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Effect of Phosphate-Solubilizing Bacteria and Fungi, Mineral Phosphate and Vermicompost Application on Major Soil Chemical Characteristics, Mineral Uptake and Growth of Coffee (*Coffea arabica L.*) Seedlings under Nursery Condition

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ይህ ጥናት የተዘጋጀው የባክቴሪያ እና የፈንገሶች ፎስፌት ማዳበሪያ ቀማሪነትና የአሚሚነት ብቃታቸውን መገምገም፣ የቡናን ምርታማነት አመላካች ባህርይ በሆነው የቡና ችግኝ ዕድገት ላይ በቡና ችግኝ ማፊያ ጣቢያ ደረጃ መፈተሸና አጅግ በጣም ጥሩ ብቃት ያሳዩትን መምረጥ ነበር። ጥናቱ የተከናወነው ከየካቲት እስከ ነሐሴ ወር 2011 ዓ.ም. በጅማ ግብርና ምርምር ማዕከል የቡና ችግኝ ማፍያ ፕላስቲክ በመጠቀም ነበር። ይህ ሙከራ የተከናወነው ፍፁም በዘፈቀደ አሠራር እያንዳንዱን ተጠኝ በሶስት ቅጅ በመደጋገም ነበር። ሙከራው 4 ስብስቦች ነበሩት፣ 1ኛው ስብስብ ሁለት ምድብ ያለው ፎስፌት ማዳበሪያ አሚሚ ባክቴሪያና ፈንገሶች ምድብ እና ፎስፌት ማዳበሪያ ከፎስፌት አሚሚ ባክቴሪያና ፈንገሶች ጋር የተጣመረ ምድብ፣ 2ኛው ስብስብ 3ባክቴሪያዎችና 3ፈንገሶች እና ሁለት ደረጃ ያለው ፎስፌት የተጣመረበት፣ 3ኛው ስብስብ በነጠላ 6ቱ ፎስፌት ቀማሪ ባክቴሪያና ፈንገሶች 1.5 ኪ..ግ አፈር ከ300ግራም ቨርሚኮምፖስት ጋር የተደባለቀ፣ 3ኛው ስብስብ ባለ2 ፋክቶሪያል ሙከራ ሆኖ (3 ደረጃ ያላቸው ፎስፌት ቀማሪ ባክቴሪያና ፈንገሶች እና 3 ደረጃ ያላቸው ፎስፌት ቀማሪ ባክቴሪያና ፈንገሶች ከቨርሚ ኮምፖስት ጋር የተጣመረበት) ነበር። ሁሉም የሙከራ ስብስቦች ኒጋቲቭ እና ፖዝቲቭ ማነፃፀሪያ የነበራቸው ሲሆን ስብስብ 3 እና 4 በተጨማሪ ቨርሚኮምፖስት ብቻውን እንደማነፃፀሪያ ነበራቸው። ባክቴሪያና ፈንገሶች ከፎስፌት ማዳበሪያ ጋር ሲጣመሩ የላቀ ትርጉም ያለው በተክሎች ዕድገት ላይ አሳይቷል። በተጨማሪም RSCF1.19 ከ3ቱ ባክቴሪያዎች (RCHVCB, RScBl.19, RMaB2.11) ጋር ተዋህደው በአንድ ላይ ፎስፌት ማዳበሪያ ጋር ሲዘሩ በቡና ችግኝ ዕድገት ላይ እጅግ የላቀ ውጤት ተመዝግቧል። በአጠቃላይ አብላጫ ያለው የተክሎች ዕድገት ባህሪይ መለኪያ የሆኑ ነገሮች በነጠላና በጥምረት አጨማመር ጊዜ ከቨርሚኮምፖስት ጋር በመጣመሩ ምክንያት ከነጋቲቭና ፖዝቲቭ ማነፃፀሪያ ጋር ሲወዳደር ጭማሪ ዕድገት አሳይቷል። በጥቅሉ ትርጉም አዘል ውጤት ያልሆነ ነገር ግን ከፍተኛ የሆነ N,P እና K አጠቃቀም በተፈጥሮ ማዳበሪያ ቀማሪ ባክቴሪያ፣ ፈንገሶችና ሰው ሰራሽ ፎስፌት ማዳበሪያ ጥምረት ጊዜ ከነጋቲቭና ፖዝቲቭ ማነፃፀሪያ ጋር ሲወዳደር ብልጫ ያለው ውጤት ተመዝግቧል። ቨርሚኮምፖስት ከRSCFl.19 እና RLVCF2 ፈንገሶች ጋር ሲጣመሩ እጅግ የገዘፈና ጤናማ የሆነ የአረቢካ ቡና ችግኝ በደቡብ ምዕራብ ኢትዮጵያ አፈር ላይ ለማሳደግና ለማምረት የተመረጠ ሆኖ ተገኝቷል።

Abstract

This study was designed to evaluate the efficiency, select the best performing bacteria and fungi isolates for phosphate solublization and yield attributes of coffee seedlings under nursery condition. The study was conducted at Jimma Agricultural Research Center during February to August 2019, in plastic pots. This study was carried out in CRD in three replications per treatments. The experiment has 4 sets where set 1 had the bio-inoculant factors and phosphate factor combined factorially. Set 2 had 3 factors (3PSF, 3PSB and 2 p levels) in factorial combinations. Set 3 has single factor (the 6 inoculants under basal VC (300g/1.5kg soil). Set 4 was a two factor trial: PSF (3 levels) and PSB (3 levels) combined factorially under basal VC. All sets had negative and positive (rec. NP) controls, and set 3 and 4 had sole VC as additional control unit. Bio-inoculant and phosphate combination were significantly responded to all growth attributes. Moreover, co-inoculation of RSCF1.19 with three bacterial isolates ($RCHVCB_1$, RScB1.19, and RMaB2.11) in combination with phosphate led to significantly higher tested growth parameters. Similar increase in growth attributes were observed in both single and dual inoculation due to VC used when compared with both positive and negative control. Non-significant but higher NPK-uptake were observed in a combination of bio-inoculants and phosphate fertilizer compared to the positive and negative control. The combination of VC with RSCF1.19 and $RLVCF_2$ fungal isolates can be recommended as bio-inoculants for solublizing inorganic phosphate and to obtain vigor and healthier coffee seedlings in south west soil of Ethiopia for coffee Arabica cultivation.

Keywords: Arabica coffee, vermicompost, bio-inoculants, growth parameter, mineral uptake, single inoculation, co-inoculation

Introduction

Coffea arabica L. is the most important world cash crop, the principal source of revenue for the Ethiopian government as well as small scale coffee producers. It is also economically more profitable than any other cash crop or cereal crop and contributes more than 35% of the total export earnings and the leading export commodity (FAO/WFP, 2008).Therefore, the local coffee producing farmers of Jimma zone districts greatly requires sustainable coffee production with low cost inputs to obtain organic products which can guarantee them to sustain their product quality in the world markets. 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). Coffee rhizosphere and vermicompost are associated with large number of beneficial microorganisms including PSB and PSF which may contribute to nutritional requirement of the plant (Kunwar *et al.*, 2018; Suhane, 2007). Indeed bacterial (*Pseudomonads and Bacilli*) and fungal (*Aspergillus* and *Penicillium* sp.) isolates exhibiting P solubilizing activity have been as best phosphate solublizing microbes and means for P nutrition of crop

(Sharma *et al.,* 2013). These fates of microorganisms are not understood in the Ethiopian coffee production and management system. 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. Since bacteria and fungi capable of solublizing limited amount of phosphate are not always present in the soil, artificial inoculation may be necessary. Although phosphates are abundant in soils, they occur mostly in insoluble forms which is not unavailable to plant nutrition and hence it is important to add inorganic chemical fertilizer to satisfy plant nutrition requirement. However since 75-90% of added phosphate is precipitated by formation of metal cation complexes (Mahantesh and Patil, 2011; Sharma *et al*., 2013), it is necessary to search other option to release by fixing the bind complexes. 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. Biofertilizer is eco-friendly and cost effective agro technology to improve crop production. 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. 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. Even though much work has been done on in vitro bacteria and fungi phosphate solublization and plant growth promotion, very few reports are available related to isolation of phosphate solublization and plant growth promoting bacteria and fungi from coffee rhizosphere (Muleta *et al.,* 2013; Kunwar *et al.,* 2018) and vermicompost (Suhane, 2007) and its impact on the plant growth particularly coffee (*Coffea Arabica L*) under field condition. Therefore, in the present study bacterial and fungal isolates were used for field application under shade net nursery condition to assess their nutrient uptake and coffee seedling growth promotion efficacy using coffee seedling as test crop.

Material and Methods

Study location

Nursery coffee seedlings raising experiment was conducted under shade net to evaluate the practical application of three selected bacterial ($RCHVCB_1$, RScB1.19, RMaB2.11 and three fungal (RSCF1.19, RCHVCF2, RLVCF2) isolates followed with all strict necessary plant care practices to determine the plant growth promotion potential of these isolates on coffee seedlings at Jimma agricultural research center.

Sources of isolates and inoculants preparation

Three bacterial and three fungal inoculums were obtained from preserved stock culture which initially isolated from coffee rhizosphere and vermicompost, were identified based on morphological and biochemical characteristics and presumptively identified as genera of *Pseudomonas (*$RCHVCB_1$) and *Bacillus* (RScB1.19 and RMaB2.11). Similarly, chosen fungal isolates were characterized and identified as genera *Penicillium* (RSCF1.19) and *Aspergillus* (RCHVCF2 and RLVCF2) has been previously deposited in the Jimma University Veterinary Medicine Microbiology laboratory (Reshid 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℃ 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^{o}C and 15 psi pressure for one 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^{o}C. For fungal inoculums preparation, phosphate solubilizing fungal (PSF) isolates were mass cultured aseptically in 90 mm diameter Petri plates each containing 15 mL of autoclaved PVK (Roychowdhury *et al.,* 2015). The plates were incubated at 28 ±2°C for 10 days. On the tenth day, spore suspensions from the fungal isolates was prepared by flooding the surface of the agar with 10 mL sterile distilled water and the culture surface 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 of fungal culture was used per 50 g of the sterilized carrier material (VC) and immediately stored at 4ºC until use (Roychowdhury *et al.,* 2015; Murali *et al.,* 2016*).*

Test crop, potting media and research design

The test crop used was Coffee variety 74110which were Coffee berry disease (CBD) tolerant, high yield bearing and released variety obtained from Jimma agricultural research center. This variety is suitable for medium altitudes and collected from Ilu ababor in Eastern Ethiopia district (Balachew *et al*., 2008). Endocarp (parchments) was manually removed (Guimarães *et al*., 2013) and the Coffee seeds were surface sterilized with 75% ethanol for 1min, followed by 1% sodium hypochlorite for 30min, and extensive wash with sterile distilled water (Collavino *et al*., 2010).

The surface sterilized seeds of *coffee Arabica L.* were placed in a sterile dish and mixed with 4ml of bacterial and fungal inoculants and left for 6 hours incubation. Moreover, these inoculated seeds of *coffee Arabica L.* stored in polyethylene bag and also inoculated with carrier based inoculums at a rate of 15g/100g seeds (Mohamed and Almaroai, 2017) after moisten with 10ml of sugar solution (1spoon table sugar per 10ml water) and thoroughly mixed with the seeds until uniformly surface coated. 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 (1934) method 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 media was recorded at the end of the trial (Prasad *et al.*, 2014).To evaluate the phosphate solublization and plant growth promotion efficiency of three bacterial and three fungal inoculums, a 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. The vermicompost was applied at the rate of 20% (300gm/plastic bag) of potting media per bags (Reshid *et al.,* 2014).The inorganic fertilizer treatment (4gm DAP/bags) for positive control was mixed with 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 are 1t ha^{-1} per year (Loga & Biscoe, 1987). Seedlings were thinned when they attained two pairs of true leaves and one good growing seedling was left. When plants have three pairs of true leaves they 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 was measured using standard procedure. The nursery assay was done with completely randomized design (CRD) in three (3) replications per treatments 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 respectively. 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 shade net nursery condition using soil as the potting media for the experiment.

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 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 and Discussion

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) (Table.1A). 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 1. Characteristics of the applied soil used for potting media

No	Measured indexes	
1	%sand	59
2	%clay	40
3	%silt	1
4	Textural class	Sandy Clay
5	pH	5.62
6	Available P (ppm)	0.28
7	Total Nitrogen (%)	0.08
8	Organic carbon (%)	0.86
9	Ex. Acidity (meq/100g	0.16
10	K(%)	102.86
11	CEC (meq/100g	13.78

The soil reaction of the study site was moderately acidic (pH 5.62) based on pH in H_2O (London, 1991), which fall in a range of acidic soil condition, which is favorable for coffee 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 satisfy crop nutritional requirements,

phosphorus is usually added to soil as chemical phosphate fertilizer. However, plants can use only a small amount 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 media 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.

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) (Table2A). 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. Phosphate 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. The increase in shoot length, root length, shoot dry weight, and root dry weight of coffee seedlings inoculated with PSF isolates (RSCF1.19+P) could be attributed to a greater absorption of nutrients, especially P (Jain *et al*., 2010). Moreover, sole inoculation of both bacterial and fungal inoculants increased growth parameters over the phosphate supplied seedlings (+Ve control). Besides, sole bacterial and fungal treated did not show a significant difference in growth parameter (Table 2A). In this nursery condition, 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.

Table 2A. Growth response of coffee seedlings to sole PSF and PSB inoculants under nursery condition

Treatments	Shoot length/plant (cm)	Root length/plant (cm)	Shoot fresh weight (gm)	Root fresh weight (gm)	Shoot dry weight (gm)	Root dry weight (gm)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)
$RCHVCB_1$	10.50h	18.33ab	5.61e	0.97d	1.65e	0.49b	8.00de	2.07f	6.38e
RScB1.19	12.10fgh	19.33ab	5.81e	1.07cd	1.87de	0.53ab	7.333e	2.28ef	8.16cde
RMaB2.11	15.17cdef	19.67ab	8.35cde	1.50abcd	2.84bcde	0.68ab	8.67cde	2.61bcde	8.55cde
RSCF1.19	12.50fgh	19.67ab	6.60de	1.23bcd	2.18cde	0.58ab	8.00de	2.30def	9.31cde
RCHVCF2	13.33efgh	19.33ab	7.32de	1.30bcd	2.58bcde	0.61ab	10.00abc	2.49cdef	8.25cde
RLVCF2	11.83gh	19.00ab	5.70e	1.37bcd	1.82de	0.58ab	9.33bcd	2.39def	7.68de
$RCHVCB_1$+P	14.67defg	18.33ab	11.17bc	1.87abc	3.27bc	0.63ab	10.00abc	2.57cde	13.94bc
RScB1.19+P	14.33defg	20.67a	9.02bcd	1.50abcd	2.75bcde	0.60ab	8.00de	2.39def	11.48bcde
RMaB2.11+P	17.50bcd	20.00ab	11.57b	1.97ab	3.70ab	0.74ab	11.33a	2.79bcd	12.50bcd
RSCF1.19 +P	19.33ab	17.33b	11.33b	1.93ab	4.74a	0.81ab	10.00abc	2.92abc	11.81bcde
RCHVCF2+P	14.00efg	18.80ab	10.42bc	1.63abcd	2.92bcde	0.68ab	10.67ab	2.55cdef	9.31cde
RLVCF2+P	20.83a	19.00ab	15.09a	2.23a	4.70a	0.87a	10.67ab	3.32a	20.20a
-ve control	16.00cde	18.67ab	8.88bcd	1.73abcd	2.95bcd	0.74ab	10.00abc	2.57cde	12.69bcd
+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
Test(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, gm=gram, -Ve=Negative, +Ve=Positive, CV= Coefficient of variance, LSD=Least significance different

During single inoculation, RLVCF2 was distinguished as the most effective candidate fungal isolates. 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 & 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).

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 RScB1.19under 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 2B). 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 (Table 2B). 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. This revealed that 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 natural niche (Bonfante and Genre, 2010). 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 2B). This shows that the positive impact of the availability of nutrients towards coffee seedling establishment and vigor development. Therefore, variations in plant growth due to bacterial and fungal inoculation may be attributed to their phosphate solubilization potential and availability of phosphate as well. The increased in plant growth parameters when combined with chemical phosphate as the sole P source indicated the presence of phosphate-solubilizing traits and availability of the P. This was justified with the finding of Sharma *et al*. (2013) and Gaind, (2016), who discovered 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.

Table 2B. Growth response of coffee seedlings to dual inoculation of PSF and PSB isolates under nursery condition

Treatments	Shoot length (cm)	Root length (cm)	Shoot fresh wt (gm)	Root fresh wt (gm)	Shoot dry wt (gm)	Root dry wt (gm)	No of leaves	Girth (mm)	Leaf area (sq.cm)
RCHVCB$_1$**+** RSCF1.19	13.50ef	19.67abc	6.24g	1.53bcdefg	2.15bcde	0.66bcdef	10.00ab	2.67abc	9.87cde
RCHVCB$_1$**+** RCHVCF2	14.33def	19.00abc	7.37efg	1.43cdefg	2.70abcde	0.65cdef	8.67b	2.43bc	6.64e
RCHVCB$_1$**+** RLVCF2	12.83f	18.33abc	6.57fg	0.80h	2.26bcde	0.39f	9.33ab	2.38c	8.63de
RScB1.19**+** RSCF1.19	14.33def	18.33abc	6.63fg	1.30efgh	2.54abcde	0.65bcdef	9.33ab	2.46bc	11.10bcde
RScB1.19**+** RCHVCF2	14.33def	19.67abc	8.22defg	1.47cdefgh	2.5abcde	0.64cdef	10.00ab	2.47abc	10.64bcde
RScB1.19**+** RLVCF2	14.33def	19.00abc	10.01cdef	1.67bcdefg	2.99abc	0.83abcde	10.00ab	2.72abc	10.23cde
RMaB2.11**+** RSCF1.19	14.67cdef	17.67bc	5.45g	1.03gh	1.92bcde	0.50ef	8.67b	2.58abc	10.31cde
RMaB2.11**+** RCHVCF2	13.67ef	18.67abc	6.96fg	1.17gh	2.30bcde	0.57def	10.00ab	2.46abc	11.09bcde
RMaB2.11**+** RLVCF2	15.17bcdef	18.00abc	5.49g	1.40defgh	2.08bcde	0.58def	9.33ab	2.43bc	13.41abcd
RCHVCB$_1$+ RSCF1.19 +P	20.17a	19.67abc	11.91abc	2.03bcd	2.88abcd	0.97abc	10.67ab	3.04ab	15.90abc
RCHVCB$_1$+ RCHVCF2 +P	18.67ab	19.00abc	13.83ab	2.13bc	3.15ab	0.81abcde	10.67ab	2.94abc	14.15abcd
RCHVCB$_1$+ RLVCF2+P	16.67abcdef	19.33abc	11.57bcd	2.20b	3.59a	0.93abcd	10.67ab	2.93abc	13.17abcde
RScB1.19+ RSCF1.19 +P	18.67ab	19.67abc	15.12a	3.84a	2.88abcd	1.13a	10.67ab	3.07a	16.40abc
RScB1.19+ RCHVCF2+P	15.33bcdef	19.67abc	9.89cdef	1.73bcdefg	1.73de	0.63cdef	10.00ab	2.71abc	13.13abcde
RScB1.19+ RLVCF2+P	16.83abcde	19.33abc	11.31bcd	1.93bcde	1.93bcde	0.80abcde	11.33a	2.96abc	18.90a
RMaB2.11+ RSCF1.19+P	14.50cdef	20.00ab	8.13defg	1.50bcdefgh	1.50e	0.60cdef	10.67ab	2.79abc	11.03bcde
RMaB2.11+ RCHVCF2+P	15.83bcdef	17.33c	11.18bcd	1.80bcdef	1.80cde	0.77abcdef	10.00ab	2.57abc	18.83a
RMaB2.11+ RLVCF2+P	18.00abcd	20.33a	11.92abc	2.07bcd	2.07bcde	1.04ab	10.67ab	3.08a	14.50abc
-ve control	16.00bcdef	18.67abc	8.88cdefg	1.73bcdefg	2.95abcd	0.74bcdef	10.00ab	2.57abc	12.69abcde
+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 ≤ 0.05, P=phosphorus, cm=centimeter, mm=millimeter, gm=gram, -Ve=Negative, +Ve=Positive, CV=Coefficient of variance, LSD=Least significance difference

These shows that the solubility and 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 due to 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. 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.*

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 2C&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 2C).

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 2D). 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. Briefly, co-inoculation of bacterial and fungal isolates with vermicompost failed to influence root length and stem girth and could not able show difference over the negative or positive controls (Table 2D). Lower response to added inoculants may be due to failure to compete with the indigenous microorganisms and the soil pH stress (Kutcher *et al.,* 2002). Moreover, colonization of soil by non indigenous microorganism 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).

Table 2C. Performance of sole inoculation of bacteria and fungi under vermicompost

Treatments	Shoot length(cm)	Root length(cm)	Shoot fresh wt(gm)	Root fresh wt(gm)	Shoot dry wt(gm)	Root dry wt(gm)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)
RCHVCB$_1$+VC	22.67ab	19.67a	19.31a	2.27abc	5.69ab	0.92a	11.33ab	3.09ab	26.96ab
RScB1.19+VC	21.33abc	18.67ab	20.70a	2.43abc	6.55ab	1.17a	12.67a	2.98ab	23.03abc
RMaB2.11+VC	24.33a	18.67ab	19.12a	3.10ab	5.64ab	1.26a	12.67a	3.59a	30.23a
RSCF1.19 +VC	21.33abc	17.33abc	19.86a	3.47a	4.57ab	1.37a	12.00ab	3.58a	16.53bc
RCHVCF2+VC	21.33abc	20.00a	19.64a	3.53a	7.47a	1.43a	12.67a	3.25ab	22.83abc
RLVCF2+VC	18.67bc	15.00c	18.67a	2.53abc	5.99ab	1.02a	10.67ab	2.97ab	21.72abc
-ve control	16.00c	18.67ab	8.88b	1.73c	2.95b	0.74a	10.00b	2.57b	12.69c
+ve control	18.33bc	19.00ab	10.59b	1.93bc	3.45ab	0.75a	11.33ab	3.08ab	17.19bc
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, gm=gram, VC=vermicompost, -Ve=Negative, +Ve=Positive, CV= Coefficient of variance, LSD=Least significance difference

Table 2D. Dual inoculation combined with Vermicompost

Treatments	Shoot length(cm)	Root length(cm)	Shoot fresh wt(gm)	Root fresh wt(gm)	Shoot dry wt (gm)	Root dry wt (gm)	No of leaves	Stem Girth (mm)	Leaf area(sq.cm)
RCHVCB$_1$+ RSCF1.19+VC	24.00ab	16.00a	25.17ab	3.63a	8.76a	1.64a	13.33a	3.32a	25.50ab
RCHVCB$_1$+ RCHVCF2 +VC	23.67abc	16.00a	20.80bc	3.17abc	6.36abc	1.23ab	12.00ab	3.42a	28.59a
RCHVCB$_1$+ RLVCF2+VC	23.00abc	19.33a	26.91a	3.50ab	8.35a	1.48a	11.33ab	3.35a	23.52ab
RScB1.19+ RSCF1.19+VC	22.67abc	19.00a	20.17bc	3.20abc	6.33abc	1.32ab	12.00ab	3.39a	26.81a
RScB1.19+ RCHVCF2+VC	25.67a	19.00a	23.91ab	2.70abc	6.77ab	1.35ab	12.67ab	3.66a	30.68a
RScB1.19+ RLVCF2+VC	24.83a	17.67a	22.10abc	3.80a	7.20ab	1.34ab	12.00ab	3.13ab	30.01a
RMaB2.11+ RSCF1.19 +VC	18.00cd	16.67a	14.22de	2.43abc	6.79ab	1.15ab	11.33ab	3.07ab	19.50ab
RMaB2.11+ RCHVCF2+VC	23.50abc	18.00a	22.14abc	3.23abc	7.47ab	1.40ab	11.33ab	3.30a	20.00ab
RMaB2.11+ RLVCF2+VC	22.00abc	19.07a	21.73bc	3.33abc	6.34abc	1.21ab	12.00ab	3.36a	22.53ab
-ve control	16.00d	18.67a	8.88f	1.73c	2.95c	0.74b	10.00b	2.57b	12.69b
+ve control (reco.NP)	18.33bcd	19.00a	10.59ef	1.93bc	2.95c	0.75b	11.33ab	3.08ab	17.19ab
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
T test(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 ≤ 0.05, P=phosphorus, cm=centimeter, mm=millimeter, gm=gram, VC=vermicompost, rec.NP=recommended urea and P, -Ve=Negative, +Ve=Positive, CV= Coefficient of variance, LSD=Least significance difference

Nutrient status of potting media

Once data collection was over, the potting media were analyzed for the physicochemical properties (pH, OC, Ex. Acidity, CEC) and nutrient status. The results are presented in Table 3A, 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 3A, B, C). This reduction 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). The vermicompost supplied treatments made the experimental soil neutral pH (Table 3C&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 3A&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 3C&D). The higher percent of 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. Moreover, 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 (Bhattacharyya and Jha, 2012; Ansari *et al.*, 2013). Several soil bacteria and fungi, notably species of *Pseudomonas, Bacillus, Penicillium, Aspergillus etc* secrete organic acids and lower the pH in their vicinity to bring about dissolution of bound phosphates in soil (Gaind, 2016; Sharma *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). 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 1A and 3A, 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 3 A&B). However, there was drastic increase in the CEC in the treatments receiving bio-inoculants amended with vermicompost (Table 3C & D).

Table: 3A: Chemical properties of potting media after destructive data taken 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		
					Available P(ppm)	TN (%)	K (ppm)	Available P (ppm)	TN (%)	K (ppm)
$RCHVCB_1$	6.06	0.80	0.38	13.00	0.22	0.05	92.86	121.25	0.31	2298.92
RScB1.19	6.30	0.76	0.34	13.12	0.27	0.08	102.00	120.21	0.30	2288.90
RMaB2.11	6.16	0.81	0.34	13.14	0.21	0.06	100.81	123.25	0.31	2198.92
RSCF1.19	6.10	0.86	0.36	13.10	0.28	0.07	99.86	124.45	0.33	2806.11
RCHVCF2	6.31	0.82	0.31	13.20	0.24	0.06	101.86	124.05	0.32	2706.16
RLVCF2	6.23	0.78	0.35	13.40	0.23	0.08	98.86	120.12	0.30	2800.00
$RCHVCB_1$+P	5.84	1.05	0.48	14.18	0.69	0.07	97.91	135.50	0.41	3277.40
RScB1.19+P	5.47	0.95	0.51	20.70	0.76	0.08	105.51	130.45	0.40	3177.44
RMaB2.11+P	5.40	1.14	0.59	13.14	1.12	0.06	103.00	129.35	0.41	3100.40
RSCF1.19+P	5.30	1.04	0.46	13.84	2.40	0.08	104.43	138.80	0.43	3299.50
RCHVCF2+P	5.06	1.00	1.06	13.9	1.78	0.07	103.25	128.82	0.40	3199.50
RLVCF2+P	5.07	1.00	1.22	13.14	2.89	0.05	103.84	129.80	0.40	3190.40
-ve control	6.33	0.72	0.26	12.34	0.20	0.08	45.97	118.32	0.21	2123.44
+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

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 4A, B). 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 (Salvioli *et al*., 2012) 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 4A,B, C,D). 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. PSM application has been reported to show an increase in the amount of available P in the rhizosphere soil in the findings of (Taalab and Badr, 2007). The available P status of the potting media was lower due to exclusion of P fertilizers from negative control. The increased availability of K in all treatments over negative control may be due to the substantial amount of K already present in the experimental soil and also may be attributed to the interaction of clay with potassium to increase the available K status of the experimental soil itself (Table 1A). 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. This may be attributed to 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 manures.

Table: 3B: Chemical properties of potting media after destructive data taken 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		
					Available P(ppm)	TN (%)	K (ppm)	Available P (ppm)	TN (%)	K (ppm)
RCHVCB$_1$**+** RSCF1.19	6.10	0.60	0.31	13.41	0.19	0.07	89.66	130.25	0.30	2290.92
RCHVCB$_1$**+** RCHVCF2	6.31	0.63	0.37	13.40	0.18	0.05	96.61	130.21	0.30	2288.90
RCHVCB$_1$**+** RLVCF2	6.30	0.61	0.38	13.38	0.17	0.06	89.64	131.25	0.31	2198.92
RScB1.19**+** RSCF1.19	6.10	0.62	0.37	13.42	0.18	0.07	96.62	134.45	0.35	2806.11
RScB1.19**+** RCHVCF2	6.00	0.59	0.35	13.41	0.17	0.07	97.45	124.45	0.32	2706.16
RScB1.19**+** RLVCF2	6.48	0.60	0.32	13.40	0.16	0.05	99.61	134.12	0.30	2800.00
RMaB2.11**+** RSCF1.19	6.20	0.63	0.36	13.42	0.17	0.06	97.66	139.05	0.36	2290.90
RMaB2.11**+** RCHVCF2	6.20	0.61	0.38	13.39	0.18	0.05	98.00	128.15	0.31	2190.91
RMaB2.11**+** RLVCF2	6.10	0.60	0.33	13.41	0.18	0.05	99.60	129.05	0.30	2150.94
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
RCHVCB$_1$+ RCHVCF2+P	5.03	1.16	1.83	14.2	2.46	0.06	97.11	154.70	0.44	3190.94
RCHVCB$_1$+ RLVCF2+P	5.36	1.57	0.67	14.4	1.58	0.06	84.01	150.71	0.46	3180.94
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
RScB1.19+ RCHVCF2+P	5.27	1.09	0.50	15.68	1.55	0.10	99.29	150.02	0.47	3103.01
RScB1.19+ RLVCF2+P	5.14	1.57	1.12	14.04	2.19	0.08	98.77	147.02	0.49	3203.02
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
RMaB2.11+ RCHVCF2+P	5.13	1.04	1.33	14.24	2.40	0.06	90.04	152.41	0.44	3208.29
RMaB2.11+ RLVCF2+P	5.10	1.25	1.35	15.76	2.04	0.07	91.46	149.46	0.40	3102.26
-ve control	6.40	0.50	0.26	12.34	0.24	0.08	46.90	128.31	0.21	2023.44
+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 3C: Chemical properties of potting media after destructive data was taken in Single inoculation combined with vermicompost and nutrient Up-take by plants

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)
RCHVCB$_1$+VC	5.95	2.36	0.37	15.98	30.89	0.11	102.35	143.82	0.30	3084.06
RScB1.19+VC	6.22	2.41	1.58	17.18	40.48	0.14	99.36	140.72	0.31	2985.16
RMaB2.11+VC	6.36	1.82	0.29	14.06	41.27	0.15	95.22	143.82	0.30	2874.46
RSCF1.19 +VC	5.89	1.48	0.26	18.12	42.46	0.08	97.96	207.65	0.38	3235.58
RCHVCF2+VC	6.42	1.82	0.26	18.58	54.39	0.21	96.64	147.60	0.38	3035.50
RLVCF2+VC	6.23	1.66	0.34	17.28	33.73	0.16	97.00	149.61	0.39	2935.58
-ve control	6.35	0.54	0.26	12.34	0.24	0.08	56.98	128.41	0.21	2123.40
+ve control	6.59	1.48	0.29	14.54	0.54	0.08	96.28	132.41	0.31	2523.44
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 3D: Chemical properties of potting media after destructive data taken 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)
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
RCHVCB$_1$**+** RCHVCF2 +VC	6.18	1.20	0.24	16.54	39.46	0.16	99.52	141.40	0.30	3154.00
RCHVCB$_1$**+** RLVCF2+VC	6.28	2.15	0.26	16.68	30.92	0.13	99.09	146.56	0.31	2954.10
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
RScB1.19**+** RCHVCF2 +VC	6.30	1.89	0.29	18.32	31.46	0.13	98.28	143.30	0.28	2996.02
RScB1.19**+** RLVCF2+VC	6.31	1.95	0.31	17.94	59.10	0.14	99.17	141.36	0.29	3096.00
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
RMaB2.11**+** RCHVCF2+VC	6.40	2.01	0.24	19.88	59.97	0.15	100.46	140.53	0.31	3140.64
RMaB2.11**+** RLVCF2+VC	6.40	2.07	0.34	16.88	67.50	0.12	97.57	139.63	0.27	2941.74
-ve control	6.34	0.50	0.26	12.34	0.23	0.08	46.98	125.41	0.20	2123.44
+ve control (reco.NP)	5.59	1.48	0.29	14.54	0.54	0.08	96.28	133.31	0.29	2823.24
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

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 3A, 3B). 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 3C, 3D). The poor growth of coffee seedlings observed 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 which is essential for establishment of externally supplied microbes in the form of bio-inoculants.

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 4A and 4C). 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 4C). 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 4A). 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 4B). 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 4D).

Table 4A: 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(gm)	Root fresh weight (gm)	Shoot dry weight (gm)	Root dry weight (gm)	No of leaves	Stem Girth (mm)	Leaf area(sq. cm)	Availabl e P (ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	**1**											
Root length/plant(cm)	0.176ns	**1**										
Shoot fresh weight(gm)	0.025ns	0.126ns	**1**									
Root fresh weight(gm)	0.174ns	0.540**	0.464*	**1**								
Shoot dry weight(gm)	0.083ns	0.382ns	0.783**	0.565**	**1**							
Root dry weight(gm)	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, gm=gram, wt=weight, g=gram, sq.cm=square centimeter, TN=total nitrogen, K=potassium, P=phosphorus, ppm=part per million

Table 4B. 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(gm)	Root fresh weight(gm)	Shoot dry weight(gm)	Root dry weight(gm)	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)	0.040ns	1										
Shoot fresh weight(gm)	-0.277ns	0.177ns	1									
Root fresh weight (gm)	-0.028	0.629**	0.374*	1								
Shoot dry weight(gm)	-0.040ns	0.560**	0.445**	0.696**	1							
Root dry weight(gm)	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, gm=gram, wt=weight, g=gram, sq.cm=square centimeter, TN=total nitrogen, K=potassium, P=phosphorus, ppm=part per million

Table 4C. Correlation coefficient for plant growth parameters and nutrient up take characters of coffee seedlings during Single inoculation +VC

Characters	Shoot length/plant (cm)	Root length/plant (cm)	Shoot fresh weight(gm)	Root fresh weight(gm)	Shoot dry weight(gm)	Root dry weight(gm)	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 weight (gm)	-0.063ns	0.398ns	1									
Root fresh weight (gm)	0.288ns	0.957**	0.390ns	1								
Shoot dry weight (gm)	0.033ns	0.438ns	0.646*	0.497ns	1							
Root dry weight (gm)	-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			
Available P (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, gm=gram, wt=weight, g=gram, sq.cm=square centimeter, TN=total nitrogen, K=potassium, P=phosphorus, ppm=part per million

Table 4D: Correlation coefficient for plant growth parameters and nutrient up take characters of coffee seedlings during dual inoculation +VC

characters	Shoot length/plant (cm)	Root length/plant (cm)	Shoot fresh wt(gm)	Root fresh wt(gm)	Shoot dry wt(gm)	Root dry wt(gm)	No of leaves	Stem Girth (mm)	Leaf area (sq.cm)	Availabl eP(ppm)	TN (%)	K (ppm)
Shoot length/plant(cm)	1											
Root length/plant(cm)	0.129ns	1										
Shoot fresh weight(gm)	-0.075ns	0.580**	1									
Root fresh weight(gm)	0.241ns	0.676**	0.703**	1								
Shoot dry weight(gm)	0.046ns	0.410ns	0.723**	0.656**	1							
Root dry weight(gm)	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			
Available P (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, gm=gram, wt=weight, g=gram, sq.cm=square centimeter, TN=total nitrogen, K=potassium, P=phosphorus, ppm=part per million

According to correlation coefficient, P and K uptake was strongly related to plant growth parameter. 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 bio-inoculants 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).

Conclusion

This study revealed that PSF isolates were more efficient in P-solubilizeation 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 inoculates indicated a promising results in dual inoculation when evaluate 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.

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