SINET: Ethiop. J. Sci., 44(2): 135–150, 2021 ISSN: 0379–2897 (PRINT)
 eISSN: 2520–7997

Date received: February 03, 2021; Date revised: May 05, 2021; Date accepted: May 10, 2021
DOI: https://dx.doi.org/10.4314/sinet.v44i2.1

Effect of phosphate solubilizing bio-inoculants and vermicompost application on mineral uptake and growth of coffee (*Coffea arbica L.)* seedlings under greenhouse condition

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ABSTRACT: Arabica coffee (*Coffea Arabica L.*) is an economically important crop with the highest export revenue in Ethiopia. This study was designed to evaluate the bacterial and fungal phosphate solublization efficacy and to determine yield attributes of coffee seedlings under glasshouse conditions. The study was conducted at Jimma Agricultural Research Center. The experiment was done with completely randomized design (CRD) in three (3) replications. Three potent bacterial isolates viz., $RCHVCB_1$, RScB1.19 and RMaB2.11 and three potent fungal isolates viz., RSCF1.19 RLVCF2 and RCHVCF2 were obtained from Jimma University, Veterinary Medicine, Microbiology Laboratory. The three bacterial isolates were tested for antimicrobial resistance pattern and for their potential to serve as bio-control agents. All the bacterial isolates showed 100% resistance to all the six antimicrobials tested. The growth of pathogenic *Fusarium xyloriodes* was slightly inhibited by RSCF1.19. Single inoculation of RSCF1.19+Phosphate fertilizer and dual inoculation of RSCF1.19 and $RCHVCB_1$ with P fertilizer significantly ($p<0.05$) increased plant height, root length, stem girth, leaf number, leaf area, fresh and dry weights of coffee seedlings. However, all the treatments combined with vermicompost showed suppressive characteristics with no seedlings emergency at all. RSCF1.19 and $RCHVCB_1$ can be recommended as bio-fertilizers after conducting necessary field trials in order to reduce the cost required for chemical fertilizers.

Keywords/phrases: Bio-inoculants, coffee seedlings, dual inoculation, phyto-beneficial microbes, single inoculation

INTRODUCTION

Arabica coffee *(Coffea arabica* L.) grows in Ethiopia, which is the place of its origin and hence its production and consumption are closely intertwined with Ethiopian history, culture and economy (Alemayehu Teshome *et al.*, 2008). Arabica coffee is the most important Ethiopian commodity and the principal source of revenue for the agricultural sector and greatly requires sustainable production with better product quality to persist in the present world market competition. The simplicity of production and low cost inputs requirements are needed for coffee production, particularly for farmers deprived of sufficient resources. The use of liquid organic fertilizers as important agricultural inputs to improve production and productivity of coffee on sustainable basis has been reported by Ormeño-Díaz et al.(2018). The use of biofertilizers in agriculture has proven to be eco-friendly, productive and accessible option to be utilized as agricultural inputs (Sheraz*et al.*, 2010). Accordingly, Tam *et al.*(2016) have isolated fungal P-solubilizers from different plant rhizosphere and carried out an investigation on their phosphate-solubilizing capabilities. In addition, Satyaprakash *et al.*(2017) have documented several strains of bacteria isolated from coffee rhizosphere that were capable of solublizing inorganic phosphate. 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

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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 *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 media 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. Among the bioelements, phosphorous is a macro-nutrient which plays an important role in plants in many physiological activities such as cell division, photosynthesis, and development of good root system and utilization of carbohydrate. Although a plenty of phosphates are applied to soil as chemical fertilizer, large portion of it becomes rapidly immobilized and becomes unavailable to plants in acidic and/or alkaline soils. This fixed phosphate becomes mobilized by the action of soil bacteria and fungi which are termed as phosphate solubilizing microorganisms. These phosphate solublizing microorganisms play a pivotal role in solublizing phosphates and make it to be available to the plants by allowing a sustainable use of phosphate fertilizers (Sundara et al., 2002). Coffee seedlings growth promotion can be attributed to many mechanisms through which rhizospheric bacteria and fungi contribute to sustainable production. For sustainable crops production, microbial phosphate solublization efficacy and their application in agriculture are getting greater attention due to their cost effectiveness and eco-friendly use. Chemical fertilizers pose health hazard and also are very expensive as agricultural inputs. On the other hand, application of phosphate solublizing bacteria and fungi in the field condition has been reported to increase crop yield. The current trends of bio-fertilizers knowledge widely focused on searching for better performing inoculants for better yield gain in farming sector. Hence, it necessitates a thorough research effort to obtain rhizosphere competent inoculants of phosphate solubilizing microbes (bacteria and fungi) for their decisive use for on-farm application.

Phosphate-solubilizing bacterial isolates (RCHVCB$_1$, RScB1.19, RMaB2.11) and fungal isolates (RSCF1.19, RCHVCF2, RLVCF2) which were isolated and screened under *in vitro* conditions (Reshid Abafita *et al.,* 2021) were used for *in vivo* trials under glasshouse condition prior to test in natural nursery environment as bio-fertilizers. Hence, the main objective of the present study was to test the bacterial and fungal phosphate solublization efficacy and their antibiotic susceptibility pattern as well as to evaluate their suppressive effect against coffee pathogenic *Fusarium xyloriodes* in order to enhance coffee seedlings growth and vigor.

MATERIALS AND METHODS

Location of study area

Glasshouse 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. It 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., 2016).

Determination of antibiotic susceptibility patterns of bacterial isolates

The microbial cultures viz., phosphate solubilizing bacteria (PSB) were obtained from Jimma University, College of Agriculture and Veterinary Medicine, Microbiology Laboratory (Reshid Abafita *et al.,* 2021) 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 24 h. 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).

Co-culture test between phytobeneficial microbes and pathogenic Fusarium xyloriodes

Co-culture assay was performed following the method described by Santiago *et al.* (2017). Each of the bacterial (RCHVCB$_1$, RScB1.19, RMaB2.11) and fungal (RSCF1.19, RCHVCF$_2$ AND 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.

Inoculums preparation

The characterized and identified phosphate solubilizing bacteria (PSB) and fungi (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˚C under shaking at 100 rpm 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˚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 (20 mL per 50 g of VC) and was incubated at 28±2˚C. For fungal noculum preparation, phosphate solubilizing fungal (PSF) isolates were mass cultured aseptically in 90 mm diameter Petri plates each containing 15 mL 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 10 mL 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 500 mL flask containing 400 mL 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 50 g of the sterilized carrier materials (VC) and immediately stored at 4^{o}C until use. The mixed and inoculated carrier material was evaluated for plant growth promotion as bio-inoculants.

Test crop, potting media, pot volume 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 Belachew *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, the 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 10 ml of sugar solution (1 spoon table sugar per 10 ml water) and thoroughly mixed with the seeds until the surfaces were uniformly coated. The nursery experiments were conducted from February to August of 2019 under controlled glasshouse condition at 28–32^{o}C in plastic pots having 19 cm height with 21 cm top and 18.5 cm bottom which were filled with 3.5 kg of sand. Vermin-compost was applied at the rate of 20% of potting media per pot (Reshid Abafita *et al*., 2014). The inorganic fertilizer treatment (4 g DAP/pots) was mixed with sand before sowing coffee seeds in the media. 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 1t ha^{-1} per year (Loga and Biscoe, 1987). Seedlings were thinned when they attained two pairs of true leaves and one uniformly growing seedling was left. The seedlings were grown in a glasshouse until the emergence of seven pairs of true leaves at a temperature of 28–32^{o}C and 85% relative humidity. 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. The glasshouse assay 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. The experiment was commenced under protected glasshouse condition using sand as the potting medium for the experiments.

Data Analysis

Data were collected in replicates 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 level using SAS was done and means were separated using Duncan multiple Range Test at 0.05 level. Data obtained from the different treatments were presented in the form of tables using Microsoft Excel 2007.

RESULTS

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 1; Figure 1). The potent isolates selected for bio-inoculants (RCHVCB$_1$, RScB1.19, RMaB2.11) were 100% resistant to all the six tested antimicrobials (Table 1).

Table 1.Effects of different antibiotics on the tested isolates.

Isolate code	Vancomycin (Van)	Ceftazidime (Ce)	Doxycycline (dxt)	Erythromycine (E)	penicilin G (PG)	Tetracycline (T)
	10 µg	10 µg	30 µg	15 µg	,10 unit	10 µg
RCHVCB$_1$	R	R	R	R	R	R
RScB1.19	R	R	R	R	R	R
RMaB2.11	R	R	R	R	R	R

Legend
R=resistance

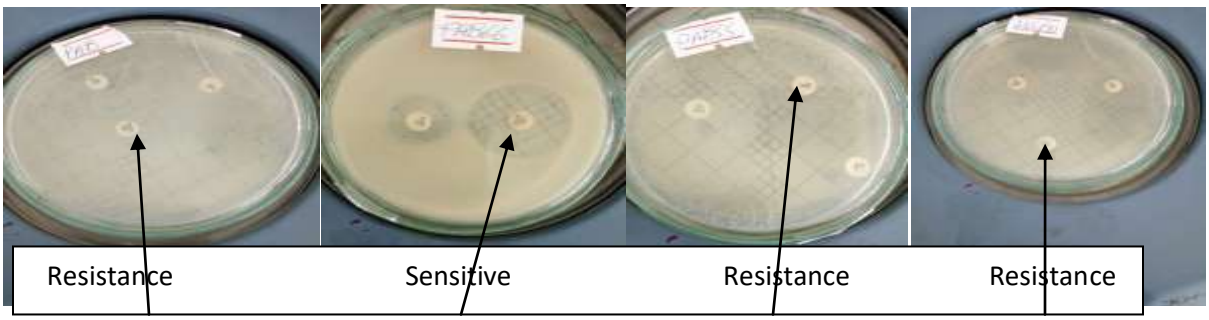


Figure 1. Pictorial presentation of antimicrobial susceptibility test results.

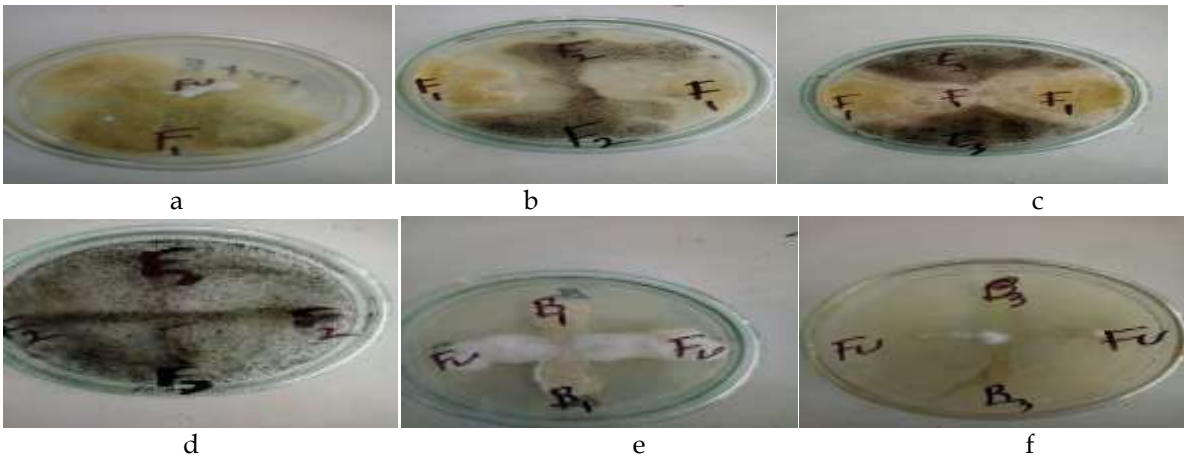
Co-culture test between Bacteria, fungi and pathogenic Fusarium xyloriodes

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 *Fusarium xyloriodes* to see whether they could co-exist or antagonistic to one another on agar plates (Figure 2). The growth of *Fusarium xyloriodes* was slightly inhibited by RSCF1.19 (Figure 2a). 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 2c), whereas the growth of RSCF1.19 was inhibited by RCHVCF2 (Figure 2b). The growth of both RLVCF2 and RCHVCF2 was not suppressed when both co-cultured on the same plates (Figure 2d), 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 2d). On the other hand, all the bacterial and fungal isolates showed no growth inhibition between each other (Figure 2e,f).

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).

Effect of bacterial and fungal inoculation on coffee seedlings

The individual application of phosphate solubilizing bacteria ($RCHVCB_1$, RScB1.19, RMaB2.1) and fungi (RSCF1.19, RCHVCF2, RLVCF2) isolates had effect on growth of coffee seedlings (Table 2). Combined inoculation of bacteria and fungi in the presence of inorganic chemical phosphate with the treatments of $RCHVCB_1$+P, RScB1.19+P, RMaB2.11+P, RCHVCF2+P and RLVCF2+P, respectively showed consistently increased growth parameters over the un-inoculated coffee seedlings. The greenhouse experiment results showed that inoculation with phosphate solubilizing fungi (RSCF1.19+P) significantly ($p \leq 0.05$) improved the growth parameters such as shoot height, stem diameter, leaf number, leaf area, fresh and dry weights compared to treatments such as $RCHVCB_1$+P, RScB1.19+P, RMaB2.11+P, RCHVCF2+P, RLVCF2+P and the controls (without bacterial and fungal inoculums and without inorganic phosphate fertilizer) (Table 2). The results showed that mean values of number of leaves were not significantly ($p \geq 0.05$) different among all the treatments except RSCF1.19+P. Generally, the effect of inoculation was comparable or greater than the chemical fertilizer and negative control. Isolate RCHVCF2+P showed significant effect on shoot length and fresh shoot biomass, dry shoot biomass but no effect on leaves, leaf area, root fresh and dry biomass as well as on stem girth of coffee seedling (Table 2).



a b c

d e f

Figure 2. Co-culture test between pathogenic Fusariumxyloriodes, fungal and bacterial isolates .

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The morphological growth performance of experimental plants was demonstrated in Figure 3. However, all the treatments combined with vermicompost showed suppressive characteristics with no any seedlings emergency (Figure 3).

Figure 3. Morphological growth performance of experimental plants, VC=Vermicompost,-Ve= Negative control ,+Ve=Positive control

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 3). 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 3).

Table 2.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
T test (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 a=5%, PSF=phosphate solubilizing fungi, PSB=phosphate solubilizing bacteria, *P=Chemical phosphate, -Ve=negative control, +Ve=positive control

Table 3. 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
T test(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 a=5%, *P=Chemical phosphate-Ve=negative control, +Ve=positive control

Table 4. 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 AvailableP (ppm)	TN (%)	K (ppm)	Nutrient uptake by plants 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,

Similarly, the co-inoculations of isolates RMaB2.11 and $RCHVCF_2$ as well as RMaB2.11 and $RLVCF_2$ in the presence of 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 ($p \leq 0.05$; Table3) over the controls, but lower plant growth parameters was obtained from both fungal and bacterial inoculations in the absence of inorganic phosphate indicating the lack of sufficient inorganic phosphate to be solubilized in potted medium.

Nutrient status of potting medium

After collecting the necessary data, the potting medium was analyzed for the physicochemical properties (pH, OC, Ex. Acidity, CEC) and nutrient status (Table 4 and 5). 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 4 and 5; Figure 3).

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 4 & 5). 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 4 and 5).

Nutrient uptake by coffee seedlings

The nutrient uptake of the coffee seedlings grown in potting medium is presented in Tables 4 and 5. 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 seedlings compared to bacterial bio-inoculants amended with inorganic chemical fertilizer treatments (Table 4). Inoculations of bacteria and fungus alone or in combination produced higher uptake of available N, P and K compared to the control (Table 5). 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.

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 6). 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 6). 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 were greatly correlated with P N uptake (r=0.448**, 0.682** and 0.457**, 0.983**, respectively; Table 7) and non-significant relationships were observed with all other growth parameters.

Table 5. 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			Nutrient uptake by plants		
						Available (ppm)	TN (%)	K (ppm)	Available P (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*

Table 6. 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)	Available P (ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	**1**											
Root length/plant(cm)	-0.149ns	**1**										
Shootfresh weight(gm)	-0.268ns	0.920**	**1**									
Root fresh weight(gm)	-0.196ns	0.967**	0.913**	**1**								
Shoot dry weight(gm)	-0.226ns	0.117ns	0.176ns	0.109ns	**1**							
Root dry weight(gm)	-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

Table 7. 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 wt (g)	Root fresh wt (g)	Shoot dry wt (g)	Root dry wt (g)	No of leaves	Stem Girth (mm)	Leaf area (sq.cm)	Available P (ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	**1**											
Root length/plant(cm)	-0.281ns	**1**										
Shoot fresh wt(gm)	-0.213ns	0.891**	**1**									
Root fresh wt(gm)	-0.233ns	0.966**	0.917**	**1**								
Shoot dry weight(gm)	-0.159ns	0.524**	0.410*	0.499**	**1**							
Root dry weight(gm)	-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**			
Available P(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**	
Available K(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

DISCUSSION

Determination of antibiotic susceptibility patterns of bacterial isolates

Isolates with multiple antibiotic resistances have greater advantage in establishing themselves as biofertilizers 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 twelve isolates RCHVCB$_1$, RScB1.19 and RM aB2.11 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-vitro co-culture test between bacteria, fungi and pathogenic Fusariumxyloriodes

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.

Effect of bacterial and fungal inoculation on coffee seedlings

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 fungus (RSCF1.19+P) compared to both positive and negative control. The higher growth parameters observed under bioinoculants 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 (Nahas, 1996; Mahadevamurthy *et al.,*2016). 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 *Pseudomonus* sp was also documented by Mamta *et al.*(2010). Research results documented by Prasad *et al.* (2014) significantly increased coffee seedlings growth when treated with *Azosprillum* sp*, Pseudomonas fluorescens* , phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal 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 bioinoculants 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 effect with no emerged seedlings. The suppressive characteristics can be attributed to the high pH value of the potting medium (vermicompost) (pH>7.5) (Reshid Abafita *et al*., 2014).

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 plant growth promoting microbes with other different beneficial properties could be the future trends of bio-fertilizers application for sustainable crop production. It is likely that phosphate solubilizing microbes (bacteria and fungi) might have helped 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*etal.,* 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.

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 *etal.* (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 biofertilizer 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 *Pseudomonqs* 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 indicate the presence of a diverse group of phosphate solubilizing microbes that dwell in the rhizosphere of coffee plants and vermicompost amendment. It is evident that phosphate solubilizing microbes are widely distributed and showed significant variations 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 *Pseudomonas* spp and *Bacillus* spp. as biofertilizers could enhance the phosphate solubilization in 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 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. Therefore, further field studies are required to confirm the application of these microorganisms to sustain maximum organic coffee yields.

CONCLUSION

The results obtained from glasshouse experiments demonstrated that addition of inorganic phosphate to the soil and inoculation with native PSB and PSF significantly increased plant growth by enhancing nutrient uptake (N, P, and K) in coffee seedlings. One of the fungal isolates, RSCF1.19 has shown better characteristics of plant growth promotion than the bacterial inoculums and thus has a greater potential for use as biofertilizer in coffee production. Moreover, co-inoculation of this fungus with *Pseudomonas* (RCHVCB$_1$+RSCF1.19) showed statistically significant growth parameters compared to the control. Similarly, the nutrient status of potting medium and nutrient uptake by coffee seedlings increased in the treatments that received both fungal and bacterial inoculants amended with inorganic P fertilizer. The results suggested that phosphate solubilizing microbes have better potential as bio-fertilizers under greenhouse conditions and needs field trials for their bio-inoculums production.

ACKNOWLEDGEMENTS

This work was supported by Ethiopian Institute of Agricultural Research and Agricultural Growth Program Component II (AGP II). We would like to thank also Jimma University, School of Veterinary Medicine for their provision of laboratory facilities.

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