JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOLOGY

Effects of phosphate solublizing rhizobacteria on growth and yield of Linseed (*Linum usitatissimum L.*) crop in Jimma zone of Oromiya Region, South West Ethiopia

By: Zegeye Zeleke

A Thesis Presented to the School of Graduate Studies of Jimma University in partial fulfillment of the requirement for the Degree of Masters of Science (Msc) in General Biology

September, 2019

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Approved by

Advisor's	**Name**	**Signature**
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List of Acronyms

ALA = Alpha linoleic acid

CRBD = completely randomized block design

CSA = Central statistical Autority

DHA= Decoxahexaenoic acid

FA = Fatty acid

LO = Lin oil

NBRIP = National Botanical research institute for Phosphate broth

OD = Optical density

PGPR= plant growth promoting rhizobia

PS= phosphate solubilizing

PSB = phosphate solubilizing bacteria

PSM = phosphate solublizing microbes

PSE = Phosphate Solublization Efficiency

PSI = Phosphate Solublization Index

PVK = Pikoviskaya

SLMP = Sustainable land management project.

TCP- Tricalcium phosphate

ABSTRACT

Phosphorus deficiency is a major constraint in crop production, since some of the chemo-fertilizers applied formed insoluble Phosphate salts. Beneficial microbes of the soil can solublize these salts into bioavailable forms. This study was undertaken with the main objective of evaluating the effects of Phosphate solublizing bacteria on growth and yield of Linseed crop *(Linum usitatissimum L).* Thirty soil samples were collected aseptically from five linseed sites (farms) of Omonada district of Jimma zone, Oromiya region, South West Ethiopia. The soil samples were serially diluted up to (10^{-6}) level, 0.1ml aliquot from each dilution was spread plated on solid Pikoviskaya's agar. Some distinct countable colonies (10-20) of various colors and morphologies were isolated and tested for in vitro phosphate solublization potential on solid medium, were purified on sterile nutrient agar and characterized on the basis of morphological, biochemical and physiological features. A total of seventy six phosphate solubilizing bacteria were isolated from the collected soil samples. Six best phosphate solublizers were selected for further liquid media solublization test using National Botanical Research Institute for P broth media, two of the six selected isolates were also marked as prime HCN-producers. Finally, the six selected isolates were inoculated on two local linseed varieties under greenhouse condition and evaluated for their effects on linseed's growth, yield and yield components. All isolates were identified following standard procedures, belonged to three main genera: 46 (60.5%) *Pseudomonas*, 18 (23.7%) *Bacillu*s and 12 (15.8%) were *Arthrobacter*. The six isolates selected for greenhouse evaluation were: *Pseudomonas* (JURB3, JURB9, JURB11 and JURB21) and *Bacilli* (JURB50 and JURB57). Both genera inoculants resulted in significantly ($p<0.05$) higher growths in both "Romae" (V_1) and "Shewa" (V_2) local linseed varieties in terms of mean root length and mean shoot height of maximum (27.27- 69.7) % and 69 %, respectively. Similarly positive responses were recorded for dry weight (g) of roots and shoots. But the ratios of dry root to shoot were either significantly lower in some or similar in others with respect to controls. All treated linseeds of both V_1 and V_2 varieties with the selected inoculants responded significantly ($p < 0.05$) greater yield and yield components. Accordingly, inoculations with *Pseudomonas* resulted in higher (89-533%) seed weight, capsule number (68-200%) and mean P-uptake of (305.5-607.5%) as compared to controls. In conclusion, as both in vitro and in vivo evaluations confirmed, *Bacillus* and *pseudomonas* were dominant genera of Linseed rhizosphere and can be used as potential source of bio-inoculants.

Key words: Bio-inoculant, Rhizobacteria, Rhizosphere, Solublization index.

CHAPTER ONE

INTRODUCTION

Linseed crop *(Linum usitatissimum L.*) is grown mainly for its oil called Lin oil (LO). It has long been used by humans as nutritional supplement sources of oil diet, the seed contains about 36 to 40 % of oil, thus served as good source of energy and protein; providing nutritionally balanced feeds (Chen *et al.,* 2006). Besides, it is used as a source of oil in animal diet and industrially as a basic component (additive) of various paints or polymers.

Linseed based diet when taken by humans in routine provides great health benefits such as high level of dietary fiber and lignin, abundant micronutrients, reduces: diabetes, incidence of obesity, blood cholesterol level and prevent cancers of colon, rectal, breast and prostate (Dineshkumar *et al.,* 2009; Madhusudhan, 2009; Miller-Colbert *et al*., 2009; Clemente *et al*., 2009). It has also antidepressant activity (EL- Beltogi. *et al.,* 2007) and used as the best source of omega- 3 and 6-fatty acids/Alpha linoleic acid (ALA) without causing significant toxic effects.

In Ethiopia oil seeds are classed within grain crop category. Linseed ranks third, next to sesame and Niger seed (nug) in terms of area of cultivation and production. Sesame, Niger seed and linseed covered 3.35 % (about 420,490.98 ha), 2.01% (252,584.38 ha) and 0.66% (82,323.86 ha**)** of the grain crop area and 1.07% (about 2,887,700.79 qui), 0.83% (about 2, 244,625.07quintals) and 0.31% (about 831,305.05 qui) of the grain production, respectively (CSA, 2014/15).

Hence, Ethiopia's latest average area of linseed cultivation was 82,325.78 hectares of land with the total crop production of 831,305.05 qui and the yield of 10.1qui./ha, during the period 2014/15 meher season (CSA, 2014/2015). In the years, 2012/13 to 2014/15, CSA reports on linseed mean cultivation of Jimma zone of Oromiya region was 4162.72 ha and 25,047.10 qui, that is, 6.02 qui/ha of yield (CSA, 2012/13-2014/15), which indicated relatively low level of cultivation in the zone. Besides, Ethiopia did not secure its demand for linseed up to now, as the supply was coming from both local and import sources. Hence, the imported quantity showed clearly the presence of gap in linseed production. Therefore the whole problem could be stated and solved as given by

"Following linseeds low production, there is a trend of increasing demand for more linseeds than ever, which then necessitated its cultivation using fertilizers. However, the sole application of chemo-fertilizers can't be the right choice, as much of these chemo-fertilizers form insoluble

phosphate salts in soils of tropics and sub tropics, besides, they are expensive financially and unfriendly ecologically whereas the phosphate solubilizing bacteria (PSB) have the potentials of solublizing the phosphate salts of the soil, making phosphate bioavailable, besides, they are financially affordable and ecologically friendly, thus, ensure sustainable agriculture."

Phosphorus is a major growth-limiting macronutrient next to nitrogen for plant growth and development (Marschner, 1996). The efficiency of P fertilizers throughout the world is around 10 - 25 % (Isherword, 1998), and where the bio-available P conc. in soil is very low, reaching the level of 1.0 mg kg^{-1} soil (Goldstein, 1994), this is due to the large amount of phosphate applied as chemical fertilizer entering in to the immobile pools via precipitation by highly reactive cations AL^{3+} and Fe^{3+}in acidic, Ca^{2+} and Mg^{2+} in calcareous or normal soils of tropics and sub tropics thus becoming water insoluble salts (Gyaneshwar *et al*., 2002; Hao *et al*., 2002).

The PSBs are among the plant growth promoting rhizobacteria (PGPR) associated to crops rhizosphere which are able to convert the insoluble phosphates in to absorbable forms by plants in various ways (Awsthi *et al.*, 2011). Inorganic forms of P is solubilized by microbes excreting organic acids that dissolve phosphatic minerals and/or chelate cationic partners of the P ions i.e. PO_4^{3-} directly, releasing P into solution (He *et al.*, 2002).

The bio-fertilizers (microbial inoculants) produce organic acids and acid phosphatases playing a major role in the mineralization of organic phosphorous in soil, thus, the PSB and PGPR together could reduce Phosphate fertilizer application by 50 % without any significant reduction of crop yield (Jelani *et al*., 2007; Yazdani *et al*., 2009). It's inferred from this that, the PSB inoculants hold great prospects for sustaining crop production with optimized Phosphate fertilization.

So far in Ethiopia studies on bio-fertilizers and their subsequent applications were limited and tests were targeted only on few plant families, such as characterizing isolated PSF on growth and yield of Tef plant was examined by Hailemariam (1993), Others evaluated the effect of PGPR on growth and yield of Tef plant by Delelegn and Fassil (2011), effects of PSM from Faba Bean (*Vacia faba*) rhizosphere reported by Fikadu (2013); Shiferaw *et al.* , (2013) and Girmaye *et al*., (2014). For so long there was no study evaluated the effects of PSB associated to oil crop plants of the study area. Thus the main objective of this study is to evaluate the effects of phosphate solubilizing bacteria (PSB) isolated from linseed rhizosphere on growth and yield of linseed crop plant.

CHAPTER TWO

2. Objectives

2.1. General objective:

To evaluate the effects of Phosphate solubilizing rhizobacteria on growth and yield of Linseed (*Linum usitatissimum*) crop

2.2. Specific objectives:

- Isolate, characterize and identify phosphate solubilizing bacteria from Linseed crop rhizosphere.
- Assess some phytobeneficial traits and Hydrogen cyanide (HCN) producing isolates under in-vitro conditions.
- Evaluate growth performances of Phosphate solublizing rhizobacteria on Linseed crop plant under greenhouse conditions.

CHAPTER THREE

3. Literature review

3.1. Phosphate as plant nutrient

3.1.1. Phosphate relevance to plants

Phosphorus (P) is absorbed by plant roots as phosphate ion (PO_4^{3-}). Unlike the case for nitrogen, there is no large atmospheric P source that made it bio-available (Ezawa *et al*.; 2002). Root development, stalk and stem strength, seed germination, flower and seed formation, improved quality, maturity, and production of fruits, vegetables and crops, Nitrogen fixation in legumes and resistance to plant diseases are all the uses of P (Shankar *et al.,* 2013). On the contrary, phosphorous deficiency leads to formation of small leaves, weak stem, and slow development (Ranjan *et al.,* 2013). Although microbial inoculants are in use for improving soil fertility during the last century, however, a meager work has been reported on P solubilization compared to nitrogen fixation. Evidence of naturally occurring rhizospheric phosphorus solubilizing microorganism (PSM) dates back to 1903 (Khan *et al.,* 2007).

The diversity and beneficial activity of the plant-bacterial association and its understanding is important to sustain agro-ecosystems for sustainable crop production (Germida *et al*., 1998). Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere (Rangaran *et al*., 2003) and also be beneficial to the plant by stimulating growth (Bloemberg *et al*., 2001; Moeinzdeh *et al.,* 2010). Many species of bacteria are able to solubilize phosphates in vitro and most of them live in the plant rhizosphere. At present, Bacilli, Rhizobia and Pseudomonads are the most studied phosphate-solubilizers (Rodriguez and Fraga, 1999). Microbial biomass assimilates soluble P, and prevents it from adsorption or fixation (Khan and Joergesen, 2009).

Microbial community influences soil fertility through soil processes viz. decomposition, mineralization, and storage / release of nutrients. Microorganisms enhance the P availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphates (Chen *et al*., 2006; Kang *et al*., 2002; Pradhan and Sukla, 2005). These bacteria in the presence of labile carbon

serve as a sink for P by rapidly immobilizing it even in low P soils (Bünemann *et al*., 2004). Subsequently, PSB become a source of P to plants upon its release from their cells.

3.1.2. Phosphate availability in rhizosphere

Phosphorus (P) is one of the major essential macronutrients for biological growth and development (Ehrlich and Einsele *et al*.; 1990). It is present at levels of 400–1200 mg kg^{-1} of soil (Fernandez *et al.;* 1988). Its cycle in the bio-sphere can be described as 'open' because there is no interchange with the atmosphere (Begon *et al*., 1990). Rehman (2004) showed that available phosphorus in plants was affected by three factors: plant available phosphorus concentration in soil solution, the amount of exchangeable phosphorus and the amount of phosphorus relative uptake in soils. Despite the high total soil P content, plants P availability is often reported to be limited, particularly in tropical soils (Collavino *et al*., 2010)

Most soil P is usually present as insoluble metal chelates (Vassilev *et al*., 2006); moreover, substantial amounts of applied chemical phosphate fertilizers are also rapidly converted into insoluble phosphate sources, (Arpana *et al*., 2002) this is due to the formation of strong bonds between phosphorous with calcium and magnesium in alkaline pH and the same bonds with iron and aluminum in acidic soils. The mobility of this element is very slow in the soil and can't respond to rapid uptake by plants. This causes the creation and development of P depleted zones near the areas of plant roots soil rhizosphere. This leads to regularly applying P fertilizers, which are not only costly, but also environmentally undesirable.

In this context, microbial solubilization of soil insoluble phosphates into soluble forms is considered as an important process in natural and agricultural ecosystems. Several bacterial and fungal species with varied potentials to solubilize inorganic phosphates, also known as phosphate solubilizing microorganisms, (PSM), have been found in the rhizosphere of plants. However, their numbers were not high enough to compete with other microbial species in the rhizosphere (Jain *et al.,* 2012). Screening of potential phosphate solubilizing isolates, which can be used as bio-inoculants to increase plant growth and yield, is recognized as an area of interest because such microbial inoculants could substantially reduce the chemical fertilizer requirement.

3.1.3. PSB abundance in the rhizosphere and their P solublization strategies

High proportions of phosphate solublizer microbes are concentrated in the rhizosphere, and they are metabolically more active than from other sources (Vazquez *et al*., 2000). Usually, one gram of fertile soil contains $10^1$to 10^{10} bacteria, and their live weight may exceed 2,000 kg ha^{-1}. Soil bacteria are in cocci (sphere, 0.5 µm), bacilli (rod, 0.5–0.3 µm) or spiral (1-100 µm) shapes. Bacilli are common in soil, whereas spirilli are very rare in natural environments (Baudoin *et. al*.2002). These PS-bacteria are ubiquitous and vary in their PS abilities with different soil properties (physical and chemical properties, organic matter, C, N and P content) and cultural activities (Kim *et al*., 1998).

The concentration of ores, temperature, and C and N sources greatly influences the PS potentials of these bacteria under in vitro conditions (Zaidi, 1999). Larger populations of PSB are found in agricultural and rangeland soils (Yahya and Azawi, 1998).

Of these, *Bacillus* and *Pseudomonas* (Illmer *et al.,* 1992; Wani *et. al*., 2007c) are the major bacteria efficient to solubilize the insoluble P.

Other bacterial genera reported recently as PSB include, *Rhodococcus, Arthrobacter Serratia Chryseobacterium, Delftia, Gordonia, Phyllobacterium Arthrobacter ureafaciens, Phyllobacterium myrsinacearum, Rhodococcus erythropolis, Delftia sp.*(Wani *et.al*.,2005) and *Azotobacter chroococcum* (Kumar *et al*.,2001), *Bacillus brevis, B. megaterium, B. polymyxa, B. sphaericus, B. thuringiensis,* .These organisms are generally isolated from rhizosphere and non-rhizosphere soils, rhizoplane, phyllosphere, RP deposit area soil and even from stressed soils using serial plate dilution method or by enrichment culture technique.

Since 1948, when Gerretsen suggested that microbes could dissolve difficultly available forms of soil P and play an important role in making P available to plants, numerous methods and media, such as Pikovskaya (Pikovskaya,1948), Bromo-phenol blue dye method,(Gupta *et al*.,1994) and National Botanical Research Institute P (NBRIP) medium (Nautiyal C.S.,1999) have been proposed. The bacterial strains possessing PS efficiency are detected by the formation of clear zones (a sign of solubilization) around the colonies (Figure 1). Due to inconsistency and variations in PS activity, the bacterial cultures are sub-cultured repeatedly in order to test the persistence of PS potential. Once the efficient PS bacteria are selected, the release of P by PS bacteria is

quantitatively assayed and the potential P solubilizers are then mass produced and tested under pot/field environments with different crops.

3.1.4. Mechanisms of Phosphate solublization

The rhizospheric bacterial populations enhance plant growth by different mechanisms. One of these is the dissolution of insoluble P whose availability in soil is influenced by changes in pH values, root exudates, variation in soil types, and plant genotypes making P available for uptake by plants. Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils (Stevenson, 2005 and Kpomblekou ,1994;). The PSB dissolve the soil P through production of low molecular weight organic acids mainly gluconic and keto gluconic acids (Deubel *et.al*, 2000 and Goldstein, 1995), in addition to lowering the pH of rhizosphere.

The pH of rhizosphere is lowered through biotical production of proton / bicarbonate release (anion /cation balance) and gaseous (O_2/CO_2) exchanges. Phosphorus solubilization ability of PSB has direct correlation with pH of the medium.

Release of root exudates such as organic ligands can also alter the concentration of P in the soil solution (Hinsinger, 2001). Organic acids produced by PSB solubilize insoluble phosphates by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil (Nahas, 1996). Inorganic acids e.g. hydrochloric acid can also solubilize phosphate but they are less effective compared to organic acids at the same pH (Kim *et. al*., 1997). In certain cases phosphate solubilization is induced by phosphate starvation (Gyaneshwar *et. al.*, 1999).

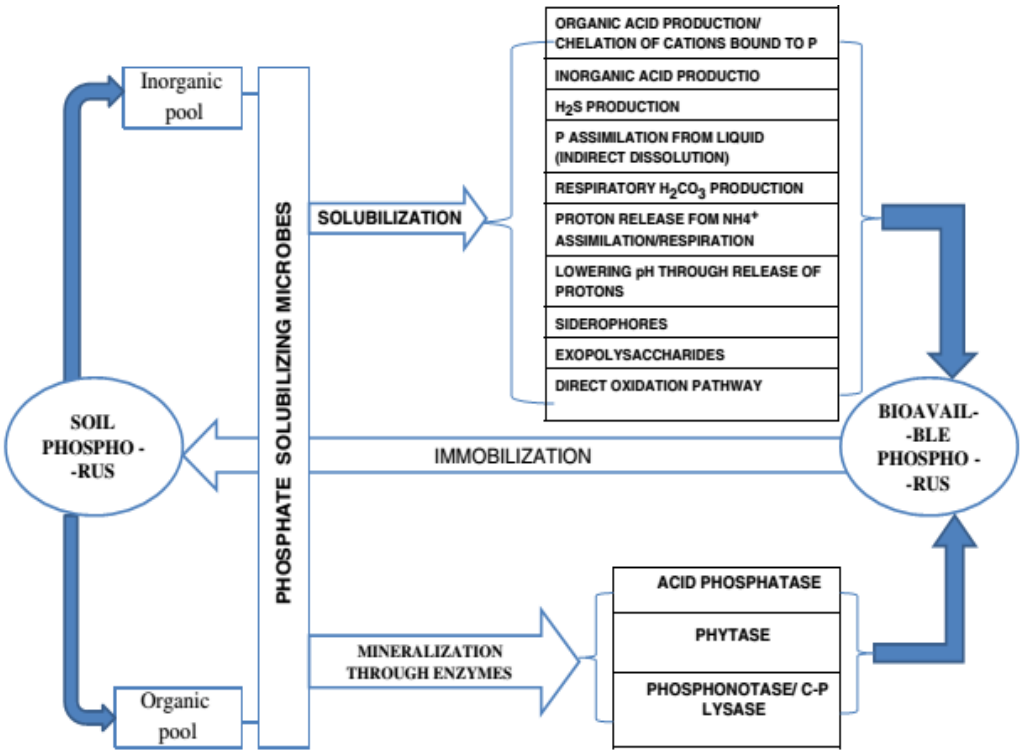


Figure.1. schematic representation of mechanisms of soil P solublization /mineralization and immmoblization by PSM (Sharma *et al*., 2013)

3.1.5. Inorganic phosphate

It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms (Halder *et al*.,1990;Duff and Wbley,1995;Sudra and Sinha,1963;banki and Dey;Cravan and Hayasaka,1982;Layvan and Berthedira,1989;Salih *et al*.,1989). Production of organic acids results in acidification of the microbial cell and its surroundings. Consequently, P_i may be released from a mineral phosphate by proton substitution for Ca^{2+} (Goldstein, 1994).The production of organic acids by phosphate solubilizing bacteria has been well documented. Among them, gluconic acid seems to be the most frequent agent of mineral phosphate solubilization. It is reported as the principal organic acid produced by phosphate solubilizing bacteria such as Pseudomonas sp. (Illimner and Schinner, 1992), Erwinia herbicola (Liu *et al*., 1992) Pseudomonas cepacia (*Goldstein et al* ,1993) Another organic acid identified in strains with phosphate solubilizing ability is 2-ketogluconic acid, which is present in Rhizobium leguminosarum (Goldstein, 1994), Rhizobium meliloti (Halder and

chakrabartty, 1993) Bacillus firmus (Banki and Dey, 1982) and other unidentified soil bacteria (Duff and Wbley, 1995).

Strains of Bacillus liqueniformis and Bacillus amyloliquefaciens were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids. Other organic acids, such as glycolic, oxalic, malonic, and succinic acid, have also been identified among phosphate solubilizers (Illimner and Schinner, 1992). There is also experimental evidence that supports the role of organic acids in mineral phosphate solubilization. (Halder *et al*., 1990) showed that the organic acids isolated from a culture of Rhizobium leguminosarum solubilized an amount of P nearly equivalent to the amount that was solubilized by the whole culture.

Alternative possibilities other than organic acids for P solubilization have been proposed based on the lack of a linear correlation between pH and the amount of P solubilized (Asea *et al.*, 1988; Eherlic, 1990).In addition, no significant amounts of organic acid production could be detected from a phosphate solubilizer fungus, Penicillium sp (Illimner and Schinner, 1992). Studies have shown that the release of H^+ to the outer surface in exchange for cation uptake or with the help of H^+ translocation ATPase could constitute alternative ways for solubilization of mineral Phosphate. Other mechanisms have been considered, such as the production of chelating substances by microorganisms (Sperberg, 1958; Duff and Wbley, 1995) as well as the production of inorganic acids, such as sulphidric (Sperberg,1958), nitric, and carbonic acid (Hopings and whitings, 1916). However, the effectiveness of these processes has been questioned and their contribution to P release in soil appears to be negligible (Vazgues, 1996).

3.1.6. Organic phosphate mineralization

Organic phosphate solubilization is also called mineralization of organic phosphorus, and it occurs in soil at the expense of plant and animal remains, which contain a large amount of organic phosphorus compounds. The decomposition of organic matter in soil is carried out by the action of numerous saprophytes, which produce the release of radical orthophosphate from the carbon structure of the molecule. The organo phosphonates can equally suffer a process of mineralization when they are victims of biodegradation (Mc Grath *et al*.; 1998).

The microbial mineralization of organic phosphorus is strongly influenced by environmental parameters; in fact, moderate alkalinity favors the mineralization of organic phosphorus (Raul and clark ,1988) The degradability of organic phosphorous compounds depends mainly on the physico-

chemical and biochemical properties of their molecules, e.g. nucleic acids, phospholipids, and sugar phosphates are easily broken down, but phytic acid, polyphosphates, and phosphonates are decomposed more slowly (Grath *et al*,1995; Ohetake *et al*.,1996; Mc Grath *et al* .,1998)

The mineralization of these compounds is carried out by means of the action of several phosphatases (also called phosphohydrolases). These dephosphorylating reactions involve the hydrolysis of phosphoester or phosphoanhydride bonds. The phosphohydrolases are clustered in acid or alkaline. The acid phosphohydrolases, unlike alkaline phosphatases, show optimal catalytic activity at acidic to neutral pH values. Moreover, they can be further classified as specific or nonspecific acid phosphatases, in relation to their substrate specificity (Rossolini *et al*.1998) recently-published a comprehensive review of bacterial nonspecific acid phosphohydrolases.

The specific phosphohydrolases with different activities include: 39-nucleotidases and 59-nucleotidases (Burns and Beachman, 1986) hexose phosphatases (Pradel and Boquet, 1988) and phytases (Consgrov *et.al*, 1970). A specific group of P releasing enzymes are those able to cleave C-P bonds from organo-phosphonates (Bujacz *et.al*., 1995; Mc Grath *et. al*., 1995; Ohetake *et al*., 1996; Mc Grath *et. al*., 1998)

Some Phosphohydrolases are secreted outside the plasma membrane, where they are either released in a soluble form or retained as membrane-bound proteins. This localization allows them to act as scavenging enzymes on organic phosphoesters that are components of high molecular weight material (i.e. RNA and DNA) and cannot cross the cytoplasmic membrane. This material can be first converted to low molecular weight components, and this process may occur sequentially i.e. the transformation of RNA and DNA to nucleoside monophosphate via RNase and DNase respectively, followed by the release of P and organic by-products via phosphohydrolases, providing the cell with essential nutrients (Goldstein, 1994).

3.1.7. Effects of phosphate on crop production

The PSB solubilize the fixed soil P and applied phosphates resulting in higher crop yields (Gull *et al*., 2004). Direct application of phosphate rock is often ineffective in the short time period of most annual crops (Goenadi *et al*., 2000). Acid producing microorganisms are able to enhance the solubilization of phosphatic rock (Gyaneshwar *et al*., 2002). The PSB strains exhibit inorganic P-solubilizing abilities ranging between 25–42 µg P ml^{-1} and organic P mineralizing abilities between 8–18 µg P mL^{-1} (Tao *et al*., 2008). The PSB in conjunction with single super phosphate

and rock phosphate reduce the P dose by 25 and 50 %, respectively (Sundara *et al*., 2002). *Pseudomonas putida*, and *P. fluorescens*, released 51 and 62 % P, respectively; with highest value of 0.74 mg P / 50 mL from Fe_2O_3 (Ghaderi *et al*., 2008). *Pseudomonas striata* and *Bacillus polymyxa* solubilized 156 and 116 mg P L^{-1}, respectively (Rodríguez and Fraga, 1999).

Pseudomonas fluorescens solubilized 100 mg P L^{-1} containing $Ca_3 (PO_4)_2$ or 92 and 51 mg P L^{-1} containing $AlP0_4$ and $FePO_4$, respectively (Henri *et al*., 2008). Use of PSMs can increase crop yields up to 70 % (Verma, 1993). Combined inoculation of Arbuscular mycorrhiza and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock (Goenadi *et al*., 2000; Cabello *et al*., 2005). Higher crop yields result from solubilization of fixed soil P and applied phosphates by PSB (Zaidi, 1999).

Microorganisms with PS- potential increase the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixation (Ponmurugan and Gopi, 2006; Kucey *et al*., 1989). *Pseudomonas spp*. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (Son *et al*., 2006). Phosphate solubilizing bacteria enhanced the seedling length of Cicer arietinum (Sharma *et al*., 2007), while co-inoculation of PSM and PGPR reduced P application by 50 % without affecting corn yield (Yazdani *et al*., 2009). Inoculation with PSB increased sugarcane yield by 12.6% (Sundara *et al*., 2002). Sole application of bacteria increased the biological yield, while the application of the same bacteria along with mycorrhizae achieved the maximum grain weight (Mehrvarz *et al*., 2008). Single and dual inoculation along with P fertilizer was 30-40 % better than P fertilizer alone for improving grain yield of wheat, and dual inoculation without P fertilizer improved grain yield up to 20 % against sole P fertilization (Afzal and Bano, 2008). Mycorrhiza along with *Pseudomonas putida* increased leaf chlorophyll content in barley (Mehrvarz *et al*., 2008).

Rhizospheric microorganisms can interact positively in promoting plant growth, as well as N and P uptake. Seed yield of green gram was enhanced by 24 % following triple inoculation of Bradyrhizobium, Glomus fasciculatuxm, Bacillus subtilis (Zaidi and Khan, 2006). Growth and phosphorus content in two alpine Carex species increased by inoculation with *Pseudomonas fortinii* (Bartholdy *et al*., 2001). Integration of half dose of NP fertilizer with bio-fertilizer gives

crop yield as with full rate of fertilizer; and through reduced use of fertilizers the production cost is minimized and the net return maximized (Jilani *et al*., 2007).

3.1.8. Bio-fertilization: An alternative and effective approach

The need to protect the natural environment is associated with advancing degradation of soils, negative impact of intensive crop production such as high level use of chemical fertilization often occurring in intensive agriculture have a negative effects on root growth and root colonization by mycorrhizal fungi (Smith and Read 2008), and the worsening effects of climate change (Dobrzyński *et al.*, 2014) constitute not only a significant cost to crop producers but are also a potential cause of eutrophication and pollution of the soil environment, water and air (Boy and Arcad 2013).

Solutions suggested to mitigate problems of both the environment and human health protections were to find out biological alternatives and implement natural technologies of plant cultivation and fertilization through applications of biofertilizers. The use of biofertilizers in agriculture has reduced chemical application for crop production (Lingua *et al*. 2013). Evidences of naturally occurring rhizospheric phosphate solublizer microbes (PSM) goes as early as 1903(Khan MS *et al.,* 2007), including the development of Arbuscular mycorrhizal fungi (AMF) (Kuwada *et al*. 2005, 2006). Besides to phosphate solublization, some of these PSM can degrade complex carbohydrates like starch and cellulose, hence can increase the soil fertility. Fertilizers enriched with beneficial strains of bacteria and fungi such as phosphate solublizing microbes (PSM), isolated from plants rhizosphere, increased effectiveness in crop production (Chen ,2006) by promoting their physiology, stimulate vegetative growth , increase in yield of crops as well as resistance to both environmental and biotic stresses (Corte *et al.* 2013, Wally *et al.* 2013) .

PSBs are being used as biofertilizer since the 1950s (Kudashev SI, 1956, and Krasilinikov, 1957). Bacteria are more effective in phosphorus solubilization than fungi (Slam S *et al*., 2002). Among the whole microbial population in soil, Phosphate Solubilizing Bacteria (PSB) constitute 1 to 50 %, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5 % in P solubilization potential (Chen. *et al*.,2006). The bacterial strain isolated from alkaline soil can solubilize phosphates at high salt, high pH and high-temperature concentration (Ronderguez H *et al*., 1999)**.** Many phosphate solubilizing bacteria are reported as plant growth promoter in many crops like tomato; rice etc (Ronderguez H *et al*., 1999, and Hafeez FY *et al*., 2004).

Protocol steps followed in isolating and developing effective inoculants of PSM based bio-fertilizer (Sharma *et al*., 2013) were: 1) Soil sample collection 2) Serial dilution 3) Inoculation on media (by pour plating/streaking) 4) Additional test on liquid media to assay P dissolution 5) Test isolates for abundant production of organic acids 6) pure culture preparation by re-inoculation 7) Study of the morphology and colony Characteristics and biochemical tests 8) Screening of best inoculants in terms of P Solublization activity 9) Identification at generic level (molecular characterization) 10) Test on a model plant 11) Developments of microphos 12) Greenhouse trails 13) Field trails 14) Standardization (quality control) and 15) Commercialization.

3.2. Linseed plant *(Linum usitatissimum L*.)

3.2.1. Botanical description

Linseed plant, with the binomial name *Linum usitatissimum*, is a member of the genus *Linum*, in the family Linacae, Order-Malphigials, among flowering plants, has chromosome number of 30. It is a food and fiber crop, known as flax, grown in cooler regions of the world. The textiles made from flax are known in the West as linen. The oil is known as linseed oil (LO). The plant species is known only as a cultivated plant, and appears to have been domesticated just once from the wild species *Linum bienne* called pale flax (Allebay *et al*.; 2005). Linseed is grown for its oil used as a nutritional supplement, ingredient in many wood finishing products, ornamental plant in gardens and for its fibers to make linen. The Latin species name" *usitatissimum'* means most useful (Mc Hughen, 1990). Flax fibers are taken from the stem of the plant and are 2-3 times as strong as those of cotton, flax fibers are naturally smooth and straight. Europe and North America depended on flax for vegetable-based cloth until the 19th century when cotton overtook flax as the most common plant used for making rag-based paper. Flax is grown on the Canadian Prairies for LO, as it is rich in linoleic acid (>66%), used as a perfect drying oil in paints, varnish and in products such as linoleum and printing inks.

It is herbaceous annual plant growing to a height of 30-120 cm. Cultivars grown for seed (oil) are usually shorter than those grown for fiber production. The root system is usually shallow. The main tap root is slender and having numerous small lateral roots, that develop in the top 30 cm soil. Stems are narrow and may branch from the base. Seed varieties possess more branches than flax. The leaves are narrow and short, alternate on the stem and sessile. The leaves are linear to lanceolate 20-40mm long and 3mm broad and blunt at the apex. The inflorescence consists of a

terminal panicle that bears numerous flowers. Flowers are usually white or blue 15-25mm diameter, complete and perfect with 5 petals, 5 sepals and fine stamens. Linseed is normally a self-pollinated crop. The fruit is round dry capsule 5-9mm diameter. These are divided into 5 lobules containing several glossy brown seeds shaped like an apple Pip (4–7 mm long. The seeds are flat, shiny and relatively small. The seed color ranges from white to shining yellow or light brown.

The soil most suitable for flax, besides the alluvial kind, is deep loams, and containing a large proportion of Organic matter. Heavy clays are unsuitable, as are soils of a gravelly or dry sandy nature. Farming flax requires few fertilizers or pesticides, within eight weeks of sowing, the plant will reach 10–15 cm (3.9–5.9 in) in height and will grow several centimeters per day under its optimal growth conditions, reaching 70–80 cm (28–31 in) within fifteen days. Moderate temperatures (21^0-26^0c), average rainfall of (45-75cm) and soil pH (5.0-7.0) are ideal; however, at times of flowering frost is harmful. At maturity the leaves become dry, the capsule turns brown and the seeds become shiny. The crop yield varies from place to place depending up on the climate, soil nature, the technology employed and varieties planted. A Well managed crop may yields 1.5-2.0 tons of seeds ha $^{-1}$. In linseed, oil to seeds crushed is 36% while cakes to seeds crushed is 67%.

3.2.2 Nutritional and Medicinal values

Linseed plant (*Linum usitatissimum Linn*.) is a multi-purpose crop; it comes in to two basic varieties: 1. brown; and 2. yellow or golden (also known as golden linseeds). Flax/Linseed produces vegetable oil known as flax/linseed oil which is one of the oldest commercial oils. It is an edible oil that contains about 36-40% of oil which obtained by expeller pressing, followed by solvent extraction. Solvent-processed flax seed oil has long been used as human and animal diets, in industry as a source of oil and as a basic component or additive of various paints or polymers. The two varieties, both produce similar omega-3Fatty acids (FAs) but the exception is a type of yellow flax called solin (trade name Linola), which has a completely different oil profile and is very low in omega-3fatty acids (Kinsella, 1991).

Recently, there has been a growing interest in the probiotic properties of flax and in its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders (Simopoulos, 2002; Haung . and Ziboh., 2001; Haung. and Mills, 1996).The beneficial effects are mostly due to flax lipids. LO is the richest plant sources of Alpha linoleic (ALA)/Omega 6 , and linolenic (Omega-3) polyunsaturated fatty acids, which are essential for humans since they cannot

be synthesized in the organism and must be ingested in food and used as precursor of Docosahexaenoic acid (DHA), which builds up the brain. ALA and DHA are both members of the omega-3 fatty acid family next to Fish oil, the richest source of DHA, which has been proved to exhibit antidepressant activity (EL- Beltogi *et al*, 2007). There is a lack of scientific data regarding the effect of LO on depression; however, recently done experiments on Swiss albino mice proved the presence of a significant antidepressant activity of LO was observed in various behavioral and pharmacological models. Which was confirmed by the elevated levels of nor epinephrine and dopamine in the brains of LO-treated animals, as compared to control animals, that seemed most likely to be mediated through an interaction with the adrenergic and dopaminergic systems Thus, use of LO can be explored as a nutritional supplement during treatment of clinical depression (Shah *et al*., 2014).

One hundred grams of ground flax seed supplies about 450 calories, 41 grams of fat, 28 grams of fiber, and 20 grams of protein (Mayo clinic, 2007). Flax seed sprouts are edible, with a slightly spicy flavor. Excessive consumption of flax seeds with inadequate water can cause bowel obstruction.

In Ethiopia, traditionally, whole flaxseed is roasted and milled in to powder for making thick paste in water and after being salted for taste will be eaten with any sort of dry meal like bread or " Injera".Yet in another way, such ground powder or raw linseed will be soaked in water for overnight which by then becomes transparent- gelatinous oily fluid still containing seeds, can be taken before any meal in the morning, believed to help as laxative in preventing constipation and solve problems associated to gastritis. Whole flax seeds are chemically stable, but ground flaxseed can go rancid at room temperature in a week time, although there is contrary evidence.

Refrigeration and storage in sealed containers will keep ground flax from becoming rancid for a longer period; under conditions similar to those found in commercial bakeries, trained sensory panelists could not detect differences between bread made with freshly ground flax and bread made with milled flax stored for four months at room temperature. (Malcolmson, 2006). Milled flax is stable to oxidation when stored for nine months at room temperature if packed immediately without exposure to air and light (Chen, 1994).

Flax seeds contain high levels of dietary fiber as well as lignans and abundance of micronutrients. Studies suggest that flax seeds taken in the diet may benefit individuals with certain types of breast

and prostate cancer (Thomson *et al*., 2005; Chen *et al*., 2006). A study done at Duke University suggests that flaxseed may stunt the growth of prostate tumors (Thomson *et al*., 2005). Flax may also lessen the severity of diabetes by stabilizing blood-sugar levels (Caligiuri ,2014).There is some support for the use of flax seed as a laxative due to its dietary fiber contents (Mayo clinic ,2006).though excessive consumption without liquid can result in intestinal blockage (Dahl *et al*.,2005). Consuming large amounts of flax seed may impair the effectiveness of certain oral medications, due to its high fiber contents (Dahl *et al*., 2005). Flaxseed has shown to lower the concentration of pro-inflammatory oxylipins in humans and reduces blood pressure in patients with peripheral arterial disease by altering circulating oxylipins via an α-linolenic acid (ALA)-induced inhibition of soluble epoxide hydrolase (caliguri , 2014).Topical flax-seed oil also showed to have positive effects on pain control and nerve function of patients with a carpal tunnel syndrome in a clinical trails (Caligiuri ,2014).Flax seeds contain Omega-3 fatty acids (mostly ALA) and Omega-6 fatty acids (Allebay *et al*., 2005).

According to chemical analysis performed to determine the levels of some Essential (macro-elements) and non-essential (micro-elements) in linseed, revealed a decreasing order concentration ranges of metallic ions from K (6494–6755 mg kg-1) > Mg (2679–3118 mg kg-1) > Ca (540–744 mg kg-1) > Na (242–614 mg kg-1) > Fe (198–242 mg kg-1) > Cu (25–45 mg kg-1) > Ni (12–16 mg kg-1) Mekebo and Chandravanishi (2014).This results indicated that linseed accumulates relatively higher amounts of K and Pb among the determined essential and nonessential metals, respectively. The non-essential heavy metal, Cd, was found to be below the method detection limit. Therefore, contents of minerals in linseed in their study was within the daily recommended level and thus advisable as healthy food for treatment of different health complications

3.2.3. Relevance to Ethiopia

Ethiopia stood seventh among the top ten countries to have produced 65,420 metric tons of linseed in the year 2011, Canada, China, Russia, India, U.K, and U.S.A are the leading six countries, Warday A.J (1967). The principal linseed growing regions in Ethiopia are located at altitudes between 1800 - 2800 meter above sea level (masl), although it occasionally grows at altitudes as low as 1680 masl or as high as 3430 masl CSA,(2012/13). Arsi, Bale, Chercher Mountains, Eastern Welega, Eastern Gojam, Tigray, Southeast Wollo, and Shoa are the major areas of production and South Gondar, Kefa, Gamogofa and Illuababor are small-scale production areas in Ethiopia.

Linseed is a major oilseed and rotational crop for barley in higher elevations of Arsi, Bale, Gojam, Gonder, Wello, Shoa and Wellega. High yields of wheat, barley and tef can be obtained following linseed (Alemayehu *et al,* 1997).

In Ethiopia oil seeds are classified with in grain crop category, in terms of area of cultivation and production, they added 6.81 % (about 855,750.22 hectares) of grain crop area and 2.81 % (about 7,600,993.24 quintals) of the production to the nation's grain total. Sesame,Niger seed (nug) and linseed covered 3.35 %(about 420,490.98 hectares), 2.01% (252,584.38 hectares) and 0.66% (82,323.86 hectares) of the grain crop area and1.07 % (about 2,887,700.79 quintals), 0.83% (about 2, 244,625.07 quintals) and 0.31 %(about 831,305.05 quintals) of the grain production, respectively, CSA (2014/15).The average area of linseed cultivation in Ethiopia was 82,325.78 hectares of land with the total crop production of 831,305.05 quintals and the yield of 10.1qui./ha, during the period 2014/15, CSA (2014/2015).

Latest reports on Linseed, at both Oromia region and Jimma zone indicated 56.9 and 2,611.01 ha area of cultivation, 497.38 and 17,110.65qui. of produce, 8.74 and 6.55 qui/ha of yield, respectively, CSA(2014/2015) meher season.

Table.1. Area/ ha, Production/qui and yield in qui/ha of Linseed by private holder peasants of Jimma Zone of Oromia region, C.S.A (2012/13, 2014/15).

Year(s)	Total area cultivation in hectares	Total production in qui.	Yield(qui/ha)
2012/13	5,714.63847	32,983.55	5.8
2014/15	2,611.01	17,110.65	6.55
Mean (2012-2015)	4,162.72424	25,047.10	6.02

Looking at the mean values of two consecutive meher seasons data of CSA (2012/13-2014/15), as indicated in (Table.1.), showed relatively low levels of linseed cultivation, production and yield, both at regional and Jimma zone levels.

CHAPTER FOUR

4. Materials and methods

4.1. Study Area Description

The soil samples were collected from Nada district named as "Burka Asandabo kebele" at Site called "Goro Warisso" of Jimma zone, Oromiya regional state of Ethiopia (Fig.2). The district is among the major but limited Linseed crop growing areas of this zone. It is located at about 283 km towards southwest from Addis Ababa, Ethiopia, between 7^0 ,40' ,0'' - 7^0 50',00" N latitude and 37^0 , 10',0"- 37^0 ,20', 0" E longitude and at altitudes of 1650 – 2200 m.a.s.l. The area was characterized mostly by hot moist tropical agro-climatic zone; known by its bimodal rainfall: with unpredictable short rains from March - April and the main season ranges over June - September. The mean minimum and maximum annual rainfall ranges from 1066 – 1200 mm with a mean annual temperature of 18-25^{O}C (SLMP, 2009).

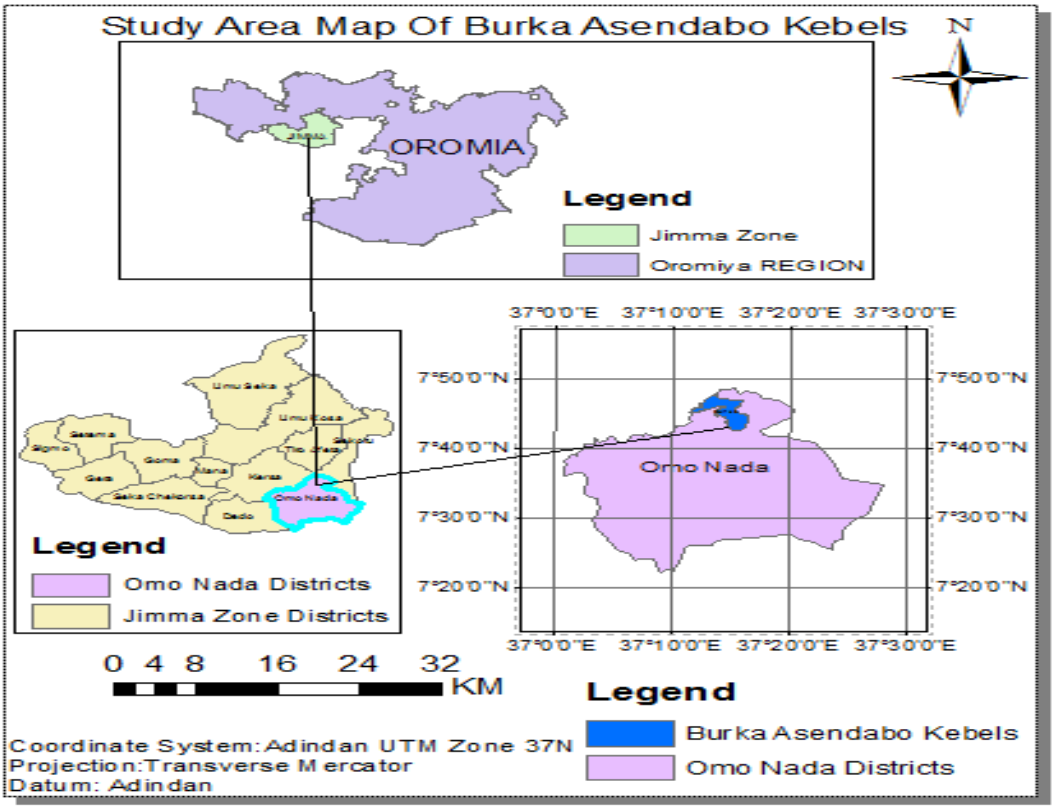


Fig. 2. Map the Study Site (ARCGIS 10.3)

4.1.1. Study area soil sample collection and analysis

A total of thirty soil samples were collected aseptically from five linseed farms (sites) of "Goro kebele". Six samples per each site (farm's) rhizosphere (10-20cm depth) which then bulked as one composite, using sterile spatula and polythene bags. The soil samples were then transported to Jimma University post graduate and research laboratory of Biology Department and kept at 4^0C until microbial analysis

4.1.2. Soil sample preparation for homogenization and serial dilution

The composite soil samples were sieved using 2 mm sieve to remove the layer particles. From each dried composite soil 10 g of sample was suspended in 90 ml sterile 0.1% buffered peptone water separately and homogenized in sterile flasks for 30 minutes on shaker of 150 rpm, then serial dilutions ranged from 10^{-2} up to 10^{-6} were made by transferring 1ml from the flasks into 9ml sterile distilled water after vortexing the diluents in each test tube(Kapoor and Shashi Paroda, 2007).

4.1.3. Screening of phosphate solubilizing rhizobacteria

From all appropriate dilutions(10^{-4} and 10^{-5}), 0.1ml aliquots were uniformly spread plated using bent glass rod on pre dried PVK agar plate, in triplicate, and then incubated at 30^0c for 5-7 days aerobically, only those plates with countable colonies (30-300) were considered as colony forming units (cfu/ml/g) soil (De Freitas *et al*.. 1997). Distinct representative colonies of various morphologies and colors (10-20) were randomly picked up and sub cultured in nutrient broth and then on to PVK agar plates repeatedly, until each culture was purified and enriched.

The screened and purified isolates were kept on nutrient agar slant at 4^0C and preserved in 80% glycerol at -22^0C as stock cultures for the subsequent studies. Every time as new cultures were made from the stock, they were incubated for 24 h at 30 ± 2^0C to activate and check their purity.

4.2. Phosphate solublization

4.2.1. Qualitative Phosphate solublization assay

All isolates were tested for their phosphate solublization activity using a selective media, PVK (Pikoviskaya, 1948) medium, composed of: (g/l: glucose (10g), Ca_3 $(PO4)_2$ (5g), $(NH_4)_2$ SO_4 (0.5g), NaCl (0.2g) KCl (0.2g), $MgSO_4$ (0.1g), $MnSO_4$ (0.002g), $FeSO_4$ (0.002g) ,Yeast Extract (0.5g), Agar (15g), at pH-7.0) agar medium. The media was autoclaved at 121^0c for 15 minutes. About 20-25 ml molten agar medium was poured into each petriplate and allowed to solidify before inoculating the isolates.

From 24 hrs old broth culture of isolates few spots were inoculated on equidistant points on PVK media in triplicates using sterile wire loop, then such plates were incubated aerobically at 30^0C for 5-7 days. The potentials of the isolates to solubilize insoluble phosphates from the precipitated Tricalcium phosphate (TCP) in an un-buffered PVK medium by forming clear zones surrounding colonies, was taken as a primary criterion of Phosphate solublization.

Colonies of efficient P solublizers were determined and recorded on the basis of their clear zones diameters measured by using transparent ruler. Determination of P solublization index was also calculated using the formula outlined in premono procedure (Premono *et al.,* 1996). PSI= Colony diameter + clear zone diameter x 100, where PSI= P- solublization index.

Colony diameter

4.2.2. Quantitative phosphate solubilization in liquid media

Two loopful bacterial suspensions were taken from 24h old cultures of candidate PSB and inoculated into 200 ml of sterilized National Botanical Research Institute for Phosphate (NBRIP) broth media (g/l: 10g of glucose 5g of Ca_3 $(po_4)_2$, 5g of $MgCl_2$ $.6H_2O$, 0.25g of $MgSO_4.7H_2O$, 0.2g of KCl, 0.1 g of $(NH_4)_2SO_4$, at $P^H = 7$) in 250 ml capacity of Erlenmeyer flask (Nutiyal, 1999). All inoculated flasks were placed on rotary shaker at 150 rpm for 8 hrs within days and incubated at 30^0c.

The amount of solubilized phosphate in broth was estimated from triplicate flasks at 5^{th}, 10^{th}, 15^{th} and 20^{th} days of incubation with sets of un-inoculated controls and the pH of the culture medium was also measured accordingly. From each culture broth, insoluble material was removed by

filtering through whatman filter paper No.1 and the filtrate was centrifuged at 10,000 rpm for 15 min.(Tennakon,2007).The available P in the supernatant was estimated by using phosphomolybodic blue color method (Yasmin and Bano, 2011), indicated as the following:

Preparation of reagents

Chloromolybdic acid was prepared from, 7.5g of ammonium molybodate was dissolved in 150 ml distilled water, which contained in 162 ml of conc. HCl and the volume was topped up to 1000 ml with distilled water .Similarly, for chlorostannous acid, 25g of $SnCl_2.H_2O$ was dissolved in 100 ml conc. HCl and the final volume was made up to 1000ml with distilled water.

Spectrophotometric reading

One ml of centrifuged supernatant culture and 10 ml of chloromolybodic acid was added into 50 ml volumetric flask and mixed thoroughly. After the volume was adjusted up to three fourth with distilled water approximately, 0.25 ml of chlorostannous acid was added to it. Immediately the volume adjusted to 50 ml with distilled water and mixed thoroughly. After 15 min., the blue color developed was read using spectorophotometer at 610 nm.

Preparation of standard curve

Quantification of solubilized phosphate was calculated by preparing a standard curve from various concentrations of standards (2 ppm P solution). A 0.2195g oven dried KH_2PO_4 at $110\ ^0C$ for 2hrs was dissolved in 400 ml of distilled water and 25 ml of 7N H_2SO4 was added. The final volume was made up to 1000 ml with distilled water (1ml=59 ppm phosphorus). Twenty ml of this was further diluted to 500 ml with distilled water to obtain 2 ppm solution (1 ml is equivalent to 2 ppm of P). Aliquots of 4, 5 ,6, 8, 10, 15, and 23 ml of the 2 ppm stock solution was transferred in to 50 ml volumetric flasks, 10ml of chloromolybodic acid were added in prepared standard in 50 ml volumetric flask and mixed thoroughly. Approximately after the volume was made up to three fourth with distilled water, a 0.25 ml of chlorostannous acid was added to it. After 10 min., the color developed was read using spectrophotometer at 610 nm. A standard graph was prepared from Phosphorus values for experimental samples were calculated after subtracted from the control.

4.3. Characterization and identification of isolates

Pure isolates from linseed rhizosphere were studied using morphological, biochemical and physicochemical tests. Isolates that formed characteristic colonies on PVK agar media were studied and assigned into their respective genera by referring to the standard procedures as outlined in Bergey's manual of determinative bacteriology (Breed *et al*., 1957).

4.3.1. Some Morphological characterization

All isolates' colony morphologies (growth forms) were considered, if colonies were flagellated / not, straight or raised / bent or circular, whether colony colors were creamy white or any other**.**

4.3.1.1. Gram staining test

Up on drops of saline (distilled) water on a clean glass slide, a sterile loop that touched pure bacterial culture was smear mixed, air dried and heat fixed gently. Crystal violet stain was flooded over for 1 minute, and then washed using running water followed by adding drops of Iodine solution, again washed by running water. Then decolorized using 95% alcohol and 5% acetone solution until the solvent color flows colorless from the slide for 5-10 seconds .Finally counter stained using safranin solution for 60s.The Gram negatives cell wall stained red color when counter stained with safranin while Gram positives retained the purple color of crystal violet. So the isolates were noted for their color and recorded as Gram positive/negative accordingly (Jason woodland, 2004). The stained cells were observed under compound microscope (Olympus BX 60) in the above, were noted to determine their Cell shape thus recorded as round, rod or spiral in shapes.

4.3.1.. Endospore test (Malachite green test)

Five days old, pure nutrient agar cultures of Gram positive isolates were taken and smeared on glass slide, heat fixed and followed by covering the smear with filter paper placed on wire gauze on a ring stand. The filter paper were saturated with Malachite green**,** placed near Benson burner until steam was seen rising from the surface, reheated while steaming up to 3 minutes. Next the paper was removed using forceps, rinsed thoroughly using running water and allowed to dry and counterstained for 45seconds with 0.5%Safranin. Eventually, it was washed, dried and examined under microscope, results depicted that spore former isolate cultures were stained red, so recorded

as positive (+) and where spores not observed, were noted as negative (-) for the test,(Jason wood land , 2004).

4.3.2. Some Biochemical characterization

Various biochemical tests were made: Gram reaction (3% KOH), Starch hydrolysis, HCN production, chitinase, Oxidase, catalase (Eckford MO, 1927; Blazevic et al., 1975), after which the isolates were identified up to Genus level.

4.3.2.1. KOH- test

Gram's reaction was determined by KOH technique (Gregerson, 1978).Visible mounts of an overnight grown cells from agar plates was smeared on $2cm^2$ area on glass slide containing a loopful of 3% aqueous KOH solution . Gram negative strains were identified as viscous gel that string out along with the loop. When there was no slime, but a watery suspension that do not follow the loop, the reaction was considered as negative and the isolate was reported as Gram positive bacteria .

4.3.2.2. Catalase test

A 24h old pure bacterial cultures of nutrient agar was taken using a sterile loop, up on a drop of 3% H_2O_2 was flooded on a microscopic slide and effervescence of gas bubble was designated as catalase positive but where such effervescence did not form within 30 seconds, were recorded as catalase negative (Jason wood land , 2004).

4.3.2.3. Oxidase test

From 24 h old pure nutrient agar culture a portion of colony was picked up using a sterile wooden tooth pick and rubbed onto Watman filter paper (90 mm Whatman® qualitative filter paper),a drop of distilled sterile water (to moisten dry colonies) and oxidase reagent was added on to colonies. Isolates that showed deep purple color that persisted within 30s were considered as reacted positive, (After 30s. reactions were discarded), if, instead of purple, light pink color retained, the isolates were noted as oxidase negative (Jason wood land, 2004).

4.3.3 Some Phytobeneficial characterization

4.3.3.1 Starch hydrolysis test

Inoculants from pure culture were streaked on to a sterile starch agar plates, then incubated at 30^0c for 24 h. Iodine reagent was added to flood the growth, Clear hallows formed around colonies were noted as a positive test result of starch, showing the isolates ability to break down starch indicated the presence of alpha amylase extracellular enzyme. The absence of such clear space around colonies means the isolates didn't use starch, thus, noted as negative for the test (Lebaffe, 2010 and MacFaddin, 2000).

4.3.3 Hydrogen cyanide (HCN) production test

A Method given by Lock (1948) was used to check HCN production by isolates. Isolates were inoculated on modified nutrient agar (nutrient agar with 0.5% (w/v) glycine). A whatman filter paper soaked in a mixture of 1% picric acid and 10% sodium carbonate was left to dry inside the laminar flow for an overnight and fixed in to the underside of plate lids were then sealed with parafilm and incubated at 30 ^{0}C from 4 to 24 hrs. The yellow to orange and finally into red-brown color change of the filter paper within the incubation period was noted as positive test result. An uninoculated plate and a plate without picric acid impregnated paper was included in each test run as control, where such color changes were absent, Lorck (1948)

4.3.4 Physico chemical growth properties of isolates.

Tolerance to salt, pH and temperature variations

The potentials of isolates to grow and subsequently solublize phosphate were evaluated under various physiochemical conditions; that is, under various salt (Nacl) concentrations, pH and temperature levels, with the purpose of finding isolates that can operate under adverse environmental conditions, like extremely saline, at very low/acidic pH and at very high temperatures (at 40^0c or more). Phosphate solubilization efficiency, PSE, (Gaur, 1990; Nguyen *et al*., 1992; Vazquez *et al*., 2000) was calculated using the formula,

$$PSE = \frac{\text{Solublization diameter}}{\text{Colony growth diameter,}} \times 100$$

Where, PSE: is phosphate solublization efficiency.

Thus each isolate's PSE was checked under various salt, pH and temperature conditions.

From A 24 hr pure broth cultures, spots of each isolate was taken using sterile wire loop and inoculated on to equidistant points on PVK media plates adjusted to varying (1, 2, 3, 4) % salt (NaCl) amended media, pH (4, 5, 7 and 9) and temperatures (20, 25, 30, 35 and 40) ^{O}C in triplicates, then incubated aerobically at 30^0C for 5-7 days and found to have solublized precipitated tri-calcium phosphate (TCP) from PVK , as confirmed by clear zone formation surrounding the bacterial colonies was measured using transparent ruler and then calculated for their mean PSE.

4.4 Greenhouse pot experiment

The experiment was conducted to evaluate the effect of PSB for improving phosphate nutrient bioavailability in potted soil amended with rock phosphate (RP), so as to see differences in the growth and yield of linseed crop. Two of the biochemical properties of isolates exhibited during laboratory session, P-solublization and hydrogen cyanide (HCN) production potentials were used to select isolates for testing on a target plant. Thus, six efficient P-solublizers JURB (**3, 9, 11, 21, 50,** and **57**) were selected due to their higher PSI values and among which two of them, JURB (**50** and **57**) were also prime HCN producers at the same time, thus were taken to greenhouse test to evaluate their effects on test plant, linseed.

The pot experiment was conducted under greenhouse condition at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) campus between the months of Aug.2016/17-Jan.2017/18. The mean minimum and maximum temperature of green house was recorded throughout the study period. Soil of each pot was inoculated with one of the selected isolate at the time of sowing, at the rate of 10^8 (1.0 ml) bacterial cells per seed for every treatment using a sterile (1.0 ml) disposable syringe (Kucey, 1988). There were five repeats of all treatments at every 20 days interval since sowing. Both the inoculated and control pots were laid out in complete randomized block design (CRBD) with two of the linseed local varieties labeled as **"**Romae"/ (V_1) and "Shewa"/ (V_2). The two linseed varieties were named locally as "Romae" (V_1) - smaller size and the "Shewa" (V_2) seeds were relatively larger. Both varieties were treated with each of the six inoculants in triplicates and the controls were only fed tap water, thus, there were totally fourteen treatments (x3 = 42 pots).

Table 2. Pot experiment treatments of PSB isolates

Ser. No	Cross with "Romae" variety /(V_1) linseed	Ser. No.	Cross with "Shewa" variety /(V_2) linseed
1	Control ($B_0 V_1$)	8	Control (B_0V_2)
2	$JURB_3$ (V_1)	9	$JURB_3$(V_2)
3	$JURB_9$(V_1)	10	$JURB_9$ (V_2)
4	$JURB_{11}$(V_1)	11	$JURB_{11}$(V_2)
5	$JURB_{21}$(V_1)	12	$JURB_{21}$(V_2)
6	$JURB_{50}$(V_1)	13	$JURB_{50}$ (V_2)
7	$JURB_{57}$(V_1)	14	$JURB_{57}$ (V_2)

JURB: Jimma university rhizobacteria; V: variety

4.4.1 Physicochemical characterization of soil samples and Rock phosphate

About 300 kg of soil was transported from "Omonada" district "kebele" (About seventy two km away from Jimma University) and Rock phosphate (RP) was also brought from Western "Wollega zone, Gimbi district -Bikilal Kebele" from an area called "Tulu Guda Gute" . Laboratory analyses of both soil and RP samples were done following procedures as outlined by Sahlemedhin and Taye (2000), in Jimma University College of Agricultural and Veterinary Medicine (JUCAVM).The soil was air dried and ground to pass through 2mm sieve and 0.5mm sieve (for total N) before analysis. The RP was also crushed, ground and sieved in 2 mm sieve as well. Soil texture was determined by a Bouyoucos hydrometer method (Black *et al*., 1965).

The pH and electrical conductivity (E.C) of the soil were measured in 1:2.5(soil: water) ratio. Organic carbon content of the soil was determined following the wet combustion method of Walkley and Black (Black *et al*., 1965). Total nitrogen (T.N) content was determined by wet oxidation (wet digestion) procedure of kjeldahl method (Sahlemedhin and Taye, 2000).The available Phosphorus content of soil was determined by Bray II method. The available potassium was determined by Morgan's extraction solution (Bray and Kurtz, 1945).

4.4.1.1. Soil Texture

Determined using the hydrometric method where by 50g of dried soil sample was weighed and added in to 1L capacity stopper plastic bottle. The organic matter was destroyed by addition of 100ml of hydrogen peroxide on weighed soil and shook it on oscillatory shaker at 360 rpm for 3hrs. The soil solution was further stirred up using cup mixture for 5 min. After turning down the solution at least 20 times the soil suspension in measuring graduate cylinder was placed on leveled bench and topped up with tap water. The unwanted soil suspension was eliminated by adding amyl alcohol, and then adjusted to 1 liter filling it with tap water. The hydrometer and temperature reading of the soil suspension was taken at 40 seconds and 2hrs intervals (Kumari *et al*., 1992).

4.4.1.2. Soil electrical Conductivity

A 10g of soil sample was added into 250ml beaker. Then, 50 ml of distilled water was added to the weighed soil and stirred for 30 min. using glass rod. The EC electrode was inserted into soil solution to take the EC reading (Denver instrument company, USA) reading was taken.

4.4.1.3. Soil pH

A 10 g air dried soil sample was transferred in to 100 ml of beaker contain 25 ml distilled water to dissolve the soil and stirred for 1 min. using glass rod. After 30 min. the suspension of supernatant was measured by pH meter (HANNA instrument, Portugal).Option pH meter 3310

4.4.1.4. Soil Available Phosphorus

A 2g of soil sample particle size < 2mm diameter was weighed and added in to 50 ml of plastic bottle with stopper. A total of 20 ml extraction solution of Bray II (mixture of 100ml of 1M HCl with 30 ml of 1 M NH_4F that diluted in 870 ml distilled water) was added to the weighed soil sample. The suspension was manually shaken for 1 min. and filtered directly through Whatman filter paper No.42 (110mm diameter, from the filtered solution and 2ppm of stock solution (KH_2PO_4), 2ml of aliquot was pipette into 100ml capacity of volumetric flasks. Thereafter 8ml of 0.5% of boric acid was added to the 2ml of standard series, samples and blanks. The suspension was mixed. Subsequently a2ml of mixed reagent (ammonium molybodate; potassium tartar ate and ascorbic acid) was added and mixed; afterwards the remaining volume mixed reagent was topped up with distilled water (Bray and Kurtz, 1945). After 30 min. the absorbance of the solution

was measured using spectrophotometer at 610 nm. The P-concentration was calculated from plotted graph of absorbance versus concentration standards of phosphorus.

4.4.1.5. Soil organic carbon

A 1g of air dried soil sample was weighed and transferred to 500ml of Erlenmeyer flask. Ten ml of 1N K_2Cr2O_7 solution, 20ml conc.H_2SO_4 and 200 ml distilled water added to both sample and blank, respectively. Both sample and blank were titrated with 0.5 N Ferrous sulphate solution drop by drop until the color flashes to green then continue to alight green endpoint (Walkey and Black, 1934)

$$\% C = N \frac{xV_1\text{-}V_2}{S} \times 0.39 \times Mcf$$

Where,

N= Normality of ferrous sulphate solution

V_1=ml ferrous sulphate solution used for blank

V_2= ml ferrous sulphate solution used for sample

S=Weight of air dried sample in gram

0.39=equivalent weight of carbon, Mcf= moisture correction factor

The organic matter of soil was calculated using its empirical factor (1.74)

% organic matter =1.74 x % carbon

4.4.1.6. Soil Total Nitrogen

Determined in accordance to Kjeldahel procedure (Kjeldahel 1883) A 1g soil sample was weighed and transferred in a digestion tube. After adding 7ml conc.H_2SO_4 the digestion with sample was heated at 300^0c for 3 hrs besides the digester block. The digested sample was transferred to macro-Kjeldahel flasks and distilled. The collected distillate was titrated with 0.1N H_2SO_4 until pink color was appeared. Then total nitrogen was calculated using the volume of the titrated sulphuric acid

$$\% N = \frac{(a-b)}{S} \times N \times 0.014 \times 100 \times mcf$$

, Where,

a= ml of H_2SO_4 required for titration of the sample

b = ml of H_2SO_4 required for titration of the blank

s = air dried sample weight in grams, N =Normality of H_2SO_4 (0.1N), 0.014 = meq weight of nitrogen in g, mcf = moisture correction factor.

4.4.1.7. Soil Available Potassium

A 10g dried soil was added in to 100 ml bottle on a shaker in to which 50 ml Morgan's extraction solution was added and sets on oscillatory shaker of at least 180 rpm for 30 min. Thereafter, the filtrate was collected in 100ml Erlenmeyer flask the extract was diluted 5-10 times using the extraction solution.100ml of potassium stock solution (100mg/L- potassium standard series and 1000 ppm K/L were pipetted out and added into 1 L volumetric flask and diluted up with distilled water. A standard series working solution of 0-2-4-6-8-10 mg/L potassium pipetted into 250ml volumetric flasks respectively of 0-5-10-15-20-25ml of the diluted 100mg/L standard solution diluted to volume with Morgan's extraction solution (Morgan, 1941). For measuring K the standard working solution was aspirated in a flame and transmittance curve was made. A similar curve was constructed by aspirating the sample solution and transmittance value was recorded.

Calculation: Curve method was used and graph was made with mg/L of potassium on Y axis versus % transmittance on X-axis, the sample conc. can be read of from the standard curve

Avail. K (mg/K soil) = a-b (50) x 1000 x df x mcf/ 1000 S

Avail. K (mg/K soil) = 50 x df x a-b x mcf, where,

a = Concentration of K measured in the sample (mg/L)

b= Concentration of K measured in the blank (mg/L)

S= air-dried sample soil weight in g (10g)

mcf= moisture correction factor, df= dilution factor, 50= ml of extract used/sample

4.4.2. Soil and Seed sample preparation

New plastic pots of 7kg soil content or volumes of (32cmx25.5cm) sized were surfaces sterilized using 95% ethanol and filled up each with the prepared soil from linseed farmlands and mixed up with properly washed and dried sand in 3:1(soil: sand) both non sterile in the ratio of 4.5 kg soil to 1.5 kg sand and 200 mg RP kg^{-1} soil (1.2g/a pot) was incorporated thoroughly in to the mixture. Twenty seeds from each of the two linseed varieties were surface sterilized using 0.5% $HgCl_2$ for 2 minutes at room temperature and thoroughly washed with distilled water (Sirikandrajah *et.al,* 1993) sown in to each of the fourteen treatments (42) pots. All pots were watered regularly with tap water. Seedling numbers were thinned down to 10 plants per pot before the second round treatment.

4.4.3. Inoculums preparation for treatments

All cultures taken from the stock were revived and sub cultured to insure purity. The inoculants were prepared in nutrient broth medium by incubating the bacterial strains to a stationary exponential growth phase followed by dilution in nutrient broth medium, from which a volume of 1ml was inoculated in to 250 ml Erlenmeyer flasks containing 200 ml nutrient broth. The flasks were incubated at 30^0c for 24 hrs, on Gallenkamp orbital shaker, up to a final cell concentration of 10^8 cfu/ml (OD 600nm =1.0) (Pilli and Swarup, 2002)

4.4.4. Measuring effects of PSB inoculation on growth and yields of linseed crop

Linseed crop plant had taken 180 days to mature and yield seeds; growth processes were evaluated by measuring fresh and dry matters (g/plant weight of roots, shoots and the ratios of dry weight of roots to shoots) recorded at harvest. For dry weight measurement, an intact roots and shoots were air dried, followed by oven drying at 72 ^{0}c for 72 hrs until constant weight is obtained, and measured separately following the standard procedures (Tenakon, 2007). Also measures of mean value data were taken from the triplicate pots: on length (cm/plant of roots from stem base up to the longest root tip, shoots), no. of capsules (fruits) and seeds/a plant, and seed weight (g)/a plant, harvested by hand threshing, were recorded and finally P-nutrient content (%) and uptake of seeds (g/plant) was calculated and computed against control.

4.4.5. Phosphorus Nutrient content determination

A method called Dry ashing, as outlined by the manual of ICARDA (International Center for Agricultural Research in Dry Areas) (Zaklouta *et. al,* 2011) for determining phosphorus was carried out on the ash solution aliquots obtained after calcinating the crop's yield (grains of linseed). The P- in solution was determined colorimetrically using metavandomolybdate reagents for yellow color development by using Spectrophotometer reading at 460nm wavelength.

Reagents were prepared by dissolving 20 g of ammonium molybdate in 25 ml distilled water of a 1 liter volumetric flask and was marked as (solution I) and1.25 g of ammonium metavanadate (NH_4VO_3) was dissolved in 300 ml of distilled water in a 1 liter beaker, heated for speedy dissolution; cooled and 425 ml of conc. perchloric acid was added, where this was marked as (solution II). Then solution II was added on solution I and brought up to volume distilled water.

Phosphorus stock solution preparation: 500 ppm: was made from about 3 g dried potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105 ^{0} C for 2 hours then cooled in desiccators. KH_2PO_4 (2.197 g) was accurately weighed into a 100 ml beaker and washed into a 1 liter volumetric flask by adding 800 ml of distilled water to dissolve and make a volume, shaken well before Stored in an amber bottle; the100 ppm P solution was made by diluting 50 ml of the 500 ppm P solution to 250 ml in a 250 volumetric flask with distilled water and the 10 ppm P solution was made by diluting 50 ml of the 100 ppm P solution to 500 ml in a 500 ml volumetric flask with distilled water.

Then phosphorus working standards of 0, 1, 2, 3, 4 and 5 ppm P, were made by pipetting 0, 5, 10, 15, 20 and 25ml respectively of the 10 ppm P solution into 50 ml volumetric flasks. Then 5 ml aliquot from the sample digest of the dry ash was pipetted into a 50ml volumetric flask in to which also 10 ml of vandamolybdate reagent was added. The volume was topped up to 50 ml with distilled water and allowed to react for 10 min. The development of yellow color was read at 460 nm from spectrophotometer. The blanks were used to set zero absorbance or 100% Transmittance. The P content concentration was read off from standard curve plotted on graph paper prepared, with absorbance on the X-axis and concentration on the Y-axis.

Calculations, $P\ (ppm) = \frac{C.\ V_1.\ V_2}{S.A} \times mcf$

Where: C = P concentration in sample ashy solution read from the curve, (ppm).V_1 = Volume of the digest (50 ml) ,V_2 = Volume of the dilution (25 ml), S = Weight of the plant material calcinated in g (1).A = Aliquot (5 ml), mcf = Moisture correction factor (1)

Phosphorus uptake, Calculated as: P-uptake (g/plant) = (%) P content x total dry weight (seed (g)/plant) (Marcante *et al*., 2016).

4.4.6. Statistical Analysis

In this study, data collected from Laboratory session and greenhouse experiment were subjected to a statistical analysis of variance (ANOVA) of "R" version (3.3.3) statistical package program for windows, and comparisons among treatments means were separated by Tukey's multiple comparisons test where differences were considered Honestly significant (HSD) at $P < 0.05$ value.

CHAPTER FIVE

5. RESULTS

5.1. Isolation, characterization and identification of PSB

5.1. Isolation for Screening PSB by Serial dilution

Bacterial colony counts were made by suspension-dilution method and dispensing each dilution on agar plated selective growth media. As a result, the average countable colonies from the triplicate plates of 10^{-4} and 10^{-5} dilutions (30-300 cfu/ml/g) (De Freitas *et al*., 1997) were considered in all samples.

The bacterial isolates screened and purified from the 1^{st} 2^{nd}, 3^{rd}, 4^{th} and 5^{th} composite soil samples were 15, 19, 11, 16 and 15 respectively, which resulted in a total of seventy six phosphate solubilizing bacteria (PSB) collected from linseeds' rhizosphere

5.2. Phosphate solublization

5.2.1. Qualitative Assay

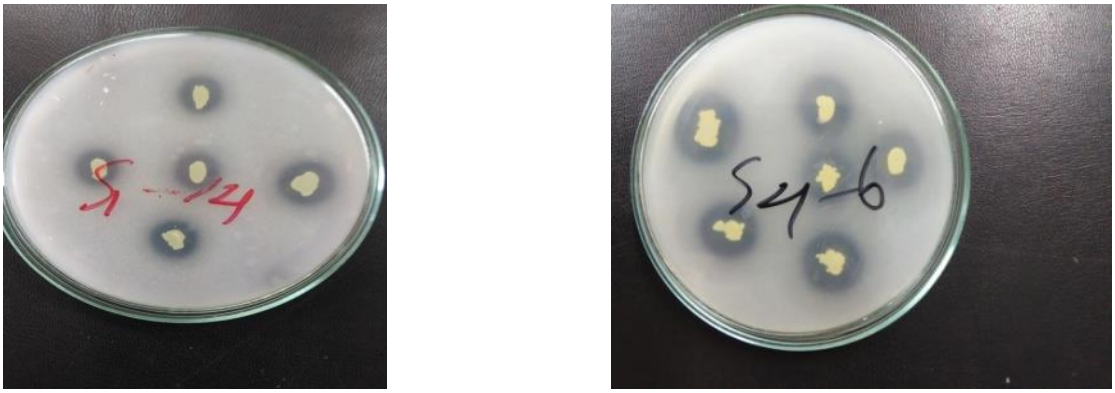
All the seventy six PSB isolates recorded PSI values ranging from 1.20 to 4.00 cm within 5-7 days of incubation period, at 30^{o}c temperatures given as in (Table 3).

Table 3 Phosphate solublization index (PSI) of PSB

PSI – ranges	No. of isolates (%)	Isolates' code
1.2-2.00	42 (55)	JURB**1,7,14-17, 22-25,29-31,33-36,38-43,45,46,51, 55,56,62-69,71,72,74-76.**
2.01- 2.5	15 (20)	JURB**2,4,6,10,20,26,27,32,37,53,59-61,70,73**
2.51-2.99	7 (9)	JURB**8, 19,28,28,44,49,52,54**.
3.00 - 4.00	12 (16)	JURB**3,5,9,11-13,18,21,47,50,57,58**.

JURB: Jimma university rhizobacteria; PSI: phosphate solublization index.

Results of Table 3 and fig.3 indicated, out of the seventy six screened isolates, $JURB_{21}$ of PSI (4.00 cm) value was the most efficient Phosphate solublizer on solid PVK agar plate, followed by $JURB_9$ of PSI (3.75cm), JURB ($_3$ and $_{11}$) both with PSI (3.5cm) and JURB ($_{50}$ and $_{57}$) both with PSI (3.4 and 3.0 cm) respectively, whereas $JURB_{71}$ recorded the smallest PSI (1.20 cm) was the least solublizer.



$JURB_9$ (After 5 days) $JURB_{21}$ (After 5 days)

Fig.3. P solublization of PSB colonies on PVK media.

5.2.2 Quantitative Assay

Six top listed PSB of superior PSI values were selected to quantify the amount of Phosphate solubilized in NBRIP (National Botanical Research Institute for Phosphate) broth. During the incubation periods, every isolate solublized significantly ($p < 0.001$) higher amounts of phosphate than the control, Bo (14.2.µg /ml). Until the 15th day, the amounts of P solublized increased in the culture media within the range of min. 30.6 µg/ml by $JURB_{57}$ and the max. 46.3 µg/ml by $JURB_{21}$. These concentrations were highly significant ($P < 0.001$) over the mean concentration of control, B_0 (14.2µg/ml) as recorded in Table 4.

Table 4 Phosphate solublized by PSB inoculants' in liquid (NBRIP) broth.

Bacterial Genus	5th day		10th day		15th day		20th day	
	pH	S. P Conc. (µg/ml)	pH	S. P conc. (µg/ml)	pH	S. P conc. (µg/ml)	pH	S. P conc. (µg/ml)
B_0 (control)	6.7^{a}	14.2^{g}	6.6^{a}	14.5^{e}	6.5^{a}	14.6^{f}	6.8^{a}	14^{f}
$JURB_3$ (*Pseudomonas*)	4.8^{cd}	32.3^{d}	4.7^{bc}	33.6^{c}	4.6^{bc}	34.2^{d}	4.5^{cd}	34^{d}
$JURB_9$ (*Pseudomonas*)	4.5^{d}	35.9^{b}	4.4^{c}	36.8^{ab}	4.2^{c}	40.6^{b}	4.3^{d}	38.4^{b}
$JURB_{11}$ (*Pseudomonas*)	4.6^{cd}	34.5^{c}	4.5^{c}	35.6^{b}	4.3^{c}	38.4^{c}	4.4^{cd}	36.8^{c}
$JURB_{21}$ (*Pseudomonas*)	4.4^{d}	36.8^{a}	4.3^{c}	38.2^{a}	4.1^{c}	46.3^{a}	4.2^{d}	40.6^{a}
$JURB_{50}$ (*Bacillus*)	5^{bc}	30.6^{e}	4.8^{bc}	32.6^{c}	4.6^{bc}	34.1^{d}	4.7^{c}	34^{d}
$JURB_{57}$ (*Bacillus*)	5.4^{b}	28.6^{f}	5.2^{bc}	29.9^{d}	5.0^{b}	30.6^{e}	5.1^{b}	30.2^{e}
P value	***	***	***	***	***	***	***	***

PSB: phosphate solublizing bacteria, JURB: Jimma University rhizobacterial isolates of linseed, S. P-Soluble Phosphate, Conc.: concentration. Mean values of similar letters within columns are not significantly different by Tukey's HSD (Honestly significant difference): P value significant: at (NS,*, **, ***- Not significant or significant at $P < 0.05$, 0.01, or 0.001 respectively).

As opposed to increasing trends of Phosphate solublization, till the 15th day, there were a correspondingly decreasing trends of pH (5, 4.2 and 4.1) as recorded for JURB (**57**, **9** and **21**) isolates in the culture media supernatants respectively, which showed significantly ($p< 0.001$) very higher decrease than the control pH (6.5), Table 4.

The greatest drop of pH (4.1) was recorded by $JURB_{21,}$ yet it was not significantly different from pH (4.2) of $JURB_{9}$, whereas the least drop in pH (5) was recorded by $JURB_{57.}$ Altogether the decrease in pH of all isolates were significantly ($p<0.001$) higher than the controls, Bo, pH (6.5) Table 4. Generally, all the six selected isolates solubilized significantly ($p < 0.001$) higher amounts of P than the control, Bo (14.6 µg/ml). Besides, isolates of *Pseudomonas* (JURB **21, 9** and **11)** solublized significantly higher amounts of P (µg/ml: 46.3, 40.6 and 38.4) respectively than the *Bacilli* (JURB**50** and **57)** solublized (µg/ml: 34.1 and 30.6) respectively

5.3 Some Morphological, Biochemical and Phyto-beneficial feature tests of Linseed rhizobacteria.

Generally, all screened isolates displayed diverse morphological, biochemical and phytobeneficial features as indicated in appendices(A and B), the profiles of six selected isolates summarized in table 6. Morphologically, they depicted variable colony growth forms on agar plates; Some 46 (60.5%) were straight and flagellated, 18 (23.7%) were chained and flagellated, the rest 12 (15.8%) were raised, circular (bent), creamy white and non-flagellated.

All isolates, 76 (100%) were rod in shape, out of which 28 (36.8 %) were Gram positive whereas 48 (63.2 %) were Gram negative. Twenty eight (36.8%) isolates were spore formers whereas 48 (63.2 %) were non-spore formers. Biochemically, for catalase test 71(93.4%), KOH test-48(63.2%) and Oxidase test 68 (89.5%) were positive.

Based on test results of morphological, biochemical and phytobeneficial characters, all isolates were identified to a genus level in accordance to Bergey's manual of determinative bacteriology (Breed *et al*., 1957). Hence, isolates were assigned into three main genera: 46 (60.5%) *Pseudomonas*, 18 (23.7%) *Bacillus* and 12 (15.8%) *Arthrobacter.*

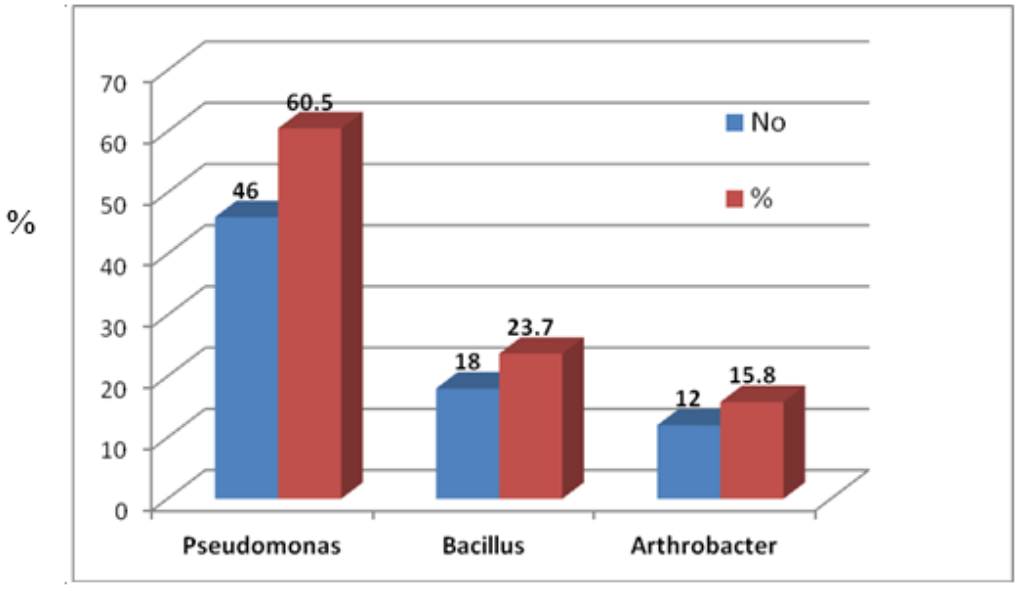


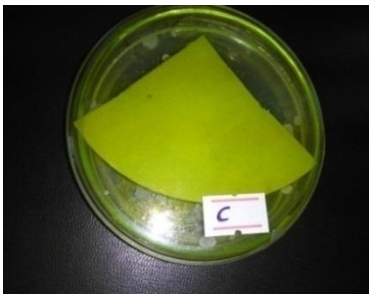
Fig.4 Genera of PSB isolates.

Some phytobeneficial features test results

Hydrogen cyanide (HCN), Amylase and Chitinase production tests

Three (4%) isolates JURB(**50, 51** and **57**) among the 76 have produced HCN metabolite, as confirmed by the color change of the filter paper from yellow to red-brown via orange-red, within 4 -24 hrs. intervals respectively. Isolates of JURB (**50** and **57**) were selected as potent bio-fertilizers for green house test on a targeted test plant.

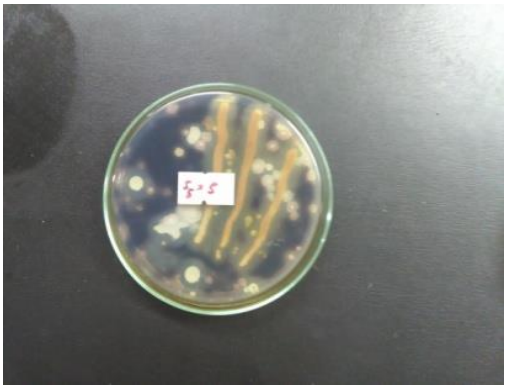
HCN- Positive (+) ($JURB_{50}$)



HCN- Negative (-) (control)

Fig.5. HCN (Hydrogen cyanide) test results.

Starch test negative



Starch test positive

Fig.6 Starch (Iodine) test results.

As for starch test, all isolates were tested for producing an enzyme amylase using iodine reagent, 29 (38.2%) isolates reacted positive (produced amylase), hence were able to degrade the complex carbohydrate starch, which was indicated by clear zone formation surrounding the bacterial colonies whereas the rest isolates, 57(75%) reacted negative (no such clearance but the blue black color of iodine remained unchanged surrounding the colonies.)

Table 5 Some Morphological, Biochemical and Phytobeneficial traits profiles of selected isolates

Isolate code	**Genera**	**Some morphological feature tests**			**Some biochemical Feature tests**			**Some phyto beneficial features tests**		
		Form	Gram stain	Endo spore Form-ation	3% KOH	Catalase 3% H_2O_2	Oxidase	PSI (cm)	Starch (I_2)	Hydrogen Cynide (HCN)
JURB3	*Pseudo-monas*	Straight flagellate	–	–	+	+	+	3.50	–	–
JURB9	*Pseudo-monas*	Straight flagellate	–	–	+	+	+	3.75	–	–
JURB11	*Pseudo-monas*	Straight flagellate	–	–	+	+	+	3.50	+	–
JURB21	*Pseudo-monas*	Straight flagellate	–	–	+	+	+	4.00	–	–
JURB50	*Bacillus*	Chained flagellate	+	+	–	+	+	3.40	+	+
JURB57	*Bacillus*	Chained flagellate	+	+	–	+	+	3.00	+	+

JURB: Jimma university rhizosphere bacteria; PSI-phosphate solublization index

5.4. Physicochemical properties of isolates

All isolates were allowed to grow at various physico-chemical conditions (saline, pH and temperatures), and were evaluated by their Phosphate solublization efficiencies (PSE) on PVK growth media, as indicated in (Table 6).

5.4.1. Tolerance to varying Salt (Nacl) concentration

All the 76(100%) isolates of 1%, 73 (96%) of the 2% and only 2(3%) of the 3% and 4% salted media responded positively by growth. The highest degree of PSE (260%) was recorded by $JURB_{21}$ in 1% salted media and the least (no solublizations) were noted for 3(4%) isolates in 2% salt and for 74 (97%) isolates in both 3% and 4% salted media. Besides, two isolates, $JURB_{11}$ and $_{12}$ were uniquely solublized Phosphate with PSE (175% and 120%) in 3% salted and PSE (120% and 100%) in 4% salted media respectively, where they seem to have developed potentials of carrying out their cellular activities under osmotic pressures (salt stressful conditions).

5.4.2. Tolerance to pH variations.

At pH (7) all the 76 (100%) isolates and at pH (5 and 9) 75 (99%) whereas at pH (4) only 12 (16%) isolates solublized precipitated Phosphate. The highest PSE (300%) was recorded at pH (7) by $JURB_{21}$ and the least (no solublizations) were recorded at pH (4) for 64(84%) and at pH (5 and 9) by 2 (3%) isolates. Besides, two rhizobia, JURB ($_{21}$ and $_{9}$), showed better solublization at pH (4) with PSE (150 % and 125%) and at pH (9) with PSE (250% and 240%) respectively

Table 6 Physiological (Physicochemical) properties of isolates

PSE range (%)	% salt (NaCl) concentration amended and number of isolates grown				pH variation and number of isolates grown				Temperature variations and number of isolates grown				
	1%	2%	3%	4%	4	5	7	9	20^0c	$25\ ^0$c	30^0c	35^0c	**40^0c**
0-50	20(26)	23(30)	-	-	3(4)	29(38)	5(7)	17(22)	41(54)	25(33)	6(8)	20(26)	-
51-100	24(32)	23(30)	-	1(1)	6(8)	24(32)	38(50)	30(39)	24(32)	27(36)	36(47)	24(32)	**1(1)**
101-150	15(20)	16(21)	1(1)	-	3(4)	11(15)	14(18)	17(22)	2(3)	13(17)	15(20)	18(24)	**3(4)**
151-200	12(16)	9(12)	1(1)	1(1)	-	10(13)	12(16)	9(12)	6(8)	8(11)	11(15)	9(12)	-
201-250	4(5)	2(3)	-	-	-	1(1)	5(7)	3(4)	2(3)	2(3)	6(8)	4(5)	-
251-300	1(1)	-	-	-	-	-	2(3)	-	-	1(1)	2(3)	1(1)	-
Total no. of isolate	76	73	2	2	12	75	76	75	75	76	76	76	**4**

PSE: phosphate solublization efficiency, Figures in brackets represents percentages of PSB

5.4.3. Tolerance to temperature variations.

Isolates reacted differently as temperature varied, when temperature increased from 20^0c to 35^0c, nearly all, 75(99%) isolates solublized precipitated TCP of PVK, whereas only 4 (5%) isolates reacted in different percentages at 40^0c.The highest PSE (300%) was recorded by *pseudomonas* ($JURB_{21}$) isolate at 30^0C and the least PSE (no solublization) was noted for 1(1%) at 20^0c and 72 (97%) isolates at 40^0c. Three isolates, JURB (**27, 49, and 57**), specifically grew and solublized P, at 40^0c with PSE (120, 133 and 125) % respectively, which seemed to be thermo-tolerants.

5.5. Greenhouse pot Experiment

5.5.1. Soil Characterization for greenhouse experiment.

The composite soil sample collected from study sites was analyzed physically and determined in to a textural class clay-loam. The mean minimum and maximum temperature of the greenhouse was 12.5 ^{0}c and 30.7^0c respectively. The various Chemical features of the soil sample is given in Table 8 and index D (Metson, 1961).

Table 7. Physical and chemical characteristics of soil and RP used for pot experiment

Sample	pH H_2O	EC (mS/c)	(%) OC	(%) TN	Av.P (ppm)	Av.K (ppm)	(%) Soil texture			
							Sand	Clay	Silt	Textural Class
Soil (compo-site)	6.8	0.158	2.31	0.115	4.52	0.905	22	38	40	Clay-Loam
RP	1.333	0.074	1.809	0.090	3.073	0.613	60	21	19	Sandy-Clay – loam

P^H- H_2O (1:2.5, soil: water), EC: electrical conductivity (1:5, soil: water), OC: organic carbon (Walkely and Black, 1934), TN: Total nitrogen (Kjeldhal); Av.P: available phosphorus (BrayII); Av.K: available potassium (Flame photometer), Texture (Hydrometer), RP: Rock Phosphate.

5.5.2. Measuring effects of PSB inoculations on growth and yields of linseed crop

Both "Romae" /(V_1) and" Shewa" /(V_2) Linseed varieties responded to PSB inoculation in terms of their growth performances, yield and yield components.

(A) (B) (C)

(D) (E) (F)

Fig. 7. Growth processes of linseed crop varieties inside greenhouse; (A): Seedling after 4- days, (B): PSB isolates of JURB inoculants ready to be inoculated, (C): Growth after 2 weeks, (D): Flowering (E): fruiting, (cropping) and (F): Capsules harvested.

Table 8 Effects of PSB inoculants on the growth of "Romae" variety (V_1) linseed crop.

Bacterial genus (PSB) Treated		Fresh matter				Dry matter			Yield and yield components				
		Root length (cm)	Root wt(g)	Shoot ht (cm)	Shoot wt(g)	Root wt(g)	Shoot wt(g)	Ratio (root to shoot)	Capsules no/a plant	Seeds no/a capsule	Total seed no/a plant	Total seed wt/a plant(g)	100 seed wt /a plant
B_0	Control	8.60^{e}	0.1^{b}	51.6^{b}	4.4^{b}	0.03^{b}	1.35^{b}	0.022^{a}	26^{d}	8^{c}	207^{d}	0.46^{c}	0.22^{f}
$JURB_3$	*Pseudomonas*	10.74^{c}	0.23^{ab}	73.43^{a}	18^{a}	0.043^{ab}	4.4^{a}	0.01^{b}	68.7^{b}	8^{c}	549.3^{b}	1.75^{a}	0.32^{b}
$JURB_9$	*Pseudomonas*	8.75^{e}	0.3^{ab}	72.5^{a}	19.3^{a}	0.09^{a}	4.6^{a}	0.02^{a}	78^{a}	9^{b}	702^{a}	1.84^{a}	0.26^{d}
$JURB_{11}$	*Pseudomonas*	14.00^{a}	0.4^{a}	69.25^{a}	16.5^{a}	0.095^{a}	4.2^{a}	0.02^{a}	70^{b}	7^{d}	490^{b}	1.7^{ab}	0.35^{a}
$JURB_{21}$	*Pseudomonas*	10.00^{d}	0.2^{ab}	67.67^{a}	14.7^{a}	0.044^{ab}	3.25^{ab}	0.013^{b}	50^{c}	7.7^{c}	400^{c}	0.87^{bc}	0.31^{c}
$JURB_{50}$	*Bacillus*	9.60^{d}	0.16^{ab}	69^{a}	12.05^{ab}	0.042^{ab}	3.13^{ab}	0.013^{b}	59^{cd}	8.7^{b}	531^{b}	1.25^{ab}	0.24^{e}
$JURB_{57}$	*Bacillus*	12.70^{b}	0.27^{ab}	71^{a}	13.83^{a}	0.075^{ab}	4.0^{ab}	0.02^{a}	72^{ab}	10^{a}	720^{a}	1.87a	0.26^{d}
P value		***	*	**	***	**	*	NS	***	***	***	***	***

JURB: Jimma university rhizobacteria; V: variety; Mean values of similar letters within columns are not significantly different by Tukey's HSD (Honestly significant difference): P value significant: at (NS,*, **, ***- Not significant or significant at $P < 0.05$, 0.01, or 0.001 respectively) ; JURB3, 9, 11 and 21 belong to Genus *pseudomonas*, $JURB_{50}$ and $_{57}$- belong to Genus *Bacillus*.

Table 9 Effect of PSB inoculants on the growth of "Shewa" variety (V_2) liseed crop**.**

Bacterial genus (PSB)Treated		Fresh matter				Dry matter			Yield and yield components				
		Root length (cm)	Root wt. (g)	Shoot ht. (cm)	Shoot wt. (g).	Root wt.(g)	Shoot weight(g)	Ratio (Root to shoot)	Capsules no/a plant	Seeds no/a capsule	Total seed no./a plant	Total seed wt/a plant (g)	100 seeds wt/a plant (g)
B_0	Control	8.25^{f}	0.15^{a}	61.25^{a}	7.35^{d}	0.027^{a}	1.25^{d}	0.023^{a}	25^{d}	7^{c}	168^{d}	0.3^{d}	0.25^{e}
$JURB_3$	*Pseudomonas*	10.25^{e}	0.173^{a}	66.13^{a}	10.33^{cd}	0.045^{a}	4^{ab}	0.014^{ab}	75^{a}	8^{b}	600^{a}	1.6^{bc}	0.27^{d}
$JURB_9$	*Pseudomonas*	12.8^{b}	0.33^{a}	75^{a}	21.08^{ab}	0.062^{a}	4.5^{a}	0.0133^{ab}	72^{a}	9^{a}	636^{a}	1.9^{a}	0.29^{c}
$JURB_{11}$	*Pseudomonas*	11.2^{d}	0.24^{a}	67.3^{a}	17.95^{bc}	0.07^{a}	4.35^{a}	0.02^{a}	70^{a}	9^{a}	634.7^{a}	1.7^{ab}	0.27^{d}
$JURB_{21}$	*Pseudomonas*	14^{a}	0.25^{a}	64^{a}	20.05^{ab}	0.06^{a}	3.75^{ab}	0.02^{b}	42^{c}	8^{b}	336^{c}	1.5^{c}	0.45^{a}
$JURB_{50}$	*Bacillus*	12.17^{bc}	0.22^{a}	70.83^{a}	17.02^{bc}	0.058^{a}	4.2^{a}	0.0133^{ab}	69^{a}	9^{a}	621^{a}	1.7^{ab}	0.27^{d}
$JURB_{57}$	*Bacillus*	11.5^{cd}	0.25^{a}	63.33^{a}	26.67^{a}	0.038^{a}	3.75^{ab}	0.01^{b}	60^{b}	8^{b}	480^{b}	1.5^{c}	0.31^{b}
P value		***	NS	NS	***	NS	*	NS	***	***	***	***	***

JURB: Jimma university rhizobacteria; V: variety; Mean values of similar letters within columns are not significantly different by Tukey's HSD (Honestly significant difference): P value significant: at (NS,*, **, ***- Not significant or significant at P < 0.05, 0.01, or 0.001 respectively) JURB3, 9, 11 and 21 belong to Genus *pseudomonas*, JURB50 and 57- belong to Genus *Bacillus*.

5.5.2.1. PSB Effects on fresh matter growth.

All treatments with *pseudomonas* inoculants, (JURB **3, 9, 11, 21**) and *Bacillus* (JURB **57**) except JURB**50** strains responded with higher increase in root lengths of both "Romae" (V_1) and "Shewa" (V_2) linseeds in the ranges of the shortest: (1.7 - 27.27) % to the ranges of the longest: (62.8 - 69.7)%, that is significantly ($p < 0.05$) greater than their respective controls. However, none of these inoculants had such similar effects on both varieties, that is, JURB**11** (*Pseudomonas*) and JURB**57** (*Bacillus*) strains promoted growth of V_1 roots whereas JURB (**3, 9, 21**) *pseudomonas* strains promoted growth of V_2 roots.

Though, insignificantly different from each other, linseeds of V_1- treated with every inoculant of JURB showed significantly ($p<0.05$) higher shoot height growths which ranged between minimum and maximum (34 - 42.3)% respectively over the control; however, these, same inoculants didn't cause similar growth difference effects on shoot height of V_2 linseeds.

5.5.2.2. PSB Effects on dry matter growth

The mean dry root weight of all treated "Romae" (V_1) linseeds, except JURB (**11** and **9**) and "Shewa" (V_2) linseeds were not significantly greater than their respective controls, B_0 (0.03 g and 0.027g), whereas the recorded mean dry shoot weights of all treated linseeds of "shewa" /V_2 and "Romae" /V_1 (except the ones treated with JURB(**21, 50,** and **57**)) were significantly ($P < 0.05$) greater than their respective controls, B_0 (V_1 (**1.35 g**) and V_2 (**1.25 g**)).

The mean dry root to shoot ratios of both "Romae" (V_1) and "Shewa" (V_2) linseeds treated with all JURB inoculants were either significantly lower in some or similar to their respective controls, B_0 (0.022 and 0.023) respectively, that is, none of the treated linseeds of both varieties recorded greater mean ratios of root to shoot, rather, conversely, the mean ratios of root to shoot of controls were significantly higher than the treated linseeds.

5.5.2.3. PSB Effects on yield and yield components

The mean grain yield (total seed weight) (g) / a plant of both "Romae" (V_1) and "Shewa" (V_2) linseeds treated with every isolate of JURB produced very significantly ($p<0.001$) higher yield over their respective controls, B_0 (g/a plant: 0.46 and 0.3 respectively). The superiority of treated linseed yields (seed weight) (g) of both (V_1) and (V_2) over their respective controls was ranged

with the lowest: (89% and 400%) noted for $JURB_{21}$ (*Pseudomonas*) isolate to the highest 305.5% of $JURB_{57}$ (*Bacillus*) and 533% $JURB_9$ (*Pseudomonas*) isolates respectively

The mean no. of capsules / a plant yield of both "Romae" (V_1) and "Shewa" (V_2) linseeds treated with PSB isolate of JURB inoculants were very significantly ($p<0.001$) greater than their respective controls, Bo (26 and 25). The (%) capsule no. of both treated (V_1) and (V_2) linseeds ranged with the minimum (92.3% and 68%) by $JURB_{21}$ (*Pseudomonas*) to the maximum (200% *Pseudomonas* ($JURB_9$ and$_3$) isolates respectively

The mean no. of seeds (grains) /a capsule yield of "shewa" /(V_2) linseeds treated with all isolates of JURB and "Romae" /(V_1) linseeds inoculated with JURB $_{57}$ (*Bacillus*) were significantly ($p < 0.05$) greater than their respective controls, Bo (8 and 7). However, the mean no. of seeds (grains) yield / a capsule of "Romae" / (V_1) linseed treated with all isolates of JURB, except $JURB_{57}$ (*Bacillus* spp) were either significantly lower (**$JURB_{11}$**) / not different ($JURB_3$ or $_{21}$) than the control, Bo (7).

Table 10 P- Nutrient content of "Romae" / (V_1) linseed yield.

Bacterial genus (PSB) treated with (V_1)	Total grain (seed) wt. (g)/a plant	P- conc. (ppm)	P-content (%)	P-uptake (g/a plant)
B_0 (control)	0.46^{c}	284.14^{b}	0.0284^{b}	0.0131^{e}
$JURB_3$ (*Pseudomonas*)	1.75^{a}	408.09^{ab}	0.0408^{ab}	0.0306^{d}
$JURB_9$ (*Pseudomonas*)	1.84^{a}	338.24^{b}	0.0338^{b}	0.0622^{ab}
$JURB_{11}$ (*Pseudomonas*)	1.7^{a}	383.93^{ab}	0.0384^{ab}	0.0653^{a}
$JURB_{21}$ (*Pseudomonas*)	0.87^{bc}	481.09^{a}	0.0481^{a}	0.0419^{cd}
$JURB_{50}$ (*Bacillus*)	1.25^{ab}	409.66^{a}	0.0410^{ab}	0.0512^{bc}
$JURB_{57}$ (*Bacillus*)	1.87^{a}	359.24^{ab}	0.0359^{ab}	0.0672^{a}
P value	***	*	*	***

JURB: Jimma university rhizobacteria; V: variety; Mean values of similar letters within columns are not significantly different by Tukey's HSD (Honestly significant difference): P values significant: at (NS,*, **, ***: Not significant or significant at $P < 0.05, 0.01, 0.001$ respectively).

Table 11. P- Nutrient content of "Shewa" / (V_2) linseed yield.

Bacterial genus (PSB) treated with V_2	Total grain (seed) wt. (g)/ a plant	P conc. (ppm)	P-content (%)	P -uptake (g/a plant)
B_0 (control)	0.3^{d}	662.82^{ab}	0.0663^{ab}	0.0199^{c}
$JURB_3$ (*Pseudomonas*)	1.6^{bc}	504.20^{b}	0.0504^{b}	0.0807^{b}
$JURB_9$ (*Pseudomonas*)	1.9^{a}	729.52^{ab}	0.0730^{ab}	0.1386^{a}
$JURB_{11}$ (*Pseudomonas*)	1.7^{cd}	749.47^{ab}	0.0749^{ab}	0.1274^{ab}
$JURB_{21}$ (*Pseudomonas*)	1.5^{c}	938.55^{a}	0.0939^{a}	0.1408^{a}
$JURB_{50}$ (*Bacillus*)	1.7^{cd}	797.27^{ab}	0.0797^{ab}	0.1355^{a}
$JURB_{57}$ (*Bacillus*)	1.5^{c}	820.96^{a}	0.082^{a}	0.1231^{ab}
P value	* * *	*	*	**

JURB: Jimma university rhizobacteria; V: variety; Mean values of similar letters within columns are not significantly different by Tukey's HSD (Honestly significant difference): P value significant: at (NS,*, **, ***: Not significant or significant at $P < 0.05, 0.01, 0.001$ respectively)

The mean P-uptake of the two linseeds "Romae"/ (V_1) and "Shewa" / (V_2) treated with every inoculant of JURB were very significantly ($p < 0.001$) greater than their respective controls, Bo (g/ a plant: 0.0131g and 0.0199g respectively). The (%) P uptakes of treated, "Romae" / (V_1) and "Shewa" / (V_2) linseeds were superior over their controls, both within the minimal ranges (133.6 and 305.5) % by $JURB_3$ **(***Pseudomonas***)** to the maximal (412.8% by $JURB_{57}$ (*Bacillus*) and 607.5% by $JURB_{21}$ (*Pseudomonas*) isolates respectively.

CHAPTER SIX

Discussion

In the current study, most screened isolates from linseed rhizosphere were efficient PSB strains of genera *Pseudomonas* and *Bacilli*, similar, dominant and efficient *Bacilli* and *pseudomonas* strains were reported from rhizospheric soil of Teff plant (*Eragrostis teff*) by (Delelegn and Fassil, 2011), Faba Bean (*Vacia faba*) reported by (Shiferaw *et.al*, 2013; Girmaye *et al*., 2014;).

Others, Sriniyasamurthy and Dayamani, (2014), isolated Gram positive PSB, *Bacillus* and *Arthrobacter,* as main strains and few others for the rest, from the rhizospheric soil of soya bean, maize, and sorghum and cow pea crops. Strains of Genus *Arthrobacter* were also isolated and included as one of the PSB (Wani *et.al*, 2005).

The PSI records of isolates ranged between 1.2cm ($JURB_{71}$) to 4.0 cm ($JURB_{21}$) this showed that, 70 (92.1%) isolates were efficient P solublizers, a results that agree with De Freitas (1997), which says "good phosphate solubilizers produce clear zones surrounding colonies with diameters of more than 1.5cm"

Variations in the sizes of clear zones formed by most PSB during qualitative assay might be due to the varying diffusion rates of organic acid products and the subsequent P-solublization potentials of the PSB, as reported previously by Johnston (1952). Besides, this study results were better than the one reported by Saida *et al.*, (2013), where only 35 (79.5%) isolates solublized Phosphate from TCP with PSI values which only ranged from 0.6 cm to 1 cm diameters of clear spaces. Similar behaviors of PSB were also reported by El-Komy (2005).

The *pseudomonas* (JURB **21, 9, 3(11)**) and *Bacilli* (JURB **50** and **57**) isolates resulted in superior PSI values of 3 cm to 4cm on solid PVK (1948) agar media were still efficient solublizers of phosphate in NBRIP broth media as they resulted in, 25mg/ml (*Bacillus*/$JURB_{57}$) to 46.3 mg / ml by *pseudomonas* ($JURB_{21}$), Similarly Alam *et al*., (2002) and Yasmin *et al*., (2012) have reported P solubilizers *Bacillus* and *Pseudomonas* isolates from NBRIP broth, where organic acid production was detected, thus decreased the pH of the culture medium, as reported by Stevenson (2005).

The fact that the *Pseudomonas* strains solubilized greater amounts of P than the *Bacilli* might suggest that they may have produced relatively more organic acids, since the pH of *pseudomonas* growth medium was more lowered than that that of the *Bacilli*, (Park *et al*., 2010).

During NBRIP broth media solublization, as the amounts of P-solublized increased steadily until the 15th day max. conc. (46.3 µg/ml) by *pseudomonas* (JURB**$_{21}$)**, its corresponding culture media pH (4.1) was the least, which indicated the presence of an inverse relationship between the amounts of P-solublized and pH of the culture media, this may also mean that, an increase in acidification of culture media might have facilitated inorganic P-solubilization, this was due to a mechanism associated with production of organic acids by the PSB isolates, very similar inverse relationships were obtained and reported by the works of Puente *et al.,* 2004; Yasmin and Bano, 2011.

The decrease in releasing rate of soluble P during the final stages of incubation period might be due to the depletion of nutrients in the culture medium, in particular, carbon source needed for the production of organic acids (Kim *et al.,* 2005; Chaiharn and Lumyong, 2009) or it might be due to the availability of soluble phosphorus in the culture medium that may have an inhibitory effect on further phosphate solubilization, or the excretory toxic products may also be responsible for such decline in P solubilization, this was in line with the reports of Varsha-Narsian *et al.* (1994).

Only few isolates, 3(4%) were hydrogen cyanide (HCN) producers, a strain of *Pseudomonas* (JURB $_{51}$) and two strains of *Bacilli* (JURB$_{50}$ and $_{57}$**)**, were important isolates, since HCN plays role in biological control (antibiosis) (Defagio *et, al*, 1990), thus, indirectly promotes plant growth by fighting against phytopathogenic microbes. The producer microbes, especially *pseudomonas,* aren't themselves affected as they are resistant to their own metabolite, reported by Bashan and de-Bashan (2005). This was also in agreement with the reports of Muleta *et al*., (2007) where *Pseudomonas spp*, produced HCN and showed remarkable inhibitory effects against the test fungal pathogen. Therefore, bio-control compounds like HCN-indirectly promote plant growth by inhibiting phytopathogens.

The fact that two of the three HCN producers were spore formers (*Bacilli spp*), provided double advantages over the non-spore formers like *pseudomona*s, since the *Bacilli* are more resistant to adverse environmental conditions, as higher temperatures and chemicals, besides to their longer

shelf life persistence as bio-products after formulation (P. Kumar *et al*., 2012). So such isolates of Phosphate solublization potentials fortified with bio-control capabilities are promising candidates to be formulated as bio-inoculants.

Some microbial isolates have the potentials of degrading and using complex carbohydrates like starch and chitin by producing enzymes as amylase and chitinase respectively. They use these complex sugars as carbon sources for their growth and reproduction, thus contribute to soil fertility. The 29 (38.2%) isolates that reacted positive with iodine reagent hydrolyzed starch by releasing their amylase enzyme, thus were efficient and competent PSB of linseeds' rhizosphere.

The superiority of the PSE (260%) in 1% salted media, (300%) at temperature of 20 to 35^{o} C and (300%) of pH (5- 9) physicochemical media displayed by,75-76 (99-100%) isolates of this study, indicated that, nearly neutral pH (7), 1% salt and temperatures (20–35)^{0}c were the optimum physicochemical growth media. Very similar optimum growth media results were obtained by Rodriguez and Fraga, (1999) and Nautiyala *et al*., (1999); Md.T.Islam *et al.,* 2007 at pH (7). However the recorded optimum growth media temperatures were slightly different from the opt. (35-40)^{0}C results records of Uma Maheswar and Sathiyavani, (2012).

In contrast to this optimum growth media, only fewer isolates were grown and solubilized Phosphate under stressful physiological conditions. Isolates of 3%, 5% and 16% were grown at: (3-4%) salted, temp. (40^{0}c) and acidic pH (4) respectively. Such results were in agreement with the one reported by (Dhanushkodi *et al.,* 2013 and Shimaila *et al.,* 2014 for *Bacillus* and *Pseudomonas* respectively),where they promoted plant growth under salt stresses, in fact, these were the microbes that have special adaptations to cope with adverse conditions, thus, can be taken as promising bio-inoculants (Yasmin and Bano, 2011).

Most isolates that tested (+) for oxidase enzyme, 65(85.5%) had cytochrome c, a respiratory protein involved in electron transport needed during energy release, often present in aerobic microbes, oxygen is used as a final electron acceptor of the respiratory path way, as reported by Mohsin *et al*., (2014), this is one of the essential characteristics of PSB, as they are expected to be highly competent and actively growing microbes of the rhizosphere.

The production of catalase enzyme by most linseed rhizosphere 64 (84.2%) isolates was important because catalase converts the toxic metabolic product, hydrogen peroxide (H_2O_2) in to nontoxic water and oxygen, so this antioxidant intracellular enzyme is essential for aerobic life, similar works were reported by Joseph and Jini (2011).

Because the available P of potted soil rated very low (4.52) (Metson, 1961) appendix D, it needed to be amended with additional source of P, Rock phosphate (RP) for testing the selected PSB equipped with useful biochemical properties on linseed crop.

The significantly ($P<0.05$) higher increase in fresh matter growths (cm/a plant: roots of both "Romae" and "Shewa", and shoots height of "Romae") could be attributed to cell elongation and multiplication induced by greater absorption of nutrients, particularly P and/or it might be due to strains' ability to produce phytohormones such as Auxins, this was partly in line with the reports of Xuan *et al.*(*2011*), where walnut seedlings inoculated with members of *Pseudomonas* (*P chlororaphis, P fluorescens) and Bacillus (B cereus*) remarkably increased plant heights of the seedlings.

Also a significant increase in shoot ht. (45%), was determined in maize plant of growth chamber inoculated with PSB strains of *Pseudomonas, (P. tolaasii*) compared to the control, as reported by Viruel, *et al*. (2014). In a pot experiment with mung bean, Sharma *et al.* (2003) reported that the *Pseudomonas* strain GRP3 increased the growth of shoots by 101% and roots by 39%. Differences in the effects of inoculants on the two linseed varieties might be attributed to variations in their genetic makeup.

In this study, the mean dry root weight (g) of all treated "Romae" (V_1) linseeds, except JURB (**11** and **9**) and "Shewa" (V_2) linseeds were not significantly greater than their respective controls. Such results explains (traces) back the reasons of earlier significantly higher growth in fresh matters (root length and shoot height), that, it should have been primarily due to the stimulatory effects of hormones to promote growth, as Collavino *et. al.,* (2010) demonstrated, plant photosynthesis affected by P deficiency can be relieved by inoculating PSB, nevertheless, the abilities to solubilize P in vitro and promote plant growth are not necessarily associated with each other. As all treated linseeds of "shewa" /V_2 and "Romae" /V_1 (except the ones treated with JURB (**21**, **50**, and **57**) recorded significantly ($P < 0.05$) greater mean dry shoot weight over their respective controls, so were walnut seedlings inoculated with strains of *Pseudomonas* and *Bacillus* remarkably increased

dry shoot weights, not only this, also in dry root weights and P-uptakes, was reported by Xuan *et al.* (*2011*). Also a significant increase in shoot dry weight (40%) was determined in maize plant of growth chamber inoculated with PSB strains of *Pseudomonas, (P. tolaasii*), while (*P. koreensis*) has remarkably increased P content (145%) as compared to the control(Viruel, *et al*. (2014).

The mean dry root to shoot weight ratios of both "Romae" (V_1) and "Shewa" (V_2) linseeds treated with PSB inoculants of JURB were either significantly lower in some or similar in others with respect to their corresponding controls, B_0 (0.022 and 0.0233 respectively), that is, none of the treatments, in both varieties resulted in significantly higher root to shoot ratios over their controls. Conversely, the controls were significantly ($P < 0.05$) recorded higher dry root to shoot ratios over the treated.

These results agree with the reports of Kim and Li, (2016), where pot experiment of green house was conducted on Lantana plants treated (supplied) with lower amounts of P, allocated more biomass (weight) to roots than to shoots, which then resulted in higher root to shoot ratios (similar to the case of this study, control over the experimental).

Though root to shoot ratios depend on particular plant environmental conditions, plant traits of relatively higher root to shoot ratio may be associated with greater extraction of P from the rhizosphere and help plants become better adapted to P- nutrient limitation, thus, higher root to shoot ratio records of control. Obviously, the control pots soil was more P deficient since not inoculated with PSB, hence more growth of control roots to extract more P from soil than the treated (which were inoculated with PSB and supposed to have solubilized P).

The significant increases of treated linseed yields (seed weight) (g) of both (V_1) and (V_2) over their respective controls, with ranged least increase (89 and 400) % noted for $JURB_{21}$ (*Pseudomonas*) isolate to the highest increase 305.5% of $JURB_{57}$ (*Bacillus*) and 533% $JURB_9$ (*Pseudomonas*) isolates respectively, was due to an increase in the development of plant biomass and grain yield of the treated linseeds.

Such superiority of treated linseeds over controls were attributed to the effects of inoculated bacterial strains that may have primarily promoted growth by the production of growth promoter hormones like auxins (IAA), which has a cascade effects on root development and nutrient uptake,

improved the P- nutritional plant status and secondly to phosphate solubilization capacity, which increased soil P availability due to their rapid colonization and solublization of P salts in rhizosphere, this was in harmony with the reports of Richardson *et al*., (2009) that early P- uptake leads to an increase in crop yield by stimulating plant growth and development.

The PSB inoculants resulted in significant (%) increase of capsule no. of both treated (V_1) and (V_2) linseeds in the ranges of least increase (92.3 and 68) % by $JURB_{21}$ (*Pseudomonas*) to the highest increase (200%) by *Pseudomonas* ($JURB_{9}$ and $_{3}$) isolates respectively, such results were in agreement with the reports of Dey *et al.* (2004) where *Pseudomonas spp* increased pod yield of peanut by 18% to 26% and Saharan *et al.* (2010) reported a 46% increase in Vigna yield using *Pseudomonas* R81.

The mean no. of seeds (grains) /a capsule yield of "shewa" (V_2) linseeds treated with all isolates of JURB and "Romae" (V_1) linseeds inoculated with $JURB_{57}$ (*Bacillus* sp.) were significantly ($P < 0.05$) greater than their respective controls, Bo (8 and 7). However, the mean no. of seeds/ grains per a capsule of "Romae" (V_1) linseed treated with all isolates of JURB, except $JURB_{57}$ (*Bacillus* spp), were either significantly lower ($JURB_{11}$**)** than or similar ($JURB_{3}$ and $_{21}$) to the control, Bo (7). The difference in effects of inoculants on the two linseed varieties might be due to their genetic makeup variations.

Despite the insignificant impact of most JURB inoculants on seed nos. /a capsule of "Romae"/ (V_1) linseed's, they recorded significantly ($p < 0.05$) greater seed biomass (g) of 100 seeds and capsules nos. /a plant, that is greater seed biomass (g) of 100 seeds and capsules nos. /a plant, which means positively contributed to the growth and development of linseeds, which might be due to the P solublization activities of these bacterial strains, thus increased the bio-availability P nutrient and its subsequent absorption by plant roots from the linseed rhizosphere. Similar results were reported on *Pseudomonas aurantiaca* where it effectively colonized the rhizosphere and internal tissues of roots of maize and promoted maize growth (Fang *et al*., 2012).

Unlike other isolates, $JURB_{57}$ (*Bacillus*) treated "Romae" (V_1) linseeds produced significantly greater no. of seeds/a capsule, which might be due to many reasons, among which the isolates' potential to prevent pathogen establishment, by its cyanogenic activity, in the rhizosphere through antibiosis, can contribute to its increased growth (Defagio *et al*., 1990).

The significantly higher (%) P- uptake by all treated, "Romae" / (V_1) and "Shewa" / (V_2) linseeds over their respective controls, both with ranged least increase of (133.6 and 305.5)% by $JURB_3$ **(***Pseudomonas***)** to the highest increase (412.8 % by $JURB_{57}$ (*Bacillus*) and 607.5% by $JURB_{21}$ (*Pseudomonas*) isolates respectively, indicated that these PSB inoculants directly. or indirectly improved phosphate solublization and absorption. Reports of similar PSB isolates inoculated from genera *Pseudomonas* (*P. fluorescens*) and *Bacillus* spp., improved the P uptake of shoot and grain in maize and peanut plants (Dey *et al.* 2004, Sahin *et al*. 2004, Hameeda *et al*. 2008).

CHAPTER SEVEN

Conclusion and Recommendations

Conclusion

When inorganic-fertilizers applied on soils of tropics and sub tropics, the phosphates largely form insoluble salts and remain unavailable to plants, such fertilizers can be used coupled with bio-inoculants. Microbiologists researched and came up with solutions like reducing the use of inorganic fertilizers by half and coupling with bio-fertilizer as PSB, without compromising crops' productivity, this is a possibility; because several crop plants treated with bio-inoculants improved phosphate uptakes, growth performances and yields.

Thus PSB inoculants have records of comparative advantages over chemo-fertilizers, like reducing costs spent on chemo-fertilizers, increased eco-friendliness, thus, insurance of sustainable agriculture. However, it shouldn't be missed that, no single microbial inoculant applied to all systems, as their effectiveness is determined by many factors like the type of plant, soil, etc.

As both in vitro and in vivo evaluations confirmed, Genera *Bacillus* and *pseudomonas* were dominant isolates of linseed rhizospere with superior efficiency of phosphate solublization potentials, thus, the two genera isolates can be taken as potential candidates to serve as bio-inoculants. Moreover, it would be even far more advantageous when such isolates will be genetically engineered for their useful traits using the high tech tools of molecular biology, pilot tested practically and transferred soon to farmers.

Recommendation

Isolation and characterization of indigenous rhizobacteria should be encouraged. The best performing rhizobacteria in the current study should be tested in the field condition as greenhouse results may not necessarily be extrapolated to field conditions.

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Appendix A. Morphological characteristics of PSB isolates

S.No	PSB isolate code	Phosphate solublization index (PSI)*	Cell morphology		Gm. stain test	Endo spore test	Genera
			Form	Shape			
1	$JURB_1$	2.00	Chained, flagellated	Rod	+	+	*Bacillus*
2	$JURB_2$	2.50	Chained, flagellated	Rod	+	+	*Bacillus*
3	$JURB_3$	3.50	Straight, flagellated	Rod	_	_	*Pseudomonas*
4	$JURB_4$	2.40	Straight, flagellated	Rod	_	_	*Pseudomonas*
5	$JURB_5$	2.50	Chained, flagellated	Rod	+	+	*Bacillus*
6	$JURB_6$	2.40	Straight, flagellated	Rod	_	_	*Pseudomonas*
7	$JURB_7$	1.90	Straight, flagellated	Rod	_	_	*Pseudomonas*
8	$JURB_8$	2.80	Chained, flagellated	Rod	+	+	*Bacillus*
9	$JURB_9$	3.75	Straight, flagellated	Rod	_	_	*Pseudomonas*
10	$JURB_{10}$	2.50	Bent, non-flagellated	Rod	+	+	*Arthrobacter*
11	$JURB_{11}$	3.50	Straight, flagellated	Rod	_	_	*Pseudomonas*
12	$JURB_{12}$	3.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
13	$JURB_{13}$	3.25	Straight, flagellated	Rod	_	_	*Pseudomonas*
14	$JURB_{14}$	1.60	Chained, flagellated	Rod	+	+	*Bacillus*
15	$JURB_{15}$	2.00	Chained, flagellated	Rod	+	+	*Bacillus*
*16	$JURB_{16}$	1.70	Chained, flagellated	Rod	+	+	*Bacillus*
17	$JURB_{17}$	2.00	Bent, non-flagellated	Rod	+	+	*Arthrobacter*
18	$JURB_{18}$	3.00	Straight, flagellated	Rod	_	_	*Pseudomonas*

19	$JURB_{19}$	2.60	Straight, flagellated	Rod	_	_	*Pseudomonas*
20	$JURB_{20}$	2.40	Straight, flagellated	Rod	_	_	*Pseudomonas*
21	$JURB_{21}$	4.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
22	$JURB_{22}$	1.70	Straight, flagellated	Rod	_	_	*Pseudomonas*
23	$JURB_{23}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
24	$JURB_{24}$	1.70	Straight, flagellated	Rod	_	_	*Pseudomonas*
25	$JURB_{25}$	1.70	Straight, flagellated	Rod	_	_	*Pseudomonas*
26	$JURB_{26}$	2.50	Bent, non-flagellated	Rod	+	+	*Arthrobacter*
27	$JURB_{27}$	2.50	Straight, flagellated	Rod	_	_	*Pseudomonas*
28	$JURB_{28}$	2.60	Bent, non-flagellated	Rod	_	_	*Arthrobacter*
29	$JURB_{29}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
30	$JURB_{30}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
31	$JURB_{31}$	2.00	Bent, non-flagellated	Rod	+	+	*Arthrobacter*
32	$JURB_{32}$	2.50	Chained, flagellated	Rod	+	+	*Bacillus*
33	$JURB_{33}$	1.70	Bent, non-flagellated	Rod	+	+	*Arthrobacter*
34	$JURB_{34}$	1.60	Chained, flagellated	Rod	+	+	*Bacillus*
*35	$JURB_{35}$	1.60	Straight, flagellated	Rod	_	_	*Pseudomonas*
36	$JURB_{36}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
37	$JURB_{37}$	2.36	Straight, flagellated	Rod	_	_	*Pseudomonas*
38	$JURB_{38}$	1.70	Straight, flagellated	Rod	_	_	*Pseudomonas*
39	$JURB_{39}$	1.33	Chained, flagellated	Rod	+	+	*Bacillus*
40	$JURB_{40}$	2.00	Chained, flagellated	Rod	+	+	*Bacillus*
41	$JURB_{41}$	1.60	Straight, flagellated	Rod	_	_	*Pseudomonas*
42	$JURB_{42}$	1.44	Straight, flagellated	Rod	_	_	*Pseudomonas*
43	$JURB_{43}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
44	$JURB_{44}$	2.67	Straight, flagellated	Rod	_	_	*Pseudomonas*
45	$JURB_{45}$	1.63	Straight, flagellated	Rod	_	_	*Pseudomonas*
*46	$JURB_{46}$	1.43	Bent, non-flagellated	Rod	+	+	*Arthrobacter*
47	$JURB_{47}$	3.00	Straight, flagellated	Rod	+	+	*Arthrobacter*

48	$JURB_{48}$	1.67	Bent, non-flagellated	Rod	+	+	*Arthrobacter*
49	$JURB_{49}$	2.83	Straight, flagellated	Rod	_	_	*Pseudomonas*
50	$JURB_{50}$	3.40	Chained, flagellated	Rod	+	+	*Bacillus*
51	$JURB_{51}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
52	$JURB_{52}$	2.57	Chained, flagellated	Rod	+	+	*Bacillus*
53	$JURB_{53}$	2.38	Chained, flagellated	Rod	+	+	*Bacillus*
54	$JURB_{54}$	2.67	Bent, non-flagellated	Rod	+	+	*Arthrobacter*
55	$JURB_{55}$	2.00	Bent, non-flagellated	Rod	+	+	*Arthrobacter*
56	$JURB_{56}$	1.39	Chained, flagellated	Rod	+	+	*Bacillus*
57	$JURB_{57}$	3.00	Chained, flagellated	Rod	+	+	*Bacillus*
58	$JURB_{58}$	3.33	Bent, non-flagellated	Rod	_	_	*Arthrobacter*
59	$JURB_{59}$	2.43	Straight, flagellated	Rod	_	_	*Pseudomonas*
60	$JURB_{60}$	2.43	Straight, flagellated	Rod	_	_	*Pseudomonas*
61	$JURB_{61}$	2.17	Chained, flagellated	Rod	+	+	*Bacillus*
*62	$JURB_{62}$	1.67	Straight, flagellated	Rod	_	_	*Pseudomonas*
63	$JURB_{63}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
64	$JURB_{64}$	1.71	Straight, flagellated	Rod	_	_	*Pseudomonas*
65	$JURB_{65}$	1.71	Straight, flagellated	Rod	_	_	*Pseudomonas*
66	$JURB_{66}$	1.67	Chained, flagellated	Rod	+	+	*Bacillus*
67	$JURB_{67}$	1.63	Straight, flagellated	Rod	_	_	*Pseudomonas*
68	$JURB_{68}$	1.71	Straight, flagellated	Rod	_	_	*Pseudomonas*
69	$JURB_{69}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
70	$JURB_{70}$	2.14	Straight, flagellated	Rod	_	_	*Pseudomonas*
71	$JURB_{71}$	1.20	Straight, flagellated	Rod	_	_	*Pseudomonas*
72	$JURB_{72}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
73	$JURB_{73}$	2.40	Straight, flagellated	Rod	_	_	*Pseudomonas*
74	$JURB_{74}$	2.00	Straight, flagellated	Rod	_	_	*Pseudomonas*
75	$JURB_{75}$	1.50	Straight, flagellated	Rod	_	_	*Pseudomonas*
76	$JURB_{76}$	1.67	Straight, flagellated	Rod	_	_	*Pseudomonas*

*PSI= (Colony diameter + Clear zone)/colony diameter, Gm-Gram stain.

Appendix B -Biochemical characteristics of PSB isolates

Serial No.	PSB isolate code	Catalase Test	KOH Test	Starch Hydrolysis test	Hydrogen Cyanide production	Oxidase test
1	$JURB_1$	+	_	+	_	+
2	$JURB_2$	+	_	+	_	+
3	$JURB_3$	+	+	_	_	+
4	$JURB_4$	+	+	_	_	+
5	$JURB_5$	+	_	+	_	+
6	$JURB_6$	+	+	_	_	+
7	$JURB_7$	+	+	_	_	+
8	$JURB_8$	+	_	+	_	+
9	$JURB_9$	+	+	_	_	+
10	$JURB_{10}$	+	_	+	_	+
11	$JURB_{11}$	+	+	+	_	+
12	$JURB_{12}$	+	+	_	_	_
13	$JURB_{13}$	+	+	_	_	+
14	$JURB_{14}$	+	_	+	_	+
15	$JURB_{15}$	+	_	+	_	+
*16	$JURB_{16}$	+	_	+	_	+
17	$JURB_{17}$	+	_	+	_	+
18	$JURB_{18}$	+	+	_	_	_
19	$JURB_{19}$	+	+	_	_	_
20	$JURB_{20}$	+	+	_	_	+

21	$JURB_{21}$	+	+	_	_	+
22	$JURB_{22}$	+	+	_	_	+
23	$JURB_{23}$	+	+	_	_	+
24	$JURB_{24}$	+	+	_	_	+
25	$JURB_{25}$	+	+	_	_	+
26	$JURB_{26}$	+	_	+	_	+
27	$JURB_{27}$	+	+	_	_	+
28	$JURB_{28}$	_	+	_	_	+
29	$JURB_{29}$	+	+	_	_	_
30	$JURB_{30}$	+	+	_	_	+
31	$JURB_{31}$	+	_	+	_	+
32	$JURB_{32}$	+	_	+	_	+
33	$JURB_{33}$	+	_	+	_	+
34	$JURB_{34}$	+	_	+	_	+
*35	$JURB_{35}$	+	+	_	_	+
36	$JURB_{36}$	+	+	_	_	+
37	$JURB_{37}$	+	+	_	_	+
38	$JURB_{38}$	+	+	_	_	+
39	$JURB_{39}$	+	_	+	_	+
40	$JURB_{40}$	+	_	+	_	+
41	$JURB_{41}$	+	+	_	_	+
42	$JURB_{42}$	+	+	_	_	+
43	$JURB_{43}$	+	+	_	_	+
44	$JURB_{44}$	+	+	_	_	+
45	$JURB_{45}$	+	+	_	_	+
$*46_4$	$JURB_{46}$	+	_	+	_	+
47	$JURB_{47}$	+	_	+	_	+
48	$JURB_{48}$	+	_	+	_	+
49	$JURB_{49}$	+	+	_	_	+
50	$JURB_{50}$	+	_	+	+	+

51	$JURB_{51}$	+	+	_	+	+
52	$JURB_{52}$	+	_	+	_	+
53	$JURB_{53}$	+	_	+	_	+
54	$JURB_{54}$	+	_	+	_	+
55	$JURB_{55}$	+	_	+	_	+
56	$JURB_{56}$	+	_	+	_	+
57	$JURB_{57}$	+	_	+	+	+
58	$JURB_{58}$	+	+	_	_	+
59	$JURB_{59}$	+	+	_	_	+
60	$JURB_{60}$	+	+	_	_	+
61	$JURB_{61}$	+	_	+	_	+
$*62_5$	$JURB_{62}$	+	+	_	_	+
63	$JURB_{63}$	+	+	_	_	+
64	$JURB_{64}$	+	+	_	_	+
65	$JURB_{65}$	+	+	_	_	+
66	$JURB_{66}$	+	_	+	_	+
67	$JURB_{67}$	+	+	_	_	+
68	$JURB_{68}$	+	+	_	_	+
69	$JURB_{69}$	+	+	_	_	+
70	$JURB_{70}$	+	+	_	_	+
71	$JURB_{71}$	+	+	_	_	+
72	$JURB_{72}$	_	+	_	_	_
73	$JURB_{73}$	_	+	_	_	_
74	$JURB_{74}$	+	+	_	_	+
75	$JURB_{75}$	_	+	_	_	_
76	$JURB_{76}$	_	+	_	_	_

Appendix C: Growth of isolates under various Physico-chemical conditions (salt, pH and temprature) as a function of their P-solublization efficiencies, PSE (%)

Serial No.	PSB isolate Code	Salt (NaCl) conc. Variation				pH variation				Temperature variation in (^{0}c)				
		1%	2%	3%	4%	4	5	7	9	20^0c	25^0c	30^0c	35^0c	40^0c
1	$JURB_1$	75	67	-	-	-	33	100	75	50	67	100	75	-
2	$JURB_2$	125	100	-	-	-	50	150	125	75	100	150	125	-
3	$JURB_3$	200	167	-	-	-	100	250	200	167	175	250	200	-
4	$JURB_4$	125	100	-	-	-	67	150	125	75	100	150	125	-
5	$JURB_5$	225	175	-	-	100	125	250	225	150	175	250	225	-
6	$JURB_6$	160	140	-	-	80	100	180	120	100	140	180	160	-
7	$JURB_7$	71	57	-	-	-	33	86	71	33	57	86	71	-
8	$JURB_8$	160	140	-	-	100	120	180	140	100	140	180	160	-
9	$JURB_9$	250	225	-	-	125	200	275	250	233	225	275	250	-
10	$JURB_{10}$	125	100	-	-	50	100	150	125	66	100	150	125	-
11	$JURB_{11}$	225	200	175	166	-	166	250	200	166	200	250	225	-
12	$JURB_{12}$	160	140	120	100	100	140	200	180	100	140	200	160	-
13	$JURB_{13}$	200	175	-	-	-	166	225	175	100	175	225	200	-
14	$JURB_{14}$	200	175	-	-	-	20	60	50	38	44	60	50	-
15	$JURB_{15}$	75	67	-	-	-	33	100	75	33	67	100	75	-
*16	$JURB_{16}$	50	40	-	-	-	20	67	60	20	50	67	40	-
17	$JURB_{17}$	80	60	-	-	-	50	100	80	40	60	100	80	-
18	$JURB_{18}$	180	160	-	-	-	125	200	160	125	160	200	180	-
19	$JURB_{19}$	140	120	-	-	-	75	160	140	100	120	160	140	-
20	$JURB_{20}$	120	80	-	-	75	100	140	120	50	80	140	120	-
21	$JURB_{21}$	260	250	-	-	150	200	300	240	250	260	300	280	-

22	$JURB_{22}$	50	33	-	-	-	20	67	50	20	33	67	50	-
23	$JURB_{23}$	80	60	-	-	-	25	100	80	25	60	100	80	-
24	$JURB_{24}$	50	33	-	-	20	33	67	50	20	33	67	50	-
25	$JURB_{25}$	50	33	-	-	20	33	67	50	20	30	67	50	-
26	$JURB_{26}$	133	116	-	-	-	20	150	116	100	116	150	133	-
27	$JURB_{27}$	133	120	-	-	-	40	150	116	80	100	150	133	120
28	$JURB_{28}$	60	-	-	-	-	80	133	100	60	100	133	116	-
29	$JURB_{29}$	80	60	-	-	-	50	100	80	50	60	100	80	-
30	$JURB_{30}$	80	60	-	-	-	50	100	80	50	60	100	80	-
31	$JURB_{31}$	80	60	-	-	-	25	100	80	50	60	100	80	-
32	$JURB_{32}$	133	116	-	-	-	80	150	116	100	116	150	133	-
33	$JURB_{33}$	50	33	-	-	-	20	60	50	25	33	67	50	-
34	$JURB_{34}$	20	-	-	-	-	25	60	40	20	25	60	40	-
*35	$JURB_{35}$	50	40	-	-	-	25	60	40	25	40	60	50	-
36	$JURB_{36}$	83	75	-	-	-	50	100	80	50	75	100	83	-
37	$JURB_{37}$	128	116	-	-	-	80	128	100	100	116	128	114	-
38	$JURB_{38}$	57	43	-	-	-	33	71	67	40	43	71	57	-
39	$JURB_{39}$	25	22	-	-	-	11	33	25	13	25	33	25	-
40	$JURB_{40}$	83	80	-	-	-	40	100	80	50	80	100	83	-
41	$JURB_{41}$	50	40	-	-	-	25	60	40	25	50	60	40	-
42	$JURB_{42}$	33	25	-	-	-	14	44	33	22	25	44	33	-
43	$JURB_{43}$	87	86	-	-	-	50	100	88	71	87	100	86	--
44	$JURB_{44}$	150	140	-	-	-	80	167	150	100	140	167	150	-
45	$JURB_{45}$	50	43	-	-	-	17	63	43	33	50	63	43	-
46	$JURB_{46}$	33	28	-	-	-	18	43	29	20	33	43	28	-
47	$JURB_{47}$	180	140	-	-	-	125	200	160	160	180	200	140	-
48	$JURB_{48}$	60	50	-	-	-	20	67	50	40	50	67	60	-
49	$JURB_{49}$	180	166	-	-	-	100	180	133	160	180	183	166	133
50	$JURB_{50}$	220	200	-	-	-	80	240	160	200	220	240	200	-
51	$JURB_{51}$	80	50	-	-	-	75	100	80	50	60	100	80	75

52	$JURB_{52}$	142	148	-	-	100	114	157	143	100	128	157	142	-
53	$JURB_{53}$	125	113	-	-	-	86	138	112	100	113	138	125	-
54	$JURB_{54}$	150	133	-	-	-	80	167	133	100	133	167	150	-
55	$JURB_{55}$	90	80	-	-	-	56	100	80	77	80	100	90	-
56	$JURB_{56}$	33	28	-	-	-	28	88	63	20	28	39	33	-
57	$JURB_{57}$	160	140	-	-	-	125	200	160	75	140	200	160	125
58	$JURB_{58}$	200	166	-	-	133	166	233	200	180	200	233	217	-
59	$JURB_{59}$	129	114	-	-	-	83	143	114	100	114	143	128	-
60	$JURB_{60}$	129	114	-	-	-	83	143	114	100	83	143	112	-
61	$JURB_{61}$	100	83	-	-	-	40	117	83	40	83	117	100	-
*62	JURB62	50	33	-	-	-	40	67	60	20	33	67	50	-
63	$JURB_{63}$	80	60	-	-	-	50	100	80	25	60	100	80	-
64	$JURB_{64}$	57	42	-	-	-	33	71	57	16	42	71	57	-
65	$JURB_{65}$	57	42	-	-	-	33	71	57	16	42	71	57	-
66	$JURB_{66}$	50	33	-	-	-	20	67	50	20	33	67	50	-
67	$JURB_{67}$	50	38	-	-	-	29	63	50	14	38	63	50	-
68	$JURB_{68}$	57	42	-	-	-	17	71	57	16	42	71	57	-
69	$JURB_{69}$	80	60	-	-	-	50	100	80	25	60	100	80	-
70	$JURB_{70}$	100	86	-	-	-	71	114	100	67	86	114	100	-
71	$JURB_{71}$	10	-	-	-	-	-	20	10	-	10	20	10	-
72	$JURB_{72}$	83	67	-	-	-	50	100	80	40	67	100	83	-
73	$JURB_{73}$	120	100	-	-	-	50	120	80	75	100	140	120	-
74	$JURB_{74}$	80	60	-	-	-	50	100	80	25	60	100	80	-
75	$JURB_{75}$	38	25	-	-	-	14	50	36	14	25	50	38	-
76	$JURB_{76}$	50	33	-	-	-	33	67	60	20	33	67	50	-

Appendix D: Soil fertility classes and their description according to soil rating (Metson, 1961)

S. No	Soil characters	Very low	Low	Medium	High	Very high
1	pH	Extremely acidic(<4.0)	Very Acidic (4.6-5.3)	Moderately acidic (5.3-6.0)	Slightly acidic (6.0-7)	Neutral 7.0-7.0
2	Electrical conductivity (salinity)	Non-saline (<2)	Slightly saline(2-4)	Moderate (4-8)	Strongly saline(8-15)	Very strongly saline(>15)
3	Organic carbon	<2	2-4	4-10	10-20	>20
4	Total nitrogen	<0.05	0.05-0.125	0.125-0.225	0.225-0.300	>0.300
5	Available phosphorus	<5	5-8	8-12	12-20	>20
6	Available potassium	<0.1	0.1-0.3	0.3-0.6	0.6-1.2	>1.2