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Inoculation effect of rhizobial strains on growth, yield and chemical composition of some legume crops in new reclaimed soil

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ABSTRACT

Two field experiments were carried out during two successive seasons of 2015 and 2016 to study growth 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 rhizobial nodule endophytic bacteria. The treatments used for each legume tested were T1) seeds without inoculation (uninoculated control); T2) seeds of each legume tested inoculated with its commercial strain T3) seeds inoculated with wild rhizobial strain 4.21; T4) seeds inoculated with wild rhizobial strain 9.17; T5) seeds inoculated with wild rhizobial strain 11.2; and T6) seeds inoculated with wild rhizobial strain 14.1. The four strains were isolated from root nodules of *Melilotus indicus* (L.) All.

Results showed that all treatments increased all vegetative growth parameters tested, shoot minerals(N%, P%, N content and total carbohydrate % and yield minerals,(N%, P%, total protein %, total carbohydrate % and total yield. For cowpea, T6) showed the best results for RL parameter significantly increased than T1) only and another parameter insignificant, (N%, P%, for shoot and N%, P% and total protein % for yield are significant than control) ; T4) for common bean (N content, N%, total protein % and total yield) are significant than control, Furthermore; T3) for pea (N content, N%, total protein %, total carbohydrate % and total yield are significant than control) and, T3) for fenugreek results indicated that (all vegetative parameters, P%, N content, total carbohydrate % for shoot, P%, and total yield are significant than control). In addition, results revealed that since isolates 4.21 and 14.1 have the ability to nodulate fenugreek and cowpea, respectively. Moreover, isolates 4.21, 9.17 and 14.1 showed plant growth promoting characteristics including IAA production and antifungal activity and showed a beneficial effect on overall plant growth. Thus, these three strains may be used as a potent biofertilizer in newly reclaimed soils.

Key words: Cowpea, *Melilotus indicus* (L.)All. , Rhizobium, inoculated, IAA.

Introduction

In most natural ecosystems, nitrogen is the primary nutrient limiting factor in plant production (Graham and Vance, 2003). About one-third of the land area of the world comprises arid and semiarid regions with very low soil fertility. Fertilization of a crop with nitrogen fertilizers represents a significant cost both economically and environmentally. Production of nitrogen fertilizers requires high amounts of non-renewable fossil energy and is one reason for the release of greenhouse gases (Jensen *et al*., 2012).

The big challenge for future agricultural expansion in the Arab countries is that most of the lands are located in deserts. Mostly, legumes played an important role necessary for building the sustainable agricultural production systems in the newly reclaimed soils (Abd El-Al, 2004).

The interest in biological nitrogen fixation and rhizobia-legumes symbioses, particularly those involving economically important legume crops in terms of food and forage is essentially for sustainable agricultural practices (Laranjo *et al.,* 2014). Rhizobium- legumes symbiosis with species is of special importance, producing 50% of 175 Tg of total biological N2 fixation annually worldwide

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(Yadav and Verma, 2014).

Legumes are responsible for a substantial part of the global flux of N_2 to fixed forms. Therefore, they are very important both ecologically and agriculturally. Legumes, through their symbiotic abilities, can play an important role in colonizing disturbed ecosystems. Rates of N_2 fixation in such environments are often low, but can still satisfy much of the legume's N needs (Graham and Vance, 2003). The symbiotic nitrogen fixation resultant from the rhizobia-legumes symbioses can allow the increase of crop yields on one hand and decreased the use of chemical fertilizers on the other hand. It may also have positive effects for subsequent crops through supplying nitrogen (N) to agro-ecosystems with the consequent reduction of environmental pollution (Lupwayi *et al*., 2004; Laranjo *et al*., 2014). 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).

Despite that grain legumes have important value, their productivity is very low (Allito *et al.,* 2015). This low productivity in grain legumes is due to the declining in soil fertility and reduced N2-fixation. Low productivity of grain legume can be improved through inoculation of adaptable effective rhizobia (Jida and Assefa, 2014; Desta *et al*., 2015). In spite of the fact that using rhizobia in inoculating legumes can achieve substantial increases in growth and yield productivity and improve soil fertility, there is no doubt that specificity exists between rhizobia strain and the legume variety, and compatibility between the two is essential for successful nodulation and nitrogen fixation (Emam and Rady, 2014; Allito *et al*., 2015).

Keeping these aspects in view, the present work carried out to test the ability of four strains of rhizobia isolated from nodules of *Melilotus indicus* (L.) All. (Wild herb) to nodulate some grain legumes,viz.,cowpea (*Vigna ungiculata* L.*),* common bean *(Phaseolus vulgaris* L.)*,* pea*s (Pisum sativum* L.), and fenugreek (*Trigonella foenum-graecum* L.) to improve their growth, productivity and chemical composition in the newly reclaimed soil.

Materials and Methods

Rhizobial strains

Four strains 4.21, 9.17, 11.2 and 14.1 were isolated from root nodules of *Melilotus indicus* (L.) All. plants grown wild in different habitats of Egypt. These strains were selected from a culture collection of 37 rhizobial-like strains isolated from root nodules of *Melilotus indicus* (L.) All., isolated bacteria were grown in yeast extract menthol (YEM) medium (Vincent, 1970) and the morphological and physiological characterization of these four isolates was found by El-Batanony *et al*. (2015). Other four-ARC rhizobial strains were used as the inoculated control for the different plants species, viz., cowpea ARC Go1 for cowpea, *Rhizobium* for common bean, *Rhizobium leguminosarum* for peas and ARC for fenugreek plants. They were kindly obtained from the Biofertilizers Production Unit of Soil, Water and Environment Research Institute, Agricultural Research Center (ARC), Giza as commercial peat inoculants (ARC inoculant). These strains were prepared by El-Batanony *et al.* (2015) according to the method of Somasegaran and Hoben (1994). Commonly, the commercial peat inoculants are used for inoculation of cowpea*,* common bean, peas and fenugreek*,* in different agricultural areas in Egypt.

Host- legumes

Seeds of cowpea (*Vigna unguiculata* L.) cv. Kaha 1,common been (*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.), cv. Giza 3 was kindly provided by Field Crops Research Institute Agricultural Research Center (ARC), Giza, Egypt.

Field trials

Two field experiments were carried out using the facilities of the Environmental Studies and Research Institute (ESRI), 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. This soil had the following physical and chemical characteristics: sand 66.5%; silt 28.5%; clay 5%; pH 7.85; electrical conductivity (EC) 1.18dSm^{-1}; organic carbon 0.141%; soluble cations: Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, N^{3+} ,and P^{3+} being 380, 147.6, 134.3, 19.5, 0.011 and 0.009 mg g^{-1}, respectively; soluble anions: CO_3^{2-}, HCO_3^{-}, Cl^{-} and SO_4^{2-} being 9, 36.6, 310.9 and 96 mg g^{-1} , respectively.

The experiments were conducted in a complete randomized block 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 with 10-15 cm distance between them and the distance between plants was 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 (fresh yield) 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 (~10^8cells ml^{-1}) using Arabic gum (40%) as an adhesive agent for 2 hours before planting. The following treatments were used for each legume tested: T1) seeds without inoculation (uninoculated control); T2) seeds inoculated with its commercial strain specific for inoculation of such legume in Egypt (inoculated control); T3) seeds inoculated with wild rhizobial strain 4.21; T4) seeds inoculated with wild rhizobial strain 9.17; T5) seeds inoculated with wild rhizobial strain 11.2; and T6) seeds inoculated with wild rhizobial strain 14.1. During soil preparation, all blots received phosphorus (70 kg P_2O_5 ha^{-1}) as super phosphate and potassium (110 kg K_2SO_4 ha^{-1}), as potassium sulphate while N (50 kg N ha^{-1}) as ammonium sulphate were added at three times after planting. The plants were grown under field conditions, the recommended agricultural practices for growing plant were applied whenever required.

Measured characters

Nodulation during plant growth was assessed 50 days after planting by counting the number of nodules (Nod. No.) in roots of 12 plants chosen randomly from each blot. Nodules were dried (60 °C for 24 h.) and nodule dry weight (Nod. DW) was determined. 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 days, the plant DW was obtained by drying the fresh samples of each treatment in an oven at 105°C.Complete drying was indicated by reaching a constant weight.

The total yield (seeds for cowpea and fenugreek and green yield for pea and common bean) of the tested legumes was recorded at harvest.

Chemical analyses

The percentage of N, P, and total carbohydrates of dried legume shoots and yield were determined for each treatment according to Jackson (1958).

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

Plant growth-promoting activities of the rhizobia isolated from wild grown Melilotus indicus L. All.

Indol acetic acid (IAA) production by the rhizobial isolates

The IAA production was measured by modifying the method reported by Scagliola *et al.* (2016). The tested strains were grown in 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 min. 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). The ethyl acetate fraction was dried with a rotary evaporator at 40 °C, resuspended in 2 ml 60% methanol and stored at -20 °C. The methanol extract was assayed 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 rhizobial isolates

The antibiosis activity of the four 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 modifications. Non-rhizobial 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 analyzed statistically as the proportion of variance explained by between-treatment differences using the general linear model of 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-nodulation related traits

For the Nod. No. and Nod. DW traits analyzed in cowpea plants, no significant variation was observed among the four different treatments for both traits. The Nod. No. in treatment T1 (uninoculated) and treatment (T2) (inoculated control) was 10.71% and 26.19% of that detected in plants treated with T6) that gave the highest Nod. No. (14 ± 6 nodules/plant), respectively. Similarly, the Nod. DW was 11.9% and 16.73% of that determined in plants treated with T6, respectively. However, Nod. No. and Nod. DW traits could not analyze in fenugreek plants because the Nod. No. was much more to be countable. On the other hand, neither common bean nor pea nodulated by any of the tested strains.

Root and Shoot growth- related traits of the four tested legumes

For the parameters RFW, PH, SHFW and SHDW traits, T6 out-yielded insignificantly both the uninoculated (T1) and inoculated controls (T2) of cowpea plants, except RL parameter significantly increased than T1) with value 22.16 (Table 1). Additionally, all treatments increased the growth trait of fenugreek plant. However, the maximum growth traits of root and shoot obtained with fenugreek plants treated with treatment T3. T3 increased growth traits of fenugreek root and shoot significantly with RL, SHFW and SHDW traits, the increase was significant with a value of 24.0, 28.31 and 4.85, respectively, while, pH parameter with T3 significantly increased than T1 only with a value of 56.50.

Neither common bean nor pea plants are nodulated by the tested rhizobial strains. However, all the measured parameters increases in proved, especially with treatments T4 for common bean plants and T3 for pea plants, but were insignificant.

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

Legume species	Treatments	R L (cm)	RFW (g/pl)	RDW (g/pl)	PH (cm)	SHFW (g/pl)	SHDW (g/pl)
***Vigna unguiculata* L.**	T1(control)	16.15	9.16	1.49	40.00	87.17	16.21
	T2(control)	20.66	7.00	1.62	31.00	69.67	12.08
	T3	17.83	8.50	1.67	23.50	60.50	11.37
	T4	13.50	7.00	1.63	31.83	74.67	11.90
	T5	19.66	7.00	1.75	34.66	86.50	15.80
	T6	22.16	10.50	1.92	40.26	91.67	17.06
	L.S.D 0.05	5.78	6.45	n.s	12.82	n.s	n.s
***Phaseolus vulgaris* L.**	T1(control)	15.16	2.15	1.75	32.00	18.47	11.13
	T2(control)	15.33	2.69	1.91	33.33	18.24	9.87
	T3	15.66	2.11	1.16	29.66	14.54	7.83
	T4	16.50	3.00	2.07	34.16	19.28	12.84
	T5	14.50	2.31	1.57	33.00	17.39	9.91
	T6	14.16	1.66	1.24	33.33	16.26	10.74
	L.S.D 0.05	2.31	0.90	0.68	2.90	3.25	3.33
***Pisum sativum* L.**	T1(control)	16.83	1.30	0.85	35.00	22.29	13.30
	T2(control)	16.16	1.30	0.80	32.00	18.00	10.25
	T3	17.83	1.40	0.97	37.16	27.34	14.56
	T4	17.00	1.25	0.59	30.50	12.90	7.95
	T5	17.33	1.40	0.91	33.16	19.71	9.26
	T6	15.50	1.52	0.73	35.00	19.66	9.70
	L.S.D 0.05	1.94	n.s	0.24	n.s	10.05	5.82
***Trigonella foenum-graecum* L.**	T1(control)	18.16	1.67	0.29	44.16	15.21	4.08
	T2(control)	20.66	2.12	0.30	50.50	18.64	3.88
	T3	24.00	3.21	0.34	56.50	28.31	4.85
	T4	17.83	1.95	0.26	54.50	18.99	3.04
	T5	20.16	1.65	0.26	52.16	25.51	3.17
	T6	21.66	3.05	0.33	52.16	24.52	3.64
	L.S.D 0.05	**3.14**	**0.90**	**0.02**	**7.06**	**8.21**	0.18

RL: Root length, RFW: Root fresh weight, RDW: Root dry weight, PH: Plant height, SHFW: Shoot fresh weight, SHDW: Shoot dry weight.

Treatments **T1**:) seeds of a legume species without inoculation (un inoculated control), **T2**: seeds inoculated with its commercial specific strain; **T3**: seeds inoculated with wild rhizobial strain A4.21; **T4**: seeds inoculated with wild rhizobial strain A9.17; **T5**: seeds inoculated with wild rhizobial strain A11.2; and **T6**: seeds inoculated with wild rhizobial strain A14.1**.**

Shoot minerals (N%; P %), 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 rhizobial strains were higher without significance in some parameters than those determined in plants not inoculated (T1) or inoculated with commercial strain (T2) for each plant species (Table 2). Cowpea plants treated with T6 showed a higher content with significant of N% and P%, of shoot than those found in uninoculated control and inoculated control of cowpea. The increases of N % and P%, were significant between plants of uninoculated or inoculated controls with a value of 2.95 and 0.591, respectively. In case of common bean plant, treatment T4 gave the highest N %, P %, nitrogen content of the shoot and total carbohydrate % than the two control but the increases are insignificant except nitrogen content of shoot showed a significant increase over the untreated control T2. They gave a value of 0.463 g N/plant. Although all the treatments improve the percentage of N%, P%, shoot nitrogen content and total carbohydrate % of pea and fenugreek plants, T3 was the best treatment for them. For pea shoot, T3 increased nitrogen content significantly over the inoculated and uninoculated controls with a value of 0.538 g N/plant. Additionally, T3 increased significantly the percentage of P% and total carbohydrate % of fenugreek plants with a value of 0.622 and 12.25, respectively.

Table 2: Nitrogen %, phosphorous%, N-content and total carbohydrate % of shoots of four traditional legume species as affected by inoculation with different wild rhizobial strains.

Legume species	Treatments	N %	P %	N-content (g N/pl)	Total carbohydrate %
***Vigna unguiculata* L.**	T1(control)	2.46	0.415	0.435	7.97
	T2(control)	2.49	0.448	0.306	7.94
	T3	2.65	0.401	0.335	7.58
	T4	2.52	0.570	0.295	6.73
	T5	2.43	0.510	0.387	7.31
	T6	2.95	0.591	0.428	7.99
	L.S.D 0.05	0.26	0.082	n.s	0.70
***Phaseolus vulgaris* L.**	T1(control)	3.59	0.597	0.398	11.52
	T2(control)	3.55	0.567	0.323	11.54
	T3	3.58	0.573	0.280	11.52
	T4	3.74	0.611	0.463	11.54
	T5	3.60	0.504	0.360	11.22
	T6	3.57	0.606	0.385	11.10
	L.S.D 0.05	n.s	n.s	0.126	n.s
***Pisum sativum* L.**	T1(control)	3.60	0.621	0.481	11.75
	T2(control)	3.57	0.572	0.359	11.84
	T3	3.72	0.620	0.538	11.95
	T4	3.64	0.577	0.289	11.68
	T5	3.63	0.529	0.358	11.43
	T6	3.60	0.613	0.345	11.36
	L.S.D 0.05	n.s	n.s	0.22	0.58
***Trigonella foenum-graecum* L.**	T1(control)	3.16	0.519	0.136	10.67
	T2(control)	3.33	0.497	0.133	10.46
	T3	3.45	0.622	0.154	12.25
	T4	2.45	0.557	0.074	11.25
	T5	3.18	0.573	0.101	11.68
	T6	3.35	0.535	0.122	10.68
	L.S.D 0.05	**n.s**	**0.062**	**0.019**	**1.68**

N: Nitrogen, P: phosphorous, pl: Plant, g: gram.
Treatments **T1**:) seeds of a legume species without inoculation (un inoculated control), **T2**: Seeds inoculated with its commercial specific strain; **T3**: seeds inoculated with wild rhizobial strain A4.21; **T4**: seeds inoculated with wild rhizobial strain A9.17; T5: seeds inoculated with wild rhizobial strain A11.2; and **T6**: seeds inoculated with wild rhizobial strain A14.1**.**

Content of minerals (N%; P %), total protein%, total carbohydrates% and total yield of the four tested legumes

The different treatments showed increases in seeds N %, P %, total protein%, total carbohydrates% and total yield (ton/ha) of the four plant species used (Table 3). T6) was the best treatment with cowpea plant. It gave significant P % over the uninoculated and inoculated controls with a value of 0.680.While it gave a significant N % and total carbohydrate % over the inoculated controls T2) with a value of 3.69 and 12.43, respectively.

For common bean plants, T4 showed a significant increase in N %, total protein% and total yield with a value of 4.20, 26.30 and 4.108 ton/ha, respectively over the two controls, while total carbohydrate % gave significant over the uninoculated controls (T1) with a value of 12.24 . Furthermore, for total yield, T5 out-yielded significantly both the uninoculated (T1) and inoculated controls (T2) of common bean plants with a value of 3.548ton /ha.

Data in Table 3 proved that T3 was the most effective treatment with both pea and fenugreek plants. It significantly increased both N % and total protein% compered to the uninoculated (T1) and inoculated controls (T2) of peas plants with a value of 4.25 and 26.61 respectively, while, total carbohydrate % and total yield significantly out-yielded the uninoculated controls (T1) with a value of 12.25 and 3.203 ton/ha, respectively. Further, T3 gave a significant increase in P % and total seeds yield of fenugreek plants over the uninoculated (T1) and inoculated controls (T2) with a value of 0.618and0.630, respectively. Also T5 treatment gave a significant increase in P % and total seeds yield over the uninoculated (T1) and inoculated (T2) controls with a value of 0.577 and 0.562, respectively.

Table 3: Nitrogen %, phosphorous %, total protein %, total carbohydrates % and yield four traditional legume species as affected by inoculation with different wild rhizobial strains.

Legume species	Treatments	N (%)	P (%)	Total protein (%)	Total carbohydrate (%)	Total Yield (ton/ha)
***Vigna unguiculata* L.**	T1(control)	3.66	0.535	22.87	11.65	1.885
	T2(control)	3.47	0.520	21.70	12.18	1.629
	T3	3.59	0.601	22.46	11.02	0.930
	T4	3.68	0.648	23.00	12.21	1.536
	T5	3.42	0.548	21.39	10.42	1.583
	T6	3.69	0.680	23.06	12.43	1.947
	L.S.D 0.05	0.18	0.129	1.16	1.78	0.74
***Phaseolus vulgaris* L.**	T1(control)	3.78	0.679	23.63	11.75	2.472
	T2(control)	3.60	0.669	22.53	12.03	2.871
	T3	3.73	0.674	23.33	11.89	3.468
	T4	4.20	0.725	26.30	12.24	4.108
	T5	3.82	0.707	23.91	11.89	3.548
	T6	3.94	0.596	24.66	11.74	2.850
	L.S.D 0.05	0.39	0.088	2.46	0.40	0.66
***Pisum sativum* L.**	T1(control)	3.77	0.679	23.57	11.81	2.205
	T2(control)	3.70	0.682	23.15	12.10	2.703
	T3	4.25	0.734	26.61	12.25	3.203
	T4	4.02	0.683	25.14	11.86	2.533
	T5	3.83	0.712	23.95	11.86	2.869
	T6	3.71	0.659	23.18	11.86	2.960
	L.S.D 0.05	0.39	n.s	2.47	0.40	0.729
***Trigonella foenum-graecum* L.**	T1(control)	3.06	0.490	19.13	11.17	0.335
	T2(control)	3.35	0.488	20.95	10.44	0.332
	T3	3.50	0.618	21.91	11.71	0.630
	T4	2.52	0.567	15.76	11.26	0.457
	T5	3.17	0.577	19.82	11.57	0.562
	T6	3.41	0.538	21.35	10.70	0.416
	L.S.D 0.05	**0.52**	**0.06**	3.24	**n.s**	**0.18**

N: Nitrogen, P: phosphorous, ton (ton= 1000Kg), ha: hectare (10000 m^2).
Treatments **T1**:) seeds of a legume species without inoculation (un inoculated control), **T2**: Seeds inoculated with its commercial specific strain; **T3**: seeds inoculated with wild rhizobial strain A4.21; **T4**:seeds inoculated with wild rhizobial strain A9.17; T5: seeds inoculated with wild rhizobial strain A11.2; and **T6**: seeds inoculated with wild rhizobial strain A14.1.

Plant growth-promoting activities of the rhizobia isolated from wild grown *Melilotus indicus* L. All.

The wild rhizobial strain 4.21, 9.17, 11.2 and 14.1 produced indole acetic acids with different concentrations (Table 4). Strains 9.17 gave the highest value of IAA (25.896 mg/l) followed by strain 4.21 (10.419 mg/l).

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

Rhizobial strain No.	IAA production mg/l
T4.21	10.419± 1.4
T9.17	25.896± 0.95
T11.2	7.001± 1.2
T14.1	9.312±1.0

In addition, the wild rhizobia strains 4.21, 9.17, 11.2 and 14.1 had highly antagonistic activity against some phytopathogenic fungi; *Pythium ultimum, Alternaria alternate and Sclerotinia rolsfi* (Table, 5). Strains 11.2 and14.1 was higher antagonizing *P. ultimum* than the other two strains. They gave inhibition percentage of 100% and 74.4%, respectively. Moreover, Strains 4.21 and 9.17 gave inhibition percentage of 56.5%, and 59.5%, respectively with *A. alternata.* Furthermore, strains 9.17and 14.1 antagonized *S. rolsfi* with inhibition zone of 51.1% and 58.5%, respectively.

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

Rhizobial strain	Phyto pathogenic Fungi	Radial growth (mm)	Inhibition %
4.21		90.0 a	00.0 c
9.17		90.0 a	00.0 c
11.2	*P. ultimum*	00.0 c	100.0 a
14.1		23.0 b	74.4 b
Control		90.0 a	00.0 c
L.S.D. 0.05		2.15	2.39
4.21		33.3 d	56.5 a
9.17		31.0 d	59.5 a
11.2	*A. alternata*	55.0 b	28.1 c
14.1		43.0 c	46.2 b
Control		76.6 a	00.0 d
L.S.D. 0.05		3.22	4.72
4.21		75.0 b	16.6 c
9.17		44.0 c	51.1 b
11.2	*S. rolsfi*	46.6 c	48.1 b
14.1		37.3 d	58.5 a
Control		90.0 a	00.0 d
L.S.D. 0.05		4.65	5.16

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

Mostly in areas where land is scarce and productivity is low due to nutrients depletion, agriculture based on *Rhizobium* inoculation for growing new legume varieties are of essential importance (Mmbaga *et al.,* 2014). Thus, the efficient utilization of biological nitrogen fixation to improve agricultural productivity requires the dependence on superior or very effective exotic rhizobia strains as inoculants (Ampomah *et al*., 2008).

Therefore, four wild rhizobial strains (4.21, 9.17, 11.2 and 14.1) obtained from root nodules of *M. indicus* (L.) All. plants grown wildly in Egypt were selected to assess their ability to nodulate cowpea, common bean, pea and fenugreek and assess their symbiotic performance with these legumes under field conditions in a new reclaimed soil compared to the uninoculated plants and to the strains commercially used for inoculation of them in Egypt.

Two legume species; cowpea and fenugreek showed nodules on their roots with all four *Rhizobium* strains. This indicates the compatibility between these legume species and strains. Furthermore, *Rhizobium* inoculation significantly increased the nodulation, growth and yield parameter of legumes. These findings are in agreement with the findings of (El Hadi and Elsheikh, 1999). The data of nodulation among the treatments indicate that some rhizobial strains possessed higher quality than others (Aliyu *et al.,* 2013). However, the other two legume species; common bean and pea failed to nodulate. It is also common to report inoculum failure to produce nodules (Mahdi, 1993).

Although cowpea is readily nodulated by the indigenous rhizobia population present in Egyptian soils, nodule number, nodule fresh weight, and nodule dry weight were lower in untreated cowpea plants than that inoculated with the different four strains. Similar results were obtained when *Cicer arietinum*, *Dolichos biflorus*, *V. mungo, V. radiata* and *Trifolium alexandrinum* were inoculated with *Rhizobium* sp. (Kala *et al*., 2011; Nishita and Joshi, 2010; Ravikumar, 2012; El-Lithy *et al.,* 2014).

Our results show that either plant species or rhizobial strains gave a different response to the studied parameters. Moreover, cowpea and fenugreek inoculation with rhizobial strains enhanced the symbiotic properties of them and gave better growth and production (Saeed and Elsheikh, 1995; Abd Elgani *et al*., 1999). Plant biomass and plant dry matter can be used as a criterion for the symbiotic effectiveness of rhizobia and is strongly correlated with nitrogen fixation (Zahran, 2009). Usually, the enhancement of plant shoot dry weight based upon inoculation and consequently an estimation of the symbiotic effectiveness of a *Rhizobium* strain was proved (Laranjo *et al*., 2014).

In addition to the nitrogen-fixing ability of the tested rhizobial strains, they had another direct and indirect growth promoting activity viz. production of phyto hormone (IAA) and suppression of plant pathogenic fungi, this was obvious in common bean plants and pea, which they were not nodulated by the tested strains and showed growth and productivity enhancement. Many authors proved that rhizobia isolated from different legumes produced IAA (Joseph *et al.,* 2007; Mandal *et al*. 2007). While others reported that rhizobia able to inhibit significantly the growth of pathogenic fungi (Estevez de Jensen *et al*., 2002; Bardin *et al*., 2004; El-Batanony *et al.,* 2007; Mazen *et al.,* 2008). Application of rhizobia on common bean reduced root rot disease caused by *Sclerotinia sclerotiorum* (Kumara *et al*., 2016).

Furthermore, N% and P % were higher in plants inoculated with the tested rhizobial strains. Both plants fresh and dry matter affected positively with the increasing N levels. Many researchers reported an increase in shoot N of legume plants inoculated with rhizobial strains (Senevirante *et al*., 2000; Zhang *et al*., 2002; FengXian *et al*. 2009; Yadav and Verma, 2014; El-lithy *et al*., 2014). The higher increase in of N and P uptake of plants treated with microbial inoculations suggest that a positive interaction exists between N and P uptake, root colonization, and growth promotion. The increased 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 (Yadav and Verma, 2014).

In present studies, inoculation of cowpea and fenugreek seeds with effective rhizobial strains significantly stimulated yield, total protein yield%, uptake of nitrogen and phosphorus and also total carbohydrate % in grains and seeds of the two plants. 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 (Hungria *et al*., 2015; Kumara *et al*., 2016). In addition, inoculation of chickpea (*C. arietinum* L.) seeds with *R. leguminosarum* BHURC04 (*Cicer* sp.) gave a significant increase in grain yield and uptake of N and P in grain over uninoculated control (Yadav and Verma, 2014). Rhizobial strains used in T3 and T6 with cowpea and fenugreek, respectively, increased the crude protein content of cowpea over the control T2) only. This may be due to the increase in N2-fixing efficiency of inoculated plants, which gave more nitrogen that is fixed and translocate to the seeds. Seed protein content of soybean, cowpea and Faba bean has been reported to be increased by rhizobial inoculation (Abd Elgani *et al*., 1999).

The national yield of cowpea is estimated at 543 kg/ha in the sole cropping systems in Senegal, the fourth producing country in West Africa (Do Rego *et al.,* 2015), thus the rhizobial strains 14.1 used in T6 considered a promising strain for inoculation as it gave 1.947 ton / ha.

Conclusion

It could be concluded that N2-fixing rhizobia–legumes symbioses are highly significant for improving soil fertility and, consequently, plant productivity. Nitrogen fixation efficiency depends on rhizobial strain, plant host, environmental factors, soil and their interaction. All of them are important in the control of N2 fixation and yield of grain legumes. Matching rhizobial strains to host legumes is the most important factor in maximizing the productivity of grain legumes. Selection of new strains of rhizobia can utilize to produce bio fertilizer inoculants that can significantly enhance legumes grain yield. In the present investigation, inoculations of rhizobia were highly effective for significant enhancement in growth, nutrient acquisition and yield of cowpea cv. Kaha 1, common bean cv. Nebraska, pea cv. Master B, and fenugreek cv. Giza3. The results of this study revealed that since isolates 4.21 and 14.1 have the ability to nodulate cowpea and fenugreek*,* respectively, in addition, isolates 4.21, 9.17 and 14.1 showed plant growth promoting characteristics including IAA production and antifungal activity and showed a beneficial effect on overall plant growth. Thus these three strains may be used as a potent biofertilizer in newly reclaimed soils. Further characterization of this strain may help in providing the interesting results.

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