Addis Ababa University

School of Graduate Studies

College of Natural Sciences

Molecular, Cellular and Microbial Biology Department

(Applied Microbiology Stream)

Evaluation of phyto-beneficial traits of indigenous phosphate solubilizing bacteria and fungi as microbial inoculants for enhancing growth and production of Coffee (*Coffea arabica*) under greenhouse and field conditions in Jimma, South west of Ethiopia

By

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DECLARATION

I declare that the Thesis, hereby submitted by me for the PhD degree in Applied Microbiology to the School of Graduate Studies of Addis Ababa University is my own work and has not been submitted by me or anybody else where.The materials obtained from other sources are duly acknowledged in the Thesis.

Signed on _____the School of Graduate Studies, College of Natural Sciences Molecular, Cellular and Microbial Biology Department (Applied Microbiology Stream) Addis Ababa University.

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LIST OF ABBREVIATIONS

AMF	Arbuscular Mycorrhizal Fungi
HPCL	High-performance liquid chromatography
PGPR	Plant growth-promoting rhizobacteria
PSM	Phosphate Solubilzing microorganisms
PSF	Phosphate solublizing fungi
TCP	Tricalcium phosphate
NBRIP	National Botanical Research Institute phosphate growth medium

VC	Vermicompost
SPP	Standard Package of Practice
INM	Integrated nutrient management
PSI	Phosphate Solublization Index
Rpm	Revolution per minute
CFU/ml	Coloney form unit per milileter
CBD	Coffee berry disease

List of publications

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GENERAL ABSTRACT

Exploitation of phosphate solubilizing bacteria (PSB) and fungi as microbial inoculants is known to promote plant growth through the supply of plant nutrients and supression of pathogens. In view of this, the present investigation was planned to assess the phytobeneficial traits of phosphate solublizing bacterial and fungal isolates recovered from coffee (*Coffee arabica*) and vermicompost to determine their potential in growth promotion of coffee seedlings under low input agriculture.. The microbes were isolated and purified following standard methods. The selected isolates were investigated for their plant growth promoting properties, eco-physiological tolerance under laboratory conditions, and further tested under greenhouse and nursery experiments. The greenhouse and nursery experiments were conducted with completely randomized design (CRD) in three (3) replications per treatments. Thus, a total of 154 bacteria and 72 fungi isolates were recovered from which twelve potent bacterial and nine fungal isolates were selected and investigated for their plant growth promoting properties. Among the twelve bacterial isolates, three of them were tentatively identified to the genera of *Pseudomonas (*$RCHVCB_1$) and *Bacillus* (RScB1.19 and RMaB2.11), and showed significant potential to solubilize Ca_3 $(PO_4)_2$ and posessed several phytobeneficial traits, viz, indole acetic acid, NH_3, HCN productions and N-fixing ability. They also exhibited remarkable tolerance to ecophysiological factors such as heavy metal, acidity and salinity, and inherent antibiotic resistance (IAR). Similarly, three fungal isolates with superior phosphate solubilizeation ability were characterized and identified as genera of *Penicillium* (RSCF1.19) and *Aspergillus* (RCHVCF2 and RLVCF2). During co-culture, RSCF1.19 (*Penicillium sp*.) slightly inhibited the test pathogen, *Fusarium xyloriodes*. The bacterial (RCHVCB1, RScB1.19, RMaB2.11) and fungal isolates (RSCF1.19, RCHVCF2, RLVCF2) enhanced rate of coffee seed germination under laboratory conditions and promoted coffee seedlings growth under glasshouse conditions. The results of inoculated seeds showed significant ($p \leq 0.05$) differences in germination rate and vigor index compared to the control. Isolates RScB1.19, RMaB2.11+RSCF1.19 and RMaB2.11 + RLVCF2 showed high germination rate (20.59%) over the control (13.33%). Moreover, a single inoculation of RLVCF2, RSCF1.19 and co-inoculation of RMaB2.11 with RLVCF2 also showed significant ($p \leq 0.05$) mean root length (1.31 cm) and mean shoot length (1.48 cm) over the control. Under greenhouse conditions, single inoculation of RSCF1.19+phosphate and dual inoculation of RSCF1.19 and $RCHVCB_1$ in the presence of inorganic phosphate fertilizer led to significantly higher plant height, root length, stem girth, leaf number, leaf area, fresh and dry weights. Due to high pH value of the potting medium (vermicompost alkaline pH-pH>7.5), all the treatments combined with vermicompost showed suppressive effect_and no any seedlings were emerged at all. Under nursery conditions, co-inoculation of RSCF1.19 with three bacterial isolates ($RCHVCB_1$, RScB1.19, and RMaB2.11) in combination with inorganic phosphate led to significantly increase the tested growth parameters. Similar increase in growth attributes was

observed in both single and dual inoculations due to vermicompost used compared with both positive and negative controls. Higher NPK-uptake was observed in a combination of bio-inoculants and inorganic phosphate fertilizer compared to the positive and negative control. In general, inoculation of RSCF1.19 and RLVCF2 isolates to coffee 74110 variety combined with inorganic phosphate fertilizer resulted in good vigor and healthier coffee seedlings (RSCF1.19, 34.42%) and (RLVCF2, 37.09%) when compared to control (28.49%). Therefore, both RSCF1.19 and RLVCF2 fungal isolates could be used as bioinoculants after field trials in coffee 74110 variety productions.

Key words: *Bioinoculant, Phosphate, phytobeneficial, eco-physiology, seed germination, Coffee seedlings, coffee Arabica.*

1. GENERAL INTRODUCTION

1.1. BACKGROUND AND JUSTIFICATION

Coffee is a perennial field crop which belongs to the genus *Coffea* in the Rubiaceae family, and is mostly grown in the tropical and subtropical regions (Berthaud and Charrier, 1988). *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* (Robusta coffee) economically dominate the world coffee trade (Damatta and Ramalho, 2006) of which, Arabica coffee represents 70% of global coffee production (Davis et al., 2012) and Arabica coffee export is forecast to reach a record of 247,200 metric tons (USDA, 2020). Besides, Barako coffee, a Liberica variety grown in the Philippines, however, production was cut short due to "coffee rust" infestation (Baconguis and Rowena, 2007), Coffea charrieriana, a caffeine-free coffee found in Cameroon (Stoffelen, et al., 2008) and Coffea stenophylla in Sierra Leone (Rudgard, 2021), which can grow at higher temperatures than Arabica and has a better flavour profile than Robusta (Magazine and Fox, 2021) are also other economically important varieties.

The main coffee producing areas in Ethiopia are west and south west, southern, eastern, and central regions (Melkamu, 2015). According to CSA (2017), the estimated area of land covered by coffee in Ethiopia was about 700474.69 ha, whereas the estimated annual national production of clean coffee was about 469091.12 tons with average productivity of 669.6 kg ha^{-1}. However, currently, it is predicted that an increase in Arabica coffee production during 2020/21 becomes 450,000 metric tons with average productivity of 0.82 ton/ha, whereas the estimated area of land covered by coffee in Ethiopia is about 540,000ha (USDA, 2020). According to Wasihun (2019), the increase in coffee production in the country is by a factor of 40,534.67 ha per year. Similarly, the number of coffee producers in the country increases significantly by the factors of 283, 169.2 holders /year.

Coffee cultivation in Ethiopia could be categorized under four broad production systems (Tadesse, 2015; Tesfu, 2012).i.e. forest coffee (8-10%), semi forest coffee (30-35%), cottage or garden Coffee (50-57%) and modern coffee cultivation (5%). It is estimated that around 280,000 metric tons of coffee produced by all production methods in which 700,000 households

participate (Petty et al., 2003). But according to Taye (2013), the forest coffee production accounts 8-10%, semi-forest coffee accounts 30-35%, garden coffee accounts 50-55% and plantation coffee accounts 5-8% of its total production involving around 15 millions people (including daily laborers).

Generally, small holder farmers account for more than 95% of total production in a low inputs-output traditional farming systems making Ethiopian coffee cultivation naturally organic (Banttee, 1995). The majority of production is on the small garden field with average less than 2 hectares with yields remaining low at around 0.7 - 0.8 metric tons per hectare (USDA, 2016).

In general, *Coffea arabica* L. is the most essential Ethiopians commodity and the principal source of revenue for coffee cultivation sectors and hence they greatly necessitate sustainable coffee cultivation with healthier product quality to remain in the present competitive market (Albertin and Nair, 2004). Although there is a tendency to increase yield via application of chemical fertilizers, it is not sustainable that necessitates other options of soil fertility management strategies for improving plant nutrients and crop productivity include the use of application of crop residues such as surface mulch, N-fixing cover crops, composts, vermicomposts and manures, and application of biofertilizer or microbial inoculants (Bhawalkar, 1996).

Bio-fertilizers are one of the important technology that create very significant impact on farmers as they are cost effective and renewable resource in making nutrients (macro and micronutrients) available to plants to substitue the chemical fertilizers for sustainable agriculture. Among macro-nutrients phosphorus (P) plays critical roles in plant nutrition (Bhat et al., 2017). Organic matter and inorganic compounds are major sources of available P in the soil (Richardson and Simpson, 2011). The insoluble and inaccessible forms of P are hydrolyzed to soluble and available forms through the process of solubilization of inorganic P and mineralization of organic P (Koch et al., 2018). Of the various chemical forms of P, plants take up only negatively charged primary and secondary orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) as nutrient, but most of P in nature exists in various organic and inorganic forms. Therefore, the availability of P

depends on the solubility of this element, which could be influenced by the activity of plant roots and microorganisms in the soil.

Phosphate-solubilizing microorganisms (PSMO) solubilize the insoluble forms of P such as tricalcium phosphate (Ca_3 $_(PO4)_2$), aluminium phosphate ($AlPO_4$), or iron phosphate (Fe_3PO_4) through the production of organic acids, siderophores, and hydroxyl ions (Sharma *et al.*, 2013). Some bacteria only solubilize calcium phosphate, while other microorganisms are capable of solubilizing other forms of inorganic phosphates at different intensities. Bacterial isolates belonging to genera *Enterobacter, Pantoea* and *Klebsiella* solubilize $Ca_3(PO_4)_2$ to a greater extent than $FePO_4$ and $AlPO_4$ (Chung *et al.*, 2005). Likewise, bacterial isolates belonging to *Serratia sp., Pantoea sp., Acinetobacter sp., Bacillus sp., Enterococcus sp., and P. fluorescens sp.* solubilize $FePO_4$ and $AlPO_4$.(Anzuay *et al*., 2017). The production of organic acids, particularly gluconic and carboxylic, is one of the well-studied mechanisms utilized by microorganisms to solubilize inorganic phosphates (Rodriguez and Fraga, 1999).

Several phosphate solublizing bacteria (PSB) have been isolated from the roots and rhizospheric soil of various plants (Souza *et al.*, 2013). Chen *et al.* (2006) have previously reported several PSB strains belonging to the genera *Bacillus***,** *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*. Later, Souza *et al.* (2013) have identified isolates belonging to the genera *Burkholderia, Cedecea*, *Cronobacter*, *Enterobacter*, *Pantoea* and *Pseudomonas* which were able to solubilize $Ca_3(PO_4)_2$. Moreover, an earlier report by Suhane (2007) showed that the abundance of phosphate solublizers in vermicompost indicating its potential to screen phytobeneficial microbes from natural pool. Biswas et al. (2018) have demonstrated the efficiency of bacteria isolated from the gut of earthworm in solubilizeation of $Ca_3(PO_4)_2$ by tolerating higher concentration of heavy metals such as Cu and Zn. Esakkiammal et al. (2015) have also reported that the fungal population is found to be significantly higher in the fresh vermicast obtained from vermicompost of *Eudrilus eugeniae* and *Eisenia fetida.* Likewise, Anastasi et al., (2005) have isolated and documented a total of 142 fungal species that are associated with vermicompost.

Penicillium and *Aspergillus* spp. are the dominant P-solubilizing filamentous fungi found in rhizosphere (Chuang,et al.2007). They are said to be the most powerful P solubilizers (Fankem *et*

al., 2006).Filamentous fungi are highly important in P solubilization. It was reported that *Aspergillus niger* and *Trichoderma harzianum* could be potential candidate for developing bio-inoculants to facilitate P supply to different crops in alkaline and acidic soils with organic and inorganic P content and also possessed plant growth promoting attributes such as auxin and sidreophore production(Gaind, 2016). Earlier reports by Pandey et al (2008) and Pindi and Satyanarayana (2012) was indicated that some *Penicillium* species and *Aspergillus niger* have a potential role in Rock Phosphate solubilizeation, P mobilization and tend to accelerate plant growth promotion using different strategies. Thus, there is a need for rigorous screening of efficient PSMO with adaptation to different soil conditions (Zaharan, 1999) with due emphasis on vermicompost and coffee rhizosphere thereby to increase organic coffee production and productivity.

The discovery of phosphate-solubilizing bacteria and fungi for solubilization of insoluble P compound in Pikovskaya's solid culture medium (Pikovskaya, 1948) opened the gate for today's thorough investigation to try out their application under field conditions. Following this finding, several strains of bacterial and fungal species have been described and investigated in detail for their inorganic phosphate-solubilizing capabilities (He *et al*., 1997). Kunwar *et al.* (2018) have investigated and reported that *in vitro* and greenhouse experiments showed a significant improvement in coffee seedlings treated with phosphate solubilizing bacteria isolated from hotspot of *Coffea arabica* rhizosphere. The ability to solubilise P in a culture medium is a potential activity but does not always guarantee biofertilizer activity in the field. Therefore, field experiments should be done with the amendment of insoluble P source to test if a potent bacterial and fungal isolates can enhance P availability under field conditions and consequently improve plant growth that indicates their potential as biofertilizers. Fertile potting medium is fundamental components in soil fertility management to establish healthy and vigorous coffee seedlings in the nursery for better coffee plantation in the future. To promote plant growth, the soil must contain both macro and micro-nutrients in the available forms to be easily taken by roots.

The current trends and progressive emerging interest in organically grown coffee needs attention to stay in viable world market. In these regards, sustainable agriculture and soil fertility enhancement is important for successful crop production in the farming system. Due to growing

demands for sustainably produced products, environmental, social and food safety reasons, the use of bio-inoculants is becoming inevitable, particularly for export market dependent commodities such as coffee.

Nitrogen and phosphorus are the two most important elements that limit plant growth and productivity in the soil. Phosphorus limitation is by far the most important production impediment for it lacks aerial phase in the geochemical cycle in nature. On the other hand, phosphorus exists in the soil by forming insoluble metallic complex with iron and/or aluminium in acidic soil or with calcium carbonate in alkaline soil. As a result, only a small fraction of phosphate is available to plants (Mahantesh and Patil, 2011). This means that the majority of soluble inorganic P is rapidly immobilized by soil fixation and becomes unavailable for plant uptake, leading to insufficient phosphorous acquisition (Kochian 2012). After application of P fertilizer, a great proportion of it forms complex compound with soil metals and becomes insoluble as a result only about 25 per cent of the phosphorus applied to the soil becomes available for plant nutrition which results in deficient soil P (Mahantesh and Patil, 2011). To alleviate P deficiency in the soil, chemical phosphatic fertilizers are widely used.

Soil bacteria and fungi employ some important mechanisms in making plant nutrient available, such as decomposition, nutrient mobilization and mineralization, nitrogen fixation and denitrification (Datta et al., 2011). They are also important in solubilization of inorganic phosphates through organic acid production (Kilian, et al., 2006). Therefore microbial mediated phosphorous management is an eco-friendly and cost effective approach and is a realistic alternative in order to lower the environmental risk and to enhance the productivity of crops (Sharma et al., 2013).

Therefore, the bioavailability of both organic and inorganic phosphate is dependent on the efficiency of phosphate solubilizing microbes in the vicinity of the coffee rhizosphere (Nyalemegbe, et al. 2009). It is the role of phosphate solubilizing microorganisms to convert the insoluble phosphate into soluble forms by production of organic acids inorder to acidify the rhizosphere, chelation and exchange reaction (Chen *et al.,* 2006; Ponmurugan and Gopi, 2006). In such complicated coffee rhizosphere, the applied chemical phosphatic fertilizers can be solubilized by beneficial soil microbes to play a pivotal role in making P available for plant

nutrition. Phosphate solubilizing bacteria and fungi promise a better alternative to the current phosphorus deficiency issues in agriculture. Bacterial and fungal biofertilizers can contribute to increase agronomic efficiency by reducing production costs and environmental pollution, once the use of chemical fertilizers can be reduced or eliminated if the inoculants are efficient both in vitro and in field condition. However, screening of pure culture isolates for those with PGP functions under in vitro alone does not guarantee their effectiveness and always may not result in isolates that promote plant growth under field conditions. This calls for thorough and continuous studies of their field applicability as biofertilizers in establishing potentially important phosphate releasing inoculants for agricultural practices to mitigate phosphorus deficiency where it is the main problem. Therefore inoculations with potent indigenous microorganisms are in accord with current views on the possible future role of plant growth-promoting and soil supporting bacteria and fungi in enhancing plant yields. It is very important that the coffee producing farmers should understand the significant role played by bacteria and fungus in releasing soluble P from inorganic and organic sources in soil through solubilization and mineralization; thus promote plant growth (Rodríguez and Fraga 1999; Wakelin, et al.2004).

Application of bio-inoculants under nursery condition is becoming an effective strategy to promote plant growth. The individual and consortia of *Azosprillum*, *Pseudomonas flurosence*, other phosphate solubilising bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) have been tried in coffee nursery for the last tenths of years (Biradar et al. 2006). Hence, there is an urgent need to use these potent bio-inoculants as biofertilizers in large scale in agronomic practices to obtain better results and to minimize the use of chemical fertilizers. Therefore, the overall hypothesis of this study was that P-solubilizing bacteria and fungi, the lower pH adapted isolates from coffee rhizosphere and alkaline pH adapted ones from vermicompost, will promote plant growth and P. nutrient uptake in coffee production.

1. 2. Statement of the Problem

For the sustainability of farm output products, Ethiopian coffee cultivaters do not have access to effective and affordable inputs, appropriate technology and information as well as functioning markets (Abrhaley, 2016). It is very cruicial that coffee growers should realize the pivotal role that soil microbes play in the transformation of major elements like nitrogen, sulfur and

phosphorus and bio-control agents. These roles of soil microbes are not understood in the Ethiopian coffee farming systems. Bearing in mind the negative effects of chemicals, microbial intervention of P-solublization seems to be an effective way to solve the P-defficiency in soil. However, the effectiveness of phosphate solublizers in the rhizospher is dependent on soil temperature, moisture, pH, salinity, and source of insoluble P, method of inoculation, (Zaidi et al. 2009). Bio-inoculants are often used to inoculate plant material without an appropriate carrier or in quantities that do not allow for efficient rhizosphere colonization under field conditions, due to competition with resident soil micro- and macro-fauna (Dangi et al., 2017). Hence, the study of phosphate solublizing microbes (PSM) activity in correlation with these factors has to be done extensively before PSM can be used as bio-inoculants with promising results.

In Ethiopia, several studies were underataken on coffee cultivation systems, mainly on comparing rhizo-biological properties relative to coffee rhizosphere (Muleta, et al., 2009; Muleta *et al*., 2013). With regard to phosphate suolubilization activity of microbes, Muleta *et al.*, (2013) have reported that over 72% of the rhizobacteria, mostly *Pseudomonas erwinia* and *P. chlororaphis* from *Coffea arabica* L were able to strongly solubilize P sources. Yin et al. (2015) have also indicated that research on phosphate solublizing bacteria and fungi insufficiently addressed in area of coffee cultivation, and few such organisms have been considered for exploitation as microbial fertilizers. Since most of the hitherto works were limited to laboratory activities, this topic deserves more investigation on the agronomic practices of these microorganisms to fully realize their role in organic coffee production.

1.3. General objective

The general objective of this study was to develop inoculants of phosphate solubiliing bacteria and fungi with additional phytobeneficial properties for their effectiveness in enhancing growth of coffee seedlings.

1.4. Specific objectives

The specific objectives of this study were:

- To isolate and screen microbes (bacteria and fungi) from the rhizosphere coffee plants (forest/home garden) and vermicompost to evaluate their phytobeneficial traits
- To evaluate the eco-physiological properties (salt, pH, antibiotics resistance, heavy metals) of the isolates so as to select them as competitive bio-inoculants when applied to the soil as bio-fertilizers and bio-insecticides.
- To develop plant/vermicompost associated microbial inoculums using suitable carrier materials (locally available ones) for coffee cultivation
- To assess the effectiveness of the potent isolates to enhance seed germination rate under laboratory condition
- To assess the combined effect of selected phosphate solubilizing inoculants and vermicompost application on growth and mineral uptake of coffee (*Coffea arabica* L.) seedlings under greenhouse condition"
- To assess the combined effect of selected Phosphate solubilizing bio-inoculants and vermicompost application on growth and mineral uptake of coffee (*Coffea Arabica* L.) seedlings under Nursery Condition.

2 . Literature Review

2.1. Role of coffee in the Ethiopian economy and its current production trends

Coffee (*Coffea* spp.) is one of the legal globally handled goods in the world following petroleum oil and it is an important agricultural product for the economy of millions of people around the world (De Los Santos-Briones and Hernández-Sotomayor, 2006; Iscaro, 2014). Countries such as Ethiopia, Uganda, Rwanda and Burundi depend on this item to obtain foreign exchange, since this crop is the main trade good of these countries with global trade sales that predictable as US$ 90 billion (Damatta et al., 2008) which leads to the constant search for improved performance. Among coffee species, only *C. arabica*, and *C. canephora* are named as the most commercially cultivated species and economically dominate the world coffee trade (Damatta and Ramalho, 2006; N'Diaye et al., 2005).

Ethiopia is the home and foundation of biodiversity of Arabica coffee seeds which still grows wild in the forest of the highlands of Ethiopia (Alemayehu, 2014). It is where *Coffea arabica*, originates and known to be the birth place of coffee Arabica, which is demonstrated by its center for origin, diversification and dissemination, variety and quality of beans (Bayetta, 2001; Thomas, et al. 2004). Predominately, Arabica coffee represents 70% of global coffee production and Robusta represents about 30% (Damatta and Ramalho, 2006; Davis et al., 2012). Ethiopia is known as producer of Arabica coffee which is considered as superior to Robusta coffee due to its fine aroma, strong body, and pleasant acidity (Zewdu, 2016).

Arabica coffee has become a major global commodity and its cultivation, processing, trading, transportation and marketing provide employment for millions of people. Arabica coffee has for centuries played an important role in the Ethiopian economy and represents the main cash crop cultivated by small scale farmers for social, economic, political and ecological sustainability (Petit, 2007). Today, coffee sustains the livelihoods for over 15 million people in Ethiopia and it contributes 25%-30% of the country's foreign exchange earnings (FDRE MOT, 2012). Coffee plays a pivotal role in the socio-economy of the country and it has been cultivated, traded and

consumed over centuries and still plays a significant role in the daily life of most Ethiopians (Stellmacher, 2007). Moreover, Arabica coffee that has origin and centre of the genetic diversity in the south western region of Ethiopia, it represents a treasure for global breeding benefits and a means of major income source to local farmers (Urich, 2005). In this way, the national production is estimated around 280,000 metric tonnes which participate 700,000 households (Petty et al., 2003). Smallholder farmers account for more than 95% of total production in a low input-output system and still its production systems continuing traditional farming methods making Ethiopian coffee production naturally organic and hence it is self-fertile and contains 0.8-1.7% of caffeine (Banttee, 1995).

The total area coverage of coffee land in the country is 1.2 million hectare, of which 900,000 hectare of land is estimated to be productive. According to some studies, about 92-95% of coffee is produced by 4.7 million small scale farmers and 5-8% large scale plantations (USAID, 2010). Recent reports show that more than 90 percent of coffee produced in the country comes from smallholder farmers, and the rest 10 percent is from medium and large scale producers (USDA, 2016). There are differences of opinion on the amount of farm size for coffee production areas by smallholder farmers. The small garden field production is a leading producer and on average less than 2 hectares with yields remaining low at around 0.7- 0.8 metric tons per hectare (USDA, 2016). This means that smallholder farmers occupied the large production portion with average about 0.67 ha. According to CSA (2017), the cultivated area of land covered by coffee in Ethiopia is about 700474.69 ha, whereas the estimated annual national production of clean coffee is about 469091.12 tons with average productivity of 669.6 kg. According to Alemseged and Getaneh (2013), Ethiopia is the world's fifth largest coffee producer and Africa's top producer, with estimated coffee production of more than 450,000 tons and marketable supply of 334,000 metric tons in farm year 2012/13.

2.1.1. Influence of Climate change on coffee cultivation

Coffee is the world's most important tropical export crop but recent studies predict severe climate change impacts on *Coffea arabica* production (Craparo et al., 2015). Agler et al. (2016) and, Lavergne et al. (2010) have stated that the influence of biotic and abiotic factors can change

species interaction and have negative impacts in agriculture. Climatic changeability has always been the main factor responsible for the reduction of coffee yields in the world and determines the future coffee production status in the coffee producing countries (Kasterine *et al*., 2010). This trend is expected to continue and worsen as climate change brings more frequent and intense extreme events, shifting rainfall patterns and rising temperatures which increases infestation by the coffee berry borer (*Hypothenemus hampeii*), predominantly where coffee grows without shade and when continuous cropping season occurrs across the year (Walyaro, 2010). Consequently, it is the most favorable for increase of coffee pest disease such as fungal disease (Coffee rust) and the coffee berry borer (Laderach *et al.,* 2010) which leads to loss of globally estimated 13% of yield reduction (Agegnehu et al., 2015). Climate change would worsen pest prevalence like "broca" (berry borer) in Eastern Africa (Jaramillo *et al*. 2011).

An increase in temperature and rainfall in Ethiopia threatens coffee at an alarming rate which may cause favourable condition for pests and diseases prevalence leading to significant yield loss (Iscaro, 2014; Kasterine *et al.,* 2010). The profoundly negative trend for the future distribution of indigenous Arabica coffee would be 65% reduction in the number of bio climatically suitable localities, and this means almost 100% reduction was projected by the year 2080 under the influence of accelerated global climate changes (Davis *et al*, 2012).

Gianessi and Williams (2011) have predicted that climate change increases the need for fungicides and leads to a renaissance of certain pests and diseases on coffee. Because of suitable areas become too warm or inclined to periodic drought due to climate variation and become unsuitable and hence smallholder farmers in Ethiopia are suffering from poor productivity of this crop (Dekens and Bagamb, 2014; Killeen and Harper, 2016). Due to profoundly increased mean temperatures and changes in precipitation regimes traditional coffee growing regions may disappear and new regions may appear (Laderach et al., 2010). Moreover, Davis *et al.* (2017) and Dekens and Bagamb (2014) have stated that coffee industry has been negatively influenced by climate variations from production to export. Arega (2006) have concluded that despite economic importance of coffee and high production potential and productivity of this crop in Ethiopia, currently producers are suffering from poor productivity and replacing it by other food crops and cash plants such as khat in most parts of Kaffa and Ilulbabor and Jimma zone as well.

2.1.2. Coffee Consumption custom and current trends in Ethiopia

Coffee in Ethiopia is not only an important export commodity but it is a part of the culture; about 50% of the produced coffee is consumed domestically and there is even a cultural ceremony connected to it. It mainly consumed during social events such as family gatherings, spiritual celebrations, and at times of mourning (Alemayehu, 2014). Ethiopians are ranked as one of the African continent in domestic coffee drinkers and largest coffee consumers in Sub Saharan Africa. Coffee has been used as income generation for about 20 percent of the populations, directly or indirectly depend for a living on coffee production and trading (Alemseged and Getaneh, 2013). Therefore, coffee represents an important economic potential and has high value as a drinking beverage in Ethiopia.

According to Tora Bäckman, (2009), total production of washed and unwashed coffee is increasing from time to time. The domestic consumers of coffee relay on small-scale farmers and coffee retailers throughout the country (FDRE-MOT, 2012). In spite of the fact that coffee supplied to the local market has low quality, the price of coffee in the local market is usually higher than export prices. Because of this price disparity, some coffee shops in most large cities have started mixing coffee with barley grain to get more profit (Birhanu, *et al*., 2009). Alemayehu (2014) has documented that the small roadside stalls serve coffee in a traditional manner in Ethiopian major cities and coffee consumption is considered as a new emerging business that involves many street venders that usually sell coffee to by-passers.

At present, this business is emerging and flourishing in major cities and towns and more popularized among coffee consumers who are frustrated by the escalating price of coffee and the deteriorating quality of coffee served in cafes and coffee shops. The unaffordable local coffee prices have also pushed some consumers, particularly those residing in non-coffee growing areas to boil and drink the husks of a coffee bean as a substitute for normal coffee.

2.2. Major constraints to coffee production in Ethiopia: an overview

Jose (2012) predicted that the major Coffee production constraints in Ethiopia include lack of competitiveness, lack of infrastructure, in adequate access to services, low value addition, and in adequate technology transfer and research. Later, Fekede and Gosa (2015) added more major coffee production constraints in Ethiopia such as diseases, insect pest, poor access to market information, lack of improved coffee variety and poor extensions services. Another emerging challenge is that small holder coffee farmers obliged to producing *khat* instead of coffee as they are increasingly attracted by the high prices and greater yield they get from cultivation of the stimulant. Therefore, due to increasing a significant number of farmers in the eastern part of the country have switched from coffee production to *khat* production the farm land for coffee is diminishing from time to time (Tolera and Gebremedin, 2015).

Variability of weather pattern such as rainfall variability on the onset of the wet season, extension of drier and hotter season is considered as another challenge of coffee production in Ethiopia (Moat *et al*., 2017). These changes in climatic conditions are anticipated to extremely influence the population dynamics and the status of agricultural insect pests on their development, reproduction and survival due to increase in temperature and occurrence of diseases (Ward and Masters, 2007).

Even though diverse agro-ecology and climatic conditions, unique distinct characters of coffee quality, a favorable national agriculture ecosystem for coffee development the country has failed to fully implement its production potential. The effect of price volatility has been a direct factor in increasing rural poverty in rural communities; (UNDP, 2012) has forecasted that due to coffee price volatility as a leading risk factor for rural communities on their farms, up to 85% of coffee farmers have been pressurized to shift away from traditional forest coffee production systems towards more immediately profitable zero-shade systems that can yield higher returns in the short term which might lead the farmers to loss their ability to maintain natural coffee landscapes assets and stable socio-economic conditions. Because of large fluctuation in market price, poor accesses to market, little market promotion, lack of incentive mechanism and benefit coffee producers are exposed to shift their production style in Ethiopia (Berhanu ,2017; Jose, 2012).

In general, factors affecting Coffee production and productivity in Ethiopia can be categorized as biotic, abiotic and technical in their nature

2.2.1. Biotic factors

Biotic factors are influencing production and productivity of Coffee negatively. Biotic factors included disease, insect pests, weeds and vertebrate animals.

2.2.1. 1. Coffee disease

Unless otherwise it is controlled, Coffee diseases cause considerable losses. Jima et al. (2017) reported that the most economically important pathogenic coffee diseases are coffee berry disease (CBD) is caused by the fungus Colletotrichum coffeanum, coffee wilt disease (CWD) is caused by the fungus Fusarium xylarioides and coffee leaf rust (CLR) is caused by fungus *Hemileia vastatrix* and coffee branch die back is caused by pseudomonas syringe and non-pathogenic agents. Cerda *et al*. (2017) forecasted that about 57% yield loss was observed worldwide by the infection of disease causing organisms on coffee crop. Kumulachew *et al*. (2016) was also reported that about 30% of national average crop losses to total harvestable coffee yield due to CBD.

Recently, Tesfaye *et al.*, (2020) documented that bacterial blight of coffee (BBC), CBD, CWD, coffee leaf blight (CLB), CLR, coffee stem drying disease (SD), leaf drier (LD) and leaf spot (LS) are the most common diseases in the Sidama, Gedeo, Gamo Goffa and Wolayta of South Nation Nationalities and Peoples Region (SNNPR). Among these the most important coffee diseases challenging the production at the location were CBD, CWD, BBC and stem drying diseases. Similar finding was also reported by Holger and Omondi (2010) that CLR occurrences in Ethiopia were observed in all regions.

The coffee berry borer *Hypothenemus hampii* feeds and reproduces inside the coffee beans and causes their quality to deteriorate (Wintgens, 2004). Moreover, as reported by Zeru *et al.*, (2005) Coffee Berry Disease (CBD), *Colletotrichum kahawae*, Coffee Wilt Disease (CWD), *Gibberellaxy larioides* and Coffee Leaf Rust (CLR), *Hemileia vastatrix* are the three major

diseases reducing production and quality of coffee in Ethiopia. A study reported by Emana (2014) in Hararghe, Eastern Ethiopia resulted in the coffee berry disease incidence ranged up to 100 %, 80 %, 95 % and 90 % was observed in Bedeno, Boke, and Habro and Darolebu districts respectively. Mechanisms of control such as dig out all parts of diseased coffee tree and burn it at the spot, disinfect machetes after cutting every diseased coffee tree, (Zinabu et al.,2017) and using resistant varieties for CBD (Gimase et al., 2019) was reported. The assessment carried out in Eastern Ethiopia indicated that insect pests are causing considerable crop losses.

2.2.1.2. Pests

In the same way, insect pests such as anthestia bug, black ant, cut worm, leaf minor, fruit fly, mealy bug (white and black), red ant, spider, stem borer, termite and weevil are the major ones causing considerable damage (Tesfaye *et al.*, 2020). Investigation results by Eshetu and Girma, (2008) indicated that OTA (Ochratoxin A), a form of mycotoxin, produced as a metabolic product of *Aspergillus Ochraceus*, *A. carbonarius* and strains of *A. niger* common to exist on coffee dried on bare ground. Fekede & Gosa, (2015) added insect pests such as green scale, coffee trips and coffee cushion scale were important coffee production constraints in the country. Similar results were reported by Fekadu et al. (2016) as coffee berry borer was found the leading pest examined during their study conducted at Gedeo Zone. Losses due to coffee pests are estimated to be 13% worldwide (Nyambo & Masaba, 1997) while in Ethiopia yield loss due to some insect pests such as Anthestia bug was reported to be 9% (Girma et al., 2008). However, Esayas and Chemeda (2007) reported that coffee blotch leaf minor never causes considerably yield loss.

The influence of insect pest is more complex in intensive coffee production system than coffee in traditional home gardens and semi forest coffee since such systems could have long traditional and culturally associated protection practices (Million**,** 1987). Coffee thrips, *Diarthrothrips coffeae (D. coffeae*) are potentially important insect pests in Ethiopia (Esayas *et al*., 2008; Million, 1987). Coffee thrips destroys coffee beans and leaves by puncturing and sucking their contents and cause grayish strips to form on the leaves hindering photosynthesis (Jaramillo *et al.*,

2011). Parvatha (2010) has shown Vertebrate pest viz. small green barbet and red whiskered Babul (pycnonotus jocosus) and monkeys cause very minimum economic damage to coffee and are also can be managed easily by chasing them away.

2.2.1.3. Weeds

It was observed that most of the weeds disturb harvesting, suppress seedling growth, and reduce yield, dry soil and harbor disease causing organisms and insect pests. Earlier reports by Nyabundi and Kimemia (1998) indicated Digitaria abbisinica (Couch grass), Commelina benghalensis (Wondering Jew), Cyperus rotundus L.(Nut grass), Cynondon dactylon (Stargrass), Pennisetum clandestinum (Kikuyu grass) are some of common weeds reducing production and productivities of coffee. In line with these earlier reports, Tesfaye *et al.*, (2020) identified the most important common weeds such as Cyprus spp (20%), Ajaratum (17%), Commelena (15%), Nicandira (11%) and others (36%) in the coffee farms of Sidama, Gedeo, Wolayta and Gamo Goffa Zones. A similar finding was reported by Coffee Research Foundation (CRF, 2003) that the yield losses due to weed effects can be over 50%. Habtamu (2015) has pointed that the widely applicable weed control mechanisms include manual, mechanical removing and use of herbicides or integrated weed management depending on the availability and farming system.

2.2.2. Abiotic factors affecting the production and productivity of coffee

Abiotic factors affecting the production and productivity of coffee were highly related to the agroclimatic condition. The most important abiotic factors were drought, reduced soil fertility, heavy rain, low moisture stress, high incidence of sun rise, snow and frost which cause singly and/or in combination, huge damages such as fruit quality reduction, wilting, flower abortion, aggravate berry disease, enhanced alternate bearing (Tesfaye *et al.*, 2020). Abiotic stresses on plants like flooding, temperature, salinity and drought affect crop production heavily, as they cause stunted growth, affects plant metabolism and thus reducing crop yield by 70% (Ngasoh et al. 2019).

Furthermore,wind, high temperature, low moisture stress, moisture shortage especially at flowering stage were considered to cause minor effect on coffee production (Ngasoh et al. 2019; Tesfaye *et al.,* 2020). These factors can be managed by using mulching, composting, providing irrigation, genetically improving the genes and transcription factors (Tesfaye *et al.,* 2020), apply phyto-hormones, signaling and trace elements, applying osmo-protectants (Hassanuzzaman et al., 2010; Wahid et al., 2007), or by employing cultural practices which includes modification and adjustment of planting time and crop density in the field (Arun-Chinnappa et al., 2017; Mirza et al., 2013).Those factors taken as technical challenges include lack of improved varieties, lack of remedies for coffee disease and insect pests, and weak linkage with the central market (Tesfaye *et al.,* 2020).

2.3. Soil fertility management strategies in coffee production

2.3. 1. Chemical fertilizers

Phosphorus (P) is a one of the most important nutrient required by the plants It is one of the major important elements that play a significant role in biological growth and development such as photosynthesis, respiration, energy storage and transfer in the living plant cells (Solangi et al., 2016). However, most soil phosphorus, approximately 95–99%, is present in the form of insoluble phosphates and hence cannot be utilized by plants (Vassilev and Vassileva, 2003). In contrast to nitrogen P is not available in the atmospheric gas and be fixed biologically for plant utilization (McVickar *et al*., 1963).

Most natural ecosystems in tropical and subtropical soils are rich in iron and aluminium and predominantly acidic and are, under the circumstances, P deficient for they fix P as insoluble phosphates of iron and aluminum (Singh & Reddy 2011). Thus, low levels of P reflect the high fixation of phosphate with other soluble components (Khan *et al.*, 2009a), such as aluminum in acid soils (pH < 5) and calcium in alkaline soils (pH > 7) (McLaughlin *et al.*, 2011). To ease P deficiency in these regions, chemical fertilizers containing soluble forms of P are widely used on large scale for crop cultivation. However, only about 25 per cent of the phosphorus applied to the soil is available for the crops (Mahantesh and Patil, 2011) which leads to excessive and repeated

application of soluble P fertilizers resulting to serious threat to groundwater (Parasanna *et al.* 2011).

Therefore, the overuse of fertilizers and their associated risks on the environmental safety calls for the search of alternative means of plant nutrient management practices such as bio-inoculants, compost, farm yard management and vermicompost(Adesemoye *et al.*, 2009).. To achieve maximum benefits in terms of fertilizer savings and better growth, the P-solublization based inoculation technology should be utilized along with appropriate levels of fertilization.

Apart from the above mentioned challenges, the scenario of future phosphate rock scarcity necessitates a shift towards low reliance on inorganic phosphate and search for locally available agricultural inputs which minimize dependence on inorganic phosphate inputs sustaining agricultural production (Hungria *et al.*, 2013). The soil solution is typically in the range of 0.01–3.0 mg per liters representing a small portion of plant needs and remains to be the main source of phosphorus supply to plants. Monobasic ($H_2PO_4^-$) and dibasic ($H_2PO_4^{2-}$) forms of phosphorus are the only absorbvable forms of soluble phosphate by which soil microbes helps release to the plants to be absorbed by plants(Bhattacharyya and Jha 2012).

Rock Phosphate (apatite), hydroxyapatite and oxyapatite could also be source of phosphorus through involvement of phosphate solubilizing microbes as well as physical processes. Moreover, organic forms such as phosphomonoesters, inositol phosphate, phosphotriesters and phosphodiesters are major deposits of P in soil, which are mineralized by microbes to make them available to plants (Gamalero et al., 2011). Due to the long lived Coffee plant on the farm a significant reduction in the amounts of nutrients available in the soil occurs over time (Willson, 1985b). To improve the soil nutrient status in soil, chemical fertilizers are widely used to support plant growth (Oruko, 1977).

Similarly, Nitrogen application enhances plant growth characteristics such as height, stem girth, length of primary branches and leaf expansion (Njoroge, 1992). According to Wintgens, (2004), the caffeine and chlorgenic acid contents of the beans are not affected by the levels of phosphorus, calcium, potassium and magnesium in the soil. Deficiency of zinc will lead to the occurrence of small light grey-colored beans, which will produce poor liquor (Wintgens, 2004).

Sustainable production and soil fertility management is important for successful coffee cultivation for both large scale small holder farmers.

Dependence on inorganic fertilizers may not be sustainable in the long term given that soils may lose microorganisms, become acidic and having unstable aggregates leading to erosion and general degradation, and this may leads to yield decline with time despite consistent use of inorganic fertilizers (Nyalemegbe, et al. 2009). Maintaining physical, chemical and biological soil properties for plant growth and environmental efficiency requires the input of organic fertilizer used as bio-inoculants for efficient utilization of nutrients by plants. It is very important that the coffee farmer should understand the subtle role played by microorganisms in the transformation of major elements like nitrogen, sulfur and phosphorus, biodegradation, neutralizing toxic wastes, bio-control agents and a host of other activities.

Coffee farmers do not realize that ammonium fertilizers tend to lower the soil pH resulting in acidity due to the microbial oxidation of ammonium to nitric acid (Nyalemegbe, et al. 2009) which tends to disturb the optimum neutral pH for the bacteria growth. Therefore, the need for effective utilization of fertilizers and renewable, locally available and cheaper options for supplying nutrient to crops is increasingly becoming important because of the need for sustainable agriculture (Nyalemegbe, et al. 2009; Zafar et al. 2011).With growing demands for sustainably produced agricultural produce for environmental, social and food safety reasons, the use of bio-inoculants is becoming inevitable, particularly for export market depended commodities such as arabica coffee.

Cost reductions, sustainability and quality improvement are now the major priorities in coffee production systems and require organic growing of coffee. Sustainable production is becoming a necessity for coffee growing sectors to remain competitive in the global trade against oversupply and price fluctuations that in some years result in coffee price crisis. However, Ethiopian farmers do not have access to effective and affordable inputs, appropriate technology and information, and functioning markets (Abrhaley, 2016). Although fertilizers have played a vital role in raising the agricultural productivity in Ethiopia over a period of time, the misuse of fertilizers is damaging human health and polluting the surrounding environment and thus violating the

sustainability of ecosystem (Karp et al., 1995). In lines with these, the cost of chemical fertilizers and their associated risks on the environmental safety calls for the search of alternative means of plant nutrient management practices, such as bio-inoculants, compost, farm yard management and vermicompost.

2.3.2. Organic fertilizer; the role of Vermicomposting

It is established that excessive application of manure can generate extreme levels of nitrogen, ammonia, and salts and reduce plant growth in the soil. As a result, research strategies have been established to maintain the drawback of manure and using earthworms to avoid problems related to it (Edwards and Bohlen, 1996). One of the strategies is exploiting the potential of vermicomposts, as plant growth media and as soil fertility improvement. Vermicomposting is a biological fertilization technique consisting of the use of earthworms' metabolism to produce humus with high nutrient content.

Vermicompost is a finely divided humus-like substance with high porosity, aeration, drainage, water-holding capacity and formed when organic matter is broken down by the joint action of earthworms and microorganisms (Lazcano, et al., 2009). The action of earthworms in this process is both physical/mechanical and biochemical. It is different from conventional compost, which is a product formed from the aerobic decomposition of organic waste like animal droppings, crop wastes and even municipal wastes (Sinha, 2009). It is cost-effective and eco-friendly waste management technology which takes the privilege of both earthworms and the associated microbes and has many advantages over traditional thermophilic composting (Asha *et al.,* 2008).

Vermicomposts require less production time than conventional composts. In addition, unlike conventional composts which contain abundant ammonium, vermicomposts contain high amounts of nitrates, which are a more readily available form of nitrogen (Sinha, 2009). It is a sustainable source of macro- and micro-nutrients, which modifies soil structure, changes in water availability, and stimulation of microbial activity and increase the activities of critical substances as a result of microbial intervention through interactions with earthworms (Atiyeh, *et al*., 2000).

The earth worms harbor P solublizers and decomposers in their intestine and release them in their excreta (Singleton *et al*. 2003). Most of these bacteria belong to the genus *Psudonomas* and *Azotobacter* (Valle *et al*., 2007), which can produce plant growth promoters, nitrogen-fixing bacteria, and phosphate solubilizers (Loreno *et al*., 2004)

An early investigation conducted by Dickinson and Lepp (1985) was indicated that the rearing of E. andrei, E. fetida and P. excavates under laboratory conditions with the three earthworm species performed fairly well as coffee pulp conversion, reproducing and transforming organic matter into an earthy stable casting. *Eisenia foetida* (Red California) and *Eudrilus eugeniae* (African Red) are the most commonly used earthworm species in vermicomposting (Chhotu 2008). According to Wintgens (2009) in the tropics P. excavates plays a crucial role in vermicomposting. A complex interaction between Earthworm's gut and rhizospheric bacteria ingested during vermicomposting and igested as vermicast influences the dynamics of soil texture which intern used to heal soil health and increase plant nutrient availability.

Vermicasts are full of microorganisms such as *Rhizobium, Pseudomonas, Azosprillium, Bacillus* and *Azotobacter* (Edwards and Bohlen 1996; Sinha et al. 2010). Suhane (2007) reported that the abundance of diversity in the vermicompost populations of phosphate solublizer and rhizobia indicating them as a large resource of natural germplasm to screen for desired characteristics present in the natural pool. Esakkiammal et al., (2015) also reported that the fungal population is found to be significantly higher in the fresh vermicast obtained from Vermicompost of *Eudrilus eugeniae* and *Eisenia fetida.* Anastasi et al., (2005) were isolated and documented a total of 142 fungal species that are associated to vermicompost.
Hence, application of vermicasts to agricultural soil are said to be inoculation of bio-inoculants which impoves the biological properties of rhizosphere soil.

In general, vermicomposts are fantabulous organic amendments with excellent physio-chemical properties and buffering ability, fortified with all nutrients in plant available forms, antagonistic and plant growth-promoting bacteria. They act as an answer for soil reclamation, enhancement of soil fertility, plant growth, and control of pathogens, pests and nematodes for sustainable agriculture. Moreover, since the availability of organic matter is the basis for the establishment

of phosphate solublizing *Pseudomonus* species in the soil, vermicompost has a dual purpose of serving as the best carrier materials for p solublizers and be used as source of plant growth promoting microbes.

Vermicompost contains available plant nutrients including N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu and B. The uptake of these nutrients has a positive effect on plant nutrition, photosynthesis, the chlorophyll content of the leaves and improves the nutrient content of the different plant components (roots, shoots and the fruits). Besides the high percentage of humic acids in vermicompost contributes to plant health, as it promotes the synthesis of phenolic compounds such as anthocyanins and flavonoids which may improve the plant quality and act as a preventive to pests and diseases (Theunissen et al., 2010). Some nutrients are immediately absorbed by the coffee plant from the vermicompost while others are only released at slow rates, ensuring long lasting nutrition (Wintgens, 2009). A research carried out by Degefe et al., (2012) in Ethiopia indicated that vermicomposting could be a good option to improve solid waste management performance of Ethiopian cities and towns through the production of excellent bio fertilizer obtained by vermicomposting Coffee Husk, Enset waste (Enset ventricosum), Khat waste (Catha edulis) and Vegetable waste amended with Cow Dung using an epigeic earthworm Eisenia Andrei for agronomic purpose.

2.3.3. Other Soil fertility management strategies in coffee production

In the course of organic coffee production system all contamination with synthetic-inorganic chemicals will be removed and to produce a sustainable system of coffee plantation management in the long term. The main organic coffee producing countries include Mexico, Guatemala, Kenya, Nicaragua, Tanzania, Brazil and Ethiopia is the main organic coffee producing countries (Wintgens, 2009)

The coffee bean is a nutrient rich fruit and its production requires a large amount of nutrients which can be provided for through organic fertilizers. The precise amount of fertilization required depends on the quantity of nutrients removed each year as coffee beans. In the shade coffee production; 53, 85 and 150 kg of N and roughly equal amounts of K must be applied per

hectare so as to correct nutrient balance of farms with per hectare coffee production of 500, 1500 and 2000 kg, respectively (Van der Vossen and Walyaro,2009). Other sources of nutrients required for coffee to increase coffee crop productivity in organic farming system, in addition to that of the widely used sources of bio-fertilizer, include bocashi, Coffee pulp, Cattle manure, Chicken manures and bio-green, Shade trees, intercropping, Mulching etc.

2.3.3.1. Bocashi and compost

It is a type of compost prepared with recommendations on the mix of variety of materials ranging from coffee pulp, household organic waste, cattle or poultry manure, ashes, molasses, yeast and stems from bean production through other organic waste. When a simple mix used for bocashi preparation it might contains only coffee pulp, cattle manure and bean stems and is commonly called compost (Valkila, 2009).

2.3.3.2. Coffee pulp

Coffee fruit contains about 40% of we weight of nutrient in the form of coffee pulp which is used as compost upon compositing by farmers to fertilize their coffee farm (Valkila, 2009). The compost made up of coffee pulp and husk was found to increase significantly coffee yield. However, still the coffee pulp is not recycled but taken away from the farm with the coffee beans (Van der Vossen and Walyaro, 2009).Therefore compost prepared from coffee processing by-products can serve as source of ample mineral nutrients required by the coffee tree for normal growth and sustained yield & enables the production of organic coffee which is of a worldwide demand today (Jafer, 2018).

2.3.3.3. Cattle manure

The nutrient content of cattle manure is estimated to be 1% N, 0.5% P and 1% K. cattle manure can be used as sources of bio-fertilizer in the organic coffee farming. Nutrient contents in Cattle manure are usually small and vary greatly depending upon source, conditions and duration of the

storage time which requires selecting of the appropriate source of cattle manure and improved management (Yadav *et al*., 2013).

2.3.3.4. Chicken manures and bio-green

Similar to cattle manure, chicken manure is rich in nutrients. Unless otherwise small-scale chicken farming is widespread, the availability of Chicken manures and bio-green comes in to question. However, few chicken farms have large quantities of manure for their own application and sale. Similarly, bio-green is poultry manure-based organic fertilizer which can be used in organic coffee farm. Bio-green is common to be used in Nicaragua (Valkila, 2009). Both chicken manures and bio-green can serve as sources of nutrients in the organic coffee farming. The highest pod yield, as well as higher biological yield and harvest index was obtained due to application of manure which shows the economic feasibility of this source as a bio-fertilizer (Yadav *et al*. 2013).

2.3.3.5. Mulching

Mulching is used as tool in organic coffee production for soil fertility management by protecting water loss, weed growth and water infiltration. Mulching materials can be prepared from by-products of coffee hulls and harvest residues such as maize and sorghum clover, bean and soya haulms. Bekeko (2013) was reported that in western Ethiopia when maize stover is used as mulch in coffee crops, it was resulted in a significant bean yield increment of 520kg up to 1070kg per hectar. Application of mulch found to reduce over bearing and dieback in arabica coffee and sustains its biological productivity for longer period of time (Yunianto, 1986). Covering the soil with different materials not only helps to preserve soil moisture and decrease soil temperature but also increase soil fertility by releasing plant nutrients on decomposition, suppress weeds, improve rainfall penetration into the ground and thus increase crop yield (Mehlich, 1965; Oruko, 1977).

2.3.3.6. Shade trees

Forests play a crucial role in conserving perfect soil properties and flora of the forest eco-system. In the semi-forest coffee production system, the interventions of coffee producing farmers is minimum to remove the dense shading and weeds, to facilitate coffee harvesting (Workafes and Kassu, 2000). The wild coffee forest soils contains high soil organic matter and major plant nutrients because of high litter fall mainly from indigenous upper canopy shade trees. These credible sources of organic matter might be lost due to deforestation and thus resulted in adverse impacts on coffee ecology and natural gene pools (Feyera,; 2006;Taye, 2006), which in turn, call for the need for site-specific forest management for enhanced organic coffee production and benefit from the premium international coffee prices (Taye, and Tesfaye,2002).

Haggar *et al.*, (2011) and Taye, (2010) underlined the contributions of vegetation cover in building up high quality soil fertility status and preventing run-off on steep slopes in order to minimize usage of chemical and thereby increase the use of shade. Usually, organic coffee grown under shade has a number of advantages. Of these, suppression of grasses and sedges, reduction of oxidation and rate of decay of organic matter in the soil, maintaining regular flowering due to reduced plant metabolism, improving soil fertility due to organic material obtained from twigs and leaf fall and fixation of atmospheric N by leguminous shade trees, reduction in Cercospora spp. and of attacks by the white stem borer are some paramount advantages of shades (Haggar *et al*., 2011). Commonly known shade trees in organic coffee production are Leucaena and leucocephala used in Malaysia while Hevea brasiliensis, rubber tree, is used in Thailand and . Banana trees, Mimosa scabrtella, Calliandra calothursus and Glividicia sepium are known in Mexico (Grossman, 2003).

2.3.3.7. Intercropping

In coffee growing areas of Ethiopia, coffee is mostly grown in multistory cropping system with shade trees, citrus, papaya and enset the upper story followed by coffee while the ground floor by cereals (maize, sorghum and teff), legumes (peas, beans and lentils), vegetables (cabbage, kale, chilly and pepper), spices (ginger, turmeric and korarima) and root crops (sweet potato and Irish

potato) (Awoke, 1997). A similar result reported by Addis *et al*., (2015), that in Ethiopia coffee is grown as garden plantation being intercropped with Korerima. Intercropped coffee with other plants provide some advantages like control of weeds, recycling of nutrients, use of unproductive areas, use of shade and extra income.

It was reported that positive net economic benefits is obtained due to intercropping young coffee with food crops. Intercropping food crops with high density coffee was only advantageous during the first year before the canopy closed up (Njoroge and Mwakha, 1994; Njoroge, 1992). During intercropping the use of the resources is more effective than a pure cropping, and the result increased yield and reduce pest and disease damage (Fujita *et al*., 1992; Jensen, 1996). Rhizobium bacteria are able to have a symbiotic relationship with plants of leguminosae family, and thereby can fixation of atmospheric nitrogen into available nitrogen for plants uptake. In this case intercropping is a form of rotation by which atmospheric nitrogen will be available for plants uptake. Intercropping non-legume plants with plants of leguminosae family increases the nitrogen content in the soil (Eskandari *et al*., 2009a; Fujita *et al*., 1992).

2.3.4. Bio-fertilizers

The current trends for ecologically and economically feasible fertilizer demands have encouraged the search for a new move towards sustainable agriculture. Sustainable agriculture comprises the most significant approach to work against the environmental quality decline by keeping up the extended ecological balance of environments (Khan *et al*., 2006). Biofertilizer is a breakthrough technology that promises very significant impact on the farmers in terms of increasing farm productivity and income. It is defined as bio-inoculants/ microbial inoculants possessing living organisms which help in nutrient availability and uptake by plant roots in the rhizosphere (Arora, 2013). Biofertilizer is the product containing carrier based (solid or liquid) (Figure.1) living microorganisms containing both micro and macro elements in the soil environment to increase the productivity of the soil and crop. The application of biofertilizer increases crop productivity, reduce production costs by reducing the volume of fertilizers applied and a better conservation of environmental resources (Sinha, *et al*., 2014).

Figure 1. Different forms of commercialized biofertilizers: AMRUTH ORGANIC FERTILIZER,malladihalli, Holalkere(T),Chitradurga-577531,Karnataka, India

Carrier material based bio-inoculants enable long-term storage, easy handling, and transportation of biofertilizers. Bio-inoculants increase and enhance the degree of availability of plant absorbable minerals in the form easily assimilated by plants (Vessey & Heisinger, 2001). Not always soil microbes dwelling in the agricultural soil are as effective as one would expect them to be and hence *in vitro* selected bio-inoculants play a very important role in nitrogen fixation and inorganic phosphate solublization effect in the agricultural soil.

Biofertilizers can be prepared using living cells of efficient microbial strains and applied through seed or soil and help crop plant to uptake the nutrients. Bio-inoculants are eco-friendly, non-hazardous and nontoxic nature. The living microorganisms, which promote plant growth by improving the nutrient status of the plant, include rhizospheric bacteria, symbiotic bacteria and non-symbiotic endophytic bacteria and rhizospheric fungi. Several microorganisms such as *Aulosira, Tolypothrix, Scytonema, Nostoc, Anabaena, Plectonema, Azolla, Rhizobium, Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Fusarium, Sclerotium, Aspergillus* and *Penicillium* are commonly used as biofertilizers (Pindi and Satyanarayana 2012; Roy and Srivastava, 2013). Rhizospheric fungi, such as arbuscular mycorrhizae and *Penicillium bilaii* are known to have plant growth-promoting effects by increasing the nutrient status of host plants (Bethlenfalvay & Barea, 1994; Sheraz *et al*., 2010).

2.3.4.1. Coffee rhizosphere associated MO and their potential uses as PGP agents

The German agronomist and plant physiologist Lorenz Hiltner first coined the word ‘rhizosphere’ for the first time to describe the plant-root interface, the area of highest bacterial activities, a word originating in part from the Greek word "rhiza", meaning root (Hartmann *et al*., 2008; Hiltner, 1904). Microbial population present in this atmosphere is comparatively diverse from that of its bulk soil because of root exudates which function as a source of nourishment for microbes (Burdman *et al*., 2000). Research has shown that several plants associated microbes can have concentrating effects on plant growth, nutrients, seed germination and disease managements (Gevers *et al.* 2012). Thus, the plant-microbe association is beneficial in nourishing the microbial population and prompting its composition and activities. The rhizosphere has often been used as the preferential site for the isolation of plant growth promoting (PGP) microorganism with potential applications as biofertilizers (Bashan *et al*., 2014; de Souza *et al*., 2013).

The term ‘’plant growth-promoting microbes (PGPM)’’ was first described by Kloepper and Schroth in the late 1970s (Kloepper & Schroth, 1978). The growth promotion includes mechanisms like the facilitation of nutrient acquisition, increased resistance against stresses and the modulation of plant gene expression (Olanrewaju *et al*., 2017; Santoyo *et al*., 2016). Several microorganisms (including bacteria and fungi) are able to display plant growth-promoting capacities and/or biocontrol abilities toward coffee pests and diseases (Muleta *et al*., 2007b). Moreover, they can enhance the growth of plants either directly by producing siderophores, phytohormones including indole-acetic acid, cytokinins, gibberellins and inhibitors of ethylene production, and organic acids, N_2 fixation, solubilization of inorganic phosphate for the easy assimilation of plants and for their own use (Goswami *et al*., 2016) or indirectly by suppression of plant pathogenic organisms, induction of resistance in host plants against plant pathogens (Vessey, 2003).

PSB are able to release soluble P from inorganic and organic sources in soil through solubilization and mineralization, thus promote plant growth (Rodrguez and Fraga, 1999).

Moreover, PSB posses phytobeneficial triat such as production of Hydrogen Cyanide (HCN), Indole Acetic Acid (IAA), ACC deaminase siderophore, antibiotics and exopolysaccarides. Even if production of HCN by PGPR was originally thought to promote plant growth by suppressing pathogens, this idea has currently been challenged by Rijavec and Lapanje (2016), who disagreed that metal chelation due to HCN ultimately amplify P availability.

Plant growth promoting microbes (PGPM) have a great influence not only in growth but also in yield of numerous crops and the living microorganisms of different groups of rhizospheric bacteria and rhizospheric fungi. Among rhizospheric bacteria, Pseudomonas is the most diversified and common genus with 11 species identified from coffee rhizosphere (Caldwell *et al.*, 2015). Besides the most commonly identified genera of the fungal community in the coffee rhizosphere are *Penicillium, Aspergillus, Fusarium* and Trichoderma (Schöps *et al*., 2020; Tkacz *et al*., 2020).

Coffee rhizosphere is associated with large number of beneficial microorganisms including PSB which may contribute to nutritional requirement of the plant (Vega, *et al*. 2020). The first mention of the microorganisms associated with coffee plants dates from the nineteenth century and the description of the arbuscular mycorrhizal fungi (AMF) colonizing the roots of C. arabica and C. liberica (Janse, 1897). Rhizosphere of *C. arabica* and *C. canephora* rhizosphere is a hotspot for high concentration of microbial populations namely, phosphate solubilizing bacteria and fungi (Kunwar, *et al*. 2018; Vega, *et al*. 2020). Vega, *et al*.(2020) concluded as no significant differences were observed, regarding the microbial populations associated with the two species of the genus *Coffea*, *C. arabica* and *C. canephora*, indicating that there was no strict specificity between these coffee species and the associated microbial populations. High population levels of bacteria, fungi and actinomycetes were found in the rhizosphere of the collection of *C. arabica* and *C. canephora*, not depending on the species (Vega, *et al*. 2020).

With current increasing demand for food production, there is a huge challenge for sustainability of current agricultural practices, and phosphorus security is emerging as a global sustainability challenge. So, there is an imperative need for a sustainable phosphorus use in agriculture (Cordell, *et al*. 2009). One of the cheap and environmentally safe alternatives for improving the

deficiency of phosphate nutrition is the manipulation of rhizospheric phosphate solubilizing bacteria (PSB) and fungi (Glick, 2012; Kunwar, *et al*. 2018; Vega, *et al*. 2020).

2.3.4.2. Phosphate solubilizing Bacteria (PSB)

The PSB are beneficial organisms that are capable of solubilizing inorganic phosphorus from insoluble compounds to soluble forms. The ability of P solublization of rhizospheric bacteria is one of the most vital characters associated with release of phosphate from complex inorganic compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate, to plant available forms (Yao *et al*., 2006). Some of these microorganisms are the genera; *Pseudomonas, Azospirillum, Burkholderia, Bacillus, Enterobacter, Erwinia, Serratia, Alcaligenes, Arthrobacter, Acinetobacter* and *Flavobacterium* (Fankem *et al.,* 2006; Zaheer *et al*., 2016).

Rhizobia, including *R. leguminosarum, R. meliloti, M. mediterraneum, B. japonicum and Bradyrhizobium sp.* are also possible P solubilizers (Afzal and Bano 2008; Zaidi, *et al*. 2014). Among these microorganisms *Bacillus megaterium, B. circulans, B. subtilis, B. polymyxa, B. sircalmous, Pseudomonas striata* and *Enterobacter are* very effective for increasing the plant available P in soil as well as the growth and yield of crops (Fankem *et al.,* 2006; Tahir *et al.*,2013). Pindi and Satyanarayana (2012) also indicated that *Pseudomonas, Bacillus, Micrococcus* and *Flavobacterium* are active in the solubilisation process. PGP bacteria are naturally occurring in soil and they are bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Yadav *et al*., 2017).

Although several phosphate solubilizing bacteria occur in soil (Zaidi *et al*. 2009), their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Therefore, inoculation of the seed with target bio-inoculants at much elevated population than that normally found in the rhizosphere is essential to take advantage of P availability for the plant growth enhancement.

2.3.4.3. Mechanisms of phosphorus solubilization by Bacteria

Phosphate Solubilzing bacteria (PSB) through various mechanisms of solubilization and mineralisation are able to convert inorganic and organic soil P respectively (Khan *et al*. 2009a) into the bioavailable form facilitating uptake by plant roots. Production of organic acid by Phosphate-solubilizing bacteria (PSB) is one of the mechanisms to reduce metal toxicity and to transform metal species to immobile forms or chelate them for mobility (Ahemad, 2015). Phosphate-solubilizing bacteria solubilize inorganic soil phosphates, such as $Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$, through the production of organic acids, siderophores, and hydroxyl (Sharma *et al.*, 2013).

The principal mechanism for phosphate solubilization is occurred in two ways. The first method is through release of inorganic phosphate from insoluble chemical form by the action of organic acids such as citrate, gluconate, oxalate and succinate, produced and released by bacteria. Release of mineral phosphate can be mediated by either organic acids as a result of exchange of PO_4^{2-} or by chelation of Aluminium and Iron ions linked to phosphate compounds (Omar, 1998). The amount of acid in liquid culture can be determined from culture filtrates by thin layer chromatography or paper chromatography or by high-performance liquid chromatography (Gyaneshwar *et al*., 1998). The other way of increasing phosphate in the soil is through mineralization. Soil bacteria produce enzymes that could mineralize free P from organic matter (Hilda and Fraga, 2000). Different enzymes have been characterised to mediate this activity such as phosphatases, phytases and phospholipases, which are key drivers in this transformation (Beech *et al*., 2001).

2.3.4. 4. Phosphate-solubilizing fungi

Different studies also showed several fungi groups solubilize inorganic phosphorus. These include; *Achrothcium, Alternaria, Myrothecium, Oidiodendron, Paecilomyces, Penicillium, Phoma, Pichia fermentans, Populospora, Pythium, Rhizoctonia, Rhizopus, Saccharomyces, Arthrobotrys, Aspergillus, Cephalosporium, Cladosporium, Curvularia, Cunninghamella,*

Chaetomium, Fusarium, Glomus, Helminthosporium, Micromonospora, Mortierella, Schizosaccharomyces, Schwanniomyces, Sclerotium, Torula, Trichoderma, and *Yarrowia* (Alori, *et al*., 2017; Richardson and Simpson, 2011;Sharma *et al*.,2013).

Phosphate solublizing fungi are more important to the solubilization of inorganic phosphate in soils than bacteria as they typically produce and secrete more acids than bacteria (Khan *et al*., 2010; Sharma *et al*., 2013). Moreover, their ability to withstand biotic and abiotic stress under soil condition makes them the potential candidate for developing bio-inoculant. *Penicillium* and *Aspergillus* spp. are the dominant P-solubilizing filamentous fungi found in the rhizosphere (Chuang, *et al*., 2007).

It was reported that *Aspergillus niger* and *Trichoderma harzianum* could be potential candidate for developing bio-inoculants to facilitate P supply to different crops in alkaline and acidic soils and also produces auxin and sidreophore helpeful for plant growth promotion (Gaind, 2016). *Aspergillus niger* and some *Penicillium* species were tested for solubilization of Rock Phosphate and other biotechnological importance such as biocontrol, biodegradation, and phosphate mobilization (Pandey *et al*., 2008).

2.3.4.5. Mechanisms of phosphorus solubilization by fungi

Phosphate solublization by fungi is undertaken with production of organic acids such as citric, gluconic, fumaric, malic, oxalic, lactic, 2-ketogluconic, malonic acids, succinic, propionic and acetic acid which results in decrease in pH of the medium. Malic acid is said to be more efficient than succinic acid and this may be due to higher number of hydroxyl group in malic acid compared to succinic acid (Gaind, 2016).

Based on the specific attribute of each fungal isolates, the amount and nature of organic acid produced may vary under the same production conditions. Hence, quantity and quality of organic acid produced is fully dependent on the type of P-solubilizing microorganisms. Therefore, considerable variation may exist among different fungal isolates for making available P from the same phosphate source (Toro *et al*., 1997).

2.4. Phytobeneficial and eco-physiological traits of phosphate solublizing microbes (PSMS)

To get the maximum benefit of inoculation under different ecological zones, there is a need to develop the bio-inoculants using a microbial strain with multifunctional attributes.

2.4.1. Phytobeneficial traits of phosphate solubilizing microorganisms (PSM)

Apart from phosphate solubilization, Plant growth promoting traits such as production of siderophores, indole acetic acid (IAA), Hydrogen cyanide (HCN) and ammonia (NH_3) are important plant growth promoting traits that enhance plant growth and plant disease control. Siderophore, iron-chelating ligands and low-molecular weight, helps a particular microorganism to compete against fungal pathogens for available iron and the role of siderophores in control of diseases has been well documented (Sharma *et al.,* 2003). .Inoculation of chickpea and soybean seeds with a siderophore-producing fluorescens pseudomonad resulted in increasing seed germination, growth, and yield of the plant (Kumar and Dube, 1992).

The direct promotion of plant growth by PSMs is as a result of the production of plant growth regulators (mainly auxin), enhanced availability of nutrients to the host plant. The dual role of IAA as plant growth promoter (Gusain, *et al*., 2015) and as biocontrol agent by inhibiting the germination of spore and development of mycelium of pathogenic fungi (Brown and Hamilton 1993) are well documented. Another study also showed that high level of IAA induced significant increase in the plant height and root length of wheat seedlings along with increase in chlorophyll content when compared with control (Mohite, 2013).

Moreover, bacterial IAA facilitates adaptation of host plants in metal-contaminated sites through activation of physiological changes in plant cell metabolism under metal stress so that the growing plants can resist high concentrations of heavy metals (Glick 2010). Microbial production of HCN has been reported as an important antifungal trait to control root infecting fungi (Ramette *et al*.2003) and a potential and environmentally compatible mechanism for biological control of weeds (Heydari, *et al.,* 2008).

2.4.2. Ecophysiological traits

Stress tolerance towards eco-physiological factors such as high salinity and soil acidity of beneficial soil bacteria may be good attribute for the selection of bioinoculaqnts (Upadhyay *et al.*, 2012). The use of halotolerant PGPRs that are selected based on both high salt tolerance and efficiency in expressing PGP traits could significantly advance our ability to grow crops in environments with natural or induced salinity (Zhu *et al.*, 2011). On the other hand, resistance to antibiotics and agrochemicals is an indicator of bio inoculant to persist and establish in the soil by resisting agro-chemicals such as pesticides, herbicides and chemical fertilizers (Silver and Misra, 1988).

To sum-up, considering the negative effects of chemical P fertilizers, microbial intervention of P-solublization seems to be an effective way to solve the phosphorus availability in soil. Microbial mediated P management is an ecofriendly and cost effective approach for sustainable development of agricultural crop (Sharma *et al*., 2013). This requires rigorous screening for efficient phosphate solublizing bacterial and fungal strains with adaptation to different soil conditions (Zaharan, 1999) from vermicomposting and coffee rhizosphere thereby to increase organic coffee production and productivity.

To achieve this, indigenous phosphate solublizing bacterial and fungal isolates can be characterized under different conditions in the laboratory and tested on coffee seedlings in the coffee nursery for their effectiveness. Although several phosphate solubilizing bacteria and fungi occur in soil (Zaidi *et al*. 2009), usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Thus, the amount of phosphate liberated by them is generally not sufficient for a substantial increase in coffee plant growth. Therefore, inoculation of the plant by a target microorganism at a much higher concentration than that normally found in soil is necessary to take advantage of the phosphate solubilization for the plant yield enhancement. Dual inoculation of phosphate solublizers with *Rhizobium* stimulated the plant growth more than their single inoculation depending upon the soil conditions (Perveen *et al.,* 2002; Zaidi *et al.,* 2003). This situation has certainly brought the subject of phosphate

solubilization to the front line and dependence on costly mineral fertilizers is going to be lessened in future.

In Ethiopia few studies of coffee management systems have focused on measuring and comparing the soil biological properties in relation to coffee rhizosphere. Muleta *et al*. (2007a) identified composition of coffee shade tree species and density of indigenous arbuscular mycorrhizal fungi (AMF) spores and their inputs in Coffee production systems as a topic that deserves more research attention. Moreover, in vitro antagonism of rhizobacteria isolated from Coffea arabica L. against emerging fungal coffee pathogens (Muleta et al.2007b), distribution of arbuscular mycorrhizal fungi spores in soils of smallholder agro-forestry and mono cultural coffee systems (Muleta et al.2008) and characterization of rhizobacteria isolated from wild Coffea Arabica (Muleta et al.2009) were documented. Recently Diriba *et al.*, assessed for P solubilising ability of isolated strains of rhizobacteria associated with *Coffea arabica* L. From a total of 395 isolates tested, over 72% mostly *Pseudomonas erwinia* and *P. chlororaphis* were able to solubilize P sources strongly and HPCL analyses showed several organic acids, with 2-ketogluconic acid dominating (Muleta *et al.*, 2013).

Nevertheless, collection of local isolates, development of effective microbial bio-inoculants for Coffee, use and importance of bio-inoculants technologies are yet to be done in Coffee production sector. Furthermore, in contrast to inorganic fertilizers, farmers' awareness towards bio-inoculants, importance and use has been very minimal or at its infant stage. In this regards, research efforts should be made to obtain appropriate formulations of microbial inoculants incorporating phosphate-solubilizing bacteria and fungi which will help in promoting the use of such beneficial bacteria and fungi in sustainable agriculture. Hence, elucidation of this phenomenon and the development of cultures for raising vigorous and healthy coffee seedlings in the nursery for establishing superior coffee plantation in a long run are major contributions of this study to organic coffee producing systems.

2.5. Success of inoculants in coffee production

The individual and consortia of *Azosprillum, Pseudomonas flurosence*, Phosphate Solubilising Bacteria (PSB) and Vesicular Arbuscular Mycorrihiza (VAM) have been tried in coffee nursery for the last tenths of years along with Standard Package of Practice (SPP) (Biradar *et al.* 2006; Glory Swarupa 1997). In addition, during our investigation, Phosphate Solubilising Bacteria (PSB) and fungi recovered from coffee rhizosphere and vermicompost have been tried in coffee seed germination and coffee nursery.We have gotten good results in coffee germination assay as well as increased growth parameters in bio-inoculated treatments (Reshid et al., 2021a; 2021b).

The PSM *Bacillus megaterium* has been commercialized as biofertilizer and can reduce phosphate fertilizer requirements of plantation crops up to 75%. Moreover, strains of P-solublizing *Pseudomonas striata, B. polymyxa*, and *B. megaterium* have also been commercialized worldwide (Mehnaz, 2016). However, research articles have been indicating that very little is known about application of inoculants for coffee cultivation. For example, identification and characterization of microorganisms associated with *Coffea arabica* in their functional characteristics towards plant growth promotion (Muleta et al., 2008; Muleta *et al*., 2007a; Muleta *et al*., 2007b; Muleta *et al*., 2009; Muleta *et al*., 2013; Satyaprakash *et al.*, 2017 **)** are few of them. Similarly few trials were done on coffee plant growth under greenhouse condition and under coffee nursery condition (Azizuddin and Krishnamurthy, 1984; Joshi *et al.* 2015; Kunwar, *et al*., 2018; Ormeño-Díaz *et al*. 2018; Prasad, *et al*., 2014). But there is no valid information regarding coffee production using inoculants in the country or other countries that indicated doses or application frequency under field conditions to produce coffee.

2.6. Formulations and application methods of inoculants in coffee production

Microbial formulation is a carrier-based preparation to provide microbes with better survival for longer duration. The formulation contains the organisms, which are useful in wide agriculture usages such as plant growth promoter, nutrient availability, and to improve soil and plant health

and which has a shelf life of two years with an initial CFU count of 10^{10} and at the end of one year not less than 10^8 at a wide temperature range of 5°C- 40^0C. Microbial formulations for plant growth comprise beneficial bacteria, beneficial fungi, and carrier materials which extends the effective life time of the microbial inoculant. The microbial inoculant is effective for increasing plant productivity in legumes, non-legumes and vegetable crops. However, there are a number of disadvantages of the application process, such as the availability of enough bioinoculant quantity for total seed surface, contact with chemicals, bacterial movement, the material used as carrier (nature, particle size, and presence of contaminating microorganisms or viability of the bacteria), and the technology used for drying and preservation of bacteria (addition of nutrients and preservatives) in the substrates.

The seed treatment is a means to apply bio-inoculants which are effective and economic. For small quantity of seeds (up to 5 kg quantity) the coating can be done in a plastic bag having size (21**"** x 10**"**) or big size can be used. The bag should be filled with 2kg or more of seeds, closed in such a way to trap the airs as much as possible, squeezed for 2 minutes or more until all the seed are uniformly wetted and then the bag should be opened, inflated again and shacked gently. Stop shaking after each seeds gets a uniform layer of culture coating. The bag is opened and the seed is dried under the shade for 20-30 minutes.

For large amount of seeds coating can be done in a bucket and inoculant can be mixed directly with hand. Seed Treatment with *Rhizobium*, *Azotobacter*, *Azospirillum*, along with PSM can be done.

3. MATERIALS AND METHODS

3. 1. Description of the soil sample collection areas

Soil samples were collected from Seka Chekorsa, Goma and Manna districts of Jimma Zone (Figure 2).

Seka chekorsa, Goma and Manna districts are among the woredas in Jimma zone, which are located at 368, 368 and 395km respectively, southwest of Addis Ababa. The microbial analysis was conducted in Jimma University, School of veterinary medicine Laboratory.

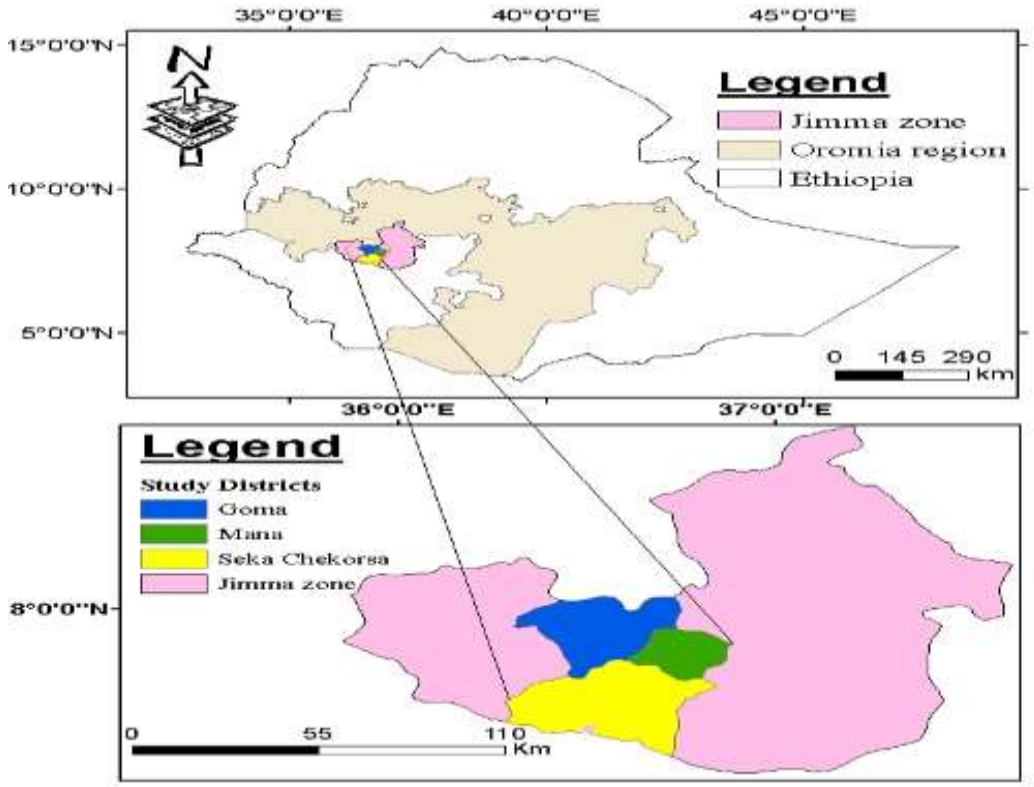

Figure 2. Soil sample collection area

3. 1.1. Vermicompost production

Green grass (Gg) was collected from JARC, chopped and subjected to initial decomposition in draining plastic containers of 45 ×35 × 15 cm sizes by sprinkling water, regular mixing and turning of the substrates for 15 days. Cow dung (CD) was collected from nearby cattle sheds in

fresh form and allowed to dry in open space for one week and used for the study. The Russian based earthworms, *Eisenia fetida*, originally collected from vermiculture of the JARC were mass multiplied in cow dung and used for the study. Each pre-decomposed Gg and CD substrates were mixed in1:2 ratio, respectively on dry weight basis by making piles in plastic containers of 45×35×15 cm size and was left for a period of 4 days for stabilization by sprinkling water daily. After 4 days, each bin containing vermibed substrate was introduced with 200 adult *E.* fetida. Vermicomposting was carried out in an environmentally controlled experimental glass house at a temperature of 26±2°C and the vermibeds were maintained to contain a moisture level of (65-75%) by sprinkling water over the surface at two days intervals until maturity.

3.1 .2. Collection of soil and Vermicompost samples

Over 150 soil samples were collected from commonly known coffee growing districts of Mana, Goma and Seka Chokorsa in Jimma Zone, Oromia Regional State. The soil samples obtained from a depth of 0-15 cm from the rhizosphere adhering to roots of coffee plants. The samples were randomly collected from coffee plantation fields/farmers managed forest coffee/ within 1 to 2 km interval between the samples. Matured vermicompost was collected and air dried under shade for the isolation of phytobeneficial bacteria and fungi as described by Kole & Altosaar (1988). All samples were placed in polythene bags, brought to the laboratory in ice boxes and were stored at 4^{o}C in refrigerator for further analysis. The pH of soil sample and vermicompost was measured from the suspension of 1:2.5 soils: H_2O by pH meter. Soil and vermicompost determination of OC was done following Wakley and Black (1934) method and available NPK was also analyzed based on Bray II procedure (Bray and Kurtz, 1945).

3. 1.3. Isolation of microbes

Primary isolation and identification of fungal and bacterial P solublizers was done on Pikovskaya's agar (PVK), containing per liter:0.5g yeast extract, 10g dextrose, 5g Ca_3(PO4)2, 0.5g (NH4)2SO4, 0.2g KCl, 0.1g, MgSO4.7H_2O,0.0001g, MnSO4.H_2O,0.0001g, FeSO4.7H_2O and 15g agar, pH 7.2) supplemented with tri-calcium phosphate (5 g/L) as an insoluble inorganic phosphate source (Pikovskaya, 1948) by plate count method (Ravina et al., 1992). 10 ml/L Rose

Bengal was added for control of bacterialgrowth at concentration 1 /1 5000 to PVK for fungal growth (Smith and Daws 1944). A 10g of soil and vermicompost was separately suspended in 90 ml sterile distilled water in Erlenmeyer flask and mixed thoroughly for 30 minutes using a mechanical shaker at 110 rpm. Then 1ml of aliquot from each was transferred with sterile pipettes for ten-fold serial dilution. From appropriate serial dilution, 0.2ml of aliquot was transferred to sterile Petri-plate containing pre-solidified PVK medium. The inoculated plates were incubated for 7–14 days at 30^0C for bacterial isolation and incubated for 6 days at25±2°C for fungal isolation. From the total colonies, only those colonies which showed clear zones were re-streaked onto PVK medium to obtain pure cultures of phosphate solublizing colonies. The pure colonies that showed clear zones around them were maintained in PVK slants at 4^0C for subsequent analysis.

3.1. 4. Invitro Phosphate Solubilization tendency of isolates

The P-solubilization index (SI) of phosphate solubilizing bacterial and fungal isolates were checked on the PVK medium supplemented with tri-calcium phosphate (5g/L) as insoluble phosphates source. A pinpoint inoculation of bacterial and fungal isolates was placed on the center of plates under aseptic conditions. The growth and solublization of insoluble phosphates in the PVK medium by forming the halo zones were determined by solubilization diameter after incubation at 30^0C for 7 days for bacteria and at 25±2° C for 6 days for fungi. Bacterial and fungal colonies surrounded by a halo, indicating phosphate removal, were visually observed and measured (Alam *et al*.,2002). Three replicate plates were used for each isolate. The solubilization index was measured according to the formula:

$$PSI = \frac{\textit{Colony diameter + halozone diameter}}{\textit{Colony diameter}}$$

3.1.5. Phosphate solubilization in bromophenol blue of bacteria

PVK medium supplemented with 2.4 mg/ml bromophenol blue was used for phosphate solubilization. The media inoculated with the isolates was incubated at 30^0C for 7days and observed for yellow color change.

3.1.6. Solubilization of inorganic phosphate in liquid medium

Pikovskaya's broth medium (125 ml) supplemented with 0.5% TCP (equivalent to 5000 mg L^{-1}) was prepared. The medium in 125 ml amount was dispensed into a 250 ml capacity Erlenmeyer flask. Three replicate flasks were used for each bacterial and fungal isolate. Sterile uninoculated medium was served as control. The pH of the medium was adjusted to 7.0 before autoclaving and each flask was inoculated with 0.1 ml of 24 h old active culture suspensions of each PS bacterial isolates with a cell density of 10^8cuf/ml. Similarly, the liquid medium in the flasks was inoculated with fungal isolate using 8 mm mycelia disks taken from 7 days old cultures.

The flasks were kept on a rotary shaker (125 rpm) for 6 days until the day of sampling for bacteria and kept for 15 days until the day of sampling for fungi. To collect solublized inorganic P by bacteria, insoluble materials in each culture broth were removed by centrifuging at 4,000 rpm for 20 minutes and filtered through Whatman filter paper No.1. To quantify solubilized P in each fungal culture, insoluble materials were removed by centrifuging at 5,000 rpm for 25 minutes and filtered through Whatman filter paper No.42. From each culture, 0.625 ml of the filtrate was transferred to a volumetric flask of 100 ml capacity and 13 ml of mixed reagent added. The volume was top upped to 100 ml with distilled water. Soluble phosphorus was determined following quantitative spectrophotometric analysis according to the method of Murphy and Riley (1962) and the pH of the cultures was also measured accordingly using pH meter (Kumari *et al*., 2010). The absorbance of blank and cultures of the inoculated treatment filtrates were read at 882 nm wave length using spectrophotometer (JENWAY 6100, JENWAY LTD, UK). A calibration curve for standard, plotting absorbance against respective P concentrations was prepared and the P concentration of treatment filtrates was read from calibration curve.

3.2. Invitro test for phytobeneficial traits

3.2.1. Production of Indole acetic acid (IAA)

Production of IAA was done based on methodology of Patten and Glick (1996).The potent bacterial cultures were inoculated into nutrient broth with L-tryptophan (5 μg/mL) and incubated at 28±2^{0}C for 5 days. After incubation, cultures were centrifuged at 3000 rpm for 30 min. A 2

mL of the supernatant was mixed with 2 drops of orthophosphoric acid and 4 mL of Salkowski's reagent (50 mL of 35% perchloric acid + 1 mL 0.5$FeCl_3$) and incubated in the dark for 25 minutes. Development of pink colour indicates IAA production.

3.2.2. Production of ammonia (NH3)

Bacterial isolates were tested for the production of ammonia in peptone water. PSB was grown for 48 h in nutrient broth (NB) medium at 36±2°C. Freshly grown cultures (100 µL of 24 h grown) were inoculated into 10 mL peptone water in each tube. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow color was taken as a positive test for ammonia production (Cappuccino and Sherman, 1992).

3.2.3. Hydrogen cyanide (HCN) production

Qualitative cyanide determination was carried out by Lorck (1948) method modified by Alstrom and Burns (1989). PSB isolates were sub cultured onto nutrient agar (NA) medium supplemented with glycine ($4.4gL^{-1}$). Whatman filter paper No.1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate fixed to the underside of the Petri-dish lids and sealed with parafilm before incubation at 28^0C for 48h. Changes in color from yellow to reddish brown was taken as an indication of strongly cyanogenic potential.

3.2.4. Nitrogen Fixing Activity

In order to screen for nitrogen fixing ability among the isolated PSB, the pure cultures were inoculated onto Jensen's medium (Jensen,1942) containing g/L: Sucrose 20.0, Dipotassium phosphate 1.0, Magnesium sulphate 0.5, Sodium chloride 0.5, Ferrous sulphate 0.1, Sodium molybdate 0.005, Calcium carbonates 2.0, Agar 15.0. The inoculated Petri plates were incubated at 37^0C for 5 days. Un-inoculated Petri plate served as control.

3.3. Invitro test for eco-physiological properties

3.3.1. Evaluation for heavy metal tolerance

Tolerance to heavy metals was measured using PVK medium (Pikovskaya, 1948) supplemented with different concentrations of various soluble heavy metal salts. Freshly prepared agar plates were amended with various soluble heavy metal salts namely Hg, Cu, Zn and Mn, at various concentrations ranging from 100 to 400 μg/ml was inoculated with overnight grown cultures in PVK medium. Heavy metal tolerance was determined by the appearance of bacterial growth after incubating the plates at 37°C for 48 h. Isolates were considered resistant when growth was observed ("+'') and ("–") for absence of growth.

3.3.2. Evaluation for salinity tolerance

PVK medium supplemented with different concentrations of NaCl (3%, 4%, 5%, 6% and 7% (w/v) were used to screen the P-solubilization efficacy of different isolates and it was incubated at 28^{o}C for 5 days (Suman *et al*, 2018). The results were recorded qualitatively as ("+'') for presence and ("–") for absence of growth indicating the salt tolerance and sensitivity level of the microbes.

3.3.3. Screening for acid tolerance

PSB isolates were tested for pH tolerance according to the method described by Hayat *et al*. (2013). To analyze whether bacteria can grow in a range of pH 4.0 to 10.0 at an increment of one unit pH, all the active strains of PSB were inoculated separately into test tubes containing 10 ml of nutrient broth (NB) at varying pH levels adjusted with sterile 0.1N HCl and 1N NaOH. Bacteria were incubated for 8 days at 25^{0}C. The results were recorded qualitatively as ("+'') for presence and ("–") for absence of growth.

3.3.4. Determination of antibiotic susceptibility patterns of Isolates

The microbial cultures viz., phosphate solubilizing bacteria (PSB) were obtained from Jimma University, College of Agriculture and Veterinary Medicine, Microbiology Laboratory which were isolated and screened under *in vitro* conditions and used to test their antibiotics susceptibility patterns. Susceptibility of the isolates to antibiotics was performed by the disc

diffusion method as described by Bauer *et al.* (1966) and Liasi *et al*. (2009) using commercially available antibiotic discs (Oxoid). Briefly, the purified bacterial colonies of the respective isolates were inoculated into nutrient broth and incubated at 36±2°C for 48 h. sterile cotton swabs were dipped into the bacterial broth suspension and evenly spread on pre-dried surfaces of nutrient agar plates. The inoculated plates were allowed to dry before placing the diffusion discs containing antibiotics. The plates were then incubated at 37°C for 24h. The commercial antibiotics used were penicillin G (PG, 10 unit), Ceftazidime (Ce,10 µg), Doxycycline (dxt, 30 µg)**,** Erythromycine (E,15 µg), Tetracycline (T,10 µg)**,** and Vancomycin, (Van, 10 µg). After incubation of the plates, inhibition zone diameters were measured by including the diameters of the discs. The isolates were classified as sensitive S (≥ 21 mm); intermediate, I (16-20 mm) or resistant, R (≤ 15 mm), respectively according to Vlková *et al*. (2006). For the purpose of data analysis, the intermediates were considered as sensitive (National Committee for Clinical Laboratory Standards 2000; Rojo-Bezares*et al.*, 2006).

3.3. 5. Identification of PSM

The selected PSB isolates were studied for their morphological, physiological and biochemical tests based on the methods defined in Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994). Bacterial colonies from purified culture were grown on PVK solid medium by streak plate method and incubated at 30°C until colonies appeared. Gram staining reaction of isolates was observed under light microscope. Morphological characters of the fungal isolates were observed by growing them on PVK agar and stained with lectophenol cotton blue stain for observation of morphological characteristics of the hyphae, spores, and conidiophores under light microscope.

3.3. 6. *In-vitro* co-culture test between bacteria, fungi and pathogenic *Fusariumxyloriodes*

Co-culture assay was performed following the method described by Santiago *et al.* (2017). Each of the bacterial (RCHVCB$_1$, RScB1.19 and RMaB2.11) and fungal (RSCF1.19, RCHVCF$_2$, RLVCF$_2$) isolates were grown in nutrient agar medium at 30°C for at least 3 days and then

streaked perpendicularly on freshly prepared nutrient agar medium; *i.e.*, after the first strain was allowed to grow at 30°C for 3 days. Thereafter, the second strain was streaked at an angle of approximately 90° going outward from the emerged colonies of the first strain and incubated also at 30°C for 3 days. Finally, photographic documentation of the agar plates was obtained including those showing colony lines and inhibition zones that appeared at the intersection of the paired strains.

3.3.7. Multiplication of bacterial and fungal isolates and inoculum preparation

Three characterized and identified phosphate solubilizing bacteria (PSB) and three fungi isolates (PSF) were used to prepare the bio-inoculants (Reshid Abafita *et al.*, 2021). Inoculums of PSB were prepared in Pikovskaya's broth medium (Pikovskaya, 1948). After multiplication of the selected elite isolates in the PVK broth by incubating at 28±2^{o}C under shaking at 100rpm for three days, the broth culture was mixed with sterilized vermicompost (VC) as carrier material. The viable count in the inoculums was kept as 1x10^{8} CFU/ml before mixing with carrier material (VC) that was sterilized at 121^{o}C and 15 psi pressure for an hour. Proper water content of the sterile carrier material (VC) was maintained and inoculated with broth cultures of phosphate solublizing isolates (20mL per 50g of VC) and was incubated at 28±2^{o}C. For fungal inoculum preparation, phosphate solubilizing fungal (PSF) isolates were mass cultured aseptically in 90 mm diameter Petri plates each containing 15mL of autoclaved PVK. The plates were incubated at 28 ±2°C for 10 days. On the tenth day, spore suspensions from the fungal isolates were prepared by flooding the surface of the agar plates with 10mL sterile distilled water and the culture surfaces were gently scraped using a sterile glass rod to dislodge the spores. The spore suspension was transferred separately to 500mL flask containing 400mL sterile distilled water. Flasks were shaken for 2 minutes to ensure that the spores were properly mixed. The cultures were filtered through Whatman No.42 filter paper into sterile glass bottle. The spore suspension of 25 ml (10^{6} spores mL^{-1}) of fungal culture was used per 50g of the sterilized carrier materials (VC) and immediately stored at 4ºC until use. The mixed and inoculated carrier material was evaluated for plant growth promotion as bio-inoculants.

3.3.8. Invitro seed germination assay

The test crop used was coffee variety 74110 which was coffee berry disease (CBD) tolerant, high yield bearing and released variety and obtained from Jimma Agricultural Research Center. This variety is suitable for medium altitudes and collected from Illu Aba Bora in western Ethiopia (Bayetta, *et al.*, 2008). The seeds were surface sterilized by immersing in 3.25% (v/v) sodium hypochlorite for 1 min, followed by 70% (v/v) ethanol for 1 min and rinsing five times in sterile distilled water. These seeds were submerged in liquid 4 ml of fungal spore suspension and bacterial cultures, both as single and dual inoculation separately for six hours incubation at room temperature (Biradar, *et al*., 2006). The seeds were dried in a laminar air-flow. The Seed germination assay was done with completely randomized design (CRD) in three (3) replications per treatments and arranged in two groups. The first group was designed with six (T_1 –T_6) treatments for single inoculation and the second group were designed with ten (T_7-T_{16}) treatments for co-inoculation. A total of 10 seeds were placed at equal distance on the sterile moist blotter paper of 8.5 cm diameter in pre-sterilized borosil glass Petri dishes of 10 cm diameter. The seeds were covered with other moist blotter paper and these Petri dishes were incubated at room temperature for 50 days. During these days, sterile distilled water was properly added to maintain wet condition. After incubation period, percent seed germination, shoot (plumule) and root (radicle) length of each seeds was recorded. The germination rate and vigor index were calculated according to the following equations (Islam *et al*., 2016):

$$\text{Germination rate (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100 \quad (1)$$

$$\text{Vigor index} = \%\ \text{Seed germination} \times [\text{Mean root (radicle) Length} + \text{Mean Shoot (plumule) Length}] \quad (2)$$

3.4. Invivo glasshouse assay and In-vivo Evaluation of selected isolates for coffee seedlings under Nursery condition

3.4.1. Description of the study area

Both glasshouse and nursery coffee seedling assay was carried out at Jimma Agricultural Research Center (JARC) during February to August of 2019. JARC is located at 363 km to the

southwest of Addis Ababa. JARC is found at 7°40'47"N latitude and 36°49'47"E longitude. The mean minimum and maximum temperature of JARC are 26.2 and 11.3°C, respectively. The elevation of the Center is 1,753 m above sea level and it receives 1,529.5 mm average annual rainfall. The total area of Jimma Zone is 18415 km^2 and located between latitudes $7^0$18'N and $8^0$56'N and longitudes $35^0$52'E and $37^0$37'E (Addis Tadsesse *et al*., 2015).

3.4.2. Soil Physical and Chemical Properties

Before the commencement of the experiment the texture of potting media (soil) was determined by the Hydrometer principle whereas soil pH was measured from the suspension of 1: 2.5 soils: H_2O by pH meter. Soil organic carbon was determined by the Wakley and Black method (Wakley and Black, 1934) and available phosphorus in the soil was determined based on (Bray II procedure (Bray and Kurtz, 1945). Nutrient uptake (N, P and K) from growth medium was recorded at the end of the trial (Prasad, *et al.,* 2014).

3.4.3. Test crop, potting medium, pot volume, treatments and research design.

The test crop used was coffee variety 74110 which was coffee berry disease (CBD) tolerant, high yield bearing and released variety and obtained from Jimma Agricultural Research Center. This variety is suitable for medium altitudes and collected from Illu Aba Bora in western Ethiopia (Bayetta, *et al.,* 2008). Endocarp (parchments) was manually removed (Guimarães *et al.,* 2013) and the coffee seeds were surface sterilized with 75% ethanol for 1 min followed by 1% sodium hypochlorite for 30 min with extensive wash using sterile distilled water (Collavino *et al.,*2010). The surface sterilized seeds of *Coffea arabica L.* were placed in a sterile dish and mixed with 4 ml of inoculants of bacterial and fungal isolates and incubated for 6 hours at room temperature (Mohamed and Almaroai, 2017). Moreover, these seeds of *Coffea arabica L.* were also inoculated with carrier based inoculums at a rate of 15 g/100 g seeds (Mohamed and Almaroai, 2017) after moistening with 10ml of sugar solution (1spoon table sugar per 10ml water) and thoroughly mixed with the seeds until the surfaces were uniformly coated. The glasshouse

experiments were conducted from February to August of 2019 under controlled glasshouse condition at 28–32ºC in plastic pots having 19 cm height with 21cm top and 18.5 cm bottom which were filled with 3.5 kg of sand. Similarly, the nursery experiments were conducted during February to August of 2019 in plastic nursery bags under natural environments. Each bag was filled with 1.5 kg of agricultural dry field soil; two inoculated seeds were sown in each nursery plastic bag. TheVermin-compost was applied at the rate of 20% of potting medium per pot (Reshid, *et al*., 2014). The inorganic fertilizer treatment (4g DAP/pots) was mixed with sand and soil before sowing coffee seeds in the medium. The fertilizer application rates for the inorganic P treatments were calculated from the published rates of inorganic fertilizer recommendations for young coffee in the field which was $1tha^{-1}$ per year (Loga and Biscoe, 1987). Two inoculated seeds were sown in each pots and nursery plastic bag. Seedlings were thinned when they attained two pairs of true leaves and one uniformly growing seedling was left. The seedlings were grown until the emergence of seven pairs of true leaves in both experiments. After completion of trial, the plants were up-rooted and washed thoroughly with water and several parameters such as shoot and root length, leaf numbers, leaf area, stem girth, fresh and dry weight of the whole plant, NPK up-take of the leaves were measured using standard procedures. Both glasshouse and nursery experiments was done with completely randomized design (CRD) in three (3) replications and designed with four groups. The first two groups were designed with fourteen (T_1 -$T_{14,}$) and twenty (T_1-T_{20}) treatments for both single and co- inoculation with and without P fertilizer. The second two groups were designed with Nine (T_1-T_9) and twelve (T_1-T_{12}) treatments for both single and co-inoculation with vermicompost.

3.5. *STATISTICAL ANALYSIS*

Data were collected in replicates of three and analyzed using SAS Statistical Package Version 8.5.0) 2010, Origin Lab Corporation. To determine the effects due to inoculation, Analysis of Variance at the 0.05 levels and Correlation coefficient using SAS was done and means were separated using Duncan Multiple Range Test at 0.05 levels (Gomez and Gomez, 1984). Data obtained from the different treatments were presented in the form of tables and using Microsoft Excel 2007.

4. RESULTS

4.1. Physico-chemical properties of soil and vermicompost samples

Physio-chemical parameters of rhizosphere soil samples and vermicompost were determined and presented in Table3.1.The soil pH of study site and vermicompost was in the range of 4.6-6.2 and 8.5-9.5 respectively (Table 1). The organic carbon and the extractable phosphorus concentration recorded from study site were in the range of 0.63-0.87% and 10.12-10.30ppm respectively, which are in the very low range (FAO., 1990; London, 1991). However, the organic carbon and the extractable phosphorus concentration recorded from vermicompost were in the high range. The potassium concentration of the study site was also in the range of 60.04-102.86% which is in the high range (Table.1) (London, 1991).The total N concentration of the study site and vermicompost were in the range of 0.04- 0.11% and 1.23-1.24 respectively (Table .1), which is in the low range (FAO., 1990; London, 1991).

Table 1 Physico - chemical characteristic of Soil samples and Vermicompost sample collected.

Samples	Sampling area/source	Sample color	CEC (meq/100g)	Organic C	% TN	AvailableP(ppm)	K(%)	pH(H_2O: 1:2.5)	Ex. Acidity (meq/100g
Rgo3.17	Goma	Light grey	13.00	0.87	0.07	10.36	65.04	6.2	0.40
RMa2.39	Manna	Light grey	12.12	0.83	0.07	13.10	102.86	5.6	0.50
RVC3	Vermicompost	black	20.86	10.83	1.23	295.998	100.33	8.5	0.59
RCHVC1	Vermicompost	black	20.84	10.83	1.24	295.998	100.33	9.5	0.67
RSC1.50	Seka chekorsa	red	14.78	0.66	0.20	169.16	60.04	4.6	0.30
RLVC 2	Vermicompost	black	20.80	10.83	1.24	295.998	89.87	9.5	0.66
RMa 2.33	Manna	Light grey	13.00	0.86	0.08	10.36	102.86	5.50	0.42
RSc1.19	Seka chekorsa	red	14.70	0.63	0.11	168.10	64.04	4.86	0.59
Rgo3.5	Goma	grey	13.18	0.81	0.05	10.30	102.86	5.52	0.41
RSc1.7	Manna	grey	14.14	0.82	0.04	13.33	102.86	6.1	0.43
RMa2.11	Manna	grey	12.36	0.85	0.08	10.12	102.86	5.59	0.40
RMa1.2	Manna	brown	15.6	0.84	0.08	13.30	102.86	5.55	0.40

4.1. 1. Isolation and enumeration of bacterial and fungal isolates

A total of PSB and fungi isolated from coffee rhizosphere and vermicompost isolated on PVK agar medium from the study area are presented in Table 2. Some of the colonies which produced halo around them on the PVK agar were considered as phosphate solubilizers and selected for characterization. A total number of 154 bacteria and 72 fungi were isolated from vermicompost

and coffee rhizosphere (Table2). Among the 154 bacterial isolates, only 12 of them showed clearly visible large transparent halo zones around their colonies on PVK agar medium after 7 days of incubation (Figure 3ABC). Similarly among 72 fungal isolates, only 9 of them showed clearly visible large halo zones around their colonies on the same culture medium after incubation at 25±2° C for 6 days (Figure 4AB).

Table.2. Total number of bacteria and fungi isolated from samples

Sources of soil samples	Bacterial Isolates			Fungal isolates	
	Number of samples	Number of PSB	Proportion of PSB(%)	Number of PSF	Proportion of PSF(%)
VC	6	54	35.07	42	58.34
Coffee rhizosphere	120	100	64.94	30	41.67
Total	126	154	100	72	100

VC=vermicompost

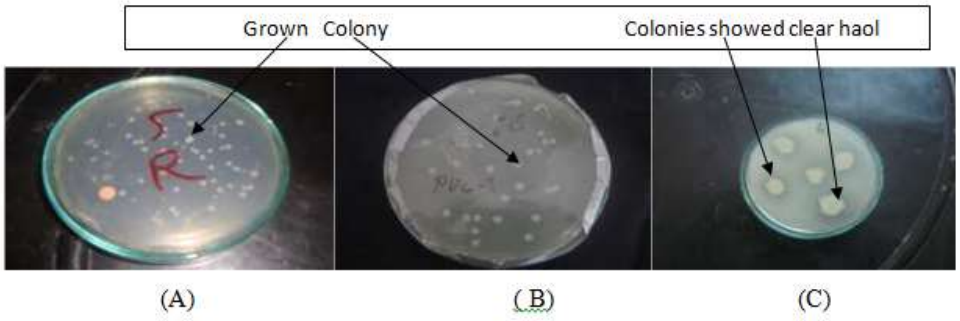

Figure 3. Colony growth and halo zone appearance on Pikovskaya's agar medium

Figure.4 .Colonies of fungi showing clearly visible large halo zones around their colonie

4.1. 2. Phytobeneficial traits of bacteria isolated from vermicompost and coffee rhizosphere

Among those twelve potent PSB isolates, isolate RMaB2.11 showed the maximum phosphate solubilizing halo zone (15.33±1.53 mm (Table 4). However, the solubilization index (SI) of the isolates RCHVCB1, RScB1.19 and RMaB2.11 were 3.87±0.12, 3.56±0.14 and 3.09±0.08, respectively (Table 4). These indicated that the isolates solubilized inorganic phosphate efficiently in the medium containing 0.5% tri-calcium phosphate (TCP).

Phosphate solubilizing bacterial isolates, RScB1.19, RCHVCB1 and RMaB2.11, produced 361.46, 340.37and 327.32μg mL^{-1} available P in the PVK broth after 6 days of incubation time respectively (Table 4).

The pH value in the uninoculated control flasks remained similar (Table 4). A gradual pH decrease from the initial value of 7.07 to 3.20 on the 6th day was recorded in PVK broth supplemented with tricalcium phosphate (Table4). Moreover, it was observed that significant ($P \leq 0.05$) increments in soluble P content along with a significant decline in the pH of the PSB-inoculated medium as evaluated with the control where it remained constant (pH 7).

The lowest pH value was recorded (3.20) when the quantity of solubilized P reached the maximum value (361. 46 μg/ml) and this highest P-solubilizing potential was exhibited by the bacterial strain RScB1.19 (361.46μg /ml). The maximum drop in pH value was significantly coincided with elevated quantity of P solubilization, where pH was declined to 3.2 from initial pH (7.07) by PSB strain RScB1.19 (Table 4).

These are also capable to solubilize inorganic phosphorus ($Ca_3(PO4)_2$) in bromophenol blue liquid medium by indicating yellow color development using TCP as a source of insoluble P(Table 4). These indicated that the isolates solubilized inorganic phosphate efficiently in the medium containing 0.5% tri-calcium phosphate (TCP).

The maximum drop in pH value was non-significantly correlated with elevated levels of phosphate solubilization, where pH was declined to 3.2 from initial pH (7.07) by PSB strain RScB1.19 (Table 4). However, strong negative correlation between phosphate solubilization and solublizetion index, as well as a strong positive correlation between coloney diameter and halo zone diameter can also be observed (Table 3)

Table 3. Correlation coefficients studied among coloney diameter, halo zone diameter, solublization index, amount of solubilized P and pH changes

Variable	coloney diameter	halo zone diameter	solublization index	amount of solubilized P(μg/ml)	pH
Coloney diameter	1				
Halo zone diameter	0.83046**	1			
Solublization index	0.18810 NS	0.13276NS	1		
Amount of solubilized P (μg/ml)	-0.17893NS	-0.07578 NS	-0.95662**	1	
pH	0.09377NS	0.27815NS	0.01684NS	-0.07792 NS	1

Legend: NS= not significant

Table 4.Qualitative phosphate solubilization efficiency of bacterial isolate

Isolate code	Colony diameter(mm)	Halo-zone diameter(mm)	solubilization index (SI)	P-solubilization in bromophenol blue
RgoB3.17	6.33±0.58abc	10.33±1.53de	2.62±0.11cde	+
RMaB2.39	7.00±1.00ab	13.33±1.16abcd	2.92±0.14bc	+
RLVCB 3	6.67±2.08abc	11.67±3.51bcd	2.81±0.61bcd	+
RCHVCB1	4.67±0.58c	14.67±1.16ab	3.87±0.12a	++
RSCB1.50	6.67±2.08abc	8.33±2.31e	2.26±0.12e	+
RLVCB 2	6.00±1.00bc	12.33±1.53abcd	3.07±0.12b	+
RMaB 2.33	8.00±1.00a	13.33±2.08abcd	2.66±0.09cd	+
RScB1.19	5.33±0.58bc	13.67±2.08abc	3.56±0.14a	+
RgoB3.5	6.67±0.58abc	11.33±1.16cde	2.70±0.02cd	+
RScB1.7	7.67±1.53a	11.33±1.53cde	2.50±0.15ed	+
RMaB2.11	7.33±0.58ab	15.33±1.53a	3.09±0.08b	+
RMaB1.2	6.00±1.00abc	11.00±1.00cde	2.86±0.30bc	+
CV	18.46	15.63	7.40	
Mean	6.53	12.22	2.91	
T test (0.05) (LSD)	2.04	3.24	0.37	

*Means followed by the same letter(s) in each column are not significantly different at $P \leq 0.05$.; '+' positive to bromophenole blue," -"Negative

Quantitatively PSB isolates, RScB1.19, RCHVCB1 and RMaB2.11, were produced 361.46, 340.37and 327.32μg mL^{-1} soluble P in the PVK broth after 6 days of incubation period, respectively (Table 5). A gradual pH reduction from the initial value of 7.07 to 3.20 on the 6^{th} day was recorded in PVK broth supplemented with TCP (Table 5). Moreover, it was observed that the amount of solubilized P increased along with the medium pH decrease (Table 5). The

lowest pH value was recorded (pH:3.20) when the amount of solubilized P reached the maximum value (361.46 μg/ml) which exhibited by the bacterial strain RScB1.19 (Table 5).The 12 superior bacterial isolates were tested for their other phytobeneficial traits such as IAA, HCN, NH3 production and nitrogen fixing ability and ecophysiological traits (Table 5). Among the isolates RCHVCB1 (gram negative) and RScB1.19 (gram positive) were found to be the best producer of IAA followed by RMaB2.11 and were selected as potential IAA producers (Table 5). On the other hand, the rest 10 isolates were found to be a medium producer of IAA (Table 5). Study on qualitative analysis of HCN indicated a strong production of HCN by isolates from soil samples and vermicompost collected in the study area. Accordingly, 9 of the isolates produced HCN but three isolates were negative for HCN production (Table 5).

All the 12 isolates produced ammonia (Table 5). Two isolates (RMaB2.39, RLVCB 2) were not able to grow on the N-free medium (Table 5). The rest ten isolates were able to grow on the N-free medium (Table 5).

Table 5. Quantitative, pH estimation and PGP traits of phosphate solubilization efficiency of bacterial isolate

Quantitative and pH analysis				Phytobeneficial Traits			
Isolate code	Incubation period (day)	P-Solublized (µg/ml)	pH after incubation	IAA	NH_3	HCN	N- Fixing
Control	6	0.00±00m	7.07±0.06a	-	-	-	-
RgoB3.17	6	174.22±0.02l	4.44±0.02b	+	+	+	+
RMaB2.39	6	192.95±0.02i	4.30±0.01d	+	+	-	-
RLVCB 3	6	219.65±0.01h	4.21±0.01e	+	+	++	+
RCHVCB1	6	340.37±0.02b	3.46±0.00j	++	+	++	+
RSCB1.50	6	260.51±0.01e	3.98±0.02g	+	+	+	+
RLVCB 2	6	228.04±0.00g	4.18±0.01f	+	+	-	-
RMaB 2.33	6	251.53±0.09f	4.16±0.01f	+	+	+	+
RScB1.19	6	361.46±0.01a	3.20±0.00k	+	++	++	+
RgoB3.5	6	186.16±0.01j	4.36±0.01c	+	+	+	+
RScB1.7	6	271.21±0.01d	3.85±0.01h	+	+	+	+
RMaB2.11	6	327.32±0.01c	3.76±0.01i	++	+	++	+
RMaB1.2	6	180.26±0.01k	4.44±0.01b	+	+	-	+
CV		0.01	0.41				
Mean		230.28	4.26				
Ttest(0.05) (LSD)		0.02	0.03				

* Means followed by the same letter(s) in each column are not significantly different at $P \leq 0.05$

4.2. Eco-physiological traits of the isolates

4.2.1. Tolerance to heavy metals

The 12 PSB isolates resisted heavy metals such as Hg, Cu, Zn and Mn, up to 400µg/ml at varying degree (Table 6 and 7). The results showed that most of the PSB isolates grew well at

low concentrations of heavy metals studied and their number drastically decreased as the concentration of Mercury (Hg), Copper (Cu) and Manganese (Mn) increased. In Zinc (Zn) medium, all the PSB isolates were able to grow at concentrations of 100-300 µg/ml, except at concentration 400 µg/ml. Among the twelve PSB isolates, RCHVCB1, RScB1.19 and RMaB2.11 showed high tolerance to all the tested heavy metals (Table 7).

Table 6. Numbers of heavy metal tolerant PSB at different concentrations of tested heavy Metals.

concentration (µg /ml)	Heavy metals			
	Mercury ($HgCl_2$)	Copper ($CuCl_2$)	Manganese ($MnCl_2$)	Zinc ($ZnCl_2$)
100	11(91.67)	9(75.00)	11(91.67)	12(100)
200	11 (91.67)	7(58.33)	9(75.00)	12(100)
300	8 (66.67)	6(50.00)	7(58.33)	12(100)
400	4(33.33)	7(50.00)	4(33.33)	11(91.67)

* Values in parenthesis indicated % of heavy metal tolerant isolates.

Table 7. Heavy metal tolerant PS bacterial isolates at different concentrations.

Isolate No	Heavy metals (µg /ml)															
	Mercury ($HgCl_2$)				Copper ($CuCl_2$)				Manganese ($MnCl_2$)				Zinc ($ZnCl_2$)			
	100	200	300	400	100	200	300	400	100	200	300	400	100	200	300	400
RgoB3.17	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	+
RMaB2.39	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+
RLVCB 3	+	+	+	-	+	-	-	-	+	+	-	-	+	+	+	+
RCHVCB1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RSCB1.50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RLVCB 2	+	+	-	-	-	-	-	-	+	+	-	-	+	+	+	+
RMaB 2.33	+	+	-	-	+	+	+	+	+	-	-	-	+	+	+	+
RScB1.19	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+
RgoB3.5	+	+	-	-	+	-	-	-	+	-	-	-	+	+	+	+
RScB1.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RMaB2.11	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
RMaB1.2	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-

"+" resistant, "-'' sensitive

4.2.2. Tolerance to salinity and pH

The results showed that most of the PSB isolates grew well at concentrations of 3%, 4% and 5% NaCl (Table 8). However, no growth was observed as the concentration of NaCl increased from 6% to 7% (Table 8). The interaction effect between isolates and NaCl concentrations was found to be effective. Generally, as the concentration of NaCl increased beyond 5%, growth decreased abruptly (Table 8). Moreover, the majority of the isolates tolerated pH in the range of 5 to 8 except isolates RSCB1.50, RScB1.19 and RMaB2.11, because they showed tolerance to pH-4. Some isolates RCHVCB1 and RLVCB2 were able to tolerate pH up to 9 (Table 8).

Table 8. Evaluation of the isolate to salinity and pH

Isolate code	Concentrations of NaCl (w/v)					Acid tolerance (pH)						
	3%	4%	5%	6%	7%	4	5	6	7	8	9	10
RgoB3.17	+	+	+	-	-	-	+	+	+	+	-	-
RMaB2.39	+	+	+	-	-	-	+	+	+	+	-	-
RLVCB 3	+	+	+	-	-	-	+	+	+	+	-	-
RCHVCB1	+	+	+	+	-	-	+	+	+	+	+	-
RSCB1.50	+	+	+	-	-	+	+	+	+	+	-	-
RLVCB 2	+	+	+	-	-	-	+	+	+	+	+	-
RMaB 2.33	+	+	+	-	-	-	+	+	+	+	-	-
RScB1.19	+	+	+	-	-	+	+	+	+	+	-	-
RgoB3.5	+	+	+	-	-	-	+	+	+	+	-	-
RScB1.7	+	+	+	-	-	-	+	+	+	+	-	-
RMaB2.11	+	+	+	-	-	+	+	+	+	+	-	-
RMaB1.2	+	+	+	-	-	-	+	+	+	+	-	-

"+" indicates presence of growth "-" indicates absence of growth

4.2.3. Determination of antibiotic susceptibility patterns of the Isolates

Resistance to antibiotics is a threat phenomenon in medical microbiology. However, in agriculture, it is considered as advantageous for bio inoculants to persist and well established in the soil by resisting agro-chemicals such as pesticides, herbicides and chemical fertilizers. Accordingly, all of the potent isolates showed resistance to antimicrobials tested (Table 9 and

Figure 5). The potent isolates selected for bio-inoculants ($RCHVCB_{1,}$ RScB1.19, and RMaB2.11) were 100% resistant to all the six tested antimicrobials (Table 9).

Table 9: The effect of different antibiotics.

S. No.	**Isolate code**	Vancomycin (Van) 10µg	Ceftazidime (Ce) 10µg	Doxycycline (dxt) 30µg	Erythromycine (E) 15µg	penici lin G (PG) ,10unit	Tetracycline (T) 10µg
1	RgoB3.17	R	R	R	R	I	S
2	RMaB2.39	I	R	S	R	R	R
3	RLVCB 3	S	S	I	R	R	R
4	$RCHVCB_1$	R	R	R	R	R	R
5	RSCB1.50	I	R	R	R	R	S
6	RLVCB2	R	R	R	R	R	R
7	RMaB 2.33	R	R	R	R	R	R
8	RScB1.19	R	R	R	R	R	R
9	RgoB3.5	R	R	R	R	R	S
10	RScB1.7	R	R	R	R	R	R
11	RMaB2.11	R	R	R	R	R	R
12	RMaB1.2	R	R	I	R	R	I
No..of resistant		9(75)	11(91.67)	8(66.67)	12(100)	11(91.67)	8(66.67)
No. of sensitive		3(25)	1(8.33)	4(33.33)	0(0.0)	1(8.33)	4(33.33)

".Values in parenthesis indicated % of tolerant and sensitive isolates"
Legend:I= intermediate; R=resistant; S=sensitive

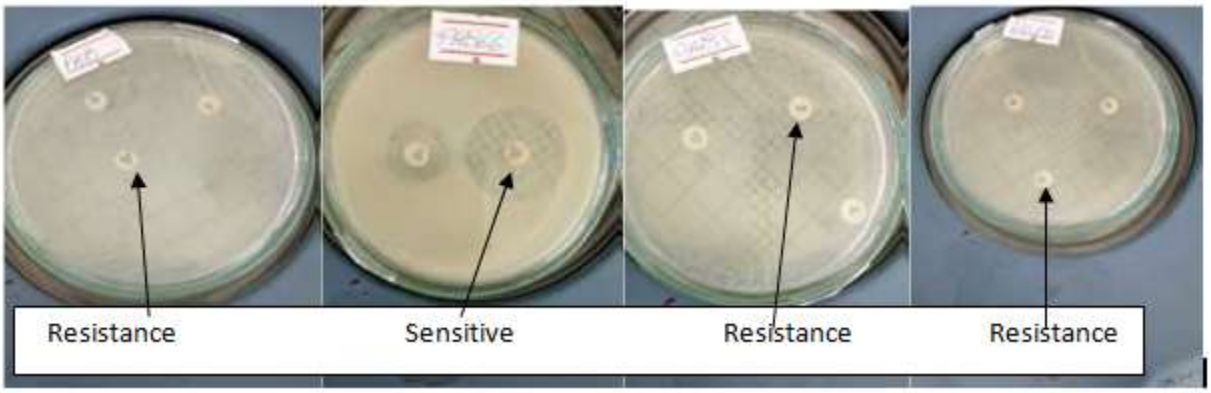

Figure 5. Pictorial presentation of antimicrobial susceptibility test results

4.2.4. Qualitative and quantitative estimations of phosphate Solubilization by Fungi

All the 9 fungi tested were able to solubilize the inorganic TCP at sixth days of incubation, showing strong average mean halo zone formation (mean SI=2.91) with a little variation in average mean colony diameter (Table 10 and Figure 6).

Table 10. Qualitative estimation of phosphate solubilized by fungal isolates

Qualitative analysis			
Isolate code	Colony diameter(mm)	Halo-zone diameter(mm)	Solublizationindex (SI)
RCDVCF4	43.33±1.53abc	90±0.00a	3.08±0.07094599bc
RLVCF2	45.00±0.00ab	90±0.00a	3.03±0.05bc
RCHVCF3	42.33±2.08bc	90±0.00a	3.10±0.13bc
RCHVCF2	43.33±2.89abc	90±0.00a	3.08±0.14bc
RCHVCF1	45.00±0.00ab	90±0.00a	3.00±0.00c
RLVCF1	41.33±3.22c	90±0.00a	3.19±0.16b
R CHVC F4	37.67±2.52d	90±0.00a	3.40±0.16a
RSCF1.19	46.00±1.73a	90±0.00a	2.96±0.07c
RMaF2.35	44.00±1.00abc	90±0.00a	3.05±0.05bc
CV	4.798	0.00	3.45
Mean	43.11	90.00	3.10
T test (0.05) (LSD)	3.58	0.00	0.19

* Means followed by the same letter(s) in each column are not significantly different at $P \leq 0.05$

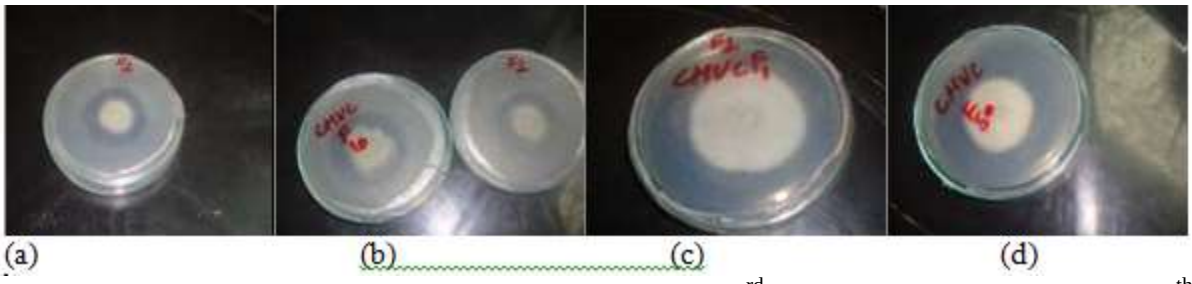

Figure 6. Clear zone formation by fungal strains (a&b) at 3rd day after inoculation (d&c) at 6th day after inoculation

The phosphate solubilization potential of the 9 fungal isolates ranged between180.26±0.01to 360.48±10.05 µg/ml (Table 11). Among all the nine PSF, the fungal isolate RSCF1.19 was found to be the best solubilizer of TCP (360.48±10.05 µg/ml). Overall, RSCF1.19, $RCHVCF_2$ and $RLVCF_2$ were found to be the best fungal solubilizers (Table 11).

Increase in phosphate solubilization coincided (r^2=0.999, p≤0.01) with the decline in pH of the medium in case of all the three fungi (Table 11).

Table 11. Quantitative estimation of phosphate solubilized by fungal isolates

Quantitative and pH estimation			
Isolate code	Incubation period (day)	P-Solublized (µg/ml)	pH after incubation
Control	15	7.02±0.06a	7.07±0.06a
RCDVCF4	15	180.26±0.01i	4.44±0.01b
RLVCF2	15	327.32±0.01c	3.76±0.01g
RCHVCF3	15	219.65±0.01g	4.21±0.01ed
RCHVCF2	15	340.37±0.02b	3.46±0.00h
RCHVCF1	15	260.51±0.01d	3.98±0.02f
RLVCF1	15	228.04±0.00f	4.29±0.12cd
R CHVC F4	15	251.53±0.03e	4.16±0.01e
RSCF1.19	15	360.48±10.05a	3.20±0.10i
RMaF2.35	15	186.16±0.01h	4.36±0.01bc
CV	15	1.35	1.26
Mean		235.43	4.29
T test (0.05) (LSD)		5.44	0.09

* Means followed by the same letter(s) in each column are not significantly different at P ≤ 0.05

4.2.5. Correlation co-efficient among coloney diameter, halo zone diameter, solublization index, amount of solubilized P and pH changes

A positive correlation between coloney diameter and halo zone diameter, as well as negative correlation between solublization index and amount of solubilized P was recorded for P

solublization. However, non-significant correlation relation between amount of P solublized and pH changes was observed (Table 12).

Table 12.The correlation co-efficient among coloney diameter, halo zone diameter, solublization index, amount of solubilized P and pH changes

Variable	cd	hzd	si	µg/ml	pH
cd	1				
hzd	0.98143**	1			
SI	0.13029NS	0.08520 NS	1		
µg/ml	-0.01341 NS	0.01933NS	-0.96953 **	1	
pH	-0.32733 NS	-0.32539 NS	-0.10785NS	-0.03980NS	1

Legend: NS= not significant

4.2.6. Characterization and Identification of isolates

Based on their phytobeneficial and ecophysiological trait profiles, the three superior bacterial isolates; $RCHVCB_1$, RScB1.19 and RMaB2.11 were selected for the seed germination assay and subjected to be characterized and identified biochemically. The results revealed that among the three isolates two of them (RScB1.19 and RMaB2.11) were negative for gelatin liquefaction, and positive for oxidase test, catalase, and starch hydrolysis (Table 13). Similarly, these two isolates were negative for sucrose utilization and positive for utilization of galactose, lactose and glucose (Table 13).

Among the total of 72 isolates of coffee rhizosphere and VC samples, 9 fungal isolates were showed clearly visible large halo zones around their colonies with different diameter on PVK agar medium after 6 days of incubation. The code numbers given to the nine (9) fungal isolates and their respective identification are as follows: RCDVCF4 (*Aspergillus sp*.), RLVCF2 (*Aspergillus sp*.), RCHVCF3 (*Aspergillus sp*.), RCHVCF3 (*Aspergillus sp*.), RCHVCF2(*Aspergillus sp*.),RCHVCF1(*Aspergillus sp*.), RLVCF1(*Aspergillus sp*.), RCHVCF4(*Aspergillus sp*.), (*Aspergillus sp*), RSCF1.19, (*Penicillium sp*.) and RMaF2.35 (*Aspergillus sp*.). The morphological characteristics of the hyphae, spores, and conidiophores of the PSF were examined by optical microscopic RSCF1.19 presented typical penicillate conidiophores with

conidia (Figure.7D), whereas *Aspergillus* group produced typical double spore production cells, which were identified as the black conidia in conidiophores and represented *Aspergillus sp* (Figure 7B).

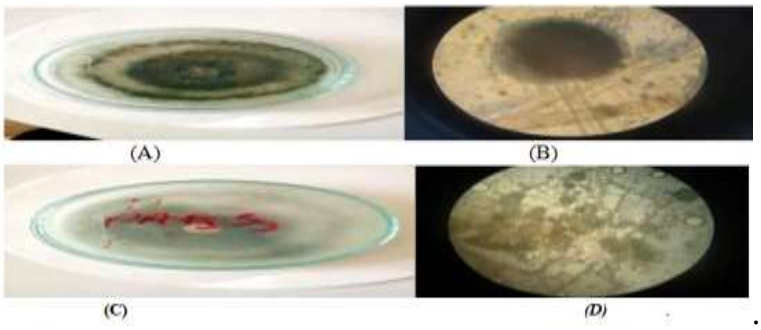

Figure7.Morphologicalcharacters*(A)*Conidiophores,*(B)*Conidia*(Aspergillusspp)(C)*Conidiophores(D)Conidia*(Penicilium sp)*

Table 13.Morphological and biochemical characteristics of phosphate solubilizing bacterial isolates

Characteristics	Bacterial isolates		
Cultural,and Morphological Characteristics	$RCHVCB_1$	RScB1.19	RMaB2.11
	Rod shaped	Rod shaped	Rod shaped
	Gram -ve	Gram +ve,	Gram +ve,
	Yellowish green Colony color	Creamy white Colony color	Creamy white Colony color
	Oval colony shape	Circular colony shape	Circular colony shape
Motility test	+	+	+
Bacterial growth at 5% NaCl	+	+	+
Catalase test	+	+	+
Oxidase test	+	+	+
Gelatin hydrolysis	+	-	-
Starch hydrolysis	-	+	+
Nitrate reduction	+	+	+
Galactose	-	+	+
Glucose	+	+	+
Lactose	-	+	+
sucrose	+	-	-

"+" indicates positive result "-" indicates negative result.

4.2.7. *In-vitro* co-culture test between bacteria, fungi and pathogenic *Fusarium xyloriodes (F. xyloriodes)*

In addition to phosphate solubilization, biocontrol is one of the most desirable traits for inoculants. To assess this, we tested co-cultures of both fungi and bacteria against pathogenic *F. xyloriodes* to see whether they could co-exist or antagonistic to one another on agar plates (Figure 8). The growth of *F.xyloriodes* was slightly inhibited by RSCF1.19 (Figure 8A). However, a clear evidence of growth inhibition was obtained when RSCF1.19 was streaked perpendicular to RLVCF2. A sufficient inhibition zone was created by RSCF1.19, which suppressed the growth of RLVCF2 (Figure 8C), whereas the growth of RSCF1.19 was inhibited by RCHVCF2 (Figure.8B). The growth of both RLVCF2 and RCHVCF2 was not suppressed when both co-cultured on the same plates (Figure 8D), which showed the co-existence of both isolates on the same plate with no trace of growth inhibition at the center where the two isolates crossed each other (Figure 8D). On the other hand, all the bacterial and fungal isolates showed no growth inhibition between each other (Figure 8E,F),

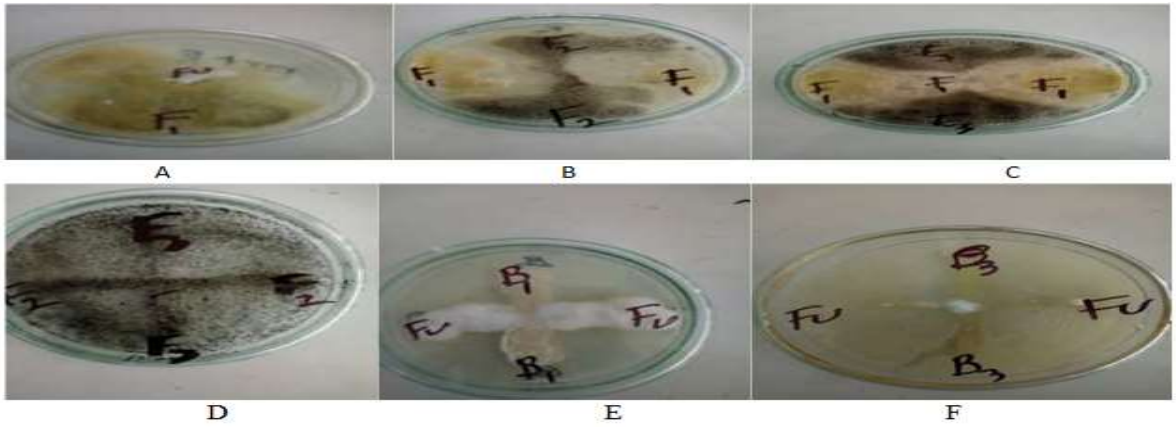

Figure 8. Co-culture test between pathogenic *Fusarium xyloriodes*, fungal and bacterial isolates
A. co-inoculation of RSCF1.19(F1) and *Fusarium xyloriodes* ,B. co-inoculation of RSCF1.19(F1) and RCHVCF2 (F2),C. co-inoculation of RSCF1.19(F1)and RLVCF2(F3),D. co-inoculation of RCHVCF2 (F2) and RLVCF2(F3), E. co-inoculation of *Fusarium xyloriodes* and $RCHVCB_1$(B1),F. co-inoculation of *Fusarium xyloriodes* and RMaB2.11(B3)

4.2.8. Effect of isolates on coffee seed germination

The Highly effective PSB (n=3) and fungal isolates (n=3) affected the germination of coffee seeds remarkably (Table 14). Germination rates and vigor index was calculated for all inoculation treatments (Table 14). In almost all the treatments RScB1.19 + RSCF1.19, RMaB2.11 + RCHVCF2, RMaB2.11 + RLVCF2, RCHVCB$_1$+RSCF1.19, RCHVCB$_1$, RScB1.19 and RSCF1.19 isolates increased seed germination index over untreated seeds (Table 14).

Table 14. Results of germination rate and Vigour Index (VI)

Treatments		Germination rate (%)	Mean Root Length(cm)	Mean Shoot Length(cm)	Vigor index
T1	RCHVCB1	23.33ab	1.50ab	1.83ab	77.69
T2	RScB1.19	30.00a	1.17ab	1.33bcd	75.00
T3	RMaB2.11	23.33ab	1.00b	1.23cd	52.03
T4	RSCF1.19	23.33ab	1.47ab	2.00a	80.96
T5	RCHVCF2	16.67ab	1.30ab	1.67abcd	49.45
T6	RLVCF2	23.33ab	1.83a	1.33bcd	42.17
T7	RCHVCB1+ RSCF1.19	26.67ab	1.50ab	1.23cd	72.81
T8	RCHVCB1+ RCHVCF2	16.67ab	0.93b	1.23cd	36.01
T9	RCHVCB1+ RCHVCF2	16.67ab	1.33ab	1.53abcd	47.73
T10	RScB1.19 + RSCF1.19	26.67ab	1.33ab	1.67abcd	80.01
T11	RScB1.19 + RCHVCF2	13.33ab	1.17ab	1.17d	31.10
T12	RScB1.19 + RLVCF2	10.00b	1.17ab	1.67abcd	28.37
T13	RMaB2.11 + RSCF1.19	16.67ab	1.17ab	1.33bcd	41.73
T14	RMaB2.11 + RCHVCF2	30.00a	1.50ab	1.50abcd	90.00
T15	RMaB2.11 + RLVCF2	30.00a	1.83a	1.50abcd	99.90
T16	-Ve control	13.33ab	1.00b	1.17d	28.88
CV		51.27	33.46	21.41	
Mean		20.59	1.31	1.48	
T test (0.05) (LSD)		17.55	0.73	0.53	

* Means followed by the same letter(s) in each column are not significantly different at $P \leq 0.05$.

4.3. Effect of bacterial and fungal inoculation on coffee seedlings under glasshouse condition

4.3.1. Effects of single inoculation on the growth of coffee seedlings

RSCF1.19 obtained from coffee rhizosphere was identified as having the highest potential as bio-inoculant phosphate solublizer. The dual inoculation of RSCF1.19 and $RCHVCB_1$ in the presence of inorganic P source significantly improved the growth of coffee seedlings over that of the un-inoculated control and other tested isolates in terms of plant shoot height, root height, stem diameter, leaf number, leaf area, fresh ($p \leq 0.05$) and dry weights ($p \leq 0.05$) (Table 15).

Accordingly, the increased levels of growth parameters over the other treatments indicated that the combined inoculation of fungi and bacteria isolates (RSCF1.19 and $RCHVCB_1$) enhanced the growth of the plant by solublizing the applied inorganic phosphate and posed better up-take by plant roots. Moreover, combined inoculation of RSCF1.19 and RMaB2.11 in the presence of inorganic P significantly caused enhanced plant growth in terms of root height, stem diameter, leaf number, leaf area, fresh weight ($p \leq 0.05$) and dry weights ($p \leq 0.05$) over treatments without inorganic phosphate and both negative and positive controls (Table 15).

Table 15. Growth response of coffee seedlings to sole PSF and PSB inoculants under glasshouse condition

Treatments		Shoot length/plant(cm)	Root length/plant(cm)	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)
T_1	$RCHVCB_1$	10.90cde	12.50cd	0.97c	0.14bc	0.48bc	0.08cd	8.00b	2.33bc	0.36i
T_2	RScB1.19	16.00abc	12.00e	2.70bc	0.60ab	0.89bc	0.25ab	10.67ab	3.33ab	1.20d
T_3	RMaB2.11	10.83cde	13.67ab	0.97c	0.14bc	0.36c	0.08d	8.00b	2.33bc	0.55gh
T_4	RSCF1.19	13.83bcde	13.00bc	2.33bc	0.37abc	0.89bc	0.18abcd	9.00b	3.33ab	0.90e
T_5	RCHVCF2	9.17de	12.17dde	1.03c	0.17bc	0.27c	0.09cd	8.00b	2.33bc	0.59gh
T_6	RLVCF2	11.83bcde	12.67c	1.73bc	0.33abc	0.57bc	0.15bcd	9.33b	2.67abc	0.86f
T_7	$RCHVCB_1$+P	13.67bcde	12.33d	1.70bc	0.20bc	0.53bc	0.16bcd	10.00b	2.67abc	0.96de
T_8	RScB1.19+P	10.67cde	13.50ab	1.23c	0.20bc	0.39c	0.09bcd	8.67b	2.00c	0.75fg
T_9	RMaB2.11+P	12.67bcde	13.83a	1.53bc	0.20bc	0.39c	0.11bcd	10.00b	2.33bc	0.94de
T_{10}	RSCF1.19 +P	21.17a	10.83def	5.13a	0.77a	1.71a	0.32a	13.00a	3.67a	2.78a
T_{11}	RCHVCF2+P	16.53ab	11.83de	3.73ab	0.57abc	1.22ab	0.24abc	10.67ab	2.67abc	2.55b
T_{12}	RLVCF2+P	14.50bcd	13.33abc	3.13abc	0.50abc	0.77bc	0.19abcd	10.00b	3.33ab	2.32c
T_{13}	-ve control	8.67e	8.00f	0.97c	0.10c	0.39c	0.07d	8.00b	2.00c	0.52gh
T_{14}	+ve control	9.00de	9.00ef	1.17c	0.30abc	0.41c	0.08cd	9.33b	3.00abc	0.86f
CV		26.05	11.10	65.13	85.68	64.76	63.54	17.10	23.55	79.55
Mean		12.82	12.02	2.02	0.33	0.66	0.15	9.48	2.71	1.19
Ttest(LSD) (0.05)		5.60	2.06	2.21	0.47	0.72	0.16	2.72	1.07	1.07

*Means followed by the same letter(s) in each column are not significantly different at α=5%, PSF=phosphate solubilizing fungi, PSB=phosphate solubilizing bacteria, *P=Chemical phosphate, -Ve=negative control, +Ve=positive control

The morphological growth performance of experimental plants was demonstrated in (Figure 9). However, all the treatments combined with vermicompost showed suppressive characteristics with no any seedlings emergence (Figure 9).

Figure 9. Morphological growth performance of experimental plants

4.3.2. Effects of co-inoculation on the growth of coffee seedlings

RSCF1.19 obtained from coffee rhizosphere was identified as having the highest potential as bio-inoculant phosphate solublizer. The dual inoculation of RSCF1.19 and $RCHVCB_1$ in the presence of inorganic P source significantly improved the growth of coffee seedlings over that of the un-inoculated control and other tested isolates in terms of plant shoot height, root height, stem diameter, leaf number, leaf area, fresh ($p \leq 0.05$) and dry weights ($p \leq 0.05$) (Table 16). Accordingly, the increased levels of growth parameters over the other treatments indicated that the combined inoculation of fungi and bacteria isolates (RSCF1.19 and $RCHVCB_1$) enhanced the growth of the plant by solublizing the applied inorganic phosphate and posed better up-take by plant roots. Moreover, combined inoculation of RSCF1.19 and RMaB2.11 in the presence of inorganic P significantly caused enhanced plant growth in terms of root height, stem diameter, leaf number, leaf area, fresh weight ($p \leq 0.05$) and dry weights ($p \leq 0.05$) over treatments without inorganic phosphate and both negative and positive controls (Table 16).

Table 16. Growth response of coffee seedlings to dual inoculation of PSF and PSB isolates under glasshouse condition

Treatments		Shoot length/plant(cm)	Root length/plant(cm)	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)
T1	$RCHVCB_1$ + RSCF1.19	11.90cde	13.50ab	1.53cdef	0.21abcd	0.52bcde	0.13b	8.67cbd	3.00a	1.03abcd
T2	$RCHVCB_1$ + RCHVCF2	12.70cd	12.50abc	1.73bcdef	0.40ab	0.58bcde	0.16b	9.33abcd	3.00a	0.40d
T3	$RCHVCB_1$+ RLVCF2	11.33cde	9.67bcd	1.73bcdef	0.33abcd	0.60bcde	0.15b	8.00cde	2.67ab	1.04abcd
T4	RScB1.19+ RSCF1.19	7.67f	13.33ab	0.67f	0.07d	0.25e	0.06b	7.33de	2.33ab	0.40d
T5	RScB1.19+ RCHVCF2	14.00b	10.17abcd	2.13bcdef	0.23abcd	0.63bcde	0.14b	10.67ab	2.33ab	1.21abcd
T6	RScB1.19+ RLVCF2	10.80cde	13.50ab	1.23cdef	0.17bcd	0.42cde	0.12b	8.67cbd	2.33ab	0.61cd
T7	RMaB2.11+ RSCF1.19	14.40bc	13.00abc	1.70bcdef	0.27abcd	0.58bcde	0.14b	10.33abc	2.67ab	0.86bcd
T8	RMaB2.11+ RCHVCF2	13.27bcd	12.17abc	2.40abcde	0.33abcd	0.74abcde	0.15b	10.67ab	3.00a	1.99ab
T9	RMaB2.11+ RLVCF2	12.67cd	13.17ab	1.87bcdef	0.30abcd	0.51bcde	0.13b	8.67bcd	2.67ab	1.04abcd
T10	$RCHVCB_1$ + RSCF1.19 +P	16.50a	12.50abc	3.80a	0.47a	1.21a	0.19ab	11.33a	3.00a	2.13a
T11	$RCHVCB_1$+ RCHVCF2+P	11.67cde	13.33ab	1.23cdef	0.14bcd	0.44cde	0.10b	8.67bcd	2.33ab	0.47d
T12	$RCHVCB_1$+ RLVCF2+P	12.17cd	14.17a	1.47cdef	0.17bcd	0.48bcde	0.36a	10.00abc	2.33ab	0.94bcd
T13	RScB1.19+ RSCF1.19 +P	11.73cde	13.83a	1.13cdef	0.17bcd	0.37de	0.09b	9.33abcd	2.00b	0.46d
T14	RScB1.19+ RCHVCF2 +P	9.50de	10.17abcd	1.37cdef	0.17bcd	0.47cde	0.09b	10.00abc	2.00b	0.47d
T15	RScB1.19+ RLVCF2+P	13.83bcd	13.33ab	2.47abcd	0.33abcd	0.79abcd	0.15b	10.67ab	2.00b	1.41abcd
T16	RMaB2.11+ RSCF1.19+P	14.83bc	13.17ab	3.23ab	0.47a	0.97ab	0.19ab	11.33a	3.00a	1.73abc
T17	RMaB2.11+ RCHVCF2+P	14.00b	12.83abc	2.67abc	0.37abc	0.87abc	0.15b	10.00abc	2.67ab	1.80ab
T18	RMaB2.11+ RLVCF2+P	11.17cde	11.67abcd	0.90ef	0.13bcd	0.30de	0.09b	6.00e	2.00b	1.24abcd
T19	**-ve control**	8.67de	8.00d	0.97def	0.10cd	0.39cde	0.07b	8.00cde	2.00b	0.52d
T20	**+ve control**	9.00de	9.00cd	1.17cdef	0.30abcd	0.41cde	0.08b	9.33abcd	3.00a	0.86bcd
CV		20.27	20.04	52.63	68.79	51.50	82.58	15.81	20.75	66.64
Mean		12.13	12.15	1.77	0.26	0.58	0.14	9.35	2.52	1.03
Ttest(LSD) (0.05)		2.03	4.02	1.54	0.29	0.49	0.19	2.44	0.86	1.14

*Means followed by the same letter(s) in each column are not significantly different at α=5%, *P=Chemical phosphate-Ve=negative control, +Ve=positive control

Similarly, the co-inoculation of strain RMaB2.11 with $RCHVCF_2$, as well as RMaB2.11with $RLVCF_2$ when combined with inorganic phosphate showed a better increase in plant growth parameters such as plant shoot height, root height, stem diameter, leaf number, leaf area, fresh ($p \leq 0.05$) and dry weights ($p \leq 0.05$) (Table 16) over the controls, and lower plant growth parameters was obtained from both fungal and bacterial inoculated but without inorganic phosphate applied, indicating the lack of sufficient inorganic phosphate to be solubilized in potted medium.

4.3.2. 1. Nutrient status of potting medium

After collecting the necessary data, the potting medium was analyzed for the physicochemical properties (pH, OC, Ex. Acidity, and CEC) and nutrient status (Table 17 and 18). The potting medium reaction was near neutral in the treatments containing bio-inoculants only and in the negative control. But, there was a slightly decreased pH value in the treatments containing bio-inoculants and P fertilizer in both singly and co-inoculated potting media. There was no emergence of seedlings in the treatments amended with vermicompost (T_1-T_9) and (T_1-T_{12}) since the potting medium reaction was near alkaline (Table 17 and 18; Figure 9).

Table 17: Chemical properties of potting medium after taking data destructively at single inoculation treatments and nutrient uptake

Treatments		pH(1:2.5)	OC(%)	Ex.Acidity(meq/100g	CEC(meq/ 100g)	Available nutrients			Nutrient uptake by plants		
						AvailableP(ppm)	TN (%)	K (ppm)	AvailableP(ppm)	TN (%)	K (ppm)
T1	$RCHVCB_1$	6.50	1.65	0.30	20.70	10.37	0.02	50.00	78.90	0.30	1680.20
T2	RScB1.19	6.51	1.60	0.31	19.78	11.33	0.03	50.01	76.91	0.30	1580.10
T3	RMaB2.11	6.41	1.61	0.29	20.68	10.31	0.02	49.02	77.89	0.29	1650.30
T4	RSCF1.19	6.40	1.65	0.30	18.78	10.36	0.01	50.03	78.80	0.31	1681.29
T5	RCHVCF2	6.52	1.67	0.33	21.00	10.35	0.02	48.00	76.90	0.30	1580.20
T6	RLVCF2	6.50	1.64	0.30	19.70	11.30	0.02	50.01	75.70	0.31	1670.30
T7	$RCHVCB_1$+P	5.30	1.93	0.38	18.14	53.50	0.04	75.05	124.04	0.32	3282.84
T8	RScB1.19+P	5.46	1.96	0.43	19.36	41.56	0.03	75.97	120.00	0.34	3182.85
T9	RMaB2.11+P	5.19	1.66	0.57	17.6	47.20	0.03	76.35	121.02	0.33	3282.80
T10	RSCF1.19 +P	5.10	1.86	0.38	17.58	88.64	0.04	75.78	214.20	0.33	3312.00
T11	RCHVCF2 +P	5.30	1.87	0.51	18.12	71.71	0.03	76.30	210.20	0.32	3212.01
T12	RLVCF2+P	5.28	1.53	0.41	18.54	56.01	0.05	73.42	212.20	0.31	3210.20
T13	-ve control	6.18	1.34	0.35	17.9	10.54	0.05	50.33	66.91	0.20	1270.10
T14	+ve control	5.18	1.64	0.46	17.48	34.82	0.04	53.76	121.02	0.25	3082.80
T15	VC	8.17	2.03	0.41	20.86	88.06	0.06	107.59			

*p=Chemical phosphate, -Ve=negative control, +Ve=positive control, VC=Vermicompost, OC=organic carbon, EX=exchangeable,

Percent of organic carbon and the Cation Exchange Capacity (CEC) of the potting soil was the same in all the treatments by receiving bio-inoculants amended with inorganic P fertilizer as well as treatments without inorganic P fertilizer (Table 17 & 18). However, available P was to some extent increased in the treatments containing bio-inoculants amended with inorganic P fertilizer compared to treatments with only bio-inoculants. But, percent organic carbon and available P were high in treatments inoculated with fungal bio-inoculants compared to bacterial bio-inoculants in the presence of P fertilizer. Similarly, available K was high in the treatments containing bio-inoculants amended with inorganic P fertilizer compared to treatments with only bio-inoculants but available K was higher in the treatments containing co-inoculated bio-inoculants both with and without P fertilizer amendments than singly inoculated treatments (Table 17 and 18).

4.3.2. 2. Nutrient uptake by coffee seedlings

The nutrient uptake of the coffee seedlings grown in potting medium is presented in Tables 17 and 18. Although not significant, the highest P and K-uptake by shoot of coffee seedlings grown in potting medium was generally observed with treatments that received bio-inoculants and inorganic chemical phosphate fertilizer compared to un-inoculated treatments and negative control. Moreover, treatments that received fungal bio-inoculants in the presence of inorganic chemical fertilizer showed increased P and K-uptake by coffee seedling compared to bacterial bio-inoculants amended with inorganic chemical fertilizer treatments (Table 17). Inoculations of bacteria and fungus alone or in combination produced higher uptake of available N, P and K compared to the control (Table 18). Thus, the higher growth parameters observed under fungal treatments can be attributed to availability and uptake of balanced and higher quantities of nutrients to coffee seedlings through inorganic phosphate fertilizer as well as bio-inoculants consortia compared to treatments that received bio-inoculant without inorganic phosphate fertilizer and negative control.

Table 18: Chemical properties of potting medium after taking data destructively at co-inoculation treatments and Nutrient uptake.

Treatments		pH(1:2.5)	OC(%)	Ex.Acidity(meq/100g	CEC(meq /100g)	Available nutrients AvailableP(ppm)	TN(%)	K (ppm)	Nutrient uptake by plants AvailableP(ppm)	TN (%)	K (ppm)
T1	$RCHVCB_1$ + RSCF1.19	6.50	1.67	0.24	18.10	12.33	0.03	70.94	78.80	0.30	1680.20
T2	$RCHVCB_1$ + RCHVCF2	6.49	1.60	0.23	18.11	13.33	0.02	63.94	76.91	0.30	1580.10
T3	$RCHVCB_1$ + RLVCF2	6.49	1.61	0.20	17.18	11.33	0.04	53.94	77.89	0.29	1650.30
T4	RScB1.19+ RSCF1.19	6.48	1.67	0.25	19.18	13.33	0.03	73.94	78.80	0.31	1681.29
T5	RScB1.19+ RCHVCF2	6.49	1.54	0.21	18.18	13.30	0.02	50.94	76.90	0.30	1580.20
T6	RScB1.19+ RLVCF2	6.49	1.67	0.20	17.18	13.13	0.02	71.94	75.70	0.31	1670.30
T7	RMaB2.11+ RSCF1.19	6.44	1.68	0.20	17.10	13.33	0.02	73.94	78.80	0.31	1680.30
T8	RMaB2.11+ RCHVCF2	6.51	1.60	0.22	18.12	11.33	0.03	70.94	77.90	0.26	1485.20
T9	RMaB2.11+ RLVCF2	6.49	1.62	0.23	17.18	13.33	0.02	71.94	76.90	0.30	1670.31
T10	$RCHVCB_1$ + RSCF1.19 +P	5.40	1.73	0.51	19.38	73.53	0.03	100.67	120.81	0.39	3274.22
T11	$RCHVCB_1$ + RCHVCF2+P	5.47	1.58	0.43	19.08	38.96	0.05	80.51	110.80	0.29	3174.22
T12	$RCHVCB_1$ + RLVCF2+P	5.45	1.60	0.38	18.24	39.37	0.03	99.27	111.71	0.29	3274.22
T13	RScB1.19+ RSCF1.19 +P	5.36	1.69	0.46	18.08	66.96	0.05	101.85	122.53	0.47	3284.00
T14	RScB1.19+ RCHVCF2+P	5.41	1.59	0.38	19.20	59.76	0.02	95.12	123.50	0.47	3184.04
T15	RScB1.19+ RLVCF2+P	5.48	1.52	0.41	19.28	50.37	0.03	102.48	121.51	0.47	3244.01
T16	RMaB2.11+ RSCF1.19+P	5.21	1.71	0.40	18.56	59.77	0.04	94.22	112.20	0.31	3157.30
T17	RMaB2.11+ RCHVCF2+P	5.14	1.45	0.39	18.32	62.99	0.04	80.44	109.22	0.41	3057.31
T18	RMaB2.11+ RLVCF2+P	4.86	1.83	0.59	18.12	50.96	0.04	81.95	110.22	0.31	3237.30
T19	-ve control	6.18	1.34	0.35	17.9	10.54	0.05	50.33	69.91	0.20	1270.10
T20	+ve control	5.18	1.64	0.46	17.48	34.82	0.04	52.76	123.02	0.23	3082.80

*p=chemical phosphate, -Ve=negative, +Ve=positive

Based on the correlation (r) analysis during single inoculation of isolates, P-uptake was greatly correlated with plant height (r=0.457*), root length (r=0.529*) , shoot fresh weight (0.550*) and shoot dry weight (0.478*; Table 19). Similarly, K uptake was correlated with root length, shoot dry weight, number of leaves per plant and stem girth (r=0.494*, 0.570**, 0.488* and 0.502*, respectively) and was not significantly ($p \geq 0.05$) related with other growth parameters (Table 19). Non-significant relationships were observed with total N uptake and all the growth parameters except in root fresh weight and leaf area (r= 0.463* and 0.794**; respectively). However, during dual inoculation, only plant height and leaf area was greatly correlated with P N uptake (r=0.448**, 0.682** and 0.457**, 0.983**, respectively; Table 20) and non-significant relationships were observed with all other growth parameters.

Table 19.Correlation coefficient for plant growth parameters and nutrient up take characters of coffee seedlings during single inoculation

characters	Shoot length/plant(cm)	Root length/plant(cm)	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)	AvailableP(ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	**1**											
Root length/plant(cm)	-0.149ns	**1**										
Shoot fresh weight(g)	-0.268ns	0.920**	**1**									
Root fresh weight(g)	-0.196ns	0.967**	0.913**	**1**								
Shoot dry weight(g)	-0.226ns	0.117ns	0.176ns	0.109ns	**1**							
Root dry weight(g)	-0.011ns	0.881**	0.785**	0.810**	0.113ns	**1**						
No of leaves	-0.095ns	0.811**	0.701**	0.838**	0.057ns	0.745**	**1**					
Stem Girth (mm)	-0.142ns	0.862**	0.886**	0.863**	0.212ns	0.888**	0.721**	**1**				
Leaf area(sq.cm)	-0.040ns	0.337ns	0.430ns	0.281ns	0.638**	0.538*	0.187ns	0.281ns	**1**			
AvailableP(ppm)	0.457*	0.529*	0.391ns	0.478*	0.215ns	0.550*	0.395ns	0.410ns	0.379ns	**1**		
TN (%)	0.183ns	0.127ns	0.163ns	0.021ns	0.463*	0.367ns	0.061ns	0.319ns	0.794**	0.400ns	**1**	
K (ppm)	-0.067ns	0.494*	0.441ns	0.570**	-0.234ns	0.443ns	0.488*	0.502*	0.182ns	0.274ns	0.137ns	**1**

Legend: ns= not significant, ppm=parts per million, wt= weight

Table 20. Correlation coefficient for plant growth parameters and nutrient up take characters of coffee seedlings during dual inoculation

characters	Shoot length/plant(cm)	Root length/plant(cm)	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)	AvailableP (ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	**1**											
Root length/plant(cm)	-0.281ns	**1**										
Shoot fresh weight(g)	-0.213ns	0.891**	**1**									
Root fresh weight(g)	-0.233ns	0.966**	0.917**	**1**								
Shoot dry weight(g)	-0.159ns	0.524**	0.410*	0.499**	**1**							
Root dry weight(g)	-0.089ns	0.507**	0.574**	0.492**	0.318ns	**1**						
No of leaves	-0.340ns	0.812**	0.683**	0.792**	0.413*	0.229ns	**1**					
Stem Girth (mm)	-0.029ns	0.731**	0.723**	0.720**	0.447**	0.372*	0.565**	**1**				
Leaf area(sq.cm)	0.406*	-0.069ns	0.208ns	0.012ns	-0.018ns	0.225ns	-0.170ns	0.162ns	**1**			
AvailableP(ppm)	0.448**	0.057ns	0.195ns	0.113ns	0.154ns	0.264ns	-0.180ns	0.097ns	0.682**	**1**		
TN (%)	0.457**	-0.074ns	0.191ns	-0.010ns	-0.012ns	0.184ns	-0.191ns	0.196ns	0.983**	0.645**	**1**	
AvailableK (ppm)	0.015ns	0.249ns	0.279ns	0.286ns	-0.242ns	0.160ns	0.245ns	0.321ns	0.183ns	-0.047ns	0.169ns	**1**

Legend: ns= not significant, ppm=parts per million, wt= weight

4.4. Effect of bacterial and fungal inoculation on coffee seedlings under nursery condition

4.4.1. Soil Physical and Chemical Properties

The soil texture of the study site was Sandy Clay. The soil pH of study soil sample was 5.62 (pH in water at soil: liquid ratio of 1:2.5). The organic carbon, total Nitrogen concentration, Ex. Acidity, the extractable phosphorus concentration and potassium recorded from study soil sample were 0.86%, 0.08%, 0.16,0.28 (ppm) and 102.86% respectively, which are in the very low range (London, 1991) (Table 21A).

Table 21A . Characteristics of the applied soil used for potting medium

No	Measured indexes	
1	%sand	59
2	%clay	40
3	%silt	1
4	Textural class	Sandy Clay
5	pH	5.81
6	Available P (ppm)	0.28
7	Total Nitrogen (%)	0.08
8	Organic carbon (%)	0.86
9	Ex. Acidity (meq/100g	0.38
10	K(%)	102.86
11	CEC (meq/100g	13.78

*Ex=exchangeable, K=potsium, CEC= Cation Exchange Capacity

4.4.1. 1. Performance of sole inoculations

Plants received recommended NP fertilizers (+Ve control) and fungal inoculums combined with P sources(RSCF1.19+P and RLVCF2+P) showed increased plant height, leaf number, root fresh weight, shoot dry weight, stem diameter and leaf number as compared to negative control (without bacterial, fungal inoculums and inorganic phosphate fertilizer),and all three bacterial inoculums + phosphate fertilizer (P) (Table 22A). There was no significant change in growth characteristics for seedlings treated with the fungal or bacterial isolates without phosphorus fertilizer applied when compared to negative control. Therefore, PSF isolates of RSCF1.19 or

RLVCF2 and P exerted more significant influence on growth characteristics of coffee seedlings than fungal isolates (RCHVCF2+P) and sole of all the three bacterial (RCHVCB$_1$, RScB1.19, RMaB2.11+P) isolates. P application to neither the two fungal isolates (RLVCF2 and (RCHVCF2) nor the bacterial sole inoculants failed to display significant root length and root dry weight difference. The results of the present investigation confirmed that the two PSF strains namely RSCF1.19 and RLVCF2 when combined with inorganic phosphorus had the ability to solubilize the inorganic phosphate to make it available to the plant nutrition. Moreover, sole inoculation of both bacterial and fungal inoculants increased growth parameters over the phosphate supplied seedlings (+Ve control).

The morphological growth performance of experimental plants was demonstrated in figure 10. There was no significant differences in both fungal and bacterial inoculants in each inorganic phosphate applied and unapplied plants interms of root length and root dry weight.

Figure 10. .Pictorial presentation of morphological growth affected by treatments.

Generally in the single inoculation experiment, the growth parameters in both bacterial and fungal inoculants increased in all the treatments over the chemical phosphate combined seedlings. Besides, treatments in both bacterial and fungal treated without P combined did not show a significant increase in growth parameter (Table 22A)

Table 22A. Growth responses of coffee seedlings to sole PSF and PSB inoculants under nursery condition

Treatments		Shoot length/plant(cm)	Root length/plant(cm)	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)
T1	$RCHVCB_1$	10.50h	18.33ab	5.61e	0.97d	1.65e	0.49b	8.00de	2.07f	6.38e
T2	RScB1.19	12.10fgh	19.33ab	5.81e	1.07cd	1.87de	0.53ab	7.333e	2.28ef	8.16cde
T3	RMaB2.11	15.17cdef	19.67ab	8.35cde	1.50abcd	2.84bcde	0.68ab	8.67cde	2.61bcde	8.55cde
T4	RSCF1.19	12.50fgh	19.67ab	6.60de	1.23bcd	2.18cde	0.58ab	8.00de	2.30def	9.31cde
T5	RCHVCF2	13.33efgh	19.33ab	7.32de	1.30bcd	2.58bcde	0.61ab	10.00abc	2.49cdef	8.25cde
T6	RLVCF2	11.83gh	19.00ab	5.70e	1.37bcd	1.82de	0.58ab	9.33bcd	2.39def	7.68de
T7	$RCHVCB_1$+P	14.67defg	18.33ab	11.17bc	1.87abc	3.27bc	0.63ab	10.00abc	2.57cde	13.94bc
T8	RScB1.19+P	14.33defg	20.67a	9.02bcd	1.50abcd	2.75bcde	0.60ab	8.00de	2.39def	11.48bcde
T9	RMaB2.11+P	17.50bcd	20.00ab	11.57b	1.97ab	3.70ab	0.74ab	11.33a	2.79bcd	12.50bcd
T10	RSCF1.19 +P	19.33ab	17.33b	11.33b	1.93ab	4.74a	0.81ab	10.00abc	2.92abc	11.81bcde
T11	RCHVCF2+P	14.00efg	18.80ab	10.42bc	1.63abcd	2.92bcde	0.68ab	10.67ab	2.55cdef	9.31cde
T12	RLVCF2+P	20.83a	19.00ab	15.09a	2.23a	4.70a	0.87a	10.67ab	3.32a	20.20a
T13	-ve control	16.00cde	18.67ab	8.88bcd	1.73abcd	2.95bcd	0.74ab	10.00abc	2.57cde	12.69bcd
T14	+ve control	18.33abc	19.00ab	10.59bc	1.93ab	3.45abc	0.75ab	11.33a	3.08ab	17.19ab
CV		12.97	9.99	19.14	30.06	25.99	31.32	12.29	11.46	33.14
Mean		15.03	19.08	9.10	1.59	2.96	0.66	9.52	2.60	11.25
Ttest(LSD) (0.05)		3.27	3.20	2.93	0.80	1.29	0.35	1.96	0.50	6.25

*Means followed by the same letter(s) in each column are not significantly different at $P \leq 0.05$, P=phosphorus, cm=centimeter, mm=millimeter,g=gram, -Ve=Negative, +Ve=Positive, CV= Coefficient of variance, LSD=Least significance difference

4.4.1. 2. Dual inoculation effects on coffee seedling

Co-inoculation of bacteria with fungal isolates under inorganic phosphorus fertilizer (P) increased all the growth parameters except plant height, shoot fresh weight, root fresh and dry weight as compared to controls. Accordingly, co-inoculation of RSCF1.19 with $RCHVCB_1$ or RScB1.19 under phosphorus fertilizer led to significantly higher shoot fresh and dry weight, root dry weight, stem girth, root length, shoot height, number leaves and leaf area than RCHVCF2 and $RLVCF_2$ (Table 22B).However, the co-inoculation of bacteria isolate$RCHVCB_1$ with fungi isolates of RSCF1.19, $RCHVCB_1$ or RCHVCF2 under phosphorus fertilizer (P) did not show a significantly different root fresh and dry weight when compared to the controls and P-lacking treatments (Table22B). Similarly co-inoculation of bacteria RScB1.19 with fungi isolate of RCHVCF2 under phosphorus fertilizer did not show significantly different plant height when compared to the negative control. Moreover, all fungal isolates positively affected the plant growth as compared to bacterial isolates and the bacterial isolates also positively affected the plant growth as compared to both non-inoculated control and plants without inorganic chemical phosphorus. The result also displayed that dual inoculation without phosphate fertilizer was unable to show significantly increased growth parameters as compared to negative control except in case of number of leaves and root length (Table 22B). This shows that the positive impact of the availability of nutrients towards coffee seedling establishment and vigor development.

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Table 22B. Growth response of coffee seedlings to dual inoculation of PSF and PSB isolates under nursery condition

Treatments		Shoot length(cm)	Rootlength(cm)	Shoot fresh wt(g)	Root fresh wt(g)	Shoot dry wt(g)	Root dry wt(g)	No of leaves	Girth (mm)	Leaf area(sq.cm)
T1	$RCHVCB_1$ + RSCF1.19	13.50ef	19.67abc	6.24g	1.53bcdefg	2.15bcde	0.66bcdef	10.00ab	2.67abc	9.87cde
T2	$RCHVCB_1$ + RCHVCF2	14.33def	19.00abc	7.37efg	1.43cdefg	2.70abcde	0.65cdef	8.67b	2.43bc	6.64e
T3	$RCHVCB_1$ + RLVCF2	12.83f	18.33abc	6.57fg	0.80h	2.26bcde	0.39f	9.33ab	2.38c	8.63de
T4	RScB1.19+ RSCF1.19	14.33def	18.33abc	6.63fg	1.30efgh	2.54abcde	0.65bcdef	9.33ab	2.46bc	11.10bcde
T5	RScB1.19+ RCHVCF2	14.33def	19.67abc	8.22defg	1.47cdefgh	2.5abcde	0.64cdef	10.00ab	2.47abc	10.64bcde
T6	RScB1.19+ RLVCF2	14.33def	19.00abc	10.01cdef	1.67bcdefg	2.99abc	0.83abcde	10.00ab	2.72abc	10.23cde
T7	RMaB2.11+ RSCF1.19	14.67cdef	17.67bc	5.45g	1.03gh	1.92bcde	0.50ef	8.67b	2.58abc	10.31cde
T8	RMaB2.11+ RCHVCF2	13.67ef	18.67abc	6.96fg	1.17gh	2.30bcde	0.57def	10.00ab	2.46abc	11.09bcde
T9	RMaB2.11+ RLVCF2	15.17bcdef	18.00abc	5.49g	1.40defgh	2.08bcde	0.58def	9.33ab	2.43bc	13.41abcd
T10	$RCHVCB_1$ + RSCF1.19 +P	20.17a	19.67abc	11.91abc	2.03bcd	2.88abcd	0.97abc	10.67ab	3.04ab	15.90abc
T11	$RCHVCB_1$ + RCHVCF2 +P	18.67ab	19.00abc	13.83ab	2.13bc	3.15ab	0.81abcde	10.67ab	2.94abc	14.15abcd
T12	$RCHVCB_1$ + RLVCF2+P	16.67abcdef	19.33abc	11.57bcd	2.20b	3.59a	0.93abcd	10.67ab	2.93abc	13.17abcde
T13	RScB1.19+ RSCF1.19 +P	18.67ab	19.67abc	15.12a	3.84a	2.88abcd	1.13a	10.67ab	3.07a	16.40abc
T14	RScB1.19+ RCHVCF2+P	15.33bcdef	19.67abc	9.89cdef	1.73bcdefg	1.73de	0.63cdef	10.00ab	2.71abc	13.13abcde
T15	RScB1.19+ RLVCF2+P	16.83abcde	19.33abc	11.31bcd	1.93bcde	1.93bcde	0.80abcde	11.33a	2.96abc	18.90a
T16	RMaB2.11+ RSCF1.19+P	14.50cdef	20.00ab	8.13defg	1.50bcdefgh	1.50e	0.60cdef	10.67ab	2.79abc	11.03bcde
T17	RMaB2.11+ RCHVCF2+P	15.83bcdef	17.33c	11.18bcd	1.80bcdef	1.80cde	0.77abcdef	10.00ab	2.57abc	18.83a
T18	RMaB2.11+ RLVCF2+P	18.00abcd	20.33a	11.92abc	2.07bcd	2.07bcde	1.04ab	10.67ab	3.08a	14.50abc
T19	-ve control	16.00bcdef	18.67abc	8.88cdefg	1.73bcdefg	2.95abcd	0.74bcdef	10.00ab	2.57abc	12.69abcde
T20	+ve control	18.33abc	19.00abc	10.59bcde	1.93bcde	2.87abcd	0.75abcdef	11.33a	2.90abc	17.19ab
CV		14.84	8.12	22.31	24.79	30.68	31.84	12.18	13.75	31.45
Mean		15.81	19.02	9.36	1.74	2.44	0.73	10.10	2.71	12.89
Ttest(LSD) (0.05)		3.88	2.55	3.45	0.71	1.24	0.39	2.03	0.62	6.70

*Means followed by the same letter(s) in each column are not significantly different at $P \leq 0.05$, P=phosphorus, cm=centimeter, mm=millimeter,g=gram, -Ve=Negative, +Ve=Positive, CV= Coefficient of variance, LSD=Least significance difference

4.4.1. 3. Effect of co-application of vermicompost with biofertilizers on coffee seedling growth

The seedlings of coffee treated with sole bacterial or fungal isolates with vermicompost resulted in vigorous plant growth parameters like the root length, shoot length, number of leaves, stem diameter and fresh and dry weight of shoot and root than control when co-applied (Table 22C&D).In this experiment inorganic phosphate fertilizer was not integrated with vermicompost and hence the vermicompost alone was integrated with both bacterial and fungal bio-inoculants. Except RLVCF2 + vermicompost, which did not show any significant change in plant height and root length, all the other inoculants performed in a similar result with the plants treated with vermicompost alone as compared to negative control (Table 22C).

Co-inoculation of RSCB1.19 +RCHVCF2+VC and RSCB1.19 +RLVCF2+VC showed significant increase in shoot length when compared with both negative and positive control as well as sole VC alone. Moreover, co-inoculation of RCHVCB1+ RLVCF2+VC, RSCB1.19 + RLVCF2+VC, RCHVCB1+RSCF1.19+VC was able to cause significant increase in shoot and root fresh weight respectively over negative or positive control and VC alone. Similarly, co-inoculation of RCHVCB1+RSCF1.19+VC and RCHVCB1+ RLVCF2+VC showed significantly increased shoot and root dry weight respectively compared with negative or positive control and VC alone (Table 22D).

Except in root length and stem girth, an overall increase in all plant growth parameter was observed in co-inoculation of vermicompost and biofertilizers when compared with both positive and negative control.

Table 22C. Performance of sole inoculation of bacteria and fungi under vermicompost

Treatments	Shoot length(cm)	Root length(cm)	Shoot fresh wt(g)	Root fresh wt(g)	Shoot dry wt(g)	Root dry wt(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)
T1 $RCHVCB_1$ +VC	22.67ab	19.67a	19.31a	2.27abc	5.69ab	0.92a	11.33ab	3.09ab	26.96ab
T2 RScB1.19+VC	21.33abc	18.67ab	20.70a	2.43abc	6.55ab	1.17a	12.67a	2.98ab	23.03abc
T3 RMaB2.11+VC	24.33a	18.67ab	19.12a	3.10ab	5.64ab	1.26a	12.67a	3.59a	30.23a
T4 RSCF1.19 +VC	21.33abc	17.33abc	19.86a	3.47a	4.57ab	1.37a	12.00ab	3.58a	16.53bc
T5 RCHVCF2+VC	21.33abc	20.00a	19.64a	3.53a	7.47a	1.43a	12.67a	3.25ab	22.83abc
T6 RLVCF2+VC	18.67bc	15.00c	18.67a	2.53abc	5.99ab	1.02a	10.67ab	2.97ab	21.72abc
T7 -ve control	16.00c	18.67ab	8.88b	1.73c	2.95b	0.74a	10.00b	2.57b	12.69c
T8 +ve control	18.33bc	19.00ab	10.59b	1.93bc	3.45ab	0.75a	11.33ab	3.08ab	17.19bc
T9 VC only	20.17abc	16.67bc	18.66a	2.47abc	4.73ab	1.20a	11.33ab	3.32ab	20.36abc
CV	15.94	8.93	10.35	29.71	45.24	39.38	12.27	14.59	30.16
Mean	20.46	18.19	17.27	2.61	5.23	1.10	11.63	3.16	21.28
Ttest(LSD) (0.05)	5.65	2.81	3.09	1.34	4.10	0.75	2.47	0.80	11.11

*Means followed by the same letter(s) in each column are not significantly different at $P \leq 0.05$, P=phosphorus, cm=centimeter, mm=millimeter,g=gram, VC=vermicompost, -Ve=Negative, +Ve=Positive, CV= Coefficient of variance, LSD=Least significance difference

Table 22D.Dual inoculation combined with Vermicompost.

Treatments		Shoot length(cm)	Root length(cm)	Shoot fresh wt(g)	Root fresh wt(g)	Shoot dry wt(g)	Root dry wt(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)
T1	RCHVCB$_1$+ RSCF1.19+VC	24.00ab	16.00a	25.17ab	3.63a	8.76a	1.64a	13.33a	3.32a	25.50ab
T2	RCHVCB$_1$+ RCHVCF2 +VC	23.67abc	16.00a	20.80bc	3.17abc	6.36abc	1.23ab	12.00ab	3.42a	28.59a
T3	RCHVCB$_1$+ RLVCF2+VC	23.00abc	19.33a	26.91a	3.50ab	8.35a	1.48a	11.33ab	3.35a	23.52ab
T4	RScB1.19+ RSCF1.19+VC	22.67abc	19.00a	20.17bc	3.20abc	6.33abc	1.32ab	12.00ab	3.39a	26.81a
T5	RScB1.19+ RCHVCF2+VC	25.67a	19.00a	23.91ab	2.70abc	6.77ab	1.35ab	12.67ab	3.66a	30.68a
T6	RScB1.19+ RLVCF2+VC	24.83a	17.67a	22.10abc	3.80a	7.20ab	1.34ab	12.00ab	3.13ab	30.01a
T7	RMaB2.11+ RSCF1.19 +VC	18.00cd	16.67a	14.22de	2.43abc	6.79ab	1.15ab	11.33ab	3.07ab	19.50ab
T8	RMaB2.11+ RCHVCF2+VC	23.50abc	18.00a	22.14abc	3.23abc	7.47ab	1.40ab	11.33ab	3.30a	20.00ab
T9	RMaB2.11+ RLVCF2+VC	22.00abc	19.07a	21.73bc	3.33abc	6.34abc	1.21ab	12.00ab	3.36a	22.53ab
T10	-ve control	16.00d	18.67a	8.88f	1.73c	2.95c	0.74b	10.00b	2.57b	12.69b
T11	+ve control (reco.NP)	18.33bcd	19.00a	10.59ef	1.93bc	2.95c	0.75b	11.33ab	3.08ab	17.19ab
T12	VC only	20.17abcd	16.67a	18.66cd	2.47abc	4.73bc	1.20ab	11.33ab	3.32a	20.36ab
CV		15.50	11.43	15.48	32.68	32.59	32.89	13.58	11.54	34.96
Mean		21.82	17.92	19.61	2.93	6.25	1.23	11.72	3.25	23.09
Ttest(LSD) (0.05)		5.73	3.47	5.14	1.62	3.45	0.69	2.70	0.63	13.67

*Means followed by the same letter(s) in each column are not significantly different at $P \leq 0.05$, P=phosphorus, cm=centimeter, mm=millimeter, g=gram, VC=vermicompost, rec.NP=recommended urea and P, -Ve=Negative, +Ve=Positive, CV= Coefficient of variance, LSD=Least significance difference

4.4.2. Nutrient status of potting medium

Once data collection was over, the potting medium was analyzed for the physicochemical properties (pH, OC, Ex. Acidity, CEC) and nutrient status. The results are presented in Table.23A, B, C and D. Accordingly, the potting media reaction was near neutral in the treatments containing both single and co-inoculation of PSF, PSB bio-inoculants with VC similar to negative control. But, there was slightly decreased pH in the treatments containing bio-inoculants amended with P fertilizer in single and dual as well as VC amended scenario (Table 23A, B,C). The vermicompost supplied treatments made the experimental soil neutral pH (Table 23C&D). Percent organic carbon, available P and K slightly increased in single and dual inoculations amended with inorganic P fertilizer compared to treatments having no P and the negative control (Table 23A&B). Similarly, percent organic carbon, available P, and K were higher in sole or dual inoculated and vermicompost supplied treatments compared to the negative control (Table 23C&D). However, available P was higher in the treatments containing bio-inoculants amended with vermicompost than treatments with bio-inoculants amended with inorganic P fertilizer. But, there were no differences in available K in all treatments without P fertilizer, amended with both P fertilizer and vermicompost, containing single or co-inoculations compared to available K in the potting medium (-Ve control) (Table 23A, B, C&D). The Cation Exchange Capacity (CEC) of the potting medium was higher in the treatment receiving bio-inoculants amended with inorganic P fertilizer than the negative control (Table 23A&B). However, there was drastic increase in the CEC in the treatments receiving bio-inoculants amended with vermicompost (Table 23C&D). Significant difference in available P content of the medium between treatments was noticed and the effect was more prominent in soil supplemented with inorganic P and bio-inoculants. We observed a positive correlation between available soil NP content and plant growth parameter in seedlings received bio-inoculants combined with inorganic P (Table 24A, B).However, significant correlation between available K and plant growth parameters was not observed in bio-inoculants +P as well as bio-inoculants +VC treatments (Table 24A,B, C,D). The combined application of bio-inoculants and P resulted in slight increase of CEC over the negative control. Moreover, the combined application of bio-inoculants and vermicompost resulted in higher increase of CEC over bio-inoculants and inorganic P fertilizer applied treatments.

Table: 23A:Chemical properties of potting medium after taking data destructively at single inoculation treatments and Nutrient uptake

Treatments		pH(1:2.5)	OC(%)	Ex.Acidity(meq/100g)	CEC(meq/100g)	Available nutrients			Nutrient uptake of seedlings		
						AvailableP(ppm)	TN (%)	K (ppm)	AvailableP (ppm)	TN (%)	K (ppm)
T1	$RCHVCB_1$	6.06	0.80	0.38	13.00	0.22	0.05	92.86	121.25	0.31	2298.92
T2	RScB1.19	6.30	0.76	0.34	13.12	0.27	0.08	102.00	120.21	0.30	2288.90
T3	RMaB2.11	6.16	0.81	0.34	13.14	0.21	0.06	100.81	123.25	0.31	2198.92
T4	RSCF1.19	6.10	0.86	0.36	13.10	0.28	0.07	99.86	124.45	0.33	2806.11
T5	RCHVCF2	6.31	0.82	0.31	13.20	0.24	0.06	101.86	124.05	0.32	2706.16
T6	RLVCF2	6.23	0.78	0.35	13.40	0.23	0.08	98.86	120.12	0.30	2800.00
T7	$RCHVCB_1$ +P	5.84	1.05	0.48	14.18	0.69	0.07	97.91	135.50	0.41	3277.40
T8	RScB1.19+P	5.47	0.95	0.51	20.70	0.76	0.08	105.51	130.45	0.40	3177.44
T9	RMaB2.11+P	5.40	1.14	0.59	13.14	1.12	0.06	103.00	129.35	0.41	3100.40
T10	RSCF1.19+P	5.30	1.04	0.46	13.84	2.40	0.08	104.43	138.80	0.43	3299.50
T11	RCHVCF2+P	5.06	1.00	1.06	13.9	1.78	0.07	103.25	128.82	0.40	3199.50
T12	RLVCF2+P	5.07	1.00	1.22	13.14	2.89	0.05	103.84	129.80	0.40	3190.40
T13	-ve control	6.33	0.72	0.26	12.34	0.20	0.08	45.97	118.32	0.21	2123.44
T14	+ve control	5.59	1.48	0.29	14.54	0.54	0.08	96.20	128.41	0.31	2993.14

*Ex=exchangeable, g= gram, OC=organic carbon, CEC= Cation Exchange Capacity, TN=total nitrogen, K=potassium, P=phosphorus, ppm=part per million, -Ve=Negative, +Ve=Positive

Table: 23B: Chemical properties of potting medium after taking data destructively at co-inoculation treatments and Nutrient uptake

Treatments		pH(1:2.5)	OC(%)	Ex.Acidity(meq/100g)	CEC(meq/100g)	Available nutrients in the soil			Nutrient uptake of seedlings		
						AvailableP(ppm)	TN (%)	K (ppm)	AvailableP(ppm)	TN (%)	K (ppm)
T1	$RCHVCB_1$+ RSCF1.19	6.10	0.60	0.31	13.41	0.19	0.07	89.66	130.25	0.30	2290.92
T2	$RCHVCB_1$+ RCHVCF2	6.31	0.63	0.37	13.40	0.18	0.05	96.61	130.21	0.30	2288.90
T3	$RCHVCB_1$ + RLVCF2	6.30	0.61	0.38	13.38	0.17	0.06	89.64	131.25	0.31	2198.92
T4	RScB1.19+ RSCF1.19	6.10	0.62	0.37	13.42	0.18	0.07	96.62	134.45	0.35	2806.11
T5	RScB1.19+ RCHVCF2	6.00	0.59	0.35	13.41	0.17	0.07	97.45	124.45	0.32	2706.16
T6	RScB1.19+ RLVCF2	6.48	0.60	0.32	13.40	0.16	0.05	99.61	134.12	0.30	2800.00
T7	RMaB2.11+ RSCF1.19	6.20	0.63	0.36	13.42	0.17	0.06	97.66	139.05	0.36	2290.90
T8	RMaB2.11+ RCHVCF2	6.20	0.61	0.38	13.39	0.18	0.05	98.00	128.15	0.31	2190.91
T9	RMaB2.11+ RLVCF2	6.10	0.60	0.33	13.41	0.18	0.05	99.60	129.05	0.30	2150.94
T10	$RCHVCB_1$+ RSCF1.19+P	5.04	1.98	0.46	14.04	2.28	0.08	95.11	158.71	0.48	3290.94
T11	$RCHVCB_1$ + RCHVCF2+P	5.03	1.16	1.83	14.2	2.46	0.06	97.11	154.70	0.44	3190.94
T12	$RCHVCB_1$+ RLVCF2+P	5.36	1.57	0.67	14.4	1.58	0.06	84.01	150.71	0.46	3180.94
T13	RScB1.19+ RSCF1.19+P	5.26	1.97	0.70	14.2	1.18	0.07	99.62	156.00	0.40	3213.00
T14	RScB1.19+ RCHVCF2+P	5.27	1.09	0.50	15.68	1.55	0.10	99.29	150.02	0.47	3103.01
T15	RScB1.19+ RLVCF2+P	5.14	1.57	1.12	14.04	2.19	0.08	98.77	147.02	0.49	3203.02
T16	RMaB2.11+ RSCF1.19+P	5.34	1.97	0.45	15.42	1.22	0.07	97.72	159.00	0.44	3302.26
T17	RMaB2.11+ RCHVCF2+P	5.13	1.04	1.33	14.24	2.40	0.06	90.04	152.41	0.44	3208.29
T18	RMaB2.11+ RLVCF2+P	5.10	1.25	1.35	15.76	2.04	0.07	91.46	149.46	0.40	3102.26
T19	-ve control	6.40	0.50	0.26	12.34	0.24	0.08	46.90	128.31	0.21	2023.44
T20	+ve control	5.59	1.48	0.29	14.54	0.54	0.08	96.28	138.40	0.31	2923.40

*Ex=exchangeable, g= gram, OC=organic carbon, CEC= Cation Exchange Capacity, TN=total nitrogen, K=potassium, P=phosphorus, ppm=part per million, -Ve=Negative, +Ve=Positive

Table 23C: Chemical properties of potting medium after taking data destructively in single inoculation combined with vermicompost and nutrient uptake

Treatments		pH(1:2.5)	OC(%)	Ex.Acidity(meq/100g)	CEC(meq/100g)	Available nutrients in the soil			Nutrient uptake of seedlings		
						Available P (ppm)	TN (%)	K(ppm)	Available P(ppm)	TN (%)	K (ppm)
T1	RCHVCB$_1$+VC	5.95	2.36	0.37	15.98	30.89	0.11	102.35	143.82	0.30	3084.06
T2	RScB1.19+VC	6.22	2.41	1.58	17.18	40.48	0.14	99.36	140.72	0.31	2985.16
T3	RMaB2.11+VC	6.36	1.82	0.29	14.06	41.27	0.15	95.22	143.82	0.30	2874.46
T4	RSCF1.19 +VC	5.89	1.48	0.26	18.12	42.46	0.08	97.96	207.65	0.38	3235.58
T5	RCHVCF2+VC	6.42	1.82	0.26	18.58	54.39	0.21	96.64	147.60	0.38	3035.50
T6	RLVCF2+VC	6.23	1.66	0.34	17.28	33.73	0.16	97.00	149.61	0.39	2935.58
T7	-ve control	6.35	0.54	0.26	12.34	0.24	0.08	56.98	128.41	0.21	2123.40
T8	+ve control	6.59	1.48	0.29	14.54	0.54	0.08	96.28	132.41	0.31	2523.44
T9	VC only	6.82	1.65	0.24	16.02	32.51	0.16	100.33	140.01	0.30	2723.44

*Ex=exchangeable, g= gram, OC=organic carbon, CEC= Cation Exchange Capacity, TN=total nitrogen, K=potassium, P=phosphorus, ppm=part per million, -Ve=Negative, +Ve=Positive

Table 23D: Chemical properties of potting medium after taking data destructively at co-inoculation combined with VC and Nutrient uptake

Treatments		pH(1:2.5)	OC(%)	Ex.Acidity(meq/100g)	CEC(meq/100g)	Available nutrients in the soil			Nutrient uptake of seedlings		
						Available P (ppm)	TN (%)	K(ppm)	Available P(ppm)	TN (%)	K (ppm)
T1	RCHVCB$_1$+ RSCF1.19 +VC	6.15	1.76	0.26	16.34	33.34	0.15	99.10	146.56	0.31	3354.10
T2	RCHVCB$_1$+ RCHVCF2 +VC	6.18	1.20	0.24	16.54	39.46	0.16	99.52	141.40	0.30	3154.00
T3	RCHVCB$_1$+ RLVCF2+VC	6.28	2.15	0.26	16.68	30.92	0.13	99.09	146.56	0.31	2954.10
T4	RScB1.19+ RSCF1.19 +VC	6.32	1.80	0.34	15.96	47.53	0.16	99.10	144.16	0.30	3396.00
T5	RScB1.19+ RCHVCF2 +VC	6.30	1.89	0.29	18.32	31.46	0.13	98.28	143.30	0.28	2996.02
T6	RScB1.19+ RLVCF2+VC	6.31	1.95	0.31	17.94	59.10	0.14	99.17	141.36	0.29	3096.00
T7	RMaB2.11+ RSCF1.19+VC	6.37	1.88	0.34	14.66	37.89	0.13	100.42	147.60	0.32	3541.74
T8	RMaB2.11+ RCHVCF2+VC	6.40	2.01	0.24	19.88	59.97	0.15	100.46	140.53	0.31	3140.64
T9	RMaB2.11+ RLVCF2+VC	6.40	2.07	0.34	16.88	67.50	0.12	97.57	139.63	0.27	2941.74
T10	-ve control	6.34	0.50	0.26	12.34	0.23	0.08	46.98	125.41	0.20	2123.44
T11	+ve control (reco.NP)	5.59	1.48	0.29	14.54	0.54	0.08	96.28	133.31	0.29	2823.24
T12	VC only	6.82	1.65	0.24	16.02	35.50	0.16	100.33	139.41	0.30	3013.34

*Ex=exchangeable, g= gram, OC=organic carbon, CEC= Cation Exchange Capacity, TN=total nitrogen, K=potassium, P=phosphorus, ppm=part per million, -Ve=Negative, +Ve=Positive

4.4.3. Nutrient uptake by Coffee seedlings

Inoculation of different bacterial and fungal isolates has resulted in different response pattern of coffee seedling as compared to the P-lacking inoculations and un-inoculated control. Non-significant but highest N,P and K-uptake by shoot of coffee seedlings was generally observed with treatments received bio-inoculants and phosphate fertilizer compared to un inoculated treatments, positive and negative control (Table 23A, 23B). However, there was no clear cut increasing in nutrient uptake among treatments received bio-inoculants combined with vermicompost by coffee seedlings compared to control (Table 23C, 3D).

The sole inoculation and VC combination scenario depicts that P-uptake was greatly correlated with shoot fresh wt, shoot dry wt, and leaf area (r=0.538**, 0.497* and 0.557*, respectively) (Table 24A and 24C). But in a single inoculation treated with VC available P was correlated only with leaf area and total N was correlated with shoot fresh wt, shoot dry wt, stem girth and leaf area (r=0.557*, 0.573*,0.876**,0.575* and 0.639* respectively). But Non-significant relationships were observed with available K uptake and all growth parameters except in plant height (r= -0.594* (Table 24C). The sole inoculation and P combination scenario, also revealed that total N uptake was correlated with shoot fresh wt, root fresh wt, shoot dry wt, stem girth and leaf area (r=0.525**,0.454*,0.584**, 0.454* and 0.879**, respectively) while available K did not correlated with any growth parameters (Table 24A). However, during dual inoculation combined with P, root length, shoot dry wt, No. of leaf, stem girth and leaf area was significantly correlated with P N uptake (r=0.444**, 0.424*, 0.542**, 0.466**, 0.853** and 0.580**, 0.631**, 0.570**, 0.487** and 0.862** respectively) (Table 24B). However, available K uptake was not correlated with any growth parameters in dual inoculation combined with inorganic P. Moreover, any correlation coefficient was not observed between growth parameters tested and available NPK uptake when the inoculants were combined with VC under dual inoculation; except in leaf area (r=0.847**) (Table 24D).

Table 24A. Correlation coefficient for plant growth parameters and nutrient up take characters of coffee seedlings (single inoculation)

characters	Shoot length/plant(cm)	Root length/plant(cm)	Shoot fresh weight(gm)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)	AvailableP(ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	**1**											
Root length/plant(cm)	0.176ns	**1**										
Shoot fresh weight(g)	0.025ns	0.126ns	**1**									
Root fresh weight(g)	0.174ns	0.540**	0.464*	**1**								
Shoot dry weight(g)	0.083ns	0.382ns	0.783**	0.565**	**1**							
Root dry weight(g)	0.090ns	0.386ns	0.444*	0.380ns	0.518**	**1**						
No of leaves	0.161ns	0.330ns	0.522*	0.599**	0.684**	0.506*	**1**					
Stem Girth (mm)	0.117ns	0.204ns	0.500*	0.460*	0.683**	0.579**	0.734**	**1**				
Leaf area(sq.cm)	-0.001ns	0.1203ns	0.680ns	0.342ns	0.598**	0.289ns	0.372ns	0.284ns	**1**			
Av.P(ppm)	0.072ns	0.098ns	0.538**	0.326ns	0.497*	0.247ns	0.291ns	0.167ns	0.913ns	**1**		
TN (%)	0.005ns	0.270ns	0.525**	0.454*	0.584**	0.383ns	0.361ns	0.454*	0.879**	0.874**	**1**	
K (ppm)	-0.178ns	0.166ns	0.019ns	0.156ns	-0.004ns	-0.221ns	-0.194ns	-0.009ns	0.127ns	0.070ns	0.256ns	**1**

*P=phosphorus, cm=centimeter, mm=millimeter, wt=weight, g=gram, sq.cm=square centimeter, TN=total nitrogen, K=potassium, ppm=part per million

Table 24B Correlation coefficient for plant growth parameters and nutrient up take characters of coffee seedlings (dual inoculation)

characters	Shoot length/plant (cm)	Root length/plant(cm)	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)	AvailableP(ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	1											
Root length/plant(cm)	0.040ns	1										
Shoot fresh weight(g)	-0.277ns	0.177ns	1									
Root fresh weight(g)	-0.028	0.629**	0.374*	1								
Shoot dry weight(g)	-0.040ns	0.560**	0.445**	0.696**	1							
Root dry weight(g)	0.171ns	0.179ns	0.260ns	0.403*	0.368*	1						
No of leaves	0.257ns	0.473**	0.148ns	0.527**	0.477**	0.614**	1					
Stem Girth (mm)	-0.128ns	0.445**	-0.052ns	0.494**	0.558**	0.317ns	0.428*	1				
Leaf area(sq.cm)	0.287ns	0.503**	0.044ns	0.530**	0.582**	0.195ns	0.579**	0.497**	1			
Av.P(ppm)	0.277ns	0.444**	-0.116ns	0.342ns	0.424*	0.216ns	0.542**	0.466**	0.853**	1		
TN (%)	0.296ns	0.580**	0.109ns	0.566**	0.631**	0.315ns	0.570**	0.487**	0.862**	0.867**	1	
K (ppm)	0.308ns	0.213ns	-0.013ns	-0.112ns	-0.170ns	-0.195ns	0.044ns	-0.244ns	0.141ns	0.099ns	0.214ns	1

*P=phosphorus, cm=centimeter, mm=millimeter, g=gram, wt=weight, sq.cm=square centimeter, TN=total nitrogen, K=potassium, ppm=part per million

Table 24C Correlation coefficient for plant growth parameters and nutrient up take characters of coffee seedlings (Single inoculation +VC)

characters	Shoot length/plant (cm)	Root length/plant (cm)	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)	AvailableP(ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	1											
Root length/plant(cm)	0.281ns	1										
Shoot fresh weight(g)	-0.063ns	0.398ns	1									
Root fresh weight(g)	0.288ns	0.957**	0.390ns	1								
Shoot dry weight(g)	0.033ns	0.438ns	0.646*	0.497ns	1							
Root dry weight(g)	-0.284ns	0.107ns	0.190ns	0.191ns	0.563*	1						
No of leaves	-0.343ns	0.317ns	0.199ns	0.360ns	0.576*	0.686**	1					
Stem Girth (mm)	-0.091ns	0.180ns	0.606*	0.237ns	0.807**	0.581*	0.505*	1				
Leaf area(sq.cm)	-0.343ns	0.143ns	0.228ns	0.319ns	0.361ns	0.232ns	0.424ns	0.084ns	1			
AvailableP(ppm)	-0.219ns	0.238ns	0.434ns	0.421ns	0.445ns	0.453ns	0.230ns	0.398ns	0.557*	1		
TN (%)	-0.097ns	0.367ns	0.573*	0.487ns	0.876**	0.446ns	0.417ns	0.575*	0.639*	0.619*	1	
K (ppm)	-0.594*	-0.086ns	-0.399ns	-0.052ns	-0.118ns	0.123ns	0.072ns	-0.328ns	0.335ns	0.203ns	0.161ns	1

*P=phosphorus, cm=centimeter, mm=millimeter,wt=weight, g=gram, sq.cm=square centimeter, TN=total nitrogen, K=potassium, ppm=parts per million

Table 24D Correlation coefficient for plant growth parameters and nutrient up take characters of coffee seedlings (dual inoculation +VC)

characters	Shoot length/plant (cm)	Root length/plant (cm)	Shoot fresh wt(g)	Root fresh wt(g)	Shoot dry wt(g)	Root dry wt(g)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)	AvailableP(ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	1											
Root length/plant(cm)	0.129ns	1										
Shoot fresh weight()	-0.075ns	0.580**	1									
Root fresh weight(g)	0.241ns	0.676**	0.703**	1								
Shoot dry weight(g)	0.046ns	0.410ns	0.723**	0.656**	1							
Root dry weight(g)	0.238ns	0.397ns	0.347ns	0.441ns	0.062ns	1						
No of leaves	0.457ns	0.701**	0.310ns	0.439ns	0.340ns	0.554*	1					
Stem Girth (mm)	0.415ns	0.471*	0.518*	0.557*	0.389ns	0.759**	0.612**	1				
Leaf area(sq.cm)	-0.270ns	0.323ns	0.559*	0.305ns	0.680**	0.103ns	0.265ns	0.166ns	1			
AvailableP(ppm)	-0.393ns	0.144ns	0.294ns	0.124ns	0.033ns	-0.102ns	-0.298ns	-0.279ns	0.423ns	1		
TN (%)	-0.354ns	0.199ns	0.361ns	0.153ns	0.341ns	0.305ns	0.209ns	0.090ns	0.847**	0.442ns	1	
K (ppm)	-0.086ns	-0.153ns	-0.205ns	0.014ns	-0.181ns	-0.080ns	-0.426ns	-0.208ns	-0.293ns	0.000**	-0.147ns	1

*P=phosphorus, cm=centimeter, mm=millimeter, g=gram, wt=weight, sq.cm=square centimeter, TN=total nitrogen, K=potassium, ppm=part per million.

5. DISCUSSION

5.1. Identification of microbes

On the basis of morphological and biochemical characteristics of the phosphate solubilizing efficacy, three bacterial isolates ($RCHVCB_1$, RScB1.19 and RMaB2.11) were identified at genus level and selected for the seed germination assay. The results obtained are consistent with the many phenotypic characteristics of the genera *Bacillus* and *Pseudomonas*. The results revealed that among the three isolates, two of them (RScB1.19 and RMaB2.11) were *Bacillus* species but one isolate identified as *Pseudomonas* sp. ($RCHVCB_1$). Accordingly, Babu *et al* (2017) have isolated both *Bacillus* and *Pseudomonas* from maize rhizosperic soils samples using morphological and biochemical parameters. The present findings are consistent with the finding of Dhurve *et al.(*2017) who verified the ability of *Pseudomonas* isolates to produce H_2S gas, liquefy gelatin but failed to hydrolyse starch. Moreover our findings are in line with reports of Karpagam and Nagalakshmi (2014) who characterized and identified Bacillus spp. based on their Vogues Proskauer test, Citrate utilization, nitrate reduction, gelatin hydrolysis, lactose and mannitol fermentation but did not produce H_2S gas.

5.1. 1. Phosphate solubilizeation tendency of the isolates

The qualitative phosphate solubilizeation tendecy through agar based bioassays supplemented with $Ca_3(PO)_2$ of our isolates exhibited the potential of these isolates for inorganic phosphate solubilizeation. Moreover, phosphate solubilization results recorded in liquid medium showed that all the isolates had the potential to solubilize the inorganic form of P as indicated by a gradual increase in the amount of soluble P in the medium. The highest decrease in pH value was related to high levels of P released by PSB isolate RScB1.19, while the pH was dropped to 3.2 from initial pH 7. Our result was similar to the earlier findings which indicated that available P released from $Ca_3(PO_4)_2$ by 3 strains of *Bacillus megaterium and Pseudomonas fluorescens* ranged from 427.7 to 489.4 mg l^{-1} and the pH values of the cultures were reduced from 7 to values between 4 and 4.4 (Jeon *et al.* 2003)

The decline in pH might indicate the production of organic acid, which suggests that acidification of the culture supernatant, might be the principal mechanism for phosphate solubilization (Chen *et al.*, 2006; Sharma *et al.*, 2013). Efficient P solubilizers are known to lower the pH of plant growing medium in which the microbes are establishing themselves. (Nautiyal *et al.*, 2000). The qualitative estimations performed through agar based bioassays exhibited the potential of fungal isolates for phosphate solubilization, while the quantitative estimations showed the involvement of various mechanisms including production of organic acids which was evidenced by decline in pH. Organic acids increase the P accessibility in the soil by forming complexes with cation on the soil mineral surface (Behera *et al*., 2017). Presently, we demonstrate quantitative P-solubiliizing capability using the spectrophotometric analysis according to the method of Murphy and Riley (1962), and hence the three PSF showed desired P-solubilizing capability in inorganic TCP broth. Similarly, the qualitative estimations performed through agar based bioassays showed a clear halo zone around the fungal colony (Gupta *et al*., 2007). Rinu and Pandey (2010) demonstrated a decline in pH was in a parallel increment with fungal phosphate solubilizeation tendency. The pH of the fermentation broth of the nine PSF generally changed with the increasing initial pH and tended to decrease with a narrow pH range (3.20±4.44) from the initial pH (7.02±0.06). This result could show that organic acids were secreted by all the nine PSF with different degrees (Son *et al*., 2006). The finding of the phosphate solubilization of the three fungal isolates (RSCF1.19, RCHVCF2 and RLVCF2) for the TCP clearly indicated an optimum and efficient solubilization potential of TCP (Chakraborty *et al*., 2010). These three phosphate solublizing fungi (RSCF1.19, $RCHVCF_2$ and $RLVCF_2$) were isolated from the coffee rhizosphere in acidic soils and alkaline vermicompost.

RSCF1.19 showed more P accumulation (360.48±10.05µg/ml) with pH range of 3.20±4.44 compared to other eight PSF which may be explained by the finding that the solubilization of the P mostly dependent on the amount of acids production (Cunningham and Kuiack 1992). The PSF secretes various organic acids, such as gluconic, malic, oxalic, tartaric, citric and butyric, etc (Li *et al*.2016). It was found that during phosphate solublization oxalic acid was mainly secreted by *Aspergillus* spp., whereas gluconic acid was mainly produced by *Penicillium* spp. *(Li et al*.2015). The lower pH (3.20) by isolate RSCF1.19 may be attributed to the higher production of organic acids compared to the rest eight PSF *(Li et al.*, 2015). *A. niger* and *A. flavus* have been studied as TCP solubilizers by several researchers whose investigations are in agreement with our findings

(Nenwani, *et al*. 2010). Several strains of PSMs solubilize TCP and make it available to plants; earlier research has also been conducted on the solubilization of TCP in liquid cultures by *A. niger* (Yu, *et al.*,2005).

5.1.2. Phytobeneficial traits of bacteria isolated from vermicompost and coffee rhizosphere

Phosphate solubilizing rhizobacteria exhibited diverse phytobeneficial triats. Accordingly, most of the rhizobacteria associated with coffee rhizosphere and vermicompost produced indole acetic acid detected qualitatively.

A characteristic pressure of Rhizobacteria in the soil environment is a release of auxin phyto-hormones to make available phosphorus in the soil (Spaepen *et al.*, 2007). Mohite, (2013) described that IAA producing rhizosphere isolates were significantly increased the plant height and root length of wheat seedlings along with increased in chlorophyll content compared to the control. Moreover, IAA enables the rhizo-bacteria to adapt and resist the high concentrations of heavy metals through activation of physiological changes in plant cell metabolism under metal stress (Glick 2010). Moreover, an increased in root length because of IAA, was reported in *Brassica campestris* plants due to inoculation with *Pseudomonas and Azotobacter* spp (Ghosh *et al.,* 2003). Production of high level of IAA by bacterial isolates has a direct influence on plant growth as they increase plant root length (Gusain *et al*., 2015). Hence the test isolates were extremely promising in enhancing root growth for the vigor of plant establishment and increased yield production. In the present study, most of the isolates produced HCN under in *vitro* conditions and they are promising in the plant growth promotion as reported in the previous study (Wani *et al.,* 2007). An investigation was conducted on the production of HCN by *P. fluorescens* to control root rot of ground nut due to *M. phaseolina* with strong suppression of the pathogen (Meena *et al.* 2001). Moreover, Ramette *et al*., (2006) have reported that growth of causative agent of black root rot of tobacco was suppressed due to released HCN in the rhizosphere by *Pseudomonas* sp. Ahmad *et al*, (2008) have also documented accumulation of HCN by *Pseudomonas* and *Bacillus spp.* in nodule forming plant rhizosphere.

All of our isolates were able to produce ammonia and this phytobeneficial trait can effect indirectly plants growth. Our result was in line with reports of Ahmed et al. (2008) who found that release of ammonia by all the isolates of *Pseudomonas and Bacillus spp.*

Among the phosphate solublizers, ten isolates including both *Bacillus* species and Pseudomonas species (83.33%) were able to grow on the N-free media. This result is consistent with the findings of Muthukumarasamy *et al* (2007) who confirmed the existence of N-fixation among the genera of *Bacillus* and *Pseudomonas.*

5.1. 3. Ecophysiological traits

In the present study, some of the potent isolates exhibited enhanced tolerance to various potentially toxic heavy metals. He *et al.* (2013) described that rhizosphere bacteria such as *Bacillus* sp. and *Pseudomonas* sp. are very promising agents due to their solubilization of insoluble and biologically unavailable Zn by secreting low molecular weight organic acids. Madhaiyan *et al*.(2004) have also added an isolates obtained from coffee plantation that could solubilize insoluble $Zn_3(PO_4)_2$. Our results also support the idea that these groups of soil bacteria play a pivotal role in monitoring the possible impact of heavy metal contamination by making it bio-available to plants. The variation in the heavy metal toxicity towards the bacterial isolates might be explained by the conditions of isolating bacteria and the nature as well as physiological characteristics of bacterial isolates (Wei and Wee, 2011).

Among our isolates, RCHVCB1 showed growth on maximum concentration of NaCl (6%) and was superior to other isolates. This bacterium was isolated from the vermicompost having pH 9.5. It was indicated that Pseudomonas aeruginosa has been shown to withstand biotic and abiotic stresses (Pandey, *et al*.2012). Salinity and pH is one of the most important abiotic traits that cause a reduction in plant growth and yield in many parts of the world (Kaya *et al*, 2007).

The absence of growth with increased concentration of NaCl can be attributed to the exposure of organisms to the conditions of hyper-osmolarity resulting in a decrease in their cytoplasmic fluid activities which influences the osmolarity of the cells (Botsford, 1984). Pal (1998) reported that the strain PAS-2 isolated from pasture and wasteland of pH 4.8 had highest acid pH-5 tolerance. The present studies indicated that the PSB isolates obtained from acidic soils of pH 3.8 to 4.8

had high acid-tolerance. Tolerance towards high salinity and pH could be important traits for rhizobacteria in a competitive rhizosphere for multiplication, survival and spread in alkaline soils.

Isolates with multiple antibiotic resistances have greater advantage in establishing themselves as bio fertilizers in natural soil conditions as well as any new ecological niche. Such isolates with high level of intrinsic antibiotic resistance have their own significance in establishing themselves in the rhizosphere with greater capability when used as bio-fertilizer in natural soil conditions. Antibiotic sensitivity/resistance assay revealed that from the all three isolates $RCHVCB_1$, RScB1.19 and RMaB2.11 showed a very high level of resistance to all the 6 antibiotics and said to be advantageous for establishing themselves in stressful environment. Resistance to antibiotics is acquired by a change in the genetic make- up of microorganisms which can occur by either a genetic mutation or by transfer of antibiotic resistant genes among microorganisms (Spain and Alm, 2003). Similar investigations on antibiotic resistance have been reported by Wani and Irene (2014).

In addition to phosphate solubilization, biocontrol is one of the most desirable traits for inoculants. Therefore, in the present study, the co-existence of isolates RSCF1.19 with $RCHVCB_1$ was confirmed by the absence of inhibition zones at the intersection of the two colonies on the same plate medium and this indicates the possibility for co-colonization on the roots of coffee seedlings. These results revealed that the combination of two bacterial and two fungal phosphate solublizing isolates could colonize coffee roots with inherent ability to solubilize inorganic phosphate in order to release plant growth-promoting hormones and easily establish themselves in the eco-physiologically stressed environments. In the present study, synergy between the compatible isolates ($RCHVCB_1$ with RSCF1.19 +P) was evidenced by the slightly increased growth parameters in the coffee plant seedlings. Our results also in agreement with the findings of Pandey *et al.*(2012) who demonstrated that microbial diversity in soil gives rise to a stable ecosystem through the synergistic interactions of compatible microbes, resulting in increased plant productivity.

5.1.4. Seed germination assay

Based on the current investigation, these three fungal isolates (RSCF1.19, RCHVCF2, and RLVCF2) and three bacterial isolates ($RCHVCB_1$, RScB1.19, RMaB2.11) that showed substantial qualitative and quantitative inorganic phosphate solublization was chosen to be tested for seed germination under laboratory condition. In this regards, three bacterial and three fungal isolates were selected for germination assay. In our investigation, a significant variation in seed germination rate and root and shoot lengths of coffee seedlings were observed in response to different PSB and fungal isolates. In general, all the isolates showed better performance with respect to seed germination and growth of seedlings. Inoculation results revealed that root and shoot lengths were significantly increased when treated with RLVCF2 and RSCF1.19 over uninoculated control, respectively. Moreover, co-inoculation of RMaB2.11 with RLVCF2 (RMaB2.11+ RLVCF2) was also significantly increased root length over uninoculated control. Results showed that both RSCF1.19 and RLVCF2 (Fungal) isolates took the highest level over bacterial inoculants in coffee seed germination and vigor index as well as root and shoot elongation. Bertrand *et al*. (2001) was also reported a similar findings that increased root length due to microbial intervention during root propagation. However, except in co-inoculation of RScB1.19 with RLVCF2 (RScB1.19+ RLVCF2), both bacterial and fungal isolates were not shown significant variation in germination rate over the control. Both co-inoculation and single inoculation of bacteria and fungi (RScB1.19+ RSCF1.19, RMaB2.11+ RCHVCF2, RMaB2.11+ RLVCF2, RCHVCB1+ RSCF1.19, RCHVCB1, RScB1.19, and RSCF1.19) showed better performance with respect to vigor index. In germination assay, coffee seedlings growth (root, shoot and vigour index) was improved (Khalid *et al*., 2004).

Likewise, PGPR enhanced the growth and germination of seeds in pot under natural condition (Yilmaz, 2003). A large body of evidence suggests that phosphate solublizing microorganisms enhance the growth, seed emergence and crop yield, and contribute to the protection of plants against certain pathogens and pests (Herma, *et al.,* 2008). On the basis of these findings, it can be assumed that the future microbial phosphate solublizers are the best alternative to chemical fertilizers and pesticides. Therefore, it was concluded that the bacterial and fungal isolates such as RSCF1.19, RCHVCF2, RLVCF2, RCHVCB1, RScB1.19 and RMaB2.11, can be used as

inoculants for development of efficient biofertilizer for field application in cultivation of *Coffea arabica* in sustainable agriculture.

5.2. Effect of bacterial and fungal inoculation on coffee seedlings under greenhouse condition

A significantly increased plant growth in terms of plant height**,** root length, stem diameter, leaf number, leaf area, fresh weight and dry weights with co-inoculation of RCHVCB$_1$+ RSCF1.19 +P as well as single inoculation of fungi (RSCF1.19+P compared to both positive and negative control. The higher growth parameters observed under bio-inoculants combined in the presence of inorganic phosphate can be attributed to the activity of phosphate solubilizing microbes in rhizosphere that could release soluble P and also through production of IAA, ACC deaminase, siderophore, antibiotics and HCN compared to the control (Zaidi *et al.,* 2014). This revealed that the potent microbes are not only phosphate solubilizers but also promote plant growth through the production of plant growth hormones (Bottini *et al.,* 2004). Similarly, increase in biomass due to treatment with phosphate solubilizing bacteria has been reported in maize (Hameeda *et al.,* 2008). However, among inoculants, single inoculation of fungal inoculums, RSCF1.19 in the presence of inorganic P showed significantly increased superior efficiency in promoting all the measured plant growth parameters except in root length compared to the bacterial inoculums. This significant increase in growth parameters of the plant is believed to be because of the fact that fungi have a greater potential to solublize insoluble phosphate compounds than bacteria and easily establish in the soil (Mahadevamurthy *et al.,* 2016; Nahas, 1996). Next to fungal inoculums, the three bacterial isolates showed better plant growth parameters when combined with P source than inoculants without P sources. A better increase in plant growth in terms of plant height**,** root length, stem diameter, leaf number, leaf area, fresh weight and dry weights with inoculation of *Pseudomonas* sp was also documented by Mamta *et al*. (2010). Research results documented by Prasad *et al.* (2014) indicate that significantly increased coffee seedlings growth when treated with *Azosprillum* sp*, Pseudomonas fluorescens,* phosphate solubilizing bacteria (PSB) and arbuscular mycorrihiza fungi (AMF).

Single application of chemical phosphate without solublizers (+Ve control) did not significantly improve plant growth parameters (shoot and root dry weight) in coffee seedlings. The poor

growth of seedlings observed in the treatments inoculated with bio-inoculants but without inorganic phosphate compared to the seedlings under bio-inoculated treatments combined with P sources that could be due to the lack of adequate inorganic phosphate which is essential to be solubilized and be available for uptake by plants for the establishment of growth parameters. In this study, vermicompost was used as carrier material to enhance easy establishment of bio-inoculants in the potting medium due to its contribution to better growth of seedlings in all combined treatments. This confirms that organic matter is a predictable ingredient of potting mixture when bio inoculants are used for raising coffee seedlings even when the soil under investigation in the potting mixture is deficient in organic matter. However, all the treatments amended with vermicompost showed suppressive characteristics and no any seedlings were emerged at all. The suppressive characteristics can be attributed to the high pH value of the potting medium (vermicomposting) alkaline pH (pH>7.5) (Reshid Abafita *et al*., 2014).

5.2.1. Nutrient status of potting medium and uptake by coffee seedlings

Bacterial and fungal phosphate solublizers, which could solubilize insoluble phosphate compounds by producing organic acids and phosphatase enzymes improve P availability in soils (Park *et al.*, 2010) and stimulate growth due to mineral uptake by plants. Consistently, our results are in agreement with findings from other researchers that indicate the importance of selection and integration of the most efficient bacterial and fungal P solublizers as bio-inoculants in the presence of chemical phosphate fertilizer to improve crop mineral nutrients in nutrient-deficient soils.

Co-inoculation of bacterial and fungal RSCF1.19 and RCHVCB$_{1)}$ isolates could promote mineral uptake and growth of coffee seedlings. Availability of P in the soil is crucial for facilitated uptake and easy utilization of it by plant roots (Vessey, 2003). Hence, higher available P due to the addition of inorganic P-fertilizer and solubilization with inoculated PSB and PSF might cause an enhancement of P uptake and plant growth. Generally, results from the present study and others findings suggest that co-inoculation of PGP microbes with other different beneficial properties could be the future trends of bio-fertilizer application for sustainable crop production. It is likely that phosphate solubilizing microbes (bacteria and fungi) might have helped in plant root development due to their ability to produce phytohormones in the plant rhizosphere to

enhance absorption of water and acquisition of nutrients such as phosphate by plant roots (Barea *et al.,* 2005). From these results we can conclude that inoculation of coffee seeds with efficient bio-inoculants significantly enhanced plant growth in glasshouse experiments. In the present study, the pronounced plant growth by these isolates could be attributed to the production of IAA, NH_3, HCN, N-fixation and solubilization of phosphate. These results are in concurrence with the findings of many authors who reported production of phytohormones and phosphate solubilization by soil microbes (Dhurve *et al.,* 2017).

The analytical data of nutrient status in the potting medium clearly indicates more nutrient availability in treatments containing bio-inoculants and inorganic P fertilizer compared to the treatments with only bio-inoculants. On the other hand, a decrease in pH in treatments containing bio-inoculants amended with inorganic P fertilizer could be due to acid production by potent microbes during P solubilization (Gaind, 2016). Percent of organic carbon and the cation exchange capacity (CEC) of the potting sand were the same in all the treatments that received bio-inoculants in the presence of inorganic P fertilizer as well as treatments without inorganic P fertilizer. These might be due to exclusion of organic amendments from the treatments which may build organic matter in the potting medium. Generally, beneficial rhizospheric and nonrhizospheric phosphate solubilizing microbes enhance growth through synthesizing particular compounds for plants or by facilitating the uptake of particular nutrient from the soil or by preventing and protecting the plants from pathogens (Yadav *et al*., 2011).The results of our study revealed that the added microbial inoculants have the advantage of making nutrients available in balanced and adequate quantities from the potting medium as seen in the present experiments. PSF and PSB inoculation increased total N and P concentration in the tissues of coffee seedlings which has a positive correlation with seedlings growth parameters such as plant height**,** root length, stem diameter, leaf number, leaf area, fresh weight and dry weight. These increments were attributed to the inherent bacterial and fungal growth-promoting abilities through diverse mechanisms.

5.2.2. Nutrient uptake by coffee seedlings

Nutrient uptake by coffee seedlings depends on availability of nutrient. Some soil fungi and bacteria are the most important phosphate solublizers. Inoculation of these microbes helped plant

to take phosphorus compare to the control. The increase in phosphorus uptake by inoculation of microbes could be attributed to availability and uptake of balanced and higher quantities of phosphorous to coffee seedlings through inorganic phosphate fertilizetion as well as consortia of bio-inoculants compared to treatments received only bio-inoculants without inorganic phosphate fertilizer and negative control. Son *et al.* (2006) have reported increased seed P content by phosphate solubilizing microorganisms. Our results are in agreement with reports of Jilani *et al.* (2007) that a combination of 50% of recommended chemical fertilizer and bio fertilizer gave equal yield as 100% of recommended chemical fertilizers. It is concluded that bioavailability of precipitated phosphorus is possible by *Fungus* such as *Penicilium* and bacteria such as *Pseudomonus* spp. Co-inoculation of both P-solubilizing fungi and bacteria has positive effects on the growth and nutrient status of the soil by providing growth hormone and increasing the NPK uptake by seedlings and thus provides healthy environment for the next crop. The results in the present investigation point to the presence of a diverse group of phosphate solubilizing microbes that dwell in the rhizosphere of coffee plants and vermicompost amendment in the southwestern Ethiopia. It is evident that phosphate solubilizing microbes are widely distributed and significant variations were noted among the microbes with respect to their phosphate solublization efficacy. The use of TCP along with the phosphate solubilizers, *Penicillium* and *Aspergillus* spp. as well as *Pseudomonus* spp and *Bacillus* spp. as biofertilizers could enhance the phosphate solubility of the soils. Bio-fertilizers are eco-friendly, free from hazardous chemicals, possess no detrimental health effects and are cost effective. Therefore, the use of vermicompost and indigenous coffee rhizospheric phosphate solubilizing bacteria and fungi can be a reliable alternative in low inputs and sustainable agriculture. These phosphate solublizers can be used in the field as efficient and potential phosphatic biofertilizers for the cultivation and growth of coffee seedlings *(Coffea arabica* L**.)**. Therefore, further field studies are required to confirm the application of these microorganisms under field conditions to sustain maximum organic coffee yields.

5.3. Effect of bacterial and fungal inoculation on coffee seedlings under nursery condition

5.3.1. Soil Physical and Chemical Properties

The soil reaction of the study site was moderately acidic (pH 5.62) based on pH in H_2O, which fall in a range of favorable acidic soil condition for coffea arabica cultivation (pH 4-7) (Rothfos, 1980). However, most of the farmers do not realize that in such acidic soil a large proportion of the soluble forms of P fertilizers is precipitated in insoluble form by forming insoluble metallic complex soon after application and becomes unavailable to plants, as a result only a small fraction of phosphate is available for the plant growth (Maheswar and Sathiyavani, 2012).

To guarantee crop dietary necessities, phosphorus is frequently supplied to soil as inorganic phosphate fertilizer. However, plants can absorb only few quantity of this phosphate due to formation of metal complexes and rapidly becomes fixed in soils (Sharma *et al* 2013). Therefore, use of phosphate solublizing microorganisms to make P available for plant utilization under low pH is recommended for profitable coffee growth. The relatively lower soil organic carbon, extractable phosphorus concentration and nitrogen of study potting medium could be attributed to the continuous cropping and cultivation, intensive tillage practice and heavy rainfall in the area. This revealed that the requirements of the use of supplementary fertilizers and organic amendments to optimize crop yields.

5.3.2. Performance of bio-inoculants

In this nursery condition, all fungal isolates positively influenced plant growth promotion as put side by side to bacterial inoculants and the bacterial inoculants also certainly influenced the plant growth as weighed against to both non-inoculated control and plants without inorganic chemical phosphorus. The increase in root length, shoot length, shoot dry weight and root dry weight of coffee seedlings inoculated with PSF isolates (RSCF1.19+Pand RLVCF2+P) could be attributed to a greater absorption of nutrients, especially P (Jain *et al*.,2010) . Hence, the fungi were more PS and enhancing plant growth as compared to bacterial isolates (Sharma *et al*., 2013). They help reduce the cost of chemical fertilizer and nourish the soil with ample supply of mineral elements

(Mohan and Rajendran, 2014). Therefore, as an alternative to the chemical fertilizer, microbial inoculants have proven role in growth enhancement in nursery conditions (Malik *et al*., 2013). Co-inoculation of *Penicillium species* with bacterial isolates is more effective than sole inoculation of either of the inoculants. The possible justification would be the fungus has developed association with the roots of higher plants in the plant cell which can enables it to easily establish stimulation of growth in the root cells as a result of mycorrhizal inhabitation and easily established in the environments to be co-existed in their ordinary niche (Bonfante and Genre.2010). Therefore, variations in coffee seedling growth due to bacterial and fungal inoculation may be attributed to their phosphate solubilization potential and accessibility of phosphate as well. The increased in coffee seedling growth parameters when combined with chemical inputs as sole P source indicated the existence of phosphate-solubilizing traits and utility of the P. The present studies also confirm that the inoculation of *Penicilium* sp. (RSCF1.19) and *Aspergillus* sp *(*RLVCF2*)* under field nursery condition is effective and in agreement with the findings of Dash *et al*. (2013) on forest trees like *Acacia auriculiformis.* This was justified with the finding of Gaind (2016) and Sharma *et al*. (2013) who discovere that fungi are more important to the solubilization of inorganic phosphate in soils than bacteria as they typically secrete more acids to liberate fixed P. These shows that the availability of P when combined with PSF in the present study has been contributed to the significantly increased growth parameters of coffee seedlings. The significant improvement in coffee seedlings growth because of the effectiveness of fungal isolates showing that the inoculated fungal isolates were more effective as compared to the pre-existing fungal and bacterial strains in the soil as well as the inoculated bacterial itself. Co-inoculation of bacterial and fungal isolates with vermicompost failed to influence root length and stem girth and could not able to show difference over the negative or positive controls. Lower response to added inoculants may be due to failure to compete with the indigenous microorganisms and the soil PH stresses (Kutcher, et al. 2002). Moreover, colonization of soil by added inoculants depends both on its interaction with indigenous flora associated with plants and its ability to utilize diverse substrates in the soil (Miethling *et al*., 2000).

5.3.3. Nutrient status of potting medium and uptake by coffee seedlings

The higher percent organic carbon and available P was recorded in the treatments receiving bio-inoculants, P fertilizer and vermicompost due to high organic matter received from addition of vermicompost in the potting medium. A slight reduction in pH in the treatments containing bio-inoculants amended with P fertilizer in single and dual as well as VC amended scenario may be attributed to the release of inorganic acids produced by bio-inoculants during solublization of inorganic P fertilizer. Similar reduction in soil pH due to interaction between P fertilization and bio-inoculants was also recorded in the earlier research (Mairan, *et al.* 2005). Quite a lot of soil microbes, particularly species of *Penicillium, Aspergillus Pseudomonas, Bacillus, etc* release organic acids and decrease the pH in their surroundings to facilitate release of complexes of P in soil (Gaind, 2016; Sharma *et al*. 2013). Secretion of phytohormone such as IAA, gibberellins, and ACC-deaminase enzyme in the need of nitrogen and carbon source is one of the mechanisms in some useful strains of PGPRs to increase percent OC and mineral phosphate solubilisation (MPS) into some useful carbon source to influence on the growth of plants (Ansari *et al*.,2013).

The decreased percent organic carbon status in the negative control may be due to its continuous removal from potting media by coffee seedling in the absence of external supply of organic matter through P fertilizer and vermicompost (Richardson and Simpson, 2011).

A positive and significant correlation between soil N P content and plant growth parameter may be attributed to the solubilizeation of NP and subsequent uptake. Besides, inorganic phosphate (Pi) transporters on fungal hyphae which help in the direct absorption of phosphate from the soil and a glutamine synthase gene found in fungi, which strengthens the possibility of nitrogen metabolism in fungal hyphae that can be transported later to the plant, could be responsible for the availability of N in the potting medium (Chialva *et al*.,2019). Higher availability of P in treatments containing bio-inoculants, P fertilizer and amended with vermicompost may be attributed to the solubilisation of P by the organic acids released from bio-inoculants, the organic vermicompost, reduction of P fixation in the potting media because of chelation of P fixing cations like Ca, Fe, Al and Mn and also due to the enhanced microbial activities. Availability of P in the rhizosphere due to involvement soil phosphate solubilizing microbe was also reported in the findings of Taalab and Badr, (2007). The available P status of the potting medium in control was lower due to exclusion of P fertilizers from negative control. The increased availability of K

in all treatments over negative control may be because of the substantial amount of K already persist in the experimental soil and also may be due to the interaction of clay with potassium to increase the available K status of the experimental soil itself. Higher increase of CEC over bio-inoculants and inorganic P fertilizer applied treatments may be because of the buildup of soil humus due to application of organic vermicompost and activities of bio-inoculants. Similar results were reported by Rajshree, *et al*. (2005), which revealed that CEC of inceptisols increased due to the increased formation of colloidal exchange complexes from organic matter obtained by application of vermicompost.

The poor uptake of nutrients by coffee seedlings obtained in the treatments received bio-inoculants without inorganic phosphate compared to coffee seedlings under negative control may be due to the lack of adequate inorganic phosphate in the soil for plant acquisition which is essential for establishment of externally supplied microbes in the form of bio-inoculants.

According to correlation coefficient, P and K uptake was strongly related to coffee seedling increment in growth parameters. Analysis of the seedlings showed that both fungal and bacterial inoculants helped in increasing the availability of P and K as compared to control and the increment were more pronounced in seedlings grown in the medium containing vermicompost compared to the medium supplemented with P fertilizer only. The low amount of P content in the tissue of un-fertilized plants indicates the unavailability of the soluble phosphorus and its subsequent utilization by the host plants, as compared to the positive control. The N content was not much affected but an increase in the medium supplemented with vermicompost was observed which provides more organic matter and bioavailable nutrients to the roots for better utilization.

Fungi and *Bacteria* are the most important phosphate solublizers. Inoculation of these microbes helped plant to take phosphorus compare to control. The increase in phosphorus uptake by inoculation of microbes can be attributed to availability and uptake of balanced and higher quantities of phosphorous to coffee seedlings through inorganic phosphate fertilization as well as bioinoculants consortia compared to treatments received only bio-inoculants without inorganic phosphate fertilizer and negative control. Therefore the bioavailability of precipitated phosphorus is possible by fungus such as *Penicilium (*Pindi and Satyanarayana, 2012) and bacteria such as *Pseudomonus* spp (Zaheer, *et al*. 2016). The factor responsible for phosphate solubilization may be organic acid as decline in pH of experimental rhizosphere soil, indicate the presence of these factors responsible for change in the pH of the medium (Gaind, 2016).

6. CONCLUSION AND RECOMMENDATIONS

Few studies in Ethiopia showed the existence of PSB in the rhisosphere soil of *Coffee arabica* L. but the present study included not only the rhisosphere soil bacteria but also the rhisosphere fungi associated with *Coffea arabica* L and vermicompost. In this investigation three potent PSB and three PSF isolate was screened from rhizosphere of coffee plants and vermicompost, showing high performance in phosphate solubilizeation. They belong to different bacterial genera: *Pseudomonas and Bacillus,* as well as fungal genera*: Penicillium and Aspergillus.*

During quantitative phosphate solublization it was observed a gradual pH decrease from the initial value of 7.07 to 3.20 on the 6th day in PVK broth supplemented with tricalcium phosphate with elevated levels of phosphate solubilizeation. The lowest pH value was recorded (3.20) when the highest amount of solubilized P reached the maximum value (361.46 µg/ml) by the bacterial isolate RScB1.19 and P-solubilizing potential was also exhibited by the fungal isolate RSCF1.19 (360.48µg /ml) next to the bacterial isolate at the same pH value(3.20). The factor responsible for phosphate solubilization may be organic acid as decline in the pH of the experimental medium.

Phosphate-solubilizing bacteria *(*$RCHVCB_1$),RScB1.19 and RMaB2.11) possessing phytobenefiecial traits viz, P solubilizeation, IAA, NH_3, HCN, N-fixing ability and tolerance to ecophysiological factors such as heavy metal tolerant, acidity tolerant, salinity tolerant and antibiotic resistance were qualified for invivo trials. Moreover, fungal isolates (RSCF1.19, RCHVCF2 and RLVCF2) showing better performance in insoluble phosphate solublization under *in vitro* conditions were choosen for in vivo trials under controlled glasshouse and lath house natural nursery environment as a biofertilizer. In almost all treatments with single and co-inoculation of RScB1.19+RSCF1.19, RMaB2.11+ RCHVCF2, RMaB2.11+RLVCF2, $RCHVCB_1$+RSCF1.19, $RCHVCB_1$, RScB1.19 and RSCF1.19 isolates showed the ability to improve coffee seed germination and growth parameters. These results indicated the positive role of bacteria and fungi in enhancing the biomass of coffee seedlings. It is clearly revealed in the present study that the efficiency of the isolates was more pronounced in fungal isolates. Therefore, a positive effect of microbial application of phosphate solubilizing nature has clearly been evident in the present study.The organisms were isolated indigenously and proved the importance and usefulness of native microbes.

The high P solubilization activity of the introduced PSB and PSF lead to the higher available P content in soil which in turn resulted in increased nutrient uptake of plants and reflected on the plant growth parameters of coffee seedlings. All tested biometric parameters showed paramount performance in mixed inocula compared to individual application. The results proves the superiority of the isolates to the standard PGPR strain *Pseudomonas* and *Penicillium* used in this study as potential microbial inoculants. The results in general provides a wide space to use these organisms as potential biofertilizers not only due to its P solubilisation traits but also due to multiple plant growth promoting attributes associated with the isolates.

This study revealed that PSF isolates were more efficient in P-solubilization than PSB. It was also concluded that NPK uptake was associated with the activities of phosphate solubilizing bio-inoculants and this could be the reason that solublizers' activity is associated with mineralization of P in soils and plays an important role in P cycling that improves plant growth by accumulating NPK in the plant tissue. Most tested inoculants indicated promising results in dual inoculation when evaluated to single inoculation. The sole inoculation and VC combination scenario depicts that P-uptake was greatly correlated with any growth parameter tested. But in a single inoculation treated with VC available P was correlated only with leaf area and total N was correlated with any growth parameters. But Non-significant relationships were observed with available K uptake. Inoculations with these potent indigenous microorganisms are in accord with contemporary views on the possible future role of plant growth-promoting and soil supporting bacteria and fungi in enhancing plant yields. Biofertilizer is eco-friendly and cost effective agro technology to improve crop production. Thus, there is pressing call for to minimize the use of inorganic fertilizers and make use of biofertilizers in large scale in agronomic practices to obtain better results.

This is a preliminary study a vast experimentation with different combination of other useful inoculants, fertilizer treatments and environmental factors are required to be done following this study. Even though the biochemical analysis in this study revealed the presence of a wide array of bacteria and fungi associated with Coffee plants and vermicompost, it is not enough to show the existing diversity of bacterial and fungal strains. Thus, further studies should focus on the use of molecular techniques in the identification of these bacteria and fungi to come out with efficient ways for incorporating the strains into biofertilizers to promote improved yield of coffee plants and sustainable agriculture

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