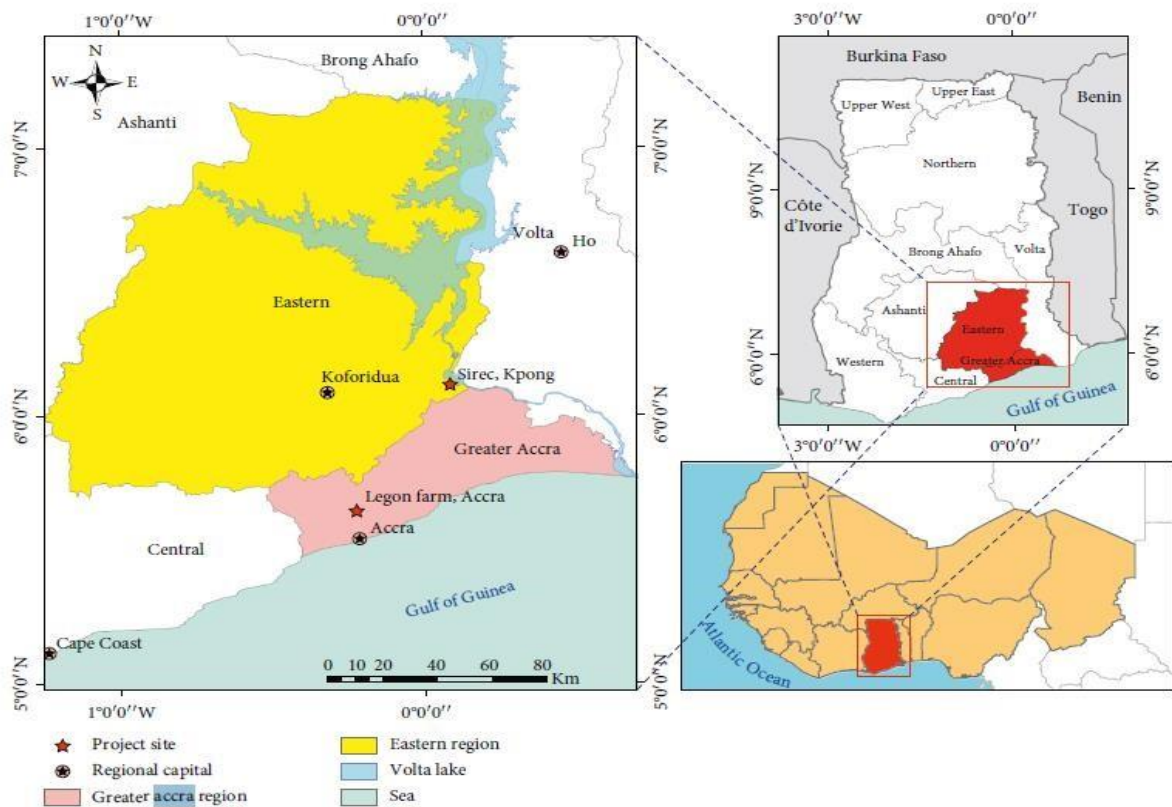


Figure 3.1: Map of Ghana showing the location of SIREC-Kpong (Map adapted from MacCarthy *et al.* (2018)).

The average annual rainfall at the experimental site ranges between 800–1326 mm (Table 3.1).

The site has a bi-modal rainfall pattern, with a brief drought in August, a minor rainy season (September–November), and another drought period (December–February) (MacCarthy *et al.*, 2018).

The average yearly temperature at the center is around 27 °C, with a high temperature of around 34 °C during the day. The Relative humidity at night ranges from 60 to 90 % and 20 to 55 % during the daytime.

The vegetation of the Research Center is savanna grassland with scattered shrubs and deciduous trees. The plants in the zone can acclimatize during drought period and harsh edaphic conditions, when the soil becomes dry and starts to crack, because of their tough deep root system (Ametekpor & Dowuona, 1995).

The soils at the Research Center belong to the Akuse series. They are heavy, poorly drained and has a soil depth of about 150 cm. The FAO-UNESCO soil classification for the soils at the site is Calcic Vertisol (Fao, 1988). It is classified as Typic Calciusert by the USDA classification system (Ametekpor & Dowuona, 1995). Garnetiferous hornblende gneiss is the soil's parent material. Due to their montmorillonitic character, the soil swells and become sticky when wet, but shrinks and breaks severely when dry.

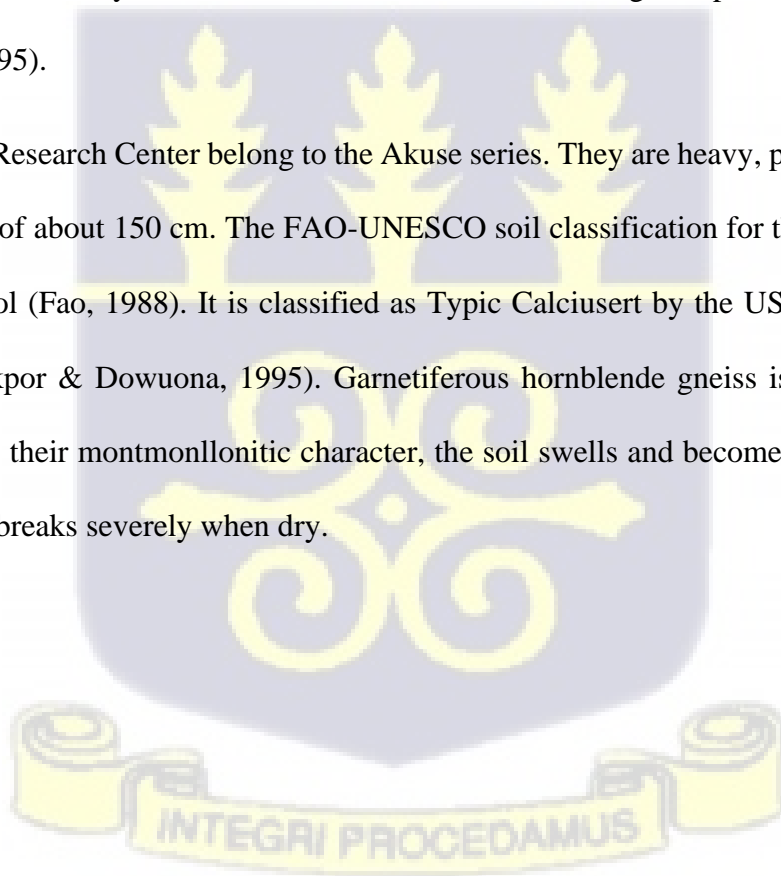


Table 3.1: Main edaphic and climatic features of experimental sites

Feature	Value
Coordinate Altitude	6°09' N 00°04' E
Altitude(m)	22
Rainfall (mm)	800–1326
Temperatures (°C), min (mean) max	22.1 (27.7) 33.3
Relative humidity, %	70–100
Soil type	Typic calciustert (tropical black clay, Akuse series)
Vegetation	Grassland
Slope (topography)	Gentle (1–5%)

NB: values in table obtained from MacCarthy *et al.* (2018).

3.1.2 Weather conditions during experimental period

Meteorological data (temperature (°C), humidity (%) and precipitation (mm)) during the period of the field experiment, from September 2020 to January 2021 was collected from a weather station on site. The data collected is presented in Table 3.2.



Table 3.2: Meteorological data registered at experimental site during experimental period

Year	Month	Maximum Temperature (°C)	Minimum Temperature (°C)	Total Rainfall (mm)
2020	September	33.8	28.5	142.2
2020	October	34	27	210.9
2020	November	34.5	31	132.2
2020	December	35	29.5	74.4
2021	January	35.2	31.5	35.2

3.1.3 Soil at Experimental field

The soil at the site of the field experiment is black to gray in color, heavy, poorly drained and sticky when wet. Clayey is the textural classification of the soil. A sample of the soil was taken at 0-20 cm depth for determination of physical and chemical properties. The soil at the site of the experiment was sampled and used for the pot experiment.

3.2 EXPERIMENTAL MATERIAL

The rice variety Legon Rice1 was used for both the pot and field experiments. The soil used for the experiment was obtained from the Soil and Irrigation Research Centre (SIREC). The variety Legon Rice1 is an aromatic rice with a maturity period of about 120 days.

Poultry manure was obtained from a nearby poultry farm at Kpong. A sample of the poultry manure was analyzed for its chemical properties.

3.3. NURSERY ESTABLISHMENT

A nursery was established for raising rice seedlings (Legon Rice1) for the field and pot experiments. A 1 m by 4 m wet nursery bed was constructed for raising the rice seedlings.

Rice seeds were pre-germinated by soaking for 24 hours in a dish of water. The water was drained and seeds were placed in shade for two days. The pre-germinated rice seeds were broadcast on the nursery bed. After Twenty- one days the seedlings were transplanted to the field and pots.

3.4 EXPERIMENT 1: POT EXPERIMENT

A pot experiment was designed to examine how the growth and yield of rice would respond to different chemical nitrogen fertilizers (Urea and Ammonium sulfate) and Zn application. The experiment was conducted in the open. Twenty-one days old rice seedlings were transplanted into plastic pots with a height of 36 cm and a diameter of 28 cm. Each pot was filled with 11 kg of soil sampled from the site of the field experiment.

3.4.1 Design and layout of experiment

A 2 x 6 factorial experiment was laid out in a completely randomised design (CRD). Nitrogen source and rate of zinc were the factors involved. The levels of nitrogen source included urea and sulfate of Ammonia (SoA). The rates of zinc included 0 kg ha⁻¹, 5 kg ha⁻¹, 10 kg ha⁻¹, 15 kg ha⁻¹, 20 kg ha⁻¹, 25 kg ha⁻¹. The 2 levels of nitrogen source were combined with the 6 rates of zinc to obtain 12 treatment combinations. Each of the treatment combinations was replicated three times to obtain 36 experimental units. Each experimental unit was represented by three pots.

Table 3.3: Treatment structure for the pot experiment

Treatment notation	N source (@ 120 Kg N /ha)	Zinc rate (kg Zn/ha)
Urea X Zn0	Urea	0
Urea X Zn5	Urea	5
Urea X Zn10	Urea	10
Urea X Zn15	Urea	15
Urea X Zn20	Urea	20
Urea X Zn25	Urea	25
SoA X Zn0	Sulphate of Ammonia	0
SoA X Zn5	Sulphate of Ammonia	5
SoA X Zn10	Sulphate of Ammonia	10
SoA X Zn15	Sulphate of Ammonia	15
SoA X Zn20	Sulphate of Ammonia	20
SoA X Zn25	Sulphate of Ammonia	25

NB: SoA = Sulphate of Ammonia

3.4.2 Soil sampling and filling of pots

Soil samples were collected from the site earmarked for the field experiment. The sampled soil was air dried and passed through a sieve with mesh size of 2.0 mm. 108 plastic pots (36 cm in height and 28 cm in diameter) were filled with 11 kg of the sieved soil each.

3.4.3 Crop establishment and pot management

Twenty-one days old seedlings of uniform sizes were transplanted into the pots. Two seedlings were transplanted to each pot. The pots were irrigated and about 3.5 cm water was maintained above the soil surface until about 10 days to harvest. The water was managed such that no flooded condition existed when fertilizer was applied. The water above the soil was able to control weeds throughout the growing period.

3.4.4 Fertilizer management

3.4.4.1 Nitrogen(N) and Zinc (Zn) fertilizer application

120 kg N/ha of nitrogen fertilizer was applied either as urea or sulphate of ammonia to each pot depending on the treatment specification of the pot (Table 3.4). The total amount of nitrogen fertilizer (120 kg N/ha) that was applied to the experimental units was split into two halves and applied separately at different times. Thus, 60 kg N ha⁻¹ was applied as basal (two weeks after transplanting) and the remaining 60 kg N ha⁻¹ applied as topdress (6 weeks after transplanting). The targeted N quantity (120 kg N/ha) of N fertilizer applied to the pots was estimated based on the bulk density and mass of soil in the pots.

Different rates of zinc were applied in the form zinc sulphate (ZnSO₄) fertilizer to the pots two week after transplanting (Table 3.4). The amount of chemical N or Zn fertilizer to be applied to the pot was determined using the following equation..

$$\text{Quantity of fertilizer per pot (kg)} = \frac{\text{mass of soil in pot (kg)}}{\text{mass of soil per hectare (kg)}} \times \text{Amount of fertilizer for a hectare (kg)}$$

$$\text{Amount of fertilizer for a hectare (kg)} = \frac{\text{Target rate } \left(\frac{\text{kg}}{\text{ha}}\right)}{(\% \text{ of nutrient in fertilizer } \times 100)} \times 100 \text{ kg of nutrient}$$



Table 3.4 Nitrogen and Zinc fertilizer specification for each treatment

Treatment	Quantity of N (kg N/h)	Quantity of N fertilizer (Urea or SOA) per pot (g/pot)	Quantity of Zn (kg Zn/ha)	Quantity of Zn sulfate applied (g/pot)
SoA x Zn 0	120	1.6	0	0
SoA x Zn 5	120	1.6	5	0.2
SoA x Zn 10	120	1.6	10	0.3
SoA x Zn 15	120	1.6	15	0.4
SoA x Zn 20	120	1.6	20	0.6
SoA x Zn 25	120	1.6	25	0.7
Urea x Zn 0	120	3.5	0	0
Urea x Zn 5	120	3.5	5	0.2
Urea x Zn 10	120	3.5	10	0.3
Urea x Zn 15	120	3.5	15	0.4
Urea x Zn 20	120	3.5	20	0.6
Urea x Zn 25	120	3.5	25	0.7

NB: % N in SoA = 21 % ; % Zn in ZnSO₄ = 22%

3.4.4.1 Phosphorus (P) and potassium (K) fertilizer application

The recommended rates of Phosphorus (P) and potassium (K) by SIREC were applied to rice seedlings. Thus, 45 kg ha⁻¹ of (P₂O₅) using triple superphosphate and 45 kg ha⁻¹ K₂O using muriate of potash were applied to each of the pots as basal fertilizer two weeks after transplanting.

3.4.5 Pest and diseases control

Some of the common pest of rice includes leafhoppers, birds and stemborers. Signs of stem borers was observed 10 weeks after transplanting. All the experimental pots were sprayed with a systemic insecticide K-Optimal (Lambda Cyhalothrine 15 g/L +Acetamipride 20 g/L EC) a day after the pests were noticed to control them.

Birds are major pest of rice and is necessary for every rice grower to control them. The activities of birds were observed at the experimental site. All experimental plots were covered with a net at the flowering stage of the rice to help prevent birds from feeding on the rice grains.

The rice plant in the experiment area did not show any incidence of diseases and as such no disease control measure was employed.

3.4.6 Data collection

3.4.6.1 Plant height:

Plant height for each pot was recorded at maximum tillering (27 DAT), booting (55DAT) and at maturity (119 DAT). The height of the plant was measured from the soil surface to the tip of the highest fully expanded leaf with a meter rule.

3.4.6.2 Number of tillers per plant:

The number of tillers in each pot was recorded at Maximum tillering (27 DAT), Booting (55DAT) and at maturity (119 DAT). The number of tillers per pot was counted manually by hand.

3.4.6.3 Number of effective tillers

The number of effective tillers for each pot was recorded at maturity (119 DAT). The number of effective tillers was determined by counting the number of tillers with panicle.

3.4.6.4 Above ground biomass

The above ground (shoot) biomass for each pot was recorded at harvesting. The plants were cut at the soil surface and oven dried at 70 °C for 48 hours to attain a uniform weight. An electronic scale was used to obtain the weights of the dried rice samples.

3.4.6.5 Days to 50 %flowering

The number of days to 50 % flowering was recorded for each pot by counting the number of days from seed emergence to the day 50 % the total number of tillers flowered.

3.4.6.6 1000-grain weight

The 1000-grains weight was recorded after seeds were air dried for 72 hours to a moisture content of 14 %. After drying, 1000 seeds were counted using the rice grain counter and weighed with an electronic scale.

3.4.6.7 Grains per panicle

The grains per panicles was determined after harvesting. Five panicles were randomly selected from each pot during harvesting. The selected panicles were threshed to separate the grains. After that the grains (including filled and unfilled) were counted using an electronic grain counter and divided by five to estimate the number of grains per panicle.

3.4.6.8 Percentage filled grains (%)

The percentage filled grains was derived from data collected from the total number of grains per panicle and number of filled grains per panicle. The number of filled grains was separated from total number grains obtained from 5 randomly selected panicles during harvest. The average for the five panicles was taken to estimate the filled grains per panicle. Mathematically the number of filled grains was calculated as;

$$\% \text{ filled gains} = \frac{\text{filled grains per panicle}}{\text{Total number of grains}} \times 100$$

3.4.6.9 Grain yield (g/pot)

All the plants from each experimental unit were harvested into separate sacks to determine the grain weight. The harvested samples were dried for two weeks and then threshed to separate the gains from the straw. The grains were dried to a moisture content of 14% and weighed on an electronic scale to determine grain yield per pot.

3.4.6.10 Straw yield(g/pot)

The straw obtained after threshing to separate the grains was oven dried at 70 °C for 48 hours. The dried samples were weighed for each treatment with an electronic scale to determine the straw yield per pot.

3.4.6.11 Panicle weight(g)

Five panicles were selected after harvest and weighed on an electronic balance to determine the average panicle weight for the five panicles.

3.4.6.12 Harvest index

Harvest index (HI) was determined using the formula;

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Grain yield} + \text{straw yield}}$$

3.4.6.13 Agronomic Zinc use efficiency

The agronomic Zn use efficiency (AZUE) was calculated using the following formula;

$$ZUE = \frac{\text{Grain yield of plots fertilized with Zn} \left(\frac{\text{kg}}{\text{ha}} \right) - \text{Grain yield of plots not fertilized with Zn} \left(\frac{\text{kg}}{\text{ha}} \right)}{\text{Amount of Zn applied (kg/ha)}}$$

3.5 EXPERIMENT 2: FIELD EXPERIMENT

A field experiment was conducted from September 2020 to January 2021 to examine how source of nitrogen and rate of zinc would influence the growth, yield, and zinc content in rice grains on a vertosol under an irrigated ecology.

3.5.1 Land preparation

The field was ploughed and puddled to break soil clods to reduce the permeability of the soil which reduces percolation losses. After puddling, the soil was levelled with a shovel and a rake. The field was be flooded with a shallow water layer, to help identify high spots during leveling.

3.5.2 Experimental design and treatment structure

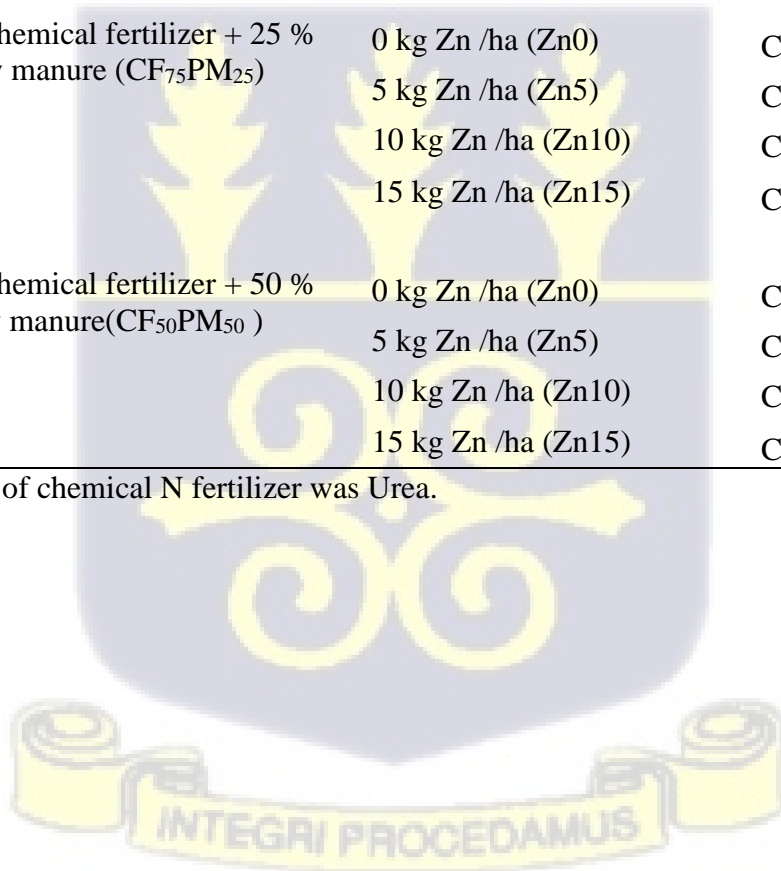
A 4 x 4 factorial experiment was conducted. The experimental design was randomized complete block design (RCBD), laid out in a split-plot arrangement. Four levels of sources of nitrogen were used as the main-plots whereas four rates of zinc served as the subplots. Each of the four levels of N sources (CF₁₀₀, CF₇₅PM₂₅, CF₅₀PM₅₀, No) was combined with 4 zinc rates (0, 5, 10 and 15 kg Zn/ha) to obtain 16 unique treatment combination made up of 4 main plots with 4 sub plots each. The experimental area was made of 48 experimental unit (plots) which comprised of 3 replicates. The details of treatment structure is specified below (Table 3.5).



Table 3.5: Description of treatment for field experiment.

N sources (Main plots)	Zinc Rates (Sub plots)	Treatment combination
No nitrogen application -Control (No)	0 kg Zn /ha (Zn0)	No x Zn0
	5 kg Zn /ha (Zn5)	No x Zn5
	10 kg Zn /ha (Zn10)	No x Zn10
	15 kg Zn /ha (Zn15)	No x Zn15
100 % N from chemical fertilizer (CF ₁₀₀)	0 kg Zn /ha (Zn0)	CF ₁₀₀ x Zn0
	5 kg Zn /ha (Zn5)	CF ₁₀₀ x Zn5
	10 kg Zn /ha (Zn10)	CF ₁₀₀ x Zn10
	15 kg Zn /ha (Zn15)	CF ₁₀₀ x Zn15
75 % N from chemical fertilizer + 25 % N from Poultry manure (CF ₇₅ PM ₂₅)	0 kg Zn /ha (Zn0)	CF ₇₅ PM ₂₅ x Zn0
	5 kg Zn /ha (Zn5)	CF ₇₅ PM ₂₅ x Zn5
	10 kg Zn /ha (Zn10)	CF ₇₅ PM ₂₅ x Zn10
	15 kg Zn /ha (Zn15)	CF ₇₅ PM ₂₅ x Zn15
50 % N from chemical fertilizer + 50 % N from poultry manure(CF ₅₀ PM ₅₀)	0 kg Zn /ha (Zn0)	CF ₅₀ PM ₅₀ x Zn0
	5 kg Zn /ha (Zn5)	CF ₅₀ PM ₅₀ x Zn5
	10 kg Zn /ha (Zn10)	CF ₅₀ PM ₅₀ x Zn10
	15 kg Zn /ha (Zn15)	CF ₅₀ PM ₅₀ x Zn15

NB: The source of chemical N fertilizer was Urea.



3.5.3 Field layout and crop establishment:

The experimental field consisted of a total of 48 plots and contained 3 replicates. Each replication comprised of 4 main-plots with four 4 sub-plots. The size of each plot was 3 m x 3 m, with a space of 1m between sub-plots and 1.5 m between main-plots. Bunds of size 250 mm x 300 mm were constructed around each plot to regulate the flow of nutrients and water outside the plots.

21 days old rice seedlings of uniform sizes were transplanted from the nursery to the plots. Two seedlings were transplanted per hill with spacing of 0.2 m by 0.2 m. This resulted to a total density of 196 hills per plot.

Details of experimental Layout:

Main-plot (N sources): 4

Sub-plot (Rates of zinc): 4

Replication: 3

Plot size: 3 m × 3 m

spacing: 0.2 m × 0.2 m between hills.

Rice variety: Legon Rice1

3.5.4 Fertilizer application

3.5.4.1 Nitrogen

Besides the control plots (No), 100 kg N/ha was applied in different forms and combinations depending on the N source specification of the plot (Table 3.6).

Table 3.6: Nitrogen fertilizer application combinations for the Main plots

Main plots - N source	Description of N Fertilizer application per plot
No	<ul style="list-style-type: none"> No nitrogen fertilizer application
CF ₁₀₀	<ul style="list-style-type: none"> 112.5 g urea (50 kg N/ha) applied 10 DAT as basal. 112.5 g urea applied at 37 DAT as top-dress
CF ₇₅ PM ₂₅	<ul style="list-style-type: none"> 2.6 kg of PM (25 kg N/ha) applied two weeks before transplanting 56.25 g urea (25 kg N/ha) applied 10 DAT as basal. 112.5 g (25 kg N/ha) of urea applied at 37 DAT as top-dress
CF ₅₀ PM ₅₀	<ul style="list-style-type: none"> 5.2 kg of PM (50 kg N/ha) applied two weeks before transplanting 112.5 g urea (50 kg N/ha) applied at 37 DAT as top-dress

NB: NB: % N in urea = 46 % ; % N in PM = 0.862 % ; *No*: No N fertilizer; *CF₁₀₀* : 100 % Chemical fertilizer (Urea); *CF₇₅PM₂₅* :75 % chemical fertilizer +25 Poultry manure (PM); *CF₅₀PM₅₀* :50 % Chemical fertilizer +50 Poultry manure (PM);

3.5.4.2 Zinc application.

Besides the control (Zn O) different rates of zinc fertilizer was applied to the sub-plots in the form of zinc sulphate (ZnSO₄) at 10 DAT.



Table 3.7: Quantities of zinc sulphate applied to sub-plots

sub-plots (rates of zinc)	quantity of zinc sulphate per plot(g)
Zn 0 kg Zn ha ⁻¹ (control)	0
Zn 5 kg Zn ha ⁻¹	20.45
Zn 10 kg Zn ha ⁻¹	40.91
Zn 15 kg Zn ha ⁻¹	61.36

% Zn in ZnSO₄ = 22%; Area of sub-plot = 9 m²

3.5.4.3 Phosphorus (P) and potassium (K) fertilizer application

The recommended rates of Phosphorus (P) and potassium (K) at research center (SIREC) were applied to the rice seedlings. Thus, 45kg ha⁻¹ of P₂O₅ using triple superphosphate and 45 kg ha⁻¹ K₂O using muriate of potash were applied to each of the plots as basal fertilizer at 10 DAT.

3.5.5 Irrigation of plots

Plots were irrigated regularly to maintain the water level around 3 cm from transplanting to the active tillering stage. The water level was gradually increased to 5-10 cm at the maximum tillering. After that the level was gradually decreased to about 2 cm after flowering. The remaining water was drained 7 days before harvesting.

3.5.6 Weed management

The plots were sprayed with a pre-emergence herbicide (Stomp) a week before transplanting to prevent weed competition with transplanted seedlings for water and nutrients. The flooding of water in plots was able to control the weeds throughout the growing period of the rice. Propanil,

a post-emergence herbicide was sprayed on the field twice at 8 and 12 weeks after transplanting to prevent the weeds from growing on the bunds.

3.5.7 Pest and diseases control

Some of the common pest rice is susceptible to includes leafhoppers, birds stemborers. Signs of stem borers and leafhoppers were observed 9 weeks after planting. All the plots were sprayed with a systemic insecticide K-Optimal (Lambda Cyhalothrine 15 g/L +Acetamipride 20 g/L EC) a day after the pest was notice in order to control them.

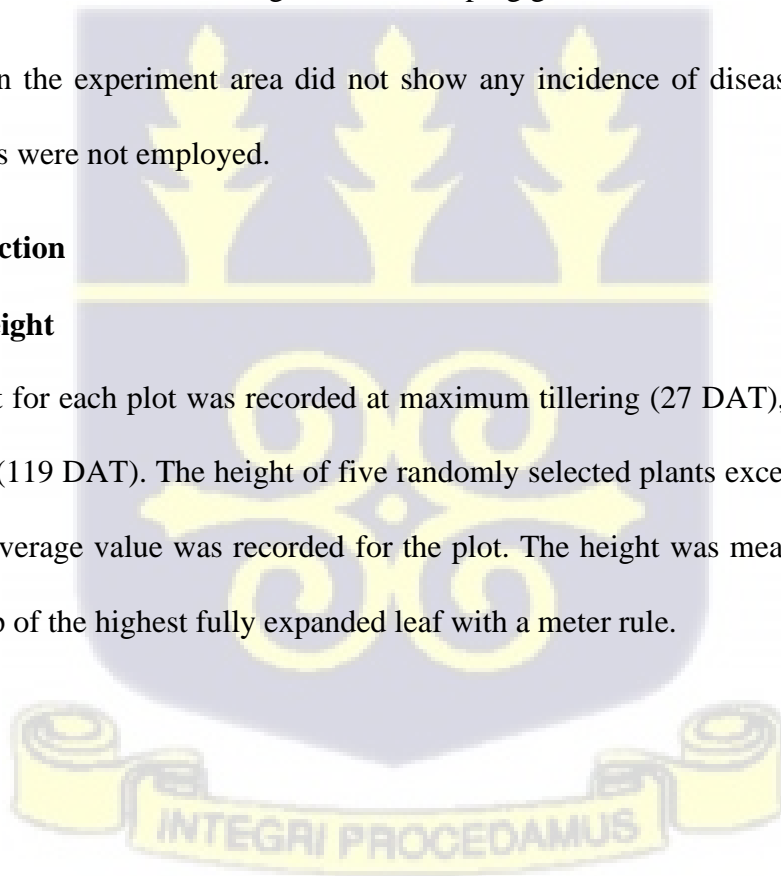
Birds are major pest in rice cultivation and is necessary for every rice grower to control them to attain optimum yield. The experimental area was covered with a net at the flowing stage of the rice to help prevent birds from feeding on the developing grains.

The rice plant in the experiment area did not show any incidence of diseases and as such disease control measures were not employed.

3.5.7 Data collection

3.5.7.1 Plant height

The plant height for each plot was recorded at maximum tillering (27 DAT), booting (55 DAT) and at maturity (119 DAT). The height of five randomly selected plants except border rows was taken, and the average value was recorded for the plot. The height was measured from the soil surface to the tip of the highest fully expanded leaf with a meter rule.



3.5.7.2 Number of tillers per plant

The number of tillers for each treatment was recorded at maximum tillering (27 DAT), booting (55 DAT) and at maturity (119 DAT). The number of tillers from five randomly selected hills was counted and the average values was recorded for the various treatment plots.

3.5.7.3 Number of effective tillers

The number of effective tillers in each pot was recorded at maturity (119 DAT). The average number for five randomly selected hills were taken. The number of effective tillers was determined by counting the number of tillers with panicle.

3.5.7.4 Above ground biomass

The above ground biomass for each plot was recorded at maximum tillering (27 DAT), booting (55 DAT) and at harvesting (120 DAT). Destructive sampling of 10 randomly selected hills except border rows were cut at the soil surface and oven dried at 70 °C for 48 hours to attain a uniform weight. An electronic balance was used to obtain weight of the dried rice samples. The weight obtained was used to estimate the dry matter yield per plant.

3.5.7.5 Days to 50 % flowering

The number of days to flowering was recorded for each pot by counting the number of days from seedling emergence to the day when 50 % of the plants in the plot flowered.

3.5.7.6 1000-grain weight

The 1000-grain weight was recorded after harvesting. 1000 grains obtained from panicles harvested from the plots during harvesting were oven dried at 70 °C for 48 hours to moisture content of 14 %. The seeds were counted afterwards using the rice grain counter and weighed with an electronic balance.

3.5.7.8 Grains per panicle

The grains per panicles was determined in the laboratory after harvesting. Five panicles were randomly selected from each plot during harvest. The selected panicles were threshed to separate the grains. After that the grains (including filled and unfilled) were counted using an electronic grain counter and divided by five to estimate the number of grains per panicle.

3.5.7.9 Percentage filled grains

Percentage filled grains was derived from data collected from the total number of grains per panicle and the number of filled grains per panicle. The number of filled grains was separated from total number grains obtained from 5 randomly selected panicles during harvest.

The average taken for five panicles was used to estimate filled grains per panicle. Mathematically the number of filled grains was calculated as;

$$\% \text{ filled gains} = \frac{\text{filled Grains per panicle}}{\text{Total number of filled grains}} \times 100$$

3.5.7.10 Grain yield

An area of 2m² excluding border rows was sampled for each plot into labeled sacks at harvest. The harvested samples were dried for two weeks and then threshed to separate the grains from the straw. Using a moisture meter, moisture content (MC) was recorded for each experimental plot after drying. Grain yield was then reported as t/ha at 14 % MC.

$$\text{Grain yield} = \frac{(100 - \text{moisture content in the grains}) \times \text{weight of grains}}{100 - \text{targeted moisture content (14\%)}}$$

3.5.7.11 Straw yield

The straw obtained after threshing to separate the grains was oven dried at 70 °C for 48 hours. The dried samples were weighed on an electronic scale to determine the straw yield per plot.

3.5.7.12 Panicle weight

Five selected panicles were harvested and weighed on an electronic balance to determine the average panicle weight for the five panicles

3.5.7.12 Panicle length

The length for five selected panicles were selected after harvest and measured with a measuring tape. The average length of the five selected panicles was recorded as the panicle length.

3.5.7.1 Plant tissue analysis

Zinc concentration in the rice straw and grains were analyzed at the University of Ghana Soil Science laboratory. The samples were first grinded into powder before drying them in the oven at 70°C. One gram of the sample was weighed and digested in a 2:1 nitric-perchloric acid solution (HNO₃:HClO₄) in a flask. The temperature of the flask was progressively raised up to 230 °C in a digesting chamber by placing it on a hot plate. The flask was heated to this temperature until brown NO₂ fumes stopped forming and thick white HClO₄ fumes formed in the flask. The contents were evaporated further until the volume was decreased to around 3–5 ml, but not to dryness. When the liquid turned colorless, it meant the digestion was finished. The flask was left to cool to about 20°C, after which 20 ml of distilled water was added to the solution in the flask. An atomic absorption spectrometer was used to determine the amount of Zn in aliquots of this solution (Wright and Stuczynski, 1996).

3.5.7.14 Plant zinc uptake

Zinc uptake was calculated using the following formular:

$$\text{Total Zn uptake} = \text{straw and grain biomass} \left(\frac{\text{kg}}{\text{ha}} \right) \times \text{Zn concentration in straw and biomass}$$

3.5.7.15 Agronomic Nitrogen use Efficiency

The agronomic Nitrogen Use efficiency (ANUE) for N fertilized plots was calculated using the following mathematical formular;

ANUE

$$= \frac{\text{Grain yield of plots fertilized with N} \left(\frac{\text{kg}}{\text{ha}} \right) - \text{Grain yield of plots not fertilized with N} \left(\frac{\text{kg}}{\text{ha}} \right)}{\text{Amount of N applied (kg/ha)}}$$

3.5.7.16 Agronomical Zinc Use Efficiency

The Zinc Use Efficiency (AZUE) for Zn fertilized plots was calculated using the following mathematical formular;

$$AZUE = \frac{\text{Grain yield of plots fertilized with Zn} \left(\frac{\text{kg}}{\text{ha}} \right) - \text{Grain yield of plots not fertilized with Zn} \left(\frac{\text{kg}}{\text{ha}} \right)}{\text{Amount of N applied (kg/ha)}}$$

3.5.7.17 Harvest index

Harvest index (HI) was determined using the formula:

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Grain yield} + \text{straw yield}}$$

3.6 DATA ANALYSIS

The data were subjected to analysis of variance (ANOVA) using GenStat statistical software, version 9.2 and Minitab. Means were separated at 5 % level of significance, if there are significant differences. Tukey's honestly significant test and Fisher's least significant difference (LSD) for means separation in the pot and field experiment respectively



CHAPTER 4

4.0 RESULTS

4.1 SOIL AND POULTRY MANURE ANALYSIS

4.1.1 Soil Analysis

Results from the analysis of the soil properties at the experimental site showed that zinc concentration (0.21 mg/kg) and total N (0.10%) was low in the soil (Table 4.1). The pH of the soil was neutral with a low Cation exchange capacity (CEC). The available Phosphorus (P) was moderate.

Table 4.1: Selected properties of the soil at the experimental site

pH in water	Organic C (%)	Total N (%)	Available P (mg /kg)	CEC (cmol /kg)	Zinc (mg/kg)
7.2	0.74	0.1	114.6	34.2	0.21

4.1.2 Poultry Manure Analysis

Results produced from the analysis of the poultry manure revealed that the N and P contents was moderate (Table 4.2). Zinc content in the poultry manure (92 mg/kg) was relatively higher than in the soil (0.21 mg/kg).

Table 4.2: Some chemical properties of the poultry manure used for the study.

Total Nitrogen (%)	Total P ₂ O ₅ (%)	Total K ₂ O (%)	Zinc (mg/kg)
0.86	1.38	1.1	92.8

4.2 RESULTS FROM POT EXPERIMENT

4.2.1 Plant height

Plant height generally increased from active tillering to harvest stage, ranging from 35.1cm to 95.5cm for all the treatments (Table 4.3). The maximum plant height was observed at maturity. The treatments did not have any impact on plant height throughout the growing period except at mid tillering. At this stage Sulphate of Ammonia (SoA) produced significantly ($p < 0.05$) taller plants than Urea. Urea treatment resulted in taller plants at maturity than SOA, although an opposite trend was observed from mid-tillering to booting.

Table 4.3: Plant height (cm) at the growth stages of rice as affected by N source and rate of Zn

Growth Stages	N Source	Zinc rate (kg/ha)						Mean
		Zn0	Zn5	Zn10	Zn15	Zn20	Zn25	
Mid tillering	Urea	39.4	36.5	39.3	40.3	35.1	39.8	38.4b
	SoA	40.7	38.0	39.9	42.7	40.9	40.9	40.5a
	Mean	40.1	37.3	39.6	41.5	38.0	40.3	
Booting	Urea	71.7	69.2	67.9	72.4	63.6	69.5	69.1
	SoA	73.3	69.2	75.5	71.3	70.4	66.3	71.1
	Mean	72.5	69.2	71.7	71.8	67.0	67.9	
Maturity	Urea	95.5	91	90.5	92.7	87.8	95	92.1
	SoA	92.5	87.3	95.3	89.0	89.2	92.7	91.0
	Mean	94.0	89.2	92.9	90.8	88.5	93.9	

Zn0, Zn5, Zn10, Zn15, Zn20 and Zn25 are 0, 5, 10, 15, 20, and 25 kg Zn /ha respectively applied in the form ZnSO₄. Mean without letters attached to them are not significantly ($p > 0.05$) different. Means with different letters are significantly different from each in the Tukey's honestly significant difference test ($p < 0.05$).



4.2.2 Number of tillers per pot

The mean tillers/pot increased from mid-tillering to booting. Afterwards average tillers/pot observed at booting declined from 23.7 to 19.35 at maturity. The maximum tiller numbers were obtained at booting. The interaction between zinc rate and nitrogen source did not have any significant ($p>0.05$) influence on tillers/pot at any of the growth stages (Table 4.4).

The response of tiller number to sulphate of ammonia was higher and significantly ($p<0.05$) different from that of urea throughout the growth period. Although zinc rate did not influence tillering at mid-tillering and at maturity, the tiller number response to Zn rate was highest at the booting stage. The response of tiller number was highest at Zn 20 and lowest at Zn 5.

Table 4.4: Response of tiller number to nitrogen source and Zinc rate at the different growth stages.

Growth Stages	N Source	Zinc rate (kg/ha)						Mean
		Zn0	Zn5	Zn10	Zn15	Zn20	Zn25	
Mid-tillering	Urea	4.3	5.3	4.7	5.7	5.7	5.7	5.2 b
	SoA	6.7	5.3	5.7	7.0	6.0	6.3	6.2 a
	Mean	5.5	5.3	5.2	6.3	5.8	6.0	
Booting	Urea	18.7	19.3	19.7	22.8	23.2	22.3	21.0 b
	SoA	26.5	23.0	23.3	29.0	28.8	27.7	26.4 a
	Mean	22.6ab	21.2b	21.5b	25.9a	26.0a	25.0ab	
Maturity	Urea	17.7	17.3	16.7	18.5	19.0	18.5	17.9 b
	SoA	20.5	20.0	20.8	21.5	20.5	21.3	20.8 a
	Mean	19.1	18.7	18.8	20	19.8	19.9	

Zn0, Zn5, Zn10, Zn15, Zn20 and Zn25 are 0, 5, 10, 15, 20, and 25 kg Zn /ha respectively applied in the form ZnSO₄. Mean without letters attached to them are not significantly ($p>0.05$) different. Means with different letters are significantly different from each in the Tukey's honestly significant difference test ($p<0.05$).

4.2.3 Number of effective tillers

Interaction between zinc rate and N source did not influence the effective tillers/pot. The highest effective tiller number was recorded at Zn 25, but this was not significantly different from the other zinc rate treatments (Table 4.5). For the main effects N source, SoA produced an average of 2.8 more effective tillers/pot than Urea. This was found to be statistically significant ($p < 0.05$).

4.2.4 Number Days to 50 % flowering

The main effect N source and Interaction between Zinc rate and N source did not have significant ($p < 0.05$) influence on the days to flowering. However, it was significantly ($p < 0.05$) affected by zinc rate (Table 4.5). Applying higher zinc rate ($> 15\text{kg Zn/ha}$) tended to delay flowering. The effect of zinc on days to 50% flowering varied in the order: Zn25 $>$ Zn20 $>$ Zn0 $>$ Zn5 $>$ Zn5 $>$ Zn10 $>$ Zn15.

Table 4.5: Effect of source of N and rate of Zn on effective tiller number, number of days to 50% flowering, above ground biomass and panicle length

Parameters	N source	Zinc rate						Mean
		Zn0	Zn5	Zn10	Zn15	Zn20	Zn25	
Effective tillers	Urea	17.3	17.2	16.5	18.3	19.0	18.5	17.8b
	SoA	20.3	19.8	20.5	21.3	20.5	21.3	20.6a
	Mean	18.8	18.5	18.5	19.8	19.8	19.9	
DAF (Days)	Urea	94.7	94.7	92.7	93.0	93.7	96.0	94.1
	SoA	93.0	92.7	94.3	92.7	95.0	96.0	93.9
	Mean	93.8ab	93.7a	93.5a	92.8a	94.3ab	96.0b	
Above ground Biomass (g/pot)	Urea	143.0	131.0	129.3	144.2	133.0	139.6	136.7 b
	SoA	138.8	145.2	151.0	149.7	140.5	160.8	147.7 a
	Mean	140.9	138.1	140.2	147.0	136.8	150.2	
Panicle length (cm)	Urea	22.9	22.9	22.7	22.1	22.3	21.6	22.4
	SoA	23.0	22.5	23.5	22.5	22.4	21.9	22.6

Mean	22.9	22.7	23.1	22.3	22.4	21.8
Zn0, Zn5, Zn10, Zn15, Zn20 and Zn25 are 0, 5, 10, 15 20, and 25 kg Zn /ha respectively applied in the form ZnSO ₄ .. Mean without letters attached to them are not significantly (p>0.05) different. Means with different letters are significantly different form each in the Tukey's honestly significant difference test (p<0.05).						

4.2.5 Above ground Biomass

Sulphate of ammonia induced greater aboveground biomass than urea (Table 4.5). Zn 25 recorded the greatest above ground biomass (150g/pot), however it was statistically (p> 0.05) similar to the other zinc rates. The interaction effect of Zinc rate and N source did not influence the above ground biomass production at maturity.

4.2.6 Panicle length.

The variation in panicle length (21.6 – 23.5 cm) in response to the various treatment was low and did not follow any pattern (Table 4.5). Neither of the main effects (N source and Zinc rate) nor the interaction between them had a significant (p<0.05) impact on the panicle length.

4.2.7 1000 grain weight

The 1000 grain ranged between 24g and 29.7g. Zinc rate, nitrogen source and interaction between zinc rate and N source did not influence the 1000 grain weight (Table 4.6).

4.2.8 Grains per panicle

N source and Interaction effect for Zinc rate and N source did not significantly (p<0.05) influence the number of grains per panicle. Zinc rate significantly (p<0.05) influenced the number of grains per panicle. Application of higher rate of zinc (>10 kg Zn/ha) reduced the number of grains produced on the panicle (Table 4.6). Grains/ panicle as affected by zinc rates followed the trend; Zn10 > Zn0 > Zn 5 > Zn 15 > Zn 20 > Zn 25.

4.2.9 Percentage filled grains

Interaction between Zinc rate and N source did not show any significant ($p>0.05$) effect on the percentage filled grains. Similarly, percentage filled grains was not significantly ($p>0.05$) influenced by N source or Zn rate. (Table 4.6)

4.2.10 Grain yield (g/pot)

N source and Interaction effect for Zinc rate and N source did not significantly ($p<0.05$) influence the number of grains per panicle. SoA produced significantly ($p<0.05$) higher yield (63.6g/pot) than Urea (56.3g/pot) (Table 4.7). The grain yield produced by the various Zinc rates were statistically ($p>0.05$) similar and followed the trend $Zn0 > Zn15 > Zn20 > Zn25 > Zn10 > Zn 5$.

4.2.11 Harvest index

The harvest index was not significantly ($p<0.05$) affected by the interaction between N source and rate of zinc application (Table 4.6). Similarly N source and rate of zinc application recorded statistically ($p>0.05$) similar Harvest index.

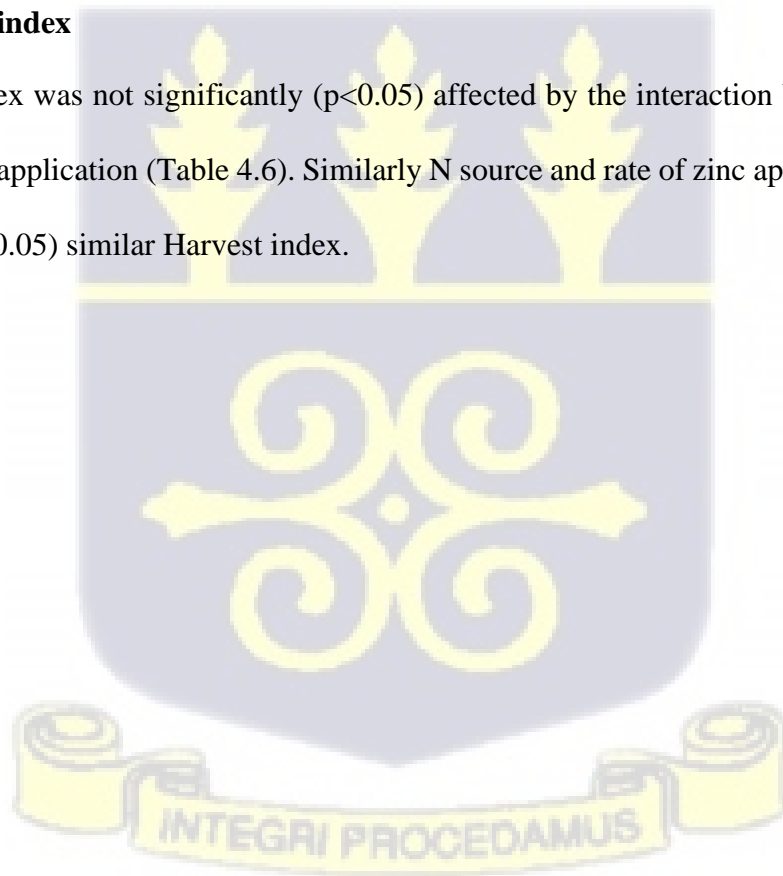


Table 4.6: 1000-grain weight, grains/panicle, percentage filled grains, grain yield and Harvest index affected by rate of zinc and source of N

Parameters	N Source	Zn						Mean
		Zn0	Zn5	Zn10	Zn15	Zn20	Zn25	
1000-grain Weight (g)	Urea	29.7	26.0	25.7	28.0	28.7	26.7	27.4
	SoA	24.7	25.7	27.7	24.7	25.0	24.0	25.3
	Mean	27.2	25.8	26.7	26.3	26.8	25.3	
Grains per panicle	Urea	108.4	102.6	105.3	85.6	93.0	70.0	94.2
	SoA	100.8	97.7	121.4	86.3	77.3	60.0	90.6
	Mean	104.6ab	100.2ab	113.4a	86.0bc	85.2bc	65.0c	
% Filled grains	Urea	68.5	61.0	66.1	60.1	63.6	61.1	63.4
	SoA	73.7	69.5	64.7	67.4	60.2	65.0	66.8
	Mean	71.1	65.3	65.4	63.8	61.9	63.1	
Grain yield (g/pot)	Urea	63.0	54.3	57.0	53.8	57.1	52.9	56.3b
	SoA	63.3	58.0	61.5	67.8	63.6	67.1	63.6a
	Mean	63.1	56.1	59.2	60.8	60.3	60.0	
Harvest index	Urea	0.44	0.41	0.44	0.37	0.43	0.38	0.41
	SoA	0.46	0.40	0.41	0.45	0.46	0.42	0.43
	Mean	0.45	0.41	0.42	0.41	0.44	0.40	

Zn0, Zn5, Zn10, Zn15, Zn20 and Zn25 are 0, 5, 10, 15, 20, and 25 kg Zn /ha respectively applied in the form ZnSO₄. Mean without letters attached to them are not significantly ($p > 0.05$) different. Means with different letters are significantly different from each in the Tukey's honestly significant difference test ($p < 0.05$).



4.3 RESULTS FROM FIELD EXPERIMENT

4.3.1 Plant height

Generally plant height increased from mid-tillering to harvest. There was no significant ($p > 0.05$) interaction effect on plant height. At mid-tillering, the nitrogen fertilized plots recorded significantly ($p > 0.05$) higher values than No (control). However, no significant ($p > 0.05$) difference was observed among treatments that received nitrogen. This trend was also observed from booting to maturity (Table 4.7).

Zinc rate did not have any influence on plant height at mid-tillering. The plants in the zinc treated plots were significantly taller than the control (Zn0) at booting. However, there were no differences between the fertilized plots. At maturity, it was observed that increasing Zinc rate tended to increase the plant height and followed the trend: Zn 15 > Zn10 > Zn5 > Zn0. Although Zn15 recorded the tallest plant height (100.5 cm) it was not significantly ($p > 0.05$) different from the control (Zn0) (98.4 cm).

Table 4.7: Plant height(cm) at various growth stages of rice affected by N source and Zinc rate

Growth Stages	N source (Ns)	Zinc Rate (Zn)					LSD (0.05)		
		Zn0	Zn5	Zn10	Zn15	Mean	Ns	Zn	Zn X Ns
Mid tillering	No	39.3	39.7	40.0	40.8	40.0	5.5	NS	NS
	CF ₁₀₀	47.1	50.5	44.8	48.8	47.8			
	CF ₇₅ PM ₂₅	45.2	49.1	47.4	50.0	47.9			
	CF ₅₀ PM ₅₀	46.8	48.1	47.8	45.8	47.1			
	Mean	44.6	46.8	45.0	46.4				
Booting	No	84.7	87.0	87.3	86.3	86.3	3.4	2.8	NS
	CF ₁₀₀	91.9	104.7	100.7	99.8	99.3			
	CF ₇₅ PM ₂₅	99.4	98.3	98.6	98.6	98.7			
	CF ₅₀ PM ₅₀	91.6	98.8	99.8	99.7	97.5			
	Mean	91.9	97.2	96.6	96.1				

Maturity	No	90.3	90.8	92.2	92.5	91.4			
	CF ₁₀₀	102.1	105.3	103.1	103.6	103.5			
	CF ₇₅ PM ₂₅	100.7	100.7	103.0	105.0	102.4	6.6	NS	NS
	CF ₅₀ PM ₅₀	100.5	99.4	99.7	101.0	100.2			
	Mean	98.4	99.0	99.5	100.5				

NS = not significant at $P > 0.05$; *No*: No N fertilizer; *CF₁₀₀* :100% Chemical fertilizer (Urea); *CF₇₅PM₂₅* :75% chemical fertilizer +25 Poultry manure (PM); *CF₅₀PM₅₀* :50% Chemical fertilizer +50 Poultry manure (PM); Zn0 Zn5, Zn10 and Zn15 are 0, 5, 10 and 15 kg Zn /ha respectively applied in the form zinc sulphate.

4.3.2 Number of Tillers

The number of tiller/m² increased from mid tillering to booting but then decreased at maturity.

Interaction between zinc and nitrogen source was significant at mid-tillering, booting, maturity for tiller/m². At mid tillering CF₇₅PM₂₅ x Zn15 produced the highest number of tillers/m² and was followed by CF₇₅PM₂₅ x Zn5 and CF₁₀₀ x Zn0. No x Zn0 recorded the lowest number of tillers/m² (134.2) at mid tillering. At booting CF₅₀PM₅₀ x Zn5 produced the greatest number of tillers/m² (336.7). At maturity CF₇₅PM₂₅ x Zn5 produced the highest (305) number of tillers/m². At this stage increasing zinc rate from 5 kg Zn to 15 kg Zn increased the tillers/m² under CF₁₀₀, however under CF₇₅PM₂₅ it decreased tillers/m².

N source significantly ($p < 0.05$) affected the number of tillers/m² at mid-tillering booting and maturity (Table 4.8). For all the growth stages, 100% urea (CF₁₀₀) recorded the highest tillers/m². At maturity CF₁₀₀ recorded the maximum number of tillers/m² and was statistically ($p > 0.05$) similar to other nitrogen combinations but significantly ($p < 0.05$) different from the control (No).



Zinc rate significantly affected number of tillers at mid tillering. Zn0, Zn5, Zn10 produced statistically ($p < 0.05$) similar number of tillers which was significantly greater than Zn15. Zinc rate did not show any significant ($p > 0.05$) influence of tillers/m² from booting to maturity (Table 4.8).

Table 4.8: Mean tiller/m² at various growth stages of rice affected by N source and Zinc rate

Growth Stages	N source (Ns)	Zinc Rate (Zn)					LSD(0.05)		
		Zn0	Zn5	Zn10	Zn15	Mean	Ns	Zn	Zn X Ns
Mid tillering	No	134.2	143.3	142.5	145.0	141.3			
	CF ₁₀₀	235.8	235.8	208.3	222.5	225.6			
	CF ₇₅ PM ₂₅	186.7	240.0	201.7	260.0	222.1	15.9	10.2	22.1
	CF ₅₀ PM ₅₀	181.7	163.3	188.3	206.7	185.0			
	Mean	184.6	195.6	185.2	208.6				
Booting	No	238.3	241.7	251.7	245.0	244.2			
	CF ₁₀₀	307.5	293.3	335.8	325.0	315.4			
	CF ₇₅ PM ₂₅	323.3	296.7	300.0	294.2	303.6	24.5	NS	28.2
	CF ₅₀ PM ₅₀	285.0	336.7	305.8	286.7	303.6			
	Mean	288.5	292.1	298.3	287.7				
Maturity	No	231.7	231.7	227.5	230.0	230.2			
	CF ₁₀₀	291.7	275.0	285.0	291.7	285.9			
	CF ₇₅ PM ₂₅	260.0	305.0	281.7	271.7	279.6	18.6	NS	25.7
	CF ₅₀ PM ₅₀	296.7	261.7	266.7	280.8	276.5			
	Mean	270.0	268.4	265.2	268.6				

NS = not significant at $P > 0.05$; No: No N fertilizer; CF₁₀₀: 100% Chemical fertilizer (Urea); CF₇₅PM₂₅: 75% chemical fertilizer + 25 Poultry manure (PM); CF₅₀PM₅₀: 50% Chemical fertilizer + 50 Poultry manure (PM); Zn0 Zn5, Zn10 and Zn15 are 0, 5, 10 and 15 kg Zn /ha respectively applied in the form zinc sulphate.

4.3.3 Number of panicles/m²

The various N sources influenced the number of panicles/m² produced at maturity. The interaction between Nitrogen source and zinc fertilization had a significant effect on the number of panicles/m². Increasing zinc rate under CF₁₀₀, increased the number of panicles/m², whereas

increasing zinc rate under the N sources containing PM (thus $CF_{75}PM_{25}$ and $CF_{50}PM_{50}$) decreased the panicles/m².

The no nitrogen treatment (No) produced significantly ($p < 0.05$) lower number of panicles/m² than plots that received nitrogen treatment. The effect of Nitrogen source on the number of panicles/m² produced followed the trend: $CF_{100} > CF_{75}PM_{25} > CF_{50}PM_{50} > Ns0$.

For the main effect zinc rate, Zn5 produced the highest number of panicles/m² at 255.8, however it was not significantly ($p > 0.05$) different from Zn0 which produced the lowest at 248.9.

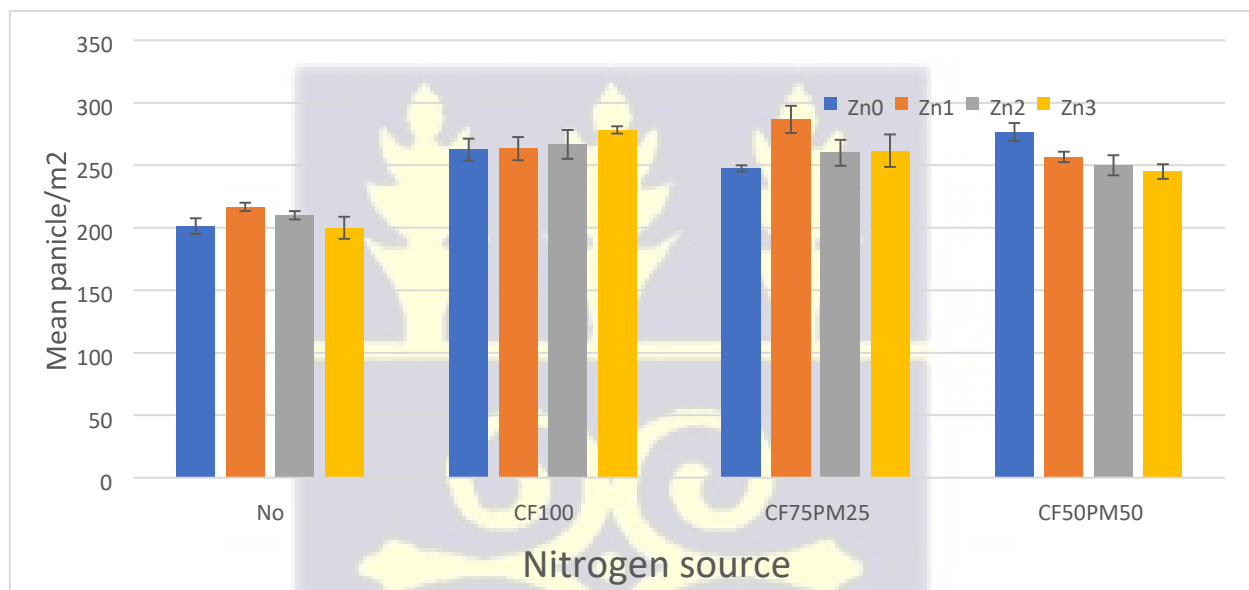


Figure 4.1: Interaction effect of N sources and Zn rates on Mean panicles/m².

No: No N fertilizer; CF_{100} : 100% Chemical fertilizer (Urea); $CF_{75}PM_{25}$: 75% chemical fertilizer +25 Poultry manure (PM); $CF_{50}PM_{50}$: 50% Chemical fertilizer +50 Poultry manure (PM); Zn0 Zn5, Zn10 and Zn15 are 0, 5, 10 and 15 kg Zn /ha respectively applied in the form zinc sulphate.

4.3.4 Above ground biomass

Interaction between the main effects zinc rate and Nitrogen source was not significant at mid tillering, booting and maturity.

The main effect N source significantly ($p < 0.05$) influenced the above ground biomass at mid tillering, booting and maturity (Table 4.9). The above ground biomass recorded for the various levels of N sources were statistically different from each other at mid-tillering. At this stage decreasing the proportion of the chemical fertilizer (CF) resulted in the decrease in biomass production. The biomass produced under CF_{100} and $CF_{75}PM_{25}$ were statistically similar to each other at booting and were significantly ($p < 0.05$) higher than $CF_{50}PM_{50}$ and No. Unlike the other growth stages, at maturity the source of nitrogen fertilizer did not influence biomass production. However, the biomass produced from the N fertilized plots were significantly ($p < 0.05$) greater than the unfertilized plots (No).

Although Zinc rate significantly ($p < 0.05$) impacted above ground biomass at mid tillering, it did not show any significant influence at booting and maturity. At mid tillering, plots that received zinc fertilization produced statistically similar above ground biomass and were significantly ($p < 0.05$) greater than the control (Zn0).

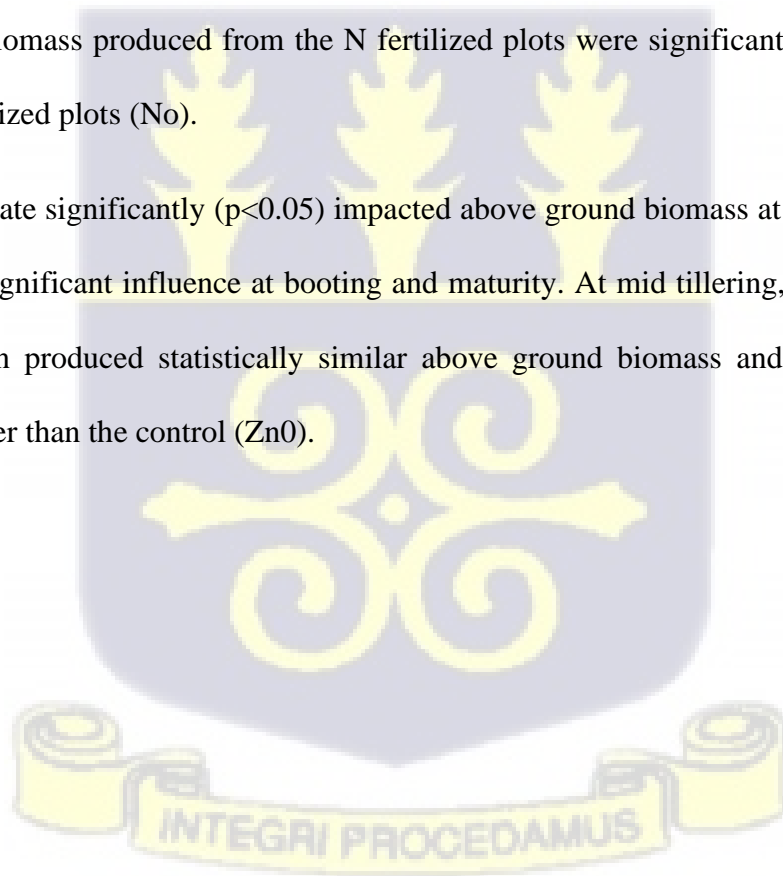


Table 4.9: Above ground (g/m²) at various growth stages of rice affected by N source and Zn rate

Growth Stages	N source (Ns)	Zinc Rate (Zn)				Mean	LSD (0.05)		
		Zn0	Zn5	Zn10	Zn15		Ns	Zn	Zn X Ns
Mid tillering	No	106.2	110.1	109.7	110.8	109.2			
	CF ₁₀₀	144.0	160.2	153.5	159.3	154.3			
	CF ₇₅ PM ₂₅	130.2	151.5	135.2	146.0	140.7	8.92	7.95	NS
	CF ₅₀ PM ₅₀	103.8	106.0	133.7	135.7	119.8			
	Mean	121.1	132.0	133.0	138.0				
Booting	No	397.9	415.0	420.2	409.1	410.6			
	CF ₁₀₀	499.0	552.8	537.3	515.4	526.1			
	CF ₇₅ PM ₂₅	514.0	630.4	565.7	490.3	550.1	48.36	NS	NS
	CF ₅₀ PM ₅₀	494.2	421.2	438.8	439.5	448.4			
	Mean	476.3	504.9	490.5	463.6				
Maturity	No	963	952	946	956	954.3			
	CF ₁₀₀	1224	1401	1420	1265	1327.5			
	CF ₇₅ PM ₂₅	1355	1266	1169	1333	1280.8	126.5	NS	NS
	CF ₅₀ PM ₅₀	1054	1307	1338	1251	1237.5			
	Mean	1149	1231.5	1218.3	1201.3				

NS = not significant at $P > 0.05$; *No*: No N fertilizer; *CF₁₀₀*: 100% Chemical fertilizer (Urea); *CF₇₅PM₂₅*: 75% chemical fertilizer +25 Poultry manure (PM); *CF₅₀PM₅₀*: 50% Chemical fertilizer +50 Poultry manure (PM); Zn0 Zn5, Zn10 and Zn15 are 0, 5, 10 and 15 kg Zn /ha respectively applied in the form zinc sulphate.

4.3.5 Days to flowering

Interaction between nitrogen source and zinc rate did not show any significant ($p > 0.05$) influence on the days to 50% flowering.

The main effect N source and zinc rate did not have any significant ($p > 0.05$) influence on the number of days to 50% flowering (Table 4.10). Zn15 took the longest number of days to attain 50% flowering at 91 days, however, it was not statistically different from the other zinc rates..

4.3.6 Grains per panicle

Interaction between Zinc rate and nitrogen source did not influence the grains/panicle. N source had a significant ($p < 0.05$) effect on the number of grains produced per panicle. The treatment that contained poultry manure (CF₇₅PM₂₅ and CF₅₀PM₅₀) produced greater number of grains/panicle than sole application of chemical fertilizer (CF₁₀₀) and the control (No) (Table 4.10). Zinc application did not show any significant ($p > 0.05$) influence on the grains/panicle.

4.3.6 Filled grains percentage

Interaction between nitrogen source and zinc rate did not have a significant ($p < 0.05$) influence on the percentage of filled grains (Table 4.10).

Nitrogen source had a significant ($p < 0.05$) impact on the percentage of filled grains. CF₁₀₀ produced the greatest percentage filled grains which was statistically similar the CF₇₅PM₂₅ while CF₅₀PM₅₀ recorded the lowest (Table 4.10). zinc rate did not have a significant ($p < 0.05$) influence on the percentage of filled grains.

4.3.6 100-grain weight

The 1000-grain weight recorded ranged from 20g to 37g. The interaction between nitrogen source and zinc rate was not significant ($p > 0.05$). The 1000-grain weight did not show any statistical ($p > 0.05$) response to the main effects nitrogen source and zinc rate (Table 4.10).

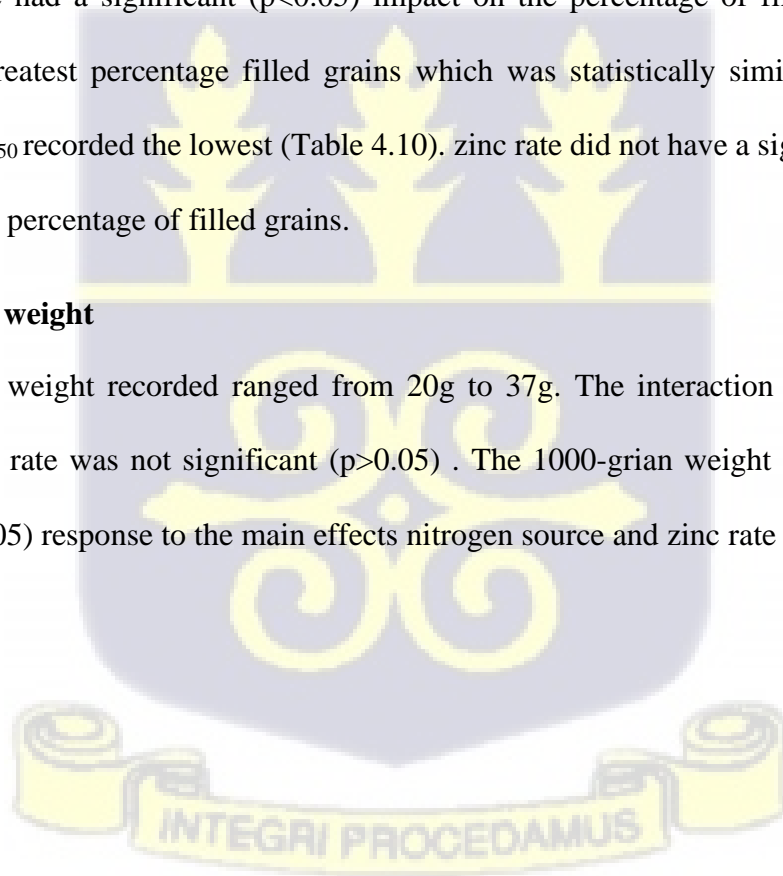


Table 4.10: Days to 50% flowering, Grains/panicle, percentage filled grains and Test Weight affected by Zinc rate and nitrogen source

Parameters	N source (Ns)	Zinc Rate (Zn)				Mean	LSD (0.05)		
		Zn0	Zn5	Zn10	Zn15		Ns	Zn	Zn X Ns
Days to 50% flowering (Days)	No	89.3	90.0	89.0	91.0	89.8			
	CF ₁₀₀	91.0	90.7	91.0	91.3	91.0			
	CF ₇₅ PM ₂₅	91.3	90.3	90.3	90.3	90.6	NS	NS	NS
	CF ₅₀ PM ₅₀	90.7	91.0	91.0	91.7	91.1			
	Mean	90.6	90.5	90.3	91.1				
Grains per panicle	No	84.7	89.7	90.0	77.0	85.4			
	CF ₁₀₀	85.5	90.1	95.5	99.7	92.7			
	CF ₇₅ PM ₂₅	94.8	105.3	89.8	96.7	96.7	7.53	NS	NS
	CF ₅₀ PM ₅₀	89.0	87.5	96.7	102.9	94.0			
	Mean	88.5	93.2	93.0	94.1				
% Filled grains (%)	No	82.0	78.7	81.7	79.3	80.4			
	CF ₁₀₀	80.7	88.0	81.0	78.6	82.1			
	CF ₇₅ PM ₂₅	80.5	85.5	78.6	88.9	83.4	1.816	NS	NS
	CF ₅₀ PM ₅₀	74.9	82.2	78.5	83.2	79.7			
	Mean	79.5	83.6	79.9	82.5				
1000-grain weight (g)	No	25.0	25.2	25.2	24.9	25.0			
	CF ₁₀₀	24.3	25.0	26.3	23.3	24.7	NS	NS	NS
	CF ₇₅ PM ₂₅	26.3	23.7	24.0	24.7	24.7			
	CF ₅₀ PM ₅₀	25.7	29.7	24.7	24.7	26.2			
	Mean	25.3	25.9	25.1	24.4				

NS = not significant at $P > 0.05$; No: No N fertilizer; CF₁₀₀ :100% Chemical fertilizer (Urea); CF₇₅PM₂₅ :75% chemical fertilizer +25 Poultry manure (PM); CF₅₀PM₅₀ :50% Chemical fertilizer +50 Poultry manure (PM); Zn₀ Zn₅, Zn₁₀ and Zn₁₅ are 0, 5, 10 and 15 kg Zn /ha respectively applied in the form zinc sulphate.

4.3.7 Yield

Interaction between nitrogen source and zinc rate did not show any significant ($p > 0.05$) influence on the grain yield. CF₇₅PM₂₅ recorded the highest grain yield and was statistically similar to CF₁₀₀ which was significantly ($p < 0.05$) higher than CF₅₀PM₅₀ (Fig 4.2 A). The rate of zinc application had a significant impact on grain yield. Increasing the zinc rate above 5 kg Zn/ha tend to decrease

the grain yield. The trend for grain yield in response to Zinc rate is as follows: Zn5>zn10>zn0>Zn15.

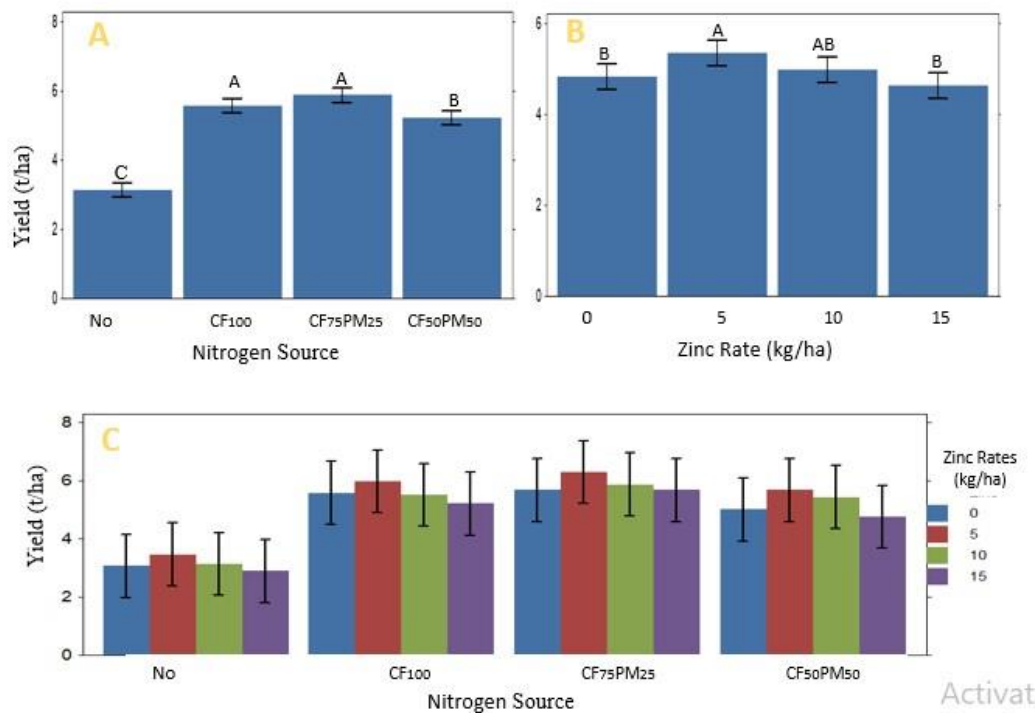


Fig. 4.2: Response of grain yield affected by Main effects N source (A), Zinc rate (B) and Interaction effect (C).

No: No N fertilizer; CF₁₀₀ :100% Chemical fertilizer (Urea); CF₇₅PM₂₅ :75% chemical fertilizer +25 Poultry manure (PM); CF₅₀PM₅₀ :50% Chemical fertilizer +50 Poultry manure (PM). Bars with the same letters are significantly (p>0) different from each other by the fishers least significant difference.

4.3.8. Harvest Index

Harvest index (HI) is an important indicator, which shows how efficiently plants convert total biomass as influenced by the treatments into grain. The highest HI was recorded in CF₇₅PM₂₅, however it was not significantly (p<0.05) different from that of CF₁₀₀ and CF₅₀PM₅₀ (Table 4.11).

Furthermore, the zinc fertilizer application rate did not influence harvest index. There was no significant ($p < 0.05$) interaction between Nitrogen source and rate of on harvest index.

4.3.9. Agronomic Nitrogen Use Efficiency (ANUE)

ANUE did not show a significant ($p < 0.05$) response to Zinc rate and interaction between the zinc rate and N source. Sole application of chemical fertilizer (CF₁₀₀) recorded a significantly ($p < 0.05$) greater ANUE than combined applications of with PM (Table 4.11) . Increasing the percentage of PM in the total N applied decreased ANUE and followed the trend CF₁₀₀ > CF₇₅PM₂₅ > CF₅₀PM₅₀.

4.3.10. Agronomic Zinc Use Efficiency (AZUE)

Interaction between the zinc rate and N source was did influence AZUE. .The source of nitrogen fertilizer did not have a significant ($p > 0.05$) influence on the AZUE (Table 4.11). The AZUE decreases as the Zinc rate was increased and followed the trend Zn5 > Zn10 > Zn15. Zn5 recorded a significantly greater AZUE than Zn 10 and Zn 15.

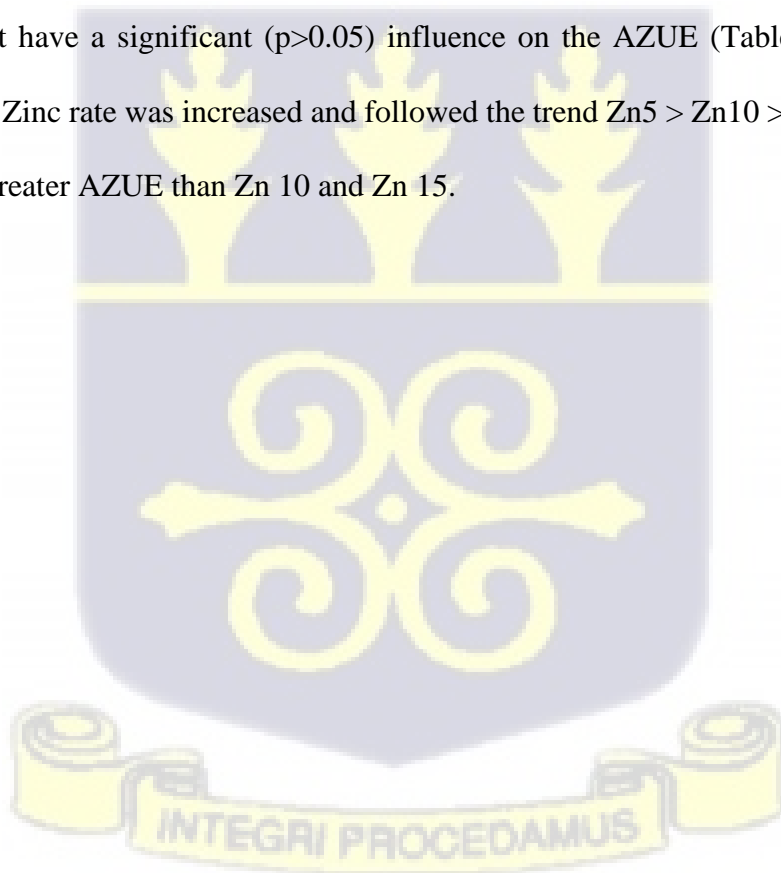


Table 4.11: Harvest index, ANUE, AZUE and ZRE affected by Zinc rate and nitrogen source

Parameters	N source (Ns)	Zinc Rate (Zn)				Mean	LSD(0.05)		
		Zn0	Zn5	Zn10	Zn15		Ns	Zn	Zn X Ns
Harvest Index	No	0.43	0.44	0.44	0.43	0.43			
	CF ₁₀₀	0.49	0.45	0.50	0.47	0.48			
	CF ₇₅ PM ₂₅	0.48	0.48	0.52	0.53	0.50	0.02	NS	NS
	CF ₅₀ PM ₅₀	0.46	0.53	0.47	0.48	0.49			
	Mean	0.46	0.47	0.48	0.48				
ANUE (kg/kg)	No								
	CF ₁₀₀	25.12	25.09	23.80	23.21	24.3			
	CF ₇₅ PM ₂₅	26.05	28.27	27.37	27.85	27.39	3.03	NS	NS
	CF ₅₀ PM ₅₀	19.45	22.06	23.00	18.67	20.80			
	Mean	23.54	25.14	24.72	23.24				
AZUE (kg/kg)	No		46.70	6.70	-11.70	13.90			
	CF ₁₀₀		57.70	-6.60	-24.40	8.90			
	CF ₇₅ PM ₂₅		75.80	19.80	0.30	31.97	NS	35.05	NS
	CF ₅₀ PM ₅₀		86.60	42.10	-16.90	37.27			
	Mean		66.70	15.50	-13.18				

NS = not significant at $p > 0.05$. No: No N fertilizer; CF₁₀₀: 100% Chemical fertilizer (Urea); CF₇₅PM₂₅: 75% chemical fertilizer +25 Poultry manure (PM); CF₅₀PM₅₀: 50% Chemical fertilizer +50 Poultry manure (PM); Zn0 Zn5, Zn10 and Zn15 are 0, 5, 10 and 15 kg Zn /ha respectively applied in the form ZnSO₄.

4.3.11. Straw Zn uptake

The straw zinc uptake was not significantly ($p > 0.05$) affected by interaction between nitrogen source and Zn rate. Zinc application did not show any significant ($p > 0.05$) impact on straw zinc uptake (Table 4.12). However, the nitrogen source had a significant ($p < 0.05$) effect on it. The highest straw Zn uptake was recorded by CF₁₀₀ followed by CF₇₅PM₂₅ and CF₅₀PM₅₀, which were statistically similar.

4.3.12. Grain Zn Uptake

Interaction between nitrogen source and zinc rate did not influence the grain Zn uptake. The grain zinc uptake for the plots that received nitrogen fertilizers was significantly greater than control (No) (Table 4.12). However, no significant differences was observed among the plots treated with nitrogen application. The highest grain Zn uptake (5.8 g/m^2) was produced by Zn5 which was significantly ($p < 0.05$) different from the other zinc rates.

The grain zinc uptake was influenced by N fertilizer application. With the same Zn application rate, grain zinc uptake was higher for Zn treatments that received N fertilizers than the control that did not receive N fertilizer (Table 4.12). However, no significant difference was observed among the Zn rate treatments. The highest grain Zn uptake (5.8 g/m^2) was observed in Zn5 which was significantly ($p < 0.05$) different from the other zinc rate treatments.

4.3.13. Total Zn uptake

Zinc rate and interaction between zinc rate and N source did not have a significant ($p > 0.05$) impact of the total zinc uptake (Table 4.12). The total zinc uptake for treatments that received N fertilization were statistically like each other but differed from the control (No). The Nitrogen source was more effective in influencing the total Zn uptake than the Zn rate. The highest total Zn uptake was observed in CF₁₀₀ and this was significantly higher the control, where no Nitrogen fertilizer was applied. Total Zn uptake was not statistically ($p > 0.05$) different among the treatments that received nitrogen fertilizers (Table 4.12). Zinc application rate did not influence total Zn uptake.



Table 4.12: Straw zinc uptake, Grain zinc uptake and total zinc uptake affected by Zinc rate and nitrogen source

Parameters	N source (Ns)	Zinc Rate (Zn)					LSD(0.05)		
		Zn0	Zn5	Zn10	Zn15	Mean	Ns	Zn	Zn X Ns
Straw zinc uptake (g/m ²)	No	22.2	29.7	28.2	27.9	27.0	9.0	NS	NS
	CF ₁₀₀	47.6	60.2	57.2	55.4	55.1			
	CF ₇₅ PM ₂₅	48.7	48.5	38.2	43.0	44.6			
	CF ₅₀ PM ₅₀	33.9	42.9	48.4	39.6	41.2			
	Mean	38.1	45.3	43.0	41.5				
Grain zinc uptake (g/m ²)	No	2.1	3.1	2.5	2.1	2.4	2.2	0.7	NS
	CF ₁₀₀	7.3	7.4	6.1	5.6	6.6			
	CF ₇₅ PM ₂₅	5.9	6.7	5.7	5.8	6.0			
	CF ₅₀ PM ₅₀	4.9	5.9	5.6	5.3	5.4			
	Mean	5.0	5.8	5.0	4.7				
Total zinc uptake (g/m ²)	No	24.3	32.8	30.7	30.0	29.5	9.6	NS	NS
	CF ₁₀₀	55.0	67.6	63.3	61.0	61.7			
	CF ₇₅ PM ₂₅	54.6	55.3	44.0	48.8	50.7			
	CF ₅₀ PM ₅₀	38.7	48.8	54.0	44.8	46.6			
	Mean	43.2	51.1	48.0	46.2				

NS = not significant at $P > 0.05$. No: No N fertilizer; CF₁₀₀: 100% Chemical fertilizer (Urea); CF₇₅PM₂₅: 75% chemical fertilizer +25 Poultry manure (PM); CF₅₀PM₅₀: 50% Chemical fertilizer +50 Poultry manure (PM); Zn0 Zn5, Zn10 and Zn15 are 0, 5, 10 and 15 kg Zn /ha respectively applied in the form ZnSO₄.

4.3.14. Straw zinc concentration

The interaction between the N source and zinc rate did not influence straw zinc uptake. N source had a significant ($p < 0.05$) effect on Zn concentration in the straw. Straw zinc concentration ranked in this order: CF₁₀₀ > CF₇₅PM₂₅ > CF₅₀PM₅₀ > No.. Zinc rate had a significant ($p < 0.05$) effect on straw zinc concentration. Zn5 recorded the highest followed by Zn 10 and Zn15.

4.3.15. Grain zinc concentration

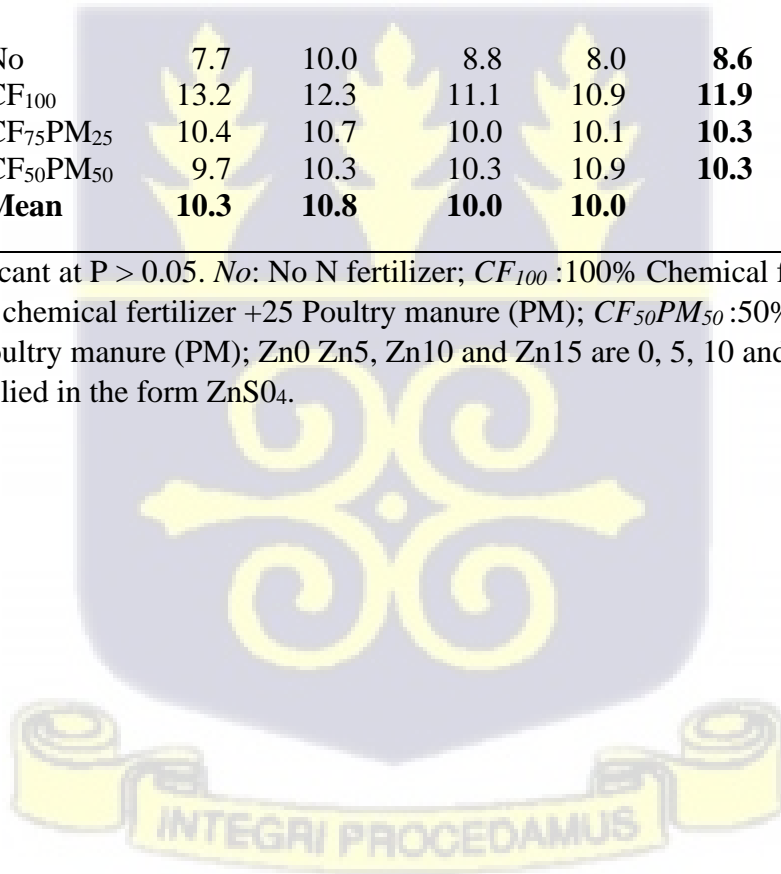
The variation in grain zinc concentration was low and ranged from 7.7 to 13.2 mg/kg. As a result neither N source, zinc rate or the interaction effect between N source and zinc rate had a

significant ($p < 0.05$) influence on the grain zinc concentration (Table 4.13). $CF_{100} \times Zn_0$ recorded the highest grain zinc concentration while the lowest was recorded at $No \times Zn_0$. For Zinc rate, Zn_5 recorded the highest zinc rate (10.8 mg/kg) whereas for N sources CF_{100} recorded the highest.

Table 4.13: Straw zinc concentration and grain zinc concentration affected by Zinc rate and nitrogen source

Parameters	N source (Ns)	Zinc Rate (Zn)					LSD(0.05)		
		Zn0	Zn5	Zn10	Zn15	Mean	Ns	Zn	Zn X Ns
Straw zinc concentration (mg/kg)	No	40.7	55.0	53.0	51.7	50.1			
	CF_{100}	76.0	78.0	81.0	81.3	79.1			
	$CF_{75}PM_{25}$	68.7	73.7	68.3	65.0	68.9	7.3	4.5	NS
	$CF_{50}PM_{50}$	59.7	69.3	67.7	60.7	64.3			
	Mean	61.3	69.0	67.5	64.7				
Grain zinc Concentration (mg/kg)	No	7.7	10.0	8.8	8.0	8.6			
	CF_{100}	13.2	12.3	11.1	10.9	11.9			
	$CF_{75}PM_{25}$	10.4	10.7	10.0	10.1	10.3	NS	NS	NS
	$CF_{50}PM_{50}$	9.7	10.3	10.3	10.9	10.3			
	Mean	10.3	10.8	10.0	10.0				

NS = not significant at $P > 0.05$. *No*: No N fertilizer; CF_{100} : 100% Chemical fertilizer (Urea); $CF_{75}PM_{25}$: 75% chemical fertilizer +25 Poultry manure (PM); $CF_{50}PM_{50}$: 50% Chemical fertilizer +50 Poultry manure (PM); Zn_0 , Zn_5 , Zn_{10} and Zn_{15} are 0, 5, 10 and 15 kg Zn /ha respectively applied in the form $ZnSO_4$.



CHAPTER FIVE

5.0 DISCUSSION

5.1 POT EXPERIMENT

5.1.1 Comparing the effects of urea and sulphate of ammonia (SoA) on growth and yield

Nitrogen fertilization plays a crucial role in the development and productivity of rice. The source of N fertilizer influences growth and grain yield due to the differences of the N sources in supplying nitrogen to the crop. In the present study, most of the yield and growth attributes of rice responded differently to the different types of N fertilizer (Urea and SoA). In the pot experiment SoA proved superior to urea in terms of both grain yield and total biomass production. The positive influence of SOA might be because it contains sulfur which has been observed to have a positive influence on tillering which is a major yield determining attribute of rice (Dillon *et al.*, 2012).

The plants fertilized with SoA were taller than plants fertilized with urea for at mid-tillering, booting and maturity. However, there was a decrease in the percentage difference between SoA and urea after top dressing during the panicle initiation stage from 5.3% to 2.9%, and then further reduced to 1.2% at maturity. This suggests the efficiency of urea might have increased with time. The Urea fertilized plants attained similar heights like SoA fertilized plant after topdressing. This phenomenon might be because N uptake from SoA is higher than Urea only for the early stages of rice development (Heenan & Bacon, 1987).

The number of tillers produced is a major yield component in rice production. The nitrogen supply by SoA was more effective than urea in inducing tiller production from mid tillering to

maturity which also reflected on the total dry matter they produced. This was due to the increased N availability and uptake from SOA when compared to that of urea. The superiority of SoA might also be due to relatively reduced N loss compared to urea which might have consequently reduced the N supplied from urea (Heenan & Bacon, 1987). Increased N availability to rice enhances the cytokinin content within the tiller nodes which boosts germination of the tiller primordium (Liu *et al.*, 2011). According to Mannan *et al.* (2012) sufficient N supply at the early development of rice is essential for maximum tiller production at maturity.

Sulphate of ammonia was superior to urea in terms the number of effective tillers and above ground biomass. The observed differences could be due to the presence of sulfur in SoA. Sulfur has been reported to improve tillering (Dillon *et al.*, 2012). Improved tillering by SoA resulted in increase in above ground biomass. In contrast with this finding, Phongpan *et al.* (1988) did not observe any differences in above ground biomass produced from urea fertilized rice plants when compared to plants fertilized with SoA.

Nitrogen has been previously reported to influence the production, filling and quality of rice grain (Blumenthal *et al.*, 2008; Wang *et al.*, 2017). From the present study, grain production and development responded similarly to urea and SoA. From results obtained, N source did not influence test weight, number of grains/panicle and percentage filled grains/panicle despite its influence on vegetative growth. This finding is contrary to what was observed in wheat by Hafez & Kobata (2012), who reported that SoA application produced significantly higher number of grains/panicle than urea application.

In a study conducted by Heenan & Bacon (1987) SoA fertilized plots produced better yields than urea because of relatively greater N loss through volatilization from urea. Similarly, in the present study, grain yield produced by SoA treated plants was found to be statistically greater than that of urea. The yield produced by pots that received SoA as N source yielded 13% more than that of urea. This reflected in the superiority of SoA over urea in terms of number of productive tillers. The yield gap between SoA and urea might be due to the presence of sulfur in SoA. For wheat cultivation, it has been reported that sulfur is needed for optimum yield (Yesmin *et al.*, 2021). The results obtained from this study suggests that SoA in the pot experiment may have outperformed urea in terms of both yield and growth because the soils used were sulfur deficient. Yesmin *et al.* (2021) claimed that growing cereals on sulfur deficient soils could decrease crop yield. Another reason for the performance of SoA might be because the N released from urea is more susceptible to volatilization losses to the environment after application to the soil than SoA. Wilson (2003) observed 5% reduction in N loss when ammonium sulfate was employed instead of urea.

5.1.2 Response of yield and growth to Zinc rates

Zinc application on zinc deficient soils generally enhances growth in rice (Ghasal *et al.*, 2016; Kandali *et al.*, 2015). It has the potential of increasing plant height owing to the role zinc plays in internode elongation (Rao *et al.*, 2019). Zinc fertilization has been reported to influence the height of rice plants grown on soils with low zinc levels (Kadam *et al.*, 2018; Kandoliya *et al.*, 2018). However, findings from the pot experiment did not follow this trend even though the zinc level (0.21 mg/kg) in the soil used was low. From the results obtained zinc fertilization did not affect plant height at any of the growth stages. The lack of response of plant height to zinc

application suggest the available zinc in the soil used for the pot experiment was already sufficient to support internode elongation. This finding differs from that of Rao *et al.* (2019), who was able to increase the height of rice plants through zinc fertilization.

The lack of response of the vegetative growth attribute such as tiller number, plant height and dry matter accumulation to zinc application suggests even though zinc was applied, there was already enough to meet the zinc required for the vegetative growth of the plant. Another reason for this tendency may be due to reduced zinc uptake as a result of the high P level (114.6 mg/kg) of the soil (Rehman *et al.*, 2012). P concentration even increased more with the application of TSP after transplanting. This finding is contrary to what was reported earlier by Rao *et al.* (2019) that application of ZnSO₄ at 25 kg/ha gave significantly higher tiller number and straw yield than the control. Kandali *et al.* (2015) also reported a significant impact on zinc application on growth attributes at 5 kg/ha in a field experiment. The results obtained on above ground biomass conforms with that of Kalala *et al.* (2016) who did not observe response of dry matter to zinc fertilization even on a soil with zinc level below 1.5 mg kg⁻¹.

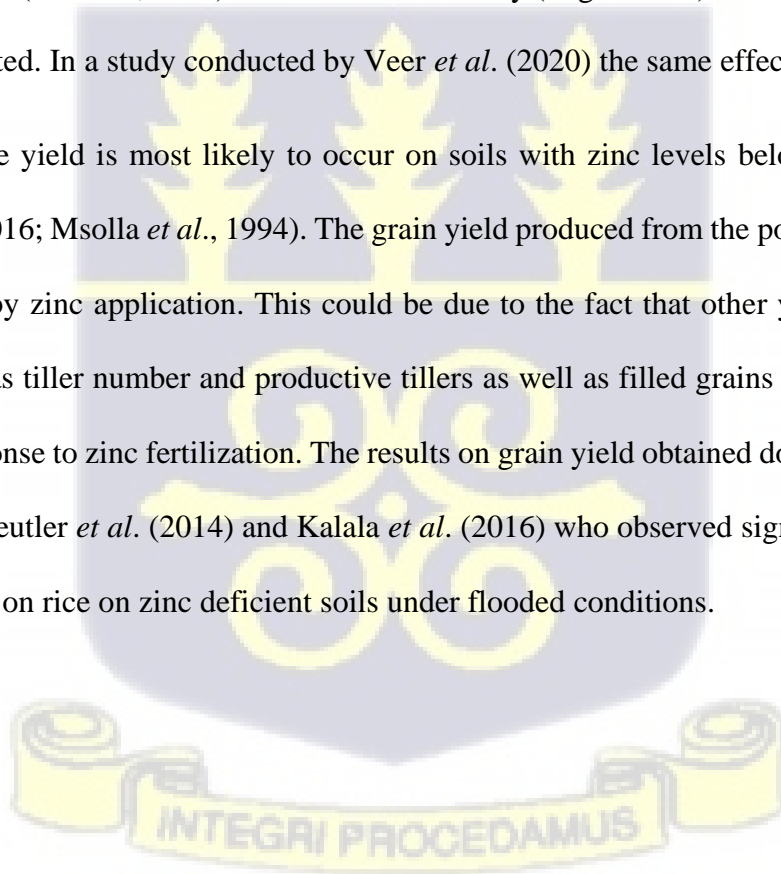
Zinc fertilization influenced the grains per panicle while it did not have any impact on the 1000-grain weight and filled grains percentage. Increasing zinc rate to 10 kg Zn/ ha produced the highest grains per panicle of 113.4. Any further increase in zinc rate resulted in a significant decrease the grains per panicle. This result suggests that higher levels of zinc in the soil might have had a toxic effect on grain production. The response of rice to zinc application was expected because zinc is actively involved in pollen formation and seed production in rice (Rao *et al.*, 2019). Jat *et al.* (2011) and Kandali *et al.* (2015) also observed the influence of zinc application on grain production. However, they did not notice any toxic effect of higher zinc

rates on grain production as it was observed in the present study (Jat *et al.* 2011 and Kandali *et al.*, 2015).

Unlike grains per panicle, there was lack of impact of zinc fertilization on percentage filled grains. This may be due to the fact that although zinc was involved in grain production it was not engaged in the grain filling (Jiang *et al.*, 2007). Jiang *et al.* (2007) claimed that zinc has a limited involvement in grain filling.

There was a lack of response of 1000-grain weight (test weight) and harvest index to zinc application which was expected due to the fact that they are genetic traits which depend mainly on the type of variety used in the study and that environmental factors such as fertilization has no influence on it (Yoshida, 1981). Since the same variety (Legon rice1) was used this tendency was to be expected. In a study conducted by Veer *et al.* (2020) the same effect was observed.

Response of rice yield is most likely to occur on soils with zinc levels below 1.0 mg Zg/ha (Kalala *et al.*, 2016; Msolla *et al.*, 1994). The grain yield produced from the pot experiment was not influenced by zinc application. This could be due to the fact that other yield determining attributes such as tiller number and productive tillers as well as filled grains did not show any significant response to zinc fertilization. The results on grain yield obtained does not agree with the finding of Beutler *et al.* (2014) and Kalala *et al.* (2016) who observed significant impact of zinc application on rice on zinc deficient soils under flooded conditions.



5.2 FIELD EXPERIMENT:

5.2.1 Evaluating the effect of Integrated N application on the yield and growth of rice

Previous studies have shown that combined application of inorganic and organic fertilizers is more effective in grain and biomass production than sole application of organic or inorganic nitrogen sources (Ismael *et al.*, 2021; Selim, 2020). The combined application is accompanied with benefits that the crop cannot benefit when compared with sole application of N. The rice plant is likely to respond differently to N from urea and PM, primarily due to their nutrient release patterns. Secondly apart from the nitrogen PM provided to the crop it also contains other micro and macro nutrients that can enhance the growth and yield of rice.

In the present study replacing 25 % of the traditionally applied chemical fertilizers with PM was found be at par with sole application of chemical fertilizer (CF₁₀₀) in most of the growth and yield attribute rice. Although the same amount of N was applied, most of the growth and yield attributes of rice responded differently based on the source and ratio of the combination of the fertilizer.

Findings from the field experiment showed that N application enhanced the growth attributes plant height, tiller/m² and above ground biomass better than unfertilized plants. Similar trends have also been reported in previous studies (El-Refaae *et al.*, 2007; Malik *et al.*, 2014). Considering the role nitrogen plays in most of the metabolic activates affecting growth, this outcome was to be expected (Shrestha *et al.*, 2020b).



Between plants that received nitrogen fertilizer application from different N sources, there was no significant differences observed in plant height. This outcome agrees with what was reported earlier by Moe *et al.* (2019) who did not observe any difference in rice plant height for crops treated with 100% urea and 50% Urea + 50% PM.

Nitrogen plays a significant role in tiller development in rice (Sakakibara *et al.*, 2006). Findings from Belefant-Miller (2007) indicated that poultry manure application increases tillering in rice over sole application of chemical fertilizer. This finding was dissimilar from what was observed. Urea 100 % as N source recorded the highest aboveground biomass. Similarly, decreasing the urea percentage in the N source treatment decreased the aboveground biomass, thus the Urea 50 % + PM 50 % recorded lower aboveground biomass compared to Urea 75 % + PM 25 %. Moe *et al.* (2019) made similar observation, reporting that the aboveground biomass produced by 50 % PM+ 50 % Urea was lower than that of 100% Urea.

The N source had an influence on yield attributes such as number of effective panicles/m², grains per panicle and percentage filled grains. Results from the field experiment showed that the number of productive tillers produced by 50 % CF + 50 % PM was markedly lower than sole application of CF. A dissimilar trend was obtained by Arif *et al.* (2014), who observed a significant increase in grains per panicle after replacing 50 % of the recommended chemical fertilizer trend with PM.

From the present study 75 % Urea + 25 % PM recorded the greatest number of grains per panicle followed by 100 % CF then 50 % CF + 50PM. A similar trend was observed for the percentage filled grains which suggests that grain production and filling was increased after replacing 25% of the chemical fertilizer with PM, however, further increase in the proportion of the PM content

negatively affected the grain filling and production. PM has reduced nutrient release ability, limit nutrient uptake and fail to meet short-term crop requirements (Iqbal *et al.*, 2019). Therefore, increasing the proportion of the CF helped to meet the short term demands of the crop. When 50% of the CF was replaced with PM, it was not able to produce/ release sufficient of N to meet the present N requirement of the plant.

In this study test weight did not respond to nitrogen fertilizer application, contrary to earlier reports by Tang *et al.* (2015) that combined application of inorganic and organic fertilizer could potentially enhance test weight of rice. On the other hand, it may be argued that rice test weight is a genetic trait and depends on the variety of rice. The finding of this study confirms the earlier report by Jamil & Hussain (2000), who observed no response of test weight to N fertilization.

Differences in grain yield was observed between the ratio of Urea and PM (75% Urea + 25%PM and 50% Urea + 50% PM). The grain yield of 75% Urea + 25% PM was greater than 50% Urea + 50% PM and was not significantly different from that of 100% Urea. This differences in yield could be explained by the fact that differences exist in the nutrient release patterns of different types of nitrogen fertilizer sources. Treatments whose nitrogen release synchronizes with the demands of the crop promotes high yields. This tendency was also observed by Karki *et al.* (2018) who reported that combining urea and Farmyard mature in ratio of 75% to 25% recorded the highest grain yield and number of effective tillers than 50% to 50% combination and sole application of urea or farmyard manure (Karki *et al.*, 2018). According to Meng *et al.* (2009) replacing 10%-20% of inorganic nitrogen with organic nitrogen could improved N consumption efficiency and consequently improved yield. However, replacing it beyond 20% may negatively affect yield. When 50% of the urea was replaced with PM, it was not able to release sufficient

N to meet the present N requirement of the plant. Due to this 50% CF + 50 %PM recorded the lowest grain yield among the N fertilized plots.

5.2.2 Effect of zinc fertilization on growth and yield on rice

Zinc is involved in metabolic and biochemical processes that influence growth and yield of crops (Rehman *et al.*, 2012). Because of this, cultivation of crops on zinc deficient soils can impair the growth of crops. The results obtained from the field experiment showed that the major growth attributes of rice, that is, plant height, tiller/m² and above ground biomass were influenced by zinc fertilization at least at one growth stage. The impact of zinc fertilization is most likely because zinc concentration in the soil (0.21 mg kg⁻¹) was below the critical zinc level needed for vegetative growth. The application of zinc on soils with zinc level below 1.4 mg kg⁻¹ was reported to have a positive influence on vegetative growth in rice (Kalala *et al.*, 2016; Msolla *et al.*, 1994).

The findings from the field experiment show that zinc fertilization affected plant height from booting up to maturity. The height of plants from plots that received zinc fertilizer application was greater than the unfertilized plots (control) at booting and maturity. However, difference in height was not observed between plots that received zinc application (Zn5, Zn10 and Zn15). The involvement of zinc in internode elongation could explain the considerable increase in height of plant of the fertilized plots over the control (Rao *et al.*, 2019). Kadam *et al.* (2018) also reported an increase in plant height after the application of zinc sulphate.

While tillering was enhanced by zinc fertilization at mid tillering, the effect of zinc fertilization was not observed at booting and maturity. The response of rice to zinc fertilization at early stage of the rice might be because of increase in zinc uptake that resulted in increase in auxin

metabolism which is a key contributing factor in tillering in rice (Khan *et al.*, 2007). The lack of response to tillering at maturity could be explained by a sudden increase in zinc availability and uptake from booting. In similar experiment Rao *et al.* (2019) observed a positive influence of zinc fertilization on tillering from active tillering to maturity. Similarly Khan *et al.* (2007) also obtained significantly higher number of tiller (17.41) after application of 10 kg Zn ha⁻¹ when compared to the control.

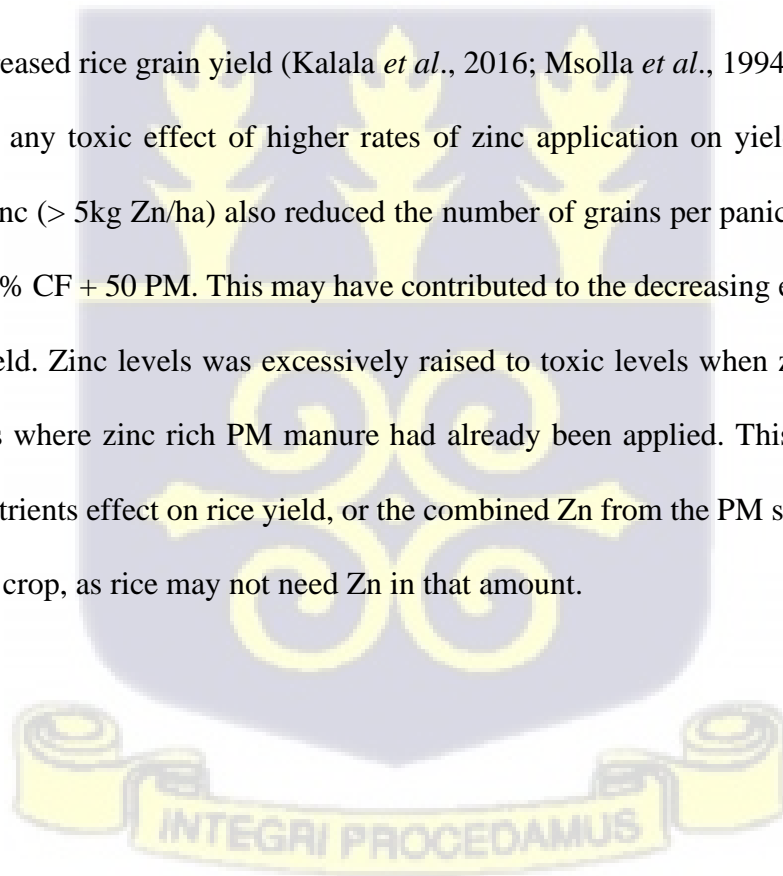
The main effect Zinc rate did not influence the number of panicles/m². This suggests that the zinc available in the soil was already sufficient for physiological activities such as photosynthesis and sucrose production which are key contributors to the production of panicle bearing tillers. In contrast Rao *et al.* (2019) reported that the application of zinc on soils low in zinc enhances the production of productive panicle bearing tillers. In the present study the effect of zinc rate on panicles/m² was different under the various N sources. Increasing zinc rate under CF₁₀₀, increased the number of panicles/m², whereas increasing zinc rate under the N sources containing PM tended to decrease the panicles/m². This suggests that applying Zinc sulphate to plots already treated with PM which contained zinc, may have increased the zinc level high enough for it to be toxic to the plants.

Finding from the field experiment showed the above ground biomass was statically influenced by zinc fertilizer at mid tillering. However, zinc fertilization did not show any influence on the above ground biomass recorded at booting and maturity. This might be because zinc is a micronutrient which the plant needs in small amount. Therefore, the amount inherent in the soil was sufficient to meet the maximum amount of zinc required to support the vegetative growth

of the plants. Similarly Kalala *et al.* (2016) did not observe response of dry matter to zinc fertilization on a soil with zinc level below 1.5 mg kg⁻¹.

The test weight of the rice grains did not exhibit any response to zinc fertilization in the present study. This is because the test weight is a genetic trait and environmental factors such as fertilization has no influence on it (Yoshida, 1981).

Previous studies have showed that cultivation of rice on zinc deficient soils can significantly reduce the yield of cereals (Cakmak *et al.*, 1997). In the present study the experiment was conducted on soil with low zinc level (0.21 mg/kg). The zinc rate 5 kg Zn/ha recorded the highest grain yield. Increasing the zinc rate above this rate resulted in the decrease in grain yield. In field experiments conducted on soils with zinc concentration below 1.0mg Zn/ha, zinc fertilization increased rice grain yield (Kalala *et al.*, 2016; Msolla *et al.*, 1994). However, they did not observe any toxic effect of higher rates of zinc application on yield. Application of higher rate of zinc (> 5kg Zn/ha) also reduced the number of grains per panicle under 75% CF + 50 PM and 50% CF + 50 PM. This may have contributed to the decreasing effect higher rates had on grain yield. Zinc levels was excessively raised to toxic levels when zinc sulphate was applied on plots where zinc rich PM manure had already been applied. This could be due to imbalance of nutrients effect on rice yield, or the combined Zn from the PM source and Zn rate was toxic to the crop, as rice may not need Zn in that amount.



5.2.3 Effect of N source and zinc rate Zinc uptake

The uptake of a nutrient measures the total amount of that nutrient in the tissues of the plant. Sufficient nitrogen nutrition enhances the uptake of other nutrients from the soils (Shahane *et al.*, 2018; Yoshida, 1981). In a field experiment Kutman *et al.* (2011) observed a significant increase in Zinc uptake for wheat after the application 225 kg N/ha. Similarly, in the present study the source of N fertilizer significantly influenced zinc uptake in both rice straw and grains. Sole application of chemical fertilizer (CF₁₀₀) recorded the highest zinc uptake by the straw which differed from the other N sources that had PM integrated with the urea (CF). However, the grain zinc uptake recorded by sole application of urea and integrated application was similar. Although nutrient uptake and partitioning are different in crops, in this study and for this parameter, the rice crop responded in same manner. The N sources contained the same amount of N at the time of harvest, they had all released similar amounts of N that boosted Zinc uptake. The total zinc uptake between the plots that received zinc fertilization were similar, however they were all significantly higher than the control. A similar trend was also observed for wheat by Kutman *et al.* (2011). This effect was because adequate nitrogen supply to the plant improved the vegetative growth and root development of the fertilized plant. This enhanced the ability of the root to absorb other nutrients including zinc from the soil.

Unlike N source, Zinc fertilization neither influenced the total zinc uptake nor the zinc uptake in the straw. However, zinc fertilization had a significant impact on the grain zinc uptake. Zn5 produced the greatest grain zinc uptake. However, increasing the zinc rate above 5kg Zn/ha significantly reduced the zinc amount in the grain. This suggests that higher rates of zinc application may have a toxic effect on the plant. Benton (1991) and Borkert *et al.* (1998) also

observed the negative response of cereals and legumes to higher zinc rates . They claimed that Zinc was phytotoxic for a number of cereals such as maize, wheat and rice.

Shahane *et al.* (2018) argued that zinc and N had synergistic effect on rice growth, yield and nutrient uptake. Nevertheless, the results obtained from the field experiment indicates that there was no interaction between zinc rate and N source on the total zinc uptake in rice. This finding does not agree with Shahane *et al.* (2018) and Arora & Singh (2004) who observed a positive interactive effect of N fertilization and Z fertilization on nutrient uptake in rice and Barley respectively.

5.2.4 Effect of zinc and nitrogen fertilization on grain zinc concentration

Increase in nitrogen uptake and nutrition improve the uptake of other nutrients from the soil. In the current study zinc and nitrogen application increased zinc concentration in the straw. This finding is in accordance with Khan *et al.* (2018), who successfully increased zinc concentration in vegetative organs of rice through zinc fertilization. However, he did not observe any difference among the grain zinc concentration produced by various N sources. Similarly, among the various nitrogen sources in this study, the zinc concentration in the straw was similar because there was sufficient N produced by the various N sources to enhance the uptake of zinc.

Increase in zinc uptake can eventually results in increase in zinc concentration in the straw (Yin *et al.*, 2016). In the present study, on the average the zinc concentration in the straw of the zinc fertilized plot was 10 % higher than the unfertilized plots. Increasing zinc rate up to 5 kg Zn/ha increased the straw zinc concentration. However, further increases of the zinc application rate did not influence it.

Unlike straw zinc concentration, grain zinc concentration was not influenced by both nitrogen and zinc fertilization, although grain zinc uptake was influenced by zinc fertilization. More than 80% of the zinc partitioned into the shoot was found in the straw. The wide gap between zinc concentration in the straw and grains may be due to certain morphological and physiological barriers preventing movement of Zinc from the straw to the grain. This implies that internal translocation of Zn from shoot to panicle or from rachis to grain is the primary cause of low concentration of zinc in the grains, rather than root uptake of Zn from the soil. A similar tendency was observed by Yin *et al.* (2016) who reported that Zinc fertilizer application increased the brown rice zinc concentration only by 20%, however there was 100% increase in zinc concentration in vegetative parts.



CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

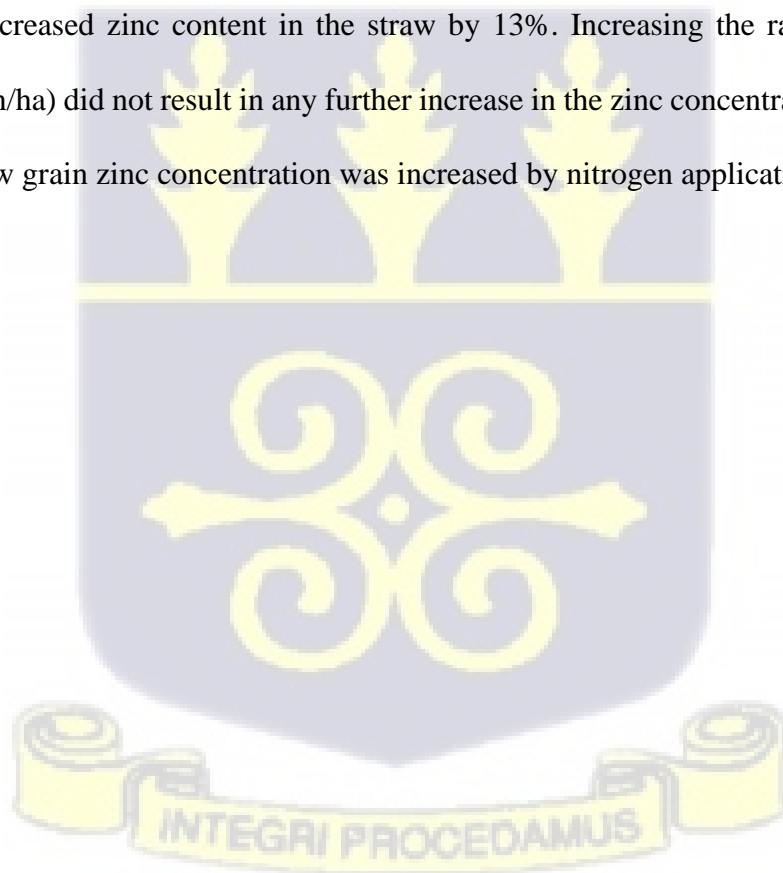
6.1 CONCLUSION

A pot and a field experiment were setup to examine the response of rice to zinc fertilization and source of nitrogen fertilizer. The following conclusions were drawn based on the results obtained from both experiment:

- SoA was a better source of inorganic N fertilizer than Urea. The response of majority of growth and yield components of rice to SoA application was superior to Urea application. SoA application produced the greatest number of tillers, productive tillers, above ground biomass and percentage filled grains. The grain yield attained from SoA was 13 % higher than urea.
- In the pot experiment growth and yield attributes such as above ground biomass, plant height, tiller number at maturity, effective tillers, panicle length, 100-grain weight and grain yield did not respond to Zinc application. For the field experiment there was response of grain yield and number of productive tillers of rice to zinc application. Applying 5 kg Zn /ha resulted in the greatest grain yield. However, increasing the rate of zinc application above this level resulted in reduction in yield and number of panicles/m².
Growth attributes such as above ground biomass plant height, and tillering did not respond to zinc fertilization.
- Replacing 25% of the recommended rate of conventionally applied chemical fertilizers with PM was found to have similar effect as sole application of chemical fertilizer

(CF₁₀₀) on most of the growth and yield attribute of rice. However, sole application of chemical fertilizer (CF₁₀₀) performed better than when the urea(CF) and PM was combined in a ratio of 50: 50 (CF₅₀ PM₅₀). CF₇₅ PM₂₅ produced the highest grain yield and was found to be statistically similar to sole application of urea. The ratio of the chemical N source to organic N source had an influence of both growth and yield.

- The source of N fertilizer had an impact on the total zinc uptake. Zinc uptake for sole application of chemical N fertilizer was markedly greater than combined application with of urea with PM.
- Zinc fertilizer did not influence zinc concentration in the grains. Application of 5kg Zn/ha increased zinc content in the straw by 13%. Increasing the rate of application (>5kg Zn/ha) did not result in any further increase in the zinc concentration in the straw. The straw grain zinc concentration was increased by nitrogen application.



6.1 RECOMMENDATION

Based on the outcomes of the field and pot experiment the following recommendations have been made:

- SoA would be better choice of N fertilizer for rice farmers. Further studies should be done to investigate the timing of application on N uptake from SoA and urea at the various growth stages. m,
- Combined application of chemical fertilizers with PM should be adopted by farmers to improve yield and soil fertility for sustainable rice cultivation.
- Farmers can apply zinc rich PM alone without any application of inorganic zinc source to correct zinc deficiency in the soils.
- The application of zinc fertilizers should be regulated since excess application of zinc may be toxic to rice.



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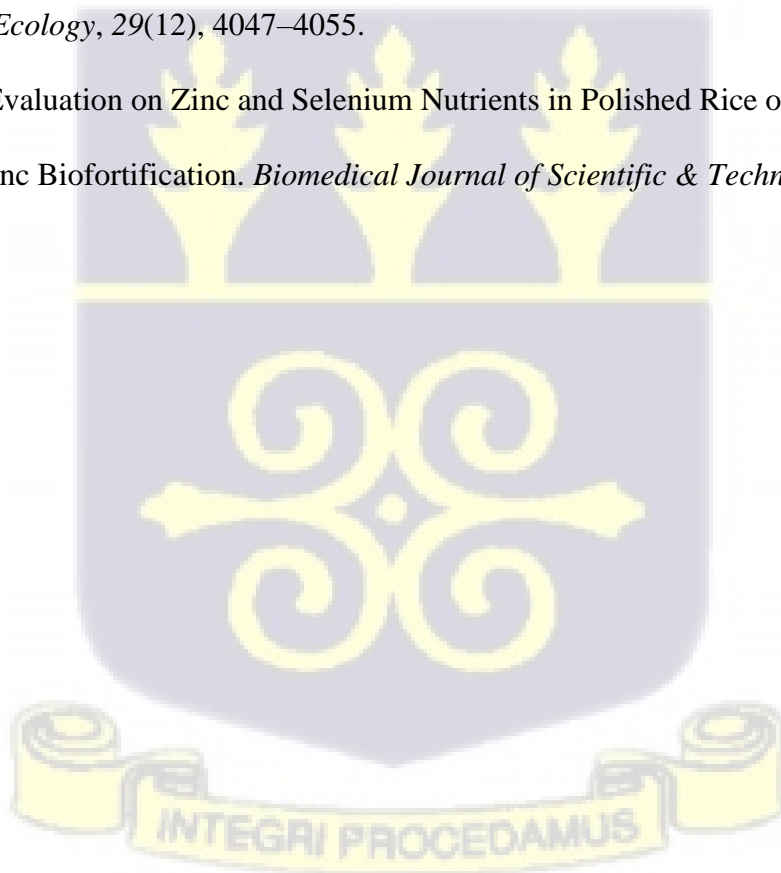
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APPENDIX

Appendix A: ANOVA tables for pot experiment

Appendix A1. ANOVA table for plant height at 27 Days After transplanting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Nitrogen	1	40.111	40.111	5.3	0.03 *
Zinc	5	73.996	14.799	1.96	0.122 NS
Nitrogen x Zinc	5	26.936	5.387	0.71	0.62 NS
Residual	24	181.507	7.563		
Total	35	322.549			

Appendix A2. ANOVA table for plant height at 55 Days After transplanting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Nitrogen	1	35.01	35.01	2.16	0.154 NS
Zinc	5	158.61	31.72	1.96	0.122 NS
Nitrogen x Zinc	5	142.1	28.42	1.76	0.16 NS
Residual	24	388.55	16.19		
Total	35	724.28			

Appendix A3. ANOVA table for plant height at 98 Day After transplanting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Nitrogen	1	10.67	10.67	0.79	0.383 NS
Zinc	5	173.1	34.62	2.56	0.054 NS
Nitrogen x Zinc	5	90.47	18.09	1.34	0.282 NS
Residual	24	324.33	13.51		
Total	35	598.57			

Appendix A4. ANOVA numbers at transplanting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
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Nitrogen	1	8.028	8.028	5.56	0.027	*
Zinc	5	5.806	1.161	0.8	0.558	NS
Nitrogen x Zinc	5	5.139	1.028	0.71	0.621	NS
Residual	24	34.667	1.444			
Total	35	53.639				

Appendix A5. ANOVA table for tiller numbers at 55 Days After transplanting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	261.361	261.361	53.38	<.001	**
Zinc	5	146.389	29.278	5.98	<.001	**
Nitrogen x Zinc	5	18.889	3.778	0.77	0.58	NS
Residual	24	117.5	4.896			
Total	35	544.139				

Appendix A6. ANOVA table for tiller numbers at 98 Days After transplanting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	72.25	72.25	15.86	<.001	**
Zinc	5	10.806	2.161	0.47	0.792	NS
Nitrogen x Zinc	5	5.417	1.083	0.24	0.942	NS
Residual	24	109.333	4.556			
Total	35	197.806				

Appendix A7. ANOVA table for Effective tillers at 98 Days After transplanting

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	72.25	72.25	14.61	<.001	*
Zinc	5	13.972	2.794	0.57	0.726	NS
Nitrogen x Zinc	5	4.833	0.967	0.2	0.961	NS
Residual	24	118.667	4.944			
Total	35	209.722				

Appendix A8. ANOVA table for above ground biomass accumulation at maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	1086.69	1086.69	13.61	0.001	**

Zinc	5	831.56	166.31	2.08	0.103	NS
Nitrogen x Zinc	5	748.75	149.75	1.88	0.136	NS
Residual	24	1915.97	79.83			
Total	35	4582.98				

Appendix A9. ANOVA table for days to 50% flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	0.25	0.25	0.15	0.704	NS
Zinc	5	35.139	7.028	4.15	0.007	NS
Nitrogen x Zinc	5	16.917	3.383	2	0.116	NS
Residual	24	40.667	1.694			
Total	35	92.972				

Appendix A10. ANOVA table for Panicle length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	0.4647	0.4647	0.55	0.465	NS
Zinc	5	6.9037	1.3807	1.64	0.189	NS
Nitrogen x Zinc	5	1.1516	0.2303	0.27	0.923	NS
Residual	24	20.2549	0.844			
Total	35	28.7749				

Appendix A11. ANOVA table for Test weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	42.25	42.25	4	0.057	NS
Zinc	5	13.81	2.76	0.26	0.93	NS
Nitrogen x Zinc	5	48.92	9.78	0.93	0.481	NS
Residual	24	253.33	10.56			
Total	35	358.31				

Appendix A12. ANOVA table for grains per panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	113.8	113.8	0.72	0.406	NS
Zinc	5	8953.9	1790.8	11.28	<.001	**
Nitrogen x Zinc	5	920.9	184.2	1.16	0.357	NS
Residual	24	3811.1	158.8			
Total	35	13799.6				

Appendix A13. ANOVA table for Percentage filled grains

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	101.7	101.7	2.51	0.126	NS
Zinc	5	313.42	62.68	1.55	0.212	NS
Nitrogen x Zinc	5	171.59	34.32	0.85	0.529	NS
Residual	24	971.08	40.46			
Total	35	1557.78				

Appendix A14. ANOVA

table for grain yield per pot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	469.05	469.05	22	<.001	
Zinc	5	156.73	31.35	1.47	0.236	
Nitrogen x Zinc	5	244.12	48.82	2.29	0.078	
Residual	24	511.77	21.32			
Total	35	1381.68				

Appendix A15. ANOVA table for harvest index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Nitrogen	1	0.003342	0.003342	3.25	0.084	NS
Zinc	5	0.013039	0.002608	2.53	0.056	NS
Nitrogen x Zinc	5	0.012105	0.002421	2.35	0.071	NS
Residual	24	0.02469	0.001029			
Total	35	0.053176				

Appendix B: ANOVA tables for Field experiment

Appendix B1. ANOVA table for plant height at mid tillering stage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
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Rep stratum	2	40.78	20.39	0.68	
Rep.Ns stratum Ns	3	532.653	177.551	5.93	0.032
Residual	6	179.763	29.961	5.71	
Rep.Ns.Zn stratum					
ZN	3	40.706	13.569	2.59	0.077
Ns.Zn	9	66.099	7.344	1.4	0.243
Residual	24	125.961	5.248		
Total	47	985.962			

Appendix B2. ANOVA table for plant height at booting stage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	641.73	320.86	28.49	
Rep.Ns stratum					
Ns	3	1352.4	450.8	40.02	<.001
Residual	6	67.58	11.26	1.04	
Rep.Ns.Zn stratum					
Zn	3	211.84	70.61	6.5	0.002
Ns.Zn	9	200.89	22.32	2.05	0.077
Residual	24	260.81	10.87		
Total	47	2735.26			

Appendix B3. ANOVA table for plant height at maturity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	17.664	8.832	0.2	
Rep.Ns stratum					
Ns	3	1076.845	358.948	8.23	0.015
Residual	6	261.776	43.629	6.69	
Rep.Ns.Zn stratum					
ZN	3	28.247	9.416	1.44	0.255
Ns.Zn	9	40.238	4.471	0.69	0.715
Residual	24	156.5	6.521		
Total	47	1581.27			

Appendix B4. ANOVA table for number of tillers/m² at Mid tillering stage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	590.9	295.4	1.16	
Rep.Ns stratum					
Ns	3	55816	18605.3	73.16	<.001
Residual	6	1525.8	254.3	1.74	
Rep.Ns.Zn stratum					
Zn	3	4548.3	1516.1	10.35	<.001
Ns.Zn	9	10386.6	1154.1	7.88	<.001
Residual	24	3516.7	146.5		
Total	47	76384.2			

Appendix B5. ANOVA table for Number of panicles/m²

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2076.04	1038.02	3.77	
Rep.Ns stratum					
Ns	3	19603.12	6534.38	23.72	<.001
Residual	6	1653.12	275.52	2.8	
Rep.Ns.Zn stratum					
Zn	3	385.42	128.47	1.31	0.296
Ns.Zn	9	4630.21	514.47	5.23	<.001
Residual	24	2362.5	98.44		
Total	47	30710.42			

Appendix B6. ANOVA table for number of tillers/m² at booting stage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	997.1	498.6	0.83	
Rep.Ns stratum					
Ns	3	37228.1	12409.4	20.57	0.001
Residual	6	3619.5	603.3	3.91	
Rep.Ns.Zn stratum					
Zn	3	840.6	280.2	1.82	0.171
Ns.Zn	9	9439.6	1048.8	6.8	<.001
Residual	24	3704.2	154.3		
Total					

47 55829.2

Appendix B7. ANOVA table for number of tillers/m² at maturity stage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2854.9	1427.5	4.12	
Rep.Ns stratum					
Ns	3	23423.4	7807.8	22.52	0.001
Residual	6	2080.5	346.7	1.78	
Rep.Ns.Zn stratum					
Zn	3	146.4	48.8	0.25	0.86
Ns.Zn	9	5963	662.6	3.4	0.008
Residual	24	4681.2	195.1		
Total	47	39149.5			

Appendix B8. ANOVA table for Above ground biomass at mid tillering stage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	730.35	365.18	4.58	
Rep.Ns stratum					
Ns	3	14833.14	4944.38	62.06	<.001
Residual	6	478.01	79.67	0.89	
Rep.Ns.Zn stratum					
Zn	3	1823.74	607.91	6.82	0.002
Ns.Zn	9	2239.75	248.86	2.79	0.022
Residual	24	2139.27	89.14		
Total	47	22244.28			

Appendix B9. ANOVA table for Above ground biomass at tillering stage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
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Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5134	2567	1.1	
Rep.Ns stratum					
Ns	3	153688	51229	21.86	0.001
Residual	6	14062	2344	1.65	
Rep.Ns.Zn stratum					
Zn	3	11444	3815	2.69	0.069
Ns.Zn	9	38178	4242	2.99	0.016
Residual	24	34085	1420		
Total	47	256590			

Appendix B10. ANOVA table for Above ground biomass maturity stage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	334467	167233	10.42	
Rep.Ns stratum					
Ns	3	1015249	338416	21.09	0.001
Residual	6	96264	16044	1.25	
Rep.Ns.Zn stratum					
Zn	3	47213	15738	1.22	0.322
Ns.Zn	9	248795	27644	2.15	0.065
Residual	24	308346	12848		
Total	47	2050334			

Appendix B11. ANOVA table for days to 50% flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.50	0.25	0.3	
Rep.Ns stratum					
Ns	3	11.75	3.917	4.7	0.051
Residual	6	5.00	0.833	0.52	
Rep.Ns.Zn stratum					
Zn	3	3.75	1.25	0.78	0.517
Ns.Zn	9	7.75	0.861	0.54	0.833

Residual	24	38.50	1.604
Total	47	67.25	

Appendix B12. ANOVA table for grain yields per panicle

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	11.85	5.93	0.1	
Rep.Ns stratum					
Ns	3	846.87	282.29	4.96	0.046
Residual	6	341.15	56.86	0.76	
Rep.Ns.Zn stratum					
Zn	3	226.1	75.37	1.01	0.405
Ns.Zn	9	1291.85	143.54	1.93	0.096
Residual	24	1787.69	74.49		
Total	47	4505.52			

Appendix B13. ANOVA table for percentage filled grains

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	172.8	86.4	26.14	
Rep.Ns stratum					
Ns	3	98.28	32.76	9.91	0.01
Residual	6	19.83	3.31	0.15	
Rep.Ns.Zn stratum					
Zn	3	140.72	46.91	2.1	0.126
Ns.Zn	9	361.52	40.17	1.8	0.12
Residual	24	535.18	22.3		
Total	47	1328.33			

Appendix B14. ANOVA table for 1000-grain weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	9.211	4.605	0.84	
Rep.Ns stratum					
Ns	3	17.158	5.719	1.04	0.439
Residual	6	32.874	5.479	0.78	

Rep.Ns.Zn stratum					
Zn	3	13.873	4.624	0.65	0.588
Ns.Zn	9	64.27	7.141	1.01	0.459
Residual	24	169.6	7.067		
Total	47	306.986			

Appendix B15 ANOVA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.2306	0.6153	5.56	
Rep.Ns stratum					
Ns	3	55.1490	18.3830	166.00	<.001
Residual	6	0.6644	0.1107	0.58	
Rep.Ns.Zn stratum					
ZN	3	3.3143	1.1048	5.83	0.004
NS.ZN	9	0.3799	0.0422	0.22	0.988
Residual	24	4.5469	0.1895		
Total	47	65.2852			

Appendix B16. ANOVA table for Harvest index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.009581	0.004791	7.98	
Rep.Ns stratum					
Ns	3	0.029848	0.009949	16.58	0.003
Residual	6	0.0036	0.0006	0.49	
Rep.Ns.Zn stratum					
ZN	3	0.002054	0.000685	0.56	0.645
NS.ZN	9	0.018283	0.002031	1.67	0.152
Residual	24	0.029231	0.001218		
Total	47	0.092597			

Appendix B17. ANOVA table for ANUE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	151.64	75.82	10.63	

Rep.Ns stratum					
Ns	2	261.07	130.53	18.3	0.01
Residual	4	28.54	7.13	0.22	
Rep.Ns.Zn stratum					
ZN	3	22.4	7.47	0.24	0.871
NS.ZN	6	32.39	5.4	0.17	0.982
Residual	18	571.12	31.73		
Total	35	1067.14			

Appendix B18. ANOVA table for AZU E

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	8157	4079	0.73	
Rep.Ns stratum					
Ns	3	5104	1701	0.31	0.82
Residual	6	33326	5554	3.39	
Rep.Ns.Zn stratum					
ZN	2	39283	19642	11.98	<.001
NS.ZN	6	2639	440	0.27	0.944
Residual	16	26237	1640		
Total	35	114745			

Appendix B19. ANOVA table for Straw zinc uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1171.43	585.71	7.15	
Rep.Ns stratum					
Ns	3	4857.03	1619.01	19.77	0.002
Residual	6	491.45	81.91	1.69	

Rep.Ns.Zn stratum					
ZN	3	331.05	110.35	2.27	0.106
NS.ZN	9	584.01	64.89	1.34	0.27
Residual	24	1164.2	48.51		
Total	47	8599.17			

Appendix B20. ANOVA table for grain zinc uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.4788	1.7394	0.37	
Rep.Ns stratum					
Ns	3	123.2671	41.089	8.83	0.013
Residual	6	27.934	4.6557	6.34	
Rep.Ns.Zn stratum					
ZN	3	7.9485	2.6495	3.61	0.028
NS.ZN	9	5.2552	0.5839	0.79	0.624
Residual	24	17.6326	0.7347		
Total	47	185.5162			

Appendix B21. ANOVA table for total uptake

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1261.07	630.53	6.84	
Rep.Ns stratum					
Ns	3	6464.24	2154.75	23.36	0.001
Residual	6	553.5	92.2	1.9	
Rep.Ns.Zn stratum					
ZN	3	400.62	133.54	2.68	0.07
NS.ZN	9	593.22	65.91	1.32	0.277
Residual	24	1196.68	49.86		
Total	47	10469.29			

Appendix B22. ANOVA table for straw zinc concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
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Rep stratum	2	129.54	64.77	1.2	
Rep.Ns stratum					
Ns	3	5222.06	1740.69	32.27	<.001
Residual	6	323.62	53.94	1.88	
Rep.Ns.Zn stratum					
ZN	3	419.56	139.85	4.88	0.009
NS.ZN	9	339.19	37.69	1.32	0.28
Residual	24	687.5	28.65		
Total	47	7121.48			

Appendix B23. ANOVA table for grain zinc concentration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	12.393	6.196	0.46	
Rep.Ns stratum					
Ns	3	63.086	21.029	1.57	0.292
Residual	6	80.499	13.416	4.42	
Rep.Ns.Zn stratum					
ZN	3	4.876	1.625	0.54	0.662
NS.ZN	9	18.16	2.018	0.67	0.732
Residual	24	72.822	3.034		
Total	47	251.835			

