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RESEARCH PAPER

Prospects of Potassium and Phosphate Solubilizing Bacteria for Nodulation Enhancement, growth and yield of Chickpea plant (*Cicer arientinum L.)*

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A B S T R A C T:

Seventy one percent of the overall P and K in Erbil governorate soils are in inorganic forms which are unavailable for use in plants. As a result, chemical fertilizer is often used to supplement the nutrient for the growth crops. To reduce the addition of chemical fertilizer to agricultural soils, this research has been conducted to isolate naturally occurring potassium solubilizing microorganisms (KSB) and phosphate solubilizing microorganisms (PSB) from Erbil soil samples and use to improve chickpea growth. Thirty nine efficient PSB isolates were selected based on their ability to clear zone formation on Pikovskaya´s agar medium of and 26 efficient KSB isolates using Aleksandro medium. Depending on microscopical, cultural, and biochemical characteristics, PSB strains belonged to *Ps.putida* while KSB strains referred to *B.circulans*. All PSB and KSB strains were screened for their solubilization efficiency on both solid and liquid medium. Bh36 strain of *Ps.Putida* was the most efficient isolate in P solubilization (94.92%,117.78mg/ml) and the most efficient *B.circulans* strain in K solubilization was Q1 (87.01%,4.81mg/ml), and they were used for P and K biofertilizers preparation, respectively. Pot experiment showed that seed inoculation with *Ps.Putida*, *B.circulans*, and combined (*Ps.Putida*+ *B.circulans*) application significantly enhanced chickpea growth. Combined (*Ps.Putida*+ *B.circulans*) inoculation recorded the highest increase in shoot height (46.29cm), root length (37.84cm), shoot dry weight (4.28g/plant), number of seed (26.37seeds/plant), number of active nodules (11.56/plant) P (6.35g/plant) and K uptake (65.75g/plant), soil available phosphorus (17.21mg/kg) and soil available potassium (314.5mg/kg). It could be assumed that local *Ps.Putida* and *B.circulans* strain can be used as biofertilizer of P and K to improve plant growth and soil fertility.

KEY WORDS: *B.circulans*, Biofertilizer, Chickpea, K-solubilizing bacteria, P-solubilizing bacteria, *Ps.Putida*.
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1. INTRODUCTION

Phosphorus (P) and potassium (K) are essential macro-nutrients that playing a significant role in plant growth and development. Plants usually need (P) for cellular bioenergetics, metabolic regulation, and vital components of essential bimolecular including RNA, DNA,ATP, phospholipids and sugar-phosphates (Plaxton and Lambers, 2015).

On the other hand, (K) plays a vital role in plant growth, metabolism and development, water retention, regulating opening, closing of stomata and enzyme activation (Pettigrew, 2008, Badr, 2006). Potassium also helps plants in faster growth, additionally to increasing plant resilience to pests, pathogens...etc (Rehm and Schmitt, 2002). Potassium deficiency causes decreased growth ,seed size and plant yield (Gupta et al., 2015). A great part of the total (P) in the soil is non-soluble and unavailable therefore cannot be utilize by plants. The major factors that hamper

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crop production globally is a lack of soluble (P) in many agriculture soils (Panhwar et al., 2012, Pei-Xiang et al., 2012). A large portion of soil microbes, called phophate solubilizing microorganisms (PSM), these organisms have ability to dissolve insoluble inorganic phosphorus and make them available to plants by varies mechanisms such as production of organic acids and phosphatase enzymes (Shaikh et al., 2016). The quantity of soluble potassium which absorb by plant is very low, which represent 2% of total potassium, while large amount of it 90-98% is exist in insoluble form (Prajapati and Modi, 2012). Potassium solubilizing bacteria can solubilize K- bearing minerals, and increase it availability due to the production of some weak organic acids such as citric, tartaric...etc which causes in plant growth and yield (Maurya et al., 2014, Meena et al., 2014a). Phosphate and potassium solubilizing bacteria have a key role in soulubilization in organic and inorganic P and K in soil which can be used as biofertilizers, because biofertilizers are low-cost and environment-friendly plant nutrient source, which improves the availability of plant nutrients and enhancing the sustainability and the health of the soil (Singh et al., 2011). Biofertilizers have beneficial effect on crop production and increasing the uptake of nutrients by plants, when they use as inoculation in seeds and soils (Singh et al., 2011).

Since there are little studies about biofertilizers in Kurdistan region for this reason this study was selected and the objects are to isolation and characterization of phosphorus and potassium solubilizing bacteria in Erbil soil and preparation of biofertilizer from isolated strain, and then use for promotion plant growth and improving of some soil properties.

2. Materials and Methods

2.1. Collection of Samples

Fourteen soil samples collected in different geographical areas in Erbil governorate/Kurdistan region-Iraq included (Qushtapa, Salahaddin, Aski-Kalak, Girda-Rasha, Sami Abdul-Rahman park, Daratoo, Shanadar Park, Bastora, Smaqool, Ainkawa, Bahrka, Kawrgosk, Bnasllawa, and Kasnazan) in October 2018. The soils were brought to Microbiology laboratory, College of Agriculture Engineering Science, University of Salahaddin-Erbil.

2.2. Isolation, Purification and Identification of Phosphate Solubilizing Bacteria

Field moist soil samples were mixed with sterile sodium chloride solution of 0.85% and shackled for 20min, an aliquot dilutions were inoculated using NBRIP (national botanical research institute phosphorous) medium containing (0.1g $(NH_4)_2SO_4$, 5g $MgCl_2.6H_2O$, 10g glucose, 0.25g $MgSO_4.7H_2O$, 5g $Ca_3(PO_4)_2$, 0.2g KCl, in 1L distilled water) using pour plate method and incubated for five days at 28±2˚C. The colonies with clear halozone were considered to be phosphate solubilizing colonies. Purification of predominant colonies was performed using re-streaking on NBRIP agar. For further study, single colonies that appeared on NBRIP agar plates were transferred in to Pikoviskaya's liquid broth (PVK) and on agar slants.

Identification of isolated phosphate solubilizing strains was performed according to their microscopical, cultural, physiological and biochemical characteristics included: colonyshape, size, and color also known as pigmentation, colony texture, cell shape, arrangement, gram reaction, cell size, motility, spore formation, production of catalase, oxidase, and gelatin hydrolysis, growth at 37˚C, 4˚C and 44˚C, and carbohydrate fermentation patterns (Brenner et al., 2005).

2.3. Efficiency of Phosphate Solubilizing Bacteria in Phosphate Solubilization in Soil

Phosphate solubilizing activity of all phosphate solublizing isolates was assayed using plate screening method and broth culture method. All the suspended colonies were screened for phosphate solubilization efficiency on PVK ager medium containing (10g glucose, 0.002g $FeSO_4.7H_2O$, 0.1g $MgSO_4.7H_2O$, 0.5g$(NH_4)_2SO_4$, 0.002g $MnSO_4.H_2O$, 0.5g yeast extract, 0.2g NaCl, 5g$Ca_3(PO_4)_2$,and 0.2g KCl,). Spot inoculation was done at the center of PVK plate then the incubation was conducted at 28±2˚C. Clear halo diameter was successively measured after 24hr, up to 7 days. Phosphate solubilization efficiency (*Ps.putida*) of each isolates was evaluated depending on the following equation (Sarikhani et al., 2019).

$$PSE = \frac{\text{Solubilization Diameter}}{\text{Growth diameter}} \times 100$$

Broth culture method was also used to evaluate phosphate solubilizing activities for bacterial isolates. Hundred ml aliquots of PVK broth was transferred in to 250ml conical flask, after sterilization, tricalcium phosphate ($0.5g.100ml^{-1}$) were added. Each flask was inoculated with 1ml of active culture suspensions of each PSB isolate. For the control treatment a sterilized non-inoculated medium has been prepared. Then all inoculated treatments and non-inoculated control treatment were kept in shaker incubator for a week. After incubation period, all the broth culture was centrifuged at 11,000 rpm for 10 minutes remove bacterial cells and other insoluble materials. The supernatant was takento determine available phosphoroususing spectrophotometerat 882nm.

2.4. Isolation, Purification and Identification of Potassium Solublizing Bacteria

Potassium solublizing bacteria (KSB) can be isolated with a serial dilution method using modified Aleksandrov medium containing (0.5g $MgSO_4.7H_2O$; 2g $AlKO_6Si_2$; 0.1g $CaCO_3$; 0.006g $FeCl_3$; 2.0g $Ca_3(PO_4)_2$; 5.0g glucose; and 20.0g agar in 1L distilled water), the plates were incubated at 28±2˚C for 5 days. The bacterial isolates were maintained by periodic transfer on Aleksandrov agar medium slants and stored at 4˚C for further use. The identification of purified KSB bacteria was performed according to their microscopical, cultural, and biochemical properties included: colony size, shape, texture and pigmentation, cell shape, motility, gram stain, and endo-spore formation, oxidase, catalase, , gelatin liquefaction, urea hydrolysis, citrate hydrolysis and carbohydrate fermentation, according to Bergey´s Manual of Systematic Bacteriology (Brenner et al., 2005).

2.5. Efficiency of Potassium Solubilizing Bacteria in Potassium Solubilization in Soil

The efficiency of potassium solubilizing isolates was performed by spot inoculation on Aleksandrov agar medium. The plates were incubated at 28±2˚C, for 5 days, the colonies exhibiting clear zones and diameter of solubilization zone were calculated using following equation (Prajapati and Modi, 2012).

$$KSE = \frac{\text{Diameter of clearzone}}{\text{Growth diameter}} \times 100$$

Selected strains were further evaluated for their potassium solubilizing activity. Potassium soilubilizing ability of selected strains was quantitatively screened on 15ml of Aleksandrov broth medium amended with insoluble K sources (Potassium Aluminum Silicate) was taken in the volumetric flask having volume of 250ml and inoculated with KSB. The incubation was done using special shaker at about 28±2˚C for a week. When the incubation time has ended, culture filtrates were centrifuged at 8000rpm for 10min. The supernatants were subjected to estimation the release of potassium by KSB using a Flame photometer(Chitra and Sharavanan, 2014). The autoclaved medium without KSB inoculation was usedas a control.

2.6. Preparation of Biofertilizer

The most efficient isolate for each of potassium soilubilizing bacteria and phosphate soilubilizing bacteria were select for biofertilizer preparation. Biofertilizers was prepared by addition of 10ml of fresh broth culture ($35*10^7$cell/ml) to 100g carrier (%20 compost + %20 charcoal + %20 $CaCO_3^+$ %20 clay +%19 sand +%1 gum). Two types of biofertilizer were prepared included phosphate solubilizing biofertilizer PSB (*Ps.putida*) and potassium solubilizing biofertilizer KSB (*B.circulans*).

2.7. Pot Experiment

The pot experiment was carried out in Girda-Rasha field College of Agriculture Engineering Science, which is about 5 km to the southwest of Erbil city, during February 6, 2019 to May 20, 2019. The pots (45cm height, 30cm diameters) were filled with unsterilized soil (silty clay loam, pH 7.65, EC 1.23ds/m, total nitrogen 2.352mg/g, $CaCO_3$ 312g/kg, organic matter 11.765 g/kg, available phosphorus 3.423mg/g, soluble ions: HCO_3^- 13.22 mml/L, Mg^{2+} 1.88mml/L, Na^+ 11.07 mml/L, K^+ 29 mml/L, Ca^{2+} 1.21 mml/L, and Cl^- 1.7 mml/L. Growth promoting effects of prepared biofertilizers were studied on chickpea plant. The surface sterilized seeds of chickpea were inoculated with prepared biofertilizer (one gram freshly biofertilizer per 100g seed) before sowing (Milani and Anthofer, 2008). There were 4 treatments with four replications included: non inoculated control (without biofertilizer), PS biofertilizer (*Ps.putida*), KS biofertilizer

(*B.circulans*), and PS biofertliaer+ KS biofertilizer in combination. The experiment was set up in randomized complete design (CRD) using four replicates per treatment. Data was recorded for dry weight of shoots (the shoots were dried at 70°C for 72hr to calculate the dry matter), shoot height, number of seeds/plant, root length, number of active nodules/plant, P and K uptake of chickpea plant, and the available potassium and phosphorus of soil rhizosphere.

2.8. Statistical Analysis

In all cases, Duncan's H.S.D. multiple range tests were applied for comparing between mean of treatments by using SPSS 12.0 (Casanova et al., 2004).

3. Results and Discussion

3.1. Isolation and Identification of Phosphate Solubilizing Bacteria

Thirty nine isolates of phosphate solubilizing bacteria have been obtained and tested from various locations of rhizosphere soil in Erbil city (Qu1, Qu2, Qu3, Sa4, Sa5, Sa6, Sa7, Ka8, Ka9, Ga10, Ga11, Ga12, Ps13, Ps14, Ps15, Ps16, Da17, Da18, Da19, Psh20, Psh21, Psh22, Bs23, Bs24, Bs25, Bs26, Bs27, Sm28, Sm29, Sm30, An31, An32, Bh33, Bh34, Bh35, Bh36, Bh37, Kz38 and Kz39) and they had marked phosphate solubilizing abilities as visualized by developing halo zone around each colony after a week from incubation on a special medium (PVK). The soils showed variations in the appearance and growth of phosphate solubilizing bacterial strain. The highest population of PSB we observed in the soil samples of Bastora and Bahrka, while the lowest number was found in the soil samples of Aski-Kalak and Ainkawa.

These isolated strains were found to be quite similar in size, both in length and diameters. All isolates were gram negative, aerobic. Their colony on agar plate appeared as round and creamy, About 65% of 39 isolates were straight curve shape and 35% were rod shape, did not spore former and not motile, could not grow at 4˚C and 44˚C but could grow very well at 37˚C, and all isolates showed positive response to catalase and oxidase, but they showed negative response to gelatin and starch hydrolysis. All isolates could utilize glucose but did not utilize arabinose, lactose; mannitol and maltose, while they were differing from sucrose, rhibose, xylose, and rhamnose. depending on some morphological, culture, different biochemical analysis and using Bergey´s manual of determinative Bacteriology isolated bacterial strain are *Ps. putida* (Brenner et al., 2005).

3.2. Phosphate Solubilizing Activities

Phosphate solubilizng activity of all isolated strains was quantitatively and qualitatively determined using both broth and solid PVK media respectively. As they give clear zone, it can be assumed that these strains have activity for solubilizing phosphorus. The findings table (1) showed that on PKV agar media plates, all isolates have ability to solubilize phosphorus. The strain Bh36 showed the highest P-solubilizing activity (94.92%), while the lowest P-solubilizing activity (20.17%) were found in Bs24 treatment, which was significantly different from other isolates.

Results of evaluating P-solubilizing activity table (1) at the end of incubation time, in liquid PVK medium it was found that all isolates released P from tricalcium phosphate ranging from (27.62 to 117.78mg/ml) with variations among different isolates. Similarly to solid plate method, Bh36 released the maximum amount of soluble-P, while Bs24 recorded the minimum amount.

The results are in agreement with the finding of (Kumar et al., 2010, Dhandapani, 2011, Khudhur, 2017). The P-solubilizing activity is related to the microbial biochemical potential to produce and release organic acids which their carboxylic groups chelate the cations (mainly Ca) bound to phosphate converting them into soluble forms (Kpomblekou-a and Tabatabai, 1994), as well as the positive correlation between soluble phosphours content and titratable acid production, suggested that acidification of the medium could facilities solubilizng of phosphorus (Park et al., 2016).Based on above results the most effective isolate of *Pseudomonas putida* chosen for most experiment.

Table 1.Qualitative and Quantitative estimation of phosphate solubilization efficiency of isolated bacterial strains using Pikovskaya's media

Isolate	Bacterial	Phosphate solubilization on	Phosphate solubilization in

	genera	Agar %	Broth (mg/ml)
Qu1	*Ps.putida*	29.7^{y}	47.61^{w}
Qu2	*Ps.putida*	50.72^{g}	38.18^{x}
Qu3	*Ps.putida*	44.5^{t-u}	70.00^{n}
Sa4	*Ps.putida*	80.90^{f-e}	89.68^{g-f}
Sa5	*Ps.putida*	39.20^{w}	52.47^{t}
Sa6	*Ps.putida*	58.17^{o}	78.19^{j-k}
Sa7	*Ps.putida*	76.55^{h-g}	81.37^{i}
Ka8	*Ps.putida*	33.19^{x}	44.78^{x}
Ka9	*Ps.putida*	48.27^{t}	62.51^{q}
Ga10	*Ps.putida*	37.70^{x}	55.69^{s}
Ga11	*Ps.putida*	42.90^{u}	60.50^{r}
Ga12	*Ps.putida*	53.10^{q}	71.58^{m}
Ps13	*Ps.putida*	71.12^{l}	91.50^{fe}
Ps14	*Ps.putida*	28.64^{y}	37.12^{x}
Ps15	*Ps.putida*	63.42^{n}	79.14^{j}
Ps16	*Ps.putida*	51.01^{r}	68.20^{o}
Da17	*Ps.putida*	75.38^{i}	97.01^{d}
Da18	*Ps.putida*	65.23^{m}	82.71^{i}
Da19	*Ps.putida*	50.00^{g}	72.85^{m}
Psh20	*Ps.putida*	74.48^{j}	92.32^{e-d}
Psh21	*Ps.putida*	59.09^{o}	82.33^{i}
Psh22	*Ps.putida*	47.01^{t}	60.44^{r}
Bs23	*Ps.putida*	$31.\ 02^{y}$	31.10^{y}
Bs24	*Ps.putida*	20.17^{z}	27.62^{z}
Bs25	*Ps.putida*	48.105^{t}	50.51^{u}
Bs26	*Ps.putida*	55.51^{p}	71.81^{m}
Bs27	*Ps.putida*	37.90^{w}	49.01^{v}
Sm28	*Ps.putida*	81.45^{e}	98.70^{b}
Sm29	*Ps.putida*	76.87^{g}	83.39^{h}
Sm30	*Ps.putida*	52.02^{r}	68.49^{o}
An31	*Ps.putida*	37.80^{w}	51.18^{u}
An32	*Ps.putida*	65.93^{m}	77.01^{l-k}
Bh33	*Ps.putida*	47.30^{t}	70.54^{n}

Bh34	*Ps.putida*	87.39[d]	93.73[d]
Bh35	*Ps.putida*	90.48[b]	99.92[b]
Bh36	*Ps.putida*	94.92[a]	117.78[a]
Bh37	*Ps.putida*	89.98[c-b]	98.13[b]
Kz38	*Ps.putida*	73.14[k]	89.10[g-f]
Kz39	*Ps.putida*	41.10[v]	64.22[p]

*Similar letter or letters means non-significant difference

3.3. Isolation and Identification of Potassium Solubilizing Bacteria

Generally a total of 26 isolates of potassium solubilizing bacterial were recognized of different place in Erbil city (Qu1, Qu2, Qu3, Sa4, Sa5, Sa6, Ka7, Ga8, Ga9, Ga10, Ps11, Ps12, Da13, Da14, Da15, Psh16, Bs17, Bs18, Sm19, An20, An21, Ba22, Kw23, Kw24, Kw25 and Kz26) had marked potassium solubilizing abilities depending on the clear zone produced around the colonies of bacteria on Aleksandro agar medium after 7 days of incubation. The soil samples were differing in the number and growth of potassium solubilizing bacteria.

These isolated organisms when studied for morphological, cultural and biochemical characteristics barely was necessary for identification. The performed tests showed that all isolates were gram positive, rod shape, endospore producing, motile, aerobic, their colony on agar plate appeared as creamy, showed positive response to catalase, gelatinase and citrate ultilization, but they showed negative response to oxidase, urea hydrolysis, could utilize maltose, while they were differ from xylose, glucose, arabinose, mannitole and sucrose. According to the previous results and depending on Bergey´s manual of systematic Bacteriology(Brenner et al., 2005), The isolated bacterial strains were identified to the species *Bacillus circulans*.

Potassium solubilizing activities of selected strains were firstly screened for clear zone formation on Aleksandrov agar medium. All chosen strains, formed clear zone around the colonies. All the bacteria tested were capable to solubilizing potassium, but not always at the same extent table (2). The Qu1 strain had the most pronounced ability of potassium solubilization (87.01%), followed by Da15 (85.11%) and Ba22 (78.4%), while Sma19 (20%) and Ga9 (21.14%) showed the least solubilization activity.

All Potassium solubilizing strains was quantitatively determined in the Aleksandrov broth medium, added by PAS (Potassium Aluminum Silicate). The results in table (2) shows all the strains of KSB could solubilize insoluble PAS very effectively under in vitro condition. Likewise, Qu1 (4.8mg/ml) strain gave the highest amount of soluble K, and Da15 (4.2mg/ml) and Ba22 (3.9mg/ml), on the other hand, Sma19 (1.1mg/ml) and Ga9 (1.2mg/ml) were the lowest among all other strains.

This agree with the results recorded by (Mikhailouskaya and Tchernysh, 2005, Zhang and Kong, 2014). The ability of bacteria to release potassium greatly depends on the nature of the mineral compound (Iakhontova et al., 1987). The variability among the bacterial strain reveals the importance of exploration of different mineral potassium solubilizing bacteria and their solubilizing mechanisms. The key mechanism of potassium solubilization it is normally noted that the action of organic acids synthesized by KSB.

3.4. Potassium Solubilizing Activities

Table 2.Qualitative and Quantitative estimation of potassium solubilization efficiency of isolated bacterial strains using Aleksandrov media

Isolate	**Bacterial genera**	**Potassium solubilizationon Agar %**	**Potassium solubilizationin Broth (mg/ml)**

Qu1	*B.circulans*	87.01^{a}	4.8^{a}
Qu2	*B.circulans*	40^{l}	2^{g-i}
Qu3	*B.circulans*	69.98^{d-e}	3.2^{e-f}
Sa4	*B.circulans*	64.00^{e}	3^{g-e}
Sa5	*B.circulans*	54.23^{h}	2.2^{i-h}
Sa6	*B.circulans*	31^{n}	1.5^{k}
Ka7	*B.circulans*	48.12^{j}	2.4^{h}
Ga8	*B.circulans*	58.18^{g}	1.9^{g}
Ga9	*B.circulans*	21.14^{p}	1.2^{m-l}
Ga10	*B.circulans*	57.6^{g-h}	2.3^{h}
Ps11	*B.circulans*	35^{m}	1.4^{l-k}
Ps12	*B.circulans*	63.00^{e}	3^{ef}
Da13	*B.circulans*	73^{d}	3.4^{d}
Da14	*B.circulans*	26.33^{o}	1.3^{k}
Da15	*B.circulans*	85.11^{a}	4.2^{b}
Psh16	*B.circulans*	75^{dc}	3.7^{c}
Bs17	*B.circulans*	29.19^{n-o}	1.4^{l-k}
Bs18	*B.circulans*	62.75^{e}	3.3^{d-c}
Sm19	*B.circulans*	20^{p}	1.1^{n}
An20	*B.circulans*	26.07^{o}	3.4^{c}
An21	*B.circulans*	62.17^{e}	1.7^{g}
Ba22	*B.circulans*	78.4^{b}	3.9^{c}
Kw23	*B.circulans*	77.01^{c-b}	3.7^{c}
Kw24	*B.circulans*	43^{k-l}	2.8^{g}
Kw25	*B.circulans*	46^{j-k}	2.8^{g}
Kz26	*B.circulans*	52^{i-h}	3.1^{e-f}

*Similar letter or letters means non-significant difference

3.5. Pot Experiment

3.5.1. Plant Growth

The results illustrate that the seed inoculated with prepared biofertilizers significantly increased growth, yield and number of active nodules, phosphorus and potassium uptake, and availability of phosphorus and potassium in soil over non-inoculated control. As can be noticed in table (3), all the treatments improved root length over non-inoculated treatment. A significant variation in root length was observed in response to different bacterial inoculums. Co-inoculation with *Ps.putida+ B.circulans* (T3) produced the highest root length 37.84cm; it means that this inoculation treatment caused 43.76% increase in root length in comparing with non-inoculated treatment (control). *Pseudomonas putida* in combination with *B.circulans* was observed that the

mostefficient inoculums for enhancement of root length. Better root developmentmay be attributed to synergistic relationship of the inoculated bacteria for enhancing root length; *B.circulans* (T1) was the least effective inoculums but still it produced17% longer roots as compared to the non-inoculated treatment (control). Similar to these results, increasing of plant root length by phosphate solubilizing strain alone and in combination with potassium solubilizing also recorded by (Han and Lee, 2005, Chaiharn and Lumyong, 2011, Liu et al., 2016, Bakhshandeh et al., 2017). The strains of P- solubilizing bacteria causes increase in root length and growth regulators production which are causing increase in nutrient and water absorption by plants or host plants(Gupta et al., 2002, Barea et al., 2005). Potassium solubilizing microorganisms have been reported to be significant organisms for plant root establishment, prototypeof rootgrowth, plant nutrition andplant competitiveness, predominantlyunder abiotic and biotic stress and conditions of nutritional imbalances(Wu et al., 2005, Meena et al., 2014a, Meena et al., 2014b).

Healthy roots help the plant for absorption of soil water and uptake of major plant nutrients as well as results in good yield. The increase in plant root size and number or depletion zone of infected roots are leading to increase in nutrient absorption and water stress of plants, which in turn could be the major factors improving plant growth (Bai et al., 2003).

The results also indicatedthat the inoculated plants with different inoculums showed shoot height ranging from (34.31cm) up to (46.29cm). All produced biofertilizer exhibited significant increase in shoot height of chickpea over control, the maximum increase (46.29cm) was shown by co-inoculation of *Ps.putida*+ *B.circulans*, this increase may be attributed to the beneficial impact of bacterial inoculums in stimulating plant growth and increasing nutrient uptake, while the plants which treated by *B.circulans* alone was report the lowest value of shoot height (40.05cm). Phosphate and potassium solubilizing micro-organisms and other beneficial rhizobacteria cause the release of nutrients in plant utilizable form and have beneficial effect on the growth of plants (Sindhu et al., 2002, Glick, 1995,Marques et al., 2010).

Data regarding to dry weight of shoot as shown in table (3) revealed that application of prepared biofertilizer caused significant increase in weight of dry matter compared with control treatment. The highest shoot dry weight (4.28g/pot) was recorded in *Ps.putida*+ *B.circulans* followed by *Ps.putida* alone (2.83 g/pot) treatments. The treatment combinations caused 152%increase in weight of shoot. Here, control and *B.circulans* showed nearly similar results (1.70 and 1.98 g/pot). The increasing on shoot dry weight for inoculated treatments compared with control treatments might be attribute to the role of bacterial in stimulating absorption of macro-nutrients (N,P and K) and some trace elements, large rate of photosynthesis, which results in increasingleaf areaand nitrogen absorption could be expected (Basak and Biswas, 2010)and the effect of hormones (Silini-Cherif et al., 2012) and increase take of CO_2 (Dotaniya, 2015).

The seed yield values provided in table (3) showed that all the tested isolates had the capability to increase the seed yield of chickpea significantly as compared to control treatment. The highest seed yield (26.37seeds/plant) was obtained in pots which received *Ps.putida*+ *B.circulans* inoculation that were noticeably greater than any other treatments. While single inoculation of *B.circulans* showed the lowest value (16.62seeds/plant)behaved similar to that of *B.circulans* for root length shoot dry weight and plant height. Inoculation with either microbe enhanced the yield in chickpea but their interactive effect was more prominent. This is in agreement with the report of similar increase in plant seed yields due to inoculation of PSB and KSB strains were observed by (Pereira and Castro, 2014, Sarker et al., 2014, Sindhu et al., 2010).

In case number of active nodules of chickpea plants shown in table (3), pot experiment gave the highest average value and the lowest value. Highest active nodules (11.56 nodules/plant) was produced with co-inoculation *Ps.putida*+ *B.circulans* treatment, followed by *Ps.putida* inoculation (8.72nodules/plant), the leghemoglobin is responsible for a pink color of active nodules. On the other hand, the lowest values were found for both controls (5.98nodules/plant) and *B.circulans* (6.00nodules/plant), the active nodules have

important effect in the fixating of atmospheric nitrogen to beneficial form for fabaceae family. Inoculations alone (*Ps.putida*) or in combination produced higher number of active nodules. the results agree with (Gull et al., 2004), they show that the PSB affects nodule formation.

Table 3.Effect of single and co-inoculation of (*Pseudomonas putid*a and *Bacillus circulans*) biofertilizer on the growth and yield of Chickpea (*Cicer arientinum* L.) plant

Treatments	Root length cm	Shoot height cm	Shoot dry Weight g/plant	No.of Seeds/plant	No. of active Nodules /plant
T0 Control (withoutbacteria)	26.32^{d}	$34.31g^{h}$	1.70^{k}	10.00^{f}	5.98^{j}
T1*B.circulans*	30.82^{c}	40.05^{f}	1.98^{j}	16.62^{e}	6.00^{i}
T2 *Ps.putida*	34.67^{b}	45.12^{e}	2.83^{i}	19.00^{d}	8.72^{h}
T3*Ps.putida*+ *B.circulans*	37.84^{a}	$46.29b^{h}$	4.28^{h}	26.37^{c}	11.56^{g}

3.5.2. Phosphorus and Potassium Uptake in Plants

Findings indicated that inoculation with PSB and KSB or in combination significantly increased P and K uptake in chickpea plant table (4). The highest phosphorus and potassiumcontent generally occurred in combined treatment, this treatment combination caused increases in phosphorus and potassium content (6.35, 65.75 mg/plant respectively), while the lowest value (1.24, 16.32mg/plant respectively) were recorded in control. Combined inoculation treatments resulted in higher growth performances and P and K uptake than those from single inoculation, and either single treatment of inoculation resulted increase in content of both phosphorus and potassium to various degrees compared to control treatment (Figs.1 and 2). likewise, phosphorus and potassium solubilizing bacteria have also been found to play a significant role in plant nutrition by increasing phosphorus and potassium content by plants (Datta et al., 1982, Nianikova et al., 2002). Phosphate solubilizing bacteria besides having ability for phosphate solubilization is also capable of promoting growth through mechanisms such as production of plant growth hormone and vitamins, enhancement of plant nutrient uptake and the suppression of pathogenic or harmful organisms (O'sullivan and O'Gara, 1992) in the rhizosphere.

Phosphate solubilizing bacteria was a more effective phosphorus uptake than potassium solubiling bacteria. *Pseudomonas putida* is recognized to be good plant growth promotes by producing organic acids and phosphatase enzymes, increases the availability of plants to the soluble phosphorus (Khan et al., 2009). The results were identical with those findings (Shwetha and Lakshman, 2013, Walpola and Yoon, 2013, Khudhur, 2017). Additionally, single inoculations with KSB showed a better K-uptake, seeds and seedling inoculation of various plants with potassium solubilizing bacteria in general commonly showed significant enhancement of germination rate, seedling vigor, plant production, yield and the uptake of potassium by plants under greenhouse and fields conditions (Anjanadevi et al., 2016, Meena et al., 2014a, Zhang and Kong, 2014). Sheng and He (2006) in wheat plant showed that seeds inoculated with *B. edaphi-cus* had the superior of potassium uptake than the non-inoculated treatments which can be attributing to the produce of organic acids by these strain.

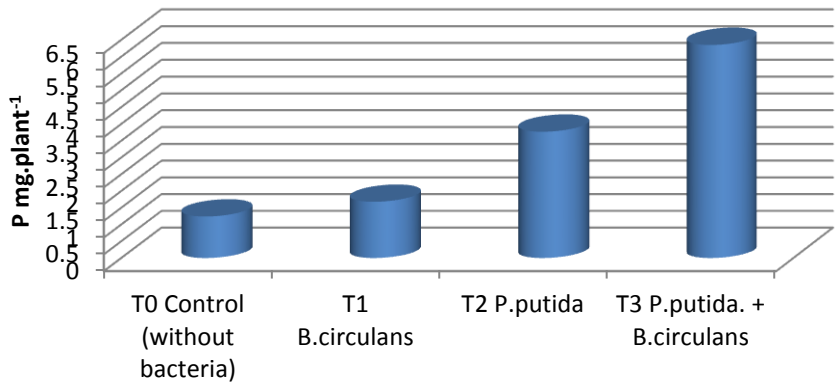

Fig.1. Effect of bacterial inoculation on Phosphorus content of chickpea plant

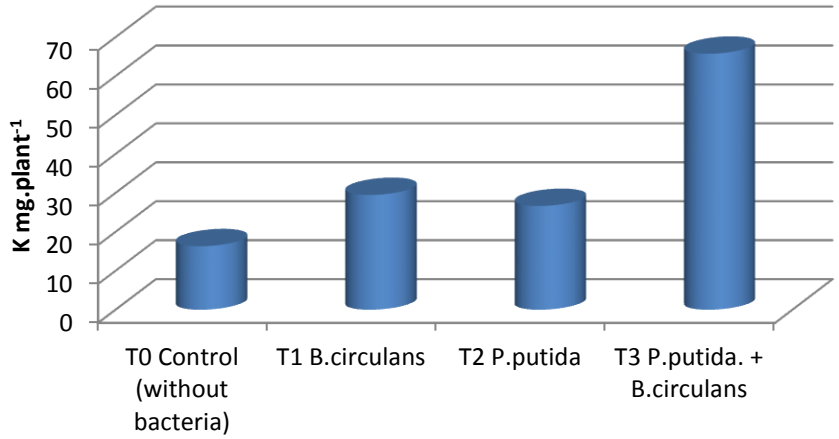

Fig.2. Effect of bacterial inoculation on Potassium content of chikpea plant

3.5.3. Available Phosphorus and Potassium Content of Cultivated Soil

The results in table (4) found that available phosphorus content of rhizosphere soil inoculated with PSB alone or in combination with KSB was found to be significantly higher than those in no – inoculated soil. The highest available P (17.21mg/kg) was obtained when *Ps.putida*+ *B.circulans* used for inoculation, while the least increase of available P-content (290.5 mg/kg) was recorded by *B.circulans* (Fig. 3). Khoshnaw and Esmail, (2020) were studied the phosphorus concentration and it balance in wheat plant, which caused an increase in grain yield and growth positively were cultivated at Girda-Rasha farm soil. therefore, PSB can also improve the availability of potassium in soil or potassium concentrations in plant tissues, additionally to increasing the availability and concentrations of phosphorus in soil and plant tissues (Bakhshandeh et al., 2017).

Potassium content of tested soil table (4) showed significant difference between treatments. The highest available K (403.5mg/kg) was recorded in the soil treated with mixture of *Ps.putida*+ *B.circulans* which was significantly higher than all other treatments. While the lowest value of available K was recorded by *Ps.putida* (279.9mg/kg) followed by non-inoculated treatment (174.8mg/kg) (Figs. 4). Inoculation with KSMs alone or co-inoculation with other plant growth promoting microorganisms (PGPMs)

enhanced K uptake, increased the availability of K in soil, and promoted growth of crops (Shrivastava et al., 2016). The results in agreement with the finding of Teotia et al. (2016) cleared that co-inoculation of PSB and KSB resulted in consistently higher P and K availability than in the control without bacterial inoculums.

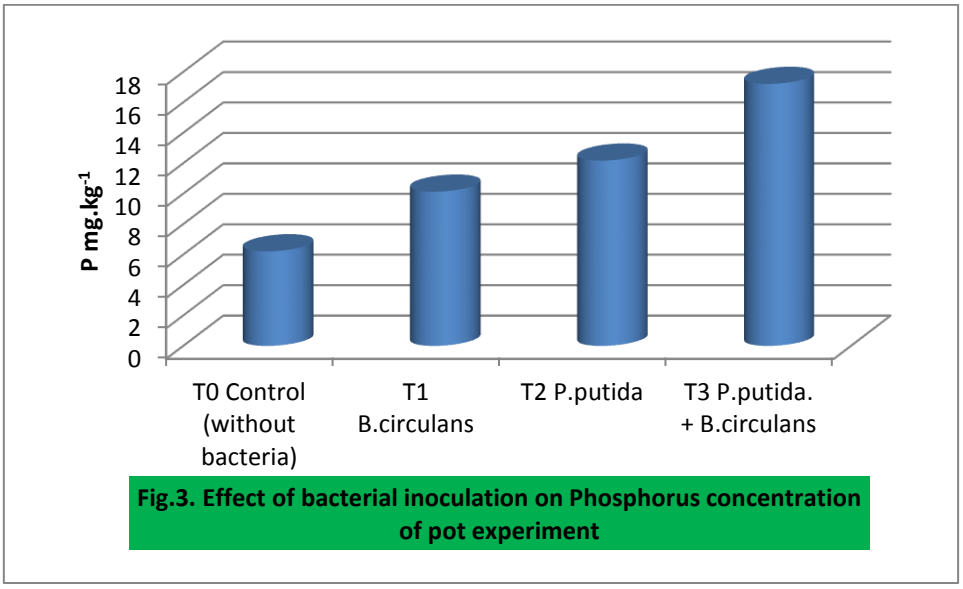

Fig.3. Effect of bacterial inoculation on Phosphorus concentration of pot experiment

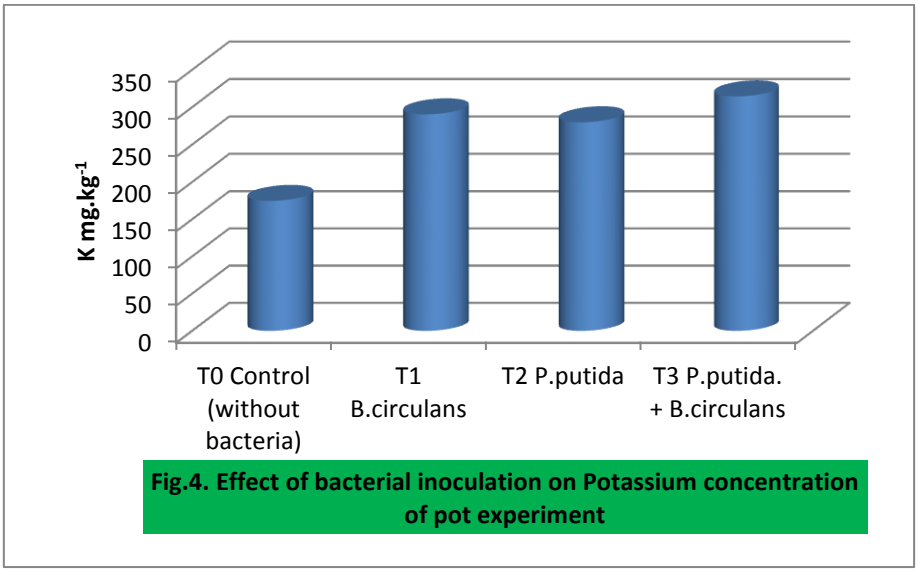

Fig.4. Effect of bacterial inoculation on Potassium concentration of pot experiment

Table 4.Effect of single and co-inoculation of (*Pseudomonas putida* and *Bacillus circulans*) biofertilizer on phosphorus and potassium uptake in Chickpea (*Cicer arientinum* L.) plant and on soil available phosphorus and potassium content

Treatments	P mg/plant	K mg/plant	P mg/kg	K mg/kg
T0 Control (withoutbacteria)	1. 24^{h}	16.32^{d}	6.21^{k}	174.8^{n}
T1*B.circulans*	1.628^{g}	29.48^{c}	10.13^{j}	290.5^{m}
T2 *Ps.putida*	3.77^{f}	26.65^{b}	12.17^{i}	279.9^{l}
T3*Ps.putida*+ *B.circulans*	6.35^{e}	65.75^{a}	17. 21^{h}	314.5^{k}

4. Conclusions

This study was conducted to prepare biofertilizer from local PSB and KSB strain and use to enhance plant growth and the nutrient uptake. Results suggested that prepared inoculums have a positive effect on chickpea plant growth and nutrient absorption when used as a single or combined inoculation of PSB and KSB. Specifically, inoculation increased several growth parameters (shoot dry weight, number of active nodules, shoot height, number of seedsand root length); the inoculation also improved available soil phosphorus and potassium, in addition to promotion of shoot phosphorus and potassium uptake compared to the non-inoculated treatments. *Pseudomonas putida* alone and in combination with *B.circulans* was found highly effective and significant enhancement in growth, nutrient content, increasing the plant available P and K in soil as well as the growth and yield of chickpea. Based on our results, local isolates of PSB and KSB can be used as biofertilizer to enhance soil fertility and plant growth.

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