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Co-Inoculation Effect of Rhizobia with Potassium and Zinc Solubilizing Bacteria for Enhancing Some Growth Parameters of *Phaseolus vulgaris* Plants

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ABSTRACT

Four strains of *Rhizobium leguminosarum* bv. *Phaseoli* (301, 3612, 1799 and 3629)*, Bacillus megaterium, Bacillus polymyxa* and *Azotobacter chroococcum* were evaluated for potassium, Zinc solubilization and exopolysaccarides production. The maximum potassium (K) liberated was recorded by *Bacillus megaterium* (33.2 µg/ml) followed by *Bacillus polymyxa* (32.4 µg/ml) and *Azotobacter chroococcum* (32.0 µg/ml). *Azotobacter chroococcum* showed the highest remarkable solubilization potential for zinc oxide (275) and was the highest in terms of EPS (7.44 g/L). *Rhizobium leguminosarum* bv. *Phaseoli* (301) showed K- liberated (10µg/ml), Zinc solubilization efficiency was (100) and EPS production was (6.36 g/L). Upon these tests *Bacillus megaterium, Azotobacter chroococcum* and *Rhizobium leguminosarum* bv. *Phaseoli* (301) were selected and tested for efficient K solubilization using various potassium sources and during different periods of incubation (5, 10 and 15 days). The highest levels of K solubilization were observed in KCl and K_2SO_4 amended medium broth compared to mica powder-containing media. The maximum release of potassium were 40, 120 and 190 µg/ml (untabulated) in *R. leguminosarm* bv *phaseoli* (301), *Azotobacter chroococcum* and *Bacillus megaterium* respectively at 15 days of incubation. A pot experiment was carried out to study the effect of coinoculation of rhizobial strain with K and Zn solubilizing bacteria and the results showed that the application of T7 of *Rhizobium* sp. strain 301+*Azotobacter chroococcum* (that enhanced for K-solubilization) exhibited the best results of vegetative growth parameters, chlorophyll contents and increased nitrogen, phosphorus, potassium and zinc contents in *phaseolus vulgaris* plants comparing the controls.

Keywords: Potassium, Zinc, solubilization, bacteria, *Phaseolus vulgaris*

1. Introduction

Crop productivity is influenced by a variety of micro- and macro-nutrients. Potassium (K) is an essential macronutrient and the most widely absorbed cation in plants, where it is involved in the growth, metabolism, and development of the organism. Plants with insufficient potassium develop poor roots, grow slowly, produce small seeds, and produce lower yields (Sparks and Huang, 1985). Zinc deficiency is a well-known topic in Egypt old soil that contain relatively moderate amounts of zinc, but the degree of its availability is low due to the different soil physical and chemical characteristics, such as high levels of calcium carbonate and pH as well as low level of organic matter content which leads to zinc deficient in plants (Hafeez *et al.,* 2013). Zn deficiency in plants retards photosynthesis and nitrogen metabolism, decreases the synthesis of carbohydrates and phytohormones, delays crop maturity leading to decrease in crop yield (Welch and Graham, 2004).

Chemical fertiliser misuse has a significant negative impact on the global economy and environmental sustainability. There is a growing need to turn back to nature or sustainable agents that promote evergreen agriculture (Kour *et al.,* 2020).

Studies have shown that a variety of soil microbes can release soluble K from K-bearing minerals such as K-feldspar, mica, and illite. Potassium solubilizing bacteria (KSB) could solubilize these insoluble sources of K to soluble or available form of K by different mechanisms which include

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secretion of organic acids, inorganic acids and polysaccharides, acidolysis, and chelation (Meena *et al.,* 2015). Under in vitro conditions, Javad *et al.* (2013) confirmed that isolates from *Bacillus megaterium* are the most efficient KSB. The structure and chemical composition of the potassium bearing mineral as well as bacterial strain were found to influence the efficiency of potassium solubilization by different bacteria (Liu *et al.,* 2006; Sheng and Huang, 2002).

Concerning Zn solubilization, several plant growth promoting rhizobacteria (PGPR) have the ability to solubilize inaccessible forms of Zn in soil, allowing plants to grow more quickly (Hussain *et al.,* 2015). Saravanan *et al.* (2007) proved that *Thiobacillus thioxidans, Thiobacillus ferroxidans, Acinetobacter, Bacillus, Gluconacetobacter* and *Pseudomonas* have been reported as zinc solubilizers.

Co-inoculation, according to several researchers, stimulates plant growth and nodulation more than separate inoculation (Kong *et al.,* 2017). When Azotobacter sp. and *Rhizobium* were inoculated into maize and wheat plants, the amount of potassium mobilised from waste mica was significantly higher and used as a source of potassium for plant growth, according to Singh *et al.* (2010). In a similar study, Korir *et al.* (2017) found that inoculating *rhizobium* strains with *Paenibacillus polymyxa* and *Bacillus megaterium* improved common bean growth. Furthermore, Raklami *et al.* (2019) confirmed the beneficial effects of rhizobia and PGPR co-inoculation in Morocco's mediterranean semi-arid regions. Similarly, Sibponkrung *et al.* (2020) found that *Bacillus velezensis* and *Bradyrhizobium* co-inoculation resulted in increased nodulation and N_2-fixing efficiency in soybean. It was previously reported that inoculating plants with KSB greatly helped their growth. (Bakhshandeh *et al.,* 2017). Sindhu *et al.* (2010) found that using a biofertilizer containing KSB not only improves soil fertility but also increases crop yield, protects against harmful diseases, and reduces the use of other chemical fertilisers.

Phaseolus vulgaris (common bean) is a grain legume that provides dietary protein to millions of people all over the world (Broughton *et al.,* 2003). Despite its nutritional and economic importance, *P. vulgaris'* productivity lags behind that of most other crop legumes (Cernay *et al.,* 2016). As a result, the current study was undertaken to screen some bacterial strains for potassium, zinc solubilization, and exopolysaccarides production, study the effect of using different forms of potassium sources and different incubation periods as well as to compare the effects of coinoculating rhizobia with potassium and zinc solubilizing bacteria on *Phaseolus vulgaris* growth, nodulation status, and nutrient uptake as compared to single inoculation.

2. Materials and Methods

2.1. Bacterial strains

Rhizobium leguminosarm bv *phaseoli* strains (301, 3612, 1799 and 3629)*, Azotobacter chroococcum, Bacillus megaterium* and *Bacillus polymyxa*, the strains were generously donated by the Biofertilizers Production Unit, Agriculture Microbiology-Soil, Water and Environment Research Institute Agriculture Research Center, Giza, Egypt.

2.2. Screening of bacterial strains for K Solubilization

The spotting method was used to investigate potassium solubilization by bacterial strains on Aleksandrov medium plates containing insoluble mica powder as a potassium source (Sindhu *et al.,* 1999). Plates were incubated for 7 days at 28°C±2. The ability of different bacterial strains to form solubilization zones was used to detect potassium solubilization.

2.3. Quantitative Estimation of Potassium Release

In 25 mL Aleksandrov medium broth, a loopful of 48 hour old grown bacterial strain was inoculated. All of the inoculated flasks were incubated for 7 days at 28°C±2. Following the incubation times, the broth cultures were filtered through Whatman No. 1 filter paper and centrifuged for 20 minutes at 12,000 rpm. The soluble K content in the supernatant was measured using flame photometer (Sugumaran and Janarthanam, 2007).

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2.4. Plate assay for Zn solubilization efficiency

A plate assay was used to test bacterial strains for their ability to solubilize zinc on Tris-minimal medium supplemented with zinc oxide (ZnO) (Fasim *et al.,* 2002). They were incubated for 7 days in the dark at 28°C to see if a clear halo zone formed around the bacterial growth. Zinc solubilization efficiency (SE) was calculated as described by Ramesh *et al.* (2014).

$$SE\ (\%) = \frac{\text{Diameter of solubilization halo zone}}{\text{Diameter of colony}} \times 100$$

2.5. Screening for exopolysaccharides

Bacterial strains were screened to estimate EPS production. The culture broth was centrifuged at 3500 rpm after incubation, and the supernatant was combined with two volumes of acetone. Centrifugation at 3500 rpm for 30 minutes was used to collect the crude polysaccharide produced. After washing with distilled water and acetone alternately, the EPS was transferred to filter paper and weighed after drying overnight at 105° C (Damery and Alexander, 1969).

2.6. Optimization of conditions for efficient k solubilization

I. Effect of using different forms of potassium sources

Three forms of potassium, KCl, K_2SO_4, or mica powder (3.0 g as potassium source) were used to prepare Aleksandrov medium broths. Three selected KSB strains were inoculated and incubated for 7 days at 28±2°C. After days of incubation, released K was determined as indicated previously.

II. Effect of incubation time on K solubilization

K- solubilization by the three selected bacterial strains was determined during different periods of incubation (5,10 and 15 days). After days of incubation, released K was determined.

III. Pot experiment

Pot experiment was conducted in greenhouse of Biological Nitrogen Fixation unit, soils, water and Environ. Res. Instit. ARC, Giza, Egypt during summer season of 2018-2019 to study the influence of slected efficient bacterial strains on plants of (*Phaseolus vulgaris* variety Samantha) grown in sandy soil. The following treatments were practiced as follow:

1) Control (Recommended doses of NPK)
2) *Rhizobium* sp. strain 301
3) *Azotobacter chroococcum*
4) *Bacillus megaterium*
5) *Rhizobium* sp. strain 301 + *Azotobacter chroococcum*
6) *Rhizobium* sp. strain 301 + *Bacillus megaterium*
7) *Rhizobium* sp. strain 301+ *Azotobacter chroococcum* (enhanced for k solubilization)
8) *Rhizobium* sp. strain 301 + *Bacillus megaterium* (enhanced for k solubilization)

The treatments were arranged in a randomized complete block design with three replicates and the experimental pots received the half dose of NPK. After 45 days of planting, the plants were uprooted and assayed for number of nodules, dry wt. of nodules and dry wt. of shoots in addition to chlorophyll contents and nutrients uptake (NPK and Zn).

IV. Soil used.

Sandy soil was collected from Ismailia Research station, ARC. Egypt. Analysis of soil was carried out according to Page *et al.* (1982). The physico- chemical analysis is shown in Table (1).

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Table 1: Physico-chemical characters of soil used

Soil characteristics	Value or (%)
Particle size distribution:	
Sand (%)	85.48
Silt (%)	11.17
Clay (%)	3.35
Texture grade	Sandy
Chemical characters:	
Saturation percent (S.P %)	13%
pH	7.58
E.C (dSm^{-1} at 25^0C)	0.57
Organic matter %	0.40
Total nitrogen %	0.021
Soluble cations meq L^{-1}	
Ca^{++}	1.58
Mg^{++}	0.82
Na^{+}	0.64
K^{+}	1.95
Soluble anions meq L^{-1}	
$Co_3^{=}$	0.00
HCo_3^{-}	0.62
Cl^{-}	0.76
$So_4^{=}$	3.61

2.7. Statistical analysis

The obtained results were statistically analyzed using the general linear models procedure of SAS, (1999). The differences were statistically tested using Duncan's multiple range tests to measure the degree of significance for these differences.

3. Results and Discussion

3.1. Screening for potassium solubilization in bacterial cultures

The ability to solubilize potassium was tested in four strains of *Rhizobium leguminosarum* bv. *Phaseoli* (301, 3612, 1799 and 3629), *Bacillus megaterium, Bacillus polymyxa,* and *Azotobacter chroococcum*. On mica powder-containing medium plates, six bacterial strains formed a significant zone of K solubilization. *Bacillus megaterium* was able to solubilize potassium effectively and recorded higher solubilization efficiency up to 275% followed by *Bacillus polymyxa* 200% and *Azotobacter chroococcum* 150% while *Rhizobium* sp. Strains 301, 3612 and 1799 showed less solubilization efficiency 75, 50 and 65% respectively on Aleksandrov medium plates. On the other hand strain of *R. leguminosarm* bv *phaseoli* (3629) failed to form zone on Aleksandrov medium plates (Fig.1) and (Table 2).

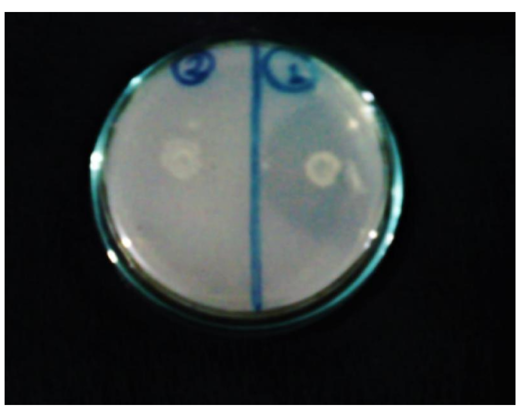


Fig. 1: Potassium solubilization on the Aleksandrov agar plate amended with mica powder

Quantitative estimation of K solubilization is performed. The results of this assay revealed that the amount of K liberated in the broth medium differed between the strains tested (Table 2) The maximum K liberated was recorded by *Bacillus megaterium* (33.2 µg/ml), *Bacillus polymyxa* (32.4 µg/ml), followed by *Azotobacter chroococcum* (32.0 µg/ml) and *R. leguminosarm* bv *phaseoli* strain (301) that give (10 µg/ml). The results obtained from this study are in agreement with Archana *et al.* (2008) who reported that KSB of *Bacillus* sp. solubilized 44.49 µg/ml from mica in liquid medium. Anjanadevi *et al.* (2016) confirmed that two potent isolates of *Bacillus megaterium* and *Bacillus subtilis* were able to solubilize potassium efficiently. Meena *et al.* (2015) also discovered that some potassium-solubilizing rhizobia (KSR) like *Rhizobium pusense* can dissolve waste mica.

Zinc solubilization

The ability for zinc solubilization is present in a large number of plant growth-promoting rhizobacteria (Ramesh *et al.,* 2014). In the present study, among seven bacterial strains, *Azotobacter chroococcum* had the highest solubilization potential for zinc oxide (275 %), followed by *Bacillus megaterium* (180 %), and *Bacillus polymyxa* (125 %), while *Rhizobium* sp. strains 301, 3612, 1799, and 3629 had the lowest solubilization efficiency (100, 60, 66, and 80 % respectively) (Fig. 2) and (Table 2).

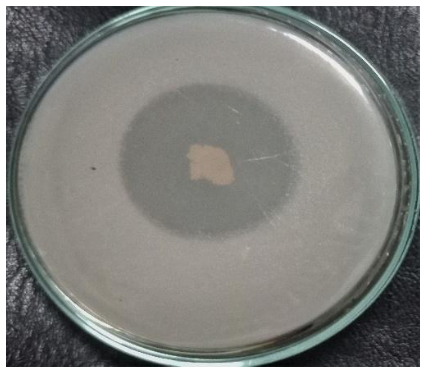


Fig. 2: Solublization of zinc in Tris-minimal agar plate supplemented with Zinc oxide.

Table 2: Screening for potassium and zinc solubilizing bacteria

Bacterial strains	Potassium solubilization: Solubilization efficiency (%)	Potassium solubilization: K-liberated (µg/ml)	Zn - Solubilization efficiency (%)	pH	EPS (g/L)
***R. leguminosarm* bv *phaseoli* (301)**	75	10^{b}	100	5.2	6.36^{b}
***R. leguminosarm* bv *phaseoli* (3612)**	50	6.8^{c}	60	5.6	4.14^{d}
***R. leguminosarm* bv *phaseoli* (1799)**	65	8.5^{bc}	66	5.5	3.62^{e}
***R. leguminosarm* bv *phaseoli* (3629)**	-	-	80	5.4	3.54^{e}
Azotobacter chroococcum	150	32.0^{a}	275	4.3	7.44^{a}
Bacillus megaterium	275	33.2^{a}	180	4.8	5.48^{c}
Bacillus polymixa	200	32.4^{a}	125	5.3	4.40^{d}

Means in the same column followed by the same letters are not significantly different (P=0.05), according to Duncan's test.

Desai *et al.* (2012) isolated *Azotobacter, Bacillus,* and *Pseudomonas* strains from various crop production systems and examined them *in vitro* for solubilization of 'Zn' from insoluble zinc (ZnO), finding that 15 strains solubilized zinc and formed a solubilization zone on solid media. Sindhu *et al.* (2019) also demonstrated that sixteen rhizobial isolates were able to solubilize zinc by forming a solubilization zone on zinc minimal medium agar plates containing insoluble zinc oxide. Ramesh *et al.* (2014) isolated *Bacillus* sp. and confirmed that the strains produced much higher levels of soluble zinc content. Zinc compound solubilization is produced by the creation of organic acids, and zinc is then released into the environment (Fasim *et al.,* 2002).

Concerning pH, the current investigation clearly showed that the pH decrease ranged from 5.6 to 4.3 and acidifcation of the media by bacterial strains directly influenced the potassium and zinc solubilization, this may because the production of organic and inorganic acids is a major mechanism for mineral solubilization (Meena *et al.,* 2015), which are capable of converting insoluble K to soluble forms of K that are easily absorbed by the plant (Meena *et al.,* 2014). Similary Desai *et al.,* (2012) confirmed that, maximal zinc availability was proportional to the medium's pH. Vaid *et al.* (2014) proved that the decrease in pH was due to the production of gluconic acid by isolates and dissolving zinc oxide. According to Liu *et al.* (2006), large volumes of organic acids are produced by the bacterium, which can form bidentate complexes with metal ions and are more effective at enhancing mineral dissolution.

In this research, the seven bacterial strains showed EPS Production, and maximum amount of EPS production was observed in *Azotobacter chroococccum* (7.44 g/L) followed by *R. leguminosarm* bv *phaseoli* 301 (6.36 g/L), *Bacillus megaterium* (5.48 g/L) and *Bacillus polymixa* (4.40 g/L) while *Rhizobial* strains 3612, 1799 and 3629 showed less EPS production 4.13, 3.62 and 3.54 g/L respectively (Table 2). These findings support Welch and Vandevivere's, (1994) hypothesis that naturally occurring polymers can affect mineral dissolution by acting as attachment structures to the mineral or rock surface. Moreover Meena *et al.* (2014) confirmed that fresh microbial EPS increases the rate of feldspar dissolution, probably by forming complexes with framework ions in solution. Sheng and He, (2006) confirmed that extracellular polymer production can lead to the K release from K-containing minerals for plant uptake.

The impact of using various potassium sources

The selected bacterial strains were further tested for efficient K solubilization using different forms of potassium sources and as shown in the results, when mica powder was replaced in the medium with other potassium sources, maximum K solubilization was observed in KCl and K_2SO_4 amended medium broth rather than mica powder containing media (Fig 3). Similarly, the efficiency of potassium solubilization by various bacteria was found to vary depending on the structure and chemical composition of potassium bearing minerals (Liu *et al.,* 2006).

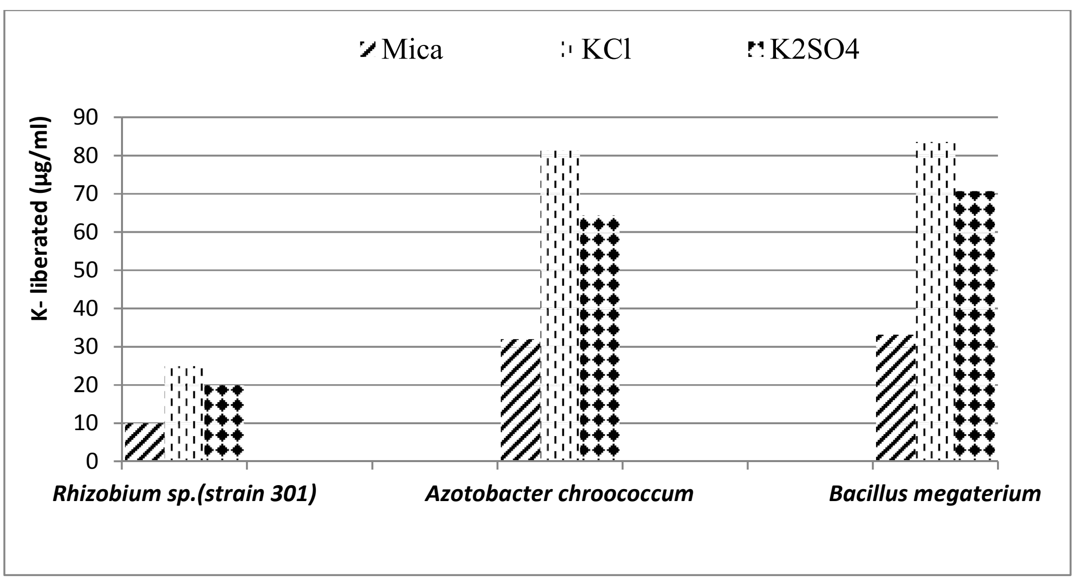


Fig. 3: K solubilization by selected bacterial strains from various potassium sources

K- solubilization as affected by incubation time

The amount of potassium released via mica in a broth media by the three selected bacterial strains were determined at 5, 10, 15 days (Fig. 4) and with increasing incubation time, the amount of K released increased by all strains. The maximum release of potassium was 40, 120 and 190 µg/ml (untabulated) in *R. leguminosarm* bv *phaseoli* (301), *Azotobacter chroococcum* and *Bacillus megaterium* respectively after 15 days of incubation.

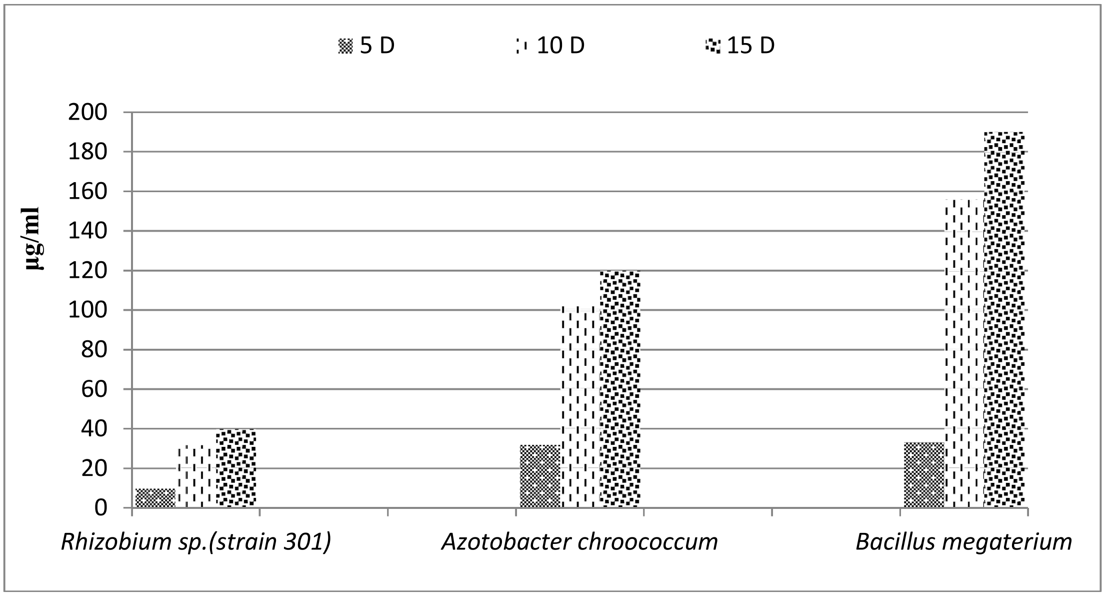


Fig. 4: Effect of incubation time on K- solubilization by selected bacterial strains

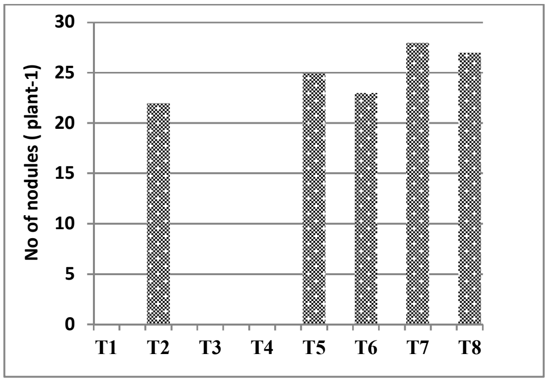
This may be due to the synthesis of organic acids or exopolysaccarides during the incubation period. Similar results observed with Brindavathy and Gopalaswamy (2014) who analyzed K dissolution rate of 14 bacterial isolates and concluded that all strains increased the amount of K released from mineral K as the incubation time increased due to production of exopolysaccharide.

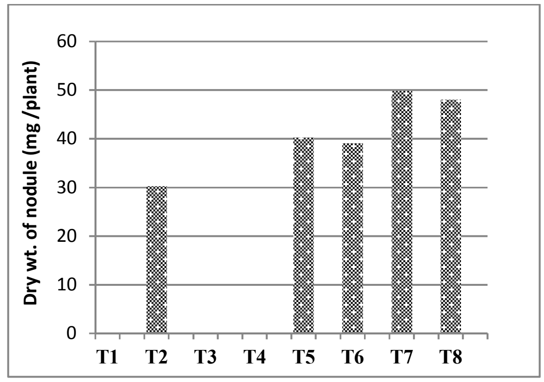
Growth parameters of bean plants

The effect of coinoculation of rhizobia with bacteria that solubilize potassium and zinc on nodulation status and dry weights (g) of shoot / plant is presented in Figure (5). Data revealed that the treatment T7 of (*R. leguminosarum* bv. *Phaseoli+Azotobacter chroococcum* that enhanced for k solubilization) was the best treatment comparing control followed by T8 (*R. leguminosarum* bv. *Phaseoli + B. megaterium* that enhanced for k solubilization) this may be because of K- and Zn-solubilizing bacteria exerted beneficial effects on growth of plants in addition to the co-inoculation could stimulate plant growth and nodulation more than individual inoculation as indicated by Kong *et al.* (2017). Many studies indicated that, the usage of KSB as bio-fertilizers in agriculture can assist to decrease the use of agrochemicals and promote environmentally friendly crop production (Archana *et al.,* 2013). Furthermore, many microorganisms, particularly those associated with roots, have the power to strengthen plant growth and productivity by increasing the supply of low-mobility mineral nutrients like Zn in the soil (Rodriguiz *et al.,* 2004). El-Hadidi *et al.,* (2016) and Kafagy *et al.,* (2017) found that the use of zinc fertiliser increased rice dry matter production and nitrogen uptake.

Photosynthetic pigments

Results (Table 3) showed that chlorophyll was improved greatly by co-inoculation treatments as compared to single strains inoculated plants especially T7 (*R. leguminosarum* bv. *Phaseoli +Azotobacter chroococcum* that enhanced for k solubilization) increased chlorophyll a and b to 2.22 and 2.02 µg/ml respectively in addition, total chlorophyll was significantly increased to 4.13 µg/ml followed by T8 (*R. leguminosarum* bv. *Phaseoli +B. megaterium* that enhanced for k solubilization) increased chlorophyll a, b and total chlorophyll to 1.79, 1.83 and 3.13µg/ml respectively. This may be due to the improvement in plant uptake of N, P, and K and the activity of KSB and ZnSB. Mikkelsen, (2008) confirmed that minerals are required for the production of chlorophyll during photosynthesis. Jayaganesh *et al.* (2011) proved that K could influence a,b and total chlorophyll content of the leaves and may also improve crop yield directly and/or indirectly through increased photosynthesis.





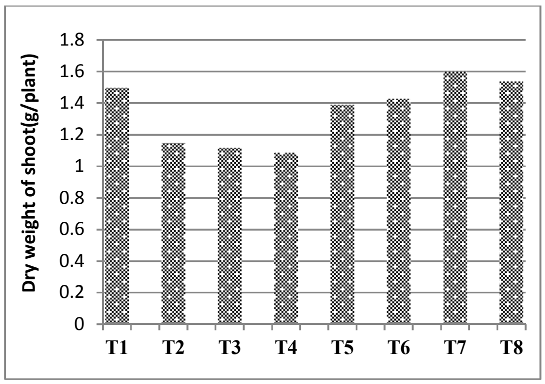


Fig. 5: No. of nodules, dry wt. of nodules and shoot dry wt. of plants as affected by coinoculation of rhizobia sp. with potassium and zinc solubilizing bacteria:

T_1: Control (Recommended doses of NPK); **T_2:** *R.leguminosarum* bv. Phaseoli; **T_3:** *Azoto. Chroococcum*; **T_4:** *Bacillus megaterium;* **T5:** *R.leguminosarum* bv. *Phaseoli + Azoto. Chroococcum*; **T_6:** *R. leguminosarum* bv. *Phaseoli + B. megaterium* ; **T_7:** *R. leguminosarum* bv. *Phaseoli + Azotobacter chroococcum* (enhanced for k solubilization); **T_8:** *R. leguminosarum* bv. *Phaseoli + B. megaterium* (enhanced for k solubilization)

Table 3: Chlorophyll contents as affected by coinoculation of rhizobium sp. with potassium and zinc solubilizing bacteria

Treatments	Chl-a µg/ml	Chl-b µg/ml	Total Chlorophyll µg/ml
Control (Recommended doses of NPK)	1.78^{b}	1.43^{bc}	2.91^{b}
R.leguminosarum* bv. *Phaseoli	0.86^{c}	1.31^{c}	2.31^{c}
Azoto. Chroococcum	0.96^{c}	1.25^{c}	1.79^{d}
Bacillus megaterium	0.89^{c}	1.24^{c}	1.74^{d}
R.leguminosarum* bv. *Phaseoli + Azoto. chroococcum	1.80^{b}	1.69^{ab}	3.01^{b}
R. leguminosarum* bv. *Phaseoli + B. megaterium	1.73^{b}	1.97^{a}	3.11^{b}
***R. leguminosarum* bv. *Phaseoli + Azotobacter chroococcum* (enhanced for k solubilization)**	2.22^{a}	2.02^{a}	4.13^{a}
***R.leguminosarum* bv. *Phaseoli + B. megaterium* (enhanced for k solubilization)**	1.79^{b}	1.83^{a}	3.14^{b}

Means in the same column followed by the same letters are not significantly different ($P<0.05$) according to Duncan's test.

Nutrients uptake content in *Phaseolus vulgaris* plants

The effect of co-inoculation of *rhizobium* sp., (strain 301) with bacteria that solubilize both potassium and zinc on the NPK and Zn content of plants after 45 days is revealed in Table (4). The results presented that the nitrogen and potassium content of plants were increased significantly by applying the coinoculation treatments as compared with the control and single inoculation. The highest values of nitrogen and potassium (3.43 and 2.66 %) respectively were obtained with co-inoculation of

R. leguminosarum bv. *Phaseoli+Azotobacter chroococcum* that enhanced for k solubilization (T7) followed by (T8) application of *R. leguminosarum* bv. *Phaseoli* + *B. megaterium* (3.22, 2.62 %). On the other hand, the lowest values of nitrogen and potassium in plants resulted from the single inoculation and control plants. P concentration in plant was increased with all treatments comparing control. The highest values of Zn concentration in plants (70 ppm) were attained by the coinoculation of *R. leguminosarum* bv. *Phaseoli* + *Azotobacter chroococcum* that was enhanced for k solubilization followed by *R. leguminosarum* bv. *Phaseoli* + *Azotobacter chroococcum* (68 ppm). These findings were in accordance with (Nasri and Khalatbari, 2011) who mentioned that the concentration of Zn, P and K increased in the plant with increasing zinc concentration in foliar spray solution.

Table 4: Coinoculation of rhizobium with bacteria solubilizing potassium and zinc on nutrients concentration in Leaves plants

Treatments	**N (%)**	**P (%)**	**K (%)**	**Zn ppm**
Control (Recommended doses of NPK)	2.85^{e}	1.16^{f}	1.74^{d}	63^{b}
R.leguminosarum* bv. *Phaseoli	2.76^{g}	1.61^{b}	1.70^{d}	33^{e}
Azoto. chroococcum	2.53^{h}	1.49^{c}	1.63^{e}	40^{d}
Bacillus megaterium	2.81^{f}	1.35^{d}	2.19^{b}	40^{d}
R.leguminosarum* bv. *Phaseoli + Azoto. Chroococcum	3.16^{c}	1.31^{e}	2.24^{b}	68^{a}
R. leguminosarum* bv. *Phaseoli + B. megaterium	2.94^{d}	1.5^{c}	1.93^{c}	45^{c}
***R. leguminosarum* bv. *Phaseoli + Azotobacter chroococcum* (enhanced for k solubilization)**	3.43^{a}	1.49^{c}	2.66^{a}	70^{a}
***R. leguminosarum* bv. *Phaseoli + B. megaterium* (enhanced for k solubilization)**	3.22^{b}	1.7^{a}	2.62^{a}	67^{a}

Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan's test.

Conclusion

Transformation of nutrients from an unavailable state to a usable state by microbes is going to be an important approach for sustainable agriculture. Co-inoculation of rhizobium strain with bacteria solubilizing both k- and Zn- enhanced the growth of *Phaseolus vulgaris* plants than single inoculation

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