Influence of Faba bean production in salt-affected soils by *Rhizobium leguminosarum bv. viciae* inoculation and Phosphorus application.

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Abstract

Faba bean (*Vicia faba* L.) represents a major source of protein for animal and human nutrition, and provides several benefits such as improved soil quality. The Giza cultivar 87 (*Vicia faba* L.) was evaluated in three different salinity levels (6.9, 8.7 and 14.8 dSm^{-1}) during two successive cropping seasons (2016-2017 and 2017-2018). The experiment was designed to analyze effect of soil salinity on nitrogen fixation, protein, chemical composition and crop productivity (for both grain and straw). Three phosphorus levels and inoculation with *Rhizobium leguminosarum bv*. v*iciae* were investigated to improve the growth of *Vicia faba* L under these conditions. Soil salinity levels reduced the grain number and straw weight of plants. Moreover, yield reductions were associated with increasing soil salinity levels confirming salinity effects on faba bean productivity. Salinity induced a significant decreased in all plant growth parameters, plant chlorophyll and grains proteins, as well as increased Na% of faba bean plants. The plants treated with *R. leguminosarum bv*. *viciae* showed significant increase in growth traits such as plant length (%), plant fresh weight (%), protein, N-content and dry weight. On the other hand, the dual treatments with *R. leguminosarum bv*. *viciae* plus phosphorus gave a great results compared with inoculation or phosphorus alone. Symbiotic nitrogen fixation inoculation enhanced the growth and yield parameters.

Key words: Salinity; Faba bean (*Vicia faba* L.); *Rhizobium leguminosarum bv*. v*iciae;* inoculation; Phosphorus

Introduction

About 20% of the world's cultivated land is affected by salinity, which results in the loss of 50% of agricultural yield [**1**], it consider the main threat to the plant production [**2**], the chief environmental worry factors that lessens growth and pointedly confines crop output [**3**] and declined growth, nodulation and N-fixation in legumes; due to increase concentration of Na^+ and Cl^- ions, salinity badly affected plant growth and development, hindering seed germination, growth and enzyme activity [**4**]. A number of dynamic physiological roles of plant growth lifeless by salinity; photosynthesis [**5**]; alimentary imbalance (lowering N, P, K^+ and Ca^{2+}) [**6**] and protein synthesis and enzymatic activity [**7**].

Due to its high protein content (27-34%) faba bean (*Vicia faba* L.) cultivated universal for human and animal feeding [**8**]. In addition, symbiotic N_2-fixation in leguminous crops is sensitive to saline conditions [**9**]. Salinity reduces the survival rate and replication of the

Rhizobium in the soil and the creation of proficient nodulation [**10, 11**]. The number and weight of nodules per plant are also reduced below saline situations [**12, 11]**.

Rhizobia comprise diverse members of the class Proteobacteria, and have large number of species (up to 180 species) [**13]**. Faba bean shoot and root dry weights, number of nodules per plant, in addition to harvest components were improved due to inoculation [14, **11**] undoubtedly, and nitrogen, sulphur, phosphorus and other enrichers are very important factors in growing crop of faba bean [**15**].

Plant growth-promoting rhizobacteria (PGPR) as helpful microorganisms [**16, 17**]; can progress seed germination, root and shoot growth, nutrient approval, and plant worry tolerance [**17**]. So far, a range of salt-tolerant rhizobacteria (e.g., *Rhizobium, Azospirillum, Pseudomonas, Flavobacterium, Arthrobacter, and Bacillus*) has exposed positive relations with plants in harassed surroundings [**18, 19]**. Environmental stresses drastically affect plant persistence and harvest, thus plants have established numerous retaliation mechanisms to adapt to the transient stresses. With respect to reproductive success, a decline of photosynthesis will finally result in restricted resource accessibility for reproduction in parental and gametophytic tissues due to a reduction in energy reserves foremost to plant hunger [**20, 21**].

Abiotic or biotic stress environments motivate proline accretion in plants [**22]** it has been perceived in plants misery from salinity tension, on top of in reaction to pathogen infection. Moreover, it has an important role in stabilization of the mitochondrial electron transport system (ETS) and other proteins associated to photosynthesis, specifying a direct link between free proline cell content and rises photosynthetic harvest further down hassle environments [**22, 23]**.

However, the use of bioinoculants PGPR has been of pronounced support in fighting this abioticclimate-encouraged alteration that bounds the total concert of plants below stress [**24, 25**]. Persistence of plants will gear toward confirming a defensible harvest and development of soil potency and construction. This is a good attitude to stress administration [**26]**.

Therefore, the aim of this study was assessed the reaction of faba bean to phosphorus fertilization and inoculation with N_2-fixing bacteria under soil salinity form.

Materials and methods

Faba bean, Giza 87 seeds were kindly provided from Department of Cereals, Field Crop Research Institute, ARC; Microbial inoculants *R. leguminosarum bv*. v*iciae* was kindly supplied from Microbiology Department of the Soils, Water and Environment Research Institute, ARC and the experimental design was conducted at the Sakha Agriculture Research Station farm, Agricultural Research Center, Kafrelsheikh, Egypt.

Media

Yeast mannitol broth for *R. leguminosarum bv*. *viciae* preparation [**27**]. That enclose the following by gl^{-1}: K_2HPO_4: 0.5, $MgSO_4$: 0.2, NaCl: 0.1, Mannitol: 10, Yeast extracts: 1 gm., Distilled water: 1000 ml, pH: 6.8-7 Autoclaved at 121◦ C for 15 min.

Medium of soil extract agar was used for total bacterial count [**28**]. The compositions are K_2HPO_4; 0.5 g and Glucose; 1.0 g were dissolved in 100.0 ml of soil extract then finalized to one liter of tap water and 15.0 g of agar was additional. The medium pH was attuned to 6.8-7.0 and sterilized at 121^oC for 15 minutes.

* Soil extract was set by heating 1000 g of garden soil with 1000 ml of tap water in the autoclave at 121^oC for 30 minutes. 2 g of calcium carbonate is supplementary and the soil suspension was filtered through a double filter paper until the extract comes clear and ready to use.

Martin's medium [**28]** was used for calculating total fungi and its composition is: Dextrose; 10.0 g, peptone; 5.0 g, K_2HPO_4; 1.0 g, $MgSO_4$; 0.5 g, Rose Bengal (1 part in 30.000 parts of medium) and agar 20 g these components were dissolved in one liter of distilled water then the medium pH was adjusted to 7.2 and was sterilized at 121^oC for 15 minutes. Streptomycin solution (30 ml per 100 ml) was supplementary to cooled medium.

Inoculum preparation for seed inoculation

Peat based cultures of rhizobia were set using the method defined by [**27]**. Cell suspensions containing $1x10^8$ (cfu ml^{-1}) were used to permeate sterilized peat at the rate of 52 ml liquid culture /100 g peat. Inoculated peat was well mixed and permitted to established at room temperature for 48 hr. Seeds soaked with 10% Arabic gum water solution as an adhesive material [**29**] were inoculated with rhizobial peat-based preparation. Seeds were allowed to air dry in the shad for 30 min. and sown immediately. Four seeds per pot were sown. Seedlings were thinned to two per pot.

Determination of proline accumulation in faba bean plants.

Proline in dry leaves was assayed at 50 days after sowing. Proline was gritty following the method described by **Batrs *et al*. [30].** Fresh weight of leaf material (0.5g) was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and then this aqueous solution was filtered through Whattman No, 2 filter paper and finally two ml of the filtrate solution were mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was read at 520 nm using Shimadzu UV 1601 spectrophotometer. Appropriate proline standers were used for the calculation of proline in the samples.

In vitro, evaluation of *R. leguminosarum bv. Viciae* to indole-3-acetic acid production under salinity effect.

R. leguminosarum bv. viciae was grown in 250 conical flask and then 1 ml of the inoculum was transferred to each Erlenmeyer flask containing 50 ml of specific medium (YMB), supplemented with 0.3 gl^{-1} tryptophan and incubated at 28° C with agitation at 100 rpm in the dark. The cultures were centrifuged at 4000 rpm for 15 min and two milliliters of the supernatant were mixed with one milliliter Pilet-Chollet reagent which consists of 12 g $FeCl_3$ l-1 of 7.9 M H_2SO_4. The mixture was kept in the dark at room temperature for 30 minutes and then analyzed. Development of pink color indicated that IAA was produced. The absorption of the mixtures was

determined at 530 nm with a photometer [**31**]. The level of produced IAA was estimated using an adjustment curve with commercially available IAA. Media was supplied with different NaCL (0.5, 0.85 and 1.2 % gl^{-1}) compared with normal media without NaCL as control.

Micronutrients.

In the digested solution, micronutrients (Zn and Cu) were measured using atomic adsorption spectrophotometer (Perkin Elmer 3300) according to **Cottenie *et al*. [32]**.

In vivo, evaluation of microbial inoculation on faba bean productivity (Green house experiment).

Inoculation method

The inoculation process involved mixing 50 g inoculant (*R. leguminosarum bv. viciae*) into a 5% sugar solution to form slurry. The slurry was then mixed with the seed until it was evenly coated. The coated seeds were lifted to dry in the shed for (30- 45) minutes and planting was done.

Pots and field experiments

Greenhouse pots and field experiments carried out at Sakha Agricultural Research Station, Kafrelsheikh, Egypt to examine the effect of inoculation with *R. leguminosarum bv. viciae* on faba bean to evaluate salinity effect season (2016-2017). The used pots were about 35 cm in diameter and 40 cm in high filled with 8.5 kg clay soil. The salinity soil of pots experiments were obtained from ARC, Sakha Agriculture research station were the field experiment was carried later season (2017-2018). Composite surface soil samples (0 -25 cm depth) were taken just before conducting the experiment. The soil samples were air dried, crushed and sieved through 2 mm sieve, and subjected to the cations analyzed in saturation soils sample experiment extracts were Ca^{++}, Mg^{++}, K^{+} and Na^{+} and the anion SO_4^{--} ($Meql^{-1}$) and EC were estimated as described by Richards, [33], while the anions $CO_3^{--}$and HCO_3^{-} were estimated by titrating with $KHSO_4$ (N/50) using phenolphthalein indicator for the former and bromocrysol green for the latter [33]. Chlorides Cl^{-} was determined by titration (5 ml of samples) against standard solution of sliver nitrate as conducted by Jackson´s methods [37] asand the results were reported in table 1.

The pots and field experiments design had a split with three salinity level, 3 phosphorus levels with or without *R. leguminosarum bv. viciae* inoculation with three replicates. Results were recorded at 70 and 120 day of sowing in the pots experiment and at the harvesting in the field experiment.

Fertilizer application.

In the pots experiment, nitrogen was added as a control with 100% from the Egyptian Ministry of Agriculture and Soil Reclamation mineral fertilizer. Nitrogen fertilizer was applied at sowing time was 37 kg ha^{-1}, 25% as aster dose for all treatment and 75% in two different times for the control. Potassium sulphate (at rate of 120 kg ha^{-1}) was added to inoculated and un-inoculated plants.

The pots were fertilized with basal rate super phosphate at a rate of

1- 240 kg ha^{-1} as 100 % phosphorus treatment.
2- 180 kg ha^{-1} as 75 % phosphorus treatment.
3- 120 kg ha^{-1} as 50 % phosphorus treatment.

Plant growth analysis.

At 70 day of sowing and the harvesting, plants were collected and subjected to the following analyses after drying in an oven at 70◦ C until a constant weight; dry weight (g plant^{-1}), total number and dry of branches, kernels, nodules and grains plant^{-1} and 100 grain weight determined.

Chemical analyses

For determination of N, K contents expressed as plant samples or grains were dried and 0.2 g were incubated in 5 ml H_2SO_4 and 1 ml perchloric acid in a conical flask for 24 h as described by **Chapman and Parker [34]**. The digested materials were completed to 50 ml H_2O and then distilled by a micro-Kjeldahl method and the nitrogen concentration of distillate was determined by titration against 0.02 normal H_2SO_4 according to **Black *et al.* [35].** Phosphorus concentration of samples was determined calorimetrically according to the methods described by **Snell and Snell [37]**. Sodium and potassium contents were determined for the digested solution by using flam photometer (No, 712700 REG. DES No, 866150) as described by **Jackson [37].** N-content was calculated according to **Black *et al*. [35]**. Total protein calculated by 6.25 x N content. Total chlorophyll was determined by a Minolta chlorophyll meter SPAD-502 for leaf of faba bean plant after 70 after sowing.

Leghaemoglobin Assay

Leghaemoglobin concentration was determined by the cyanmethemoglobin method by **Wilson and Reisenauer [38].** 50 to 100 mg nodules were collected and it was crushed in 9 volumes of Drabkin's solution in a microfuge tube with a glass rod, and then the tube was centrifuged at 12,000 for 15 min. Supernatant was filtered through a 0.2 µm syringe filter. The filtrate was taken in a micro cuvette and its absorbance is noted in a spectrophotometer at wavelength 540 nm.

Establish of microbes

Microbial (colony forming unit) counts were determined by approximation of total number of microbes in the rhizosphere soil according to **Allen [28].**

Statistical analysis

The collected data were subjected to statistical analysis, using the analysis of variance (ANOVA). LSD range tests were used to compare differences between the means **[39].**

Results and Discussion

R. leguminosarum bv. viciae was investigated to IAA production. The Table 2 it showed variation in IAA production under effect of different salinity levels. Normal media without NaCl gave the best IAA production compared with other salinity levels treatment (9.9, 6.1, 3.2 and 1.8 ug ml^{-1}, respectively). These results agreed with **Zimmer and Bothe [40]** that designated that tryptophan is the precursor of IAA biosynthesis and used to stimulate its production. IAA is responsible for the improvement of plant growth by motivation of apical dominance and development of a highly organized root system by which uptake of nutrients becomes more effective [**41, 42].** Also, **Alami *et al*. [43]** and **Elakhdar [11]** indicated that *Rhizobium* sp. can produced IAA under both salinity stress and normal condition. On the other hand, IAA production reduced with increasing salinity levels. These results agreed with **Ravikumara *et al.* [44]** and **Elakhdar [11]** who indicated that IAA production was declined with increasing salinity levels, because salt do as main stress to microbes.

The pot experimental soils obtained from different sites of Sakha agriculture research station and it showed variation in Ec. Salinity had negative influence on faba bean growth factors (Table 3) with increase its level. Inoculation of faba bean plants with *R. leguminosarum bv*.

viciae improved faba bean branches compared with un-inoculated one at low salinity level. While, under high salinity level the 75% phosphorus application + inoculation were positive effect on branches compared with the other treatments with 5.3 branch plant^{-1}.

With increasing salinity levels the plant length were decreased. While inoculation + 50% phosphorus treatment gave the best faba bean length compared with the other treatment under all salinity and phosphorus levels where it was 90.2 cm plant^{-1} Picture 1, 2. Fresh weight of plants under phosphorus and inoculation treatment was affected by salinity. Inoculation + P-application were positively affected on fresh weight compared with un-inoculated treatments. 100% Phosphorus plus inoculation with *R. leguminosarum bv*. v*iciae* gave the best treatment of fresh weight.

At 8.7 dSm^{-1} salinity level the dry weight of plants was the best under 100%P plus inoculation with *R. leguminosarum bv*. v*iciae* compared with other treatments and it was 4.6 g plant^{-1} Table 3. This agreed with **Marcar *et al*., [45]; Katerji *et al*.,[46] and Arafa *et al*., [19]** they showed inoculation progress the faba bean growth parameter below salt worry. In this context, Salinity reduced plant photosynthesis due to the complex effects of osmotic, ionic and nutritional interactions and this decrease in photosynthetic activity might also disturb N_2-fixation by legumes under salt stress as signposted by **Georgiev and Atkias, [48] and Arafa *et al*., [19].**

Study of salinity levels, phosphorus and inoculation on nodules number and its dry weight were presented in Table 3 and showed a significant variation with negatively affected. The treatment 100% phosphorus + inoculation with *R. leguminosarum bv. viciae* gave the best number and nodules dry weight (133.3 and 1.44 g plant^{-1}, respectively). In this respect, **Arias *et al*. [49]; Ibibijen *et al*. [50]; Vaquez *et al*. [13]** and **Arafa *et al*., [19]** reported that *Rhizobium* symbiosis is dependent on high concentrations of P nutrition and consequently increased nodulation and N_2-fixation. Also**, Elakhdar [11]; Katerji *et al*., [46]** and **Arafa *et al*., [19]** showed that stress conditions are long and/or strong enough, formation of nodules will completely inhibit. This may be due to (1) loss of turgor of the nodule peripheral cells, (2) altering nodule zonation, (3) moved infection thread increase and expansion, (4) caused disorders in bacterial release from the infection threads and (5) induced mixture of electron dense material (EDM) and its deposition in vacuoles.

The salinity levels effect on faba bean plants at harvesting in the pots experiment after 120 day of sowing were given in Table 4 under inoculation with *R. leguminosarum bv*. *viciae* with or without phosphorus treatments. Number of kernels per pot were increased with 75% P + inoculation with *R. leguminosarum bv*. *viciae* treatment at low salinity level with (15 pot^{-1}). With increasing salinity level the number of kernels decreased but a treatment with 75% P + *R. leguminosarum bv*. v*iciae* gave positive effect compared with other treatment. At *R. leguminosarum bv*. v*iciae* +100%P treatment, the dry weight of kernels was the best at low salinity level. While increasing salinity led to decreased in the kernels dry weight. High salinity level and 50% P without inoculation gave the lowest dry weight of faba bean it was 4.8 g plant^{-1}. Also, Number of seeds per pot was studied. At *R. leguminosarum bv*. v*iciae* inoculation + 75%P the seeds number was the greatest compared with un-inoculated ones under all salinity levels Table 4.

At 70 of sowing leghemoglobin concentration and chlorophyll were studied Table 4 Leghemoglobin at high P dose (100%P) under inoculation treatment gave the highest amount

compared with un-inoculated treatment in the presence of low salinity level, while with increased salinity levels; the low P treatments gave high leghemohlobin this may due to activity of nodules depends on leghemoglobin. Saline stress lowered the reduction in cytosolic protein legheamoglobin (specifically legheamoglobin) content of the nodules may be another reason of low N_2-fixation under salt stress [**51, 46]**.

The chlorophyll content was assayed in pots experiment at 70 days of sowing Table 4. Salinity has deleterious effect on chlorophyll content with rising salinity, the chlorophyll content increased. Inoculation with N-fixing bacteria+75%P fertilization under low salinity level gave the best chlorophyll content it was 49.4. While, un-inoculated treatment with 75%P at the high salinity level (14.8 dSm^{-1}) was the lowest value with 44.6. In this context, **Netondo *et al*., [52]** showed that **s**alinity also affects photosynthesis mainly through a reduction in leaf area, chlorophyll content and stomatal conductance, and to a lesser extent, through a decline in photosystem II efficiency. Chlorophyll decreased with increasing salinity level and inoculation impact the chlorophyll content in the presence of P compared with un inoculated treatment at chlorophyll was declined with amplified the salinity and inoculated with N_2-fixing bacterial were the best in the presence or absent of P. this agreed with **Delgado *et al*., 1993** [**51]; Elakhdar [11]; Katerji *et al*., [46] and Arafa *et al*., [19].**

At the 70 days of sowing samples were taken from the rhizosphere faba bean plants for total microbial count. The results in figures 1, 2 and 3 showed that there was a decline in the microbial counts with increasing the salinity concentrations. Inoculation with *R. leguminosarum bv. viciae* showed an increased in microbial numbers matched with un-inoculated control. Rhizosphere of the plants roots inoculated with *R. leguminosarum bv. Viciae* + 75% P had the highest viable microbial numbers of actenomycin at the salinity level with EC 6.9 dSm^{-1}. P-dissolving bacteria gave the highest number at 75 %P with lower salinity level. Fungi gave the best number at 100% P with inoculated with *R. leguminosarum bv. viciae*. Inoculated treatment gave the best bacterial number at all salinity level and P treatments compared with un-inoculated. This agreed with **Elakhdar [11] and Katerji *et al*., [46]** they showed that inoculation with *R. leguminosarum bv. viciae* increased total microbial count in the soil, because *R. leguminosarum bv. viciae* improving nutrient uptake and N_2-fixation by faba bean roots which reflect on the healthy status of the faba bean plants. The faba bean plant produces beneficial substance in the rhizosphere area which supports the soil microbes to multiple and growing as a symbiotic relationship.

The data of chemical composition (macro-microelements) of faba bean plants in pots experiment at harvest were reported in table 5. N% of faba bean plants was estimated and it gave high value at inoculation with *R. leguminosarum bv. Viciae* + 100% P-fertilizer compared with un-inoculated ones at the different salinity levels. With increasing salinity levels N% was negatively affected. At 75%P fertilizer + inoculation treatment gave the best P% compared with the other treatments. Phosphorus% decreased with increasing salinity concentration while 75% fertilization gave the best P% in the presence of *R. leguminosarum bv.* v*iciae* inoculation compared with un-inoculated ones at all salinity levels. Similarly, **Bargaz *et al*., [53]; Arafa *et al*., [19]** found that increasing salinity concentrations led to a lessening in P absorption process. K is one of the most important elements for plant growth. Salinity reduced the value of K with increased while inoculation treatment enhanced its concentration. Study of **Bargaz *et al* [53];**

Arafa *et al.*, [19] was in accordance with the present results, where they reported that increasing salinity concentration led to decrease of K concentration. Decreasing K concentrations in plants by salinity was attributed to the antagonism between Na^+ and K^+. The enlarged P absorption by plants might be due to a solubilizing effect of acidic exudates produced by the microbes prominently present in the rhizosphere and effective *Rhizobium* isolates (54).

Na % was increased with rise salinity levels while inoculation with *R. leguminosarum bv*. v*iciae* gave the lowest value. These results agreed with **Tejera *et al.* [55]; Bargaz *et al.*, [53]** and **Arafa *et al.*, (19)** who showed that inoculation with the salt-tolerant rhizobia reduced tissue Na solute compared to control un-inoculated.

Microelements Zn^+ and Cu^{++} ppm also estimated in the faba bean plants and it was negatively affected by increasing salinity level. Inoculation and P treatment enhanced microelements %. Effect of copper deficiency led to reduction of N_2-fixation **[56].** Effect of Zinc shortage led to lessening in number and size of nodules because its possible role involved in leghaemoglobin synthesis **[57].** Insufficiencies in these nutrients negatively affected progress of bacteria or plants and may cause decreases in the numbers and size of nodules as well as the amount of N_2-fixed **[58, 53, 19].**

Field experiment was carried at Sakha agriculture research station to evaluation salinity effect on faba bean growth parameter under P- fertilizers and inoculation with *R. leguminosarum bv. viciae* were studied and reported in table 6. It was showed declined in plant length and number of kernels with increased salinity while inoculation + 100%P treatment gave the best plant length and kernels number with 94.3 cm 14.6, respectively compared with other treatments. In the presence of high salinity level with low P-fertilizer dose and without inoculation the plant height and number of kernels were the lowest 31.3 cm and 4.3, respectively.

It was showed decrease in the dry weight of plants as well as dry weight of seeds per plant with increasing salinity concentrations. On the other hand, inoculation with *R. leguminosarum bv. viciae* +100% P-fertilizer treatment was the best compared with other treatments at the lowest salinity level (46.3 and 20.7 g $plant^{-1}$, respectively). At the high salinity level (14.8 dSm^{-1}) the dry weight was 7.3 g $plant^{-1}$ at 50%P application without inoculation and dry weight of plant was 10.4 g $plant^{-1}$ with inoculation. At the same condition, inoculation increased dry weight of seeds from 3.4 to 5.3 g $plant^{-1}$. At the end, the dry weight of 100 grains of faba bean plants inoculated with *R. leguminosarum bv. viciae* gave the best dry weight under all salinity levels (6.9 dSm^{-1}, 8.7 dSm^{-1} and 14.8 dSm^{-1}) compared with un-inoculated ones. These results agree with those of **Lauchli [59]** who showed that legumes are classified as salt sensitive crop species and their production is particularly affected by salt stress since these plants depend on symbiotic N_2-fixation for their nitrogen requirement. In addition, this limitation in productivity is associated with a lower growth of the host plant, poor symbiotic development of root-nodule bacteria [**48, 53]** and consequently a reduction in the N_2-fixation capacity (**51**). Inoculation of faba bean with *Rhizobium* increased the yield and the quality of seeds compared to un-inoculated treatment [**53 and 19]**. Moreover, due to rhizobia capacity to grow on nitrogen-poor soils, they can be efficiently used for successful saline soil fertility and help to reintroduce agriculture to these lands **[60, 46, 61].** There are several reports on the application of effective rhizobial on common bean improved growth and caused yield enhancement in different climatic and soil conditions [**62, 63**].

In the same experiment, protein and N-contents and were reported in table 7. Salinity usually has detrimental effects on two parameters, while inoculation with *R. leguminosarum bv. viciae* at all P-fertilizer levels (100, 75 and 50%) were the greatest compared with un-inoculated ones under all salinity levels (6.9 dSm^{-1}, 8.7 dSm^{-1} and 14.8 dSm^{-1}). Similar results were reported **Benidire *et al*., [64]** and **Ismael *et al*., [42].** Further studies are needed to investigate the growth promoting ability of the diverse indigenous rhizobial strains in the Egyptian soil.

In the field experiment of this study at 80 day of sowing, proline was studied as showed in table 7. The proline concentration was increased in faba bean plant with increasing salinity levels. Inoculation with *R. leguminosarum bv. viciae* at high salinity level (14.8 dSm^{-1}) increased proline content compared with un-inoculated treatment at the same condition. Inoculation may increase plant tolerance and induced proline synthesis. In accordance with **Ozturk and Demir [65]** concluded that proline is known to occur widely in the higher plants and normally accumulates in large quantities in response to environmental stress**. Sheteawi and Tawfik [66]** indicated that proline content generally increased in plants due to stress and the accumulation of proline may improve the cytoplasmic osmoregulation and thus, increase plant tolerance and biofertilized plants revealed higher values of these metabolic products than non-fertilized plants as response to their ameliorating and stimulating effect. It has also been shown that P addition to salt-affected soils improved crop growth and yield in 34 of 37 crops studied, but did not increase salt tolerance of crops [67].

Conclusion

Salinity is the main threat to the plant production in many countries in all over the world. Application of 75% phosphorus from the recommended dose with *R. leguminosarum bv. viciae* inoculation increase faba bean salinity tolerance and gave best growth green parameters and production under slat stress which is mean limitation of phosphorus improved the salt-tolerant of faba bean. Inoculation with *R. leguminosarum bv. viciae* could improve the N-fixed and nutrient up-take from the soil. *R. leguminosarum bv. viciae* have the ability to produce IAA which play a vital role in the growth stimulation and health states of the plant. It should be used *R. leguminosarum bv. viciae* and 75% phosphorus from recommended dose of ministry of agriculture and soil reclamation of Egypt as duel effect to reduce mineral fertilizer and increase productivity under soil salinity conditions.

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Table 1: Soil characterization of the pots and field experiments.

Parameter	6.9 dSm^{-1}	8.7 dSm^{-1}	14.8 dSm^{-1}
Some physical properties and Particle size distribution			
Clay%	51.6	49.0	58.6
Silt%	25.1	27.7	28.4
Coarse sand%	4.9	7.4	7.9
Fine sand%	15.6	19.6	22.4
Texture grade	Clayey	Clayey	Clayey
Some chemical properties			
pH (1:2.5 water suspension)	8.7	8.9	9.3
EC (dSm^{-1} in soil paste extract)	6.9	8.7	14.8
Soluble cations, meq/L			
Ca^{++}	15.9	18.2	33.2
Mg^{++}	12.8	14.8	27.8
Na^{+}	39.8	44.5	77.5
K^{+}	0.51	0.48	0.39
Soluble anions, meq/L			
CO_3^{--}	0.0	0.0	0.0
HCO_3^{-}	11.2	17.5	25.0
Cl^{-}	23.8	38.6	67.9
SO_4^{--}	29.7	29.7	57.9
Available macro elements, ppm			
N	41.6	35.4	32.6
P	11.5	7.1	4.9
K	440	330	342

Table 2 showed effect of NaCL with different concentration on IAA (µg ml^{-1}) production

IAA (µg ml^{-1})				
Salinity levels of NaCL gl^{-1}				
Treatments	**Control**	**0.5%**	**0.85%**	**1.2%**
	9.9	6.1	3.2	1.8

Table 3 showed effect of inoculation and phosphorus fertilization on faba bean growth parameters (N of branches, plant length, fresh weight, dry weight, No. of nodules and dry weight of nodules) at days of sowing.

Treatments			No. of branches	Plant length (cm)	Fresh weight g pot^{-1}	DW plant g pot^{-1}	No. of nodules	DW of nodule g plant^{-1}
S1	P1	I	3.66bcd	85.3ab	173.6a	4.633a	133.3a	1.44a
		U	2.33d	70.3cd	150.5b	2.816bcde	19.67efg	0.06g
	P2	I	4.00abc	83.3ab	186.5a	3.850ab	111.67b	1.22b
		U	2.66cd	63.3de	104.8de	3.383b	12.33fg	0.06g
	P3	I	3.66bcd	92.0a	127.2c	3.016bc	95.0c	1.09bc
		U	2.66cd	63.6de	82.1fg	2.083cdef	10.33fg	0.04g
S2	P1	I	4.00abc	77.6bc	147.3b	2.816bcde	82.33c	1.03c
		U	2.33d	54.0fgh	105.6de	2.000cdefg	9.0g	0.043g
	P2	I	4.33ab	82.6b	111.6cd	2.883bcd	13.67fg	0.66de
		U	4.00abc	52.0ghi	105.1de	1.750defg	5.0g	0.043g
	P3	I	3.66bcd	73.6c	126.2c	1.463bcd	25.0ef	0.80d
		U	3.66bcd	62.6def	89.4ef	0.920fgh	8.0g	0.043g
S3	P1	I	4.66ab	58.6efg	110.2cd	1.656efgh	25.33ef	0.36f
		U	3.33bcd	46.3hij	88.9ef	0.750h	8.0g	0.03g
	P2	I	5.33a	63.3de	93.8def	1.633efgh	53.3d	0.70d
		U	4.66ab	43.6ij	51.4hi	0.830gh	9.66fg	0.056g
	P3	I	4.66ab	53.0gh	66.9gh	1.036fgh	30.3e	0.53e
		U	3.33bcd	38.6j	42.0i	0.730h	9.0g	0.025g
LSD	0.01		1.81	11.77	25.34	1.59	21.24	0.21
	0.05		1.35	8.78	18.87	1.18	15.82	0.15

S1: Salinity level 6.9 dSm^{-1} S2: Salinity level 8.7 dSm^{-1} S3: Salinity level 14.8 dSm^{-1} P1, 2, 3: Phosphorus lev (100, 75 and 50%) DW: dry weight I: inoculation U: Un-inoculated

Table 4 showed effect of inoculation and phosphorus fertilization on faba bean growth parameters (No. of kernels, dry weight of kernels, No. of seeds and dry weight of seeds, chlorophyll and Leghemoglobin) at harvesting after 120 day of sowing in pot experiment.

Treatments			DW of g plant^{-1}	No. of kernels	DW of kernels g pot^{-1}	No of plant g pot^{-1}	DW of seeds g pot^{-1}	Leg (gl^{-1})	Chl
	P1	I	6.966e	7.67c	17.6a	45.67bc	14.8ab	0.55a	49.23ab
		U	4.300h	4.0f	14.16cde	28.3gh	11.8de	0.31b	48.9abc
S1	P2	I	9.833bcd	15.0a	16.5ab	81.0a	14.3bc	0.28b	49.4a
		U	6.500ef	7.67c	12.7ef	24.67ghi	10.1fg	0.11d	48.84abc
	P3	I	11.133ab	13.3a	17.8a	51.0b	15.9a	0.26bc	49.2ab
		U	6.533ef	7.0cde	12.6ef	23.3hij	9.8fgh	0.096d	48.83abc
	P1	I	9.366cd	11.0b	15.9abc	52.67b	13.9bc	0.11d	49.0ab
		U	4.566h	5.0ef	12.0fg	22.33hij	9.0ghi	0.076d	48.4abc
S2	P2	I	12.200a	10.66b	14.7bcd	38.0de	12.8cd	0.11d	48.5abc
		U	10.633d	7.33cd	12.0fg	24.3ghi	8.3hijk	0.10d	48.2abc
	P3	I	8.900d	5.67cdef	13.0def	42.3cd	10.9ef	0.31b	48.1abc
		U	6.266efg	4.33f	8.7hij	21.0ij	7.3jkl	0.17cd	48.0abc
	P1	I	7.166e	7.67c	10.2gh	35.67def	8.9ghij	0.47a	47.75abc
		U	4.666gh	5.67cdef	7.16jkl	16.67jk	5.7lm	0.28bc	47.44abc
S3	P2	I	9.166cd	10.b	9.3hi	31.67efg	8.1ijk	0.54a	47.4abc
		U	5.133fgh	5.33def	6.4kl	16.67jk	5.3m	0.45a	44.60d
	P3	I	7.266e	7.0cde	8.2ijk	30.6fg	7.0kl	0.55a	47.3bc
		U	4.866gf	4.33f	6.2l	12.3k	4.6m	0.44a	46.94c
LSD	0.01		2.17	3.09	2.55	9.56	2.18	0.155	2.73
	0.05		1.61	2.31	1.90	7.12	1.62	0.114	2.03

S1: Salinity level 6.9 dSm^{-1} S2: Salinity level 8.7 dSm^{-1} S3: Salinity level 14.8 dSm^{-1} P1, 2, 3: Phosphorus levels (100, 75 and 50%) Leg: Leghemoglobin DW: dry weight Chl: chlorophyll U: un-inoculated I: Inoculation

Table 5 showed effect of inoculation and phosphorus fertilization on faba bean chemical composition (N, P, K, Na, Cu and Zn) at harvesting of 120 days in pots experiment.

Treatments			N %	P %	K %	Na %	Cu ppm	Zn ppm
	P1	I	1.68a	0.126ab	2.14a	0.095h	7.09bc	36.33a
		U	1.58bc	0.120ebc	2.023bc	0.093h	7.176ab	35.30ab
S_1	P2	I	1.69a	0.136a	2.09ab	0.101fgh	7.66a	36.966a
		U	1.58bc	0.113bcd	1.84def	0.096h	6.9bc	36.400a
	P3	I	1.64ab	0.123abc	1.936cd	0.093gh	7.153ab	36.766a
		U	1.50d	0.110cde	1.816ef	0.096h	7.026bc	35.366ab
	P1	I	1.59bc	0.128ab	1.816ef	0.110efg	6.566cd	33.500bc
		U	1.53cd	0.110cde	1.793efg	0.12cde	6.533cd	31.466d
S_2	P2	I	1.58bc	0.120abc	1.860de	0.113def	6.266de	32.933cd
		U	1.50d	0.106def	1.810ef	0.116cde	6.066def	31.60cd
	P3	I	1.59bc	0.110cde	1.823ef	0.113cde	6.166def	31.50d
		U	1.58bc	0.106cdef	1.813ef	0.123bcd	6.09def	32.03cd
	P1	I	1.54cd	0.113bcde	1.806ef	0.120cde	5.996def	26.10e
		U	1.52cd	0.100def	1.693ghi	0.133ab	5.70efg	24.33e
S_3	P2	I	1.56cd	0.106cdef	1.743fgh	0.136abc	5.993def	25.86e
		U	1.41e	0.113bcde	1.676hij	0.123abc	5.623fg	24.80e
	P3	I	1.41e	0.096ef	1.616ij	0.133ab	5.74efg	25.37e
		U	1.38e	0.09f	1.573j	0.146a	5.340g	24.76e
LSD	0.01		0.108	0.022	0.147	0.012	0.77	2.60
	0.05		0.07	0.017	0.110	0.013	0.57	1.93

S1: Salinity level 6.9 dSm^{-1} S2: Salinity level 8.7 dSm^{-1} S3: Salinity level 14.8 dSm^{-1} P1, 2, 3: Phosphorus levels (100, 75 and 50%) U: un-inoculated I: Inoculation

Table 6. Showed effect of inoculation and phosphorus fertilization on faba bean growth parameters

(plant length, no. of kernels, dry weight of kernels, dry weight of plant, dry weight of seeds plant $^{-1}$ and dry weight of 100 seeds at harvesting in field experiment.

Treatments			Plant length	No. of kernels	DW of plant	DW of seeds g plant^{-1}	DW of 100 seed g
	P1	I	94.33a	14.67a	46.3a	20.7a	89.7a
		U	84.33cd	11.33bc	28.2bcde	11.3cdef	87.0a
S1	P2	I	89.67ab	12.0b	38.5ab	14.8abc	92.0a
		U	82.33d	10.33bcd	33.8b	14.1cd	57.96cdef
	P3	I	89.0bc	11.00bc	30.2bc	16.3abc	82.0ab
		U	81.0d	10.33bcd	20.8cdef	13.2cde	81.16ab
	P1	I	75.33e	9.00cde	28.2bcde	14.3bcd	79.9ab
		U	69.67fg	8.00def	20.0cdefg	10.2cdef	76.1abc
S2	P2	I	73.0ef	8.00def	28.8bcd	20.6ab	85.2a
		U	65.0gh	7.67ef	17.5defgh	11.9cde	80.6ab
	P3	I	73.0ef	7.67ef	14.6fgh	6.9efgh	79.46ab
		U	61.0h	6.67efgh	9.2fgh	4.5gh	74.5abc
	P1	I	43.0i	8.0def	16.5efgh	8.0defgh	64.0abc
		U	38.0ijk	5.0gh	7.5h	3.9gh	62.3bcd
S3	P2	I	39.67ij	7.0efg	16.3efgh	8.3defg	49.86bcde
		U	34.0kl	6.0fgh	8.3gh	4.0gh	48.9def
	P3	I	35.0jkl	5.0gh	10.4fgh	5.3fgh	43.6ef
		U	31.33l	4.3h	7.3h	3.4h	38.26f
LSD	0.01		6.85	3.19	15.9	8.4	26.5
	0.05		5.10	2.37	11.8	6.3	19.79

S1: Salinity level 6.9 dSm^{-1} S2: Salinity level 8.7 dSm^{-1} S3: Salinity level 14.8 dSm^{-1} P1, 2, 3: Phosphorus levels (100, 75 and 50%) DW: dry weight U: un-inoculated I: Inoculation

Table 7 showed effect of inoculation and phosphorus fertilization on faba bean growth chemical composition (N-content, Proline and protein) at harvesting in field experiment.

Treatments			N-content	Proline mg g^{-1} dry weight	Protein
	P1	I	0.27a	0.35h	1.73a
		U	0.17abcdef	0.33h	1.1abcdef
S1	P2	I	0.25abc	0.35h	1.55abc
		U	0.22abcd	0.34h	1.37abcd
	P3	I	0.26ab	0.34h	1.64ab
		U	0.15bcdefg	0.33h	0.91bcdefg
	P1	I	0.22abcd	0.44fg	1.41abcd
		U	0.15bcdefg	0.43g	0.94bcdefg
S2	P2	I	0.21abcde	0.46e	1.31abcdef
		U	0.13cdefg	0.45efg	0.83cdefg
	P3	I	0.12defg	0.47e	0.73defg
		U	0.067fg	0.45ef	0.41fg
	P1	I	0.12cdefg	0.52d	0.77defg
		U	0.06fg	0.51d	0.37fg
S3	P2	I	0.076fg	0.61c	0.79cdefg
		U	0.09efg	0.62bc	0.56efg
	P3	I	0.076fg	0.69a	0.48fg
		U	0.46g	0.63b	0.29g
LSD	0.01		0.165	0.039	1.02
	0.05		0.125	0.020	0.76

S1: Salinity level 6.9 dSm^{-1} S2: Salinity level 8.7 dSm^{-1} S3: Salinity level 14.8 dSm^{-1} P1, 2, 3: Phosphorus levels (100, 75 and 50%) U: un-inoculated I: Inoculation

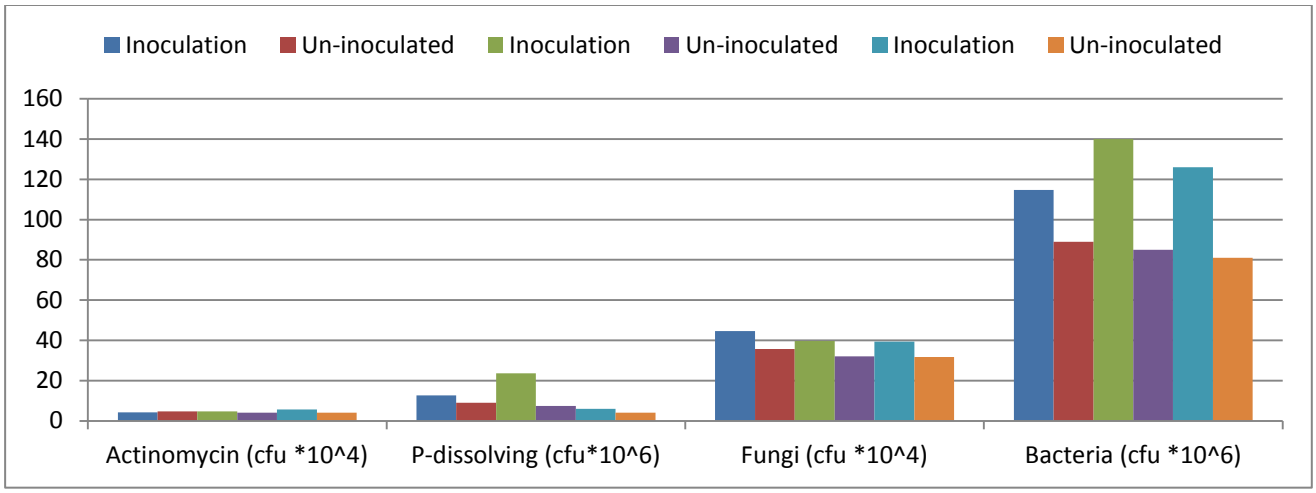


Fig: 2 showed effect of inoculation and phosphorus fertilization on log microbial count of faba bean rhizosphere root at 70 days in pots experiment at salinity level 6.9 dSm^{-1}.

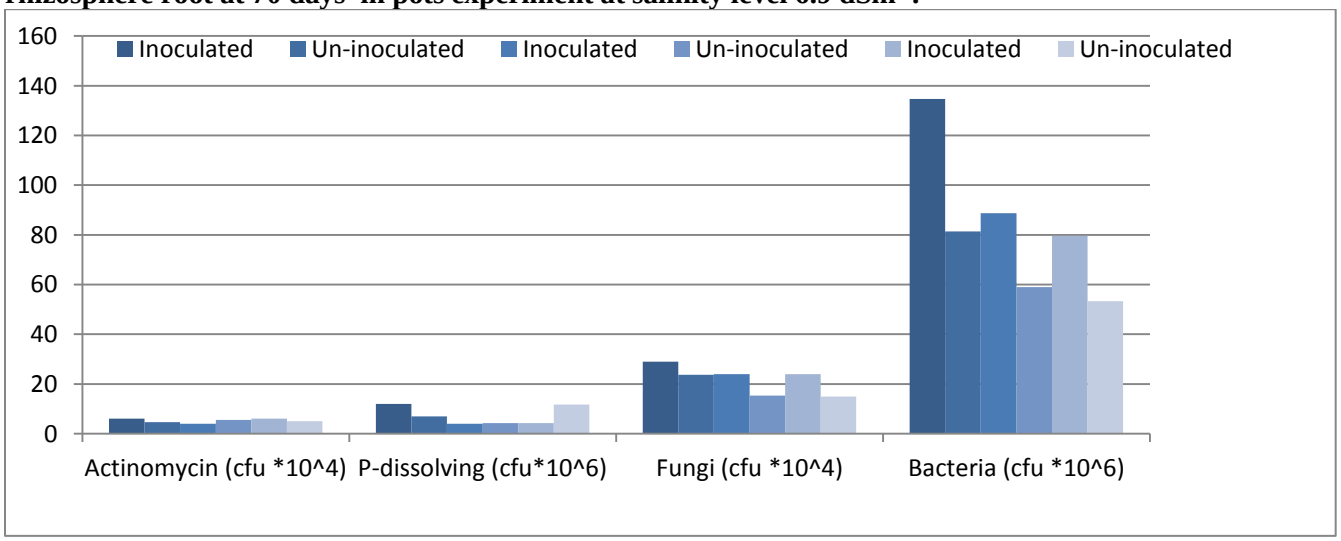


Fig: 3 showed effect of inoculation and phosphorus fertilization on microbial count of faba bean rhizosphere root at 70 days in pots experiment at salinity level 8.7 dSm^{-1}.

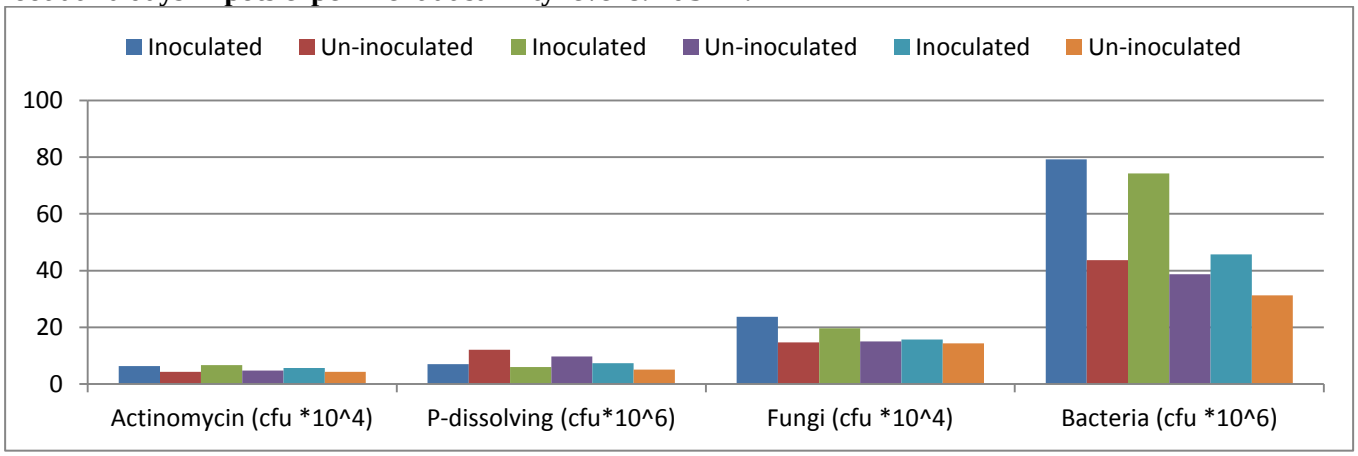
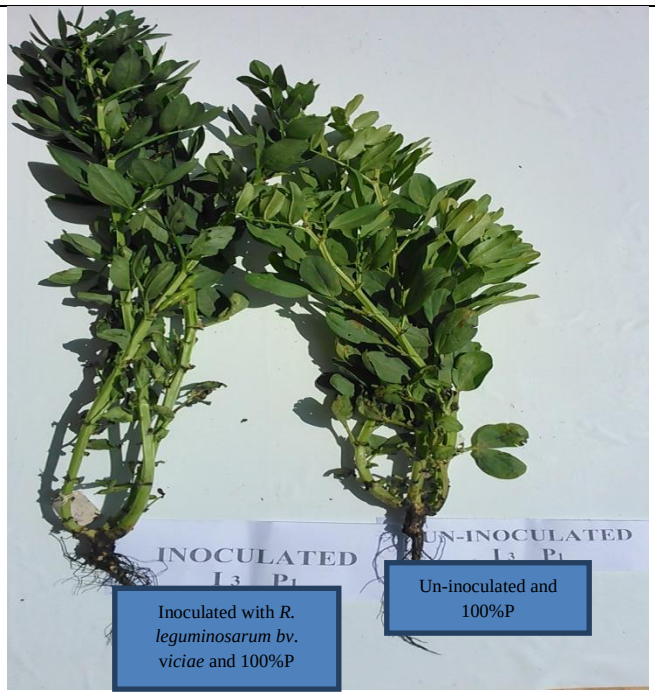


Fig: 1 showed effect of inoculation and phosphorus fertilization on microbial count of faba bean rhizosphere root at 70 days in pots experiment at salinity level 14.8 dSm^{-1}.



Picture 1: showed the difference between inoculated and un-inoculated faba bean plants with *R. leguminosarum bv. viciae* and 100% P at 6.9 dsm^{-1} soil salinity in the field.

Picture 2: showed the difference between inoculated and un-inoculated faba bean plants with *R. leguminosarum bv. viciae* and 75% P at 6.9 dsm^{-1} soil salinity in the field.