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POSSIBILITY OF DECREASING CONSUMPTION OF CHEMICAL FERTILIZERS WITH USING PHOSPHOROUS AND POTASSIUM SOLUBILIZING BACTERIA INOCULATION ON FENNEL

El-Saied R.M.[1], Reham S. Abd Elhamed [2] and Hassanein W.A.[3]

[1]Plant nutrition department, Soils, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt.
[2]Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.
[3]Botany Department, Faculty of Science, Zagazig University, Egypt.

Abstract

Phosphorus and potassium are the main macronutrients essential for plant growth and development, therefore they are commonly added as fertilizers to optimize yield. A total of 14 phosphate solubilizing bacteria from agricultural soil were isolated on the NBRIP agar medium. The isolates showing clear halo zones around the bacterial growth were considered as phosphate solubilizers. Out of them, 8 isolates were able to solubilize potassium on Alexandrov medium. The isolates showing clear halo zones around the bacterial growth were considered as potassium solubilizers. Among these potent isolates, isolate no. 6 showed highest phosphate and potassium solubilization indices (3.06 and 3.05) on NBRIP and alexandrov agar media respectively. Drop in pH during growth indicated the production of organic acids. Its identification was confirmed by 16s rDNA gene sequences as *Enterobacter cloacae*. A soil experiment was conducted to evaluate the potential of bacteria with full or half recommended dose chemical PK fertilizers during two seasons, in soil planted with fennel seeds to study growth, yield characters, chemical composition and seed oil content of fennel plant. The results showed that the integration of 50% recommended dose of chemical fertilizers with P and K-solubilizing bacteria were significantly enhanced growth parameters, fruit yield/ha (3.02 ton) and essential oil yield (48.32 l/ha) for 1st season while 100%NPK with biofertilizers enhanced N, P, K and protein (1.82%, 0.501%, 2.40% and 11.38%) respectively. Therefore, the direct application of half PK chemical fertilizers with solubilizing bacteria will be a promising, sustainable to the use of classical fertilizers.

***Keywords*:** Bacillus polymyxa, *Enterobacter cloacae*, super phosphate, potassium sulfate, fennel.

Introduction

Plant nutrition has role in increasing plant production. Phosphorus helps in the metabolism of the plant, cellular energy transfer, respiration and photosynthesis (Usada and Shimogawara, 1993). It is a structural component of gene and chromosome nucleic acids and of many coenzymes, phosphoproteins, and phospholipids. Therefore, an adequate supply of P is essential in the early stages of plant growth. As a result, P deficiency includes decreased plant height, delayed leaf emergence and reduced tillering, dry matter yield and seed production (Hoppo *et al.,* 1999). Potassium is one of the main nutrients and helps in the growth and development of plants. It has a role in various physiological processes and the absorption of other nutrient elements (Mengel and Kirkby, 1982). It also aids in the activation of more than 60 enzyme systems in plants. On the other hand, potassium deficiencies become a problem because they decrease easily in soils due to crop absorption, runoff, leaching and soil erosion (Sheng *et al.,* 2002). So recently world attended to use biofertilizers which contain efficient strains of nitrogen fixing bacteria, phosphate and potassium solubilizing bacteria to reduce plant and soil contamination with chemical mineral fertilizers. Moreover, these bacteria cells increased the availability of nutrient in form which can be easily assimilated by plants (shaaban *et al*., 2015), improving crop efficiency and quality with decreasing pollution and achievement sustainable agriculture (Rahimi *et al*., 2019). Phosphate Solubilizing Bacteria (PSB) solubilize the organic substance in the soil and convert organic phosphorus into inorganic phosphorous (Pi) that can be absorbed and used by plants (Lü and Huang, 2010), increase the plant's physiological activities, quality and seed quantity (El Sebai *et al.,* 2019). Application of K solubilizing bacteria (KSB) increasing the amount of K available in the soil and promoting the mineral content in the plant (Abou-el-Seoud and Abdel-Megeed 2012 and Zhang and Kong, 2014). Inoculation of *E. cloacae* showed decreased pH, increased auxin content and enzyme activities in rhizosphere soils, nutrient concentration in shoots and roots of soy bean and wheat, increased shoot and seed weight compared to un-inoculated control (Ramesh *et al*., 2014). Application of biofertilizers with chemical fertilizers increased vegetative growth, yield parameter and essential oil content in the fennel compared to chemical fertilizer treatments only (Zaki *et al*., 2019).

Fennel (*Foeniculum vulgare* Mill.; Family, Umbelliferae) is native to North Africa, the Mediterranean region, southern Europe and Asia, it grows well in Egypt (Abd El- Wahab and Mehasen 2009). Fennel is one of the most important medicinal and aromatic plants due to its pharmaceutical properties, as carminative, diuretic, anti-inflammatory, antimicrobial, antioxidants and galactogogue (Evans, 1989 and Mahfouze and sharaf-Eldine (2007). Fennel is used to increase milk production in humans and other animals; it is also used to promote lactation, aid weight loss and longevity. Furthermore, volatile fennel oils are used to control flatulent dyspepsia and colic in children (Facciola, 1990).

Material and Methods

Source of bacteria:

Bacteria were isolated from the soil and rhizosphere of roots of wheat, cowpea, maize and trifolium plants growing in fields, Sharkia, Daqahlia and Giza districts, Egypt.

Isolation of phosphorous solubilizing bacteria:

All the bacterial isolates were checked on National Botanical Research Institute's phosphate growth medium

(NBRIP) (($MgCl_2.6H_2O$ (5g l^{-1}), $MgSO_4.H2O$ (0.25 g l^{-1}), KCl (0.2 g l^{-1}), $(NH4)_2SO_4$ (0.1 g l^{-1}), $Ca_3(PO_4)_2$ (5 g l^{-1}) and agar (18 g l^{-1}) amended with glucose (10 g l^{-1}) (Nautiyal, 1999) as a selective medium for phosphorus solubilization. Solubilization index was evaluated according to the ratio of the total diameter (colony + halo zone) and the colony diameter (Edi-Premono *et al.*, 1996). PSB showed zone of solubilization on NBRIP agar were further examined for their ability to release Pi from Tri Calcium Phosphate (TCB) in broth medium. Cultures were harvested after growth periods in order to record the change in pH and concentration of Pi released in the medium. Available phosphorus in broth cultures was estimated according to (Rouser *et al*., 1970).

Isolation of potassium bacteria

All the bacterial isolates were tested for their ability to solubilize insoluble inorganic potassium on Alexandrov agar plates as a selective medium ($MgSO_4.7H_2O$)(0.005 g l^{-1}), ($FeCl_3$) (0.1 g l^{-1}), ($CaCo_3$)(2.0 g l^{-1}), Potassium mineral (2.0 g l^{-1}), Ca_3HPO4 (2.0 g l^{-1}) and agar (18 g l^{-1}) amended with glucose (5 g l^{-1}) (Hu *et al.*, 2006). The colonies that form clear zone were further examined for their ability to release K from broth medium (supplemented with 1% muscovite mica). The amount of K released in the broth and pH was estimated for five days of incubation. The available K content was determined by flame photometer (Sugumaran and Janarthanam, 2007).

Purified isolates of *Bacillus polymyxa* were kindly obtained from Microbiology section, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

Identification of the selected bacterial isolate: The identification of the most potent PSB and KSB bacterial isolates were confirmed using 16S rDNA sequencing. The experiment was carried out in sequence unit, Sigma Company, Giza, Egypt.

Antagonistic activity between the *Bacillus polymyxa* and *Enterobacter cloacae*: The antagonistic activity of nitrogen fixing bacteria and most potent PSB and KSB isolates was carried out using method described by Huang and Hoes (1976).

Treatments and experimental design:

Experimental field was carried out in a private farm at Belqas village, Daqahlia governorate during the two successive seasons on October 2017/2018 and 2018/2019 to study the efficiency of bacterial strains to utilizing of chemical fertilizers in soil on growth, yield, chemical composition and essential oil content of fennel *(Foeniculum vulgare* Mill). Healthy and homogenous size of fennel seeds were surface sterilized with 2% sodium hypochlorite and soaked in double volume of sterile distilled water containing bacterial strain. After 2 hr., the bacterial suspension was drained off and the seeds were dried under shade for 30 min. (Commare *et al.*, 2002). A single inoculation seed harbored about (10^8 cfu/ml) on its surface.

Rates of fertilizers and time of application as recommendation of (Ministry of agriculture, 2015). Phosphorus fertilizer as calcium super phosphate (15.5% P_2O_5) was added at a rate of 200 kg P_2O_5 fed^{-1} during the preparation of soil. Nitrogen fertilizer at a rate of 300 kg N fed^{-1} as ammonium nitrate (33.5% N) was split into two equal doses before the first and the second irrigation. Potassium fertilizer was applied in the form of potassium sulphate (48 % K_2O) at the rate of 100 kg fed^{-1}. in two equal doses with nitrogen fertilizer doses.

The experiment was conducted using completely randomizing block design with three replicates and six treatment, (T1) Control, (T2) "super phosphate + potassium sulfate" at 100% recommended dose, (T3) "super phosphate + potassium sulfate" at 50% recommended dose, (T4) Control+ bacterial inoculation, (T5) "super phosphate + potassium sulfate" at 100% recommended dose+ bacterial inoculation and (T6) "super phosphate + potassium sulfate" at 50% recommended dose + bacterial inoculation.

The plot area was 10.5 m^2 (3×3.5 m), the distance between hills was 40 cm; the distance between the rows was 60 cm. After complete germination the seedlings were thinned to one plant/hill. The random samples were collected for morphological and chemical analysis at vegetative stage after 45 days from planting. At fruiting stage, the plants were harvested during the two seasons to determine the yield parameters and seed oil content.

Statistical analysis: statistical analyses were carried out using MSTAT-C Program version 2.10 (1991). Least significant difference (LSD) was employed to test for significant difference between treatments $p \leq 0.05$ (Gomez and Gomez, 1984)

Table 1 : Physical and chemical characteristic of experimental soil:

Physical properties				Organic matter%	E.C. dsm^{-1}	pH	SP (%)	Metal conc. (ppm)		
Clay	Silt	Sand	Texture					N	P	K
29.9	36.9	33.2	Sand clay loam	1.09	0.89	7.84	53.5	48.7	5.12	225

Sampling and collecting data:

(A) External morphology:

Plant height (cm), number of branches/plant, number of leaves/plant, fresh and dry weight of shoots (g)/plant were estimated.

(B) Yield and yield components:

Weight, number of umbels and umblets /plant, fruits yield / plant (g) and fruit yield/ha (ton) were determined.

(C) Chemical analysis of plant:

Pigments content was determined according to (Metzner *et al*., 1965). Carbohydrates were estimated according to enthrone method (Sadasivam and Manickam, 1996). Total nitrogen content in shoots was determined by micro-kjeldahl apparatus was employed for total N-determination as described by (Jones *et al.*, 1991). Crude protein was calculated by multiplying N content by 6.25 for fennel leaves (A.O.A.C., 1970). Total phosphorus was determined spectrophotomitrically by Peters *et al.* (2003).

Total potassium was estimated flame photometerically (Peters *et al.,* 2003).

(D) Essential oil

Essential oil from fennel fruits was isolated by hydro distillation in order to extract the essential oils using the method of Guenther (Guenther, 1961). Oil percentage of the fruits was determined according to (British Pharmacopoeia, 1963). Essential oil yield (ml/ plant) and (l/ha) were calculated by multiplying oil (%) by fennel fruits yield plant (g) and yield/ ha (ton) respectively.

Results and Discussion

Nutrients are soluble and available to the plant with NPK chemical fertilizers. So, its effect is direct and fast (Chen, 2006). However, the price of NPK chemical fertilizers recently became very expensive and pollutes the environment. Much attention has recently been directed to the use of biofertilizers (microbial inoculation) to reduce contamination of the plant and soil with chemical fertilizers, so biofertilizers are used to reduce dependence on chemical fertilizers that are not only dangerous for human consumption, they can also upset the ecological balance and providing sustainable agriculture (Dawwam *et al*., 2013 and Bhardwaj *et al*., 2014). In the present investigation, 14 isolates of bacteria were isolated from the soil and rhizosphere of roots of plants. Isolated bacteria were further screened for phosphate and potassium solubilization on NBRIP and Alexandrov agar media respectively. Isolation of phosphate and potassium bacteria was previously carried out by Mardad *et al*. (2014) and Tan *et al.* (2014).

Phosphorous solubilization of tested bacteria

Data in (Table 2) described the solubilization zones of different isolates were found to vary from (0.53 cm to 1.28 cm). Moreover the solubilization Index (SI) of the tested phosphate-solubilizing bacterial strains ranged between 1.61 to 3.06. The extent of Pi solubize by the selected isolates in the broth NBRIP medium during five days were presented in (Table 3).The results indicated the release of Pi was different among strains. Moreover it was found that, by increasing incubation period, Pi amount increased. Isolate PSB6 was the most potent during five days and recorded highest data ranged between (39.88 µg/ml) in first day of incubation to (96.86 µg/ml) in the last day. This is attributed to the release of organic acids by various bacteria (Lal, 2002). The phosphorus release capacity could be evaluated from the levels of this released element, which was dissolved in the supernatant in the form of orthophosphate. The release of soluble orthophosphate from TCP by microorganisms generally involves the production of organic acids and a decrease in the pH of the culture medium (Carrillo *et al*., 2002 and Puente *et al.,* 2004). pH of the cultural broth samples dropped significantly as compared to the control where it remained constant around pH 7.0 (Table 4). Isolate PSB6 caused decrease in pH from 6.90 at the beginning to 4.90 in the end of incubation and this attributed to the diffusion of different organic acids secreted by bacterial isolate. The pH drop in P- solubilizing bacteria in liquid cultures resulted in present study was supported by Ramesh *et al*. (2014) who stated that *Enterobacter* sp. has ability to solubilize P from inorganic and organic P sources and secrete different organic acids as succinic, acetic, glutamic, oxaloacetic, pyruvic, malic and fumaric acids, a newly detected and identified organic acid was the alpha-ketoglutaric acid (Mardad *et al*., 2014).

Potassium solubilization of tested bacteria

Data showed that 6 isolates cannot grow on Alexandrov medium (Table 5). While others isolates can grow and formed a halo zone around the growth (Fig. 1). The solubilization zones of different isolates were found to vary from (0.10 cm to 1.23 cm) after incubation. In the same respect, the solubilization indexes of different isolates were varying from (1.13 to 3.05) (Table 6). Isolate PSB6 was most potent in releasing of K from muscovite mica during five days and recorded highest data ranged between (6.00µg/ml) in first day of incubation to (14.00µg/ml) in the last day. The extent of K released by the tested isolates in the broth Alexandrov medium during five days was presented in (Table 7). The results indicated the release of K was different among strains. Moreover the results showed that, by increasing incubation period, K amount increased. The mechanism of potassium released from minerals is still not clear. The bacteria might produce acids, alkalis or chelants which enhance the release of elements from potassium bearing minerals such as muscovite mica (Tan *et al.*, 2014 and Zhang and Kong, 2014). The pH of the cultural broth samples (Table 8) dropped significantly as compared to the control where it remained constant around pH 7.0. Isolate PSB6 caused decrease in pH from 6.75 at the beginning to 4.70 in the end of incubation and this attributed to secrete different organic acids. This is consistent with the findings of previous studies (Tan *et al.*, 2014 and Zhang and Kong, 2014).

Identification and antagonistic activity

Among the 14 isolates, only one bacterium was selected due to high amount of phosphate and potassium solubilizing capacity and it was identified as *Enterobacter cloaceae* by 16s rDNA gene sequences (Fig. 2). It was found that, there is no antagonistic activity between *Bacillus polymyxa* and *Enterobacter cloaceae* (Fig. 3) and this attributed to they did not produce antibacterial metabolites (Liu *et al*., 1995 and Glick *et al.,* 1999). Thus *Bacillus* strains inoculation might play an important role in protein biosynthesis, either by direct nitrogen supply (through fixation of nitrogen) or indirectly by the accumulation of nitrite and its subsequent increased the plant yield (Hussein and Arafa, 2009 and Yazdani *et al.*, 2009). *Enterobacter cloacae* have not only been shown to fix nitrogen but also have phosphate solubilizing properties (Mauricio *et al*., 2009) and potassium solubilizing ability (Ramesh *et al.*, 2014) so have effect on accelerate growth parameters, yield criteria, nutrient concentration and enhanced seed oil content.

Growth parameters

The data were presented in (Table 9) showed that biofertilizers (*Bacillus polymyxa* and *Enterobacter cloacae*) with chemical fertilizers significantly improved the morphological parameters at vegetative stage (45 days) during two seasons. The highest values of plant height were obtained by T5 with (39.8 cm) in 1st season. Results also recorded significant increase in number of branches (4.0 branches/plant), leaves number (24.8/plant), fresh weight (8.15 g) while dry weight recorded (0.90 g.) for 1st season; the 2nd season has the same trend. These matched with many investigators as fennel plants (Zaki *et al.,* 2010 and Zaki *et*

al., 2019). It noticed that, Interaction treatments reflected significant differences on all the measured vegetative growth parameters. These enhancements in growth parameters by the combined applications of biofertilizers (mixed strains) with chemical fertilizers may be due to the ability of bacteria to produce hormones, especially IAA (Sheng and Huang, 2001). *Enterobacter cloacae* have not only been shown to fix nitrogen but also have phosphate solubilizing properties (Mauricio *et al*., 2009) and potassium solubilizing ability (Ramesh *et al.,* 2014), so have effect on accelerate growth parameters. Phosphorus and nitrogen are known to play an important role in the molecular structure of nucleic acids, DNA and RNA resulting in increased protein synthesis and protoplasm formation with increasing in vegetative growth (Mengel and Kirkby, 1987 and El-Shanshoury, 1995). This is attributed to creating favorable conditions for bacteria for the root system to absorb and translocate water and nutrients to the green parts of the plant and promoting photosynthetic activities that result in denser vegetative growth. (Zaki *et al*., 2019)

Yield parameters

In the present study the yield parameters were significantly increased with the combined application of mixed strains and chemical fertilizers during two seasons (Table 10), where the increase in number of umbles (10.25 umbels/plant), number of umblets (130 umblets/plant) fruit yield/plant (72.45 g), fruit yield/ha (3.02 ton/ha) during 1st season were recorded by 50% PK + biofertilizers. It worth noting that 2nd season has same direction. Synergetic effects of biofertilizers and chemical fertilizers have also been reported in various crops such as hot pepper (Supanjani *et al*., 2006), sweet fennel (Zaki *et al*., 2010 and Zaki *et al.,* 2019), fennel (Dadkhah, 2012). These increases in yield parameters may be due to an increase in the solubilization of chemical fertilizers, which increase the P and K available in the soil and, therefore, stimulate the growth and absorption of minerals by plants (Park *et al*., 2003). Furthermore, it can be attributed to the increase in nitrogen fixation by *Bacillus polymyxa* that improves vegetative growth and finally the yield of fennel plants (Vessey, 2003 and Premsekhar and Rajashree, 2009).

Total chlorophyll and total carbohydrate

The application of dual inoculation with chemical fertilizers (50% or 100%) were significantly favored higher accumulation of chlorophyll a, b and carotenoid contents, respectively (0.421 mg/ml, 0.292 mg/ml and 0.093 mg/ml), recorded by (T5) for 1st season as well as enhanced total carbohydrate (22.86%) (Table 11). These results support the previous findings in different medicinal and aromatic plants (Ali and Hassan, 2014 and El- Mokadem and Sorour, 2014). The improvement in chlorophyll and carbohydrate content may be due to increased absorption and translocation of essential metal ions by bacterial inoculation, which leads to acceleration of metabolic rates related to the synthesis of these constituents. The greater height increase registered with the inoculated plants could be due to a greater absorption of inorganic nutrients (Cooper, 1984) and higher photosynthesis rates (Allen *et al*., 1981). In addition, it may be due to the promotion of cytokinins secreted by N_2 fixatives are known to delay senescence of plant tissues through their effect in reducing chlorophyll loss (Gaballah, 1995). Also, phosphate solubilizing bacteria stimulate chlorophyll synthesis through stimulation of pyridoxal enzyme formation that plays an important role in amino levulinic acid synthetase as a primary compound in chlorophyll synthesis. Different authors recorded similar results (Al-Fraihat *et al.,* 2011 and Hassan and Ali, 2013). A positive relationship has been reported between leaf carbohydrates and leaf pigments (Hassan and Ali, 2013). Therefore, the synthesis of photosynthetic pigments in leaves may be an induced factor for carbohydrate synthesis, hence the carbohydrate percentage was increased in fennel.

Chemical analysis of shoots

Data showed in (Table 12) the different applied biofertilizers (*Bacillus polymyxa* and *Enterobacter cloaceae*, chemical fertilizers (50%, 100% PK) and their interactions significantly increased chemical constituents in leaves of fennel plants at vegetative stage (45 days) for 1st and 2nd season. Where (T5) recorded highest data (1.82 %, 0.501%, 2.40% and 11.38%) for N,P,K and protein content, respectively. These results were agreed with the work on sweet fennel (Zaki *et al*., 2010 and Zaki *et al*., 2019). These data were probably due to the fact that the presence of bacterial strains led to increased solubility and availability of nitrogen in the soil, which consequently increased the amount of absorption by the roots; As a result, the percentage of nitrogen in the leaves would increase. The percentage of P and K in leaves could be increased may be due to that *Enterobacter cloacae* secrete organic acids which soluble phosphate and potassium (El- Mokadem and Sorour, 2014).

Essential oil yield

Data presented in (Table 13) illustrated the effect of chemical fertilizers (100%, 50% recommended dose) and/ or biofertilizers on essential oil content. It is noticed that, the highest significant content was obtained in presence of dual inoculation of both inoculants where soil amended with 50% PK (1.16 ml/plant, 48.32 l/ha) for 1st season, the 2nd season has the same trend. Results have been agreed with Dadkhah (2012) for fennel, Hellal *et al*. (2011) for *Anethum graveolens* L., Aly *et al.* (2015) for dill and Amer *et al*. (2019) for *Salvia officinalis*. This increase in oil content may be due to increment number of umbels, umbelets per plant, fruit yield/ha. and essential oil percentage (Eisa, 2016), enhancing vegetative growth parameters, plant chemical composition such as nitrogen, phosphorus, potassium and total carbohydrates coinciding with increase the yield and essential oil components (Abdelaziz *et al.,* 2007).

Conclusion

It can be concluded that combining dual bacterial inoculation (*Bacillus polymyxa* as nitrogen fixer and *Enterobacter cloacae* as phosphate and potassium dissolving bacteria) with chemical fertilizers (superphosphate and potassium sulfate) exhibited an enhancement in growth, yield, chemical composition as well as seed oil yield of fennel plant**.** Moreover, using biofertilizers reduce the application of chemical fertilizers by 50% without any reduction in growth and yield of fennel plants**.**

Table 2 : Phosphorous solubilization index of the tested bacteria cultivated on NBRIP medium.

Isolate Number (PSB)	Halo zone diameter (cm)	Total diameter (colony+halozone) (cm)	Colony diameter (cm)	Solubilization index (SI)
1	0.53 l	1.40 n	0.87 d	1.61 j
2	0.84 i	1.59 j	0.75 h	2.12 g
3	1.00 e	1.82 f	0.82 f	2.22 f
4	0.90 h	1.84 e	0.94 b	1.96 h
5	1.10 d	1.80 g	0.70 j	2.57 c
6	1.28 a	1.90 d	0.62 l	3.06 a
7	0.90 h	1.64 i	0.74 i	2.22 f
8	0.98 f	1.56 k	0.58 n	2.69 b
9	0.76 j	1.96 c	1.20 a	1.63 j
10	0.93 g	1.53 l	0.60 m	2.55 c
11	0.98 f	1.66 h	0.68 k	2.44 e
12	0.67 k	1.50 m	0.83 e	1.81 i
13	1.11 c	2.01 b	0.90 c	2.23 f
14	1.23 b	2.04 a	0.81 g	2.52 d
$LSD_{at\ 5\%}$	0.01	0.02	0.01	0.02

Different letters within a column indicate a significant difference at $P \leq 0.05$, n=5

Table 3: Amount of released Pi (µg/ml broth) by tested phosphorous solubilizing bacteria during five days of incubation.

Isolate Number (PSB)	Released Pi (µg/ml broth)				
	1 day	2 days	3 days	4 days	5 days
1	8.14 n	13.83 k	15.46 k	16.14 m	18.27 k
2	28.05 e	40.82 f	46.44 f	60.98 f	65.26 e
3	11.40 k	16.28 i	21.98 h	30.93 g	31.74 f
4	9.72 l	14.13 k	18.70 i	22.79 j	30.22 g
5	30.11 d	43.38 d	62.48 c	65.88 d	68.83 c
6	39.88 a	62.67 a	80.58 a	90.35 a	96.86 a
7	19.70 g	22.40 g	23.93 g	28.06 h	30.65 g
8	32.56 b	49.65 b	72.44 b	80.58 b	83.83 b
9	8.40 m	12.96 l	16.18 j	16.49 m	17.64 l
10	30.94 c	45.55 c	59.59 e	66.51 c	67.90 d
11	12.47 j	13.24 l	16.22 j	18.14 l	19.69 j
12	15.40 h	21.78 h	21.78 h	23.61 i	27.42 h
13	13.35 i	15.50 j	18.90 i	21.76 k	22.40 i
14	26.65 f	42.80 e	60.90 d	63.06 e	65.18 e
$LSD_{at\ 5\%}$	0.24	0.35	0.48	0.53	0.55

Different letters within a column indicate a significant difference at $P \leq 0.05$, n=5

Table 4 : Change in medium pH by the tested phosphorous solubilizing bacteria during five days of incubation.

Isolate number	1 day	2 days	3 days	4 days	5 days
1	7.00 a	6.80c	6.00d	5.47f	5.47ef
2	6.97abc	6.83bc	5.95de	5.48f	5.42f
3	6.90de	6.45f	5.95de	5.45fg	5.27g
4	6.97abc	6.52e	5.70g	5.66d	5.01i
5	6.85ef	6.20g	5.70g	5.40gh	5.00 ij
6	6.90 de	6.00 h	5.30 i	5.00 j	4.90 k
7	6.98ab	6.55e	6.17c	6.01b	5.98b
8	6.91cde	6.00h	5.78f	5.35h	4.98ij
9	6.81f	6.70d	5.95de	5.28i	5.16h
10	7.00ab	6.43f	5.55h	5.46f	5.12h
11	7.03a	6.87b	6.48b	6.00b	5.78c
12	6.93bcd	6.52e	5.90e	5.70d	5.55d
13	7.00a	6.70d	6.00d	5.86c	5.50de
14	6.98ab	6.23g	5.79f	5.55e	4.94 jk
Control	7.00a	7.00a	7.00a	6.98a	6.98a
$LSD_{at\ 5\%}$	0.06	0.07	0.06	0.06	0.06

Different letters within a column indicate a significant difference at $P \leq 0.05$, n=5

Table 5 : Potassium releasing ability of the tested isolates on Aleksandrov medium

Isolate number (PSB)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
K- released by PSB	–	+	+	–	+	+	–	+	–	+	+	–	+	–

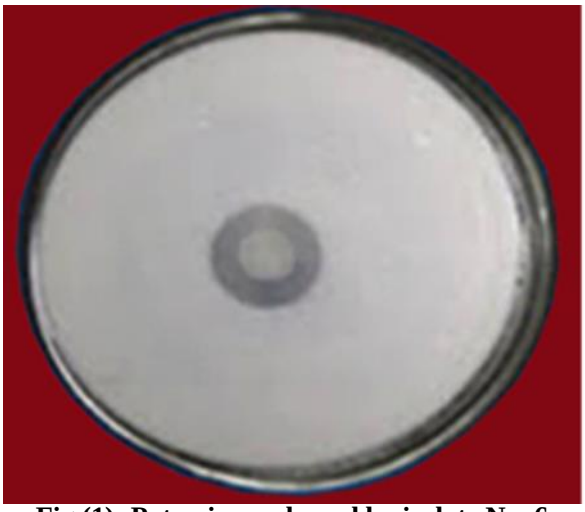

Fig.(1): Potassium released by isolate No. 6.

Table 6 : Potassium solubilization index of the tested bacteria cultivated on Aleksandrov medium.

Isolate Number (PSB)	Halo zone diameter (cm)	Total diameter (colony+halozone) (cm)	Colony diameter (cm)	Solubilization index (SI)
2	0.70 c	1.45 c	0.75 d	1.93 c
3	0.47 d	1.10 d	0.63 c	1.75 cd
5	1.26 a	1.91 a	0.65 c	2.94 ab
6	1.23 ab	1.83 ab	0.60 c	3.05 a
8	0.70 c	1.75 b	1.05 a	1.67 d
10	1.20 ab	1.85 ab	0.65 c	2.85 b
11	0.10 e	0.90 e	0.80 b	1.13 e
13	1.16 b	1.03 de	0.87 b	1.18 e
$LSD_{at\ 5\%}$	0.09	0.14	0.08	0.19

Table 7 : Released potassium by the tested bacterial isolates during five days of incubation.

Isolate Number (PSB)	Released potassium (µg/ml)				
	1 day	2 days	3 days	4 days	5 days
2	5.80 a	8.00 a	9.50 c	10.60 bc	11.00 bc
3	4.10 cd	5.70 c	7.00 e	9.90 c	10.30 cd
5	6.00 a	8.30 a	11.00 b	11.00 b	11.50 b
6	6.00 a	8.50 a	12.06 a	13.50 a	14.00 a
8	4.50 bc	4.50 d	8.00 d	9.00 d	9.40 d
10	4.90 b	7.00 b	9.14 c	10.60 b	11.20 bc
11	4.00 d	4.20 d	5.00 f	7.50 e	8.00 e
13	4.10 cd	4.50 d	5.70 f	8.00 de	8.00 e
$LSD_{at\ 5\%}$	0.23	0.29	0.38	0.46	0.47

Table 8 : Change in medium pH by the tested potassium solubilizing bacteria during five days of incubation.

Isolate number	1 day	2 days	3 days	4 days	5 days
2	6.85a	5.31bc	5.08b	4.87b	4.75b
3	6.90a	5.40bc	5.25b	4.98b	4.90b
5	6.85a	4.95c	4.95b	4.87b	4.70b
6	6.75a	5.00c	4.88b	4.75b	4.70b
8	6.95a	5.55b	5.00b	4.87b	4.84b
10	6.87a	5.03bc	4.91b	4.83b	4.79b
11	6.90a	5.35bc	5.08b	5.08b	5.00b
13	6.83a	5.55b	5.33b	5.00b	4.94b
Control	7.00a	7.00a	6.98a	6.98a	6.96a
$LSD_{at\ 5\%}$	N.S.	0.54	0.52	0.51	0.50

Different letters within a column indicate a significant difference at $P \leq 0.05$, n=5

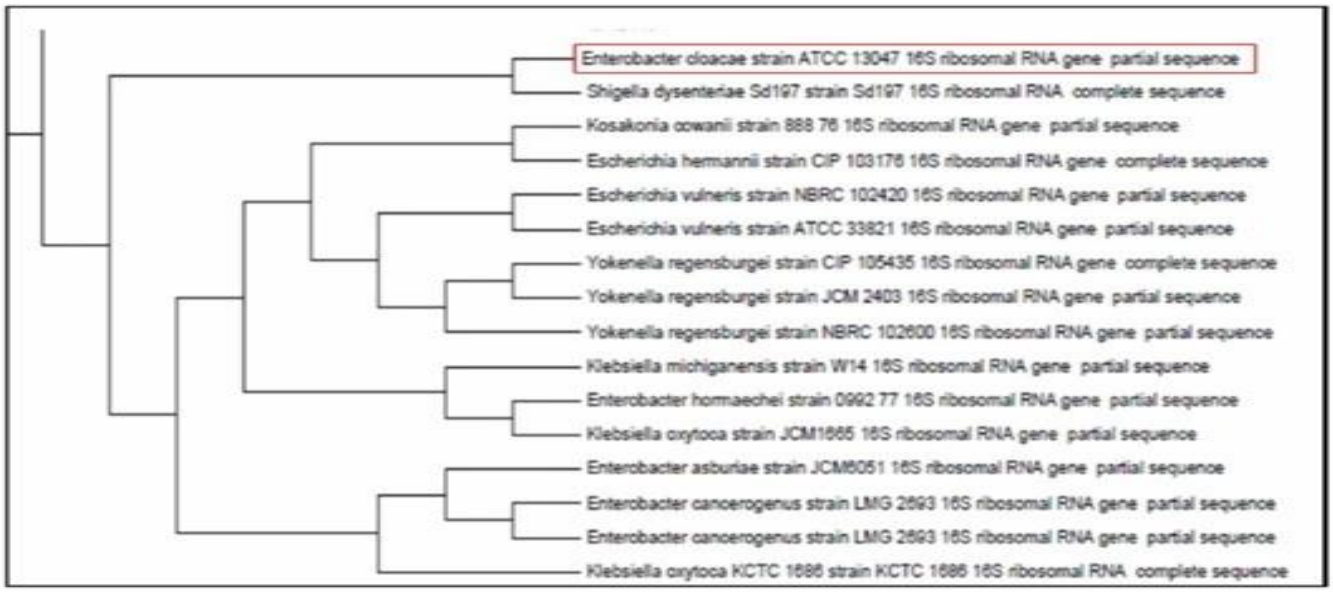

Fig. 2 : Phylogenatic tree represented the DN similarities of the obtained 16S rDNA gene sesquences.

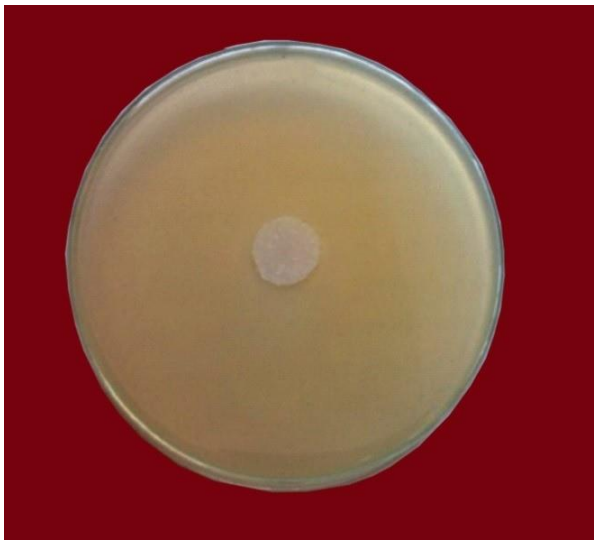

Fig. 3 : The antagonistic activity between *Paenibacillus polymyxa* and *Enterobacter cloacae*.

Table 9 : Effect of biofertilizers (*B. polymyxa* and/or *E. cloacae*) with chemical fertilizers (100 and 50% recommended dose) on growth parameters of fennel plants at 45 days.

Treatments	Stem (cm)	Branch (No.)	Leave (No)	F.wt shoot (g/plant)	D. wt shoot (g/plant)
First season					
T1	24.50f	2.15e	7.0f	4.20f	0.52c
T2	37.75c	3.50b	20.8c	7.15c	0.83a
T3	34.5d	3.00c	15.9d	6.08d	0.64b
T4	28.5e	2.50d	13.2e	4.50e	0.59bc
T5	39.85a	4.00a	24.8a	8.15a	0.90a
T6	39.00b	4.00a	23.0b	7.80b	0.88a
LSD $_{at\ 5\%}$	**0.21**	**0.21**	**0.22**	**0.19**	**0.08**
Second season					
T1	25.20e	2.25d	7.0f	4.00f	0.50c
T2	36.00b	3.50b	18.5c	7.00c	0.83a
T3	33.00c	3.05c	17.5d	5.75d	0.67b
T4	26.50d	3.00c	12.5e	4.30e	0.53c
T5	39.75a	4.15a	22.5a	8.80a	0.89a
T6	38.50a	4.00a	20.7b	7.44b	0.86a
LSD $_{at\ 5\%}$	**0.18**	**0.19**	**0.20**	**0.17**	**0.07**

T1: control, T2:supersulfate+sulfate potassium (100%), T3: supersulfate+sulfate potassium (50%), T4: bacteria inoculation, T5: supersulfate+sulfate potassium(100%) + bacteria, T6: supersulfate + sulfate potassium(50%)+ bacteria.
Different letters within a column indicate a significant difference at $P \leq 0.05$, n=3

Table 10 : Effect of biofertilizers (*B. polymyxa* and/or *E. cloacae*) with chemical fertilizers (100 and 50% recommended dose) on yield parameters of fennel plants.

Treatments	Number of umbels / (plant)	Number of umbelets / (plant)	Fruit yield/plant (g)	Fruit yield/ha (ton)
First season				
T1	4.00e	16.00f	10.40f	0.43d
T2	8.50b	97.70c	67.50c	2.81a
T3	7.00c	79.00d	54.10d	2.25b
T4	4.75d	34.00e	23.80e	0.99c
T5	10.00a	121.00b	70.75b	2.95a
T 6	10.25a	130.00a	72.45a	3.02a
LSD $_{at\ 5\%}$	**0.28**	**0.28**	**0.83**	**0.21**
Second season				
T1	4.50e	18.00f	11.18f	0.47d
T2	8.90c	85.50c	67.00c	2.79a
T3	7.75d	73.00d	55.00d	2.29b
T4	4.50e	30.00e	25.08e	1.04c
T5	9.65b	109.00a	70.00b	2.92a
T 6	10.00a	106.00b	71.45a	2.98a
LSD $_{at\ 5\%}$	**0.18**	**0.31**	**0.24**	**0.20**

Different letters within a column indicate a significant difference at $P \leq 0.05$, n=3

Table 11 : Effect of biofertilizers (*B. polymyxa* and/or *E. cloacae*) with chemical fertilizers (100 and 50% recommended dose) on chl a, b, total chlorophyll and total carbohydrate of fennel plants at 45 days.

Treatments	Chl a (mg/g)	Chl b (mg/g)	Total chlorophyll (mg/g)	Cartenoid (mg/g)	Total carbohydrate (%)
T1	0.246e	0. 178e	0.424f	0.073b	18.20e
T2	0.404b	0.277b	0.681b	0.086a	22.55b
T3	0.363c	0.248c	0.611d	0.072b	20.30c
T4	0.326d	0.213d	0.539e	0.069b	19.00d
T5	0421a	0.292a	0.713a	0.093a	22.86a
T6	0.414a	0.288a	0.702a	0.090a	22.73ab
LSD $_{at\ 5\%}$	**0.007**	**0.008**	**0.008**	**0.007**	**0.24**
Second season					
T1	0.300f	0.190d	0.490f	0.071c	18.44e
T2	0.372c	0.260b	0.632c	0.087b	22.61b
T3	0.341d	0.240c	0.581d	0.079bc	20.05c
T4	0.327e	0.242c	0.569e	0.074c	19.12d
T5	0.397a	0.273a	0.670a	0.094a	22.81a
T6	0.381b	0.267a	0.648b	0.089b	22.70ab
LSD $_{at\ 5\%}$	**0.008**	**0.006**	**0.006**	**0.008**	**0.19**

Different letters within a column indicate a significant difference at $P \leq 0.05$, n=3

Table 12 : Effect of biofertilizers (*B. polymyxa* and/or *E. cloacae*) with chemical fertilizers (100 and 50% recommended dose) on chemical constituents of fennel plants.

Treatments	Nitrogen (%)	Phosphorous (%)	Potassium (%)	protein (%)
First season				
T1	1.54b	0.401e	2.12d	9.63b
T2	1.77a	0.472b	2.32bc	11.06a
T3	1.61b	0.436c	2.25c	10.06b
T4	1.59b	0.422d	2.15d	9.94b
T5	1.82a	0.501a	2.40a	11.38a
T6	1.79a	0.493a	2.38ab	11.19a
LSD $_{at\ 5\%}$	**0.07**	**0.010**	**0.07**	**0.46**
Second season				
T1	1.52d	0.422d	2.11c	9.50d
T2	1.72b	0.475b	2.35a	10.75b
T3	1.63c	0.454c	2.24b	10.19c
T4	1.60c	0.447c	2.17c	10.00c
T5	1.79a	0.496a	2.39a	11.19a
T6	1.77ab	0.492a	2.38a	11.06ab
LSD $_{at\ 5\%}$	**0.06**	**0.008**	**0.07**	**0.40**

Different letters within a column indicate a significant difference at $P \leq 0.05$, n=3

Table 13 : Effect of biofertilizers (*B. polymyxa* and/or *E. cloacae*) with chemical fertilizers (100 and 50% recommended dose) on percentage and Essential oil yield in seeds of fennel plants.

Treatments	Essential oil yield		Percentage %
	ml/ plant	L/ha	
First season			
T1	0.06e	2.62f	0.61d
T2	0.96b	39.90c	1.42b
T3	0.74c	30.60d	1.36b
T4	0.19d	7.72e	0.78c
T5	1.10a	46.02b	1.56a
T6	1.16a	48.32a	1.60a
LSD at 5%	**0.05**	**0.08**	**0.07**
Second season			
T1	0.06e	2.68f	0.57e
T2	0.96b	39.90c	1.43b
T3	0.70c	29.31d	1.28c
T4	0.20d	8.22e	0.79d
T5	1.11a	46.43b	1.59a
T6	1.15a	47.98a	1.61a
LSD at 5%	**0.06**	**0.09**	**0.10**

Different letters within a column indicate a significant difference at $P \leq 0.05$, n=3

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