POTENTIAL OF PHOSPHATE SOLUBILISING BACTERIA FOR ENHANCING MINJINGU ROCK-PHOSPHATE SOLUBILITY AND PHOSPHORUS-USE EFFICIENCY FOR SUSTAINABLE MAIZE PRODUCTION IN ACID SOILS

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EXTENDED ABSTRACT

Maize is a primary staple crop for over 80% of Tanzanians, accounting for more than 70% of the country's starch requirements. Small-scale farmers produce the majority of the crop, with production unpredictable and inconsistent with agricultural requirements. Despite the fact that total maize production has increased over the last decade, owing primarily to extensification, yields per unit area remain low, averaging around 1.6 Mg/ha and translating to just 17.8 to 26.7 percent of the feasible yield. In order to fulfill the demands of the country's fast rising population, maize production must be increased. Low soil fertility, which has been related to inadequate fertilization and poor soil fertility management practices, is one of the reasons for low maize production. Soil phosphorus (P) insufficiency is one of the most critical soil fertility concerns in most Tanzanian soils, owing to the preponderance of soils with high Phosphorus-fixing capacity. Water-soluble P fertilizers (WSF) and local rock phosphates (RP) have been advised to ease the P deficit problem. While WSF are agronomically very effective, their application is limited due to their expensive cost, whereas directly applied RP are associated with delayed agronomic effectiveness and low plant phosphorus consumption efficiency.

A potent solution to the low reactivity of RP is its co-application with phosphate-solubilising microorganisms (PSMs) in an appropriate carrier or carbon source. PSMs are soil microorganisms, capable of converting the insoluble P sources into its plant available forms. When co-applied with RP, PSMs could help solubilize the insoluble P in RP. However, there is limited knowledge on PSMs characterized and selected based on their ability to solubilize local phosphate rocks while utilizing locally available, naturally occurring organic carbon sources.

To evaluate potentials of PSB-RP combination of maize productivity, the first specific objective was set to characterize phosphate solubilising bacterial (PSB). Forty (40) PSBs were previously isolated from various agricultural fields in eastern and southern highlands of Tanzania. Eleven (11) Isolates were selected, molecularly identified, screened for plant-growth promoting traits such as (1) ability to solubilize three P-sources namely ferric-, tricalcium-, and hard Minjingu rock phosphate, (2) Indole Acetic Acid (IAA) production, (3) Siderophore production, (4) Ammonia gas production, (5) Hydrogen cyanide (HCN) gas production, and (6) Activity against phytopathogenic fungi. The second objective was to examine the influence of organic carbon sources and fertilizer-grade nitrogen sources on phosphate solubilising abilities of PSBs. Three organic carbon sources namely Molasses (ML), sunflower seedcake (SC), and filter mud (FM) were initially evaluated for their physicochemical properties according their comparative potentials to enhance solubilisation of Tri-calcium phosphate (TCP) and Ferric phosphate (FP) by ten PSB isolates. In place of glucose, 20 g per liter of either ML, SSC or FM were added in Pikovskaya Agar (PVK) broth. Accordingly, a double-modified synthetic minimal media (dSMM) was prepared which contained all components of the Pikovskaya broth, except the carbon and Nitrogen source. The third objective focused on evaluating the influence of organic carbon sources and fertilizer-grade nitrogen sources on antifungal efficiencies of PSB isolates against phytopathogenic fungi. Each of the PSB isolate was grown in the modified synthetic minimal agar medium supplemented separately with either ML, SSC, FM, or glucose as the sole source of carbon. Accordingly, agar plates were spot-inoculated with different PSB isolates on one end of the 9 cm diameter Petri dish and test fungi on the other end. Antifungal efficiency (AE) of each PSB under each treatment was determined. Lastly, influence of co-application of MPR and PSBs in a molasses-based carrier was evaluated for enhancing maize yield and phosphorus use efficiency. Field experiments were laid out in randomized complete block design in

quadruplicates under two sites, Madaba and Magadu, respectively located in the southern highlands and eastern zones of Tanzania. The soils of each study area were characterized for soil fertility parameters. Experimental treatments were PSB inoculum co-applied with varying P rates (0, 20, 40, 60, and 80 kg P /ha) from MPR. Two P rates without PSB inoculum (0 and 40 kg P /ha) were set respectively as absolute and positive control. Soil available P, maize grain yield, biomass yield, phosphorus uptake, and use efficiency for each treatment were evaluate at harvest maturity.

The results indicated that six bacterial isolates had the highest homology with the known strains of the genus *Klebsiella*, two isolates related to the strains of genus *Burkholderia*, while one isolate is still unidentified. All PSB isolates were positive for ammonia production test with highest amount being 168.3 ug/ mL produced by *Klebsilla sp*.-NA19a while none of the isolates exhibited ability to produce hydrogen cyanide gas. The highest amount of IAA produced was 24.32 ug/ mL by *Klebsilla sp*.- SI-SP1. Similarly, *Klebsilla sp*-NA5 showed a significantly (P≤ 0.05) bigger solubilisation index on ferric phosphate agar plate while *K. variicola spp.*-MdG1 showed highest solubilisation index on $ZnCO_3$ agar plates. All the eleven isolates inhibited growth of *F. proliferatum* on agar plates with the highest inhibition of 47.7% by *Burkholderia sp*.-MK10.

Among organic carbon sources tested, molasses significantly ($P \leq 0.05$) enhanced solubilisation of both tri-calcium and ferric-Phosphates compared to filter mud or sunflower seedcake while urea-containing media resulted into a significantly ($P \leq 0.05$) lower amount of P-solubilized as compared to fertilizer-grade or laboratory-grade ammonium sulfate (SA) as N source. The evaluation of antifungal activity showed that the use of molasses as carbon source retains most of antifungal activity of isolates but filter mud and sunflower seedcake do not. Fertilizer-grade SA enhanced the antifungal

activity of most bacterial isolates than urea. Furthermore, the bacterial isolates under study retained their antifungal efficiencies whether yeast extract is included in the growth media.

A study on field evaluation of RP-PSB co-application revealed the following. The characterized soils of both study sites were acidic and deficient of nitrogen, phosphorus, and potassium. The data collected at harvest time indicated that soil available P of both sites responded positively to increasing P rates from Minjingu rock phosphate + phosphate solubilising bacteria (Bio-rock). Unlike Magadu soil, sole application of PSB inoculum in soils of Madaba increased soil available P to values statistically comparable to applying either sole MPR at 40 kg P /ha or 20 kg P /ha + PSB inoculum. Compared to applying sole MPR at 40 kg P /ha, maize grain yield, P uptake and phosphorus use efficiency (PUE) were improved by inoculation of PSB combined with either 20 kg P /ha or 40 kg P /ha. It is recommended, for enhanced maize productivity and efficient use of phosphorus, co-application of PSB inoculum and Minjingu RP at 20 kg P /ha for the soils of Madaba and 40 kg P /ha for the soils of Magadu.

DECLARATION

I, Damiano Raphael Kwaslema, 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 been submitted nor being concurrently submitted in any other institution for degree award.

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Dr. Hamisi J. Tindwa Date

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Dr. Nyambilila Amuri Date

(Supervisor)

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Al	Aluminium
BD	Bulk Density
BS	Base Saturation
C: N	Carbon to Nitrogen ratio
Ca	Calcium
Ca^{2+}	Calcium ion
CEC	Cation Exchange Capacity
CEC_{clay}	Cation Exchange Capacity of clay
CEC_{soil}	Cation Exchange Capacity of soil
Cl-	Chloride ion
cm	centimeters
$cmolkg^{-1}$	centimole (+) per kilogram
CO_3^{2-}	Carbonate ion
$dS\ ^{m-1}$	deciSiemens per meter
EC	Electrical conductivity
EC1:2.5	Electrical conductivity of the soil to water suspension
ECa	Electrical conductivity measured on the bulk soil
ECe	Electrical conductivity of the saturated paste extract
ECw	Electrical Conductivity of water
ESP	Exchangeable Sodium Percentage
et al.	and others
FAO	Food and Agriculture Organization
Fe	Iron
g	gram

GDP	Gross Domestic Product
ha	hectare
HCO^{3-}	Bicarbonate ion
IDW	Inverse Distance Weighting
IUSS	International Union of Soil Sciences
K^{+}	Potassium ion
kg	kilogram
m.a.s.l.	metres above sea level
Mg	Megagram
mm	millimetre
MPa	megaPascals
MSc	Master of Science
N	Nitrogen
Na+	Sodium ion
NO3−	Nitrate ion
O	Oxygen
^{o}C	Celsius centigrade
OC	Organic carbon
OM	Organic Matter
P	Phosphorous
pH	Potential hydrogen
PSA	Particle Size Analysis
r	Correlation coefficient
R^2	Coefficient of determination
S	Sulfur
SO_4^{2-}	Sulphate ion

SUA	Sokoine University of Agriculture
t	tone
TN	Total Nitrogen
USDA	United States Department of Agriculture
XRF	X-Ray Fluorescence

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Phosphorus (P) availability for plant growth is a significant barrier to crop production in many parts of Tanzania, owing mostly to the occurrence of soils with high P-fixing capacity and, at times, low intrinsic soil P levels (Szilas, 2002; Okalebo *et al.,* 2006). This issue has been repeatedly noted in Tanzania's extensively weathered acid soils in the warm to sub-humid tropics of the southern highlands and eastern zones (Msanya *et al.,* 2004; Szilas *et al.,* 2007; Senkoro *et al.,* 2017; Mtama, 2018; Mohamed *et al.,* 2021).

In these soils, phosphate ions are adsorbed on to the surfaces of kaolinitic silicate clays, hydroxyoxides of and/or soluble aluminum, iron, and/or manganese ions which make P un-available for plant uptake (Weil and Brady, 2017; Sanchez’s, 2019). Furthermore, inadequate phosphorus recovery from applied fertilizers is a prevalent situation in soils with high P fixing capabilities since most of the applied P is firmly held by the soil matrix and becomes inaccessible for plant uptake (Balemi and Negisho, 2012; Weil and Brady, 2017; Sanchez's, 2019).

This necessitates the extended application of P to soils. Water-soluble P fertilizer requirements and high costs make it prohibitive for resource-limited farmers. Direct application of locally available rock phosphate (RP), particularly Minjingu Rock Phosphate (MPR), has been a low-cost alternative under indicated favorable conditions such as low soil pH, heavy rainfall, and phosphorus and calcium deficits (Szilas *et al.,* 2007). Direct application of RP, on the other hand, is associated with delayed agronomic effectiveness for up to a third year following application (Szilas *et al.,* 2007). To farmers who are producing annual crops, such as most staple crops, delayed response of crops to

directly applied local RP is a major reason which limit their widespread use (IFDC, 2018). Recently, low Phosphorus Use Efficiencies (PUE) of the directly applied MPR has been reported (Mwangi *et al.,* 2020). Taking into consideration of the finite nature of rock phosphate reserves, there is a need of improving the use efficiency of this resource (Roberts and Johnston, 2015). Therefore, it is important to develop innovative, low-cost technologies that could sustainably intensify crop production by increasing soil phosphorus availability with minimal use of rock phosphate reserves (Taddese, 2019).

A potent solution to the low reactivity of RP is inclusion of Phosphate-Solubilising Microorganisms (PSMs) in the application package (Kalayu, 2019). PSM are soil microorganisms which can increase the solubility of otherwise insoluble P sources such as tricalcium phosphate, rock phosphates, or fixed P in the soil. The principal mechanism employed by these microbes is the production of organic acids which through chelation, increases P bio-availability (Khan *et al.*, 2014). The increased solubility of local rock phosphates (minjingu RP and Panda Hill RP) by PSM from local environment under laboratory-based experiments has been reported (Simfukwe and Tindwa, 2018). Furthermore, a study by Mwangi *et al.* (2020) reported that treating the minjingu rock phosphate with lemon juice, which principally have citric acid, could increase its solubility and phosphorus use efficiency by carrot plants as compared to directly applied MPR. Since citric acid is among the organic acids produced by PSMs, there is no doubt that co-application of MPR and PSMs in a suitable formulation will increase RP solubility, P uptake and P use efficiency by plants. However, there is limited knowledge on PSMs characterized and selected based on their ability to solubilize local rock phosphates while using locally available organic carbon sources.

1.1.1 Phosphorus (P) as a plant nutrient

Phosphorus is one of the seventeen essential plant nutrients and is needed in large quantities for optimal plant growth and production (Taiz *et al.,* 2014). Percentage P composition in healthy plant leaves ranges from 0.2 % to 0.4 %. P accounts for about 0.2 % to 0.8 % of plant's total dry weight (Balemi and Negisho, 2012; Sharma *et al.,* 2013; Weil and Brady, 2017). It is essential in every aspect of plant growth, from the molecular level to many physiological and biochemical process. It is at the center of plant metabolism (Marschner and Marschner, 2012). Phosphorus is an integral part of the most important energy compound, ATP, nucleic acids (DNA and RNA), co-enzymes, and phospholipids in plants (Barker and Pilbeam, 2007; Shen *et al.,* 2011). Moreover, phosphorus plays various indispensable roles in plants physiological and biochemical activities such as cell division, photosynthesis, respiration, and energy transfer (Marschner and Marschner, 2012; Kalayu, 2019). Adequate supply of P is essentially important for enhancing all aspects of plant growth including root formation and elongation, proper plant maturation and stress mitigation flowering, seed production, fruiting, and plant disease resistance (Vance *et al.,* 2003; Walpola *et al.,* 2012; Satyaprakash *et al.,* 2017; Kalayu, 2019). In leguminous plants, P enhances biological nitrogen fixation (Weil and Brady, 2017; Zhu *et al.,* 2017). Furthermore, P influences plant protein contents and starch quality and quantity (Mengel *et al.,* 2001; Fernandes *et al.*, 2015). Inadequate supply of phosphorus in plants typically results into reduced plant growth and productivity (Marschner, 2002). Generally, P deficiency in plants is associated with stunting, thin-stems, root growth reduction, purplish to purplish-red coloration on the margin of older leaves, and in case of severe deficiency, burning and dearth of leaves (P. Kumar, 2013). Finally, P-deficient plants form small ears with few grains and therefore ending up with poor yield (Mengel *et al.,* 2001).

1.1.2 Chemical forms and bio-availability of phosphorus in soils

In most soils, a small part of total phosphorus exists as water-soluble anions, namely, dihydrogen phosphate ($H_2PO_4^-$) and monohydrogen phosphate (HPO_4^{2-}) in soil solution. Amounts of plant-available P species in most soils rarely exceed 0.001 % of soil's total P. Their relative abundances are determined by soil pH, in which the $H_2PO_4^{2-}$ predominate as soil pH decreases (< pH 7) while HPO_4^{2-} increases as pH increases (> pH 7.2). A substantial portion of total phosphorus exists as P species bound on organic compounds or minerals. Soil organic P are found in plant residues, composts and microbial tissues. Organic compounds that hold most of soil P include inositol phosphate, which make up to 60 % of total soil organic phosphorus. Nucleic acids and phospholipids which together account only about 1 to 2 % of total soil organic P. However, there are other unidentified specific soil organic compounds that hold phosphorus. Through the action of soil microorganisms which decompose organic compounds in the soils, P held in organic compounds get released to the soil solution (mineralized) which may then be taken up by plants roots or perhaps fixed on the surfaces of clays, Ca, Fe, and/or Al minerals. However, the decomposition of P-containing organic matter in the soil may not necessarily lead to net P mineralization, particularly when the C: P ratio of the decomposing material exceeds 300:1. Unless the C: P ratio of the decomposing material is less than 200:1, microorganisms incorporate all the P into their cell biomass.

The inorganic P in the soils is held in the minerals containing either calcium or iron and aluminum ions. The relative abundance and solubility of phosphatic minerals in the soil is primarily dependent on soil reaction. Calcium-bound P minerals occur in larger quantities when the soil pH increases (neutral to alkaline soils) whereas their solubility increase in the as the soil pH decreases. Examples of calcium phosphate compounds in the soils include fluorapatite, carbonate apatite, hydroxyl apatite, oxyapatite, tricalcium phosphate,

octacalcium phosphate, dicalcium phosphate, and monicalcium phosphate. Of all the calcium-bounded P minerals, apatite is the least soluble in soils. The inverse is true for ferric- and/or aluminum-bound P minerals which are predominant in acid soils and more soluble and unstable under higher pH conditions. The most common minerals of this group are strengite ($FePO_4.H_2O$) and variscite ($AlPO_4.H_2O$).

1.1.3 Mobility of inorganic phosphorus in soils

Mobility of soil phosphorus is generally very low compared to other essential nutrient elements like nitrogen (Batjes, 2011). Primary reasons to low P mobility in soils is due to low solubility of P containing minerals and/or sorption of phosphate anions onto surfaces of clays and Ca, Fe, and/or Al (Tan, 2011). The retention of phosphate anions results into reduced surface exchange for phosphate ions compared to that of other nutrients (Sposito, 2008). However, the retention extent of inorganic P species in the soil is highly dependent on soil mineralogy. P retention is generally higher for the soils with high contents of kaolinitic clays, amorphous iron and/or aluminium ions, hydroxioxides of iron and/or aluminium, and calcium ions. This is true for the highly weathered soils such as oxisols and ultisols, and the soils developed out of volcanic ash (andisols) which together account for a considerable proportion of arable land in Tanzania (Mlingano Agricultural Research Institute, 2006). Considering the tropical soils in general, about 24 % of the land is having the soils with high P sorption capacities (Sanchez's, 2019). According to Juo and Franzluebbers (2003), the problem of phosphorus deficiencies in soils is primarily due to prevalence of soils with remarkably high P fixing soils rather than absolute lower P levels. Generally, the processes responsible for phosphorus retention in the soils are categorized as follow;

1.1.3.1 Adsorption onto the surfaces of hydrous oxides and silicate clays

Surface reactions of inorganic phosphate ions ($H_2PO_4^{2-}$) with kaolinitic clays (1:1 silicate clays) and the insoluble oxides of iron, aluminium, and manganese are among the important mechanism for P fixation in acid soils. According to Borggaard *et al.* (2004), phosphate fixation capacities of hydrous oxides depend on their crystallization levels; being higher in poorly crystalline oxides since they have exceptionally large specific surface areas. Furthermore, the relative contribution of 1:1 silicate clays and hydrous oxides varies along the soil pH range. P fixation by kaolinitic clays is believed to take place over wide range of pH values while P fixation by hydrous oxides become prominent when the soil pH is around 5.5 to 7 (Asomaning, 2020).

1.1.3.2 Precipitation by iron, aluminum, and manganese ions

In strongly acid soils where the concentrations of soluble aluminium, iron, and manganese ions are typically high, most of soluble P anions become precipitated, resulting into formation of insoluble hydroxyl-phosphate precipitates (Juo and Franzluebbers, 2003). When soluble phosphate fertilizer is added to strongly acidic soils with higher concentrations of Al^{3+} and Fe^{3+} ions, precipitation is the first process that occurs (Sanchez's, 2019). According to Weil and Brady (2017), the time lapse after the occurrence of precipitation reaction is important, since the freshly precipitated P is somehow available for plant uptake as compared to older precipitates.

1.1.3.3 Absorption into oxides and hydroxyoxides matrices in the soil

Phosphate absorption refers to the penetration or occlusion of phosphate anions into the oxides of iron and aluminium (Juo and Franzluebbers, 2003). Typically, absorption of P into matrices of oxides and hydroxyoxides is a slow process which could take up to two years to reach the equilibrium following first surface adsorption of P. It is common in well-aggregated soils having high iron and aluminium contents (Sanchez's, 2019).

1.1.4 Phosphorus deficiency as a threat to crop production and food security in Tanzania

According to Sanchez's (2019), the extreme deprivation of P for plant uptake in soils of some tropical regions of Africa has caused farmers to engage with improving soluble P in soil even before improving availability of N in the soil. About 1.7 billion hectares of land, which constitute 45 % of land in tropical region, are predominantly phosphorus-limited (Sanchez's, 2019). In most soils of Tanzania, phosphorus is the second most limiting nutrient, only next to nitrogen (N) (Szilas, 2002; Msanya *et al.*, 2004; Senkoro *et al.*, 2017; Mtama, 2018). Pedological characterization of some soils in the hot-humid and sub-humid areas of Tanzania reveled that most of the soils have low available P contents and have appreciable P fixing capacities (Szilas, 2002). The recent soil acidity data for Tanzania show that, about 4.7 million hectares of arable lands have acidic soils with pH<5.6, and therefore likely to be P deficient (African Soil information System AfSIS). Therefore, production of staple crops such as maize and thus food security in a face of country's growing population is laid on the line by low soil P, among other factors. However, Szilas *et al.* (2007), noticed unsatisfactory response of maize yield to application of P fertilizers and the observation was ascribed to possible dominance of constraints other than P deficiency, such as moisture stress, soil acidity, and other nutrient deficiencies. Therefore, based on law of minimum, these other constraints should, along with improving availability of P to plants, be corrected to achieve optimal plant response to applied fertilizer (Marschner and Marschner, 2012). Weil and Brady (2017), stated clearly that unless otherwise soil P deficiencies are corrected, food security in sub-Saharan Africa, Tanzania inclusive, is less likely to be attained.

1.1.5 Existing phosphorus management strategies

1.1.5.1 The use of water-soluble phosphatic fertilizers

To address the challenge of phosphorus deficiencies, the application of plant available P is essential (Szilas *et al.,* 2007). The most promising option in terms of effectiveness on promoting plant growth and crop yield is the use of water-soluble P fertilizers such as Diammonium Phosphate (DAP), Single Super Phosphate and Triple Super Phosphate (Senkoro *et al.,* 2017; IFDC, 2018). However, the use of water-soluble fertilizer by smallholder farmers in Tanzania is limited by high prices and poor accessibility (Benson *et al.,* 2012; IFDC, 2012). According to TFRA (2020), indicative price of 50 kg bag di-ammonium phosphate (DAP) ranged from 63 246 Tsh to 70 957 Tsh for all agricultural zones. These prices are beyond the affordability scale of small-scale farmer in Tanzania. In addition to industrial manufacturing costs, the high fertilizer prices are due to the costs of offloading, storing, transporting and distributing which adds up to 30-40 percent of the final farm-gate price (IFDC, 2012; Cameron *et al.,* 2017).

1.1.5.2 Direct application of locally available rock phosphates

One other approach in addressing P deficiency in soils involves the direct application of phosphate rocks which are locally available in some areas of Tanzanian (Szilas, 2002). Although, it is indicated that, the restoration of soil P-status can only be achieved using phosphate fertilizers, several studies have shown the feasibility of using rock phosphate for direct application (Msolla *et al.,* 2005; C. Szilas *et al.,* 2007). However, the direct application of rock phosphates is again limited by its sparse solubility and its dependency on very acidic conditions which renders it being not very effective in non-acid soils (Szilas *et al.,* 2007). Msolla *et al.* (2005), conducted a study on the direct application of Minjingu phosphate rocks in comparison with TSP in some acidic soils, and the results were not promising for the instant nutrient supply (early seasons) to the maize crop but

the relative agronomic effectiveness (RAE) of MPRs increased from 50 – 70% in the first year to 80 – 95% in year three. The recommendation from this study were to improve the poor performance of MPR for the first year of application (Msolla *et al.,* 2005). A study by Kalala (2011), have also shown the performance of Minjingu PR in maize plant growth as being increasing over time due to its high residue property. To this end, there is a need to find a way of improving the early season's agronomic effectiveness of these locally available Phosphate Rocks in Tanzania. Recent findings by Mwangi *et al.* (2020), reported low Phosphorus Use Efficiencies (PUE) of the directly applied MPR in humic andosols and orthic acrisols soils in Kenya. Over again, improvement of PUE from the applied RP is very essential due to three major reasons highlighted by Syers *et al.* (2008). First, unlike nitrogen, which can infinitely be fixed by soil microorganisms or synthesized from atmosphere through Haber-Bosch process, phosphate reserves are finite and non-renewable. Therefore, it should be sustainably used. Secondly, to meet the requirement for improving soil P status for optimal crop growth and production. Thirdly, to reduce the risk of environmental pollution, particularly eutrophication of surface and ground water which might result from un-efficiently utilized excess phosphorus from agriculture soils.

1.1.6 Rock phosphates (RP) reserves in Tanzania

Phosphate rocks are any geologic materials containing phosphate components between 5 and 50% by volume that can be used as raw material for the phosphate fertilizer industry or as a directly applied phosphorus source in agriculture (Chien *et al.,* 1977). Phosphate rocks are primarily composed of the apatite group in association with a wide assortment of accessory minerals, mainly fluorides, carbonates, clays, quartz, silicates and metal oxides. As with the rest of the world, Tanzania has four categories of phosphate rock deposits, namely; (i) igneous phosphates, associated with carbonatites, (ii) lacustrine

phosphates in rift valley sediments, (iii) metamorphic phosphates, (iv) guano deposits (Van Straaten, 2000). In Tanzania there are several rock phosphates deposits, the minjingu rock phosphate being the most exploited for soil fertilization, directly or following industrial acidulation process (Appleton, 2002; Van Straaten, 2002). Another deposit of PR that could potentially be used to boost agricultural production and enhance food security is Panda Hill PR (Msolla *et al.,* 2005).

In addition to Minjingu and Panda Hill, other rock phosphate deposits present in Tanzania are the Sangu-Ikola located at Lake Tanganyika, Ngualla in northwest of Mbeya region, the Serengi Hill, Songwe Scarp, Mbalizi and Nachendezwaya carbonatites in Mbeya (Strateen, 2002).

1.1.6.1 Minjingu phosphate rocks

Minjingu Hill is a most exploited rock phosphate reserve in Tanzania due its promising agronomic effectiveness. The deposit was discovered in 1956 and it is believed to have formed during Pleistocene because of biogenic accumulation from large bird colony that rested/ nested on small inselberg located near lake Manyara, Northern Tanzania (Szilas, 2002; Van Straaten, 2002). Two types of minjingu phosphate ores are known; the soft ore with about 22-25% P_2O_5, and another having 24% P_2O_5 (Van Straaten, 2002). Based on detailed agronomic evaluation, the Minjingu PR has been recommended for direct application in acidic soil soils with low P and Ca contents, particularly in areas experiencing high annual rainfall (Szilas *et al.,* 2007). In 1980s to early 1990s the minjingu rock phosphate was used for acidulation with average production of about 20 000 Mg/year (Appleton, 2002; Van Straaten, 2002). Currently, there is about 50 000 000 to 100 000 000 Megagram of minjingu phosphate deposit with the projected lifespan of 50 - 100 years based on current exploitation rates (Minjingu Mines and Fertilizer

Company, 2020).

1.1.7 Potential for enhancing rock phosphate solubility and PUE

1.1.7.1 Rock P Bio-fertilizers; incorporating phosphate rock and phosphate solubilising microorganisms

In present scenario, there has been a focus toward the use of bio-fertilizers in agriculture which are environmentally friendly, sustainable and cost effective when rationally formulated (Khan *et al.,* 2014; Giro *et al.,* 2016; Dominguez-benetton *et al.,* 2018; Kitam *et al.,* 2019). Bio-fertilizers are defined as the formulations containing living microorganisms or latent cells having the potential of colonizing roots of crops plants and promote the growth by improving nutrients availability and acquisition (Hassan and Bano, 2016). Phosphate based bio-fertilizers are made by combining the phosphate rocks, Phosphate Solubilising Organisms (PSMs) and appropriate carrier materials (Khan *et al.,* 2014; Giro *et al.,* 2016). The PSMs in the mixture could solubilize the inorganic P minerals such as those in RP through various mechanisms including the release of organic acids (acetate, lactate, oxalate, tartarate, succinate, citrate, gluconate, ketogluconate, glycolate, etc.), lowering the pH of the micro-environment and chelation, while the organic carbon source will sustain the life of these microorganisms (Alori and Babalola, 2018). According to Dash *et al.* (2017), application of phosphatic biofertilizers is associated not only with the enhanced phosphorus nutrition but also increased uptake of other nutrients and water, root development, vegetative growth and nitrogen fixation. Also, due to their tremendous positive influence on plant's nutrient uptake ability, PSMs can effectively enhance plant's nutrient use efficiency of the applied fertilizers (Malusá and Ciesielska, 2012). The requirements for developing phosphatic biofertilizers can be perfectly met in Tanzania's agricultural

environment, mainly because the phosphate rock deposits are present and the possibilities of finding the efficient PSM strains is higher (Van Straaten, 2000; Simfukwe and Tindwa, 2018). Therefore, developing the potable bio-fertilizer packages through rational formulation may be the promising approach to cost reduction (Orhan *et al.*, 2006).

1.1.7.2 Phosphates Solubilising Microorganisms (PSMs)

Phosphate solubilising microorganisms (PSM) are the soil microorganisms, mostly rhizospheric, capable of solubilising the insoluble inorganic phosphates like tricalcium phosphate, rock phosphate, bone meal and fixed soil P into plant available forms (Nisha, *et al.*, 2014). PSM can improve the overall plant perfomance (Nisha *et al.*, 2014). According to Kudoyarova *et al.* (2015), plant growth promotion by these rhizospheric microorganisms is attributed to three microbial properties; (1) their ability to increase bio-availability of mineral plant nutrients such as conversion of insoluble P (rock phosphates and fixed P in the soils) to plant-available forms, nitrogen fixation, zinc, and potassium solubilisation, (2) synthesis of plant growth regulators (phytohormones) such as indole-3-acetic acid (IAA) and cytokinins, and (3) suppressing plant pathogenic organisms and increasing plant's resistance to pathogens (Kannahi and Senbagam, 2014; Khan *et al.*, 2014). Phosphate solubilising abilities of bacterial isolates have been widely reported in the literature. According to Arjjumend *et al.* (2017), bacterial isolate with average performance can solubilize 20-30% of insoluble phosphate and crop yield may increase by 10 to 20% when the conditions are favourable. The PSMs solubilize the soil phosphates by producing organic acids which lead to (1) lowering the pH in rhizosphere or (2) chelation of the cations responsible for precipitation of P, (3) competing with P for sorption sites on the soil, and (4) forming soluble complexes with the metal ions associated with insoluble P compounds (phosphates of Ca, Al, and Fe). Several species of bacteria, fungi and actinomycetes have been reported to solubise the insoluble phosphate. These include the species of *Pseudomonas, Klebsiella, Bacillus, Rhizobium,*

Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aereobacter, Aspergillus, Flavobacterium and Erwinia (Khan *et al.,* 2014).

In Tanzania, Simfukwe and Tindwa (2018) reported on the rock phosphate solubilising potentials of the PSM isolates from Minjingu and Panda hill; species of *Aspergillus* (fungi), *Stenotrophomonas*, and *Bacillus* (bacteria) were the best performers. The same study concluded on the possibility of field application of phosphate solubilising microbes for enhancing crop production. Given their positive influence on plant and soil health, phosphate solubilising microorganisms can be incorporated into bio-fertilizer (microbial inoculants) as the biological alternative to compensate agro chemicals and to sustain environment friendly crop production (Saharan and Nehra, 2011). Several studies have shown the success of PSMs bio-fertilizer in crop growth promotion under specific agricultural settings. Mondal (2017) conducted a field trial with bio-fertilizer made of PSB strains and soaked into maize seeds and observed a marked difference in soil fertility, increased plant height, number of tassels observed in the maize plant. In the trial with wheat crop, Hassan and Bano (2016) formulated the *Bacillus cereus* and *Pseudomonas moraviensis* strains with maize straws and sugarcane husk into a bio-fertilizer which increased plant height and fresh weight by 18-30% and protein, proline, sugar contents and antioxidant activities by 25-40%.

1.1.7.3 Other microbial-mechanisms for plant growth-promotion

(a) Phytohormones production

Apart from availing phosphorus to plant roots, some PSBs also exhibit other multifunctional properties that benefit plants such as synthesis of phytohormones (indole-3-acetic acid (IAA), cytokinins, ethylene and gibberellic acid), which could positively influence plant growth (Alori *et al.,* 2017). Among others, bacterial-derived IAA has been mostly studied and it is reported that more than eighty percent of soil bacteria are capable

of synthesising IAA through tryptophan dependent pathway (Khan *et al.,* 2014). Furthermore, some rhizospheric bacterial strains such as *Burkholderia pyrrocinia* JK-SH007 are known to synthesis IAA through tryptophan-independent pathways synthesis mechanisms (Liu *et al.,* 2019). Plant growth-promotion by IAA derived from rhizospheric soil bacteria such as those from genera Enterobacter, Pseudomonas, Azospirillum, Xanthomonas and Rhizobium is known (Primo *et al.,* 2015). Generally, bacterial-derived IAA plays a very significant role in rhizobacteria-plant interactions thereby influencing several aspects of plant growth and development including stimulation of plant seeds and tuber germination, root formation, xylem development, and plants defense responses to pathogens and abiotic stresses (Ahemad and Kibret, 2014).

(b) Production of antimicrobials

Antimicrobials are secondary metabolites produced by microorganisms which can kill or antagonize the growth of other microbes (Chandra and Kumar, 2017). Microorganisms produce antimicrobial agents in response to the need to adapt specific the environmental conditions or to protect themselves from enemies (Vicente *et al.,* 2003). Some Phosphate-solubilising bacteria are also known to produce plant protectant chemicals against pathogens. These include, Gram-negative *P. fluorescens, P. aeruginosa* and *Chromobacterium violaceum,* which secrete antibiotics and provide protection to plants against soil-borne fungi (Khan *et al.,* 2014). Since fungal diseases are more significant in crop plants (Agrios, 2005), PSMs capable of producing antifungal agents are of interest in agricultural settings. Antifungal agents are the types of antimicrobial agents that destroys or prevents the growth of fungi (Vicente *et al.,* 2003). The mechanisms of action of these antifungal agents vary considerably from one bioactive substance to another. However, most of them inhibit synthesis of cell walls, as well as protein and nucleic acids (Gonelimali *et al.,* 2018). The use of these microbes-derived antifungal agents against

fungal diseases in crop plants has been reported as eco-friendly and cost-effective for sustainable agriculture (Kim and Hwang, 2007).

(c) Siderophore production

Siderophores are the iron binding low molecular weight proteins that scavenge iron from the iron stressed environment and make it available to the microbial cell and excess to plant roots (Kannahi and Senbagam, 2014). Iron is essential for almost all life for processes such as respiration and DNA synthesis. Despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron in many environments such as the soil is limited by the very low solubility of the Fe^{3+}. Most of agricultural soil conditions are aerobic and therefore iron exists in its oxidized forms, mainly as ferric oxides, ferric hydroxides, and/or ferric hydroxyoxides, all being less bio-available (DeLaune and Reddy, 2008). Production of siderophore compounds by soil microorganisms is therefore an adaptive mechanism to suffice their iron requirements (Saharan and Nehra, 2011). Siderophores are responsible for solubilising iron from various minerals and organic compound through chelation processes which lead to formation of soluble complexes (Khan *et al.,* 2014).

(d) Ammonia production

The role of rhizospheric microbe-derived ammonia ranges from its direct supply of plant-available form of nitrogen to its antagonistic effect on phytopathogens invasion (Mahmud *et al.,* 2021). Microbe-derived ammonia has been reported to cause significant nitrogen accumulation and enhanced plant root growth, and increased biomass accumulation (Dutta and Thakur, 2017). However, Weise *et al.* (2013), reported a contrasting result where by higher levels of bacterial ammonia caused a significant inhibition of plant growth. In a Petri dish-based experiment with *Arabidopsis thaliana*, the ammonia produced by *Serratia odorifera* 4Rx13 resulted into media pH elevation and concomitant plant growth reduction (Weise *et al.,* 2013).

(e) Production of hydrogen cyanide (HCN) gas

Some phosphate solubilising bacteria are capable of producing hydrogen cyanide, a secondary metabolite which is ecologically important and it provides a selective advantage to the producing strains (Khan *et al.,* 2014). Several phosphates solubilising bacterial species such as *Pseudomonas spp.*, *Bacillus spp.* and *Rhizobium spp.* produce hydrogen cyanide gas through oxidative decarboxylation of glycine, glutamate or methionine (Khan *et al.,* 2014). However, according to Rijavec and Lapanje (2016), the number of HCN-producing rhizobacteria is limited. HCN produced by these microorganisms acts not only as a bio-control against phyto-pathogens but it also increases solubility of otherwise insoluble phosphate compounds through substitution of phosphate anions by CN^- anions (Rijavec and Lapanje, 2016).

(f) Exopolysaccharides production (EPS)

Microbial exopolysaccharides (EPSs) are the extracellular polysaccharides synthesized by some bacterial and fungal species and released to the outer cell membrane or on the outer side of their cell walls to extracellular medium (Khan *et al.,* 2014). Typically, the trait for EPS production is associated with some abiotic stress such as salinity, drought, or heavy metal contaminations in the habitats of microorganisms (Singh, 2013). According to Yi et *al.* (2008), microbial-exopolysaccharides are also responsible for mediating phosphorus solubilisation. The EPS production is associated with bacterial ability to solubilize P from their insoluble sources such as tricalcium phosphate where by the EPS producing bacterial strains *Azotobacter sp. AzHy*-510, *Enterobacter sp.* EnHy-401, *Arthrobacter sp. ArHy*-505 had higher P solubilising abilities than non-EPS-producing strains under the study (Yi *et al.,* 2008).

1.1.8 Carbon and energy sources for phosphate solubilising microorganisms

Essentially, the survival of microorganisms in the soils is limited by carbon and energy (Hobbie and Hobbie, 2013). Heterotrophic microorganisms, including phosphate solubilising bacteria need organic resources as source of carbon and energy to sustain their live for extended periods enough to unlock the fixed phosphates (Rosenberg *et al.,* 2006). Carbon is the building block of organic molecules required for the formation of new microbial cell material and it makes up to 50% of the bacterial biomass while the energy is used for growth and metabolism. In fact, all the organic substances in microorganisms contain carbon in some form, whether they are proteins, fats, carbohydrates, or lipids (Rosenberg *et al.,* 2006). Therefore, for the phosphate solubilisation to take place in the soil there must be an adequate supply of the soil organic matter. However, in most soils, especially the highly weathered acidic soils of the tropics, the supply of the organic resource is extremely low (Juo and Franzluebbers, 2003). This impart a challenge to direct application of phosphate solubilising microbes to these soils since their survival and activities will be limited by the low supply of carbon resources. Therefore, during the formulation of the microbial inoculants, the inert materials are required as carbon sources and as carrier to keep the organisms alive and aid in application (Gaskin *et al.,* 2013).

To this end various carbon sources have been studied for this purpose but unfortunately most of these studies have concentrated on the conventional laboratory chemicals like glucose, lactose, maltose, sucrose and fructose as carbon sources for enhancing phosphate solubilisation (Costa *et al.,* 2002; Barroso *et al.,* 2006; Srividya *et al.,* 2009; Sagervanshi *et al.,* 2012; Ahemad and kibret, 2014; Mardad *et al.,* 2014; Bhakthavatchalu and Shivakumar, 2018). However, the locally available organic resources like agriculture waste materials can potentially be used in low-cost bio-fertilizer development (Stamenković *et al.,* 2018).

The findings from the study conducted by Giro *et al.* (2016) suggest that the application of both PSM and humic acids with rock phosphate may be a suitable method for reduction of soluble P fertilizer demands without compromising maize plant yields. A wheat-based study by Mukhtar *et al.* (2017) Have indicated that bacterial isolates having plant beneficial traits such as P solubilisation are more promising candidates as bio-fertilizer when used with biogas sludge and soil as the carrier materials (carbon source). Sugarcane wastes like molasses have been tested to substitute glucose in a conventional PVK media and it the ability of the PSM to solubilize P was retained (Haile *et al.,* 2016).

The carriers are the means of delivering viable microbes from the factory to the field. According to (Khan *et al.,* 2014), a good carrier should support growth and survival of microbes, high in organic matter, non-toxic, mixable, packageable, inexpensive and locally available. The locally available organic resources such as sugarcane molasses (Bakari, 2018), can be used as the carrier.

1.1.8.1 Locally available organic carbon sources

(a) Sugarcane molasses

Sugarcane molasses is the dark-colored and viscous syrups that remain as a by-product of sugarcane crystallization when no more sugar can be economically extracted (Dahot and Simair, 2013). According to Kishimba (2000), the molasses yield in Tanzania was about 40 000 megagram per annual. Molasses yields are reported to increase simultenously with the increased sugar production, about 2.5%–3% of the milled cane (Grumezescu and Holban, 2019). Due to the reported potential of increasing sugarcabe production in the country (Massawe and Mhoro, 2017), the yields of sugarcan by-products will thereby simulteniously increase. Molasses has high content of dry matter, consisting of 47-48% sugars (sucrose, glucose, fructose), amino acids, fatty acids, and mineral elements

(potassium, sodium, calcium, magnesium, iron, and copper) which could stimulate the bacterial growth (Nikodinovic-Runic *et al.,* 2013; Šarić *et al.,* 2016; Kobra *et al.,* 2016). Molasses have been reported to enhance phosphate solubilisation when incorporated in to the media of phosphate solubising fungi (*Aspergillus nigre*) and some bacteria isolates (C. Barroso and Nahas, 2007; Kobra *et al.,* 2016).

(b) Sugarcane filter-cake or the press mud

Sugarcane filter-cake or the press mud is another sugarcane by-product produced when the sugar mud is separated from the crush. During clarification and filtration of sugar juice, press mud is produced as a waste product (Ivanova *et al.,* 2016). According to Devia-Orjuela *et al.* (2019), about one to seven megagram of press mud is produced per every hundred megagram of sugarcane crushed. Despite of its highly variable chemical compositions, press mud has been often reported to contain high contents of organic compounds (cellulose, hemicelluloses, protein, wax, etc.) and the appreciable amounts of macro- and micro- nutrients (Kumar *et al.,* 2017). Due to its nutrient composition, sugarcane filter cake is reported to improve soil health and general microbial activities of the soils, when used as an amendment composite (Kumar *et al.,* 2017).

(c) Sunflower seedcake

Sunflower seedcake is a by-product of sunflower oil seed processing, which contain an appreciable amount of organic matter and nutrient elements. It has a narrow C/N ratio of less than 18:1 which imply an excellent quality for microbial decomposition to take place and release nutrients easily (Mbewe, 2015). According to Xingfei (2015), Tanzania is one of the top ten sunflower oilseed producers in the world. The annual average production of sunflower oilseed was 2 876 333.3 megagram in past five years (Kombe *et al.,* 2017). The seedcake is reported to be a potential organic fertilizer for improving soil health due its organic matter contents and nutrients it supplies (Mbewe, 2015).

1.1.9 Potentials for increasing bio-availability of fixed soil phosphorus reserve

Improving the availability of native soil P reserves for plant uptake is among the avenues that should be looked at for enhanced crop productivity. Despite of low available P in most soils, the total soil phosphorus in top 50 cm is relatively sufficient; ranging from less than 500 kg /ha to 10,000 kg /ha (Weil and Brady, 2017). Globally, it has been shown that soil total P are highest in some young little-weathered soils, particularly found in deserts and mountainous regions, whereas the highly weathered soils of humid tropics are generally having low total soil P (Juo and Franzluebbers, 2003). In a face of depleting soil P reserves mainly due to unhealthy soil fertility management practices, and the possible depletion of rock phosphate reserves, there is a need for sustainably using P resource for livelihood of future generations (Mitra, 2017). According to López-Arredondo *et al.* (2017), the problem of phosphorus in soils is further complicated by extremely low PUE from the applied phosphatic fertilizers where by only 20 % to 30 % are annually recovered in crops.

It has been reported based on theoretical estimation in soils with high total P that, if the existing soil P reserve is sustainably availed for plant uptake, it could sustain crop production for over hundred years (IPNI, 2019). Therefore, there is a need for innovative technologies that could increase crop productivity from sustainably enhanced soil P availability, PUE, and minimized exploitation of rock phosphate reserves (Taddese, 2019).

The most promising strategy is currently the use of phosphatic biofertilizers containing soil microorganisms that could enhance bio-availability of fixed soil P and the P from directly applied rock phosphate (IPNI., 2019). Effective soil microorganisms for this purpose have been reported in the literature. Osorio and Habte (2014), reported that a

fungal strain of *Mortierella sp.* could effectively desorb enough phosphorus from both kaolinite-dominated and montmorillonite-dominated soils. Organic acid production by soil microorganisms and/or plant roots can dissolve the native soil P compounds, including the water-insoluble apatite, strengite, and variscite. The reported rate of dissolution is about 2 Tg phosphorus per year in cultivated soils (Sanchez's, 2019). However, depending on the soil P sorption capacity, the released inorganic P may be short-lived due to P sorption reactions and perhaps incorporation into microbial biomass (formation of soil organic P) (Weil and Brady, 2017). Besides, as the soil microbial biomass increases, a significant amount of soluble P is availed over time, since the recycling of microbial biomass P is more efficient than availing the mineral-fixed P, such that, microbial P biomass cycling goes up to 100 mg P/kg/soil/year (Dash *et al.*, 2017). Bacillus, Pseudomonas, Rhizobium, Aspergillus, Penicillium, and Arbuscular Mycorriza fungi are the most efficient P solubilizers for increasing bioavailability of P in soil. However, combinatory application of PSMs and rock phosphates is preferable a combination of rock phosphate with PSM terms of minimizing soil P depletion if PSMs are solely applied over long-term (Kalayu, 2019).

1.2 Problem Statement and Justification

Maize is a major staple crop for over 80 % of the people in Tanzania, and is accounting for over 70 % of national starch requirement (Mtaki, 2019). Major growers of maize in the country are small-scale farmers whose production levels are both unreliable and do not meet the crop requirements (Cugala *et al.*, 2012; Wilson and Lewis, 2015). Phosphorus deficiency is among other factors, a serious constraint to production of maize in acid soils of hot-humid to sub-humid tropics in southern highlands and eastern zones of Tanzania (Mlingano Agricultural Research Institute, 2006a; Senkoro *et al.*, 2017). The existing phosphorus management strategies are not in widespread use by resource-poor

farmers. This is mainly due to high costs of water-soluble P fertilizers and delayed agronomic effectiveness along with low phosphorus use efficiency of otherwise cost-effective locally available rock phosphates for direct application (Szilas *et al.,* 2007; IFDC, 2018; Mwangi *et al.,* 2020). A potent solution to the low reactivity of RP is the inclusion of phosphate-solubilising microorganisms (PSMs) in the application package (Simfukwe and Tindwa, 2018; Taddese, 2019). Phosphate based bio-fertilizers are made by combining the phosphate rocks, phosphate solubilising organisms (PSMs) and appropriate carriers or carbon sources (Khan *et al.,* 2014; Giro *et al.,* 2016). The PSMs in the mixture could help solubilize otherwise insoluble P in RP and even the fixed P in soil while organic carbon source will sustain the life of these microorganisms. These conditions can be perfectly met in Tanzania's agricultural environment, mainly because the phosphate rock deposits are present (Szilas, 2002; Msolla *et al.,* 2005) and the possibilities of finding the efficient PSM strains is higher (Simfukwe and Tindwa, 2018). However, there is limited knowledge on PSMs characterized and selected based on their ability to solubilize local phosphate rocks while utilizing locally available, naturally occurring organic carbon sources. Conceivably, effective RP-solubilising microorganisms would present a low-cost alternative to water-soluble P fertilizers in for alleviating phosphorus deficiencies in acid soils.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to evaluate the potential of phosphate solubilising bacteria (PSB) from selected agricultural soils of Tanzania for enhancing minjingu rock phosphate solubility and phosphorus use efficiency for sustainable production of maize in acid soils of Madaba, Ruvuma and Magadu, Morogoro.

1.3.2 Specific objectives

The above main objective of the study was realized through the following specific objectives: -

i. To characterize phosphate solubilising bacterial (PSB) through molecular identity techniques and selected- biochemical and plant growth-promoting traits
ii. To examine the influence of organic carbon sources and fertilizer-grade nitrogen sources on the abilities of PSB isolates to solubilize ferric-, and tri-calcium-phosphate.
iii. To evaluate the influence of organic carbon sources and fertilizer-grade nitrogen sources on antifungal efficiencies of PSB isolates against phytopathogenic fungi.
iv. To evaluate effect of co-applying PSBs and varying MRP rates on maize yield, phosphorus uptake, and phosphorus use efficiency (PUE) on acid soils of Madaba, Ruvuma and Magadu, Morogoro.

1.4 Organization of the Dissertation

Chapter one is about general introduction to the study, highlighting the theoretical background information together with problem statement and justification to the study. This chapter also covers a thorough review of literature on issues allied with phosphorus nutrition in plants and soils, indicating the state of phosphorus fertility in soils of Tanzania and tropics at large with more emphasis put on acid soils, existing phosphorus management strategies including, and potentials for sustainably improving phosphorus availability in soils. Moreover, the general and specific objective of this study is stated in this chapter.

Chapter two covers the laboratory-based experiments on characterizing and molecularly identifying potential phosphate solubilising bacteria, determination of

efficient bacterial isolates that could solubilize various phosphate sources including minjingu rock phosphate, determining the effectiveness of locally available organic carbon and nitrogen sources on phosphate solubilisation and antifungal activity of selected bacterial species, which were previously isolated from agricultural soils of Tanzania.

Chapter three is based on field trials for determining the potential of phosphate solubilising bacteria co-applied with varying P rates from Minjingu rock phosphate on soil available P, maize P uptake, maize yield, and phosphorus use efficiency.

Chapter four highlights the general conclusion and recommendations derived from specific findings of the entire study.

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CHAPTER TWO

Manuscript 1

Influence of Organic Carbon and Nitrogen sources on Phosphate solubilisation and Antifungal Activity of species of klebsiella and Burkholderia isolated from Agricultural Soils of Tanzania

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2.1 Abstract

Forty bacterial isolates from various agricultural fields in eastern and southern highlands of Tanzania were screened for their abilities to utilize three insoluble sources of phosphorus-Ferric, tricalcium and hard Minjingu rock phosphates while growing on locally available organic sources of carbon and fertilizer-grade nitrogen. Among them, eleven isolates showing greatest phosphate solubilisation potential were molecularly identified and further subjected to screening for other plant-growth promoting traits namely, activity against phytopathogenic *Fusarium proliferatum*, Production of Indole Acetic Acid (IAA), siderophores, ammonia and hydrogen cyanide. Results indicated that, all PSB isolates were positive for ammonia production test with highest amount being

168.3 ug/ mL produced by *Klebsilla sp*.-NA19a while none of the isolates exhibited ability to produce hydrogen cyanide gas. The highest amount of IAA produced was 24.32 ug/ mL by *Klebsilla sp*.- SI-SP1. Similarly, *Klebsilla sp*-NA5 showed a significantly ($P \leq 0.05$) bigger solubilisation index on ferric phosphate agar plate while *K. variicola*-MdG1 showed highest solubilisation index on $ZnCO_3$ agar plates. All the eleven isolates inhibited growth of *F. proliferatum* on agar plates with the highest inhibition of 47.7% by *Burkholderia sp*.-MK10. Among carbon sources tested, molasses significantly ($P \leq 0.05$) enhanced solubilisation of both tri-calcium and ferric-Phosphates compared to filter mud or sunflower seedcake while urea-containing media resulted into a significantly ($P \leq 0.05$) lower amount of P-solubilized as compared to fertilizer-grade or laboratory-grade ammonium sulfate ($(NH_4)_2SO_4$) as N source. The evaluation of antifungal activity showed that the use of molasses as carbon source retains most of antifungal activity of isolates but filter mud and sunflower seedcake do not. Fertilizer-grade $NH_4)_2SO_4$ enhanced the antifungal activity of most bacterial isolates than urea. Furthermore, the bacterial isolates under study retained their antifungal efficiencies whether yeast extract is included in the growth media. Overall, a combination of molasses and fertilizer-grade ammonium sulphate can be used to replace relatively expensive laboratory-grade glucose and ammonium sulphate as sources of carbon and nitrogen, respectively in bacterial cultures for enhanced phosphate solubilisation and antifungal activity.

Keywords: Phosphate solubilising bacteria, organic carbon sources, fertilizer grade nitrogen, antifungal activity

2.2 Introduction

Soil fertility and plant diseases are among the major challenges hindering crop production in most parts of the world (Lobulu *et al.,* 2019). Among other plant nutrients, phosphorus (P) deficiency has been widely reported as a constraint to crop production. Despite its abundance in soils, phosphorus availability in most tropical soils, including Tanzania is limited due its fixation in kaolinitic clays and sesquioxides (Msolla *et al.,* 2005; Mtama, 2018). Again, the replenishment of phosphorus through water-soluble fertilizers is not always cost-effective for resource-poor farmers.

Beside soil fertility constraints is the frequent occurrence of plant diseases and insect pests (biotic factors) that have led not only to yield losses but also crop quality reduction in the country. In some part of the country, severeness of abiotic factors have prompted farmers to apply overdoses of incompatible pesticide combinations, leading to a negative health effect in the soil–plant–human nexus (Ngowi *et al.,* 2007; Massomo, 2019; Matowo *et al.,* 2020).

The use of plant growth-promoting rhizobacteria (PGPR) in agriculture has, therefore been proposed as an alternative, eco-friendly, and economically plausible approach for improving and sustaining plant nutrient availability and controlling the deleterious effects of phytopathogenic organisms (Singh, 2013; Timmusk *et al.,* 2017; Mącik *et al.*, 2020). The ability of these beneficial rhizospheric microorganisms to increase the bioavailability of otherwise hardly available nutrients, including phosphorus, can be fetched and utilized in P-deficient soils. When such microbes have additional plant-growth promoting traits, their use in developing dual-purpose biofertilizer products is more rational and urgently needed now than ever before.

Studies, however, have reported the inconsistent performance of these microorganisms under field conditions (Bashan *et al.,* 2014). The type and nature of carrier material used

to deliver these inoculums to the field are still considered the most important determinant factor for successful inoculation, among others (Bashan, 1998; Ibrahim *et al.,* 2017; Mącik *et al.,* 2020). According to Rathore (2014) and Mardad *et al.* (2014), the phosphate solubilising potential of soil bacteria is affected by the carbon and nitrogen sources in the media. Organic carbon sources contain varying compositions of sugars and nutrients, which are essential for the growth and activities of bacteria. It is, however, clear that the appropriateness of the material depends on the species or strain of the organism and the specific trait to be fetched from that organism (Reddy and Saravanan, 2013; Vassilev *et al.*, 2020).

The objective of the reported in this paper was to evaluate the influence of the locally available organic carbon sources and fertilizer-grade nitrogen on phosphate solubilisation and antifungal activity of bacteria isolated from agricultural soils in Tanzania.

2.3 Materials ad Methods

2.3.1 Materials and test organisms used

Test microorganisms were isolated from fresh soil samples collected from agricultural soils in eastern and southern highlands of Tanzania following methods described by Khan *et al.* (2014) and stored at -80 ^{o}C in the Soil Science Laboratory at Sokoine University of Agriculture until when used for this study. Three organic-carbon sources used in this study namely molasses, filter-cake, and sunflower seedcake were sourced from Mtibwa sugar factory and sunflower oil processing mini-factory in Morogoro municipality, respectively. Minjingu phosphate rock samples were obtained from the Minjingu Mines and Fertilizer Company in Arusha, Tanzania.

2.3.2 Biochemical characterization of PSB isolates

2.3.2.1 Various sugars fermentation test

Abilities to ferment glucose, lactose and sucrose by the isolates were tested using the

Klingler's Iron agar (KIA) and Triple Sugar Iron (TSI) test methods as described by Mcdade and weaver (1959). Accordingly, KIA media contained lactose (10 g), glucose (10 g), and peptone, while TSI contained, in addition to KIA's ingredients, 10 g of sucrose per litter of distilled water. In each media, 0.024 g of phenol red and 0.2 g of ferrous sulphate were added as the indicators of acidification and H_2S formation, respectively. Briefly, 10 ml of each medium were dispensed into culture tubes each, heated for 30 minutes then autoclaved at 121 ^{0}C (15 psi) for 15 minutes. Tubes were allowed to cool while bent to an angle to allow slant formation. Then a loopful of bacterial isolates for each of those under study was inoculated into the tubed media and incubated at room temperature for 48 hours after which the results were observed. A yellow butt was taken to indicate acid production, a red pink slope indicated the fermentation of glucose only while a red pink slope and butt was taken to indicate lack of fermentation of both sugars.

2.3.2.2 Starch hydrolysis test

Starch hydrolysis was tested according to the method used by Iverson and Millis (1974). Briefly, starch agar medium was prepared by adding peptone 5 g, sodium chloride 5 g, yeast extract 1.5 g, beef extract 1.5 g, starch soluble 2 g, and agar 15 g into 1 L of distilled water. The final media pH was adjusted to 7.4 before autoclaving at 121C and 15 psi for 20 minutes. The overnight bacterial cultures in nutrient broth were streaked onto the surfaces of starch agar media and incubated for 48 hours at 28°C. The presence of a clear zone around the line of bacterial colonies after the addition of iodine solution was considered a positive test for starch hydrolysis.

2.3.2.3 Antibiotic sensitivity test

The agar well diffusion method (Balouiri *et al.*, 2016) was used to test isolates' antibiotic sensitivity. 100 µL of overnight bacterial cultures were spread-plated on Mueller Hinton

agar plates after which 8 mm holes were then punched on the inoculated plates by using sterile tips, and then 100 µL of either penicillin and streptomycin solution at a concentration of 50 µM were added to the wells, separately. The plates were incubated at 28 °C for 24 hours before making observations of inhibition zones. The interpretation of inhibition zones was done based on standards described in (Dolinsky, 2020).

2.3.3 Identification of bacterial and fungal isolates

Bacterial and fungal isolate were identified through molecular techniques. Briefly, bacterial and fungal DNAs were extracted from pure cultures by using Quick-DNA™ Fungal/Bacterial Miniprep Kit (Inqaba Biotec East Africa Ltd, Tanzania Branch). The 16S rDNA of bacteria was amplified by using universal primers (27F forward primer 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R reverse primer 5'-TACGGYTACCTTGTTACGACTT-3') (Frank *et al.,* 2008). The PCR reaction mixture was comprised of OneTaq® Quick-Load® 2X Master Mix with Standard Buffer, primers, and the genomic DNA and then incubated at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 15 s, and extension at 72 °C for 45 s. Amplification of the ITS1-5.8S-ITS2 rDNA of fungi was done using universal primers ITS-1F and ITS-4R which included an initial denaturation at 94 °C for 10 minutes followed by 35 cycles each for 45 seconds at 94 °C, 30 seconds at 55 °C, and 1 minute at 72 °C, and a final extension at 72 °C for 10 minutes. Separation of the amplified rDNA fragments was done by1.5 % agarose gel electrophoresis and visualized under UV Transilluminator after staining with SafeView™ Classic, -a nucleic acid gel stain. Afterward, the sequences of the insert were directly determined using a BrilliantDye™ Terminator v3.1 and ABI 3500XL Genetic Analyzer, POP7™ (Inqaba Biotechnical Industries Ltd, SA). To establish the identity of the isolates, the were analyzed using the BLASTn tool of the NCBI database was used to analyze the 16S rRNA gene sequences to

establish identity of the isolates. The taxonomic tree and the evolutionary distances of the phosphate solubilising bacterial isolates were calculated using MEGA-X software and Kimura's two-parameter model, after aligning the sequences with Clustal W.

2.3.4 Characterization of PSB isolates for other plant growth-promoting traits

The production of IAA by bacterial isolates used in this study was determined as described by (Khan *et al.,* 2014). Pure cultures of bacterial isolates were grown in the nutrient broth amended with or without tryptophan and incubated at 36 ±2 °C for five days. The cultures were then centrifuged at 7 500 x g for 30 min, after which, 2 ml of the supernatant was mixed with two drops of 10 mM orthophosphoric acid and 4 mL of the Salkowski reagent (50 mL, 35% of perchloric acid, 1 mL 0.5 M ferric chloride solution) and incubated at 28±2 in darkness for one hour. The development of pink colour indicated positive for IAA production. IAA quantification was done spectrophotometrically by comparing absorbance values of culture supernatants to those of standard solutions read at 530 nm on an iCE 3300 Thermo-scientific atomic absorption spectrophotometer.

The modified Nesslerization method (Heonsang *et al.,* 2013) was used to quantify ammonia production by isolates. Bacterial isolates were grown in peptone water (g/l: peptone 10; NaCl 5; pH 7) and incubated at 28 ±2 °C for 4 days. After incubation, the cultures were centrifuged at 2 000 × g for 10 minutes. Separately, standard solutions (0, 10, 20, 40, and 80 mg/L of ammonia) were prepared from 1 000 mg/L NH_4Cl stock solution. Then, 0.2 ml EDTA were added to each supernatant and standard solution to eliminate calcium and magnesium interference. Then, 0.4 ml of Nesler's reagent was added to each standard and supernatants and the intensity of yellow colour was read by spectrophotometry at 420 nm. Bunt and Rovira agar medium containing zinc carbonate was used to screen for zinc solubilising abilities of bacterial isolates (Bunt and Rovira,

1955). The bacteria were inoculated onto the media in triplicates and then incubated for 7 days at 28 ± 1 °C. Zinc solubilisation index (ZSI) was calculated according to Sadiq *et al.* (2014). While modified chrome azurol S (CAS) agar assay was used to detect siderophore production by isolates (Milagres *et al.*, 1999).

2.3.4.1 Antifungal activity of bacterial isolates

A fungal pest was previously isolated from infected maize plant leaves following a procedure described by McMullen (1983) and later identified as *Fusarium spp*. After rRNA gene sequencing and Blastn search (citation). *Fusarium proliferatum—fg1*. isolated here was then used to test antifungal properties of all PSB isolates used in this study. Amended Cross Streak method (Lertcanawanichakul and Sawangnop, 2008) was used to screen for antagonistic activities of PSB isolates against *Fusarium proliferatum—fg1*. Briefly, Potato Dextrose Agar (PDA) was prepared and spot inoculated with different PSB isolates in one end of the 9 cm diameter Petri dish (2 cm away from the margin). The plates were incubated at 28 ± 1°C for 24 hours after which a PDA plug of the actively growing *Fusarium proliferatum—fg1*was placed on the opposite and the equidistant end of each Petri dish. All treatments were in triplicates. On the seventh day of incubation, the diameter of fungal colony in each plate was measured and antifungal efficiency (AE) was determined according to a method used by *Li et al.* (2012).

2.3.5 Influence of fertilizer-grade N sources on phosphate solubilisation by PSB isolates

Urea and Sulphate of ammonium (SA) fertilizers were tested against the conventional laboratory-grade Ammonium sulphate (LSA) as a source of N to PSB isolates. For the Qualitative test, the PSB isolates were inoculated on the Pikovskaya (PVK) agar media (positive control), and on the modified SMM agar supplemented with either 0, 0.25, and

0.5 g of each fertilizer-grade urea or SA, with or without yeast extract. Media were adjusted to a pH of 7± 0.2, autoclaved at 120 ^{0}C and 20 psi for 15 minutes. Cooled media plates were inoculated with each of 10 PSB isolates and incubated for 9 days at 28 ± 20C (Paul and Sinha, 2016). The diameter of the clearance zone was measured successively after 24 hours and the solubilisation index (SI) was calculated according to a method suggested by Premono *et al.* (1996). Similarly, modified SMM broth was prepared by replacing the lab-grade ammonium sulphate of the PVK medium composition with fertilizer-grade urea or fertilizer grade sulphate of ammonia. Ten PSB isolates were separately inoculated into these media and incubated in an orbital shaker at 28 0C and 150 rpm for 5 days and the amount of P solubilized in the culture supernatant was quantified using the colorimetric molybdate blue method (Olsen *et al.*, 1982).

2.3.6 Influence of organic carbon sources on solubilisation of calcium and ferric phosphates by PSB isolates

Molasses (ML), sunflower seedcake (SC), and the filter mud (FM) were initially evaluated for their physicochemical properties according to methods described by Peters *et al.* (2003) and Sullivan (2014). Afterwards, the three organic materials were evaluated for their comparative potentials to enhance solubilisation of Tri-calcium phosphate (TCP) and Ferric phosphate (FP) by ten PSB isolates. Accordingly, a double-modified synthetic minimal media (dSMM) was prepared which contained all components of the Pikovskaya broth (Pikovskaya, 1948), except for the carbon and Nitrogen source. In place of glucose, 20 g per liter of either ML, SSC or FM were added. Each media was adjusted to a pH of 7 and then autoclaved at 121 °C for 15 minutes. After cooling, the ten PSB isolates were separately inoculated to each media and incubated in an orbital shaker at 28 ^{0}C and 150 rpm. Un-inoculated medium was used as a control. The amount of soluble P in the culture supernatant was quantified using the colorimetric molybdate blue method (Olsen *et al.*,

1982) and pH was recorded, after 12, 24, 48 and 120 hours of incubation for each culture.

2.3.7 Effect of molasses concentrations on solubilisation of hard Minjingu rock phosphate (HMRP) by PSB isolates

The synthetic minimal media (SMM) was made through modification of conventional PVK agar media. Tri-calcium phosphate was replaced by 5 g HMPR powder per litter as the P source. Experimental treatments were the SMM with varying concentrations of molasses; 5 ml, 10 ml, and 20 ml per litter. SMM with 10 g glucose per litter was used as a positive control. Each media was adjusted to a pH of 7 and then autoclaved at 121 °C and 15 psi for 15 minutes. PSB isolates were separately and aseptically inoculated to each media and then incubated on an orbital shaker at 150 rpm and 28± 2 °C for 5 days (Paul and Sinha, 2016). The un-inoculated media was used as a negative control. Each culture was centrifuged at 4 000 rpm for 10 min and the amount of soluble P in the culture supernatant was quantified using the colorimetric molybdate blue method (Olsen *et al.*, 1982) and pH was recorded, after 12, 24, 48, and 120 hours of incubation for each culture.

2.3.8 Effect of molasses, sunflower seedcake and filter mud on antifungal efficiencies (AE) of PSB isolates against *F. proliferatum*

Each of the PSB isolate was grown in the modified synthetic minimal agar medium supplemented separately with either molasses, sunflower seedcake, filter mud, or glucose as the sole source of carbon. Accordingly, agar plates were spot-inoculated with different PSB isolates in one end of the 9 cm diameter Petri dish (2 cm away from the margin). The plates were incubated at 28 ± 1°C for 24 hours after which a PDA plug of the actively growing *F. proliferatum*-fg1 was placed on the opposite but equidistant end of each Petri dish. All treatments were in triplicates and incubated for seven days. At the end of the incubation period, diameter of fungal colony in each plate was measured and

antifungal efficiency (AE) was determined according to a formula used by Li *et al.* (2012).

2.3.9 Effect of molasses concentrations on antifungal efficiencies of PSB isolates against *F. proliferatum-fg1*

Bacterial isolates with potential antifungal activities were singly inoculated into agar plates containing the synthetic minimal media supplemented with four separate levels of molasses; 5 ml, 10 ml, 20 ml, and 30 ml of molasses per liter of media. Media with 10 g glucose per liter was used as a positive control. The fungal pest was co-inoculated into each plate at equidistance from the bacterial isolates. The plates inoculated with the fungi alone were used as the negative control. The cultures were incubated at 28 °C for 7 days. The zones of inhibition of the fungi from each bacterium were measured.

2.4 Statistical Analysis

The quantitative data were subjected to the Analysis of Variance (ANOVA) to evaluate the influence of carbon and nitrogen sources on phosphate solubilising abilities of the PSB isolates. Treatment means separation was done according to Duncan's New Multiple Range Test (DNMRT) at the 0.05 level of significance.

2.5 Results

2.5.1 Selected morphological, biochemical and molecular characteristics of studied isolates

Table 2.1 shows selected biochemical properties of microbial isolates used in this study. All isolates formed round-raised colonies with smooth and shiny surfaces and entire margins. Except for Mk10 and NA19a that formed brownish yellow colonies, the rest of the isolates' colonies were whitish and creamy. Results show further that isolates NA19a,

MdG1, and MbMz1 were capable of glucose, lactose, and sucrose fermentation. Furthermore, isolates SUApp3 and NA5 fermented glucose and sucrose, SL-Sp1, NA4a, and NA4b fermented glucose only while isolates Mk10, Kjm3, and MdE4 were incapable of fermenting any of the three sugars. All isolates studied were negative for hydrogen sulphide production while all were positive for catalase activity test. Two isolates- MdG1 and NA5 showed resistance to both penicillin and streptomycin while the rest were susceptible to at least one of the two antibiotics.

Table 2.1: Selected biochemical properties of bacterial isolates used under this study

		SL-Sp1	NA19a	NA4a	NA4b	SUApp 3	MdG1	Mk10	MbMz1	NA5	MdE4	Kjm3
Starch hydrolysis		-	-	+	+	+	+	-	-	-	-	+
Catalase activity		+	+	+	+	+	+	+	+	+	+	+
Sugar fermentation	Glucose	F*	F	F	F	F	F	NF**	F	F	NF	NF
	Lactose	NF	F	NF	NF	NF	F	NF	F	NF	NF	NF
	Sucrose	NF	F	NF	NF	F	F	NF	F	F	NF	NF
Zone of bacterial growth inhibition due to antibiotics (mm)	PEN	23	47	21	23	25	12	18	25	0	25	18
	STR	0	38	0	41	0	0	0	0	0	44	47
Gas Production	CO_2	-	-	-	+	-	-	-	-	+	-	-
	H_2S	-	-	-	-	-	-	-	-	-	-	-

F* = fermentor, NF** = Non-fermentor, (-) = non gas producer, (+) = Gas producer, PEN=Penicillin, STR= Streptomycin

Molecular identity results are presented in Table 2.2. The results indicated that most of bacterial isolates had high nucleotide identity (>90 %) to other known species in the American National Institutes of Health (NIH) NCBI genetic sequence database (GenBank). Accordingly, eight out of eleven bacterial isolates had the highest homology with the known strains of the genus *Klebsiella*, two isolates related to the strains of genus *Burkholderia*, while one isolate is still unidentified.

Table 2.2: Identity of isolated RP-solubilising species based on nucleotide database on American National Institutes of Health (NIH) NCBI genetic sequence database (GenBank)

Isolate	Species	Accession Number	Nucleotide Identity	Country	Source rhizosphere
Fg1	*Fusarium proliferatum*	MZ497514	100	Tanzania	Maize
SL-Sp1	*Klebsiella sp.*	MZ502674	99.8	Tanzania	Sweet potato
Mk10	*Burkholderia sp.*	MZ502221	99.9	Tanzania	Maize
NA19a	*Klebsiella sp.*	MZ502673	99.8	Tanzania	Maize
NA4a	*Unidentified*			Tanzania	Irish potato
NA4b	*Klebsiella sp.*	MZ502671	99.8	Tanzania	Irish potato
SUApp3	*Klebsiella sp.*	MZ502675	99.7	Tanzania	Sweet pepper
MdG1	*Klebsiella variicola*	MZ502670	99.8	Tanzania	Banana
Kjm3	*Burkholderia sp.*	MZ502220	99.9	Tanzania	Maize
MbMz1	*Klebsiella sp.*	MZ502668	99.8	Tanzania	Maize

NA5	*Klebsiella variicola*	MZ502672	99.8	Tanzania	Sweet potato
MdE4	*Klebsiella variicola*	MZ502669	100	Tanzania	Common bean

Table 2.3: Selected biochemical properties of organic carbon sources under study

Parameter	**pH**	**pH-BC**	**DM**	**OC**	**TN**	**P**	**K**	**Ca**	**Mg**	**Zn**	**Cu**	**Fe**	**Mn**	**CN ratio**
Unit		**Mol H^+/kg**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	
Molasses	5.3	0.3	72.6	23.1	1.23	0.26	4.06	0.78	1.84	0	0.01	0.35	0.03	18.8
Sunflower seedcake	6.5	0.18	94.4	28	4.4	5.74	1.65	0.57	0.48	0.04	0.03	0.36	0.04	6.4
Filter mud	7.4	0.1	54.2	31	0.82	4.56	0.02	0.08	0.04	0.05	0.01	3.57	0.26	37.8

*DM-dry matter, OC-organic carbon, TN-total Nitrogen, pH-BC = pH buffering capacity

2.5.2 Phosphate solubilisation and other plant-growth promoting traits

Table 2.4 shows results for selected plant-growth promotion traits of bacterial isolates used in this study. Hundred percent (100 %) by number of studied bacterial isolates could form noticeable halo zones around colonies on the media containing either tricalcium phosphate, ferric phosphate, zinc carbonate or hard Minjingu rock phosphate. Solubilisation index values were generally higher on medium containing $Ca_2 (HPO_4)_3$ and lower on medium containing $FePO_4$. Ironically, one isolate- *Klebsiella variicola*-NA5 exhibited highest SI on the media containing $FePO_4$ than on the media containing other insoluble phosphate minerals. All isolates also showed abilities to solubilize $ZnCO_3$ on solid medium.

All eleven isolates were positive for IAA and ammonia production but negative for hydrogen cyanide production. Furthermore, nine of the eleven isolates tested positive for the siderophore production on solid media. Results show further that there were significant ($P<0.001$) differences in the quantity of ammonia produced with highest and lowest amounts produced by *Klebsiella sp.* NA19a and *Burkholderia sp.* MK10, respectively. Production of IAA-like substances varied significantly ($P < 0.001$) among bacterial isolates, ranging from 1.68 µg mL^{-1} produced by *Klebsiella sp.* Sl-Sp1 in broth culture without tryptophan to 24.32 µg mL^{-1} produced by *Klebsiella variicola*- MdE4 in broth culture amended by 1% tryptophan. In all but three isolates- *Klebsiella sp.* NA19a, *Klebsiella sp.* NA4b and *Burkholderia sp.* Kjm3, amendment of broth cultures with 1 % tryptophan significantly ($P<0.001$) increased quantity of IAA produced. Table 2.4 also presents results antifungal properties of bacterial isolates against *Fusarium proliferatum*- a pathogenic fungus isolated from maize plant leaves. The highest antifungal efficiency (AE) was recorded with the isolate *Burkholderia sp.*-Mk10 exhibited the *F. proliferatum* growth inhibition at an antifungal efficiency of 47.7 % while the lowest antifungal efficiency was 27.2 % exhibited by with *Klebsiella variicola* -MdG1.

Table 2.4: Selected plant growth-promoting traits possessed by phosphate solubilising bacterial isolates

	Isolate	*Klebsiella sp.*-SLSp1	*Klebsiella sp.*NA19a	*Klebsiella sp.*NA4b	*Klebsiella sp*-SUApp3	*K.variicola* MdG1	*Burkholderi a sp. Mk*10	*Klebsiella sp. MbMz*1	*K.variico la* NA5	*K.variicola* MdE4	*Burkholderi a sp* Kjm3	Contl
	$FePO_4$	#2.4	2.9	2.3	1.7	2.5	1.9	2.4	3.1	1.8	2.2	0
SI	$Ca_2(HPO4)_3$	2.7	3.3	2.7	2.7	2.6	2.6	3.5	2.9	3.8	2.5	0
	$ZnCO_3$	7.3	8.6	8.4	7.3	10.1	5.8	6.0	1.0	9.9	7.9	0
IAA	1% Trp.	^8.3fgh	8.6fg	5.2ghijkl	14.1cd	20.5ab	17.3bc	13.2de	*nd.	22.8a	3.6ijkl	1.4l
(µg/ml)	Without Trp.	1.680l	6.0ghijk	2.2ijkl	6.2ghi	10.6ef	6.0ghij	3.2ijkl	nd	1.8jl	4.4hijkl	1.3l
Ammonia (µg/ml)		147.9ab	168.3a	114.5b	119.0b	119.3b	15.9c	143.4ab	140.6ab	113.7b	134.1ab	9.9c
Antifungal Efficiency (%)		38.5b	32.3c	39.1b	34.5c	27.2d	47.7a	22.7e	27.4d	38.5b	32.8c	-
Siderophore test”		-	+	+	-	+	+	+	+	+++	+	-
HCN Production		-	-	-	-	-	-	-	-	-	-	-

Trp. = Tryptophan, Siderophore test”; + weak; ++, moderate; +++, strong, #Values are treatment means of three replications, ^The treatments that share a

later are not significantly different, Contl = Control

2.5.3 Efficiency of isolates' phosphate solubilisation in double modified synthetic minimal medium is comparable to that of Pikovskaya broth

Properties of molasses, sunflower seedcake and filter mud used to modify the PVK medium as alternative sources of C are presented in Table 2.3. Overall, molasses gave the best fit both in terms of levels of micronutrients available and pH buffering capacities. This is because the pH of the carbon source influences the media pH which determines its suitability for bacterial growth and most soil bacteria grow preferably in the nearly neutral pH (Cho *et al.*, 2016). Appendices 2.1 and 2.2 present results on the influence of nitrogen source on phosphate solubilisation in solid and liquid media, respectively. Amendment of PVK by replacing laboratory grade ammonium sulphate with fertilizer grade sulphate of ammonia at 5 g/L gave the highest solubilisation index in most of the isolates. Accordingly, isolate *Klebsiella variicola*-MdE4 gave the highest SI value of 6.1. Addition of yeast extract in the medium of growth exerted a significant ($P \leq 0.01$) negative influence on the phosphate solubilisation index of isolates under study. Inclusion of yeast in the modified medium reduced SI values for all isolates studied. In broth conditions, the highest amounts of soluble P were 425.4 and 419.4 mg/L produced by *Klebsiella sp.*-NA4b in fertilizer-grade and laboratory grade ammonium sulphate-amended dSMM medium, respectively after at least 72 hours of incubation.

Figure 2.1 and Figure 2.2 show the results on the effects of double modified synthetic minimal medium (dSMM) on phosphate solubilisation. We show that of the three carbon sources used to develop dSMM molasses resulted into highest soluble P in liquid culture than filter mud and sunflower seedcake, respectively. The increase in the amount of soluble P from either Tricalcium phosphate (TCP) or Ferric phosphate (FP) was significantly ($P \leq 0.01$) higher in cultures supplemented with molasses than in those containing either sunflower seedcake or filter mud. After 120 hours (about 5 days) of

incubation, highest value of 367.35 g of soluble P per litre from TCP was recorded in molasses-amended cultures of *Klebsiella sp*.-MbMz1. Similarly, 163.62 g/L from FP was recorded in molasses-amended cultures *Klebsiella variicola*-NA5 after 72 hours (about 3 days) of incubation. Therefore, most isolates used in this study solubilized TCP much better than they did with FP. Separately, results show that phosphate solubilisation efficiency was related to a reduction pH of the growth medium. Of the three organic sources of carbon tested in the optimization of the dSMM, molasses showed a higher ability in reducing medium pH than filter mud and sunflower seedcake. To optimize the molasses for use in the dSMM as a source of carbon, we tested various levels of added molasses against solubilisation of hard Minjingu Rock Phosphate (HMRP). Results of the effect of various amounts of molasses in dSMM on HMRP solubilisation are presented in Appendix 3. Overall, replacing the 10 g of glucose with 20 mL of molasses did not negatively affect phosphate solubilisation efficiency of most studied isolates in dSMM cultures.

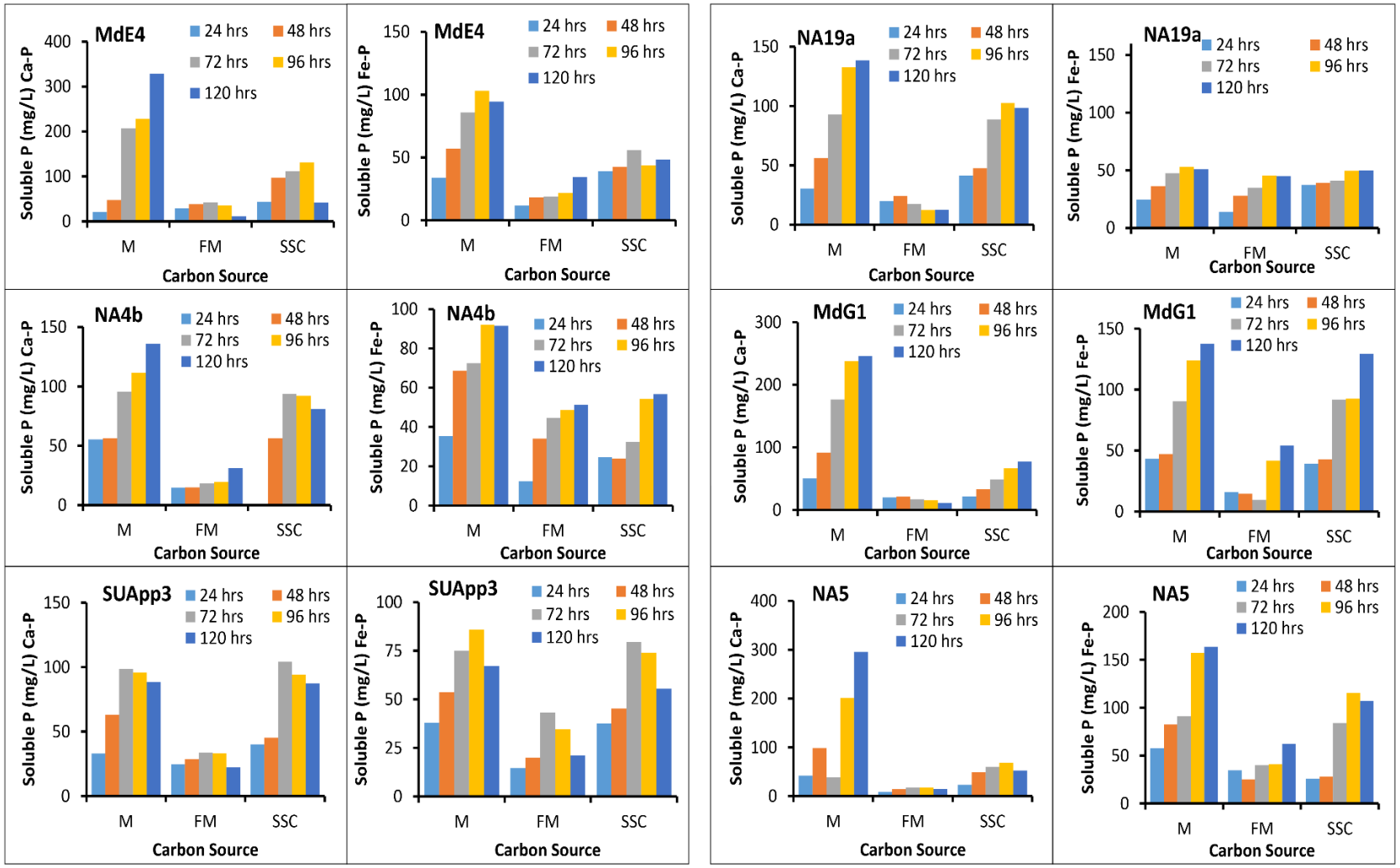

Figure 2.1: Influence of organic carbon sources on the abilities of the selected phosphate solubilising bacteria to solubilize Ferric and Tricalcium phosphates

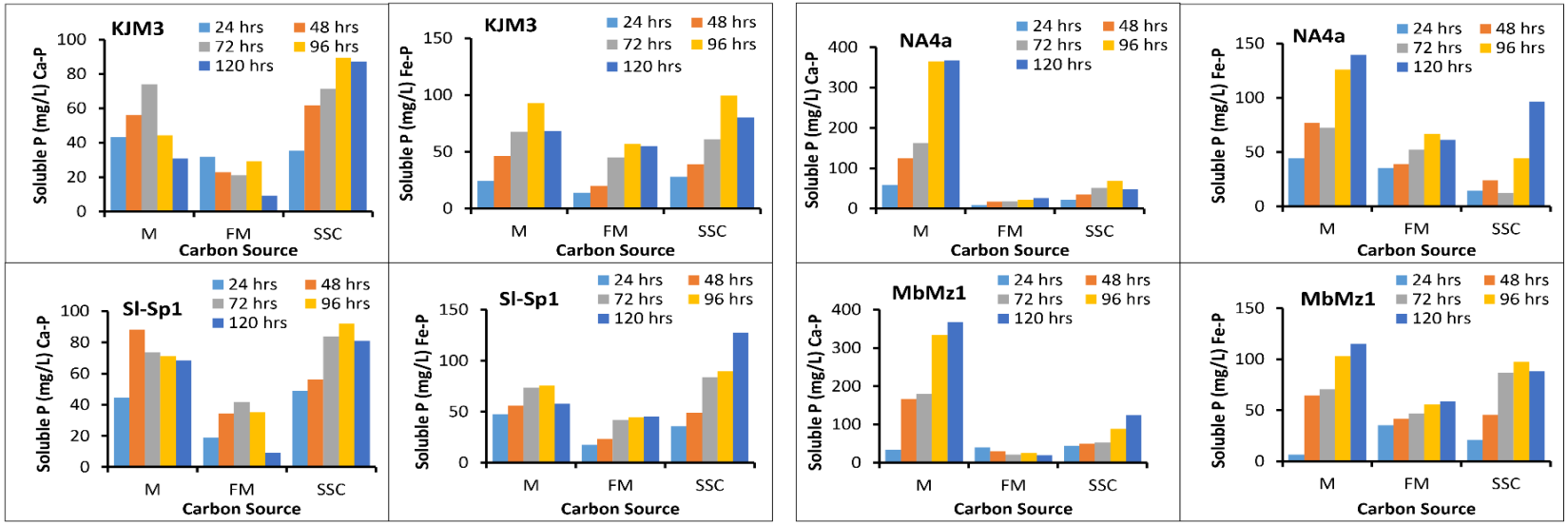

Figure 2.2: Influence of organic carbon sources on the abilities of the selected phosphate solubilising bacteria to solubilize Ferric and Tricalcium phosphates

2.5.4 Inclusion of molasses in dSMM retains most of antifungal activity of isolates but filter mud and sunflower seedcake do not

Results for antifungal activities of various isolates used in the current study against *F. proliferatum* are presented in Table 2.5. Antifungal efficiencies of most isolates growing on molasses amended dSMM were as high as when the isolates were grown on glucose-amended dSMM (Table 4). Compared to filter mud and sunflower seed cake, molasses amended dSMM resulted in significantly ($P \leq 0.01$) higher values of antifungal efficiencies. Accordingly, varying the concentration of molasses in the dSMM had a significant ($P \leq 0.05$) effect on the antifungal efficiencies of tested isolates against *F. proliferatum* Figure 2.3. Regardless of bacterial isolate and incubation period, the antifungal efficiency was highest in the media containing 30 ml/L of molasses. However, the interaction effect of molasses concentration and the time at which AE was measured is significant. On the third day, the AE was highest on the media containing 30 ml/L molasses and lowest on the media with 5 ml/L molasses, for all isolates. On the tenth day, the AE was highest on the media with 5 ml/L molasses and lowest on the media with 30 ml/L molasses, for all isolates. Results on Table 2.5 show further that the isolate *Burkholderia sp.*-MK10 exhibited the highest antifungal activity against the *F. proliferatum* on solid dSMM agar. It attained an antifungal efficiency of 45.9 and 41.6 while growing on glucose amended- and molasses amended medium, respectively- values that were higher than those of any other isolates under the same conditions of growth.

Table 2.5: Antifungal efficiencies of PSB isolates against *F. proliferatum* under different carbon sources

Carbon Source	SL-Sp1	NA19a	NA4a	NA4b	SUApp3	Mk10	MdE4
ML	34.9 ^{abcdef}	36.1 ^{abcdef}	30.9 ^{abcdef}	29.3 ^{bcdef}	34.1 ^{abcdef}	41.6 ^{abcd}	25.9 ^{defg}
GL	42.5 ^{abc}	43.9 ^{ab}	32.5 ^{abcdef}	28.6 ^{bcdef}	38.6 ^{abcdef}	45.9 ^{a}	39.7 ^{abcde}
FM	6.7 ^{i}	9.7 ^{hi}	5.3 ^{i}	0.9 ^{i}	11.5 ^{ghi}	6.6 ^{i}	24.2 ^{efgh}
SSC	25.3 ^{cdef}	23.1 ^{fgh}	22.2 ^{defg}	29.7 ^{bcdef}	23.5 ^{fgh}	28.6 ^{bcdef}	31 ^{abcdef}

Values presented are the means of three replications

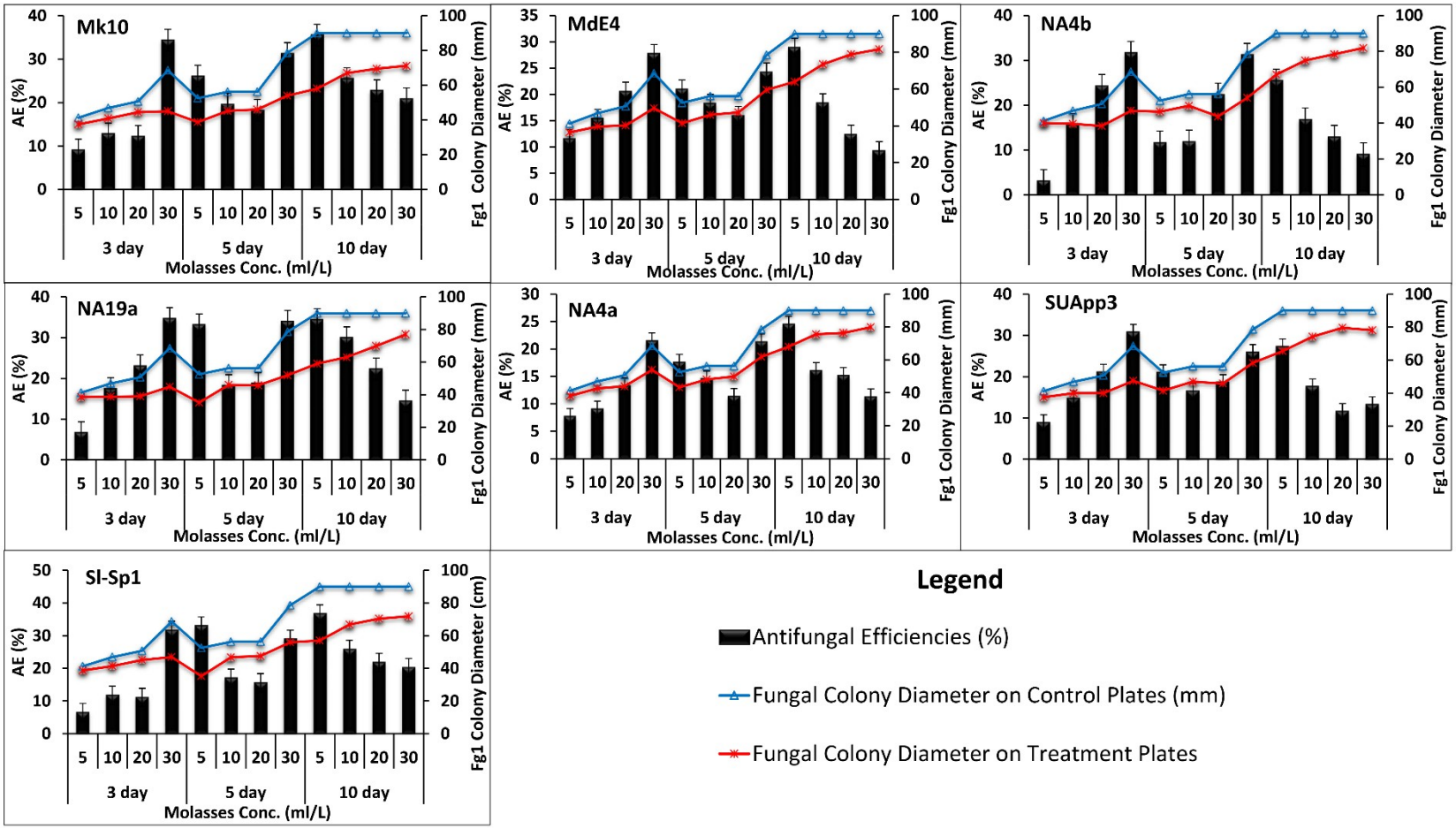

Figure 2.3: Effects of varying concentrations of molasses on antifungal activity of bacterial isolates against *F. proliferatum*

2.5.5 Effect of fertilizer-grade nitrogen on antifungal activity of PSB isolates

Table 2.6 presents results on the effect of varying nitrogen source and concentrations of nitrogen on antifungal activities of various isolates used in the current study against *F. proliferatum* on solid dSMM agar. Amendment of dSMM with the fertilizer grade sulphate of ammonia at either 2.5 g/L or 5 g/L resulted into higher antifungal efficiency of most isolates. The bacterial isolates under study demonstrated relatively lower antifungal efficiencies when grown on dSMM agar media amended with fertilizer grade Urea at either 2.5 g/L or 5 g/L. Furthermore, the inclusion of yeast extract dSMM agar was found not to affect the antifungal efficiencies of the isolates under study. Generally, the highest antifungal efficiencies were recorded with the isolate Mk10 grown on dSMM agar media with fertilizer-grade ammonium sulphate at 0.5 g/L, with or without yeast extract.

Table 2.6: Antifungal efficiencies of PSB isolates against *F. proliferatum* under different nitrogen sources and concentrations

Yeast Extract (g/L)	N-fertilizer (g/L)	SL-Sp1	NA19a	NA4a	NA4b	SUApp3	Mk10	MdE4
0.5	0.25 SA	35.9 abcdef	34.9 bcdefg	26.5 hijklmno	29.5 efghijklm	30.3 efghijkl	40.1 abcd	31.4 efghij
	0.5 SA	36.7 abcde	37.5 abcde	29.6 efghijklm	29.2 efghijklm	35.6 bcdef	43.8 a	34.8 bcdefg
	0.25 Urea	37.0 abcde	21.8 mnop	19.5 op	20.6 nop	27.9 fghijklmn	25.6 ijklmnop	25.9 hijklmno
	0.5 Urea	35.9 abcdef	22.1 mnop	23.2 jklmnop	23.2 jklmnop	23.2 klmnop	17.6 p	20.2 nop
	0	5.3 qr	5.7 qr	5.7 qr	2.8 qr	6.2 qr	10 q	5.7 qr
0	0.25 SA	37.7 ab	33.9 cdefgh	32.7 cdefghi	33.9 cdefgh	35.2 bcdef	42.2 ab	40.0 abcd
	0.5 SA	34.1 bcd	27.9 fghijklmn	26.8 ghijklmno	31.3 efghijk	32.5 defghi	43.8 a	37.4 abcde
	0.25 Urea	32.7 cdefghi	26.7 hijklmno	20.8 nop	19.9 nop	24.6 ijklmnop	25.4 ijklmnop	22.5 lmnop
	0.5 Urea	31.3 efghijk	25.2 ijklmnop	21.5 mnop	23.4 jklmnop	21.9 mnop	21.5 mnop	21.5 mnop

2.6 Discussion

This study was conceived with the intention of exploring rhizosphere bacteria, which, in addition to other plant-growth promotion traits, could perform efficient phosphate solubilisation in soil and be mass-produced using organic, cheap- and locally available carbon and nitrogen sources in culture medium. We have shown in the current study that studied isolates possessed traits responsible for enhancing plant growth and production in several ways including the increased bioavailability of soil phosphorus to plants, production of plant growth hormones such as IAA and helping plants to fight against pathogenic microorganisms (Khan *et al.,* 2014; Etesami and Maheshwari, 2018; Cueva-Yesquén *et al.,* 2021).

The observation that higher amounts of IAA were produced by some isolates in the presence of tryptophan as opposed to its absence is attributable to the role of tryptophan as the main precursor for the biosynthesis of IAA (Spaepen and Vanderleyden, 2011; Khan *et al.,* 2014). Furthermore, the observation that some of isolates of the present study did produce substantial amounts of IAA in the absence of tryptophan is related to the fact that there are other known pathways of IAA biosynthesis that are independent of tryptophan (Spaepen and Vanderleyden, 2011), as such bacterial strains can synthesize the precursors needed for IAA production by themselves (Numan *et al.,* 2018). We note here that it is advantageous to have strains with tryptophan independent IAA-producing capabilities when developing biofertilizer formulations for direct field application. Positive results from the inoculation of IAA-producing microbes have been reported in various crops, including maize (Bumunang and Babalola, 2014), peanut (Dey *et al.,* 2004), moth bean (Sachdev *et al.,* 2009), and wheat (Mohite, 2013).

Isolates in the current study have shown varying abilities to solubilize various insoluble forms of phosphorus namely ferric phosphate, tricalcium phosphate, and hard Minjingu

rock phosphate as well as insoluble zinc carbonates compounds. Other researchers have proposed that mechanisms by which microorganisms solubilize otherwise insoluble compounds may include, among other things, the production of complexing or mineral dissolving molecules such as low molecular weight organic acids, siderophores, hydroxyl ions, and carbon dioxides that lowers the rhizospheric soil pH, chelates with phosphate precipitating cations (Ca, Al, and Fe), and compete with P for sorption sites on the P fixing clay surfaces (Sharma *et al.,* 2013; Thakur *et al.,* 2014; Numan *et al.,* 2018). However, among all, the production of organic acids is accepted as the main and widely distributed mechanism (Khan *et al.,* 2014).

The observed ammonia- and siderophore-producing abilities of studied isolates are an addition to the qualities important for isolates' inclusion into a biofertilizer formulation project. Ammonia production can directly improve supply of N and thus plant growth as ammonia is one of the plant-available forms of N. Siderophores on the other hand, can increase the bioavailability of iron in the soil through the formation of soluble Fe^{3+} complexes (Tian *et al.,* 2009; Khan *et al.,* 2014; Ariole *et al.,* 2019). Under the condition of iron deficiency, such as in most aerobic environments, where iron accumulates as the insoluble oxides and hydroxides, these bacteria can be used to increase the availability of iron to plant roots (Numan *et al.,* 2018). Additionally, siderophore and ammonia production among rhizosphere microorganisms have been attributed to a reduction of deleterious effects of phytopathogenic fungi in agriculture (Rodrigues *et al.,* 2016; Numan *et al.,* 2018).

We have demonstrated in the present study, that in addition to all the plant-growth promoting traits of the studied microorganisms, over 70 % of the isolates exhibited ability to inhibit growth of phytopathogenic fungi, *Fusarium proliferatum*, previously isolated

from an infected maize plant. Biocontrol of phytopathogenic fungi by using antagonistic microbes has been considered a viable strategy (Fishal *et al.,* 2010). However, the effectiveness of the biocontrol agent is reported to be influenced by its ability to colonize the target site (rhizosphere) and survive for long enough to express its trait to suppress the soil-borne phytopathogens (Li *et al.,* 2012). Further studies are, therefore, recommended to evaluate the field condition survivorship, colonization, and biocontrol strength of the bacterial isolates under the current study.

The comparatively higher influence of molasses over filter mud and sunflower seed cake on phosphate solubilising activity of bacterial isolates observed in this study is probably due to higher enough pH lowering abilities of involved PSBs that could not offset the otherwise higher buffering capacity of the molasses as an important component of the growth medium (Hameeda *et al.,* 2006). The phosphate solubilising activity of bacteria is mechanistically related to the quantity and the nature of organic acids produced, of which the carbon source, bacterial species, and population have significant influences (*Khan et al.* (2014). The recorded relatively lower influence of sunflower seedcake on phosphate solubilisation by the isolates despite its narrow C: N ratio and adequate supply of nutrients, may be related to the presence in it of some microbial growth-inhibitory organic molecules such as linoleic and oleic fatty acids (Kelsey *et al.,* 2006; Akkaya, 2018). On the other hand, the C:N ratio of filter mud is generally too wide to allow sufficient nutrient supply for the phosphate solubilising bacterial community, hence the observed little influence on phosphate solubilising activities of studied isolates.

A logical manipulation of bacterial fermentation conditions aiming at enhancing antimicrobial production requires a better understanding of nutrient requirements for the optimal biosynthesis of secondary metabolites by specific bacterial strains (Tamreihao *et*

al., 2016; ˆPirttilä *et al.*, 2021). Glucose, followed by molasses as the carbon sources were noted as optimal for enhancing the production of antifungal substances by bacterial isolates under this study. Although glucose and other rapidly utilizable carbon sources interfere with biosynthesis of antimicrobial secondary metabolites by most microbes (Bruckner and Titgemeyer, 2002; Ruiz *et al.*, 2010), some studies have reported otherwise for some microbial species including *Gymnopilus spectabilis* (Vahidi *et al.*, 2006) and *Pseudomonas fluorescens strain CHA0* (Shaukat and Siddiqui, 2003).

Furthermore, the antifungal efficiencies of studied bacterial isolate was relatively lower on the dSMM agar media amended with organic carbon sources, with molasses giving higher AE, among others. According to Ruiz *et al.* (2010), when the growth media contain a mixture of slowly and rapidly utilizable carbon sources, the microbe would first use the later for growth with little or no production of secondary metabolites. Upon the depletion of the most preferred carbon source, the second-best carbon is used for production of secondary metabolites (Romero *et al.*, 2010). Therefore, since the OCs used in this study (molasses, sunflower seed cake, and filter mud) contain a mixture of various simple and complex sugars and nitrogen compounds (Geneau-Sbartaï *et al.*, 2008; Nikodinovic-Runic *et al.*, 2013), it is possible that the biosynthesis of antimicrobial secondary metabolites was interfered by some unidentified components in these OCs. As we further tried to optimize molasses concentration for higher antifungal efficiencies of bacterial isolates, we concluded that lower molasses concentrations enhance the production of antifungal substances. Despite of the recorded higher inhibition of *Fusarium proliferatum* on dSMM agar media with higher molasses concentration (30 ml/L) by the 3rd and 5th incubation days, the antagonism cannot be attributed to the production of antifungal substances since the fungi was later (10th day) able to able to grow and cover the zones of the agar media previously thought to have accumulated the

antifungal substances. Nevertheless, because the antimicrobial secondary metabolites are normally produced during the stationary phase of bacterial growth, the increased bacterial AE on the media with lower molasses concentration can be attributed to the increased production of antifungal substances over the incubation period (Vicente *et al.,* 2003). We assume the involvement of readily utilizable carbon metabolite, probably contained in molasses, which repress the biosynthesis of antifungal substances (Pham *et al.,* 2019). Therefore, the depletion of this repressor carbon over time resulted in the increased biosynthesis of antifungal substances and the subsequent antifungal efficiencies of bacterial isolates (Romero *et al.,* 2010).

2.7 Conclusions

The study conclude generally that a combination of molasses and fertilizer-grade ammonium sulphate can be used to replace relatively expensive laboratory-grade glucose and ammonium sulphate as sources of carbon and nitrogen, respectively in bacterial cultures for enhanced phosphate solubilisation and antifungal activity.

2.8 Recommendations

Based on the findings of this study, the following are recommended;

i. For preparation of Minjingu rock phosphate-based biofertilizers, incorporation of molasses at rate of 20 ml/L is required as a carbon source for achieving optimal P solubilisation by PSBs.
ii. Fertilizer-grade ammonium sulphate at rate of 0.5 g/L can be used as the nitrogen source for rock phosphate biofertilizers preparation.
iii. Further studies are recommended to investigate the ability of rock phosphate and PSB combination (Bio-rock P) on enhancing plant growth and phosphorus nutrition.

2.9 Acknowledgments

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2.10 References

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CHAPTER THREE

Manuscript 2

Co-applying Phosphate-Solubilising Bacteria, Minjingu Rock-phosphate, and Molasses (Bio-rock P) Enhances Maize Yield and Phosphorus Use Efficiency in Acid Soils

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3.1 Abstract

A study was conducted to evaluate the influence of co-applying phosphate solubilising bacteria (PSBs) and Minjingu rock phosphate (MPR) in molasses-based formulation for enhancing maize yield and phosphorus use efficiency. Field experiments were laid out in randomized complete block design in quadruplicates under two sites, namely Madaba and Magadu. Selected soil fertility parameters and P-sorption capacities were determined for each site. Experimental treatments were PSB inoculum co-applied with varying P rates (0, 20, 40, 60, and 80 kg P /ha) from MPR. Absolute and positive controls were 0 and 40 kg P /ha without PSB inoculum, respectively. Soil available P, maize grain yield, biomass yield, phosphorus uptake, and use efficiency were evaluated at harvest maturity. The results indicated that, soils of both study sites were acidic and deficient of nitrogen, phosphorus, and potassium. Magadu soil had higher P sorption capacities than Madaba

soils. Soil available P of both sites responded positively to increasing P rates from the applied RP+PSB. Unlike Magadu soil, sole applied PSB inoculum on Madaba soils increased soil available P to values statistically comparable to applying either sole MPR at 40 kg P /ha or 20 kg P /ha + PSB inoculum. The observation was attributed to PSBs ability to avail sorbed soil P and differences in soil P sorption capacity for the sites. This study concludes that, co-applied MPR and PSBs results into improved maize grain yield, P uptake and phosphorus use efficiency (PUE) compared to sole MPR application for both sites. PUE of maize plants is enhanced at lower RP rate (< 40 kg P /ha) for Madaba soils and at higher RP rate (≥ 40 kg P /ha) for Magadu soils. PSB inoculums can be used to enhance PUE of the applied RP and increase maize productivity in acid soils.

Keywords; Phosphate Solubilising Bacteria, Minjingu Phosphate Fertilizer, Maize Yield, Phosphorus Use Efficiency

3.2 Introduction

Maize is a major staple crop for over 80 % of the people in Tanzania, and is accounting for over 70 % of national starch requirement (Mtaki, 2019). Major growers of maize in the country are small-scale farmers whose production levels are unreliably meeting the crop requirements (Cugala *et al.*, 2012; Wilson and Lewis, 2015). In spite of the increasing trend in total production over a past decade, mainly due to extensification, the yields per unit area are still low. Current yields average about 1.6 Mg /ha and equates only to about 17.8 % to 26.7 % of attainable yield (FAOSTAT, 2019). With regard to the increasing maize consumption in the country, mainly due to rapid population growth (Mtaki, 2019), and the potential of Tanzania to be a breadbasket for the whole east African region (FAO, 2013), production of maize needs be elevated to meet these demands. However, there are several factors that are hindering intensification of maize production in Tanzania and sub-Saharan Africa, at large. Among others, low soil fertility has been frequently reportedly to constrain crop production for smallholder farms in sub-Saharan Africa, including Tanzania (Khosro and Yousef, 2012; Lobulu *et al.*, 2019).

Phosphorus deficiency has been among the serious challenge to crop production in most part of the Tanzania (Semoka and Kalumuna, 2000), mostly due to prevalence of soils with high Phosphorus-fixing capacities (Szilas, 2002; Msolla *et al.,* 2005). The implication from high P-fixing capacities of these soils is that, higher P doses are required to saturate P fixing matrices of the soil and to avail the excess for plant uptake (Wogi *et al.,* 2015; Margenot *et al.,* 2016). Therefore, the requirement of copious quantities plus the higher costs of water-soluble phosphate fertilizers possesses a problem of inadequate P replenishment, particularly in smallholder agriculture.

Correspondingly, direct application of locally available rock phosphate (RP) is not always agronomically effective due to its sparse solubility. Unless otherwise partially acidulated, the lower phosphorus use efficiencies and delayed agronomic effectiveness of the directly applied rock phosphate has been reported elsewhere (Bationo and Kumar, 2002; Margenot *et al.,* 2016). A potent solution to the low reactivity of RP is the inclusion of phosphate-solubilising microorganisms (PSMs) in the application package (Simfukwe and Tindwa, 2018; Taddese, 2019). In present scenario, there has been a focus toward the use of bio-fertilizers in agriculture which are environmentally friendly, sustainable and cost effective when rationally formulated (Khan *et al.,* 2014; Giro *et al.,* 2016; Dominguez-benetton *et al.,* 2018; Kitam *et al.,* 2019).

Bio-fertilizers are defined as the formulations containing living microorganisms or latent cells having the potential of colonizing roots of crop plants and promote the growth by improving nutrients availability and acquisition (Hassan and Bano, 2016). Phosphate based bio-fertilizers are made by combining the phosphate rocks, phosphate solubilising organisms (PSMs) and appropriate carriers or carbon sources (Khan *et al.,* 2014; Giro *et al.,* 2016). The PSMs in the mixture could help solubilize the insoluble P in RP and even the fixed P in soil while organic carbon source will sustain the life of these microorganisms.

These conditions can be perfectly met in Tanzania's agricultural environment, mainly because the phosphate rock deposits are present (Szilas, 2002; Msolla *et al.,* 2005) and the possibilities of finding the efficient PSM strains is higher in most P deficient soils (Simfukwe and Tindwa, 2018). However, there is limited knowledge on PSMs characterized and selected based on their ability to solubilize local phosphate rocks while utilizing locally available, naturally occurring organic carbon sources. Conceivably,

effective RP- solubilising microorganisms would present a low-cost alternative to water-soluble P fertilizers in the Tanzanian agricultural system. This research is therefore evaluated the effect of co-applying PSBs and minjingu rock phosphate in a molasses based-based formulation as a carrier.

3.3 Material and Methods

3.3.1 Study sites and soils

The locations of study areas and site characteristics are shown in Figure 3.1. Magadu site is located at the foot slopes of the Uluguru Mountain in Morogoro municipality in the Eastern Plateau and Mountain blocks of Tanzania (De Pauw 1984). Soils of the area are formed from gneiss parent material overlain with coastal sands on undulating to rolling topography and situated on low altitudes of 200-1000 m.a.s.l. (De Pauw, 1984).

The climate of Magadu is characterized as a warm sub-humid tropical type with bimodal rainfall distribution. The long rainfall season runs from March to May and the short rainfall season running from November to January. The average annual rainfall is around 950 mm, and the mean annual air temperature is about 24 °C.

Madaba site is in the southern highlands of Tanzania. The soils of the area are formed from gneiss parent material on hilly topography, and situated on high altitudes of 1 500-2 000 m.a.s.l., (De Pauw, 1984). Climate of Madaba district is generally described as warm sub-humid tropical with unimodal rainfall. The rains are reliable and fall between 800 mm and 1 200 mm per annum. The mean and maximum temperature ranges between 15 °C and 27 °C.

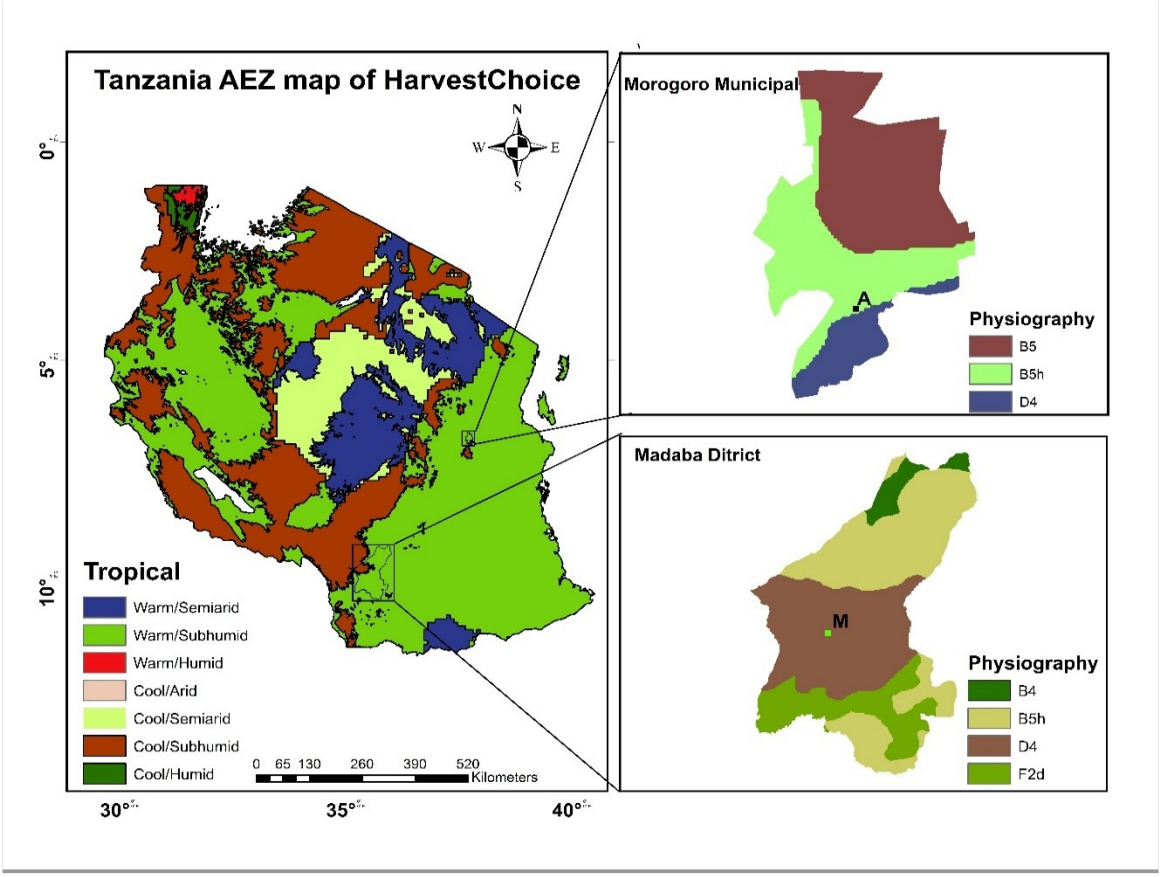

Figure 3.1: Locations of study areas overlain on the physiographic regions maps, and zoomed out from Tanzania Agroecological Zones (AEZ) map of Harvest Choice. Letter A indicate the location of Magadu farm and letter M indicate location of Madaba farm.

From the physiographic map of Tanzania, **B5** has gneiss parent material overlain with coastal sands on undulating to rolling topography, and situated at 200-1 000 m.a.s.l., **B5h** has gneiss parent material overlain with coastal sands on undulating with isolated hills topography, and situated at 200-1 000 m.a.s.l., **D4** has gneiss parent material on hilly topography, and situated at 1 500-2 000 m.a.s.l., **B4** has gneiss parent material overlain with coastal sands on undulating with gently undulating topography, and situated at 200-1 000 m.a.s.l., and **F2d** has sandstone and shale parent material on undulating with steeply dissected topography, and situated at 500-1 000 m.a.s.l., (De Pauw 1984).

3.3.1.1 Soil sampling, preparation, and analysis

Soil samples for general fertility evaluation were collected from the two studied sites. From each site, ten soil sub-sample portions were collected randomly from 1 ha area at the rooting depth of 0-30 cm and mixed to make 1 kg composite sample. The samples were air dried, ground and sieved through a 2 mm sieve.

The parameters measured include the soil pH, available phosphorus, particle size distribution, organic carbon content, CEC, exchangeable potassium, calcium, magnesium, and total nitrogen. The electrometric method was used to measure soil pH in 1:2.5 (weight/volume) soil: water suspensions (Okalebo, 1993). Available phosphorus was determined through Bray 1 extraction technique and colorimetric quantification by phosphomolybdate blue method as described by (Okalebo, 1993). Total nitrogen was determined by micro-kjeldah method. Particle size distribution was determined by the hydrometer method after dispersion with 5% sodium hexametaphosphate (Gee and Bauder, 1986) and soil textural class was determined using the United State Department of Agriculture (USDA) soil textural class triangle (United State Department of Agriculture, 1975). CEC and exchangeable bases (Ca^{2+} Mg^{2+}, K^{+} and Na^{+}) were determined by 1 M (pH 7) NH4 –acetate saturation method followed by displacing adsorbed NH^{4+} using 1 M KCl. CEC was then determined by quantifying the NH_4^{+} displaced by 1 M KCl whereas the Exchangeable Ca^{2+} and Mg^{2+} were quantified using an atomic absorption spectrophotometer (AAS) and exchangeable K^{+} using a flame photometer (Thomas, 1996). Organic carbon was determined by the Walkely and Black wet oxidation method (Nelson and Sommers, 1996).

3.3.1.2 Determination soil's phosphorus adsorption-desorption behaviour

The standard procedure developed by Nair *et al.* (1984) was used to study phosphate

sorption behaviour of Magadu and Madaba soils. Into a series of 50 ml plastic bottles, one gram of air-dry soils were added, separately for each sample. Twenty-five millilitres of solutions containing 5, 10, 20, 40, 60, 80, 100 mg/L P (prepared from KH_2PO_4 stock solution containing 100 ppm P) were added separately to each bottle. To each bottle, 0.001 M $CaCl_2$ solution and three drops of toluene were added to mimic the ionic conditions of the soil and to inhibit microbial activities, respectively. The mixtures were then allowed to equilibrate by shaking on an orbital shaker at 300 rpm for 24 hours. After equilibration, soil suspension was filtered through Whatman No. 42 filter paper and then P content of each filtrate was determined through colourimetric molybdate blue method (Murphy and Riley, 1962). The experiment was carried out in triplicate for each soil. The quantity of sorbed phosphate was calculated as the differences between quantities of P initially present in the solution and the P concentration in equilibrated solution. The adsorption data were fitted to the Langmuir equation and adsorption parameters, b (sorption maxima) and K_L (Affinity parameter) were estimated by using the linear least-squares regression, as described in (Essington, 2005).

$$\frac{Ceq}{q} = \frac{1}{bKL} + \frac{Ceq}{b} \quad \text{.....equation 3.1}$$

Where by, q is the concentration of sorbed P per unit soil weight (mg P/kg soil), Ceq is the equilibrium concentration of phosphorus in solution (mgL^{-1}), b is adsorption maxima (a constant related to soils sorption capacity), K_L is phosphate sorption energy (the affinity factor).

3.3.2 Materials used under this study

3.3.2.1 Phosphate Solubilising Microorganisms (PSMs)

PSMs are the soil microorganisms capable of transforming the insoluble phosphate (fixed P in soils and rock phosphates) into plant-available forms (Nisha *et al.*, 2014). In this

study, the previously isolated phosphate solubilising bacteria in Chapter 1 were used, as shown in Table 3.1. Generally, PSB isolates used in this study possess multiple plant growth-promoting traits, ranging from the ability to solubilize various insoluble phosphate sources such as tricalcium phosphate, ferric phosphate, and hard-minjingu rock phosphate, and to solubilize the insoluble compounds such as zinc carbonate. Other traits possessed by these isolates include; production phytohormone indole acetic acid (IAA) either in presence of tryptophan precursor, or for some isolates, in absence of tryptophan, production of iron-chelating compounds of siderophore type which may increase the bioavailabity of iron, production of ammonia which is one among the plant available nitrogen forms, and un-identified antifungal compounds which may safeguard plant protection against phytopathogenic soil-borne fungi (Ciancio *et al.,* 2019).

3.3.2.2 Minjingu phosphate rock (MPR)

The beneficiated hard minjingu phosphate rock was obtained from Minjingu Mines and Fertilizer Company located in Arusha region, Tanzania. The deposit for Minjingu phosphate is in the Eastern Rift Valley (Figure 1), near Lake Manyara around the foot slopes of a small inselberg that served as resting/nesting place for a large bird colony sometimes during Pleistocene (Szilas 2002). The deposit has two major ores, the hard and soft rock phosphate ore.

3.3.2.3 Sugarcane Molasses

Sugarcane molasses is the dark-colored and viscous syrup that remain as a by-product of sugarcane crystallization (Dahot and Simair, 2013). It has a high content of dry matter, with 47-48% sugars (sucrose, glucose, fructose), amino acids, fatty acids, and mineral elements which could stimulate bacterial growth (Nikodinovic-Runic *et al.,* 2013). The carriers are the means of delivering viable microbes from the factory to the field (Bashan,

1998). According to Khan *et al.* (2014), a good carrier should support the growth and survival of microbes, high in organic matter, non-toxic, mixable, packageable, inexpensive, and locally available. The locally available organic resources such as sugarcane molasses (Bakari, 2018), are the potential carriers.

3.3.2.4 Preparation of bio-rock P inoculum

The inoculum was prepared at the Soil Science Laboratory of the Sokoine University of Agriculture. For this, PSB isolates were retrieved from deep freezer (-80 ˚C) and aseptically inoculated into nutrient broth media in 50 ml capacity flasks and incubated on a shaking incubator at 28 ± 1 ˚C for five days. For the purpose of multiplication of PSBs, the starter bacterial cultures were prepared. Briefly, each bacterial isolate was separately and aseptically inoculated into 100 ml capacity flasks containing previously prepared sterile double modified synthetic minimal medium (dSMM). dSMM was prepared by adding molasses 20 ml, sulphate of ammonia fertilizer 0.5 g, hard minjingu rock phosphate 10 g, $MgSO_4$ 0.1 g, KCl 0.2 g 0.00001 g, $FeSO_4$ and MnSO4 0.00001 g, per liter of distilled water and its pH adjusted to 7 ± 0.2. The inoculum was incubated in a shaking incubator for five days and then transferred to larger (5 L) capacity flasks at the rate of 2 % of the medium and then re-incubated for 2 days. Thereafter, a co-culture of all isolates was prepared by transferring 5 % of each PSB culture into single 10-L capacity bottles containing a previously and then re-incubated for 7 days.

Table 3.1: Identity of isolated RP-solubilising species based on nucleotide database on American National Institutes of Health (NIH) NCBI genetic sequence database (GenBank)

Isolate	Species	Accession Number	Nucleotide Identity	Country	Source rhizosphere
Fg1	*Fusarium proliferatum*	MZ497514	100	Tanzania	Maize
SL-Sp1	*Klebsiella sp.*	MZ502674	99.8	Tanzania	Sweet potato
Mk10	*Burkholderia sp.*	MZ502221	99.9	Tanzania	Maize
NA19a	*Klebsiella sp.*	MZ502673	99.8	Tanzania	Maize
NA4a	*Unidentified*			Tanzania	Irish potato
NA4b	*Klebsiella sp.*	MZ502671	99.8	Tanzania	Irish potato
SUApp 3	*Klebsiella sp.*	MZ502675	99.7	Tanzania	Sweet pepper
MdG1	*Klebsiella variicola*	MZ502670	99.8	Tanzania	Banana
Kjm3	*Burkholderia sp.*	MZ502220	99.9	Tanzania	Maize
MbMz1	*Klebsiella sp.*	MZ502668	99.8	Tanzania	Maize
NA5	*Klebsiella variicola*	MZ502672	99.8	Tanzania	Sweet potato
MdE4	*Klebsiella variicola*	MZ502669	100	Tanzania	Common bean

3.3.3 Field experiments

Field experiments were carried out in two sites; one in Madaba, Ruvuma region and another site in Magadu, Morogoro region. At both sites the influence of Bio-rock P fertilizer on maize plant growth, yields, phosphorus uptake end use efficiency were evaluated. The experiments were laid out in a randomized complete block design (RCBD) with treatments comprised of varying P rates from Minjingu rock phosphate and PSB inoculum. The treatment structure was 0 kg P /ha (absolute control), 0 kg P /ha + PSB inoculum, 20 P /ha + PSB inoculum, 40 kg P /ha (positive control), 60 P /ha + PSB inoculum, and 80 P /ha + PSB inoculum. The granulated rock phosphate was applied through placement to the planting holes, buried 2 cm deeper than the sowing depth. Bacterial inoculum containing 10^9 cells per ml was applied at the rate of 10 L of per ha.

The inoculum was first diluted in 1:20 (inoculum: water ratio), and then 5 m*l* of the mixture was applied to each planting hole. The seeds of certified Aminika maize variety were planted with the spacing of 75 cm × 30 cm. To control other nutritional factors, basal nutrients were applied based on the results of soil analysis. Yara Amidars (40 % N and 5.5 % S) was used as the source of nitrogen and sulphur, which were deficient in the soils. The available phosphorus content of the soil, total P uptake by the maize plants, maize grain yield, and biomass yield were determined at the harvest maturity of the crop. The phosphorus use efficiency (PUE) of maize crop under each treatment was calculated through Partial Nutrient Balance (PNB) (Syers *et al.,* 2008) as indicated in Equation 3.2.

$$PUE = \frac{UP}{FP} \times 100\,\% \text{.....equation 3.2}$$

Whereby UP is total P in the crop, and FP is the amount of P supplied, expressed as a percentage (Syers *et al.,* 2008).

3.4 Statistical Analysis

The GenStat statistical package was used for the Analysis of Variance (ANOVA) and comparison of means for soil available P, plant P uptake, and yields was done by Duncan New Multiple Range Test (DMRT) at 5 % significance level.

3.5 Results and Discussion

3.5.1 The selected physicochemical properties of Magadu and Madaba site soils

The selected properties of the soil used in pot experiment are shown in Table 3.2. The soil textural classes of Magadu and Madaba soils were assigned accordingly to clayey and sandy clay, based on soil textural triangle of the United State Department of Agriculture (1975). In comparison, sandy clay soils are more suitable for growth of maize plants since it allows easy root penetration and water movement whereas while clayey soils have very high-water holding capacity, the plant root penetration and water movement may be

impaired (Weil and Brady, 2017).

The pH of the studied soils was very strongly acidic for Magadu and strongly acidic in Madaba. Refereeing to maize crop requirement, soil pH of Magadu site was not suitable and of Madaba site was marginally suitable for growth and production of maize (Landon, 1991). Acidic soil reactions are reportedly associated with interference of not only soil microbial activities but also essential nutrients bio-availability, including phosphorus, molybdenum and exchangeable bases (Weil and Brady, 2017). Therefore, soil amendments aiming at raising pH are recommended. According to Msolla *et al*. (2005), Szilas *et al.* (2007), and Kalala (2011), direct application of minjingu rock phoshate has a liming effect on soil pH and so recommended.

The studied soils of both experimental sites had low available phosphorus contents. P is probably is fixed on the surfaces of sesquioxides and kaolinitic clays which are reportedly abundant in acid soils of Tanzania (Szilas *et al.,* 2007). Total nitrogen content of both soils was very low and for that reason, application of basal N fertilizer was considered essential during field experiments.

The organic carbon content of Magadu soil is low and therefore marginally suitable for production of growth and production of maize. For Madaba soil the OC was in the medium range. According to Yang *et al.* (2019), organic matter content of the soil affects the bio-availability of phosphorus to plants through reduction of soil P sorption capacity. Moreover, optimal soil organic matter carbon, and so organic content, has an implication on soil microbial activities. According to Kumar and Rai (2020), abundance and activities of phosphate solubilising bacterial are dependent by soil organic carbon content.

Table 3.2: Selected properties of the soils of Magadu and Madaba sites, used in this study

Site	Clay	Silt	Sand	Texture	pH H2O	OC	*Av. P	TN	K	Ca	Mg	CEC
Unit	(%)	(%)	(%)		(1:1.25)	(%)	mg/kg	(%)	mg/kg	mg/kg	mg/ kg	$Cmol_c\ kg^{-1}$
Magadu	56	9	35	Clayey	4.9	0.9	3.34	0.08	1.41	1.29	1.20	10.70
Madaba	43	11	46	Sandy Clay	5.2	1.4	3.90	0.09	2.75	1.80	1.08	17.40

*Available P based on Bray 1 extraction method

3.5.2 Phosphorus adsorption behaviour of studied soils

From the experimental data, the phosphate adsorption maxima (b) of soils were determined through linear least square method. Figure 3.2 shows P adsorption properties of the studied soils. Table 3.3 show the adjustable parameters of the fitted Langmuir model for phosphorus adsorption isotherms of both experimental sites. Soil of Magadu had relatively higher adsorption maxima (769.23 mg P/kg) as compared to the soil of Madaba (185.185 mg P/kg). Higher adsorption maxima indicate higher capacity of soils to adsorb most of the phosphate ions from soil solution or from the applied phosphate fertilizer (Syers *et al.*, 2008).

Based on adsorption interpretation by Weil and Brady (2017), Magadu soil is considered a high phosphorus fixing soil since it removes more than 350 mg P/kg of soil while Madaba soil are considered low P-fixing. The relatively higher adsorption capacity of Magadu soil can be attributed to higher contents of kaolinite clays, oxides and hydroxyoxides of iron and aluminium, previously reported by Szilas (2002) and Msanya *et al.* (2004).

Phosphorus adsorption characteristics of the Magadu soil have been previously determined by (Szilas, 2002) where by the experimentally determined adsorption maxima (528 mg P/kg) was significantly different from the adsorption maxima estimated through Borggaard pedotransfer function (896 mg P/kg). Therefore, the estimated adsorption maxima under this study are more comparable to Borggaard pedotransfer function (769.23 mg P/kg). However, the observed variations are ascribed to methodological differences.

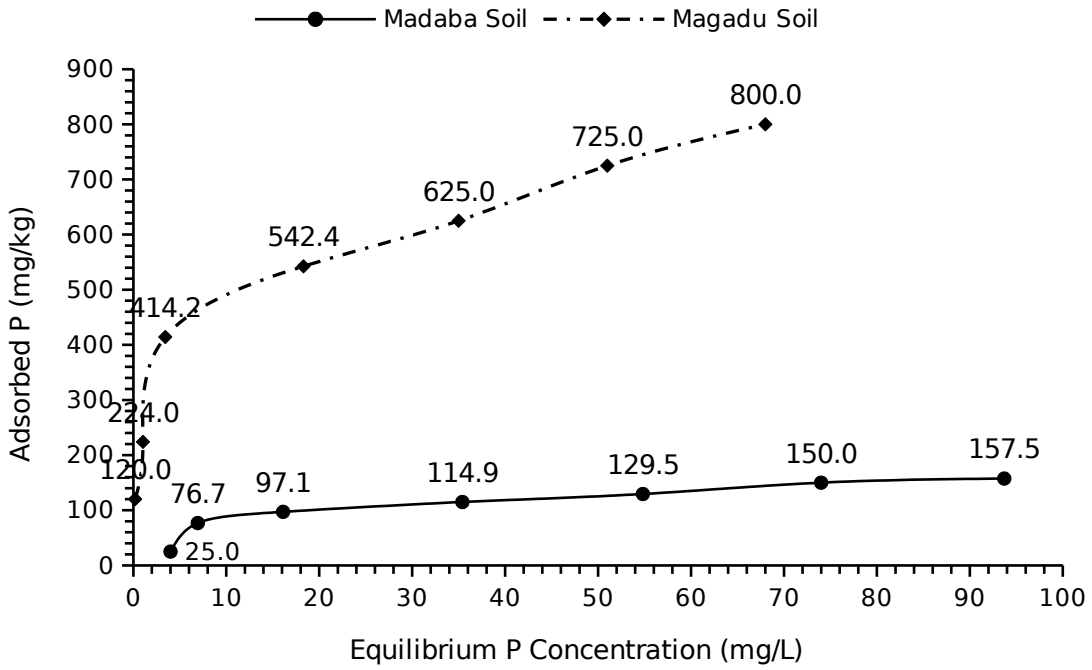

Figure 3.2: **Phosphorus adsorption properties of soils in Madaba and Magadu experimental sites**

Table 3.3: Phosphate sorption isotherm parameters for surface soils of Madaba and Magadu

Site	Slope	Y-intercept	R^2	B* (mg/kg)	K_L (Lkg^{-1})
Madaba	0.0054	0.0985	0.981	185.185	0.0548
Magadu	0.0013	0.0054	0.975	769.23	0.2407

*Where by, b is the adsorption maxima, and K_L is the affinity parameter of the soils in each site

According to Weil and Brady (2017), phosphorus adsorption capacity of the soil is a function of clay content of the soil, clay mineralogy, soil pH, and organic matter content. The soils with higher clay content typically have higher phosphorus adsorption capacity compared with the low clay soils assuming similar clay mineralogy. For acidic soils, phosphorus adsorption capacity is higher with the lowering of soil pH.

Organic matter is known to reduce soil P fixation capacity. In the present scenario, the properties of Magadu soil are in favour of high P fixation as compared to Madaba soil. The magnitude of the initial slope of the isotherms, which describes the relative affinity of soils to phosphorus, were estimated as the affinity parameter (K_L) of the fitted Langmuir models. Similar to the adsorption maxima, soils of Magadu had relatively higher affinity to phosphorus than the soils of Madaba.

3.5.3 Effect of Bio-rock P rates on soil available phosphorus after a growing season

The available phosphorus content of the soils increased significantly ($p < 0.05$) in the plots treated with any P dose from bio-rock phosphate fertilizer as compared to the absolute control at both experimental sites (Figure 3.3). For both sites, the soil available P of control plots were low.

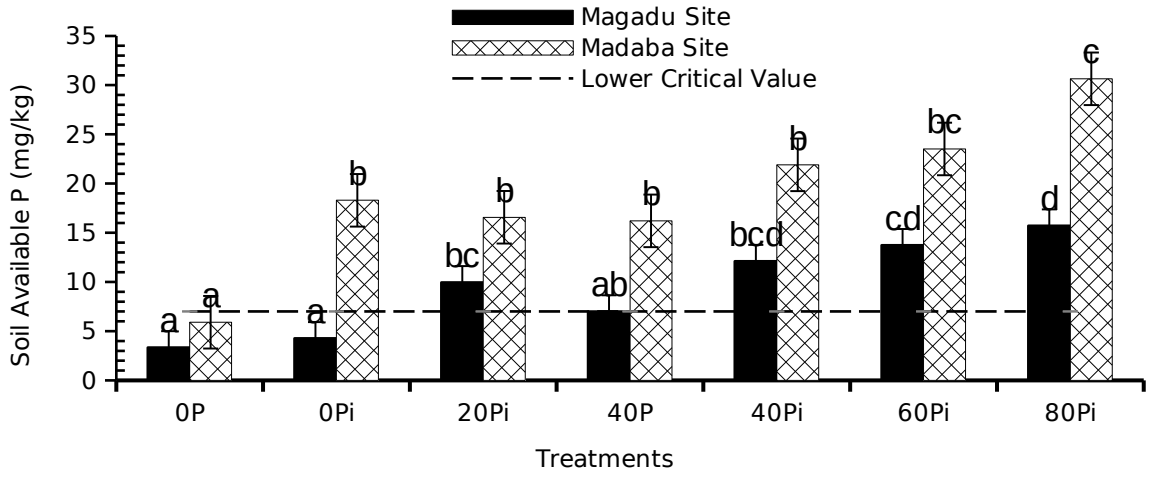

Figure 3.3: **Effects of application of P levels as Bio-rock P on soil available P content (kg P kg–1) (mean values) after a growing season at Madaba and Magadu sites.**

Critical values used here are based on Landon, (1991). Treatment comparisons was done separately for across sites and treatments which share at least one letter in each bar graph are not statistically different at 0.5 % significance level. Treatment definitions; 0P = 0 kg P /ha (absolute control), 0Pi = 0 kg P /ha + PSB inoculum, 20Pi = 20 P /ha + PSB inoculum, 40P = 40 kg P /ha (positive control), 60Pi = 60 P /ha + PSB inoculum, and 80Pi = 80 P /ha + PSB inoculum.

Besides, soils of Madaba site demonstrated a significantly higher available P as compared to soils of Magadu site, at all rate of applied P. The observation can be ascribed to differences in soil's phosphorus adsorption capacities, where by Magadu soil has higher phosphate maxima (769.23 mg/kg) than the soils of Madaba site (185.185 mg/kg). Therefore, a large portion of the applied phosphate plus that released from microbial activities in Magadu soil became plant unavailable, due to strong adsorption on surfaces of ferric and aluminum oxides and kaolinitic silicate clays (Essington, 2005) which are dominant in soils of Magadu (Szilas, 2002; Msanya *et al.,* 2004). Since soil of Magadu is highly P-fixing, it maintains low phosphorus concentration in soil solution (low available P).

The study also showed that, application of PSB inoculum alone or combined with rock phosphate at lower rate (20 kg P /ha), results into higher soil available P that is not significantly different ($p < 0.05$) from the positive control (recommended rate, 40 kg /ha of P from Minjingu fertilizer) at Madaba site.

In unforeseen scenario, the soil available phosphorus of the plots treated with PSB inoculum alone were as higher as those treated with the combination of the inoculum and RP at either 20 kg P /ha or 40 kg P /ha. We ascribe this observation to the ability of these bacteria to solubilize ferric phosphate invitro (as demonstrated in Chapter 1) which is one among the fixed forms of P in acid soils (Weil and Brady, 2017).

However, the soil available P of Magadu site did not respond significantly to PSB inoculum alone until when combined with the Minjingu rock phosphate at 20 kg P /ha. The observed disparity of microbial ability to increase available P for to studied soils can be attributed to the differences in inherent soil properties. It is however difficult to point

out the specific soil parameters. Most probably, the differences in soil P adsorption capacities may have affected microbial ability to avail fixed soil P; the bacteria could not effectively avail the tenaciously held P in soils of Magadu. Furthermore, factors that could enhance microbial survival i.e., soil organic matter content and availability of other nutrients, may have affected the ability of bacteria to unfix the otherwise unavailable P in soils. Therefore, further studies are recommended to investigate the observed gaps.

3.5.4 Effect of P rates from Bio-rock phosphate fertilizer on maize P uptake and use efficiency (PUE)

Total phosphorus uptake and its accumulation in straw and grains by maize plants were evaluated. Generally, there were significant differences in the total P uptake between the treatments ($p < 0.001$), in both experimental sites (Table 3.4). All levels of rock phosphate application (with or without PSB inoculum) resulted into significant increase in total P uptake compared to the absolute control (0 kg P /ha). Furthermore, total P uptake was increasing along with the increasing rates of P from Bio-rock phosphate. For both Madaba and Magadu soils, the highest P uptake by maize plants, 25.35 kg P /ha and 36.12 kg P /ha, were obtained plots treated with 80 kg P /ha + PSB inoculum, respectively for the sites.

Table 3.4: Effects of application of different rates of P, 20, 40, 60, and 80 kg P ha–1 as Bio-rock Phosphate on grain P, biomass P and total P uptake (mean values) at Madaba and Magadu sites

Site	Madaba^			Magadu		
Treatment	**Grain P**	**Straw P**	**Total P uptake**	**Grain P**	**Straw P**	**Total P uptake**
unit	(%)	(%)	(kg /ha)	(%)	(%)	(kg /ha)
»0P	0.15[a #]	0.05[a]	9.99[a]	0.17[a]	0.034[a]	5.41[a]
0Pi	0.27[bc]	0.06[ab]	21.42[c]	0.26[ab]	0.037[a]	11.59[b]
20Pi	0.24[b]	0.06[ab]	22.25[cd]	0.33[bc]	0.049[a]	15.77[b]
40P	0.22[b]	0.06[ab]	17.47[b]	0.26[ab]	0.053[a]	13.63[b]
40Pi	0.24[bc]	0.06[ab]	22.57[cd]	0.44[cd]	0.052[a]	24.74[c]
60Pi	0.25[bc]	0.08[bc]	23.75[cd]	0.53[de]	0.059[a]	28.68[c]
80Pi	0.28[c]	0.09[c]	26.35[d]	0.62[e]	0.105[b]	36.12[d]
s.e.d	0.019	0.0118	1.875	0.07	0.017	2.52
Significance	*	*	*	*	*	*

*Significant differences between treatments at 0.5 % significance level,

[#]Treatments which share at least one letter in each column are not statistically different.

^Treatment comparisons were done separately for across sites

» Treatment definitions; 0P = 0 kg P /ha (absolute control), 0Pi = 0 kg P /ha + PSB inoculum, 20Pi = 20 P /ha + PSB inoculum, 40P = 40 kg P /ha (positive control), 60Pi = 60 P /ha + PSB inoculum, and 80Pi = 80 P /ha + PSB inoculum

In comparison with the maize grown on soils of Madaba, the trend of P uptake was steeply increasing with the increase in P rates in soils of Magadu (Table 3.4). The disparity between the two sites is due differences in response of soil available P to the applied fertilizer (Figure 3.2). Due to high P sorption capacities and bonding energies of Magadu soils (Figure 3.1), the additional effect of applied RP on soil available P (Figure 3.2) was very high. However, in the soils of Madaba, lower RP rates combined with PSB inoculum resulted into sufficient soil P contents and therefore any addition of higher P rates had little effect on plant P uptake.

The influence of PSB inoculum on total P uptake was evaluated by comparing the results of the plots treated with sole RP at 40 kg P /ha and that of the plots with equal P rate but treated with PSB inoculum. For both Madaba and Magadu sites, P uptake varied significantly; being higher in the plots with inoculum (22.57 and 24.74 kg P /ha) and lower in the plots without the inoculum (17.47 and 13.63 kg P /ha), respectively for the experimental sites. Yet, unlike for Magadu site where the increase was very slight, applying RP at 20 kg P /ha + PSB inoculum resulted into higher P uptake than application of sole RP at 40 kg P /ha. Therefore, the influence of PSB inoculum on increased P uptake by maize plants is credited. We ascribe the observed effect of PSB inoculum not only to their ability of increasing phosphorus bio-availability through RP and soil reserve P solubilisation but also their stimulating effect on plant growth through production of phytohormones (Khan *et al.,* 2014; Kalayu, 2019).

The results for the grain and straw phosphorus accumulation by maize plants are presented in 3.4. The grain and straw P contents of maize plants responded significantly to the increasing P doses from Bio-rock. In case of grain P contents, the highest value was registered in plots treated with 80 kg P /ha + PSB inoculum in the soils of Magadu. Furthermore, the highest straw P contents were recorded in the plots treated with 80 kg P /ha + PSB inoculum, in Magadu. From this observation, it is apparent that for the soils of Magadu, application of higher P rates results in to larger P accumulation by plants. However, from the economic point of view, it is tricky when the nutrient accumulation is not associated with yield increment. Therefore, evaluation of nutrient use efficiency, phosphorus in this case, was thought to be necessary.

Phosphorus use efficiency by maize plants as influenced by the increasing P rates from bio-rock was assessed and compared to the sole application of Minjingu phosphate rock. The results are indicated in Figure 3.4, and PUE was found to vary significantly with different treatments in both experimental sites.

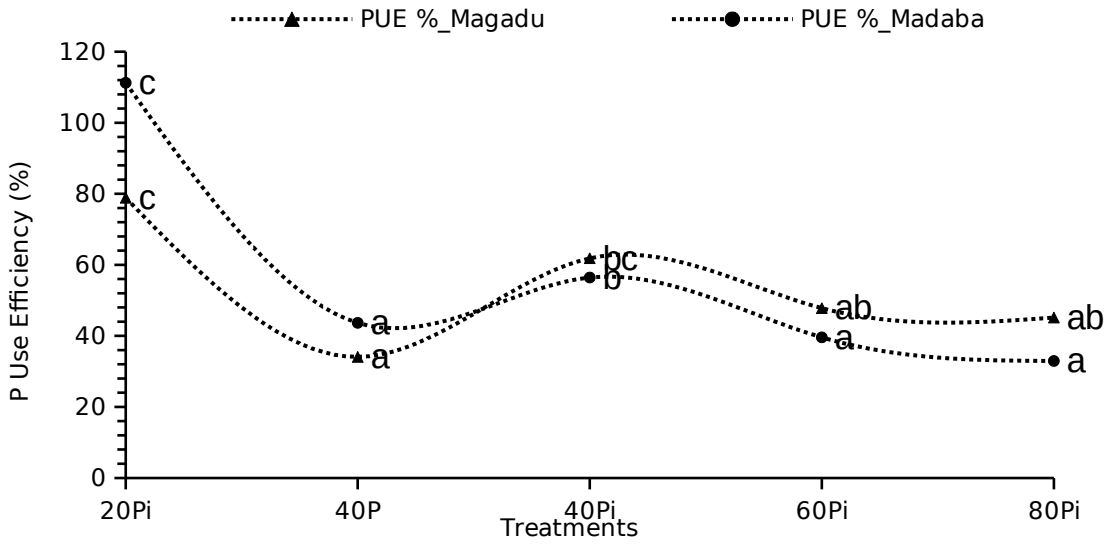

Figure 3.4: **Effects of application of different rates of P, 20, 40, 60, and 80 kg P /ha as Bio-rock maize Phosphorus Use efficiency (PUE based on PNB method) (mean values) in two experimental sites.**

Treatment comparisons were done separately across sites and treatments which share at least one letter in each line graph are not statistically different at 0.5 % significance level. Treatment definitions; 0P = 0 kg P /ha (absolute control), 0Pi = 0 kg P /ha + PSB inoculum, 20Pi = 20 P /ha + PSB inoculum, 40P = 40 kg P /ha (positive control), 60Pi = 60 P /ha + PSB inoculum, and 80Pi = 80 P /ha + PSB inoculum.

Generally, by comparing the P rates from Bio-rock, the PUE was higher at lower P rate (20 kg /ha) and it was decreasing with an increasing P application rate in both sites. Moreover, comparison of PUE of two sites reveals that lower P rate from bio-rock (< 40 kg P /ha) results into higher PUE of the maize plants grown on soils of Madaba and lower PUE for maize plants grown on Magadu soils. The converse is true for the higher bio-rock P rate (≥ 40 kg P /ha), where the PUE of Madaba was increasingly lower and that of Magadu was increasingly higher when compared to each other.

According to López-Arredondo et al. (2017), plant's macro-nutrient use efficiency is a highly complex trait determined by various factors, ranging from intrinsic plant factors,

environmental factors e.g., rainfall, temperature, soil reaction, soil texture, and availability of other essential nutrients. Therefore, the observed disparities in PUE of two studied sites can be attributed to effect of factors other than soil and applied P.

At Madaba site, application of 20 kg P /ha resulted into highest PUE (111.24 %), a value higher than 100 %. According to Syers *et al.* (2008), when the PUE value derived through PNB method surpasses hundred percent then the plants should have accumulated more P from soil reserves than it is from the applied fertilizer. This has an implication on the depletion of soil P reserves, when the reserve is low. These results also align with the observation of higher P uptake by maize plants grown in the plots treated with PSB inoculum without RP (Table 3.4). Therefore, it is essential to consider the analysis of soils' total P and subsequent replenishment when the reserve is not enough. In a view of the finite nature of global rock phosphate reserves (Kisinyo and Opala, 2020) and the projected likelihood of their depletion (Walan *et al.,* 2014), we spot out the observed increase of soil available P, plant P uptake, and maize yield in the plots treated with sole PSB inoculum as an untapped opportunity for increasing bio-availability of fixed P in tropical soils.

Compared to sole RP at 40 kg P /ha, co-application of PSBs and equal RP rate (40 kg P /ha + PSB inoculum) increased phosphorus uptake and use efficiency significantly, in both experimental sites. This is an important piece of evidence to the beneficial effects of phosphate solubilising bacteria on enhancing both P acquisition and efficient utilization by maize plant. Several mechanisms deployed by PSBs to increase phosphorus availability and utilization by plants have been reported in the literature. The direct strategy is through increasing the bio-availability of otherwise insoluble phosphates, both from the applied rock phosphate and from the soil reserve (Khan *et al.,* 2014). The

bacteria achieve this by releasing to the rhizospheric micro-environments, various low molecular weight organic acids, exopolysaccharides, siderophores, and protons that either through chelation to phosphate-associated metal ions (Fe and/ Al) or lowering the pH, frees the phosphate ions for plant uptake (Khan *et al.*, 2014; Alori *et al.*, 2017; Kalayu, 2019; Mącik *et al.*, 2020). The indirect mechanisms used by PSBs include production of phytohormones, particularly indole acetic acid (IAA), which stimulate plant root growth and therefore increasing the ability of plants to acquire nutrients from the soil (Ramaekers *et al.*, 2010). Besides, PSBs increase plant competence by helping to fight against various soil-borne harmful phytopathogenic fungi (Ciancio *et al.*, 2019). Plant physiological competence and vigour are among the factors influencing internal nutrient utilization (including phosphorus) and therefore use efficiency (Raghothama and Karthikeyan, 2005; Richardson *et al.*, 2011; Hawkesford *et al.*, 2014; Noushahi *et al.*, 2019; Cristina *et al.*, 2020). Therefore, we attribute the increased maize phosphorus use efficiency in the plots treated with PSB isolates to the indict effect of these bacteria on enhancing plant growth through production of IAA, siderophore, ammonia, and antifungal substances, since these traits are possessed by PSBs under current study (citing first paper).

3.5.4 Maize grain yield and biomass yield responses to varying P rates from Bio-rock phosphate fertilizer

The results for the response of maize grain yield and biomass yield responses to bio-rock P fertilization are indicated in Figure 3.5 and Table 3.5 respectively. In both experimental sites, maize grain yields increased significantly ($p < 0.05$) for all rates of P as compared to absolute control. Generally, the yields at Madaba site were higher for all treatments as compared to the yield at Magadu site. The variation in maize yield performances in these sites can be attributed to factors other than soil phosphorus availability, since maize productivity is dependent on multiple factors such as availability of moisture and other

essential plant nutrients (Taiz *et al.*, 2015; Weil and Brady, 2017).

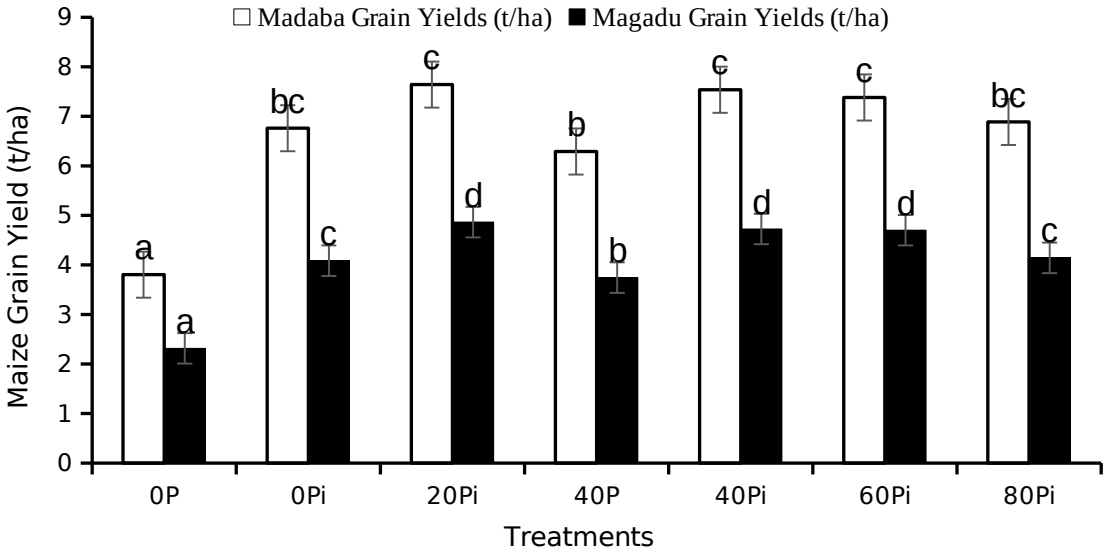

Figure 3.5: **Effect of varying P rates from Bio-rock P maize grain yield at harvest maturity.**

Treatment comparisons were done separately for across sites and treatments which share at least one letter in each bar graph is not statistically different at 0.5 % significance level. Treatment definitions; 0P = 0 kg P /ha (absolute control), 0Pi = 0 kg P /ha + PSB inoculum, 20Pi = 20 P /ha + PSB inoculum, 40P = 40 kg P /ha (positive control), 60Pi = 60 P /ha + PSB inoculum, and 80Pi = 80 P /ha + PSB inoculum

Furthermore, application of PSB inoculum alone or combined either 20 kg P /ha or 40 kg P /ha from MPR resulted into grain yields which are significantly higher than the yield of positive control (sole MPR at 40 kg P /ha). In summary, co-applying PSB inoculum and RP at either 20 kg P /ha or 40 kg P /ha increased maize grain yield by double and quarter when compared respectively to the absolute control and positive control (sole RP at 40 kg P /ha) in both experimental sites. This demonstrates the beneficial effect on phosphate solubilising bacteria on promoting plant growth and yield, through increased availability of soil P and other nutrients or through the release of plant growth promoting substances, such as IAA (Cueva-Yesquén *et al.*, 2021).

Table 3.5: Effects of application of different Bio-rock P rates on maize straw weight, cob weight and above ground biomass yields (mean values) at Madaba and Magadu sites

Site	Madaba^			Magadu		
Treatment	Straw Weight (t /ha)	Cob Weight (t /ha)	Biom. Yield t /ha	Straw Weight (t /ha)	Cob Weight (t /ha)	Biom. Yield t /ha
0P	2.8[a *]	1.38[a]	4.2[a]	3.42[a]	1.17[a]	4.6[a]
0Pi	4.3[b]	1.83[b]	6.1[b]	3.58[ab]	1.20[a]	4.8[a]
20Pi	4.5[b]	1.89[b]	6.4[bc]	3.94[b]	1.21[a]	5.1[ab]
40P	4.1[b]	1.69[b]	5.8[b]	3.89[b]	1.35[a]	5.4[b]
40Pi	5.7[c]	1.91[b]	7.6[d]	4.91[c]	1.39[a]	6.3[c]
60Pi	5.3[c]	1.73[b]	7[cd]	4.94[c]	1.43[a]	6.4[cd]
80Pi	5.8[c]	1.76[b]	7.6[d]	5.4[d]	1.44[a]	6.9[d]
s.e.d	0.303	0.102	0.328	0.191	0.13	0.264

*Significant differences between treatments at 0.5 % significance level,

[#]Treatments which share at least one letter in each column are not statistically different.

^Treatment comparisons were done separately for across sites

[»] Treatment definitions; 0P = 0 kg P /ha (absolute control), 0Pi = 0 kg P /ha + PSB inoculum, 20Pi = 20 P /ha + PSB inoculum, 40P = 40 kg P /ha (positive control), 60Pi = 60 P /ha + PSB inoculum, and 80Pi = 80 P /ha + PSB inoculum

The above ground biomass yields and straw weights of both experimental sites varied significantly in an increasing tendency with additional P rates from Bio-rock. The increased biomass production by maize plat is associated with optimal nutrient availability, uptake, and utilization. Physiologically, phosphorus is involved in plant biomass production through its involvement in various metabolic activities such as photosynthesis, energy storage and transfer, and being an essential constituent of nucleotides, nucleic acids, and phospholipids (Marschner, 2002). The response to plants to P inadequacy typically ranges from biochemical to morphological level (Weissert and

Kehr, 2017). According to (Kumar, 2013), phosphorus in plants leads to reduced plant photosynthetic activities which may lead to reduced biomass production. By linking the variability of soil available P contents in Figure 3.2 and maize plant biomass accumulation in Table 3.4, the role of supplied P can be credited.

Furthermore, maize biomass yields in plots with sole Minjingu fertilizer at 40 kg P /ha and that of plots treated with bacterial inoculums, either alone or combined with 20 kg P /ha, were statistically comparable. This observation can over again be attributed to the ability of PSB isolates to increase plant-availability of insoluble P from the applied RP and from soil reserve. Several studies have reported the effect of PSB inoculation on increasing plant biomass production (Hameeda *et al.,* 2008; Khan, Zaidi, Musarrat *et al.,* 2014; Mukhtar *et al.,* 2017).

3.6 Conclusions

i. Co-application of Minjingu rock phosphate and PSB inoculums (Bio-rock) results into increased soil available P, maize P uptake, P use efficiency and subsequently maize yields in acid soils of Magadu and Madaba.
ii. Application of sole PSB inoculum in the soils of Madaba enhances the bio-availability of native P in soil reserves, and accordingly increasing plant P uptake and yield.
iii. P use efficiency of maize plants is enhanced at lower RP rate (< 40 kg P /ha) for Madaba soils and at higher Bio-rock rate (≥ 40 kg P /ha) for the soils of Magadu.
iv. The ability of bacterial isolates to avail the otherwise fixed soil P is variable among different soils, due to differences in soil properties, particularly phosphorus adsorption capacity.

3.7 Recommendations

i. It is recommended, for enhanced maize productivity and efficient use of phosphorus, co-use of PSB inoculum and Minjingu RP at 20 kg P /ha for the soils of Madaba and 40 kg P /ha for the soils of Magadu.

ii. However, due to the observed large disparities on maize crop performances in two study sites, we recommend multi-seasonal tracking of treatments effects in the current study areas and further field trials on different soil types.

iii. Economic analysis of using Bio-rock P has not been covered by the current study, and therefore recommended.

iv. Moreover, to unleash the potential of using sole PSB inoculums application in Tanzania's soils with high P sorption capacities, we recommend further studies on PSB's ability to un-fix P from the surfaces of laboratory grade P-fixing minerals that are dominant in tropical soils and from native soil P-fixing soils.

3.8 Acknowledgement

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CHAPTER FOUR

GENERAL CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

i. Bacteria from the rhizosphere of various crop plants are possessing multi-functional plant growth-promoting traits, ranging from the ability to solubilize various P sources, phytohormones production, siderophore production, and antifungal activity.

ii. The locally available organic carbon sources, molasses in this case, and fertilizer-grade sulphate of ammonium sulphate can be used to replace relatively expensive laboratory-grade glucose and ammonium sulphate, respectively as sources of carbon and nitrogen in bacterial cultures for enhanced phosphate solubilisation.

iii. Bacterial isolates could maintain their antifungal activities against phytopathogenic *Fusarium polyferatum fg1* when grown on agar media with molasses and fertilizer grade nitrogen as the sole carbon and nitrogen sources, respectively.

iv. Co-application of Minjingu rock phosphate and PSB inoculums (Bio-rock) results into increased soil available P, maize P uptake, P use efficiency and subsequently maize yields in acid soils of Magadu and Madaba.

v. Application of sole PSB inoculum in the soils of Madaba enhances the bio-availability of native P in soil reserves, and accordingly increasing plant P uptake and yield.

vi. P use efficiency of maize plants is enhanced at lower RP rate (< 40 kg P /ha) for Madaba soils and at higher Bio-rock rate (≥ 40 kg P /ha) for the soils of Magadu.

vii. Soil properties, particularly phosphorus adsorption capacity can influence the ability of PSB to avail the fixed P in soils.

4.2 Recommendations

i. It is recommended, for enhanced maize productivity and efficient use of phosphorus, co-application of PSB inoculum and Minjingu RP at 20 kg P /ha for the soils of Madaba and 40 kg P /ha for the soils of Magadu.

ii. However, due to the observed large disparities on maize crop performances in two study sites, we recommend multi-seasonal tracking of treatments effects in the current study areas and further field trials on different soil types.

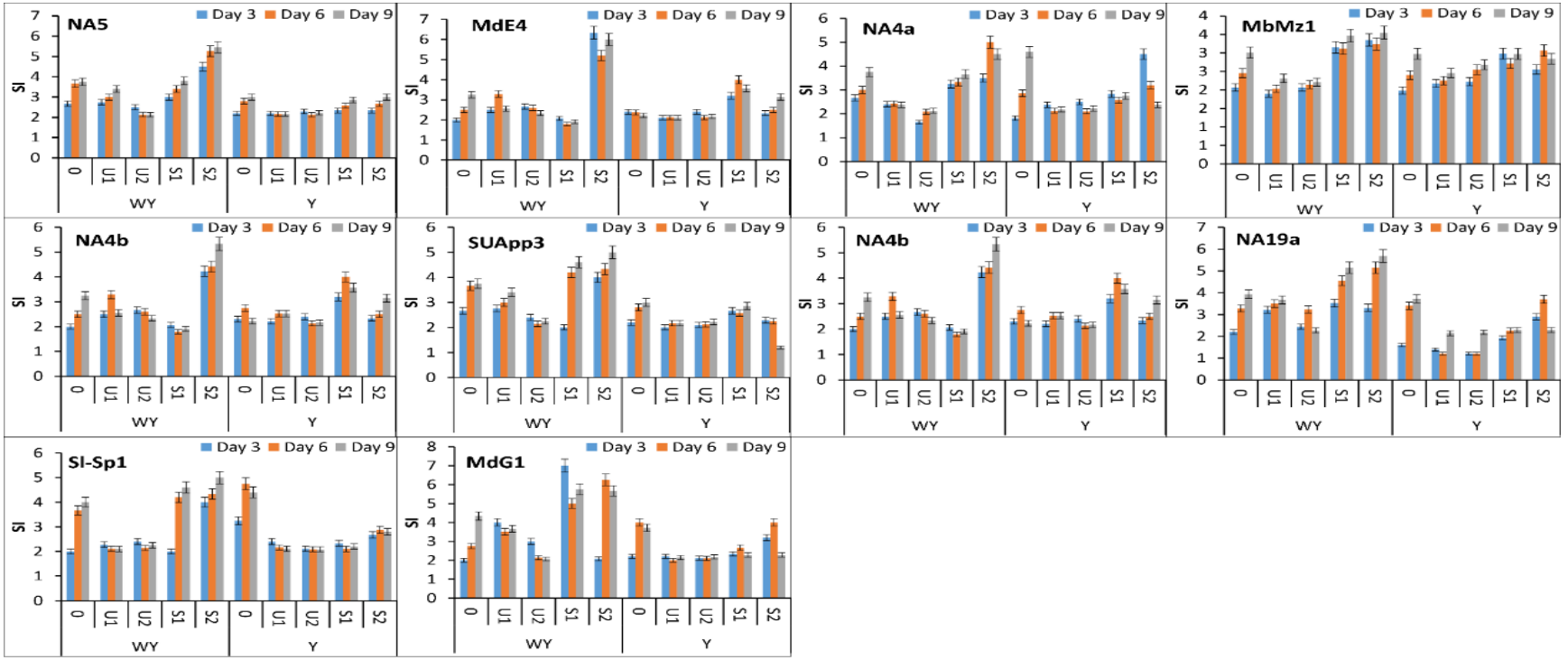
iii. We recommend the establishment of the spin-off biotech plant for mass production of Bio-rock P fertilizers for general supply to consumer farmers.

iv. Economic analysis of using Bio-rock P has not been covered by the current study, and it is therefore recommended.

v. Moreover, to unleash the potential of using sole PSB inoculums application in Tanzania's soils with high P sorption capacities, we recommend further studies on PSB's ability to un-fix P from the surfaces of laboratory grade P-fixing minerals that are dominant in tropical soils and from native soil P-fixing soils. This however, should be conducted in terms that consider sustainable use of soil P reserves by improving and maintaining soil P status through addition of recommended rates of P from local Rock phosphate.

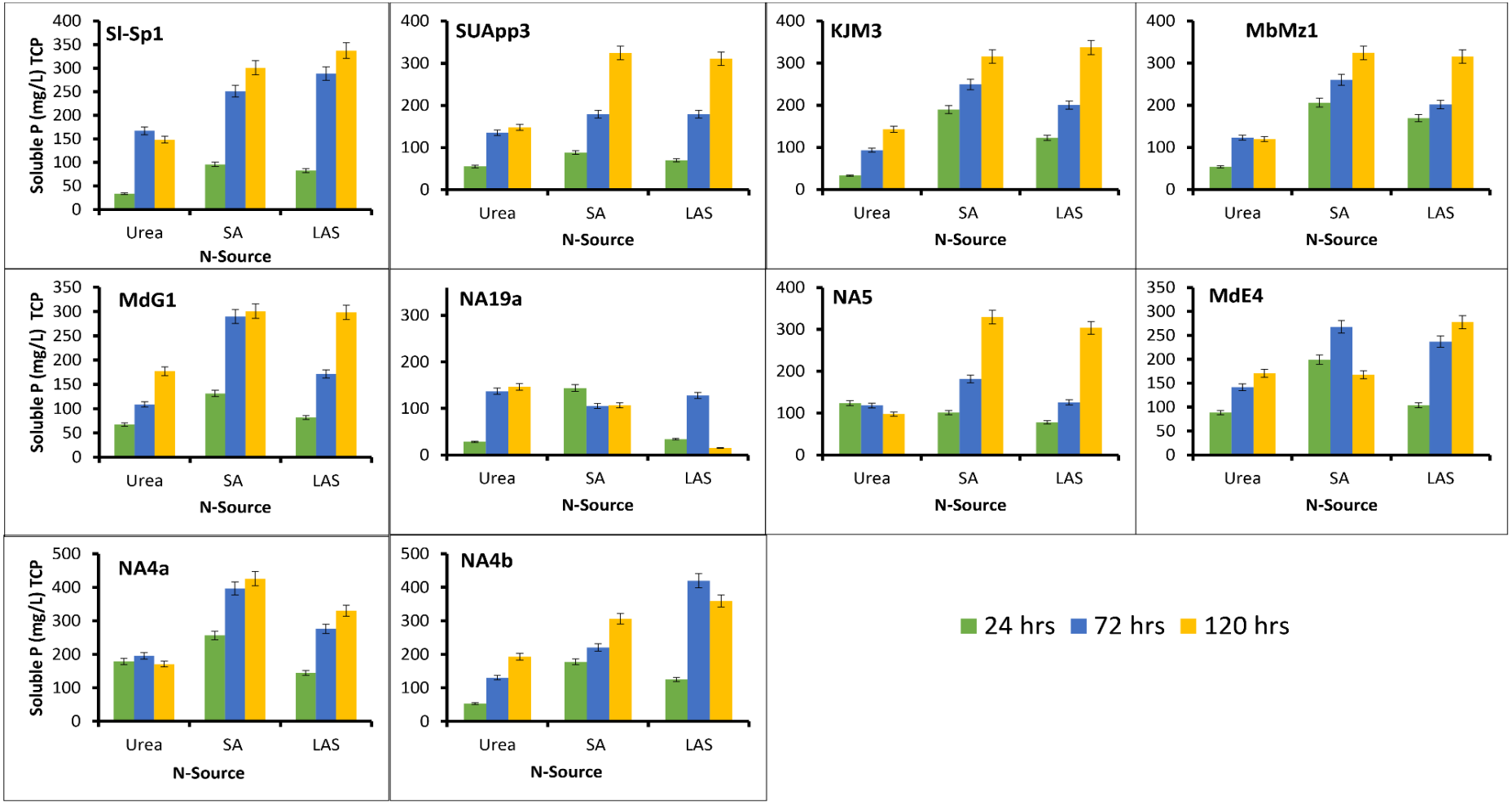
APPENDICES

Appendix 1: Solubilisation indices of PSB isolates on the modified PVK media containing different N-Sources and Levels.

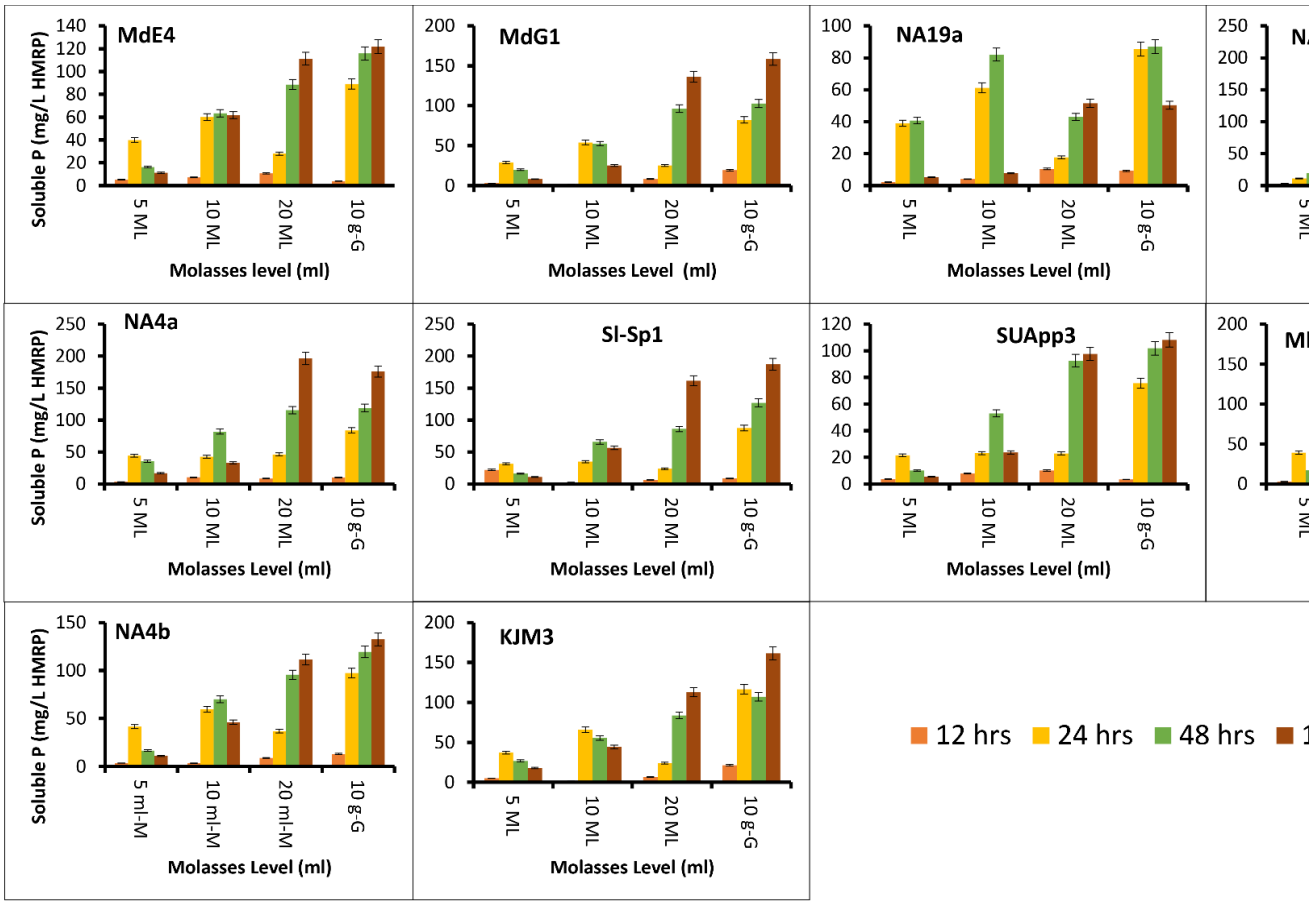


Where by; Y = Yeast extract added, WY = without Yeast extract, U1 and U2 = 0.25 and 0.5 g/L Urea, respectively, S1 and S2 = 0.25 and 0.5 g/L SA, respectively, 0 = no urea/SA added.

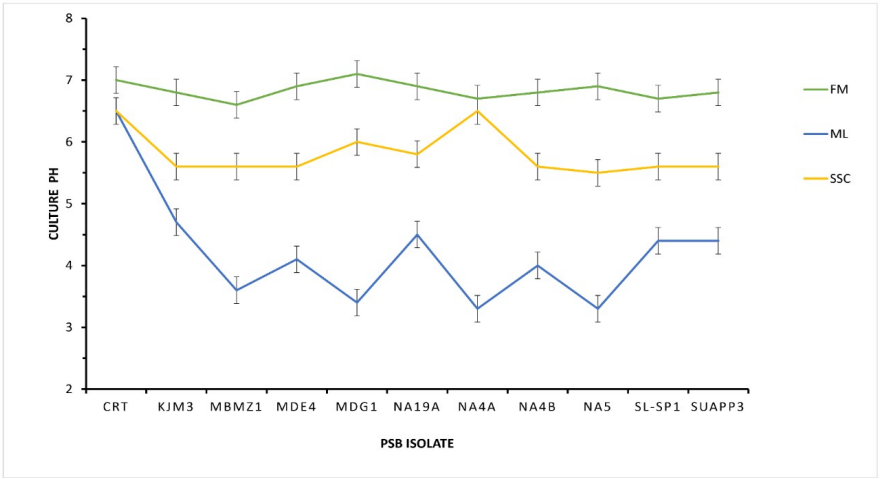
Appendix 2: The influence of Fertilizer-grade N-sources on the abilities of the selected PSBs to solubilize Tricalcium phosphates (TCP)



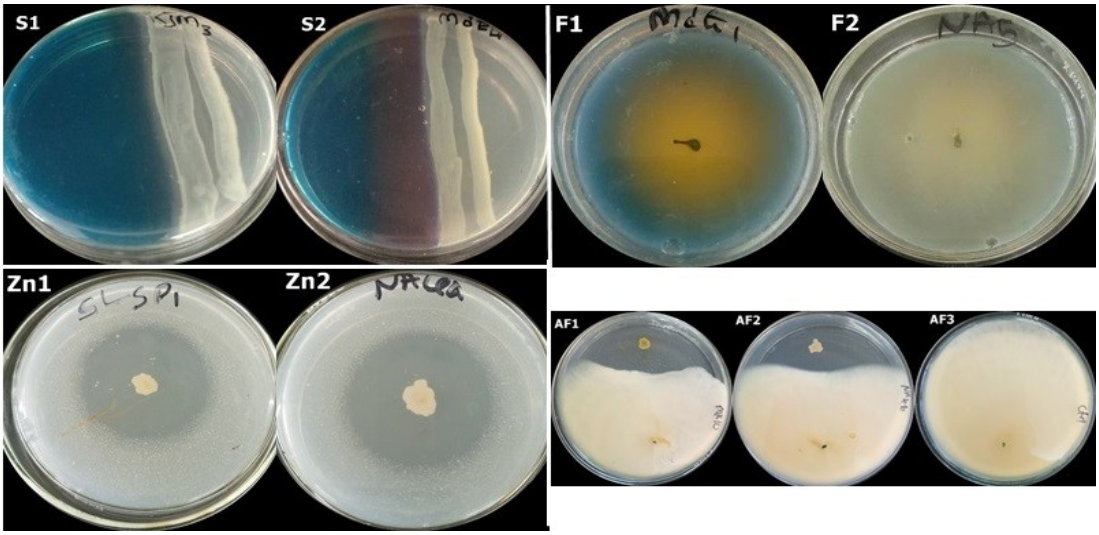
Appendix 3: Abilities of PSB isolates to solubilize the hard Minjingu rock phosphate in the media containing different levels of molasses (ML) and 10 g-glucose/L (10 g-G)



Appendix 4: Effect of organic carbon source on the PSB's ability to lower the culture pH. Where by FM is filter mud, ML is molasses, and SSC is sunflower seedcake

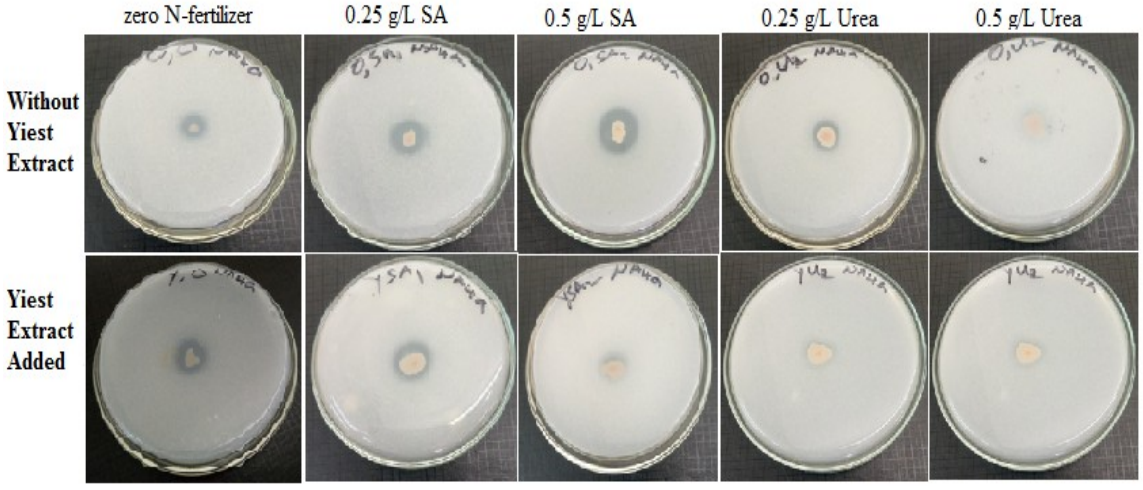


Appendix 5: Representative images of the results for PGPT tests

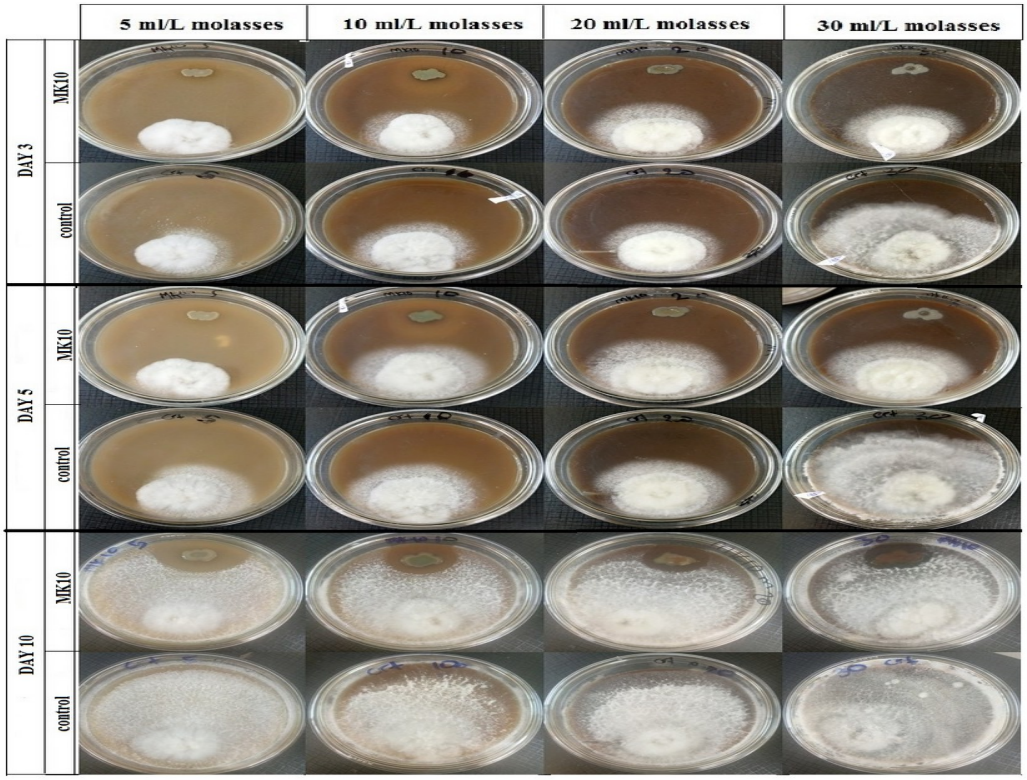


Where by S1 and S2 show the isolates KJm3 and MdE4 on CAS-media, respectively; plates F1 and F2 show solubilisation zones of MdG1 and NA5, respectively on ferric phosphate-containing media; plates Zn1 and Zn2 show solubilisation zones of Sl-Sp1 and NA4a, respectively on zinc carbonate-containing media; and Plates AF1 and AF2 show the inhibition of *Fusarium proliferatum* by bacterial isolates Mk10 and NA4b, respectively in comparison to the control plate (plate AF3).

Appendix 6: The solubilisation of Tricalcium phosphate by PSB isolates NA4a on the modified PVK media containing different N sources and concentrations



Appendix 7: Growth inhibition of *F. proliferatum* by bacterial isolate MK10 grown on the media containing varying concentrations of molasses; 30 ml/L, 20 ml/L, 10 ml/L, and 5 ml/L, against their respective controls



Appendix 8: Growth inhibition of *F. proliferatum* by bacterial isolates Sl-Sp1 grown on dSMM with different nitrogen sources and concentrations