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Growth, Yield and Chemical Composition Response of some Legume Crops to Inoculation with Non-rhizobial Endophytic Bacteria from *Melilotus indicus* (L.) All. Nodules

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ABSTRACT

Two field experiments were carried out during two successive seasons of 2015 and 2016, to study the growth response and yield as well as chemical composition of some legume plants vis., cowpea (*Vigna unguiculata* L. cv Kaha 1), common bean (*Phaseolus vulgaris* L. cv. Nebraska), peas (*Pisum sativum* L. cv. Master B) and fenugreek (*Trigonella foenum-graecum* L., cv. Giza 3) to inoculation with non-rhizobial endophytic bacteria, strains ,vis., *Enterobacte*r *sp*.(strain No.1), *Pseudomonas sp*.(strain No.2) and *Pseudomonas sp*.(strain No.3) which were isolated from root nodules of *Melilotus indicus* (L.) All. Results showed that almost all treatments increased all vegetative growth parameters tested, vis., shoot minerals, nitrogen content and total carbohydrate and seeds minerals, total protein, total carbohydrate and total yield (seeds and green yield) of the four tested legumes. Further, the three bacterial strains used in this study showed high growth promoting activities. They gave high production of IAA and showed highly antagonistic activity against some phytopathogenic fungi. Therefore, the best results were obtained with treatments T2 for cowpea and fenugreek and T4 for common bean and pea. So, it could be recommended to use these bacterial strains as inoculants for cowpea, fenugreek, common bean and pea crop legumes.

Keywords: legumes, Plant growth-promoting, strain, newly reclaimed soils, environment,.

INTRODUCTION

The random and excessive application of chemical fertilizers has led to health and environmental threats, agronomists are worried to find alternative strategies that can ensure competitive yields while protecting the health of soils. Therefore, there has been an ever-increasing interest in the use of an safety alternative for environment to further applications of mineral fertilizers (Khan *et al*., 2007) to improve plant health and productivity while ensuring safety for human consumption, and protection of the environment, particularly for the developing countries (Avis *et al*., 2008).

Owing to this capacity, legumes are a major source of food, fodder, timber, phytochemicals, phyto medicines, nutria ceuticals, and nitrogen fertility in agro systems (Graham and Vance, 2000 and 2003).

Leguminosae or Fabaceae is the third most populous family of flowering plants and is the second only to the Gramineae in their importance to humans. Legumes colonize several ecosystems (from rain forests and arctic/alpine regions to deserts and produce seed pods (Graham and Vance, 2003; Morel *et al*., 2012). Legumes are responsible for a substantial part of the global flux of N2 to fixed forms. Therefore, they are very important both ecologically and agriculturally. As consequences of legume N2 fixation, they increased plant protein levels and soil fertility through reduced depletion of soil N reserves (Allito *et al*., 2015). Legumes, through their symbiotic abilities, can play an important role in colonizing disturbed ecosystems. Rates of N2 fixation in such environments are often low, but can still satisfy much of the legume's N needs (Graham and Vance, 2003). Legumes are important crops in providing food for humans worldwide. They are a primary source of amino acids and a third of processed vegetable oil for human feeding (Graham and Vance, 2003; Kudapa *et al*., 2013; Allito *et al*., 2015). Grain legumes alone contribution are 33% of the dietary protein nitrogen (N) needs of humans (Graham and Vance, 2003).

Bacterial endophytes have an important role for host plants. They can offer several benefits mainly growth promoters and protecting agents for pathogens. Further, bacterial endophytes are efficiently communicate and interact with the plant more than rhizospheric bacteria under diverse environmental conditions (Coutinho *et al*., 2015; Santoyo *et al*., 2016). Endophytes play an important role for plant growth promotion; either directly through facilitation the acquisition of resources from the habitats including nitrogen, phosphorous and iron or modulate plant growth through regulation of various plant hormones including auxin, cytokinin or ethylene. The indirect role of the endophyte promotion occurs when limits or prevents the plants damage that caused by various pathogenic agents including bacteria, fungi, and nematodes (Santoyo *et al*., 2016). A large number of common mechanisms are used by the endophytes in the indirect plant growth promotion, including the production of antibiotics, degrading enzymes for cell wall, decreasing plant ethylene levels, encouraged systemic resistance, lowering the amount of iron obtainable to pathogens, and the synthesis of volatile compounds that inhibit pathogens. In agriculture it would obvious to be valuable to utilize endophytic bacteria to facilitate plant growth, horticulture and forests as well as cleanup of environment, because they are much more probable to persist in different environments (Santoyo *et al*., 2016). Thus, in future, endophyte technology holds the key for a potential gateway to sustainable agriculture development (Wani *et al*., 2015). In the present study, we assessed the ability of three nonrhizobial endophytic isolates from nodules of wild Melilotus indicus to support growth and productivity of some grain legumes, viz, Vigna ungiculata L., Phaseolus vulgaris L., Pisum sativum L., and Trigonella foenum-graecum L. under field conditions in the new desert soil.

MATERIALS AND METHODS

Non-rhizobial endophytic strains

Three bacterial strains, viz., *Enterobacter sp*. (strain No.1), *Pseudomonas sp*.(strain No.2 and No.3) were isolated from root nodules of *Melilotus indicus* (L.) All. plants grown wild in different habitats of Egypt. These strains were provided kindly from a culture collection of Dr. Nadia H. El-Batanony, Professor of Microbiology at ESRI, University of Sadat City. They prepared according to the method of Somasegaran and Hoben (1994).

Host- legumes

Seeds of cowpea (*Vigna unguiculata* L.) cv. Kaha 1, common bean (*Phaseolus vulgaris* L.) cv. Nebraska and peas (*Pisum sativum* L.) cv. Master B were kindly provided by the Vegetable Research Institute, Agricultural Research

Center (ARC), Egypt, and fenugreek (*Trigonella foenum-graecum* L.) Giza 3 was generously provided from the Field Crops Research Institute, ARC, Giza, Egypt.

Field trials

Two field experiments were applied at ESRI farm, University of Sadat City, Egypt (30º 23 07 N 30º 30 55 E) during 2015 and 2016 summer and winter seasons on a sandy loamy soil. Physical and chemical characteristics of the soil were: 66.5% sand; 28.5% silt; 5% clay; pH 7.85; 1.18dSm−1 electrical conductivity (EC); 0.141% organic carbon; the soluble cations were: Ca2+ 380 mg g-1, Mg2+147.6 mg g-1, Na 134.3 mg g-1, K+19.5 mg g-1, N3+ 0.011 mg g-1, and P3+ 0.009 mg g-1,; the soluble anions were: CO32− 9 mg g−1, HCO3− 36.6 mg g−1, Cl− 310.9 mg g−1and SO42− 96 mg g−1.

The experiments were managed in a complete randomized blot design where the blot size was 3 m length × 0.9 m width in 3 replicates and the seeds were sown in one side of the ridge with plant space 25 cm and two seeds per hill, while, fenugreek was planted in lines the distance between them is 10-15 cm and the distance between the plants is 3-4cm, drip irrigation was used. All tested crops were planted separately but in the same field conditions. The date of planting for cowpea was in the second week of April and for common bean and pea in the second week of October, while fenugreek was planted in the first week of November during the two seasons. The seeds of each legume tested were sterilized as described by Vincent (1970) and then coated independently with each bacterial suspension (~108cells ml−1) using Arabic gum (40%) as an adhesive agent for 2 h before planting. The following treatments were used for each legume tested: T1) seeds without inoculation (uninoculated control); T2) seeds inoculated with wild nonrhizobial endophytic strain No.1; T3) seeds inoculated with wild nonrhizobial endophytic strain No.2; and T4) seeds inoculated with wild nonrhizobial endophytic strain No.3. When preparing the soil, phosphorus was added as super phosphate to all blots as (30 kg P2O5 /fed.) and potassium (45 kg K2SO4 /fed.) as potassium sulphate, while N was added as ammonium sulphate (21 kg N /fed.) at three times after planting. The plants were grown under open field, the recommended agricultural practices for growing plant were applied whenever required.

Measured characters and chemical analysis

Growth parameters of tested legumes, viz., plant height (PH); shoot fresh (SHFW) and shoot dry weight (SHDW); root fresh (RFW) and root dry weight (RDW) were recorded at 50 d. Plant dry weight obtained by drying the plant fresh samples in an oven at 105◦C. Complete drying was obtained when reaching a constant weight.

The total seed yield of cowpea and fenugreek was recorded at harvest, the green yield of common bean and pea were collected at harvest during growth interval.

The percentage of N, P, and total carbohydrates of dried legume shoots and seeds were determined for different treatments according to the methods described by Jackson (1958).

According to the assumption that nitrogen is derived from protein containing 16 % nitrogen (AOAC, 1984); the crude protein (CP) percentage was calculated for yield of each plant species by multiplying the nitrogen percentage by 100/16 or 6.25.

The growth-promoting activities of non-rhizobial strains isolated from wild grown *Melilotus indicus* L. All.

Indol acetic acid (IAA) production by the non-rhizobial strains

The IAA production was measured by the method reported by Scagliola *et al.* (2016) after modification. The strains were grown in flasks containing 100 ml YEB supplemented or not (control) with 200 µgml-1 of L-tryptophan. The flasks were inoculated with one ml of rhizobial culture at exponential phase (106 cell/ml). After an incubation period of 72 h on a rotary shaker (120 rpm) at 28 °C, cell-free supernatant was harvested by centrifugation at 10 000 rev min-1 for 10 mino and were acidified to 2.5–3.0 pH with 1 N HCl followed by extraction twice with ethyl acetate at double the volume of supernatant (2:1). Using rotary evaporator, the ethyl acetate fraction was dried at 40 °C, resuspended in 2 ml 60% methanol and stored at -20 °C. The methanol extract was examined by HPLC using a C18 reverse-phase column. The mobile phase was 60:40 methanol: water (%) containing 0.5% of acetic acid at a flow rate of 1 ml/min. The eluted phase was detected at 280 nm. Pure IAA (Sigma-Aldrich) was used as standard.

The antimicrobial activities of the non-rhizobial strains

The antibiosis activity of the four non-rhizobial isolates were screened against three soil born pathogenic fungi (*Sclerotonia rolsfi* - from plant pathology department Faculty of Agricultural Mansoura University, Egypt), *Pythium ultimum* AUMC 4413, and *Alternaria alternata* AUMC 10301) by the method described by Kucuk (2013) with some modification. Non-rhizobium strains were grown in 100 ml yeast extract broth at 28°C in a rotary shaker at 120 rpm for 3 days. Two ml of the culture suspension (106 cell/ml) was mixed with 20 ml of PDA. After solidification of PDA, a mycelial fungal plug was placed on the agar surface. The mycelial radial growth was recorded in mm after five days of incubation at 28°C, compared with control plates (fungal growth without bacteria). Then, the inhibition % was calculated from the following formula: Inhibition % = [(normal activity - inhibited activity) / (normal activity)].

Statistical analysis

The data were statistically analyzed as the proportion of variance explained by between-treatment differences using the general the statistical package of SAS version 9.1 (SAS, 2003).

RESULTS

The statistical analysis proved that the difference between the data of the two years was insignificant. Therefore, the results discussed here are the mean of the data obtained in the two years.

Root and Shoot growth related traits of the four tested legumes

All treatments increased the growth traits of the four legume plants. Cowpea and fenugreek plants maximum growth traits of root and shoot obtained with treatment T2). For some growth traits tested, T2 out-yielded significantly the uninoculated control T1) of cowpea plants. The maximum RDW was observed with T2, a value that is 2.69 (Table, 1). Furthermore, T2 increased growth traits (RL, RDW, PH, SHFW, and SHDW) of fenugreek root and shoot significantly with a value of 22.16cm, 0.35 g/plant, 56.16 cm, 40.91 g/plant and 5.45 g/plant, respectively.

However, treatment T4 increased the growth trait of common bean and pea plants (Table, 1). Some significant increases in the measured parameters were proved; the only significant increase was in RDW for common bean with a value of 1.85 g/plant. While of pea all parameters are insignificantly.

Table 1. Vegetative growth parameters of four traditional legume species as affected by inoculation with different wild nonrhizobial endophytic strains.

Legume species	Treatments	Root length (cm)	Root fresh weight (g/pl)	Root dry weight (g/pl)	Plant height (cm)	Shoot fresh weight (g/pl)	Shoot dry weight (g/pl)
Vigna unguiculata L.	T1(control)	16.17	9.17	1.50	37.17	91.07	16.16
	T2	21.00	12.17	2.69	40.00	138.00	23.70
	T3	19.66	8.00	1.71	27.33	75.33	13.59
	T4	19.50	11.16	2.38	36.00	100.67	17.45
	L.S.D 0.05	n.s	n.s	0.99	12.37	53.70	9.13
Phaseolus vulgaris L.	T1(control)	15.16	2.00	1.19	32.00	18.27	11.03
	T2	14.00	2.35	1.31	32.83	17.70	11.20
	T3	14.66	1.61	1.31	33.16	16.49	10.21
	T4	15.83	2.55	1.85	33.66	19.54	11.28
	L.S.D 0.05	n.s	0.79	0.41	n.s	n.s	n.s
Pisum sativum L.	T1(control)	17.13	1.38	0.77	30.00	22.09	11.30
	T2	16.00	1.20	0.76	30.83	15.61	8.39
	T3	15.00	1.09	0.68	27.00	19.08	11.59
	T4	17.90	1.49	0.98	35.50	22.42	13.35
	L.S.D 0.05	2.55	n.s	n.s	n.s	n.s	n.s
Trigonella foenum-graecum L.	T1(control)	18.10	1.57	0.31	44.26	15.28	4.88
	T2	22.16	2.66	0.35	56.16	40.91	5.54
	T3	20.06	1.84	0.27	54.83	20.05	3.05
	T4	20.50	2.64	0.32	55.16	25.53	3.33
L.S.D 0.05		2.95	n.s	0.02	7.49	12.61	0.21

Treatments T1: seeds without inoculation (uninoculated control), T2: seeds inoculated with nonrhizobial endophytic strain *Enterobacter* sp.(strain No.1), No.1; T3: seeds inoculated with nonrhizobial endophytic strain *Pseudomonas* sp.No.2; and T4: seeds inoculated with nonrhizobial endophytic strain *Pseudomonas* sp. No.3.

Shoot minerals, nitrogen content and total carbohydrate of the four tested legumes

For the four different legume species, the percentage of N, P, shoot nitrogen content and total carbohydrate % in plants inoculated with tested non-rhizobial endophytic strains were not higher than those determined in plants not inoculated (T1) for each plant species (Table 2). Cowpea plants treated with T2 showed a higher content of P%, of the shoot and total carbohydrate than those found in uninoculated control of cowpea. Cowpea plants treated with T2 gave a significant increase in P% and total carbohydrate over uninoculated control with a value of 0.586 and 8.65, respectively. In case of the common bean and pea plants, treatment T4 gave the highest N %, P %, nitrogen content of shoot and total carbohydrate % than the control T1 but it was insignificant increase. Although all the treatments improve fenugreek plants, T2 was the best treatment for them. For fenugreek plants, T2 increased significantly the shoot nitrogen content with a value of 0.166 g N/Pl.

Table 2. Nitrogen percentage, phosphorous percentage, N-content and total carbohydrate % of shoots for four traditional legume species as affected by inoculation with different nonrhizobial endophytic strain.

Legume species	Treatments	N %	P %	N-content (g N/pl)	Total carbohydrate %
Vigna unguiculata L.	T1(control)	2.36	0.405	0.430	7.90
	T2	2.74	0.586*	0.651	8.65*
	T3	2.54	0.450	0.342	8.01
	T4	2.45	0.468	0.432	8.37
	L.S.D 0.05	n.s	0.087	0.261	0.44
Phaseolus vulgaris L.	T1(control)	3.49	0.590	0.382	11.49
	T2	3.42	0.533	0.384	11.15
	T3	3.51	0.555	0.370	11.42
	T4	3.62	0.619	0.392	11.67
	L.S.D 0.05	n.s	n.s	n.s	0.516
Pisum sativum L.	T1(control)	3.58	0.618	0.400	11.70
	T2	3.53	0.532	0.297	11.26
	T3	3.55	0.567	0.458	11.56
	T4	3.83	0.629	0.461	11.93
	L.S.D 0.05	n.s	n.s	n.s	0.55
Trigonella foenum-graecum L.	T1(control)	3.06	0.509	0.148	10.61
	T2	3.43	0.575	0.166*	10.73
	T3	3.37	0.515	0.111	9.43
	T4	3.03	0.521	0.114	10.72
L.S.D 0.05		n.s	n.s	0.020	n.s

N: Nitrogen, P: phosphorous, Pl: Plant, g: gram. Treatments T1: seeds without inoculation (inoculated control), T2: seeds inoculated with nonrhizobial endophytic strain *Enterobacter* sp.(strain No.1), No.1; T3: seeds inoculated with nonrhizobial endophytic strain *Pseudomonas* sp.No.2; and T4: seeds inoculated with nonrhizobial endophytic strain *Pseudomonas* sp. No.3.

Content of yield minerals, total protein, total carbohydrate and total yield of the four tested legumes

The different treatments showed increases in seeds N %, P %, total protein%, total carbohydrates% and total yield of the four plant species used (Table 3). T2 was the best treatment with cowpea plant. It gave significant P over the uninoculated control with a value of 0.675.

Table 3. Nitrogen percentage, phosphorous percentage, total protein %, total carbohydrate % and total yield of yield four traditional legume species as affected by inoculation with nonrhizobial endophytic strain.

Legume species	Treatments	N %	P %	Total protein %	Total carbohydrate %	Total Yield (ton/ha)
Vigna unguiculata L.	T1(control)	3.56	0.545	22.80	11.60	1.881
	T2	3.64	0.675*	22.89	12.63	1.933
	T3	3.27	0.655	20.44	11.45	1.766
	T4	3.39	0.581	21.19	10.78	1.301
	L.S.D 0.05	n.s	0.116	n.s	1.79	n.s
Phaseolus vulgaris L.	T1(control)	3.71	0.677	23.53	11.65	2.475
	T2	3.44	0.578	21.55	11.80	2.495
	T3	3.71	0.646	23.19	11.17	3.136
	T4	3.88	0.793*	24.27	12.70	3.818*
	L.S.D 0.05	0.31	0.063	1.94	n.s	0.78
Pisum sativum L.	T1(control)	3.74	0.579	23.50	11.71	2.200
	T2	3.48	0.603	21.76	11.88	2.309
	T3	3.75	0.630	23.43	11.69	2.120
	T4	3.95	0.853*	24.68	12.32*	2.896
	L.S.D 0.05	0.31	0.072	1.99	0.48	0.75
Trigonella foenum-graecum L.	T1(control)	3.00	0.495	18.13	11.02	0.330
	T2	3.30	0.588	20.94	11.20	0.485*
	T3	3.27	0.518	20.46	9.40	0.445
	T4	3.15	0.524	19.38	9.72	0.425
L.S.D 0.05		n.s	0.075*	n.s	1.35	0.069

N: Nitrogen, P: phosphorous, Treatments T1: seeds without inoculation (uninoculated control), T2: seeds inoculated with nonrhizobial endophytic Enterobacter sp.(strain No.1), No.1; T3: seeds inoculated with nonrhizobial endophytic strain Pseudomonas sp.No.2; and T4: seeds inoculated with nonrhizobial endophytic strain *Pseudomonas sp.* No.3. *Plant growth-promoting activities of the Non-rhizobial endophytic strains isolated from wild grown Melilotus indicus L. All.*

For common bean plants, T4 showed a significant increase in P % and total yield with a value of 0.793% and 3.818 ton/ha, respectively, over the control. Inoculation of cowpea and fenugreek with non-rhizobial endophytic strains in T2 increase the seeds total yield of them by about one and half times over uninoculated control.

Data in Table 3 proved that T4 was the most effective treatment with pea plants. For P% and total carbohydrates% out-yielded significantly the uninoculated (T1) control of pea plants with a value of 0.853and 12.32, respectively. However, T2 was the most effective treatment with fenugreek plants. It gave a significant increase in P % and total yield of fenugreek plants over the uninoculated (T1) control with a value of 0.588 and 0.485 ton/ha, respectively.

The three non-rhizobial endophytic strains (No.1, No.2, and No.3) produced indole acetic acid with different concentrations (Table 4). Strain No.1 as well as strain No.3 produced IAA more than strain No.2. They gave 15.456 and 13.236, respectively.

Table 4. Quantitative estimation of indole acetic acid production of different wild non- rhizobial strains

Non-Rhizobial endophytic strain no.	IAA production mg/l
T1	15.456±1.0
T2	4.712±1.18
T3	13.236±1.1

In addition, the three non-rhizobial endophytic strains (No.1, No.2, and No.3) had highly antagonistic activity against some phytopathogenic fungi, vis., *Pythium ultimum, Alternaria alternate and Sclerotnia rolsfi* (Table 5). The three strains inhibit completely *P.ultimum.* The inhibition percentage of *A. alternata* by strains No.1 and strains No.3 was 55.6% and 55.1%, respectively. Furthermore, inhibition percentage of *S. rolsfi* was 58.5% and53.7% by strains No.2 and No.3, respectively.

Table 5. Antibiosis effect of different wild rhizobial strains on some phyto pathogenic fungi.

Non-rhizobial endophytic strain	Phyto pathogenic Fungi	Radial growth (mm)	Inhibition %
1	*P. ultimum*	00.0 b	100.0a
2		00.0b	100.0a
3		00.0b	100.0a
Control		90.0a	00.0b
L.S.D. 0.05		00.0	00.0
1	*A. alternate*	34.0c	55.6a
2		52.6b	31.2b
3		34.3c	55.1a
Control		76.6a	00.0c
L.S.D. 0.05		3.26	3.22
1	*S. rolsfi*	51.0a	43.3c
2		37.3d	58.5a
3		41.6c	53.7b
Control		90.0a	00.0d
L.S.D. 0.05		1.21	1.35

P. ultimum: *Pythium ultimum*; *A .alternata* : *Alternari alternata; S. rolsfi: Sclerotonia rolsfi.*

The results are the mean (n = 3). Means with the same letter are not significantly different at p value 0.05 by ANOVA.

DISCUSSION

For a sustainable agriculture system, it is necessary to utilize renewable inputs, which can maximize the ecological benefits and minimize the environmental hazards. In recent years endophytic bacteria received more attention as they play a significant role in plant growth promotion and also prevent pathogenic organisms from colonizing host plant (Wahla and Shukla, 2017). The results of this study evaluating the influence of three-plant growth promoting nonrhizobial endophytic bacteria isolated from root nodules of *Melilotus indicus* (L.) All. plants on the growth and productivity of four legume plants,vis., cowpea, common bean, peas and fenugreek under field conditions in a new reclaimed soil compared to the uninoculated plants. All the tested endophytic non-rhizobial strains improve the growth and increased yield of the four legume plants. This means that endophytes bacteria are appropriate for inoculation, indicating the ability of the inoculated bacteria to colonize plant and to perform the beneficial effect (Souza *et al*., 2015; Rolli *et al*., 2017). We found that bacterial strains consistently increased one or more of plant growth parameter. The observed effect on the growth parameters and yield were statistically significant in most parameters. Khan and Khan (2015) proved that phosphate-solubilizing bacteria were shown to be effective in enhancing maize yields. A few decades ago, a large group of bacteria associated with different plants, have demonstrated plant growth-promoting properties. Associated *Azospirillum* might promote the growth, yield and nutrient uptake of different plant species of wheat and maize (Hungria *et al*., 2010). Also under field conditions in Argentina, inoculants containing *Azospirillum* have been tested and they gave positive results regarding plant growth and/or grain yield (Souza *et al*., 2015). Endophytic bacteria play important role in plant growth promotion through increased germination rates, chlorophyll content, biomass, leaf area, shoot and root length, nitrogen content, protein content, yield and tolerance to abiotic stresses (Wahla and Shukla, 2017). A total of 60 endophytic bacteria from surface sterilized nodules of field pea were isolated, 41 endophytic nodule isolates showed enhanced plant growth, high shoot dry weight and shoot N contents (Narula *et al*., 2013). Inoculation of chickpea with nonrhizobial endophytes HE-5 or HM-2 alone showed an increase in nodulation and plant growth yield when compared with uninoculated control (Priyanka and Leelawati, 2015).

Since indole-3-acetic acid (IAA) is the most important native auxin (Ashrafuzzaman *et al*., 2009) endophytic bacteria produced a phytohormone commonly. Producing auxin like compounds increases seed production and germination along with increased shoot growth and tillering (Kevin, 2003). The three non-rhizobial endophytic strains (No.1, No.2, and No.3) used in this study produce indole acetic acid with different concentrations. The ability to produce indole compounds is widely distributed among plant-associated bacteria. Recently, studies showed that high indole compound production observed in *Endophytic bacteria*, demonstrating in the Entero bacteriaceae; Costa *et al*. (2014). Hung and Annapurna (2004) found that 56 isolates out of 65 bacterial endophytes isolated from stem, root, and nodule of two soybean varieties, *Glycine max* and *Glycine soja* and were capable of producing IAA in different concentrations.

Nowadays bacterial endophytes are widely used as biocontrol agent as they have the ability to prevent a plant from adverse effects of pathogenic organisms through the production of allelochemicals, antibiotics or hydrolytic enzymes (Wahla and Shukla, 2017). The three non-rhizobial endophytic strains (No.1, No.2, and No.3) used in this study have a great ability to antagonize the phytopathogenic fungi; *Pythium ultimum, Alternaria alternate and Sclerotnia rolsfi*. Researchers reported that *Bacillus cepacia* has been destroyed *Rhizoctonia solani, R. rolfsii* and *Pythium ultimum* by producing b -1,3-glucanase. Directly application of endophytic bacteria B. cereus 65 to soil has been reported to protect cotton seedlings from root rot disease caused by *Rhizoctonia solani* (Wahla and Shukla, 2017). Endophytic *Enterobacter* and *Pantoea* species isolated from cotton was found to protect the plants against fungal pathogen *Fusarium oxysporum f. sp. Vasinfectum* by secretion of protease and chitinase (Li *et al*. 2010). The interaction of endophytic bacteria with plants, improve the immune response of plants for the future attack by pathogens, a phenomenon called as induced systemic resistance (Wahla and Shukla, 2017).

CONCLUSION

It could be concluded that legume endophytic non-rhizobial bacteria have the ability to improve plant growth by a different mechanism of action. These nodule endophytes perform different functions and could prove to be good inoculants for enhancing crop productivity The importance of using such beneficial microorganisms in the field of agriculture is the reduction of use of different agrochemicals such chemical fertilizers, and other artificial chemicals etc. which would make agriculture more productive and sustainable. The most important goal is to be able to manage microbial communities to favor plant colonization by beneficial nonrhizobial bacterial endophytes. Finally, Searching endophytic bacteria for the development of new and efficient inoculants for agriculture that increase agricultural production without damaging the environment is a current biotechnological trend (Schlaeppi and Bulgarelli, 2015). Although larger and longer field trials under different environmental conditions, on different cultivars and in different soils are needed. The results of this study revealed that since isolates No.1 and No.3 showed most of the plant growth promoting characteristics including IAA production and antifungal activity and showed the beneficial effect on overall plant growth. Thus these two strains may be used as a potent bio fertilizer in newly reclaimed soils. Further characterization of this strain may help in providing the interesting results.

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استجابة النمو الخضري و المحصول والتركيب الكيماوي لبعض المحاصيل البقولية الملقحة بالبكتريا الغير عقدية المعزولة من العقد الجذرية لنبات الحندقوق البري

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تم زراعة تجربتين حقليتين خلال موسمي النمو 2015 و 2016 لدراسة التاثير علي النمو الخضري ،المحصول والتركيب الكيماوي لاربعة محاصيل من المحاصيل البقولية منها اللوبيا صنف قها 1 ، الفاصوليا صنف نبرسكا، البسلة صنف ماستر بي والحلبة صنف جيزة 3 والتي تم تلقيحهم باستخدام ثلاثة سلالات من البكتريا والتي تم عزلها من العقد الجذريه لنباتات الحندقوق البري بالاضافة الي معاملة الكنترول . أكدت التنتائج ان معظم معاملات اضافة البكتريا كانت لها نتائج معنوية علي كل من النمو الخضري (طول الجذر،الوزن الطازج للجذر ، الوزن الجاف للجذر، طول النبات ، وكلامن الوزن الطازج والجاف للمجموع الخضري /نبات) المحصول (طن/ هكتار) والتركيب الكيماوي لكل من النبات والمحصول متمثل في النسبة المئوية للنتروجين ، والنسبة المئوية للفوسفور، المحتوي النيتروجيني للنبات والنسبة المئوية للكربوهيدرات الكلية والنسبة المئوية للبروتين الكلي بالبذور بالمقارنة بمعاملة الكنترول وكانت اعلي النتائج المتحصل عليها باستخدام المعاملة 2 (سلالة البكتريا رقم 1، انتروبكتريا) لكل من اللوبيا والحلبة والمعاملة 4(سلالة البكتريا رقم 3 ، بازيدوموناس) لكل من الفاصوليا والبسلة للصفات محل الدراسة. كما أوضحت النتائج ان هذه السلالات البكتيريه لها القدرة على انتاج محفزات النمو مثل إندول حامض الخليك بكميات كبيرة وكذلك ثبت ان هذه السلالات لديها المقدرة على مقاومة بعض الفطريات الممرضة للنباتات بدرجة عالية جدا. وعلي ذلك يمكن التوصية باستخدام بكتريا السلالة رقم 1 لتلقيح كلا من اللوبيا والحلبة والسلالة رقم 3 لتلقيح كلا من الفاصوليا والبسلة .