



Prospection of Plant Growth-promoting Rhizobacterium *Enterobacter cloacae* and N₂-fixing *Rhizobium leguminosarum* *bv.* *viciae* Effect on Faba Bean Evaluation in Sustainable Agriculture

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Authors' contributions

This work was carried out in collaboration between both authors. Author IEA designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Author AG managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Biofertilizers are relying on ecofriendly approaches as sustainability and environmental safety of agricultural for crop production. The multiplicity of beneficial effects of microbial inoculants, particularly plant growth promoters (PGP) and N₂-fixing bacteria strengthened to use biofertilizers in modern agriculture. Prospection study of *Enterobacter cloacae* KX034162 as PGPR was carried out in this study. Based on *in vivo* assays for PGPRs characterization; Exopolysaccharides production (EPS), biofilm formation, Phosphate, Zinc carbonate and zinc oxide solubilization and productivity of indole acetic acid (IAA) *E. cloacae* KX034162 was used. The synergistic inoculation effect of two microbial on faba bean (*Vicia faba* L.) growth parameters and productivity were evaluated in two successful winter seasons. The results showed that the dual inoculation of both microbes have a significant positive effect on the estimated parameters compared with the control. *E. cloacae* KX034162 produced IAA (47.0 mg l⁻¹), polysaccharides (6.4 g l⁻¹) and solubilized Zn. The dual

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inoculation enhanced nutrients absorption, nodulation, growth parameters, N% (it was 1.42 and 1.44 in the first and second season compared with 0.29 and 0.30 as un-inoculated treatments, respectively) and at the end faba bean production increased.

Keywords: IAA; *Rhizobium leguminosarum* bv. *viciae*; *Enterobacter cloacae* KX034162; faba bean; polysaccharide.

1. INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the greatest globally vital legume crops and the production of seeds in 2014 was 4.1 million tons, which is around 21% better than in 1994 [1]. The fresh and dry seeds of faba bean are exceedingly nutritious of protein content, a decent source K, Ca, Mg, Fe and Zn [2,3], polyphenols [4], carotenoids [5] and carbohydrates [5] so, it used for human and animal consumption. The addition of faba bean in cropping systems improves soil fertility by *Rhizobium* bacteria which concomitant biological nitrogen fixation (BNF), increased soil biological activity and reduced the need for fertilizer input in arable lands [6]. Plant growth-promoting rhizobacteria (PGPR) able to stimulate plant growth through enrichment of BNF, production of growth regulators soil nutrient solubilization (as P and K) [7] and improving nutrient uptake [8,9]. PGPR facilitate the nutrient uptake through phytohormone production (e.g., auxin, cytokinin and gibberellins), enzymatic lowering of plant ethylene levels and/or production of siderophores [10,11,12,13]. It also improved formation of lateral roots and root hairs causing an enhancement of plant tolerance to abiotic stress [14] and increased plant growth and enlargement [15,7]. *Rhizobium* symbiosis with legumes creates 50% of 175 million tons of total BNF annually worldwide [16], while faba bean can fix up to 200 kg N ha⁻¹ [1] and its needy on the faba bean cultivar, local farming practices, soil stuffs, and the presence of active rhizobia in the soil [17,18]. Plant roots colonized with the PGPR species such as *Azospirillum brasilense* Sp6, *Enterobacter cloacae* UW5 and *Pseudomonas putida* GR12-2 displayed increases in root hair formation, lateral and primary, depending on bacterial IAA production [19]. IAA affects plant cell division, extension, differentiation, encourages seed germination, xylem, initiates lateral and adventitious root formation and affects pigment construction [20]. PGPR produce exopolysaccharides which is considerable in biofilm development as well as root colonization and contact with microbes. [21,22,23]. Phosphorus is a vital element in the nutrition of plants, presented in both organic and

inorganic forms and have role in metabolic progressions [24]. 95-99% P⁺ present in the insoluble, immobilized and precipitated form, so plants cannot employ it [25]. Plant can absorb the two soluble forms of phosphorus; the monobasic and the dibasic ions [15]. The use of this environmentally friendly attitude could be among the most efficient methods for minimizing the use of chemicals, which can harmfully impression human health directly or indirectly. Screening of new PGPR isolates for inoculant production pointing to enhance plant growth and BNF comprise an essential stage of in vitro phosphate solubilization analysis [26,27,28,29]. The microbial activity in rhizosphere soil expressed by total microbial counts, CO₂ evolution, *Azotobacter* and Phosphate dissolving bacteria counts and Enzymatic activities exhibited a positive response to biofertilizer compared to un-inoculated control as conducted by Abd El-Gwad and Salem [30]. The aim of this study is the prospection for PGPR and N₂-fixed microorganisms for improving faba bean productivity.

2. MATERIALS AND METHODS

2.1 Microbes Used

R. leguminosarum bv. *viciae* and *E. cloacae* KX034162 were kindly supplied from Microbiology Department of the Soils, Water and Environment Research Institute, Agricultural Research Center, Sakha Agriculture Research Station, Kafrelsheikh, Egypt.

2.2 Media Used

1- Yeast mannitol broth for *R. leguminosarum* bv. *viciae* preparation [31]. That comprise the following by g l⁻¹: K₂HPO₄: 0.5, MgSO₄: 0.2, NaCl: 0.1, Mannitol: 10, Yeast extracts: 1 g., Distilled water: 1000 ml, pH: 6.8-7 Autoclaved at 121°C for 15 min.

2- Nutrient agar and broth medium was used for *Enterobacter cloacae* growth and preparation.

2.3 *Enterobacter cloacae* KX034162 Screening as PGPRs

The ability of gram negative *E. cloacae* KX034162 to classifying as a PGPR (Indole acetic acid IAA production, Exopolysaccharides synthesis, solubilization of phosphate and biofilm formation) were carried out in vitro. IAA production, as designated by Sarwar et al. [32], EPSs synthesis pronounced by Ashraf et al. [33], The solubilization of phosphate are described by methods of [34] and production of cyanide (HCN) were measured as defined by Bakker and Schippers [35].

2.4 Antagonistic Assay between *E. cloacae* KX034162 and *R. leguminosarum* bv. *viciae*

E. cloacae KX034162 was streaked as a striate line at one side of nutrient agar plate then incubated at 32°C. After 46 h on the same plate, *R. leguminosarum* bv. *viciae* was streaked orthogonally with *E. cloacae* KX034162. The striking plates were incubated again at 32°C for 70 h and appraised [36].

2.4.1 Effect of faba bean seeds exudates on *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 colony formation

The tested microorganisms were streaked on the specific media. Faba bean seeds were surface sterilized according to the method described by Allen [37]. Nutrient agar plates were embedded into the center with sterilized seeds. The plates were keep at 8°C for 2h then at 30°C for 48 h. Inhibition zones around the seeds were investigated as recommended by Thompson [38].

2.4.2 Influence of *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 on faba bean growth and productivity (Lizemeter experiment)

Two field experiments were carried out during the two winter seasons (2017-2018 and 2018-2019) at lizemeter of the Agricultural Research Center, Sakha Agriculture Research Station, Kafrelsheikh, Egypt. The lizemeter composed of 20 units each of 70×70 cm, 20 g of faba bean seeds (Saka1 cultivar) were sown in three rows for each lizemeter unit.

2.5 Soil Characterization

Soil samples (0-25 cm depth) were taken just before accompanying the experiment and it were

air dehydrated, rumped and sieved through 2 mm sieve, and imperiled to EC according to Richards [39]. Lizemeter soil was clay with 54.6% clay, silt 22.1%, fine sand 17.6%, and coarse sand 5.7%, with a pH 7.1 and EC dSm⁻¹ 3.36. Soluble elements as cations and anions (meq l⁻¹): Cations: K⁺ 0.2, Na⁺ 9.24, Mg⁺⁺ 8.18 and Ca⁺⁺ 4.38. Anions: SO₄⁻ 11.53, Cl⁻ 6.72.13, HCO₃⁻ 3.75, CO₃⁻ 0.00.

2.6 Seeds

Faba bean (*Vicia faba* L.) Sakha1 cultivar seeds were kindly brought from department of Cereals, Field Crop Research Institute, Agricultural Research Center, Sakha Agriculture Research Station, Kafrelsheikh, Egypt.

2.7 Fertilization of the Experimental Lizemeter Soils

Nitrogen was added to the lizemeter soil with the rate of 31 kg urea fed⁻¹. N fertilizer added at sowing time as starter dose (25% from the recommended dose). Super phosphate fertilizers applied as a basal super phosphate at the rate of 200 Kg fed⁻¹. K fertilizer was added with the rate of 50 Kg fed⁻¹. This was added to inoculated and un-inoculated treatments. Results were recorded at 60 days from sowing and at harvesting. The experiments design had distributed randomly with 4 treatments and five replicates for each treatment as following:

1. Control without inoculation.
2. Inoculation with *R. leguminosarum* bv. *viciae*.
3. Inoculation with *E. cloacae* KX034162.
4. Inoculation with both *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162.

2.8 Inoculum Preparation and Inoculation

Bacterial cultures (*R. leguminosarum* bv. *viciae* mature in YEM Broth or *E. cloacae* KX034162 developed in nutrient broth) at logarithmic phase (7* 10⁹ and 50*10⁹ cfu g⁻¹ respectively) were carried on (1:1) vermiculite: beat moss using Arabic gum as adhesive agent to form slurry. The slurry was then varied with the seed until it was evenly covered. The dusted seeds were lifted to arid in the shack for (30- 45) minutes and implanting was done.

2.9 Estimated of Growth and Production Parameters

Plant biometrics were estimated at 60 days of sowing and at harvest, the estimated parameters

at 60 days were plant height (cm), nodules numbers, NPK% of plant and dry weight of nodules (g) and plant (g). The following items were dignified at the harvest stage: plant height (cm), plant dry weight (g plant⁻¹), seed income (g plant⁻¹), NPK of seeds, pods number plant⁻¹, pods dehydrated weight plants⁻¹ and 100 seed weight (g).

2.10 Statistical Analysis

Experimental principles are given as means. Statistical significance was strong-minded by one differences (one ways) analysis (ANOVA) by CoStat computer database type 6.303. Variances at $p < 0.05$ were measured to be significant. The experiments were applied at three replicates [40].

3. RESULTS AND DISCUSSION

Advancement of *E. cloacae* KX034162 as PGPR was carried out in this study. The best properties of PGPR are polysaccharide and IAA production, phosphate solubilization, and biofilm formation.

E. cloacae KX034162 had the ability to formed IAA in the nutrient broth media provided with tryptophan which worked as a precursor (Table 1). In this context, IAA synthesis via the intermediates indole-3-acetamide; Most phytopathogens, such as *A. tumefaciens* and *P. syringae* pv. *Savastoni* [41,42,43] or indole-3-pyruvate including PGPR species, *A. brasilense* and *E. cloacae* [44,45], this was widespread among IAA-producing bacteria. Also, Julie et al. [19] described that amassing of IAA in the culture medium of wild-type *E. cloacae* UW5 arisen only in the occurrence of tryptophan.

Biofilm formed by *E. cloacae* KX034162 as a PGPR was assessed in this study. Biofilm may play a vital part in the adhesion of N₂-fixion bacteria and *E. cloacae* KX034162 to the faba bean roots. Activity of beneficial microbes at the root sites augmented and improved the growth parameters. PGPR have an abundant technique

to transform un-available P to available one. *E. cloacae* KX034162 had tested for phosphate liquefying zinc carbonate and zinc oxide and it had the ability to dissolve the both elements. In this context, Sharma et al., [46] explained the solubilization process by two mechanisms (a) release of complexes e.g. organic acid anions, protons, OH ions, CO₂, (b) biological P-mineralization.

Effect of faba been seeds exudates on *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 growth was reported as summarized in Fig. 1. Sterilizer faba bean seeds were immersed on a plates which streaking by *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162. A clear inhibition zone was found in the plates inoculated with *R. leguminosarum* bv. *viciae*. This may be due to the seeds exudate phytohormone ethylene which had been known to influence rhizobial growth and sometime repressed nodulation in numerous legumes. In this contexts, Peters and Crist-Estes [47] and Lee and LaRue [48] and Heidstra et al., [49] showed that the phytohormone ethylene inhibit nodulation in various legumes.

On the other hand, faba been seeds exudates had no effect on *E. cloacae* KX034162 growth. *E. cloacae* KX034162 may be produce some chemicals or utilize the phytohormone ethylene (1-aminocyclopropane-1-carboxylate) which produced by faba been seeds. In this contexts, Glick et al., [50] and Glick et al., [51] demonstrated that PGPR decrease ethylene ranks in plants by take up some of the enzyme ACC exuded from the plant and degrade it through the action of ACC deaminase which rehabilitated ACC to ammonia and α -ketobutyrate. In order to possess the balance between internal and external ACC value, extra ACC is displayed by the plant pinched away from the ethylene biosynthesis passageway [50,52]; this mechanism successfully lessens the quantity of ethylene go forward by the plant.

Table 1. Showed estimation of *E. cloacae* KX034162 as a Plant Growth-Promoting Rhizobacterium (PGPR)

| | |
|--|-------------------------|
| IAA production | 47.0 mg l ⁻¹ |
| Polysaccharide production | 6.4 g l ⁻¹ |
| Biofilm formation | +ve |
| Phosphate solubilization | +ve |
| Zink carbonate and Zink oxide solubilization | +ve |
| HCN production | -ve |

Table 2. Influence of *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 on faba bean growth (Lizemeter experiment) at 60 days of sowing in two successful seasons

| Treatments | Plant height (cm) | | No. nodules plant ⁻¹ | | Nodules DW | | Plant DW (g) | |
|--------------|-------------------|-----------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd |
| Control | 70.00 | 71.33 | 26.00 | 27.33 | 0.23 | 0.23 | 15.93 | 16.27 |
| <i>R</i> | 86.67 | 88.33 | 48.00 | 49.33 | 0.35 | 0.36 | 17.60 | 17.90 |
| <i>E</i> | 71.33 | 72.67 | 45.33 | 46.33 | 0.31 | 0.33 | 17.89 | 17.95 |
| <i>R + E</i> | 75.67 | 77.00 | 77.33 | 78.67 | 0.41 | 0.42 | 18.64 | 18.74 |
| L.S.D 0.05 | 6.17** | 7.59** | 5.3** | 3.68** | 0.072** | 0.051** | 1.32** | 0.71** |

R. leguminosarum bv. *viciae*, *E. cloacae* KX034162, *R. leguminosarum* bv. *viciae*.+ *E. cloacae* KX034162. DW: Dry weight 1st first season 2nd second season

Table 3. Influence of *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 on faba bean plants NPK% (Lizemeter experiment) at 60 days of sowing in two successful seasons

| Treatments | N% in plant | | P% in plant | | K% in plant | |
|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd |
| Control | 1.22 | 1.25 | 0.26 | 0.27 | 1.41 | 1.41 |
| <i>R</i> | 1.33 | 1.35 | 0.31 | 0.32 | 1.89 | 1.88 |
| <i>E</i> | 1.41 | 1.43 | 0.29 | 0.31 | 1.96 | 2.04 |
| <i>R + E</i> | 1.50 | 1.53 | 0.37 | 0.39 | 2.59 | 2.61 |
| L.S.D 0.05 | 0.074** | 0.039** | 0.04** | 0.03** | 0.66** | 0.50** |

R. leguminosarum bv. *viciae*, *E. cloacae*, *R. leguminosarum* bv. *viciae*.+ *E. cloacae* KX034162. 1st first season 2nd second season

Table 4. Influence of *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 on faba bean growth and productivity (Lizemeter experiment) at harvesting

| Treatments | Plant height (cm) | | Plant dry weight (g plant ⁻¹) | | Seed yield (g plant ⁻¹) | |
|--------------|-------------------|-----------------|---|-----------------|-------------------------------------|-----------------|
| | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd |
| Control | 107.93 | 108.60 | 33.97 | 34.30 | 12.60 | 12.92 |
| <i>R</i> | 131.67 | 133.83 | 37.67 | 38.00 | 16.44 | 16.85 |
| <i>E</i> | 138.03 | 139.03 | 40.10 | 40.77 | 18.72 | 19.15 |
| <i>R + E</i> | 143.17 | 144.70 | 46.23 | 46.67 | 23.14 | 23.45 |
| L.S.D 0.05 | 10.96** | 8.8** | 2.78** | 1.78** | 2.97** | 2.04** |

R. leguminosarum bv. *viciae*, *E. cloacae* KX034162, *R. leguminosarum* bv. *viciae*.+ *E. cloacae* KX034162. DW: Dry weight 1st first season 2nd second season

Table 5. Influence of *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 on faba bean growth and productivity (Lizemeter experiment) at harvesting in two seasons

| Treatments | Pods number plant ⁻¹ | | Pods DW plants ⁻¹ | | 100-seed g | |
|--------------|---------------------------------|-----------------|------------------------------|-----------------|-----------------|-----------------|
| | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd |
| Control | 5.00 | 5.67 | 20.19 | 20.62 | 49.43 | 49.77 |
| <i>R</i> | 11.67 | 12.33 | 24.65 | 25.30 | 59.95 | 60.38 |
| <i>E</i> | 12.67 | 13.33 | 26.02 | 26.55 | 53.80 | 54.37 |
| <i>R + E</i> | 15.00 | 15.33 | 32.61 | 33.24 | 68.00 | 58.80 |
| L.S.D 0.05 | 2.24** | 1.72** | 3.1** | 1.61** | 3.22** | n.s |

R. leguminosarum bv. *viciae*, *E. cloacae* KX034162, *R. leguminosarum* bv. *viciae*.+ *E. cloacae* KX034162. DW: Dry weight 1st first season 2nd second season

Table 6. Influence of *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 on faba bean NPK% in seeds (Lizemeter experiment) at harvesting

| Treatments | N% in seed | | P% in seed | | K% in seed | |
|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd |
| Control | 2.35 | 2.38 | 0.23 | 0.24 | 1.12 | 1.16 |
| <i>R</i> | 3.12 | 3.18 | 0.31 | 0.32 | 1.22 | 1.23 |
| <i>E</i> | 4.35 | 4.38 | 0.37 | 0.37 | 1.35 | 1.36 |
| <i>R + E</i> | 4.63 | 4.72 | 0.47 | 0.48 | 1.42 | 1.44 |
| L.S.D 0.05 | 0.39** | 0.33** | 0.07** | 0.04** | 0.04** | 0.05** |

R. leguminosarum bv. *viciae*, *E. cloacae*, *R. leguminosarum* bv. *viciae*.+ *E. cloacae* KX034162. DW: Dry weight
1st first season 2nd second season

Table 7. Influence of *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 on faba bean growth and productivity (Lizemeter experiment) at harvesting

| Treatments | N% in straw | | P% in straw | | K% in straw | |
|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd |
| Control | 0.29 | 0.30 | 0.09 | 0.10 | 0.24 | 0.25 |
| <i>R</i> | 0.41 | 0.42 | 0.12 | 0.12 | 0.42 | 0.42 |
| <i>E</i> | 1.32 | 1.35 | 0.16 | 0.17 | 1.07 | 1.13 |
| <i>R + E</i> | 1.42 | 1.44 | 0.22 | 0.23 | 1.29 | 1.32 |
| L.S.D 0.05 | 0.097** | 0.05** | 0.03** | 0.03** | 0.1** | 0.04** |

R. leguminosarum bv. *viciae*, *Enterobacter cloacae* KX034162, *R. leguminosarum* bv. *viciae*.+ *E. cloacae* KX034162. 1st first season 2nd second season

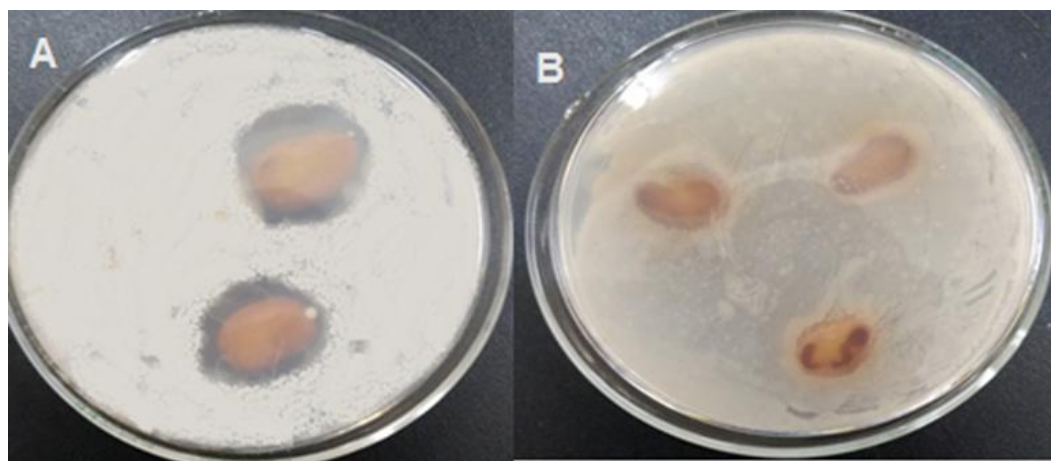


Fig. 1. Effect of faba bean seed diffusible substance on *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 distinct inhibition zone formed around the seed in case *R. leguminosarum* bv. *viciae*, while *E. cloacae* KX034162 not affected

Antagonistic assay between *E. cloacae* KX034162 and *R. leguminosarum* bv. *viciae* was reported as shown in Fig. 2. It showed no antagonism between the two microbars when grown together in the same plates. In this regard, the routine of PGPR has initiated a possible part in rising sustainable structures in crop yield [52]. A variety of symbiotic (*Rhizobium* sp.) and non-symbiotic bacteria (*Azotobacter*, *Azospirillum*, *Bacillus* and

Klebsiella sp., etc.) are used to enrich plant output [53].

Influence of *R. leguminosarum* bv. *viciae* and *E. cloacae* KX034162 on faba bean growth parameters (lizemeter experiment) in two successive seasons (2017-2018 and 2018-2019) at 60 days of sowing were reported in Table 2. Inoculation with the PGPR and N₂-fixing bacteria or mixed inoculation increased the

height of faba bean plants in the two separated seasons. The dual inoculation with *R. leguminosarum* *bv. viciae* and *E. cloacae* KX034162 gave the greatest plant height. A positive highly significance nodulation effect was appeared due to dual inoculation (77.33 and 78.67 nodules plant⁻¹, respectively in the two seasons) compared with the other treatments and inoculation treatments with microbes increased the nodules numbers per plants compared with control. Dehydrated weight of nodules per plant increased with inoculation with *R. leguminosarum* *bv. viciae* more than and *E. cloacae* KX034162 and *E. cloacae* KX034162 increased dry weight more than control. Inoculation with *R. leguminosarum* *bv. viciae* and *E. cloacae* KX034162 gave the highest nodules dry weight, the same results reported in the both seasons. The dry weight of plants was detected in Table 2 after 60 days of sowing it was a positively affected by inoculation with of *R. leguminosarum* *bv. viciae* and *E. cloacae* KX034162 compared with control (un-inoculated). Chemical compositions (NPK%) of faba bean plants were estimated in Table 3. Inoculation with PGPR (*E. cloacae* KX034162) and or *R. leguminosarum* *bv. viciae* increased NPK% compared with control. The dual effect of inoculation reported the highest NPK% value compared with other treatments. (It therefore funds that plant's root exudates can impact the diversity of occupant microorganisms' rhizosphere and these microbes can stimulus the plants by freeing regulatory ingredient [54]. Since plants are regarded as metaorganisms [55]. Gupta et al., [7] presentation of K solubilizing PGPRs as biofertilizer reduced agrochemicals submission and provision ecofriendly crop yield) need revision.

At the harvesting in the same experiment, the growth parameters were conducted in the two seasons (Table 4). The plant height was increased due to inoculation compared with control and inoculation with the two microbes was positively affected compared with other treatments. Dry weight of plants increased due do inoculation compared with control. In this context, Julie et al., [19] demonstrated that plant roots occupied with the PGPR *E. cloacae* UW5, and other types enhanced root hair number, length and formation. Inoculation with PGPR *E. cloacae* KX034162 along with N₂-fixation *R. leguminosarum* *bv. viciae* mediated synergism effect improves the nutrient absorption and productivity of faba bean.

Single or dual inoculation of faba bean plants with the studied microorganisms were influenced seeds dry weight compared with un-inoculated and dual inoculation was the best treatment. Number of pods, dry weight of pods and 100 seeds increased due inoculation with *R. leguminosarum* *bv. viciae* and *E. cloacae* KX034162.

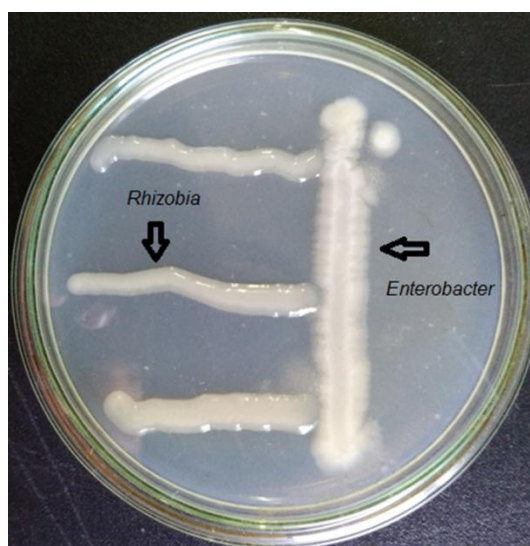


Fig. 2. Showed there is no antagonist between *R. leguminosarum* *bv. viciae* and *E. cloacae* KX034162 where the growth of the two microorganisms are continued and attached

Inoculation with the studied microbe's increased NPK% of seeds and plants compared with un-inoculated treatment this treatment is not among your treatments. The dual inoculation gave the NPK% value compared with the other treatments in the two seasons. IAA, biofilm formation, P-solubilization and polysaccharides production had a positive influence on the roots deviation and elongation and augmented mineral absorption which amended the faba bean plants growth items and output. P-solubilizing enhanced plant growth and yield [15]. Inoculations with P-solubilizing bacteria used alone or in mixture with other rhizospheric microbes have favorable properties on plant development [56]. EPSs have the facility of biofilm creation which helps the microbes to circulating essential nutrient to the plant from the soil for growth and development [21,22,23]. The increase in dehydrated weigh of plants, seeds number and dry weight of pods was due to microbial application where, PGPR reduced difusate effect of faba bean root on *R.*

leguminosarum *bv. viciae* and increased adhesion of *R. leguminosarum* *bv. viciae* to the roots. The production of IAA and polysaccharides increased root hairs and this effect on the nutrients absorption from the rhizosphere root area.

4. CONCLUSION

PGPR *E. cloacae* KX034162 and *R. leguminosarum* *bv. viciae* plays a vital part in rhizospheric soil processes and productivity of faba bean. The synergistic interaction of the *E. cloacae* KX034162 and *R. leguminosarum* *bv. viciae* along with the rhizospheric beneficial microorganisms stimulate faba bean growth and development through enhanced mineral nutrition acquisition and improved environment conditions. Furthermore, enhancement of roots proliferation and elongation by PGPR *E. cloacae* KX034162 along with N₂-fixation *R. leguminosarum* *bv. viciae* mediated synergism which improves plant NPK% and faba bean productions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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