EFFECTS OF COMMERCIAL CHEMICAL AND MICROBIOLOGICAL PRODUCTS IN SOIL ON MAIZE GROWTH AND YIELDS

DEODATUS STANLEY KIRIBA

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN SOIL SCIENCE AND LAND MANAGEMENT OF THE SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

A study was conducted under screenhouse and field conditions at Sokoine University of Agriculture to evaluate the effects of commercial chemical and microbiological products on growth and yields of SITUKA maize variety grown on an Ultisol soil. The study soil had medium organic matter, low available phosphorous (1.02 mg/kg) and very low extractable zinc (0.34 mg/kg) hence the soil was of medium fertility status and only moderately suitable for maize production. Commercial products evaluated were Teprosyn, P-solubilizers and N_2-fixers, in each experiment using the randomized complete block design (RCBD) with three replications. Six treatments, namely: i.control (without commercial product and P-fertilizer), ii.commercial product alone at manufacturer's recommended rate, iii.commercial product alone at double rate, iv.commercial product + 10 kg P ha^{-1}P-fertilizer, v.10 kg P ha^{-1} P-fertilizer and vi.20 kg P ha^{-1} P-fertilizer, were used. In the screenhouse, Teprosyn, P-solubilizers and N_2-fixers did not result in significantly ($P<0.05$) different growth performance or biomass yields relative to those in the control. However, these products in combination with P-fertilizer (at half recommended rate) resulted in significant ($P<0.05$) increase in growth parameters and biomass yields. Teprosyn increased biomass yields from 0.96 g/plant (Control) to 1.89 g/plant (K_2HPO_4 at 20 kg P ha^{-1}), P-solubilizers from 1.01 g/plant (Control) to 1.71 g/plant (K_2HPO_4 at 20 kg P ha^{-1}) and N_2-fixers from 0.95 g/plant (Control) to 2.19 g/plant (K_2HPO_4 at 20 kg P ha^{-1}). Under field experimentation, Teprosyn, P-solubilizers and N_2-fixers had no significant ($P<0.05$) effect on maize growth performance. Only the P-solubilizers, either alone or in combination with P-fertilizer, produced significantly ($P<0.05$) higher grain yields than the control increasing grain yields from 2.04 t ha^{-1} (Control) to 3.53 t ha^{-1} (YaraMila Cereal at 20 kg P ha^{-1}). It is recommended that Tanzania fertilizer regulatory authority should require manufactures to improve the quality standards of before the commercial products are accepted in the country and they should further be tested in other P-deficient soils.

DECLARATION

I, Deodatus Stanley Kiriba do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither being submitted nor being concurrently submitted in any other institution.

Deodatus Stanley Kiriba	Date
(MSc. Candidate)	

The above declaration is confirmed

Prof. Ernest Semu	Date
(Supervisor)	

Dr. Susan Ikera	Date
(Co-supervisor)	

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DEDICATION

This work is dedicated to the ones who bought me the first pencil I ever used in my life: my parents Paulina Clavery and Stanley Nshashi.

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LIST OF ABBREVIATION AND SYMBOLS

AAS	Atomic absorption spectrophotometer
ABA	Abscisic acid
AEZ	Agro-ecological zone
AM	Arbuscular mycorrhizal
ANOVA	Analysis of variance
BNF	Biological nitrogen fixation
BS	Base saturation
C/N	Carbon nitrogen ratio
CEC	Cation exchangeable capacity
CEC_{clay}	Cation exchange capacity of clay
CEC_{soil}	Cation exchange capacity of soil
CFU	Colony forming unit
CM	Crop Museum
$cmol_c$ (+)/kg	Centimole(+) per kilogram
CV (%)	Coefficient of variation (in percentage)
DAP	Days after planting
DAS	Days after sowing
DTPA	Diethylenetriaminepentaacetic acid
EM	Effective microorganisms
FAO	Food and Agriculture Organization of the United Nations
GPS	Global positioning system
HRR	Half recommended rate
IAA	Indole acetic acid
ISFM	Integrated Soil Fertility Managment

LSD	Least significant differences
m.s.l	Mean Sea Level
NA	Nutrient Agar
NSS	National Soil Service
OC	Organic carbon
P<0.05PSB	Phosphate solubilizing bacteria
PSFs	Phosphate solubilizing fungi
Psi	Pounds per square unit
PSMs	Phosphate solubilizing microorganisms
RCBD	Randomized Complete Block Design
SEA	Soil extract agar
SSS	Soil Survey Staff
SUA CM-P	Sokoine University of Agriculture crop museum profile
TCP	Tricalcium phosphate
TEB	Total exchangeable bases
TFRA	Tanzania Fertilizer Regulatory Authority
TSP	Triple Super Phosphate
USDA	United State Department of Agriculture
WRB	World Reference Base

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

For optimum crop growth, development and productivity, soil nutrients must be available sufficiently and balanced quantities (Chen, 2006). The most important constraint limiting high crop yields in developing countries, and especially among resource-poor farmers, is low soil fertility status (Mohamadi and Sohrabi, 2012).

Enhanced and sustainable soil fertility can be attained, among other things, through cultivation of crops capable of biological nitrogen fixation (BNF) (Gothwal *et al*., 2007). Use of commercial chemical and microbiological products (commonly known as commercial products) is also proposed as one of the technologies for the sustenance of high soil fertility and increased farm productivity (Woomer, 2012).

Microbiological products are composed of living microorganisms such as nitrogen (N) fixers, potassium (K) solubilizers and phosphorus (P) solubilizers, and molds or fungi which, when applied to seed or soil, colonize the rhizosphere and promote growth by converting nutritionally important elements (N and P) from unavailable to available forms through biological processes such as nitrogen fixation or solubilization of insoluble phosphates (Rokhzadi *et al*., 2008). Microbiological products are cost-effective inputs for farmers (Hameeda *et al*., 2006), and have been used to increase crop yields in several countries. For example, in Cuba, several microbiological products composed of strains of *Azotobacter, Rhizobium, Azopirillum* or *Burkhoderia* are commercially produced and used in the cultivation of different crops. These microbiological products have proved to increase

root and shoot elongation as well as yields of rice, beans, wheat, maize and sorghum (Ahmed, 2010).

Arbuscular mycorrhizal (AM) fungi improve plant growth by increasing the supply of mineral nutrients, particularly P and other minerals like zinc (Zn) and copper (Cu). Further, some microorganisms known as phosphate solubilizing microorgnainsms (PSMs) (Khan *et al*., 2007) have been found to associate with the roots of crop plants, thus playing an important role in increasing P-availability to plants and thereby increasing the growth and yields of the crop plants (Kamlesh *et al*., 2010). The phosphate solubilizing microorganisms include bacteria of the genera *Pseudomonas*, *Bacillus* and *Enterobacter*, along with fungi like *Penicillium* and *Aspergillus* (Tilak *et al*., 2005).

Similarly, N_2-fixing bacteria, mainly members of the genera *Azotobacter* and *Azospirillum,* have been isolated from the rhizospheres of various cereals and tested as bio-fertilizers to increase yields of the cereals and legumes through fixing atmospheric nitrogen (Bakulin *et al*., 2007; Gupta, 2004). Furthermore, Kaya *et al*. (2006), as cited by Pholo (2009), reported that treating seeds with commercial chemical products containing micro- and macro-nutrients has proved to improve germination and seedling establishment of wheat, soybean, sunflower and maize. However, the efficacy and quality of these products have not been sufficiently evaluated under local farming conditions of Tanzania, although they have been introduced in the Tanzanian market. To continue using these products calls for their quality to be high.

The Tanzania Fertilizer Regulatory Authority (TFRA) established in 2009 (URT,2009) is the national agency charged with all matters relating to quality of fertilizers and other amendments. The findings from this study will assist in recommending the most effective

commercial products for adoption by small-scale farmers in Tanzania. Furthermore, the results from this study will assist the TFRA in developing a legal framework to monitor, inspect and control the quality of these products within the country to ensure that farmers get effective products for increased crop yields.

1.2 Justification

Low soil fertility and high nutrient mining are among the main factors limiting crop yields in Sub-Saharan Africa (Adu-Gyamfi *et al*., 2007). Some soil fertility management technologies being pursued to address low soil fertility in Tanzania include use of organic soil amendments (e.g., crop residues, animal manures, agroforestry tree pruning) and inorganic (fertilizers, agro-minerals) resources, improved fallows (Kwesiga and Coe, 1994) and commercial products (bio-fertilizers and chemical products). Bio-fertilizers have increased crop yields in some countries. For example, in Cuba, *Azospirillum spp* and *Bacillus spp* bio-fertilizers have been shown to increase yields in maize, sorghum, and wheat (Ahmed, 2010). Products such as Twin-N from Australia, Penshabao from China, SKAF from USA, and EM technology as commercial products have, to some limited extent, been used in Tanzania, with promising results. Furthermore, commercial chemical products (containing micro-and macro-plant nutrients) have improved germination and seedling establishment of wheat, soybean, sunflower and maize.

Since 2010, some commercial chemical and microbiological products have entered the Tanzania market, each being claimed to have high effectiveness in improving soil fertility and increasing the productivity of various crops. However, their efficacy and quality have not been evaluated under Tanzanian farming conditions. The study prosed herein will test some of these products to ascertain their efficacy on improving crop growth and yields.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to increase maize yields through use of some commercial chemical and microbiological products in an Ultisol in Morogoro.

1.3.2 Specific objectives

The specific objectives were

(i) To determine the fertility status of an Ultisols soil occurring in the Sokoine University farm.

(ii) To verify the quality of a commercial chemical product (Teprosyn) and microbiological products (Bio-soil crop booster and Bio-soil Nitro+) so as to ascertain their nutrient contents and/or microbial population densities.

(iii) To evaluate the effectiveness of these commercial chemical products (with macro- and micro-nutrients) on maize growth and yields under glasshouse and field conditions.

(iv) To determine the effectiveness of P-solubilizers (Bio-soil crop booster) on maize growth and yields.

(v) To assess the effectiveness of free living N_2-fixers (Bio-soil Nitro+) on maize growth and yields.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutrients Essential for Crops Growth and Development

Crops, like all other living organisms, need some nutrient elements for their growth and development. Crops require 16 essential elements, in sufficient levels, for their physiological growth and development (Marschner, 1995).

Nutrient requirements for crops are categorized as the macro-and micro-nutrients, based on their total amounts needed. Macro-nutrients are required in large quantities while micro-nutrients are required in smaller quantities (Marschner, 1995). However, if one or more of these nutrients, whether macro- or micro-nutrients, are deficient, and do not satisfy physiological needs of crops, its deficiency leads to adverse crop growth effects. Alnwick (1996) reported that the deficiency of macro-nutrients in crops is visible and striking. For instance, nitrogen deficiency in crops leads to yellowing, stunting, and significantly low grain yields while deficiencies of micro-nutrients such as Cu, Zn, Mn, Fe, and B are not always visible, except under extreme cases of deficiencies.

Of all macronutrients, nitrogen and phosphorous are the most serious limiting nutrients for crop productivity (Christianson and Vlek, 1991; Manu *et al*., 1991; Takow *et al*., 1991). Furthermore, Wilding and Hossner (1989) reported that aluminium toxicity and calcium and magnesium deficiencies also limit not only the growth and yields but also quality of legumes and cereals.

2.2 Sources of Nutrients Essential for Crop Growth and Development in soils

Crop nutrients are normally abundant in the soil. However, due to long term cropping practices and poor soil management, reserves of nutrients in soils are depleting in many

arable soils. The depletion of nutrients in soils results in nutrient deficiencies that have been linked to low crop yields across southern Africa, including Tanzania (Adu-Gyamfi *et al*., 2007).

Three essential nutrients (carbon, hydrogen and oxygen) required for growth and development of crops are derived from the atmosphere and soil water while the remaining essential elements usually grouped as primary nutrients, secondary nutrients and micronutrients including (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum and chlorine) are supplied either from soil minerals and soil organic matter or by organic or inorganic fertilizers (Silva and Uchida, 2000). The primary nutrients (N, P and K) are commonly found in blended N-P-K fertilizers such as 10-10-10, or other grades. Primary nutrients are utilized in the largest amounts by crops, and, therefore, are applied at higher rates than secondary nutrients and micronutrients (Tucker, 1999).

The secondary nutrients (Ca, Mg and S) are supplied by the soil or supplemented in the soil via use of dolomitic lime (aglime), although these nutrients are also available from a variety of fertilizer sources. However, sulfur is also available in fertilizers such as potassium and magnesium sulfate, gypsum (calcium sulfate), minjingu mazao and elemental sulfur (Tucker, 1999).

Rock types determine the natural abundance of nutrients, particularly micronutrients. For example, igneous ultramafic and mafic rocks (pyroxenites, basalts) and sediments such black shales contain generally higher amounts of Cu, Co, Fe, Mn, B and other trace elements and B than silicate-rich granites (Van Straaten, 2002).

A significant amount of micronutrients, especially in surface soils, may arise from industrial and urban pollution through the use of untreated sewage and industrial effluents for irrigation purposes, use of agricultural sprays as well as through fertilizers. Agricultural chemicals used to control pests may increase the concentration of some metals such as Zn and Cu in surface soils when used continually for long periods of time, particularly in horticultural crops (Nayyar *et al*., 2001) or in coffee.

Furthermore, the macro-nutrient fertilizers may sometimes contain appreciable amounts of micronutrients to benefit crop growth. For example, triple phosphates (TSP) contain 418.0, 49.3 and 3488 ppm of zinc, copper and iron, respectively, and urea contains 4.0, 0.6 and 36 ppm of Zn, Cu and Fe, respectively (Nayyar *et al*., 2001). Therefore, the essential nutrients are supplied either from soil minerals and soil organic matter or by organic or inorganic fertilizers.

The ultimate source of all mineral nutrients in the soil is soil parent material, as the micronutrients are abundant in the earth's crust (Nube and Voortman, 2006). However, these minerals may not always be soluble in soil. Thus, other than use of fertilizers, supplementation by commercial chemical products, or inoculation with microbial strains capable of solubilising the minerals, increases availability of some nutrients to plants.

2.3 Occurrence of Phosphate Solubilizing Bacteria

A great proportion of phosphate solubilizing bacteria (PSB) is concentrated in the rhizosphere, where they are metabolically more active than in soil further away (Vazquez *et al.*, 2000). Kim *et al*. (1998) reported that the PSB are ubiquitous, with variations in forms and populations in different soils, depending on soil properties (physical and chemical

properties, organic matter and P content) and crop cultural practices. However, the larger populations of PSB are found in agricultural and rangeland soils (Yahya and Azawi, 1998). Many phosphate solubilizing microorganisms (PSMs), including the genera of *Pseudomonas putida, Rahnella aquatitis, Serratia marcescens, Klebsiella pneumoniae, Burkholderia cepacia, Rhizobium sp., Aspergillus niger, Penicillium sp*. (Whitelaw, 2000), have been widely utilized in the field. These PSMs have played an important role in supplementing phosphorus to crops, increasing soil nitrogen through biological nitrogen fixation and increasing the availability of Fe, Zn, etc. through production of plant growth promoting substances (Kucey *et al*., 1989), hence increasing crop yields. For example, in Argentina, corn inoculated with PSMs of the genus *Azospirillum* (*lipoferum*) showed double the number of seeds per ear, an increase in seed dry weight by 59% , and a significant stimulation in root development (Fulchieri and Frioni, 1994).

In India, two PSMs isolates, namely *Aspergillus* sp. and *Penicillium* sp., were tested for their tricalcium phosphate (TCP) solubilization efficiency in liquid media. *Aspergillus* sp. was seen to solubilize 480 g/ml of phosphorus, while *Penicillium* sp. solubilized 275 g/ml of phosphorus from 0.5% tricalcium phosphate after 4 and 3 days of incubation, respectively. Thus, such microorganisms may not only compensate for higher cost of fertilizers but may also mobilize the fertilizers added to soil (Pradhan and Sukla, 2005).

Similarly, in Brazil, PSMs were isolated from soils basing on the solubilization efficiency of inorganic and organic phosphate sources in a modified Pikovskaya's liquid medium culture containing sodium phytate (phytic acid), soybean lecithin, aluminum phosphate ($AlPO_4$), and tricalcium phosphate ($Ca_3(PO_4)_2$). Among the isolates identified, strains of *Bacillus sp*. and *Burkholderia sp*. were the most effective, mobilizing respectively 67% and 58.5% of the total P (from $Ca_3(PO_4)_2$) after 10 days, and were isolated from the rhizosphere

of the P efficient L3 maize genotype, under P stress (Oliveira *et al*., 2009). In Ghana, the use of 19.67 kg/ha P (as TSP) + PSM in rice field trials out-yielded the treatment whereby only 19.67 kg/ha P (TSP) alone were applied to the rice crop (Asuming-Brempong, 2014).

Phosphate solubilizing bacteria (PSB) inoculants containing strains from genera such as *Pseudomonas, Bacillus, Enterobacter, Agrobacterium radiobacter* and *Azospirillum lipoferum* have been used to increase plant yields worldwide, and commercial products are currently available in the market. For example, in Cuba, several biofertilizers of *Azotobacter, Azospirillum, Burkholderia spp, Pseudomonas, Bacillus* and *Rhizobium strains* are commercially produced and applied with different crops (Hilda and Fraga, 2000). Similalarly, in Egypt, use of the biofertilizer *Azospirillum brasilense* with half N rate (144 kg N/ha) caused a significant increase in yields of maize (Mohammed *et al*., 2001)

Kloepper *et al*. (1991) as cited by Eid *et al.* (2009), reported wheat yield increase up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculants. Brown (1974) reported 10–20% yield increase in wheat yields in field trials using a combination of *Bacillus megaterium* and *Azotobacter chroococcum*.

Furthermore, Chabot *et al*. (1993), as cited by Mohamed (2012), reported an increase of root and shoot weight with dual inoculation of PSB in maize, while grain yields of the different maize genotypes treated with *Azospirillum* spp. varied between 1700 and 7300 kg ha^{-1} (Salmone and Dobereiner, 2004).

2.4 Mechanisms of Phosphorus Solubilization by PSB

Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus (Hilda and Fraga, 2000). Phosphorus solubilization is carried out by a

large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms (Whitelaw, 2000), organic acid production and proton extrusion (Dutton and Evans, 1996; Nahas, 1996). For example, inorganic P was solubilized by the action of low molecular weight organic acids, mainly gluconic and keto-gluconic acids (Goldstein, 1995; Deubel *et al.*, 2000), by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil (Nahas, 1996).

Organic acids produced by PSB can also solubilize insoluble phosphates by lowering the pH of the rhizosphere through biotical production of proton (Kim *et al*., 1997), chelation of cations (Al, Fe, Ca) and competing with phosphate for adsorption sites in the soil (Nahas, 1996). Inorganic acids, e.g. hydrochloric acid, can also solubilize phosphate; however, the inorganic acids are less effective compared to organic acids at the same pH (Kim *et al.*, 1997). A general sketch of P-solubilization in soil is shown in Fig. 1.

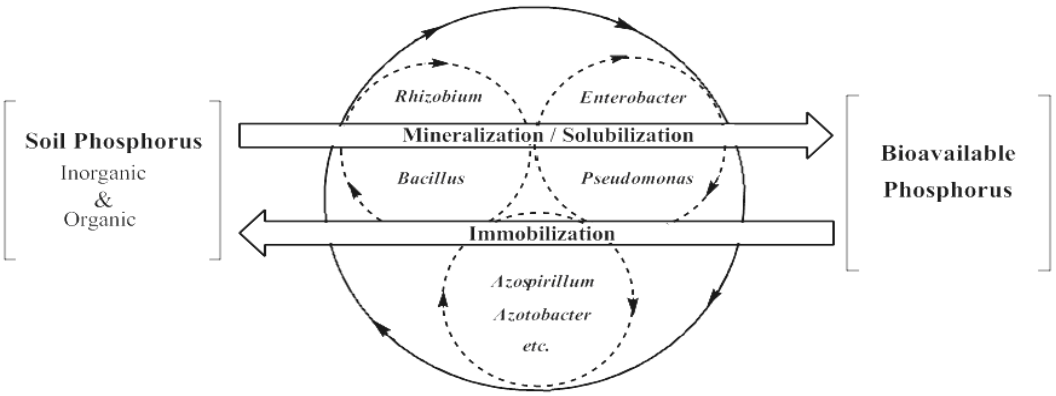

Figure 1: Schematic diagram of soil phosphorus mobilization and immobilization by bacteria
Source: Khan *et al*. (2009)

2.5 Effects of Phosphate Solubilizing Bacteria (PSB) on the Physiology of Crops

Phosphorous is essential for growth and productivity of plants as it plays an important role in plants in many physiological activities such as cell division, photosynthesis, and development of good root systems and utilization of carbohydrate. Phosphorous deficiency

results in the leaves turning purple accompanied by small leaves, weak stems and slow development (Sharma *et al*., 2011). Phosphate solubilizing bacteria (PSB) are bacterial strains that play an important role in supplying phosphate to plants, thus promoting plant growth (Khan *et al*., 2007) and reducing disease or insect damages (Chen *et al*., 2006) as root development, stalk and stem strength, flower and seed formation, crop maturity and production, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition (Khan *et al*., 2009).

In addition to increasing P supply, PSB produce and increase the synthesis of plant growth regulators, namely auxins, gibberellins, cytokinins, abscisic acid (ABA) and ethylene (Zahir *et al*., 2004), which play a vital regulatory role in plant growth and development. Of these hormeones, auxin is the predominant and active and is known to stimulate both rapid (e.g. increase in cell elongation) and long term (e.g. cell division and differentiation) responses in plants (Hagen, 1990).

A number of bacteria such as *Azospirillum* and *Azotobacter* are known to produce gibberellic acid, which is primarily responsible for stem elongation (Dobbelaere *et al*., 2003). Phosphate solubilizing bacteria also play a role in lowering the ethylene (an effective plant growth regulator that affects many aspects of plant growth, development and senescence) levels in plants, thus preventing some deleterious consequences of high ethylene concentration (Saleem *et al*., 2007).

2.6 Occurrence and Importance of Endophytic Nitrogen Fixing Bacteria

Endophytic bacteria are those bacteria that colonize the internal tissue of the plant, showing no external sign of infection or negative effect on their host (Ryan *et al*., 2008). Several

species of endophytic diazotrophs including *Acetobacter diazotrophicus, Herbaspirillum seropedicae, Herbaspirillum rubrisubalbicans, Burkholderia* sp., *Enterobacter* sp. and *Klebsiella* sp. were discovered in sugarcane plants (Boddey *et al*., 2003). These diazotrophs are able to use atmospheric nitrogen through a process known as biological nitrogen fixation (BNF), which is the conversion of atmospheric N_2 to NH_3, a form that can be used by plants (Lam *et al.*, 1996; Franche *et al.*, 2009).

Biological nitrogen fixation is a complex process that involves a number of functional and regulatory gene products (Triplett *et al*., 1989). The actual reduction of N_2 is performed by the nitrogenase enzyme complex, which consists of two metalloproteins: the nitrogenase, or nitrogenase molybdenum-iron protein (MoFe protein), and the nitrogenase reductase or nitrogenase iron protein (Fe protein) (Rees *et al*., 1998).

The Fe protein is responsible for shuttling electrons to the MoFe protein using at least two MgATPs per electron (Halbleib *et al*., 2000). The molybdenum-iron-sulfur-homocitrate clusters of the MoFe protein are the actual sites of binding and reduction of the substrate N_2, and other alternative substrates, such as acetylene, protons and many others (Postgate, 1982). Biological nitrogen fixation, thereofere, plays an important role in nitrogen availability, but it is an intensive energy input process which is tightly regulated on several levels and by different factors.

In addition, these bacteria significantly affect plant growth by increasing nutrient cycling, suppressing pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic substances and/or by producing biologically active substances such as auxins and other plant hormones such as indole acetic acid (IAA) which are known to to increase root growth and root length, resulting in greater root surface area which enables the plant to

access more nutrients from soil (Khalid *et al.,* 2004; Boiero *et al*., 2007). Recently, it was also found that IAA induces an increased level of protection in plants against external stress conditions (Bianco and Defez, 2009) and auxins of microbial origin in the interior of plants could evoke a physiological response in the host plant.

2.7 Effects of Nitrogen fixing Bacteria on Crop Growth and Yields

A range of nitrogen fixing bacteria has been found to interact with various crops (e.g. rice, wheat, maize, sugarcane and cotton), thus significantly increasing their vegetative growth and grain yields (Kennedy *et al*., 2004). Strains of *Azospirillum*, a nitrogen fixing organism living in close association with plants in the rhizosphere, have been observed to increase water and mineral uptake, and, to a good extent, BNF, thus enhancing the development and yields of rice, maize, sorghum, barley and wheat (Richa *et al*., 2007). These bacteria fix appreciable amounts of nitrogen within the rhizosphere of the host plants. Efficiencies of 52 mg N_2 g^{-1} malate have been reported (Wagner, 2011).

Fulchieri and Frioni (1994) observed that maize inoculated with *Azospirillum* had enhanced dry weight of seed by 59 percent and also had yield which was similar to that from 60 kg urea N ha^{-1}.

Burkholderia spp., which are nitrogen fixing bacteria (Vandamme *et al*., 2002), have been used to inoculate rice in a field trial, where they showed significant ($P<0.05$) increase of grain yields up to 8 t ha^{-1} (Tran Vân *et al*., 2000), thus the strain was found to be capable of saving 25-30 kg N ha^{-1} in rice. There is also evidence that these organisms can produce substances antagonistic to nematodes, thus protecting the plants from these deleterious microbes (Meyer *et al*., 2000).

Herbaspirillum, an endophytic bacterium which colonies sugarcane, rice, maize, sorghum and other cereals (James *et al*., 2000), can fix up to 31- 45 % of the plant's total N requirement in 30-day-old rice seedlings (Baldani *et al*., 2000). A study in a greenhouse revealed a significant increase in rice yields, up to 7.5 g plant^{-1} (Mirza *et al*., 2000), when rice was inoculated with *Herbaspirillum spp*.

2.8 Effects of Commercial Chemical Products on Physiology of Crops

Van der Watt (2005) proposed that treating seeds with a variety of commercial chemical products which consist of mixtures of macro-and micro-elements play a pivotal role in plant morphological and physiological growth through early seedling establishment, establishing strong root systems during seedling growth, and early development of crop plants such as wheat, soybean, sunflower and maize. Improvement of plant growth may be due to, among others,' the presence of phosphorous (P) which is one of the 17 essential nutrient elements required for plant growth. In its phosphate (HPO_4^-) form it plays a role in an array of processes including energy generation via cell respiration, enzyme activation as well as nitrogen fixation (Kaya *et al*., 2006).

Zinc on other hand, is an essential micro-nutrient playing a vital role in the synthesis of the essential aromatic amino acid tryptophan, and is also involved in enzymatic reactions where it acts as an inorganic co-factor and in the activation of the enzyme starch synthase (Kaya *et al*., 2006). Kaya *et al*. (2006) further demonstrated that a maize cultivar susceptible to Zn deficiency showed suppressed activity of starch synthase and finally the number of starch grains in kernels.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the the Study Site

The study site for which soil samples for pot experiments were collected and in which the field experiments were carried out is located in the Crop Museum within Sokoine University of Agriculture (SUA) farm (located at latitude 06° 50′ 34.4″ S and longitude 37° 38′ 50.3″ E at an altitude of 534 m above sea level), about 2 km from Morogoro municipal . It is bordered on the east by Morogoromunicipality, to the south-east by the Uluguru Mountains and to the west and north-west by the Mindu Hills and Lugala Hills, respectively (Msanya and Maliondo, 1998).

An extensive account of the climate of the study area (SUA Farm) has been documented (Msanya, 1980; Moberg *et al*., 1982 and Kaaya *et al*., 1994). The climate at SUA farm is of a sub-humid tropical type. The area experiences a bimodal rainfall distribution with two rainfall peaks in a year. The short and lighter rains last from November to January with a peak in December. These rains are followed by a short dry period which normally occurs in mid-January or February. The long and heavier rains start in March and end in May, with a peak in April. The onset and distribution of the rainfall are irregular and unreliable. Rainfall ranges from 800 to 1000 mm per annum while average annual temperatures ranges from 18 to 30^{o}C.

3.2 Soil Sampling for Determination of Fertility Status of the Study Site

One composite sample was collected from three different points sampled randomly at the depth 0-20 cm from the study site whose area was about 1230 m^2 and was uniform visually.

The composite sample was dried, ground and passed through a 2 mm sieve ready for laboratory analysis.

The composite sample was analyzed at the Department of Soil Science Laboratory at Sokoine University of Agriculture (SUA). Some portions of the soil samples were also stored in a refrigerator without processing for detecting the natural abundance of microorganisms.

3.3 Soil Analysis for Fertility Assessment of the Study Site

The chemical and physical parameters of the composite soil collected were determined, which included pH, particle size distribution, total nitrogen (%), organic carbon (%), extractable phosphorous (mg/kg), cation exchange capacity ($cmol_c$ (+)/ kg), exchangeable bases and extractable micronutrients.

3.3.1 Soil pH

Soil pH was determined in a 1:2.5 soil:water suspension by the potentiometric method (McLean, 1986). To 10 g of soil, 25 ml of distilled water were added and shaken on a reciprocating mechanical shaker for 30 minutes. The pH of soil samples was determined using a pH meter.

3.3.2 Particle size analysis

Particle size analysis was determined by the Bouyoucos hydrometer method after soil dispersion in sodium hexametaphosphate as described by NSS (1990). The textural class was determined using the USDA textural class triangle.

3.3.3 Total nitrogen

Total nitrogen was determined by the micro-Kjedahl digestion-distillation method according to the procedure described by Bremner and Mulvaney (1982). One gram of soil was digested with concentrated H_2SO_4 in the presence of a catalyst (K_2SO_4 + $CuSO_4$ + selenium powder, mixed in the ration of 10:10:1 by weight). The digest was distilled in the presence of 40% NaOH. The NH_3 liberated was collected in 4% boric acid (with mixed indicator) and titrated against standard 0.05M H_2SO_4. Then the titre was used to calculate the total nitrogen content of the soil sample.

3.3.4 Organic carbon

Organic carbon was determined using the Walkey-Black method (Nelson and Sommers, 1982). To a 1 g soil sample, 10 ml of 1 M $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4 were added and allowed to stand for 30 minutes to oxidize the soil's organic carbon. Two hundred ml (200 mls) of water were added to cool the mixture followed by addition of 10 ml of phosphoric acid (85% H_3PO_4) to sharpen the end point. The amount of excess dichromate reduced was used to estimate the organic carbon (OC) content of the soil following titrating of excess dichromate against 0.5 N ferrous sulphate solution using diphenylamine indicator.

3.3.5 Extractable phosphorous

Extractable P was determined using the Bray 1 procedure (Bray and Kurtz, 1945) because the soil sample had pH less than seven (pH<7). In the Bray 1 method the extracting solutions containing 0.03 M NH_4F and 0.025 M HCl was used. A sample of 3 g air-dried soil was placed in a plastic bottle and 20 ml of extracting solution was added, shaken by hand for 1 minute and filtered using Whatman No. 2 filter paper into a dry plastic vial. Five ml of filtrate aliquots were used for colour development in a 50 ml volumetric flask using

the molybdenum blue method (Murphy and Riley, 1962). The extractable P was determined by a spectrophotometer at the wavelength of 884 nm.

3.3.6 CEC and exchangeable bases

The CEC of the soil was determined by the ammonium acetate saturation method as described by Chapman (1965). Five gram of the soil was saturated with neutral (pH 7.0) 1 N NH_4OAc solution, shaken for 30 minutes and filtered. The filtrate was used to determine the exchangeable bases.Exchangeable Ca and Mg in the ammonium acetate leachate were determined by atomic absorption spectrophotometry, while exchangeable K and Na were determined using a flame photometer.

Excess NH_4OAc in the samples was removed by washing twice using methanol. The $NH4^+$ saturated soil was equilibrated with 4% KCl, shaken for 30 minutes and filtered. The filtrate was used for determination of $NH4^+$ by distillation in the presence of 40% NaOH and the NH_3 liberated was collected in 4% boric acid (with mixed indicator) and titrated with standard 0.1 N H_4SO_4. The titre was used for estimation of CEC.

3.3.7 Extractable micronutrients

DTPA-extractable micronutrients were determined using the procedure by Lindsay and Norvell (1978). The extractant contained 0.005 M DTPA (diethylenetriamine pentaacetic acid), 0.01 M $CaCl_2.2H_2O$ and 0.1 M TEA (triethanolamine) adjusted to pH 7.3. Twenty gram of air dried soil were placed into a 100 ml plastic bottle and mixed with 40 ml of the extracting solution. The contents were shaken using a reciprocating mechanical shaker for exactly 2 hrs and filtered using Whatman No. 2 filter paper into a dry plastic vial. The micronutrients Zn, Cu, Fe and Mn were determined by atomic absorption

spectrophotometer (AAS), using appropriate wavelengths of hollow cathode lamps specific to specific elements, using appropriate standards.

3.4 Soil Sampling for Pot Experiment

Soil from study site within SUA farm was used for pot experiment. A bulk surface sample was collected from the site at a depth of 0-20cm. The soil sample was air dried, ground and passed through a 8 mm sieve for pot experiments.

3.5 Classification of Soil from the Study Site

Field reconnaissance survey was carried out using transect walks, auger observations and descriptions to establish representative study site on the basis of landform and other physiographic attributes. At the observation site, data on landform, soil morphological characteristics, elevation, slope gradient, parent material (lithology), vegetation and land use/crops were collected. These data were filled in special forms adopted from the FAO guidelines for soil description. Based on the information collected from the reconnaissance survey, one representative soil profile was identified and excavated to represent the representative soil of the study area. The profile pit was laid out in the east-west direction using GPS compass and dug to a depth of 140+ cm. Geo-referencing of the soil profile was done using Global Positioning System (GPS) (model OREGON 400t). The soil profile pit was studied and described according to FAO Guidelines for Soil Profile Description (FAO, 2006). Soil colour was determined by using Munsell color Chart (Munsell Color Company, 1992). From the profile pit, disturbed (bulk) and undisturbed (core samples) were taken from each horizon for physical and chemical analyses in the laboratory. A total of seven (7) soil samples representing the soil genetic horizons, and three (3) core samples from three segments of the profile (0 – 15 cm, 23 – 68 cm and 94 – 140 cm) were collected for laboratory studies.

3.6 Laboratory Analyses for Soil Classification

Chemical and physical analyses were determined in the Soil Science Department Laboratory. The collected soil samples were air-dried, ground and passed through a 2-mm sieve for laboratory analyses. Undisturbed core samples were used for the determination of bulk density and percentage moisture. Bulk density was determined by the core method (Black and Hartage, 1986). Percentage moisture was calculated by dividing the weight of the lost moisture to the dry weight and multpiled by one hundred.

The disturbed soil samples were used for determination of physical and chemical properties of soils. Particle size analysis was determined by the hydrometer method after dispersion with sodium hexametaphosphate 5% (NSS, 1990). Textural classes were determined using the USDA textural class triangle (USDA, 1975).

Soil pH was measured potentiometrically in water and 1 N KCl at a ratio of 1:2.5 soil water and soil-KCl, (McLean, 1986). To 10 g of soil samples, 25 ml of distilled water or KCl respectively, were added and shaken on a reciprocating mechanical shaker for 30 minutes. The pH of soil samples was determined using a pH meter. Organic carbon was determined by the Walkey and Black wet oxidation method (Nelson and Sommers, 1982). Organic carbon obtained was converted to organic matter by multiplying by a factor of 1.724 (Duursma and Dawson, 1981). Total N was determined using micro-Kjeldahl digestion-distillation method as described by Bremmer and Mulvaney (1982). Available phosphorous was determined using filtrate extracted by the Bray and Kurtz-1 method (Bray and Kurtz, 1945) and determined by spectrophotometer at 884 nm following color developed by the molybdenum blue method (Murphy and Riley,1962; Watanabe and Olsen, 1965).

Cation exchange capacity of the soil (CEC_{soil}) and exchangeable bases were determined by saturating soil with neutral 1 M NH_4OAc (ammonium acetate). The NH_4^+ was displaced by using 1 M KCl and then determined by distillation for estimation of CEC (Chapman, 1965).

Cation capacity of clay (CEC_{clay}) was calculated using the formula outlined by Baize (1993) which corrects for the CEC contributed by organic matter (OM) as follows:
CEC_{clay} = ({CEC_{soil} – (% OM * 2)}/ % clay) * 100. The exchangeable bases (Ca^{2+}, Mg^{2+}, Na^+ and K^+) were determined by atomic absorption spectrophotometer (Thomas, 1982). The total exchangeable bases (TEB) were calculated arithmetically as the sum of the four exchangeable bases (Ca^{2+}, Mg^{2+}, Na^+ and K^+) for a given soil sample. The electrical conductivity was determined in 1:2.5 soils:water suspension using an electrical conductivity meter as per the method of Moberg (2000). Base saturation was calculated as follows: Percent BS = { Total exchangeable bases (TEB) / CEC_{soil}} * 100, and the carbon to nitrogen ration was calculated by dividing the organic carbon values to total nitrogen values.

3.7 Classification of the Soil of the Experimental Site

Using field and laboratory data (Table 1), the soil from the study site was classified to the TIER-2 of the FAO Word Reference Base (IUSS Working Group WRB, 2007) and to family level of the USDA Soil Taxonomy (SSS, 2006)**.** The interpretation of both physical and chemical data was based on some standard guidelines (Msanya, 2012)**.**

Table 2 represents a summary of the diagnostic criteria, defines the prefix and suffix qualifiers and classifies the soil up to TIER-1 category of the FAO World Reference Base Classification Scheme (IUSS Working Group WRB, 2007). Similarly, Table 2 represents the diagnostic horizon and features for classifying the soil according to the Soil Taxonomy (SSS, 2006) and the soil have been classified up to the family level of soil Taxonomy.

Table 1: Profile description and physico-chemical characteristics of the studied profile (SUA CM-P1)

Profile number: SUA CM- P1 Mapping unit: 24C1 (ET)

Agro-ecol. zone: Eastern Zone, Region: Morogoro District: Morogoro

Map sheet no. : 183/3 Coordinates : 37° 38′ 50.3″ E / 6° 50′ 34.4″ S

Location: SUA FARM, 2km North of Main Campus-Round Top Cafetaria

Elevation: 534 m asl. Parent material: colluvium derived from mafic metamorphic rocks (hornblende pyroxene granulites) of the Uluguru mountains. Landform:Alluvial colluvium plains; slightly undulated. Slope: 2%; straight/linear

Surface characteristics: Sealing; none. Run off: very slightly Erosion: slight sheet erosion. Deposition: none. Natural drainage class:well drained. Infiltration: Moderate, Natural vegetation: Monocot grasses, *Mimosa pudica*, *Acacia kirkii*, *Croton sylvaticus* (forest croton), *Lantana camara*, *Leucaena leucocephala*, *Annona muricata* , Asthma weed, *Sida acuta*, *Sida alba*, *Corchorus spp*, *Mucuna spp*, Cyperaceae grasses.

Land use systems: maize, millet, sunflower,water melon and legumes. Human influences: cuttivation, tree cutting and Irrigation. Soil Fauna: Termites

STR: isohyperthermic SMR: ustic

Described by B.M. Msanya, S.K. Deodatus and M. Johari on 30/1/2014

Ah 0 - 15 cm: dark brown (7.5YR3/3) dry, very dark brown (2.5YR2.5/3) moist; clay; slightly hard to hard dry, friable moist, sticky plastic wet; moderate, corse to medium subangular blocks and moderate, medium to fine crumbs;few medium and many fine pores; common medium, few medium and many fine and very fine roots; clear wavy boundary to

BAt 15 - 23 cm: yellowish red (5YR4/6) dry, dark reddish brown (5YR3/4) moist; clay; slightly hard dry, friable moist, sticky and plastic wet; moderate, common to medium sub angular blocks; few faint clay cutans; many fine and very fine pores; few medium and common fine and very fine roots; diffuse smooth boundary to

Bt1 23-68 cm: reddish yellow (5YR5/8) dry, yellowish red (5YR4/6) moist; clay; soft dry, very friable moist, sticky and plastic wet; moderate, medium to fine subangular blocks; many, disticnt clay cutans; many fine and very fine pores; few coarse, common fine and very fine roots; diffuse smooth boundary to

Bt2 68-78cm: strong brown (7.5YR5/8) dry, yellowish red (5YR4/6) moistt; clay; soft dary, very friable moist, slightly sticky and plastic wet; moderate, medium to coarse subangular blocks; common, faint clay cutans; many fine and very fine pores; few medium and common fine and very fine roots; diffuse smooth boundary to

Bt3 78-94cm: strong brown (7.5YR5/8) dry, strong brown (7.5YR4/6) moist; clay; slightly hard dry, friable moist, slightly sticky and plastic wet; moderate, fine subangular blocks; common faint clay cutans; few fine and many very fine pores; few very fine roots; diffuse smooth boundary to

Bt4 94-140cm: strong brown (7.5YR5/8) dry, strong brown (7.5YR4/6) moist; clay; slightly hard dry, friable moist, sticky and plastic wet; moderate, medium and fine subangular blocks; many dinstinct clay cutans; few medium, many fine and very fine pores; few very fine roots; clear smooth boundary to Btc 140^{+} cm: dark red (2.5YR3/6) moist; clay gravely; soft dry, friable moist, sticky and plastic wet; moderate coarse, medium and fine subangular blocks; many distinct clay cutans; few medium, many fine and very fine pores; many medium, moderate round, iron and manganese nodules; few very fine roots.

ANALYTICAL DATA FOR PROFILE: SUA CM – P1

Horizon	**Ah**	**BAt**	**Bt1**	**Bt2**	**Bt3**	**Bt4**	**Btc**
Depth (cm)	0-15	15-23	23-68	68-78	78-94	94-140	140+
Clay (%)	53.76	63.76	69.76	71.76	67.76	69.76	61.76
Silt (%)	19.28	11.28	7.28	1.28	5.28	7.28	5.28
Sand (%)	26.96	24.96	22.96	26.96	26.96	22.96	32.96
Texture class	C	C	C	C	C	C	C
Silt/clay ratio	0.36	0.18	0.10	0.02	0.08	0.10	0.09
Bulk density (g/cc)	1.1	nd	1.1	nd	nd	1.1	nd
pH H_2O	5.86	5.88	5.81	6.1	6.56	7.1	7.93
pH KCl	5.96	5.85	5.72	5.99	6.51	6.81	7.33
Organic C (%)	2.014	0.988	0.891	0.60	0.31	0.465	0.368
Total N (%)	0.196	0.126	0.112	0.112	0.105	0.105	0.084
C/N	10.28	7.84	7.96	5.36	2.95	4.43	4.38
Avail. P (mg/kg)	5.856	1.725	2.156	4.406	0.263	0.006	1.819
CEC NH_4OAc ($cmol_c$ (+)/ kg)	20	19.4	17	19.8	19.4	16.2	18.7
Exch. Ca ($cmol_c$ (+)/ kg)	6.89	5.34	4.18	2.52	2.19	1.87	3.70
Exch. Mg ($cmol_c$ (+)/ kg)	3.59	3.46	4.40	4.39	4.15	3.57	2.87
Exch. K ($cmol_c$ (+)/ kg)	1.62	0.28	0.10	0.08	0.08	0.07	0.61
Exch. Na ($cmol_c$ (+)/ kg)	0.46	0.30	0.97	1.32	1.25	1.46	2.01
TEB (cmol(+)/kg)	12.56	9.39	9.64	8.31	7.67	6.98	9.19
Base saturation (%)	62.81	48.38	56.71	41.97	39.56	43.09	11.47
CEC clay ($cmol_c$ (+)/ kg)	24.29	25.08	19.97	24.71	27.05	20.92	27.40
Moisture (%)	7.47	nd	8.35	nd	nd	11.48	nd
Ca/Mg ratio	1.92	1.54	0.95	0.57	0.53	0.52	1.29
Extractable Zn (mg/kg)	1.36	0.96	0.56	0.66	0.66	0.76	0.76
Extractable Cu (mg/kg)	1.58	2.10	1.32	1.06	1.06	1.06	1.84
Extractable Mn (mg/kg)	8.27	4.27	8.27	4.27	2.93	2.93	6.93
Extractable Fe (mg/kg)	32.61	16.85	4.12	1.09	4.73	5.33	1.09

Table 2: Summary of morphological and diagnostic features of the studied soil (Pedon SUACM-P1) and its classification

Diagnostic properties, horizons and features		Classification according to USDA Soil Taxonomy (SSS, 2006)				
		Order	Suborder	Greatgroup	Subgroup	Family
USDA Soil Taxonomy (SSS, 2006)	Very deep, medium to strongly acid, ustic SMR, iso-hyperthermic STR, slope 2%, pedon clayey throughout its profile, presence of many faint and distinct clay cutans in the subsoil, appreciable clay gradient between eluvial and illuvial horizon, low CEC (<24 cmol(+)/kg clay in a great portion of the subsoil); *ochric* epipedon overlying *argillic* subsurface horizon	Ultisols	Ustults	Paleustults	Typic Paleustults	*Very deep, clayey, medium to slightly acid, iso-hyperthermic Typic Paleustults*
Diagnostic horizons, properties and materials		Classification according to FAO- WRB (IUSS Working Group WRB, 2007)				
IUSS Working Group WRB (2007)	*Argic B horizon; Qualifiers: Cutanic, Haplic (Humic, Profondic , Clayic)*	TIER-1		TIER-2		
		Acrisols		*Haplic Cutanic Acrisols (Humic, Profondic, Clayic)*		

3.8 Enumeration of Natural Abundance of Microorganisms from the Soil

The unprocessed soil portion (stored in the refrigerator) collected during soil sampling for general soil fertility assessment of the study site was used for determining the natural abundance of microorganisms.

The enumeration of total number of microorganisms in the soil was determined by the pour plate method using soil extract agar (SEA) medium. This medium was made by boiling for 1 hour and cooling a mixture of 1 kg of air-dry soil with 1 litre of distilled water. The mixture was then filtered to separate the liquid (soil extract) from the soil solids. The soil extract agar medium was then formulated as follows: agar 15 g, 100 ml of soil extract and 900 ml of distilled water. The medium was then sterilized at 1.05 kg/cm^2 (15 pounds per square inch (psi)) and 121^{o}C for 15 minutes in portions of 15 ml in small screw-cap glass bottles.

Ten grammes of soil (moist) from the study site were placed, in three replicates, into bottles containing 90 ml of sterized distilled water and shaken thoroughly to detach microbial cells from the soil particles into the soil suspension. This was the 10^1 suspension. 1 ml of the above soil suspension was aseptically transferred to a bottle carrying 9 ml of sterile water then shaken to mix well to make, successively, 10^2 to 10^6 dilutions.

Using a fresh sterile pipette, 1 ml of aliquots from 10^{-6} to 10^{-1} dilutions were poured into petri dishes. About 15 ml of molten Soil Extract Agar (SEA) medium (maintained at about 50^{o}C) was poured in each petri dish and the petri dishes were gently swirled clockwise, then anticlockwise (three times each) and once forwards and once backwards to mix the soil suspension and the agar medium. Then the petri dishes were left to stand until the agar medium had solidified. The petri dishes were incubated, up-side down, at 27^o C, for one

week, after which colonies were counted from dilution levels showing a good distribution of colonies. The number of colonies from the three replicate plates was determined and the number of total microorganisms (CFU/g) of soil was calculated using the following formula in equation 1.

$$\text{Number of CFU/ g soil} = \frac{\text{Number of CFU counted}}{\text{Weight of soil (g)}} \times \text{total dilution} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

3.9 Verification of Quality of the Commercial Chemical and Microbiological Products in the Laboratory

3.9.1 Characterization of commercial chemical product (Teprosyn)

The total nutrient contents (macro and micronutrients) in teprosyn product, mainly total N, P and Zn, were determined using established laboratory procedures, as summarized below.

3.9.1.1 Determination of total nitrogen

Total nitrogen in the teprosyn product was determined by the micro-Kjedahl digestion-distillation method according to the procedure described by Bremner and Mulvaney (1982), as for soils (Section 3.3.3). Three different weights of 1.000 $\pm$ 0.100 g of teprosyn product, each were digested with 20 ml of concentrated H_2SO_4 in presence of a catalyst (K_2SO_4 + $CuSO_4$ + selenium powder, mixed in the ratio of 10:10:1 by weight). Different weights of teprosyn product were weighed since the product was viscous to pass through the pipette. The digest was distilled in the presence of 40% NaOH. The NH_3 liberated was collected in 4% boric acid (with mixed indicator) and titrated against standard 0.05M H_2SO_4. Then the titre was used to calculate the total nitrogen in the teprosyn product.

3.9.1.2 Determination of total phosphorous

Total P of the teprosyn product was determined through weighing three different weights as for total nitrogen (Section 3.9.1.1). Three different weights of 2.000 $\pm$ 0.200 g of teprosyn,

each dissolved into 10 ml of 6 N HCl and filtered. The filtrate was then diluted to 25 ml with distilled water and used to determine total phosphorous by pipetting 0.1 ml of the filtrate into a 200-ml conical flask, and ascorbic acid was also added for colour development (Murphy and Riley, 1962), followed by distilled water up to the mark (200 ml). After 30 minutes, the total P was determined using a UV-Visible spectrophotometer at the wavelength of 884 nm.

3.9.1.3 Determination of total zinc

The total zinc in teprosyn was determined by weighing three different weights (1.000 $\pm$ 0.1000 g) of the teprosyn product, each dissolved into 10 ml of 6 N HCl and filtered. The filtrate obtained was diluted to 25 ml with distilled water and Zn determined by atomic absorption spectrophotometer (AAS) at the wavelength of 213 nm.

3.9.2 Characterization of microbiological products (Bio-soil crop booster and Bio-soil Nitro+) in the laboratory

3.9.2.1 Enumeration of total number of microorganisms in the Bio-soil crop booster product

The total number of microorganisms in Bio-soil crop booster were enumerated using the pour plate method in both undiluted and diluted aliquots of the product, using nutrient agar (NA) medium.

The NA medium was prepared by dissolving 3 g of beef extract, 5 g of peptone and 20 g of agar into 1000 ml of distilled water and the pH of the sunspension adjusted to 7.0. The medium was well mixed and dispensed into portions of 15 ml in small screw-cap glass bottles. The media (in the bottles above) were sterilized by autoclaving at 1.05 kg/cm^2 (15 pounds per square inch (psi)) and 121^{o}C for 15 minutes, then left to cool in a water bath

maintained at 50^{o}C ready for plating. Petri dishes and pipettes were sterilized in an oven at 170^{o} C for 24 hours prior to use.

Ten ml of the Bio-soil crop booster was placed, in three replicates, into bottles containing 90 ml of sterilized distilled water and shaken vigorously to mix the contents. This was the 10^{-1} suspension. 1 ml of the above soil suspension was aseptically transferred to a bottle carrying 9 ml of sterile water then shaken to mix well. This was the 10^{-2} suspension. Similarly, dilutions up to 10^{-6} were made. Using a fresh sterile pipette, 1 ml of aliquot from 10^{-6} to 10^{-1} dilutions, as well as of the undiluted Bio-soil crop booster, was poured into each petri dishes. Molten NA medium (maintained at about 50^{o}C) was poured in each petri dishes, the petri dishes were swirled clockwise, then anticlockwise (three times each) and once forwards and once backwards to mix the soil suspension and the agar medium. Then the petri dishes were left to stand until the agar medium solidified. The petri dishes were incubated, up-side down, at 27^{o}C, for one week after which colonies were counted from dilution levels showing a good distribution of colonies. The average number of colonies were used to determine the number of microorgnaisms (CFU/ml) of Bio-soil crop booster product. The number of colonies from the three replicate plates was determined and the number of total microorganisms (CFU/ml) of Bio-soil crop booster was calculated using the following formula in equation 2.

$$\text{Number of CFU/ ml Bio-soil crop booster} = \frac{\text{Number of CFU counted}}{\text{Volume plated (ml)}} \times \text{total dilution} \quad \ldots..(2)$$

3.9.2.2 Enumeration of total number of microorganisms in the Bio-soil Nitro+ product

The total number of microorganisms in Bio-soil Nitro+ were enumerated using the pour plate method of both undiluted and diluted aliquots of the product, using nutrient agar (NA) medium. Preparation of the NA medium and sterilization of petri dishes and pipettes were

done as for enumeration of total number of microorganisms in the Bio-soil crop booster product (Section 3.9.2.1).

Ten ml of the Bio-soil Nitro+ was placed, in three replicates, into bottles containing 90 ml of sterilized distilled water and shaken vigorously to mix the contents. This was the 10^{-1} suspension. 1ml of the above soil suspension was aseptically transferred to a bottle carrying 9 ml of sterile water then shaken to mix well. This was the 10^{-2} suspension. Similarly, dilutions up to 10^{-6} were made. Using a fresh sterile pipette, 1 ml of aliquot from 10^{-6} to 10^{-1} dilutions, as well as of the undiluted Bio-soil Nitro+, was poured into each petri dishes. Molten NA medium (maintained at about 50^{o}C) was poured in each petri dishes, the petri dishes were swirled clockwise, then anticlockwise (three times each) and once forwards and once backwards to mix the soil suspension and the agar medium. Then the petri dishes were left to stand until the agar medium solidified. The petri dishes were incubated, up-side down, at 27^{0} C, for one week after which colonies were counted from dilution levels showing a good distribution of colonies. The average number of colonies were used to determine the number of microorgnaisms (CFU/ml) of Bio-soil Nitro+ product. The number of colonies from the three replicate plates was determined and the number of total microorganisms (CFU/ml) of Bio-soil Nitro+ was calculated using the following formula in equation 3.

$$\text{Number of CFU/ ml Bio-soil Nitro+} = \frac{\text{Number of CFU counted}}{\text{Volume plated (ml)}} \times \text{total dilution} \quad \ldots\ldots\ldots\ldots (3)$$

3.10 Preparation of Commercial Products for Pot and Field Experiments

The volume of teprosyn product recommended to coat 1 kilogram maize seeds was 8 ml (8 ml kg^{-1}) and/ or 4 ml / 0.5 kg^{-1}. The seeds were placed in a small plastic bag and teprosyn was added. The mixture was agitated rigorously for 1 minute. Subsequently, the treated

seeds were placed on a sheet of filter paper and allowed to dry indoors for 30 minutes, before being planted in the pots or plots.

The bio-soil crop booster inoculant was prepared in a plastic bucket by mixing 2.5 ml of the concentrated bio-soil crop booster with 2 litre of water and 2.5g of sugar and left for 12 hours. This is equivalent to the manufacturer's instruction to mix 250 ml of product with 200 l water and 250 g sugar. Thereafter, 10 ml of the mixture was used to inoculate one kilogram of maize seeds half an hour before planting. Similarly, the Bio-soil Nitro+ product was prepared by dissolving 2.5ml of Bio-soil Nitro+ product into 2 litre of water. Thereafter, 10 ml of the mixture was used to inoculate one kilogram of maize seeds half an hour before planting.

3.11 Evaluation of the Effectiveness of Teprosyn, P-solubilizers and Free N_2-fixers on maize growth and yields

The effectiveness of commercial chemical product (teprosyn), P-solubilizers (Bio-soil Crop booster) and N_2-fixers (Bio-soil Nitro+) were evaluated both in pot experiments and under field conditions as detailed below.

3.11.1 Pot experiments

3.11.1.1 Effects of teprosyn on maize growth and yields

The treatments for evaluating teprosyn product were:

1. Control (No teprosyn + no P fertilizers)
2. Recommended rate of teprosyn (4 ml / 0.5 kg seed)
3. Double recommended rate of teprosyn (8 ml / 0.5 kg seed)
4. Half recommended P rate (10 kg P ha^{-1}) + Recommended rate of teprosyn (4 ml / 0.5 kg seed)

5. Half recommended P rate (10 kg P ha^{-1}) + No teprosyn
6. Recommended P rate (20 kg P ha^{-1})

The pot experiments were conducted in the screenhouse of the Department of Soil Science, SUA in Morogoro region, located at latitude of 06 ° 50 ´S and longitude 37°38'E at an altitude of 525 m above sea level.

Three kilogram soil samples from the site were placed into 4-kg plastic pots, arranged in a the randomized complete block design (RCBD) with three replications. The six treatments (above) were randomly assigned in each pot.

At planting, in order to avoid contamination, all pots acquiring un-inoculated seeds were first planted, followed by pots whose seeds were inoculated with commercial chemical and microbiological products. The seeds were cleaned by immersing in water so as to remove storage chemicals before inoculation. Three seeds of certified untreated SITUKA maize variety were planted on the potted soils and were thinned to two plants per pot 12 days after sowing (DAS). Rates of 0.26 g N from urea ($CO(NH_2)_2$) and and 0.0528 g Zn per pot from zinc sulphate ($ZnSO4.7H_2O$) were applied to potted soils. To satisfy the suggested rates of P (10 and 20 kg P ha^{-1}) in treatments for evaluating the commercial products, 0.2245 g P and 0.449 g P respectively, from K_2HPO_4 were applied to potted soils.

With exception of N, as Urea (recommended rate of 60 kg N ha^{-1}), which was split applied at planting time and 28 days after planting, all treatments for teprosyn and the other fertilizers were applied at planting time.The soils in the pots were maintained at about field capacity throughout the experimental period. Plant heights were measured to the nearest

centimeter from the base to plant tops at 21, 28 and 35 days after sowing (DAS) from the two maize plants from each pot and an average was obtained.

At 35 days after planting (DAP), shoot dry matter was also determined after cutting the shoots of the two plants at 1 cm above the soil surface, drying in an oven at 65˚C to constant weight. The dried plant samples were cut into small pieces, grounded and passed through a 0.5 mm sieve for chemical analysis to determine the plant uptake of N, P and Zn.

3.11.1.2 Effects of Bio-soil crop booster on maize growth and yields

Pots were prepared, arranged and seeds planted as described in section 3.11.1.1. The treatments for evaluating Bio-soil crop booster product were:

1. Control (No Bio-soil crop booster + no P fertilizer)
2. Recommended rate of Bio-soil crop booster (5 ml / 0.5 kg seed)
3. Double recommended rate of Bio-soil crop booster (10 ml / 0.5kg seed)
4. Recommended rate of Bio-soil crop booster (5 ml / 0.5 kg seed) and half recommended P rate (10 kg P ha^{-1})
5. Half recommended P rate (10 kg P ha^{-1}) + No Bio-soil crop booster
6. Recommended P rate (20 kg P ha^{-1})

3.11.1.3 Effects of bio-soil nitro+ on maize growth and yields

Pots were prepared, arranged and seeds planted as described in section 3.11.1.1.

The treatments for evaluating Bio-soil Nitro+ product were:

1. Control (No Bio-soil Nitro + no P fertilizer)
2. Recommended rate of Bio-soil Nitro+ alone (5 ml / 0.5 kg seed)
3. Double recommended rate of Bio-soil Nitro + alone (10 ml / 0.5 kg seed)

4. Recommended rate of Bio-soil Nitro+ (5 ml / 0.5 kg seed) and half recommended P rate (10 kg P ha^{-1})
5. Half recommended P rate (10 kg P ha^{-1}) + No Bio-soil Nitro+
6. Recommended P rate (20 kg P ha^{-1})

3.11.2 Field experiments

3.11.2.1 Experimental Site, Design and Treatments

The field experiment, using the RCBD, replicated three times, was laid out at the study site at SUA farm to study the effects of commercial chemical and microbiological products in soil on maize growth and yields. The experimental unit sizes were 3 m x 3 m and the treatments for each product were as described for the pot experiments in section 3.11.1. Rates of 202.5 g P and 405 g P per plot from YaraMila Cereal fertilizer was applied to satisfy the recommended rates of 10 kg P ha^{-1} and 20 kg P ha^{-1} respectively. Similarly, 12 g Zn per plot in form of zinc sulphate ($ZnSO4.7H_2O$) was also applied to all plots.

3.11.2.2 Planting and crop husbandry practices in the field

At planting, in order to avoid contamination, the plots acquiring un-inoculated seeds were first planted and followed by plots whose seeds were inoculated with commercial chemical and microbiological products. The seeds were cleaned by immersing in distilled water so as to remove storage chemicals before inoculation.

Maize seeds of SITUKA variety were sown on 28 March, 2014 at 30 cm within a row and 75cm between rows, giving 40 maize plants per plot. Two seeds were sown, and the plants were thinned to one plant per hill on 10 April, 2014, 13 days after planting.

In conjuction with the commercial products being evaluated, other sources of plant nutrients applied in the field experiment included YaraMila Cereal fertilizer (containing NPK, Zn and Mg), Zinc sulphate ($ZnSO_4.7H_2O$) and urea ($(CONH_2)_2$) (46% N). With exception of N, which was split-applied at planting time and the second split later on (when maize plants were at knee high) for all treatments in each product, the other fertilizers were applied only once at planting time.

Nitrogen was applied at the recommended rate (60 kg N ha^{-1}) to all treatments in two splits, whereby 50 kg N ha^{-1} of the total amount of N was supplied by YaraMila Cereal fertilizer during planting and the remaining 10kg N ha^{-1} was applied as urea on 29 April, 2014 when maize plants were at knee high. Zinc was applied (as $ZnSO4.7H_2O$) at 13.33 kg Zn ha^{-1} at planting.

The maize plants were weeded twice on 8 and 26 April, 2014 while the protection of maize plants against pests was carried out using Endosulfan pesticide on 10 April and 9 May, 2014 through pouring the powder on the emerging leaves of each maize plant in all plots.

Plant height (cm) and the number of leaf data were collected at 3, 6, 9 and 12 weeks from four maize plants in two inner rows of each plot after sowing. Similarly, plant girth data was collected at 6, 9 and 12 weeks from four maize plants in two inner rows of each plot after sowing.

At tasseling stage, four maize plants from each plot were sampled (destructive sampling) and used for biomass determination. Eight plants in one inner row in each plot were left to grow to maturity stage and harvested for grain yield determination.

3.12 Plant Analysis

Both pot and field experiments plant samples were analysed in the Laboratory for plant nutrient concentrations. Plant samples were air-dried followed by oven drying at 65^{o}C. Samples were ground using a Wiley laboratory mill. 1 g of plant sample was digested using H_2O_2-$HClO_4$-HF in tubes in a block digester at 200^{o}C for 2 hours. The digest was cooled and made up to volume (50 ml). The nutrients contents (P and Zn) of the plants extract were determined as for soils (Section 3.3 above). Similarly, 0.2 g of plant sample was digested using H_2O_2-$HClO_4$-HF in tubes in a block digester at 200^{o}C for 2 hours. Thereafter, the digest was cooled, made up to volume (50 ml) and used for the determination of N content of the plants extract as for soils (Section 3.3 above).

3.13 Statistical analysis

In both pot and field expeiments, the RCBD was used. The analysis of analysis of variance (ANOVA) at $P<0.05$ on plant height, number of leaves, plant girth, plant biomass, shoot dry matter, ear leaf nutrient contents, as well as nutrient availability in soil in response to application of teprosyn, Bio-soil crop booster, Bio-soil Nitro+ and YaraMila Cereal fertilizer was carried out using the GenStat Discovery 15th edition computer software. Treatment means separation was done using Least significant difference (LSD) Test at the 5 % level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Some Physical Properties of the Soil from the Study Site

The properties of the bulk soil from the study site are given in Table 3. According to the USDA textural class triangle (Brady and Weil, 2002), the textural classes for selected site from SUA farm was clay (C). The soil will therefore have high water and nutrient retention capacity, and would be more suitable for production of many crops if other soil factors are not limiting.

The pH of the soil of the study site, at 6.33, would be suitable for most crops (Landon, 1991). Organic carbon content in soil of the study site (1.72%) and the total N (0.14 %) were medium (Landon, 1991). The medium levels of soil total N observed in this soil could be due to medium organic carbon contents.

The low Bray-1 extractable P for the soil may be due to high phosphorous fixation by high levels of Fe^{3+} and Mn^{2+} (Schwertman and Herbillon, 1992) in this soil (Table 1). Exchangeable aluminium was not determined because at pH 6.33, it would be very low. The studied soil is derived from the colluvial materials originating from the Uluguru Mountains with rocks which essentially are hornblende-pyroxene granulites containing plagioclase and quartz-rich veins (Kessaba *et al*., 1972), which are rich in these elements. The low levels of P in this soil could also be probably due to inherent low P in the soil parent material. The other elements, with the exception of Na and Zn, were medium to very high, implying that they would not be limiting for crop (maize) production.

Table 3: The physico-chemical properties of the experimental soil (SUA farm)

Property, unit	Value	Remarks
Sand, %	41.0	
Silt, %	3.0	Clay
Clay, %	56.0	
pH (H_2O)	6.33*	Medium
Organic carbon, %	1.72	Medium
Total nitrogen, %	0.14	Low
Bray 1 P, mg/kg	1.02	Low
Exchangeable Ca, $cmol_c$ (+)/kg	6.35	Medium
Exchangeable Mg, $cmol_c$ (+)/ kg	3.22	High
Exchangeable Na, $cmol_c$ (+)/ kg	0.26	Low
Exchangeable K, $cmol_c$ (+)/ kg	1.10	Medium
CEC, $cmol_c$ (+)/ kg	23.0	Medium
DTPA extractable Fe, mg/kg	38.0	Very high
DTPA extractable Mn, mg/kg	67.5	Very high
DTPA extractable Zn, mg/kg	0.34	Very low
DTPA extractable Cu, mg/kg	2.43	High

Texture of soil: clay

* The ratings of the soil properties were according to Landon (1991) and Motsara and Roy (2008), where VL= Very low, L= low, M= Medium, H = High, and VH = Very High

4.2 Characterization of Commercial Products

4.2.1 Nutrient contents in Teprosyn

Results on total nutrient contents (macro and micronutrients) in Teprosyn, as determined in the present study, are presented in Table 4. Only the total nitrogen content resembled that in the label of the product package while the total phosphorous (as P_2O_5) and zinc were much lower than the 15% phosphorous pentoxide (P_2O_5) and 18% zinc as quoted in the label. This indicates that the product would, therefore, fall short in performance and lead to poor growth and yields of crops. Therefore, TFRA should require manufacturers to meet the specified quality standards, and should monitor and ascertain the quality, before this product

is accepted for use in the country. This action will be as specified by the fertilizer Act (2009) under Part V of the act. Additionally, further research should be done by manufacturer to specify whether the product is best applied to soil or foliage for best results.

Table 4: Nutrient contents of Teprosyn

Parameter	Content as determined in laboratory	Content as specified by manufacturer
% N	9.32 $\pm$ 0.15	9
% P_2O_5	2.72 $\pm$ 0.08	15
% Zn	5.54 $\pm$ 0.11	18

4.2.2 Microbiological populations of the microbiological commercial products and in the experimental soil

The microbiological populations (colony forming units, CFUs) of the various commercial products and in the experimental soil are shown in Table 5. The Laboratory values of CFU determined for each microbiological product were lower than the CFU specified by the manufacturer as quoted in the label. This indicates that the products would, therefore, lead to poor growth and yields of the crops. Therefore, TFRA should require manufacturers to meet the quality standards before these products are accepted in the country.

Table 5: Populations of microorganisms in the commercial products and in the study soil

Product	Microbiological populations, CFU	
	Determined in laboratory	Specified by manufacturer
Bio-soil crop booster (CFU/ ml)	1.9×10^4	1.0×10^8
Bio-soil Nitro+ (CFU/ ml)	1.1×10^2	1.0×10^8
Experimental soil (CFU/ g)	6.3×10^6	-

4.3 Greenhouse Experiments

4.3.1 Effects of Teprosyn on maize growth, biomass yields and shoot nutrient concentrations

The effects of Teprosyn on maize growth performance and biomass yields are presented in Table 6. In the early days of plant growth at 28 DAP, plant height (cm) and plant girth (cm) did not show any significant differences across treatments. However, the number of leaves were significantly ($P<0.05$) different across the treatments, with the highest leaf number (7) recorded under the K_2HPO_4 treatments, especially at the rate of 10 kg P ha^{-1}. Teprosyn treatments alone or in combination with K_2HPO_4 (10 kg P ha^{-1}) did not differ much amongst themselves and sometimes not with the control in terms of plant height and plant girth.

In the subsequent periods of plant growth at 35 DAP, the treatments with the combination of Teprosyn (4 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1}) and K_2HPO_4 treatments alone, especially at the rate of 20 kg P ha^{-1}, resulted in significantly ($P<0.05$) higher plant height and plant girth. However, the number of leaves did not show significant differences across treatments. Teprosyn treatments, either alone or in combination with K_2HPO_4 at 10 kg P ha^{-1}, somehow differed amongst themselves and sometimes with the control in terms of plant heights and plant girth.

At harvest, biomass yields showed significant ($P<0.05$) differences across treatments, with the K_2HPO_4 (20 kg P ha^{-1}) treatment resulting in significantly ($P<0.05$) higher biomass yields. The Teprosyn treatments alone did not differ much amongst themselves and sometimes not with the control. Concentrations of P in shoot (as expression of nutrient uptake) did not show significant ($P<0.05$) differences across treatments (Table 7). Phosphorous in shoots varied from 0.06 % (Control) to 0.19 % (Teprosyn at 4 ml/0.5 kg seed). However, the concentration of Nitrogen and Zinc in the shoot showed significant

($P<0.05$) difference across some treatments (Table 7). Nitrogen varied from 2.81 % (Control) to 3.57 % (K_2HPO_4 at 10 kg P ha^{-1}) and zinc ranged from 45.08 mg/kg (Teprosyn at 4 ml/0.5 kg seed) to 70.48 mg/kg (Teprosyn at 4 ml/0.5 kg seed + K_2HPO_4 at 10 kg P ha^{-1}).

The trends of no significant differences ($P<0.05$) as observed in the early stage of plant growth, may be attributed to the fact that the plants were still young and developing their root systems to be able to absorb the nutrients released by the applied fertilizers. However, significant differences in plant heights and plant girth observed in the subsequent periods of plant growth, especially under Teprosyn (4 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1}) and K_2HPO_4 (20 kg P ha^{-1}) treatments may be attributed to the nutrients (N, P, K and Zn) from in Teprosyn and K_2HPO_4, respectively. The increase in maize shoot yields (Table 6) in the reference treatment (K_2HPO_4 at 20 kg P ha^{-1}) confirms that the soils used were infertile and thus responsive to nutrient inputs; hence, the conditions were favourable for the various products to show their effects.

The results of the present study, indicating that Teprosyn treatments alone had no significant ($P<0.05$) effect on plant height and plant girth in early growth or shoot biomass and nutrient (P) concentration at harvest, are in contrast with the findings by Munyahali (2012). This author observed significant positive effect of Teprosyn on maize height in the early growth stages, but also reported insignificant effects of Teprosyn on maize shoot biomass at harvest.

Table 6: Effects of Teprosyn on plant height, number of leaves, plant girth and biomass yields

Treatment	21 DAP			28 DAP			35DAP			
	Plant Height (cm)	No. leaves/plant	Plant Girth (cm)	Plant Height (cm)	No. leaves/plant	Plant Girth (cm)	Plant Height (cm)	No. leaves/plant	Plant Girth (cm)	Biomass yields, (g/plant)
Control	32.5	5.2	0.6	39.6	6.0	0.6	43.3	7.0	0.6	0.96
Teprosyn (4 ml/0.5 kg seed)	36.2	4.8	0.6	34.8	5.8	0.7	36.3	7.0	0.7	0.95
Teprosyn (8 ml/0.5 kg seed)	39.4	5.0	0.6	39.7	6.0	0.6	38.6	7.3	0.7	1.05
Teprosyn (4 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1})	34.9	5.0	0.5	39.9	6.3	0.6	54.7	7.3	0.7	1.42
K_2HPO_4 (10 kg P ha^{-1})	42.6	5.0	0.5	33.6	6.8	0.7	51.6	7.7	0.8	1.74
K_2HPO_4 (20 kg P ha^{-1})	34.8	5.0	0.6	41.3	6.3	0.7	45.1	7.7	0.8	1.89
LSD	12.96	0.29	0.06	10.38	0.74	0.09	13.31	0.88	0.11	0.41
CV (%)	19.4	3.2	5.9	15.0	6.5	7.4	16.3	6.6	8.3	17.0

Table 7: Effects of Teprosyn on nutrient concentrations in maize shoot and soil after harvest

Treatment	Shoot P (%)	Shoot N (%)	Shoot Zn (mg/kg)	Soil N (%)	Soil P (mg/kg)	Soil Zn (mg/kg)
Control	0.06	2.81	63.09	0.17	0.72	1.43
Teprosyn (4 ml/0.5 kg seed)	0.19	2.89	45.08	0.16	0.57	1.14
Teprosyn (8 ml/0.5 kg seed)	0.05	2.92	60.32	0.18	0.65	1.31
Teprosyn (4 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1})	0.10	3.26	70.48	0.16	0.82	1.64
K_2HPO_4 (10 kg P ha^{-1})	0.11	3.57	53.86	0.17	0.82	1.64
K_2HPO_4 (20 kg P ha^{-1})	0.14	3.26	53.39	0.12	1.13	2.25
LSD	0.18	0.58	15.93	0.66	0.19	0.38
CV (%)	92.1	10.3	15.2	22.6	13.1	13.1

Similarly, Peltonen-Sainio *et al*. (2006) evaluated the effect of P seed coating on oat and found that P seed coating enhanced early growth of oats but without increasing yields. Further, Karanam and Vadez (2010) reported an increase in shoot biomass of two- and four-week-old seedlings due to P seed coating of pearl millet compared with non-coated treatment. This author observed significant positive effect of Teprosyn on maize height in the early growth stages, but also reported insignificant effects of Teprosyn on maize shoot biomass at harvest. Similarly, Peltonen-Sainio *et al*. (2006) evaluated the effect of P seed coating on oat and found that P seed coating enhanced early growth of oats but without increasing yields. Further, Karanam and Vadez (2010) reported an increase in shoot biomass of two- and four- week-old seedlings due to P seed coating of pearl millet compared with non-coated treatment.

With regard to N, P and Zn contents in the shoots (at harvest), the study findings showed that P concentration in all treatments fell far below the sufficiency range (0.4 - 0.8 %) established for shoot maize plants 30 to 45 days after emergence (Lockman, 1969;

Vandamme, 2008). It has been observed (Table 4) that the P content in the Teprosyn product was low, which, together with low soil P levels, was not sufficient to improve plant growth and plant nutrient contents. Rebafka *et al*. (1993) similarly found no effect of P seed coating on shoot P concentrations at 40 DAP in pearl millet grown on an acid sandy soil. The study, however, reported higher P concentrations in the pearl millet shoots at 20 DAP (at five-leaf stage).

The Zn concentrations in other treatments (except the treatment with combination of Teprosyn at 4 ml/0.5 kg seed + K_2HPO_4 at 10 kg P ha^{-1}, that was above the sufficiency range) were within the sufficiency range (20 – 50 mg Zn kg^{-1}) established for shoot maize plants 30 to 45 days after emergence (Lockman, 1969; Vandamme, 2008). This implies that the maize plants could have taken up some more amounts of Zn, however not in large quantities to result in much improvement in plant growth or influence shoot biomass yields. The N concentrations in other treatments (except the combination of Teprosyn at 4 ml/0.5 kg seed + K_2HPO_4 at 10 kg P ha^{-1}) were below the sufficiency range (3.5 – 5.0 %) established for shoot maize plants 30 to 45 days after emergence (Lockman, 1969). It may be noted that in Table 7, and subsequent Tables, that the CV(%) is sometimes very high. This high CV % may be related, among others, to possible unforeseen differences in distribution of the applied P and Zn in the pot situation.

4.3.2 Effects of Teprosyn on nutrient availability in soil

The effects of Teprosyn on nutrients (P, N and Zn) availability in soil after harvest are presented in Table 7. The concentrations of N in soil did not show significant ($P<0.05$) differences across treatments (Table 7). Nitrogen varied from 0.12 % (K_2HPO_4 at 20 kg P ha^{-1}) to 0.18 % (Teprosyn at 8 ml/0.5 kg seed). However, the concentration of P and Zn in

the soil showed significant ($P<0.05$) difference across treatments.P increased from 0.57 mg/kg (Teprosyn at 4 ml/0.5 kg seed) to 1.13 mg/kg (K_2HPO_4 at 20 kg P ha^{-1}) and Zn from 1.14 mg/kg (Teprosyn at 4 ml/0.5 kg seed) to 2.25 mg/kg (K_2HPO_4 at 10 kg P ha^{-1}).

Teprsoyn was considered as an N, P and Zn supplement but could not provide sufficient N, P or Zn for adequate crop growth and biomass yields, until when supplemented with P (K_2HPO_4) at half the recommended rate in the soil. The results of this study indicate that the quantities of N, P and Zn contained in the Teprosyn product are too little to meet the needs of the plant when applied to soil / seed and should be supplemented with external sources of N, P and Zn to enhance plant growth and yields in P-deficient soils. Therefore, the product tested in the present study was of low quality as compared to cited values.

4.3.3 Effects of P-solubilizers (Bio-soil Crop booster) on maize growth, biomass yields and shoot nutrient concentrations

Table 8 shows the effects of P-solubilizers on maize growth performance and biomass yields. In the early days of plant growth at 35 DAP, plant height and some other growth parameters did not show any significant differences across treatments. However, the plant girth at 35 DAP were significantly ($P<0.05$) different across the treatments, with the highest value (0.8 cm) recorded under K_2HPO_4, at the rate of 10 kg P ha^{-1}. Bio-soil crop booster treatments alone or in combination with K_2HPO_4 (10 kg P ha^{-1}) did not differ significantly amongst themselves and sometimes not with the control in terms of plant height, number of leaves or plant girth.

At harvest, biomass yields showed significant differences across treatments with the treatment of K_2HPO_4 at the rate of 20 kg P ha^{-1} and the treatment with combination of Bio-

soil crop booster (5 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1}) resulting in significantly ($P<0.05$) higher biomass yields.

The Bio-soil crop booster treatments alone did not differ much amongst themselves and sometimes not with the control. Concentrations of N, P and Zn in shoot (as expression of nutrient uptake) were significantly ($P<0.05$) different across treatments (Table 9). Nitrogen increased from 2.22 % (K_2HPO_4 at 10 kg P ha^{-1}) to 3.00 % (K_2HPO_4 at 20 kg P ha^{-1}), P from 0.06 % (Control and Bio-soil crop booster at 5 ml/kg seed) to 0.12 % (K_2HPO_4 at 20 kg P ha^{-1}), and Zn ranged from 45.54 mg/kg (Bio-soil crop booster at 5 ml/0.5 kg seed + K_2HPO_4 at 10 kg P ha^{-1}) to 56.63 mg/kg (Bio-soil crop booster at 5 ml/0.5 kg seed and 10 ml/0.5 kg seed, respectively).

The trends of no significant differences ($P<0.05$) observed across treatments in terms of plant height and some other growth parameters, at 35 DAP, may be attributed to the fact that the plants were still young to be able to absorb large quantities of the nutrients released by the applied fertilizers. However, the increase in the plant girth at 35 DAP influenced by K_2HPO_4, especially at the rate of 10 kg P ha^{-1}, may be attributed to nutrients (K and P) available in the K_2HPO_4 fertilizer. The increase in maize shoots biomass in the treatments of K_2HPO_4 at the rate of 20 kg/ha and the treatment with combination of Bio-soil crop booster (5 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1}) indicate that plants had fully developed their root systems and absorbed the nutrients released by K_2HPO_4 fertilizer and / or the P-solubilized by the microorganisms present in the Bio-soil crop booster, making it available in the soil.

These findings contradict the results of Umashankar *et al*. (2012) who reported significant increase in heights of silver oak plant at 30 DAP due to inoculation with P-solubilizing fungi in comparison to the control and the observations of Domenech *et al*. (2006) working

on *Mathiola incana*, a flower crop, on inoculation with P solubilizing bacteria coupled with application of chemical fertilizer.

The present study shows that numbers of leaves per plant at 35 DAP did not differ significantly ($P<0.05$) due to seed inoculation with P-solubilizing bacteria and also due to different levels of P applications. This is in line with the findings of Umashankar *et al*. (2012) who observed insignificant ($P<0.05$) increase in number of leaves of silver oak plant at 30 DAP due to seed inoculation by P-solubilizing fungi. Since the soil used in the present study had low level of P, this implies that even if the P were solubilized, it would not be in large enough quantities to result in much improvement in plant growth.

The current study findings show higher biomass yields at harvest in the treatment of K_2HPO_4 at the rate of 20 kg/ha and the treatment with combination of Bio-soil crop booster (5 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1}). Bano and Afzal (2008) observed significant increase in shoot weight of wheat due to inoculation with phosphate solubilizing bacteria together with phosphate fertilizer (P_2O_5). Montañez and Sicardi (2013) similarly observed significant increase in dry matter yield of maize due to P-solubilizing bacteria.

Table 8: Effects of P-solubilizers on plant height, number of leaves, plant girth and biomass yields

	21 DAP			28 DAP			35DAP			
Treatment	**Plant Height (cm)**	**No. leaves/plant**	**Plant Girth (cm)**	**Plant Height (cm)**	**No. leaves/plant**	**Plant Girth (cm)**	**Plant Height (cm)**	**No. leaves/plant**	**Plant Girth (cm)**	**Biomass yields, (g/plant)**
Control	32.0	5.0	0.6	32.5	6.3	0.6	43.2	6.7	0.7	1.01
Bio-soil crop booster (5 ml/0.5 kg seed)	33.1	5.5	0.6	38.4	5.8	0.6	44.4	7.3	0.6	1.12
Bio-soil crop booster (10 ml/0.5 kg seed)	35.8	4.8	0.6	39.6	6.0	0.6	39.3	6.8	0.6	0.86
Bio-soil crop booster (5 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1})	33.9	5.3	0.6	43.0	6.3	0.6	51.1	7.5	0.8	1.65
K_2HPO_4 (10 kg P ha^{-1})	34.8	5.5	0.6	38.0	6.2	0.6	47.8	7.3	0.8	1.45
K_2HPO_4 (20 kg P ha^{-1})	39.3	5.0	0.6	40.2	6.2	0.6	48.9	7.0	0.8	1.71
LSD	10.11	0.74	0.09	10.90	0.94	0.06	20.59	0.99	0.12	0.36
CV (%)	16.0	7.8	8.8	15.5	8.4	5.4	24.7	7.7	9.3	15.2

Table 9: Effects of P-solubilizers on nutrient concentrations in maize shoot and soil after harvest

Treatment	Shoot P (%)	Shoot N (%)	Shoot Zn (mg/kg)	Soil N (%)	Soil P (mg/kg)	Soil Zn (mg/kg)
Control	0.06	2.77	52.47	0.15	1.04	2.09
Bio-soil crop booster (5 ml/0.5 kg seed)	0.06	2.78	56.63	0.17	2.39	4.78
Bio-soil crop booster (10 ml/0.5 kg seed)	0.08	2.80	56.63	0.18	1.49	2.98
Bio-soil crop booster (5 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1})	0.08	2.77	45.54	0.17	1.55	3.11
K_2HPO_4 (10 kg P ha^{-1})	0.10	2.22	55.24	0.17	1.55	3.11
K_2HPO_4 (20 kg P ha^{-1})	0.12	3.00	49.24	0.12	1.92	3.84
LSD	0.03	1.09	9.06	0.04	1.45	2.90
CV (%)	22.8	22.0	9.5	13.9	48.0	48.0

With regard to N, P and Zn contents in the shoot (at harvest), the present study findings show that P concentration in all treatments fell far below the sufficiency range (0.4 - 0.8 %) established for shoot maize plants 30 to 45 days after emergence (Lockman, 1969; Vandamme, 2008). It is probable that the crop was P-deficient at harvest or due to low amounts of P solubilized by the microorganisms present in the Bio-soil crop booster product. The current study findings show the inoculated treatments had low P content of maize shoot compared to the control. These results contrast the observations by Qureshi *et al*. (2012a) who recorded significant enhancement of plant P content of mash bean plant biomass due to bacterial inoculation than in the control.

The N concentrations in all treatments were below the sufficiency range (3.5 – 5.0 %) established for shoot maize plants 30 to 45 days after emergence (Lockman, 1969). The current study findings show insignificant ($P<0.05$) increase in shoot N content due to inoculation of P-solubilizer (Bio-soil crop booster). These findings contradict the findings of Iman and Azouni (2008), Gune *et al*. (2009) and Ahmad *et al.* (2012), who observed

increase in N content in soyabean, strawberry (*Fragaria ananassa*) and cotton respectively, and due to inoculation with P-solubilizing microorganisms.

The Zn concentrations in all treatments were within the sufficiency range (20 – 50 mg Zn kg^{-1}) established for shoot maize plants 30 to 45 days after emergence (Lockman, 1969; Vandamme, 2008). This implies that the maize plants could have taken up some substantial amounts of zinc (Kucey *et al*., 1989), resulting improvement in plant growth or influencing the shoot biomass yield.

4.3.4 Effects of P-solubilizers (Bio-soil Crop booster) on nutrient availability in soil

The effects of P-solubilizers (Bio-soil Crop booster) on nutrients (P, N and Zinc) in soil after harvest are presented in Table 9. Nitrogen increased significantly ($P<0.05$) from 0.12 % (K_2HPO_4 at 20 kg P ha^{-1}) to 0.18 % (Bio-soil crop booster at 10 ml/0.5 kg seed). However, the concentration of P and Zn in the soil did not show significant ($P<0.05$) difference across treatments (Table 9): P ranged from 1.04 mg/kg (Control) to 2.39 mg/kg (Bio-soil crop booster at 5 ml/0.5 kg seed) and Zn varied from 2.09 mg/kg (Control) to 4.78 mg/kg (Bio-soil crop booster at 5 ml/0.5 kg seed). The present study findings of insignificant ($P<0.05$) increase of available P in soil at harvest due to P-solubilizer (Bio-soil crop booster) inoculation are similar to those observed by Patil *et al.* (1979). The present study findings, however, contradict the findings of Qureshi *et al*. (2012b), and Dorahy *et al.* (2008), who observed significant increase in the available P in soil at different growth stages of cotton when phosphate solubilizer microorganisms were used.

4.3.5 Effects of free living N_2-fixers (Bio-soil Nitro+) on maize growth, biomass yields and shoot nutrient concentrations

The effects of free living N_2-fixers (Bio-soil Nitro+) on maize growth performance and biomass yields are presented in Table 10. In the early days of plant growth at 28 DAP, plant height (cm) and some other growth parameters did not show any significant ($P<0.05$) differences across treatments. However, from 28 DAP to 35 DAP, the treatments showed significant differences specially for plant girth at 28 DAP as well as plant height and some other growth parameters at 35 DAP. The number of leaves and plant girth at 35 DAP differed significantly ($P<0.05$) across treatments with highest number of leaves (8) and girth value (0.9 cm) respectively, resulted by K_2HPO_4, especially at the rate of 20 kg P /ha. The Bio-soil Nitro+ treatments alone did not differ much amongst themselves and sometimes not with the control.

At harvest, biomass yields showed significant differences across treatments, with the treatment of the K_2HPO_4 (20 kg P ha^{-1}) resulting in significantly ($P<0.05$) higher biomass yields. The Bio-soil Nitro+ treatments somehow differed amongst themselves and sometimes with the control.

Concentrations of P in shoot (as expression of nutrient uptake) were significantly ($P<0.05$) different across treatments (Table 11). Phosphorous varied from 0.06 % (Control and Bio-soil Nitro+ at 5 ml/0.5 kg seed) to 0.15653 % (K_2HPO_4 at 20 kg P ha^{-1}). However, the concentration of N and Zn in the shoot did not show significant ($P<0.05$) differences across treatments. Zinc varied from 61.24 mg/kg (Bio-soil Nitro+ at 10 ml/0.5 kg seed and K_2HPO_4 at 10 kg P ha^{-1}) to 71.40 mg/kg (Bio-soil Nitro+ at 5 ml/0.5 kg seed) and N ranged from 2.65 % (Control and Bio-soil Nitro+ at 5 ml/0.5 kg seed) to 2.91 % (K_2HPO_4 at 10 kg P ha^{-1}).

The trends observed in plant growth at 35 DAP, especially increase in plant height and some other growth parameters might be attributed to nutrients (K and P) available in K_2HPO_4 fertilizer. These findings are in agreement with the findings recorded by Hadi *et al*. (2014) who indicated that plant heights were not affected by the inoculation of basil seeds (*Ocimum basilicum*) by nitrogen free-living fixing bacteria. However, the findings of the present study contrast the findings of Baral and Adhikari (2013) and Naik *et al*. (2007) respectively, who observed significant increase in plant height of maize, mulberry plant and *Adathoda vasica* Nees crop on inoculation with N_2 fixing bacteria (*Azotobacter)* over non-inoculated treatments. Similar trends have been observed by Khan *et al*. (2010), Das and Saha (2007), Elkoca *et al*. (2008), Darzi and Hadi (2012) respectively, who observed significantly increased plant height compared with the control treatment when *brassica juncea, maize,* chickpea and dill (*Anethum graveolens*), a spice crop of the carrot family, were inoculated with bio-fertilizer containing N_2 fixing microorganisms. The increase in plant height might be due to enhanced nitrogen content and the rate of photosynthesis (Migahed *et al*., 2004) as the result of improvement of nitrogen fixing bacteria' activities in soil.

Table 10: Effects of free living N_2-fixers on plant height, number of leaves, plant girth and biomass yields

Treatment	21 DAP			28 DAP			35 DAP			
	Plant Height (cm)	**No. leaves/plant**	**Plant Girth (cm)**	**Plant Height (cm)**	**No. leaves/plant**	**Plant Girth (cm)**	**Plant Height, cm**	**No. leaves/plant**	**Plant Girth (cm)**	**Biomass yields (g/plant)**
Control	31.8	6.2	0.6	32.8	6.2	0.6	33.6	7.3	0.7	0.95
Bio-soil Nitro+ (5 ml/0.5 kg seed)	29.8	6.5	0.6	34.8	6.5	0.7	35.3	7.2	0.7	1.04
Bio-soil Nitro+ (10 ml/0.5 kg seed)	31.4	6.5	0.6	29.7	6.5	0.6	34.8	7.2	0.7	0.88
Bio-soil Nitro+ (5 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1})	34.0	6.5	0.6	34.5	6.5	0.7	47.4	7.5	0.8	1.51
K_2HPO_4 (10 kg P ha^{-1})	37.8	6.8	0.6	34.5	6.8	0.6	46.2	7.5	0.7	1.28
K_2HPO_4 (20 kg P ha^{-1})	32.2	6.8	0.6	33.5	6.8	0.7	51.7	8.0	0.9	2.19
LSD	8.33	0.96	0.08	8.68	0.96	0.10	9.15	0.66	0.08	0.33
CV (%)	14.0	8.1	7.1	14.3	8.1	8.7	12.1	4.9	6.2	13.8

Table 11: Effects of free living N_2-fixers on nutrient concentrations in maize shoot and soil after harvest

Treatment	Shoot P (%)	Shoot N (%)	Shoot Zn (mg/kg)	Soil N (%)	Soil P (mg/kg)	Soil Zn (mg/kg)
Control	0.06	2.65	61.70	0.17	1.16	2.31
Bio-soil Nitro+ (5 ml/0.5 kg seed)	0.06	2.65	71.40	0.18	1.06	2.13
Bio-soil Nitro+ (10 ml/0.5 kg seed)	0.07	2.72	61.24	0.17	0.96	1.92
Bio-soil Nitro+ (5 ml/0.5 kg seed) + K_2HPO_4 (10 kg P ha^{-1})	0.07	2.72	67.71	0.17	1.39	2.78
K_2HPO_4 (10 kg P ha^{-1})	0.12	2.91	61.24	0.16	1.29	2.58
K_2HPO_4 (20 kg P ha^{-1})	0.16	2.73	67.24	0.17	1.68	3.36
LSD	0.06	0.34	17.62	0.02	0.22	0.44
CV (%)	35.2	6.8	14.9	7.2	9.7	9.7

The present study indicates that the number of leaves were similar in the control and in the treatments inoculated with the Bio-soil Nitro+. These results contradict the findings of Hadi *et al*. (2014) who demonstrated that number of branches per plant in basil (*Ocimum basilicum*) plants were significantly influenced by the inoculation of seeds by nitrogen fixing bacteria. Similar trends have been observed by Yasari and Patwardhan (2007) working on canola plants where application of *Azotobacter* and *Azospirillum* strains increased number of branches.

The present study findings show insignificant ($P<0.05$) increase in biomass yields across treatments at harvest as was also observed by Canbolat *et al.* (2006) and Elkoca *et al. (*2008), who reported non-significant ($P<0.05$) difference in shoot biomass of barley or biomass of shoots of chickpea, respectively, when inoculant alone or fertilizer alone was used. However, the present study contradict the observations by Turan *et al*. (2010) who

observed that inoculation of N_2-fixing strains alone and with fertilizer applications significantly increased total biomass yields, macro and micro nutrients of different parts of the wheat plant, Khan *et al*. (2010) who observed that inoculation of N_2-fixing strains (*Azotobacter and Azospirillum*) alone significantly increased total biomass yields of *Brassica juncea* plant, Darzi and Hadi (2012) who observed significant effect of bio-fertilizer containing N_2 fixers on biomass yield of dill (*Anethum graveolens*) and Montañez and Sicardi (2013), who observed that inoculation of N_2 fixing bacteria significantly increased maize dry matter yield.

With regard to N, P and Zn contents in the shoot (at harvest), the study findings showed that P concentration in all treatments fell far below the sufficiency range (0.4 - 0.8 %) established for shoot maize plants 30 to 45 days after emergence (Lockman, 1969; Vandamme, 2008). It is probable that the crop was P-deficient at harvest, which possibly did not increase the ability of plants to absorb water and soil nutrients by increasing the effective absorbing surface area of root systems. The nitrogen concentrations in all treatments were below the sufficiency range (3.5 – 5.0 %) established for shoot maize plants 30 to 45 days after emergence (Lockman, 1969).

The current study findings show insignificant increase in shoot N content due to inoculation with N_2 fixers (Bio-soil Nitro+). These findings contradict the findings of Vendan and Sundaram (1997), who reported increased N-uptake in rice due to inoculation of *Azotobacter* and/or *Azospirillum,* in the presence of inorganic nitrogen fertilizer. The Zn concentrations in all treatments were above the sufficiency range (20 – 50 mg Zn kg^{-1}) established for shoot maize plants 30 to 45 days after emergence (Lockman, 1969; Vandamme, 2008). This implies that the maize plants could have up-taken some substantial

amounts of Zn released by the fertilizers or present in the soil, that caused improvement in plant growth or influencing the shoot biomass yield.

4.3.6 Effects of free living N_2-fixers (Bio-soil Nitro+) on nutrient availability in soil

The effects of N_2-fixers (Bio-soil Nitro+) on nutrients (P, N and Zinc) in soil after harvest are presented in Table 11. The concentrations of N in soil did not show significant ($P<0.05$) differences across treatments (Table 11). Nitrogen varied from 0.1610 % (K_2HPO_4 at 10 kg P ha^{-1}) to 0.1750 % (Bio-soil Nitro+ at 5 ml/0.5 kg seed). However, the concentrations of P and Zn in the soil showed significant ($P<0.05$) differences across treatments (Table 12): P increased from 0.96 mg/kg (Bio-soil Nitro+ at 10 ml/0.5 kg seed) to 1.68 mg/kg (K_2HPO_4 at 20 kg P ha^{-1}) and Zn from 1.92 mg/kg (Bio-soil Nitro+ at 10 ml/0.5 kg seed) to 3.36 mg/kg (K_2HPO_4 at 20 kg P ha^{-1}).

The present study findings show insignificant ($P<0.05$) increase of N in soil at harvest due to N-fixers (Bio-soil Nitro+) inoculation. This contradicts the observations of Kader *et al*. (2002), who reported that inoculation of N_2 fixing bacteria (*Azotobacter*) increased N availability in the soil at harvest of wheat and Chattopadhyay *et al*. (2009) who observed higher concentration of total N in inoculated soils than un-inoculated soils at final harvest of teak and Indian redwood.

Therefore, the results of this study indicate that the population of N_2 fixing bacteria in the product (Table 5) and there multiplication in soil, was low, to sufficiently fix appreciable amounts of N in the soil for adequate maize growth until when the N_2 fixing bacteria were supplemented with external P at half the recommended rate (HRR) in the soil. Thus, the product tested in the present study was of low quality vis-a-vis the cited values. Thus,

TFRA should require manufacturers to meet the quality standards before their products are accepted in the country as described by the fertilizer Act (2009) (URT, 2009).

4.4 Field Experiments

4.4.1 Effects of Teprosyn on maize growth performance

The effects of Teprosyn on maize growth performance are presented in Table 12. In the early days of plant growth (up to 21 DAP), plant height (cm) and number of leaves did not show any significant ($P<0.05$) differences across treatments. However, in the subsequent periods of plant growth, the YaraMila Cereal fertilizer treatments alone, at 84 DAP, resulted in significantly ($P<0.05$) higher plant heights and some other growth parameters, especially at the rate of 20 kg P ha^{-1}. The Teprosyn treatments, either alone or in combination with YaraMila Cereal fertilizer at 10 kg P ha^{-1}, did not differ significantly ($P<0.05$) amongst themselves and sometimes not with the control.

At tasseling, biomass yields showed significant ($P<0.05$) differences across treatments with the YaraMila Cereal fertilizer treatments alone or in combination with Teprosyn (4 ml/0.5 kg seed) resulting in significantly ($P<0.05$) higher biomass yields. The Teprosyn treatments alone did not differ significantly amongst themselves and sometimes not with the control. Concentrations of N, P and Zn in ear leaf (as expression of nutrient uptake) did not show significant ($P<0.05$) differences across treatments (Table 13): N varied from 1.80% (Control) to 2.14 % (YaraMila Cereal at 10 kg P ha^{-1}), P from 0.19 % (Control) to 2.61 % (Teprosyn at 8 ml/ 0.5 kg seed), and Zn from 15.70 mg/kg (YaraMila Cereal at 20 kg P ha^{-1}) to 19.57mg/kg (Teprosyn at 8 ml/ 0.5 kg seed).

The trends of no significant ($P<0.05$) differences as observed in the early stage of plant growth, may be attributed to the fact that the plants were still young and developing their

root systems to be able to absorb the nutrients released by the applied fertilizers. However, significant differences in plant heights and some other growth parameters observed in the subsequent periods of plant growth, especially under the reference treatment (YaraMila Cereal fertilizer at 20 kg P ha^{-1}), may be attributed to the nutrients (N, P, K, Mg, S and Zn) available in the fertilizer. The increase in maize shoot biomass in the reference treatment (YaraMila Cereal fertilizer at 20 kg P ha^{-1}), confirms that the soils used were infertile and thus responsive to nutrient inputs; hence the conditions were favourable for the fertilizer to show its effects.

The results of this study indicate that, Teprosyn treatments alone had no significant ($P<0.05$) effect on plant height in the early growth, shoot biomass and nutrients (N, P and Zn) uptake at tasseling. These results contrasts the findings of different studies performed with various strategies of nutrient application on maize or with other crops. Peltonen-Sainio *et al*. (2006) evaluated the effect of P seed coating on oat and found that P seed coating enhanced early growth of oat without increasing yields. Further, Karanam and Vadez (2010) reported an increase in shoot biomass of two- and four- week-old seedlings due to P seed coating of pearl millet compared with no-coated treatment.

However, the present study findings show that when seeds were pre-treated with Teprosyn in combination with YaraMila Cereal fertilizer (10 kg P ha^{-1}), significantly ($P<0.05$) higher values of shoot biomass were observed compared to the control. This is in line with the findings by Zelonka *et al*. (2005) on barley crop, indicating similarities in increased yield when the seeds were treated with P coupled with N. This can be explained by the fact that the supply of N enhances the production of small roots and root hairs, which in turn facilitated the high absorbing capacity per unit of dry weight (Hussaini *et al.*, 2008). Beside the direct nutritional effects of nutrient dressing, it is possible that the production of root

and root hairs improves the access to soil nutrients and water-use efficiency by the plants (Ros *et al*., 2000).

Teprsoyn was considered as N, P and Zn supplement but could not provide sufficient N, P and Zn for adequate crop growth, until when supplemented with P (YaraMila Cereal fertilizer) at half the recommended rate (HRR) in the soil. Therefore, the results of this study indicate that the quantities of N, P and Zn contained in Teprosyn product are too little to meet the needs of the plant and should be supplemented with external N, P and Zn to enhance plant growth and yields in P-deficient soils. Therefore, the results of this study indicate that the quantities of N, P and Zn contained in Teprosyn product are too little to meet the needs of the plant and should be supplemented with external N, P and Zn to enhance plant growth and yields in P-deficient soils. The product tested in the present study was of low quality and would, therefore, not qualify for sole use in Tanzania.These quantities could possibly result in better plant performance if the outlook for their use would be to contribute more to nourish soil microorganisms, thereby improving the microorganisms' capacity to mineralize additional quantities of nutrients from soil organic matter. Otherwise, the absolute qualities of these nutrients in these commercial products are too low to make a substantial direct impact on plant growth.

4.4.2 Effects of Teprosyn on maize grain yields

The effects of Teprosyn on maize grain yields and nutrient concentrations in soil after harvest are presented in Table 13. The maize grain yields at harvest showed significant ($P<0.05$) differences across treatments with YaraMila Cereal fertilizer, especially at the rate of 20 kg P ha^{-1} resulting higher grain yields. The Teprosyn treatments, either alone or in combination with YaraMila Cereal fertilizer (10 kg P ha^{-1}), did not differ significantly amongst themselves and not with the control. These findings are supported by the results of

Dogan *et al*. (2008), using the wheat crop, observed insignificant seed yields at harvest on treatment wheat seeds with zinc compound (Teprosyn F-2498). However, the current study findings contradict results of Yilmaz *et al.* (1997) who reported increase in wheat seed yield on treatment with similar zinc product.

Further, Masuthi *et al*. (2009) reported significant increase in seed yield in cowpea (*Vigna unguiculata L*.) over control due to pelleting seed with ZnSO4 product. Similarly, Singh (2007) reported improvement grain yield of sunflower, maize, wheat, soybean and groung nut on coating with Teprosyn-ZnP or Teprosyn- Zn products.

The present study findings indicated decrease in grain yields when higher rate (above the recommended rate) of Teprosyn was used (Teprosyn at 8 ml/kg 0.5 seed). Dogan *et al*. (2008) similarly observed decrease in wheat seed yields when higher rates of zinc compound (Teprosyn F-2498) above the recommended rate were used.

Table 12: Effects of Teprosyn on plant height, number of leaves, plant girth and biomass yields

Treatment	21 DAP Plant Height (cm)	No. leaves/plant	42 DAP Plant Height (cm)	No. leaves/plant	Plant Girth (cm)	63 DAP Plant Height (cm)	No. leaves/ plant	Plant Girth (cm)	84 DAP Plant Height (cm)	No. leaves/plant	Plant Girth (cm)	Biomass at tasseling, g/plant
Control	26.8	7.3	61.1	9.3	1.6	186.2	13.3	1.7	241.5	12.1	1.7	206.80
Teprosyn (4 ml/0.5 kg seed)	25.6	7.3	65.7	10.0	1.9	195.6	13.7	1.8	254.2	12.6	1.9	214.80
Teprosyn (8 ml/0.5 kg seed)	27.5	7.3	77.0	11.0	2.2	215.8	15.0	2.0	245.7	12.9	2.0	238.60
Teprosyn (4 ml/0.5 kg seed)+ YaraMilaCereal (10 kg P ha^{-1})	27.9	8.0	80.4	10.7	2.2	218.8	14.3	1.9	260.4	13.0	2.1	312.60
YaraMilaCereal (10 kg P ha^{-1})	29.3	7.7	87.6	11.0	2.3	225.8	14.0	2.0	263.5	13.3	2.1	315.60
YaraMilaCereal (20 kg P ha^{-1})	29.5	7.7	96.0	11.7	2.4	245.2	15.7	2.3	275.5	13.5	2.3	371.50
LSD	4.33	1.23	15.91	2.02	0.36	31.18	1.10	0.31	28.72	1.21	0.40	75.98
CV (%)	8.3	8.9	11.2	10.4	9.5	8.0	4.2	8.9	6.1	5.2	11.0	15.1

Table 13: Effects of Teprosyn on maize grain yields and nutrient concentrations in maize ear leaf and soil after harvest

Treatment	Ear leaf N (%)	Ear leaf P (%)	Ear leaf Zn (mg/kg)	Soil N (%)	Soil P (mg/kg)	Soil Zn (mg/kg)	Maize grain yields (t/ha)
Control	1.80	0.19	18.60	0.15	1.33	3.43	2.06
Teprosyn (4 ml/0.5 kg seed)	1.84	0.22	17.64	0.17	1.41	4.38	2.11
Teprosyn (8 ml/0.5 kg seed)	2.11	0.26	19.57	0.17	1.39	5.23	2.18
Teprosyn (4 ml/0.5 kg seed) + YaraMila Cereal (10 kg P ha^{-1})	2.07	0.24	19.09	0.17	2.25	6.58	1.93
YaraMila Cereal (10 kg P ha^{-1})	2.14	0.22	17.15	0.17	2.79	4.83	2.82
YaraMila Cereal (20 kg P ha^{-1})	2.01	0.30	15.70	0.17	5.02	3.43	3.39
LSD	0.34	0.05	5.98	0.04	0.10	3.09	0.99
CV (%)	9.3	23.3	18.3	11.7	23.2	35.8	22.5

The decrease of grain yields may be due to antagonistic effects with other nutrients in soil, making zinc absorbed to be insufficient. Antagonistic effects of Zn and P have been documented (Christensen and Jackson, 1981; Singh *et al*., 1986). It may be noted that in Table 13, and subsequent Tables, that the CV(%) is sometimes very high. This high CV % may be related, among others, to possible unforeseen differences in distribution of the applied P and Zn in the field situation.

4.4.3 Effects of Teprosyn on nutrient availability in soil

At harvest, the concentrations of N and Zn in soil did not show significant ($P<0.05$) differences across treatments (Table 13). Nitrogen varied from 0.15 % (Control) to 0.17 % (Teprosyn at 4 ml/0.5 kg seed and 8 ml/0.5 kg seed, Teprosyn at 4 ml/0.5 kg seed +

YaraMila Cereal fertilizer at 20 kg P ha^{-1}, YaraMila Cereal fertilizer at 10 kg P ha^{-1} and 20 kg P ha^{-1}) and Zn ranged from 3.43mg/kg (Control and YaraMila Cereal fertilizer at 20 kg P ha^{-1}) to 6.58mg/kg (Teprosyn at 4 ml/0.5 kg seed + YaraMila Cereal fertilizer at 10 kg P ha^{-1}). However, the concentration of P in the soil showed significant ($P<0.05$) difference across treatments (Table 13): P ranged from 1.33 mg/kg (Control) to 5.02 mg/kg (YaraMila Cereal fertilizer at 20 kg P ha^{-1}).

However, the present study findings showed significant ($P<0.05$) increase of available P in soil at harvest due to Teprosyn inoculation. The amount of P in the product could not increase maize grain yields confirming that the quantities of N, P and Zn contained in Teprosyn product are too little to meet the needs of the plant. Therefore it is suggested that the Teprosyn product should be supplemented with external N, P and Zn to enhance plant growth and yields in P-deficient soils.

4.4.4 Effects of P-solubilizers (Bio-soil crop booster) on maize growth performance

Table 14 shows the effects of Bio-soil crop booster, a P solubilizer preparation, on maize growth performance. Since the early days of plant growth (21 DAP), plant height (cm) and number of leaves showed significant ($P<0.05$) differences across treatments, with highest values associated with YaraMila Cereal fertilizer, especially at the rate of 20 kg P ha^{-1}. Bio-soil crop booster treatments alone or in combination with YaraMila Cereal fertilizer at 10 kg P ha^{-1}, did not differ much amongst themselves or with the control.

However, in the subsequent periods of plant growth, the YaraMila Cereal fertilizer treatments alone at 84 DAP resulted in significantly ($P<0.05$) higher plant heights and some other growth parameters, especially at the rate of 20 kg P ha^{-1}. The Bio-soil crop booster treatments alone somehow differed amongst themselves and sometimes with the control.

Similarly, the Bio-soil crop booster treatment in combination with YaraMila Cereal fertilizer at 10 kg P ha^{-1}, did differ somehow with the control in terms of plant heights and some other growth parameters.

At tasseling, biomass yields showed significant ($P<0.05$) differences across some treatments with the treatment with combination of Bio-soil crop booster (5 ml/0.5 kg seed) + YaraMila Cereal fertilizer (10 kg P ha^{-1}) resulting in significantly higher biomass yields. The Bio-soil crop booster and YaraMila Cereal treatments did not differ significantly ($P<0.05$) amongst themselves and sometimes not with the control. Concentrations of P and Zn in ear leaf (as expression of nutrient uptake) did not show significant ($P<0.05$) differences across treatments (Table 15).

Phosphorous ranged from 0.19 % (Bio-soil crop booster at 5 ml/0.5 kg seed) to 0.23 % (Bio-soil crop booster at 5 ml/0.5 kg seed) + YaraMila Cereal fertilizer at 10 kg P ha^{-1}). Zinc varied from 14.73 mg/kg (Control) to 15.21 mg/kg (Bio-soil crop booster at 5 ml/0.5 kg seed and YaraMila Cereal fertilizer at 10 kg P ha^{-1} and 20 kg P ha^{-1}). However, the concentration of N (ranging from 1.70 % to 2.24 %) in the ear leaf showed significant ($P<0.05$) difference across treatments with highest values observed in the YaraMila Cereal fertilizer treatment, especially at the rate of 20 kg P ha^{-1} and in the treatment with combination of Bio-soil crop booster (5 ml/0.5 kg seed) + YaraMila Cereal fertilizer (10 kg P ha^{-1}). The Bio-soil crop booster treatments alone did not differ amongst themselves and sometimes not with the control.

The trends observed in the early stage of plant growth, may have been attributed by the nutrients (N, P, K, Mg, S and Zn) available in the fertilizer and substantial amounts of soil P solubilised by the microorganisms present in the Bio-soil crop booster.

The increase in plant heights and some other growth parameters at 84 DAP influenced by the YaraMila Cereal fertilizer treatments, either alone or in combination with Bio-soil crop booster (5 ml/0.5 kg seed), indicate that plants had fully developed their roots systems and absorbed the nutrients released by YaraMila Cereal fertilizer and / or the P-solubilised by the microorganisms present in the Bio-soil crop booster making it available in the soil. These findings are supported by the results of Patil *et al*. (2012), also using the maize crop on soil with medium P levels whereby plant height increased among different doses of phosphorus application and P-solubilizing fungi inoculation at 30 and 60 DAS and at harvest of the crop. Further, Ojaghloo *et al*. (2007) observed that biological fertilizer with 50% of chemical fertilizers (N, P and K) led to an increase in plant growth, plant height, branch number of safflower (*Carthamus tinctorius*) plants in comparison with applying chemical fertilizers alone. In addition, the study by Elkoca *et al*. (2008) pointed out that plants inoculated with P-solubilizing *Bacillus megaterium* had the highest plant height in comparison with the control.

The present study showed that numbers of leaves per plant at 63 DAP and 84 DAP did not differ significantly due to seed inoculation with P-solubilizing bacteria and also due to different levels of P applications. This is in line with the findings of Patil *et al*. (2012) who, under soil with medium P levels, observed insignificant number of leaves per of wheat plant due to seed inoculation by P-solubilizing fungi and also due to different levels of P (DAP and P_2O_5 sources) at 30 and 60 DAP. This implies that the amount of P solubilized the P-solubilizer fungi would not result into substantial effects as the soil available P was optimal for plant growth. Since the soil used in the present study had low level of P (Table 3), this implies that even if the P was solubilized, it would not have resulted in large quantities to result in much improvement in plant growth.

The current study findings show higher biomass yields at tasseling in the treatment with combination of Bio-soil crop booster (5 ml/0.5 kg seed) + YaraMila Cereal fertilizer (10 kg P ha^{-1}). Patil *et al. (*1979) similarly observed significant increase in dry matter yield of cowpea due to application of rock phosphate along with P-solubilizing microorganisms. Hassan-zadeh *et al*. (2006) also reported increase in dry matter in barley due to phosphate-solution bacteria along with chemical P fertilizer.

With regard to N content in the ear leaf (at tasseling), the study findings show significant increase in N content due to YaraMila Cereal fertilizer (20 kg P ha^{-1}) and in the combination of Bio-soil crop booster (5 ml/0.5 kg seed) + YaraMila Cereal fertilizer (10 kg P ha^{-1}). These results can be supported by the findings made by Patil *et al*. (2012) who observed significant increase of nutrient contents in maize leaves at tasseling due application of PSFs in combination with various P_2O_5 levels. This increase in nutrient contents in the present study might have been due to the increased availability of P by increased solubilization by these P-solubilizers. Increase in the percentage of N content has been observed in other crop plants, for example soyabean and strawberry (*Fragaria ananassa*) due to inoculation of P-solubilizing microorganisms (Iman and Azouni, 2008 and Gune *et al*., 2009, respectively), as well as in cotton (Ahmad *et al*., 2012).

Table 14: Effects of P-solubilizers on plant height, number of leaves, plant girth and biomass yields

Treatment	21 DAP		42 DAP			63 DAP			84 DAP			
	Plant Height (cm)	No. leaves/ plant	Plant Height (cm)	No. leaves/ Plant	Plant Girth (cm)	Plant Height (cm)	No. leaves/ plant	Plant Girth (cm)	Plant Height (cm)	No. leaves/ plant	Plant Girth (cm)	Biomass at tasseling, g/plant
Control	27.5	7.0	71.9	9.8	1.9	199.3	14.0	5.5	246.8	14.1	1.7	206.80
BCB (5 ml/0.5 kg seed)	28.7	7.7	77.9	10.7	2.1	181.5	13.7	5.5	244.1	13.7	1.8	249.00
BCB(10 ml/0.5 kg seed)	26.0	6.7	67.3	10.3	1.9	160.9	13.3	5.7	250.3	13.7	1.8	147.00
BCB (5 ml/0.5 kg seed)+ YaraMila Cereal (10 kg P ha^{-1})	31.8	8.0	93.2	11.7	2.4	246.8	14.3	6.2	264.2	14.0	2.1	352.70
YaraMila Cereal (10 kg P ha^{-1})	29.8	7.3	95.3	11.0	2.4	225.0	15.0	6.6	280.6	14.4	2.1	306.00
YaraMila Cereal (20 kg P ha^{-1})	32.1	7.7	103.8	13.0	2.5	256.9	15.0	6.3	282.4	14.4	2.3	324.10
LSD	5.41	0.92	12.45	1.51	0.38	56.21	1.75	1.57	30.72	1.20	0.51	173.3
CV (%)	10.2	6.8	8.1	7.5	9.6	14.6	6.8	14.4	6.5	4.7	14.4	36.0

Table 15: Effects of P-solubilizers (Bio-soil Crop Booster) on maize grain yields and nutrient concentrations in maize ear leaf and soil after harvest

Treatment	Ear leaf N (%)	Ear leaf P (%)	Ear leaf Zn (mg/kg)	Soil N (%)	Soil P (mg/kg)	Soil Zn (mg/kg)	Maize grain yields (t/ha)
Control	1.76	0.20	14.73	0.16	2.23	8.17	2.04
Bio-soil Crop Booster (5 ml/0.5 kg seed)	1.70	0.19	15.21	0.16	1.74	7.93	3.26
Bio-soil Crop Booster (10 ml/0.5 kg seed)	1.70	0.23	14.73	0.16	1.07	4.77	1.71
Bio-soil Crop Booster (5 ml/0.5 kg seed) + YaraMila Cereal (10 kg P ha^{-1})	2.09	0.23	15.21	0.17	3.53	5.78	2.61
YaraMila Cereal (10 kg P ha^{-1})	2.00	0.20	14.97	0.17	5.64	6.03	2.19
YaraMila Cereal (20 kg P ha^{-1})	2.24	0.23	15.21	0.18	5.86	6.14	3.53
LSD	0.26	0.06	2.77	0.02	3.60	4.33	1.08
CV (%)	7.5	14.3	10.1	7.2	59.2	36.8	23.3

Therefore, the results of this study indicate that the P-solubilising bacteria in the product could not sufficiently solubilise appreciable amounts of P in the soil for adequate crop growth and yields, until when supplemented with external P [at half the recommended rate (HRR)] in the soil. This supports the observation (Table 5) that the product tested in the present study was of low quality, in terms of the number of P-solubilizing bacteria, as opposed to the values cited by the manufacturer and would, therefore, not qualify for use.

4.4.5 Effects of P-solubilizers (Bio-soil crop booster) on maize grain yields

Table 15 shows the effects of Bio-soil crop booster, a P solubilizer preparation, on maize grain yields and nutrient concentrations in soil after harvest. The maize grain yields at harvest showed significant ($P<0.05$) differences across treatments, with YaraMila Cereal

fertilizer, especially at the rate of 20 kg P ha^{-1}, resulting higher grain yields. The Bio-soil crop booster treatments alone, either used alone or in combination with P-fertilizer (YaraMila Cereal fertilizer at 10 kg P ha^{-1}), showed significant ($P<0.05$) differences amongst themselves and with the control, with Bio-soil crop booster (5ml/0.5 kg maize seed) resulting in significantly ($P<0.05$) grain yields. The increase in grain yields could be attributed to the fact that P-solubilizers increased the amount of available major and minor nutrients besides certain growth promoters which might have provided a suitable medium for the enhanced growth and hence yields. However, the lower value of grain yields observed in treatments which received only P-sources may be due to the slow rate of release of P from applied P fertilizer to meet the P requirement of crops at its critical growth periods. This is in agreement with the findings recorded by Rokhzadi *et al*. (2008) who indicated significant increase in grain yield of chickpea due to inoculation with PSB. Similar results have been observed by Tyagi *et al*. (2003) working on soybean and Bano and Afzal (2008) working on wheat.

Further, promotions in yields of various crop plants in response to inoculation with PSB were reported by other workers (Vaishya *et al.,* 1996; Kozdroja *et al*., 2004; Shaharoona *et al*., 2006 andGravel *et al*., 2007). The present study findings, however, do not agree with the results of Janagard *et al*. (2013) who recorded that inoculation of PSB alone had no significant effect on soybean grain yields while the inoculation of PSB in combination with chemical fertilizer that resulted in significantly ($P<0.05$) higher soybean grain yields, which is the reverse in this for this study. Similar trends have been recorded by Patil *et al*. (2012) and Mousavi *et al*. (2011), also working on maize with P-solubilizers and P fertilizer and Tiwari *et al*. (1989) working on chickpea. The increase in grain yields with the application of P_2O_5 + P-solubilizer might be attributed to the fact that P-solubilizers increased the amount of available P in soil. Lower yield in treatments receiving only P sources was due to

the slow rate of release of P from applied P fertilizer to meet the P requirement of crop, possibly due to absence of high numbers of native P-solubilizing bacteria in the soil.

4.4.6 Effects of P-solubilizers (Bio-soil crop booster) on nutrient availability in soil

At harvest, the concentrations of P, N and Zn in soil showed significant ($P<0.05$) differences across some treatments (Table 16). Phosphorous varied insignificantly ($P<0.05$) from 1.07 mg/kg (Bio-soil crop booster at 10 ml/0.5 kg seed) to 5.86 mg/kg (YaraMila Cereal fertilizer at 20 kg P/ha). Nitrogen ranged significantly ($P<0.05$) from 0.16 % (Control) to 0.18 % (YaraMila Cereal fertilizer at 20 kg P/ha) and Zn varied from 4.97 mg/kg (Bio-soil crop booster at 10 ml/0.5 kg seed) to 8.17mg/kg (Control). The current study findings showed insignificant increase of available P in soil at harvest due to P-solubilizers (Bio-soil crop booster) inoculation as opposed by Qureshi *et al*. (2012b), and Dorahy *et al.* (2008), who observed significant ($P<0.05$) increase of the available P in soil at different growth stages of cotton when phosphate solubilizer microorganisms were used. However, the observation (Table 5) showed that the product tested in the present study was of low (bacterial numbers) quality compared to the values provided by the manufacturer.

4.4.7 Effects of free living N_2-fixers (Bio-soil Nitro+) on maize growth performance

The effects of Bio-soil Nitro+, free N_2- fixing microorganisms, on maize growth performance are presented in Table 16. In the early days of plant growth (21 DAP), plant height (cm) did not show any significant differences across treatments while the number of leaves were significantly ($P<0.05$) different across. However, the number of leaves were significantly ($P<0.05$) different across treatments, with highest (8 leaves) recorded with YaraMila Cereal fertilizer, especially at the rate of 20 kg P ha^{-1}. In the subsequent periods of plant growth, the treatments with the combination of Bio-soil Nitro+ (5 ml/0.5 kg seed) + YaraMila Cereal (10 kg P ha^{-1}) and YaraMila Cereal fertilizer treatments alone, especially

at the rate of 20 kg P ha^{-1} at 42 DAP, resulted in significant higher plant heights (cm) and some other growth parameters. The Bio-soil Nitro+ treatments alone did not differ much amongst themselves and sometimes not with the control. However, from 63 DAP to 84 DAP, both YaraMila Cereal fertilizer treatments and Bio-soil Nitro+ treatments, either alone or in combination with YaraMila Cereal fertilizer (10 kg P ha^{-1}) did not differ significantly ($P<0.05$) amongst themselves and sometimes not with the control in terms of plant heights (cm) and other growth parameters. However, the number of leaves at 63 DAP differed significantly ($P<0.05$) across the treatments with highest values resulted by YaraMila Cereal fertilizer, especially at the rate of 20 kg P ha^{-1}. Similarly, Bio-soil Nitro+ treatments, either alone or in combination with YaraMila Cereal fertilizer at 10 kg P ha^{-1}, did not differ significantly ($P<0.05$) amongst themselves and sometimes not with the control.

At tasseling, biomass yields and concentrations of N, P and Zn in ear leaf (as expression of nutrient uptake) did not show significant ($P<0.05$) differences across treatments (Table 17). Plant N varied from 1.79 % (Bio-soil Nitro+ at 10 ml/0.5 kg seed) to 2.15 % (Bio-soil Nitro+ at 5 ml/0.5 kg seed + YaraMila Cereal at 10 kg P ha^{-1}) , P from 0.20 % (Bio-soil Nitro+ at 10 ml/0.5 kg seed and YaraMila Cereal at 10 kg P ha^{-1}) to 0.24 % (Bio-soil Nitro+ at 5 ml/0.5 kg see 10 kg P ha^{-1}+ YaraMila Cereal at 10 kg P ha^{-1}) and Zn from 17.64 mg/kg (YaraMila Cereal at 10 kg P ha^{-1}) to 20.30 mg.kg (YaraMila Cereal at 20 kg P ha^{-1}). The biomass yields could not be increased due to low uptake of nutrients by the plants.

The trends observed in the early stage of plant growth, especially increase in number of leaves, may be attributed to the nutrients (N, P, K, Mg, S and Zn) available in the YaraMila Cereal fertilizer. This is in line with the findings reported by Hadi *et al*. (2014) who indicated that plant heights were not affected by the inoculation of seeds by nitrogen free-

living fixing bacteria. However, the results demonstrated that number of branches per plant was significantly influenced by the inoculation of seeds by nitrogen fixing bacteria. Similar results observed by Yasari and Patwardhan (2007) where application of *Azotobacter* and/or *Azospirillum* strains increased number of branches of canola plants.

Increase in plant heights (cm) and some other growth parameters in the subsequent periods of plant growth may be attributed to the nutrients (N, P, K, Mg, S and Zn) available in the fertilizer and, possibly, due to additional of appreciable amounts of N fixed by the microorganisms. These findings are supported by the results reported by Elkoca *et al*. (2008) and Pandey *et al*. (2012), respectively, who observed that inoculation of Chickpea with N_2-fixing *Bacillus subtilis* significantly increased plant height and pod number compared with the control treatment, equal to or higher than those N, P and NP treatments and shoot length in rice and millets. Similarly, Darzi and Hadi (2012) observed significant effect on plant height (cm) of dill (*Anethum graveolens*) crop when inoculated with bio-fertilizer containing N_2 fixing microorganisms.

Table 16: Effects of free living N_2-fixers on plant height, number of leaves, plant girth and biomass yields

Treatment	21 DAP		42 DAP			63 DAP			84 DAP			
	Plant Height (cm)	No. leaves/plant	Plant Height (cm)	No. leaves/plant	Plant Girth (cm)	Plant Height (cm)	No. leaves/plant	Plant Girth (cm)	Plant Height (cm)	No. leaves/plant	Plant Girth (cm)	Biomass at tasseling, g/plant
Control	22.4	6.3	75.0	10.0	2.0	208.8	13.7	2.1	264.2	14.9	2.0	215.20
Bio-soil Nitro+ (5 ml/0.5 kg seed)	24.8	7.0	79.1	10.7	2.1	216.8	14.0	2.0	264.8	14.3	1.9	237.80
Bio-soil Nitro+ (10 ml/0.5 kg seed)	24.4	7.3	76.4	10.0	2.0	214.2	14.7	2.1	265.5	15.1	2.0	263.50
Bio-soil Nitro+ (5 ml/0.5 kg seed)+ YaraMila Cereal (10 kg P ha^{-1})	26.3	7.7	95.3	11.3	2.2	227.5	14.3	2.2	272.8	14.2	1.9	273.70
YaraMila Cereal (10 kg P ha^{-1})	26.4	7.7	83.4	11.0	2.0	231.2	14.3	2.0	264.7	15.2	2.0	274.40
YaraMila Cereal (20 kg P ha^{-1})	26.2	8.0	95.2	11.7	2.6	237.3	15.0	2.3	252.3	14.4	1.8	332.40
LSD	5.88	0.88	15.10	1.47	0.35	32.21	1.20	0.36	37.08	1.26	0.31	146.9
CV (%)	12.9	6.6	9.9	7.5	8.9	8.0	4.6	9.3	7.7	4.7	8.7	30.3

Table 17: Effects of free living N_2-fixers maize grain yields and nutrient concentrations in maize ear leaf and soil after harvest

Treatment	Ear leaf N (%)	Ear leaf P (%)	Ear leaf Zn (mg/kg)	Soil N (%)	Soil P (mg/kg)	Soil Zn (mg/kg)	Maize grain yields (t/ha)
Control	2.02	0.22	19.57	0.16	2.20	6.47	2.53
Bio-soil Nitro+ (5 ml/0.5 kg seed)	1.80	0.21	18.60	0.16	1.48	6.23	1.93
Bio-soil Nitro+ (10 ml/0.5 kg seed)	1.79	0.20	21.51	0.16	1.35	6.24	2.33
Bio-soil Nitro+ (5 ml/0.5 kg seed) + YaraMila Cereal (10 kg P ha^{-1})	2.15	0.24	18.12	0.16	3.44	5.18	3.02
YaraMila Cereal (10 kg P ha^{-1})	1.95	0.20	17.64	0.16	3.38	6.43	1.46
YaraMila Cereal (20 kg P ha^{-1})	2.07	0.21	20.30	0.17	5.55	7.31	2.76
LSD	0.45	0.05	6.59	0.03	3.03	3.07	1.77
CV (%)	12.8	12.4	18.8	11.0	57.4	26.7	41.7

The bio-fertilizer increased plant height by enhancing the nitrogen content and the rate of photosynthesis (Migahed *et al*., 2004) as the result of improvement of nitrogen fixing bacteria' activities in soil. Similar trends have been observed by Naik *et al*. (2007) working with *Adathoda vasica* (Nees crop) inoculated with *Azotobacter chroococcum*.

The present study findings showed insignificant increase in biomass yields and concentrations of N, P and Zn in ear leaf (as expression of nutrient uptake) across treatments at tasseling, as was also observed by Canbolat *et al.* (2006) and Elkoca *et al. (*2008), who reported no significant ($P<0.05$) difference in shoot biomass of barley or biomass of shoots of chickpea, respectively, when inoculant alone or fertilizer alone was used. However, the present study disagrees with the observations by Turan *et al*. (2010) who observed that inoculation of N_2-fixing strains alone and with fertilizer applications

significantly ($P<0.05$) increased total biomass yields, macro and micro nutrients of different parts of the wheat plant, and by Darzi and Hadi (2012) who observed significant effect of bio-fertilizer containing N_2 fixers on biomass yield of dill (*Anethum graveolens*).

Therefore, the results of this study indicate that the population of N_2 fixing bacteria in the product (Table 5) and multiplication in soil was low, to sufficiently fix appreciable amounts of N_2 in the soil for adequate maize growth even when the N_2 fixing bacteria were supplemented with external P at half the recommended rate (HRR) in the soil. Thus, the product tested in the present study was of low quality *vis-a-vis* the specified values. Since the present study indicate that a single inoculation of N_2-fixers had less effect on growth and biomass yields of maize plant, adoption of dual or triple combinations of N_2-fixers and P - solubilizing bacteria, with or without P as proposed by Elkoca *et al*. (2008), Turan *et al*. (2010) and Kannapiran and Ramkumar (2011) should be further tested.

4.4.8 Effects of free living N_2-fixers (Bio-soil Nitro+) on maize grain yields

The effects of Bio-soil Nitro+, free N_2- fixing microorganisms, on maize grain yields and nutrient availability in soil after harvest are presented in Table 17. The maize grain yields did not show significant ($P<0.05$) differences across treatments. The Bio-soil Nitro+ treatments, either alone or in combination with YaraMila Cereal fertilizer (at 10 kg P ha^{-1}) did not differ much amongst themselves and sometimes not with the control. These results contradict the findings recorded by Baral and Adhikari (2013) and Chattopadhyay *et al*. (2009) who respectively, observed significant ($P<0.05$) increase in yields of maize grains and teak and Indian redwood on inoculation with N_2 fixing microorganism. Similar responses on yields of rice and maize grains due to inoculation of *Azotobacter* and/or *Azospirillum* along with recommended dose of chemical N fertilizer were also reported by Vendan and Sundaram (1997) and Das and Saha (2007), respectively.

4.4.9 Effects of free living N_2-fixers (Bio-soil Nitro+) on nutrient availability in soil

At harvest, the concentrations of P in soil showed significant ($P<0.05$) differences across treatments (Table 17). Phosphorous ranged from 1.35 mg/kg (Bio-soil Nitro+ at 10 ml/0.5 kg seed) to 5.55 mg/kg (YaraMila Cereal fertilizer at 20 kg P ha^{-1}). However, the concentration of N and Zn in the soil did not show significant ($P<0.05$) difference across treatments: N ranged from 0.16 % (Control, Bio-soil Nitro+ at 5 ml/0.5 kg seed and 10 ml/0.5 kg seed, Bio-soil Nitro+ at 5 ml/0.5 kg seed + YaraMila Cereal fertilizer at 10 kg P ha^{-1} and YaraMila Cereal fertilizer at 10 kg P ha^{-1}) to 0.17 % (YaraMila Cereal fertilizer at 20 kg P ha^{-1}) and Zn varied from 5.18 mg/kg (Bio-soil Nitro+ at 5 ml/0.5 kg seed + YaraMila Cereal fertilizer at 10 kg P ha^{-1}) to 7.31 mg/kg (YaraMila Cereal fertilizer at 20 kg P ha^{-1}). The current study findings showed significant ($P<0.05$) increase of total N in soil at harvest due to N_2 fixers (Bio-soil Nitro+) inoculation as was also observed by Das and Saha (2007) who reported that inoculation of *Azotobacter* highly stimulated the availability of inorganic and organic fractions of nitrogen in the rhizosphere soils of rice which resulted in greater yield of the crop.

Therefore, the results of this study indicate that the population of N_2 fixing bacteria in the product (Table 5) and multiplication in soil was low to sufficiently fix appreciable amounts of N in the soil for adequate maize growth even when the N_2 fixing bacteria were supplemented with external P at half the recommended rate (HRR) in the soil. Thus, the product assessed in the present study was of low quality. Since the results of the present study indicate that a single inoculation of N_2-fixers had less effect on growth and biomass yields of maize, adoption of dual or triple combinations of N_2-fixers and P - solubilizing bacteria (Elkoca *et al*., 2008; Turan *et al*., 2010; Kannapiran and Ramkumar, 2011) should be tested, as already proposed (Section 4.4.7).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The fertility status (general) of the experimental soil was medium, moderately suitable for maize production.

Regarding the quality of these products, the total phosphorous and zinc contents (except nitrogen content) in Teprosyn and the microbiological populations (colony forming units, CFUs) determined for each microbiological product were lower than the values specified by the manufacturer as quoted in the products' labels.

Teprosyn

Under screenhouse conditions, Teprosyn, a seed N, P and Zn coating product, can be considered to be of low quality as it did not result in increased maize growth performance and biomass yields until when supplemented with P-fertilizer (at half recommended rate HRR). Similarly, Teprosyn did significantly ($P<0.05$) not increase either shoot P content or soil N content. However, Teproyn led to a significantly ($P<0.05$) increase in shoot N content, shoot Zn content and the concentrations of available P and Zn in the soil after harvest.

Under field experiments, the Teprosyn alone had no significantly ($P<0.05$) effect on growth performance in the early days of maize growth (21 DAP) until in the subsequent periods of plant growth at 84 DAP. Similarly, Teprosyn alone did not significantly ($P<0.05$) increase biomass yields, the concentrations of N, P and Zn in ear leaf or the concentrations of soil N, P and Zn at harvest, until when supplemented with P-fertilizer (at HRR). The Teprosyn

alone at recommended rate showed a significant ($P<0.05$) increase in grain yields, however, the Teprosyn alone at a double rate or in combination with the P-fertilizer (at HRR) resulted in significant ($P<0.05$) decrease of maize grain yields.

P-solubilizers (Bio-soil crop booster)

The P-solubilizers (Bio-soil crop booster), under screenhouse conditions neither did it significantly increase maize growth performance (plant height or other growth parameter) nor did it result into increased maize biomass yields until when supplemented with P-fertilizer (at HRR). Bio-soil crop booster, however, increased concentrations of N, P and Zn contents in maize shoot and the soil N content. On the other hand, the Bio-soil crop booster had no effect on the extractable P and Zn in the soil by harvest time.

Under field experiments, the Bio-soil crop booster had no significant effect on maize growth performance in the early days of maize growth (21 DAP) until in the subsequent periods of plant growth at 84 DAP. The bio-soil crop booster alone or in combination with P-fertilizer had no effect on biomass yields at harvest. Similarly, Bio-soil crop booster did not significantly increase the concentrations of N, P and Zn in ear leaf at tasseling. However, this product increased the concentrations of P, N and Zn in soil at harvest and the final maize grain yields.

Free living N_2-fixers (Bio-soil Nitro+)

The product of N_2-fixers (Bio-soil Nitro+), under screenhouse conditions, had no effect on some maize growth parameters but somehow increased the biomass yields. More biomass yields were obtained when P-fertilizer (at HRR) was coupled with the Bio-soil Nitro+. Of the shoot nutrients (N, P and Zn) and availability of nutrients (N, P and Zn) in soil after

harvest, the N_2-fixers (Bio-soil Nitro+) did only increase the concentration of shoot P and the concentration of available P and Zn in the soil.

Bio-soil Nitro+ under field conditions had no effect on maize growth performance, did not result in increased biomass yields or concentrations of N, P and Zn in ear leaf at tasseling and the concentrations of N and Zn in soil at harvest. Bio-soil Nitro+, however, increased the concentration of available P in the soil at harvest. Similarly, the Bio-soil Nitro+ alone or in combination with P-fertilizer did not increase maize grain yields over the control.
All these observations imply that these commercial products were not outstandingly effective.

5.2 Recommendations

In view of the results obtained herein, the following are recommended:

i. For the reason that the microbial populations in the microbiological products (Bio-soil crop booster and Bio-soil Nitro+) and the total nutrients (macro-and micro-nutrients) in the chemical product were low, TFRA should require manufacturers to strive to improve this aspect of quality.

ii. Due to the fact that the products had no documentations on guaranteed handling method or on transportation / storage conditions *vis-à-vis* shelf life, more documentations regarding guarantee analysis method and handling of these products for maintaining the products' long shelf life should be provided by the manufacturers to be able to test whether the microbial population in the products remains viable for long time when stored, or during transportation, from one environmental condition to another.

iii. For the reason that the quantities of N, P and Zn contained in Teprosyn product are too little to satisfy the plant requirements, external sources of N, P and Zn should always be supplemented to enhance plant growth and yields in P-deficient soils if TFRA allows it to be sold in the market.

iv. Due to the reason that the application of Bio-soil crop booster showed positive effects on available P and increased maize grain yields, it can be used in ISFM for improved maize productivity; however, TFRA should require manufactures to improve the quality standards before their products are accepted in the country and it should further be tested in other P-deficient soils.

v. For the reason that single inoculation of N_2-fixers had less effect on growth and biomass yields of maize, adoption of dual or triple combinations of N_2-fixers and P-solubilizing bacteria, with or without P should be further tested.

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