

Growth Response of Peanut (*Arachis hypogaea* L.) to Inoculation with *Bradyrhizobium* Conjugated with *Rhizobacteria* under Different Levels of Organic Fertilization on Sandy Soil

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TWO FIELD experiments were conducted at the Agricultural Research Farm of the Higher Institute for Agriculture Co-Operation during 2008 and 2009 to study the response of peanut to inoculation with *Bradyrhizobium* either individually or in combination with PGPR (*Pseudomonas fluorescens*) under different levels of organic compost.

Regarding the response to inoculation with bacteria, results showed that there were significant increases in all peanut vegetative traits, due to inoculation with any tested bacterial strain. However, using the mixture strains (*Bradyrhizobium* + *Pseudomonas fluorescens*), surpassed other inoculated or uninoculated treatments.

The response of groundnut vegetative growth, to increasing the rate of organic fertilizer was significant. Using higher rate of compost 15-ton/fed (1 hectare = 2.4 feddan) showed higher values of all tested traits under the investigation in both seasons.

Spraying groundnut plants with humex significantly increased all tested traits under investigation compared with the non-sprayed plants in both seasons.

The effect of the first order interactions, *i.e.* compost x humex, compost x biofertilizers, biofertilizers x humex as well as the effect of the second order interaction (compost x humex x biofertilizers) on all studied traits of groundnut growth, were significant.

Keywords: Peanut (*Arachis hypogaea* L.), *Bradyrhizobium*, *Pseudomonas fluorescens*, Nodulation and growth.

Groundnut or peanut (*Arachis hypogaea* L.) is considered to be one of the most important edible legume crops in Egypt, due to its seeds has high nutritive value for human and the produced cake as well as the green leafy hay for livestock (Abdalla *et al.*, 2009). In addition, its seeds oil is very important for industrial

purposes. Groundnut seeds contains about 50% oil, 25-30% protein, 20% carbohydrates and 5% fiber (Fageria *et al.*, 1997). The cultivated area of groundnut in Egypt during 2010 season was about 151853 fed (Batan, 2010). Moreover groundnut is considered one of the most important exporting crop, more than 70% of groundnut seed production is exported. Most of the cultivated area is of sandy soil using high rates of NPK chemical fertilizers aiming to maximize seed yield for human feeding and straw yield for animal feeding. Since, oil consumption in Egypt is about 1.1 million ton/year with a shortage of 89%, thus increasing the production of oil seed crops is an important aim in the Egyptian agriculture (El-Kramany *et al.*, 2007).

Due to the intensive farming, Egypt is known as a heavy consumer of chemical fertilizers (El-Egami, 2011). This intensive farming has caused negative effects on soil environment over the past decades, *i.e.* loss of soil organic matter, soil erosion and water pollution. The use of chemical fertilizers has been doubled during the last two decades. Thus the coincident application of organic manures and bio-fertilizers is frequently recommended, firstly for improving biological, physical and chemical properties of soil and secondary to get high and clean agricultural yield produced free from undesirable high doses of heavy metals and other pollutants.

Plant growth promoting rhizobacteria (PGPR) have the ability to enhance plant growth either directly, by phytohormones production, N₂-fixation and siderophores production...ect., or indirectly, through biological control of pathogens or induction of host defense mechanisms (Dey *et al.*, 2004; Zahir *et al.*, 2004 and Verma *et al.*, 2010).

Enhancement of nodulation and biological nitrogen fixation by co-inoculation legumes with PGPR are becoming a practical way to improve nitrogen availability in sustainable agricultural production system (Bai *et al.*, 2002 and Abdel-Wahab *et al.*, 2008). The most commonly implicated mode to stimulate legume-*Rhizobium* symbiosis is phytohormones inducing stimulation of root growth, to provide more sites for rhizobial infection and nodulation (Vessey & Buss, 2002).

Therefore, the objective of this work is to investigate the effect of co-inoculation with *Bradyrhizobium* and rhizobacteria on nodulation and growth of groundnut plant under different levels of compost in sandy soil.

Materials and Methods

The present work was conducted to investigate the effect of organic and biofertilizations on groundnut growth under different levels of compost in sandy soil.

Peanut seeds of variety (Ismaelia 1) were kindly provided by the Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt at May 2008 & 2009.

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Organic fertilizer (Compost)

Compost was used as a source of organic materials . It was obtained from Moshtohor factory in Qalubia Governorate , its main chemical and biological traits are given in Table 1. Compost was applied at the following two levels, *i.e.* 8 and 15 tons/feddan.

TABLE 1. The main chemical traits of the compost used during 2008 and 2009 years.

Traits	Seasons	
	2008	2009
pH	7.71	7.62
E.C(ds/m)	4.68	3.81
Organic-C (%)	20.18	19.89
Total-N (%)	1.31	1.26
C/N ratio	15.40	15.78
Organic matter %	34.71	34.21
Total -P%	0.78	0.59
Total -K %	1.75	1.68
Total Soluble-N (ppm)	143.70	132.8
Available- P (ppm)	163.80	146.7
Available-K (ppm)	574.80	518.6
Dehydrogenase activity (µg TPF/g)	90.81	90.81
Seed germination index for cress at 48 hr	114.6	108.7

Canda Humex

Canda humex is an extract from humic, fulvic and active humeins, applied at a rate of 2L/fed split into three equal doses applied at 15, 30,45 days from sowing as a foliar application.

Inoculated bacteria

Bradyrhizobium sp. (strain USDA 3456) and *Pseudomonas fluorescens* (strain IFO 2034) were kindly obtained from the Biofertilizers Production Unit, Agric. Microbiol. Dept., Soils, Water and Environ. Res. Inst. (SWERI), ARC,Giza, Egypt.

Bradyrhizobium sp.was cultured in a yeast extract mannitol broth medium (Vincent, 1970) and *Pseudomonas* was grown in king's medium B (Atlas, 1995). Cultures were incubated at 28°C for three days on a rotary shaker unit early log phase to ensure population density 10^9 cfu/ml culture. Powdered vermiculite supplemented with 10% Irish peat was packed into polyethylene bags (200 g carrier per bag), then sealed and sterilized with gamma irradiation (5.0 x 10 rads). Each bacterial culture (120 ml of log phase growing culture) was injected into a sterilized carrier to satisfy 60% of the maximal water holding capacity of the carrier mixture and mixed thoroughly.

Field experiments

Two field experiments were conducted under drip irrigation system at the Experimental Station, Agricultural Research Higher Institute for Agriculture Co-operation during the 2008 and 2009. These experiments aimed to study the effect of inoculation with *Bradyrhizobium* sp. either alone or combined with (PGPR) on nodulation, growth, yield and yield components of groundnut under different levels of organic fertilizers (compost and Canda humex) in sandy soil. The physical and chemical properties of the experimental soil are presented in Tables 2.

TABLE 2 . Physical and chemical traits of used soil in the first and second seasons.

Traits	1 st season		2 nd season	
	15-cm deep	30-cm deep	15-cm deep	30-cm deep
Particle size distribution (%)				
Coarse sand	11.30	11.14	12.10	11.90
Fine sand	74.95	76.66	74.30	75.40
Silt	9.75	8.58	8.70	8.50
Clay	4.00	3.62	4.90	4.20
Texture grade	Sandy	Sandy	Sandy	Sandy
Bulk density (g/cm ³)	1.28	1.33	1.30	1.32
CaCO ₃ (%)	1.84	1.74	1.68	1.62
Saturation percent	23.00	24.0	24.20	24.80
pH (Soil paste)	7.50	7.65	7.46	7.62
EC (dS/m)	0.24	0.43	0.31	0.52
Soluble cations and anions (meq/L):				
Ca ⁺⁺	0.88	0.72	0.82	0.76
Mg ⁺⁺	0.19	0.45	0.24	0.49
Na ⁺	1.06	2.26	1.16	2.42
K ⁺	0.32	0.71	0.42	0.98
Co ₃ ⁼	---	---	---	---
HCO ₃ ⁻	1.00	1.10	1.20	1.36
Cl ⁻	0.35	0.70	0.41	0.79
SO ₄ ⁼	1.10	2.34	1.03	2.50
Total soluble- N (mg kg ⁻¹)	15.00	20.0	22.00	26.00
Available -P (mg kg ⁻¹)	3.40	5.0	4.300	6.60
Available -K (mg kg ⁻¹)	88.00	96.0	92.50	98.60
Total -N (mg kg ⁻¹)	243.00	252.0	261.30	273.00
Organic matter %	0.42	0.33	0.40	0.30
*DTPA-extractable. Fe (mg kg ⁻¹)	1.07	1.20	1.22	1.31
Mn (mg kg ⁻¹)	0.55	0.60	0.52	0.66
Zn (mg kg ⁻¹)	0.25	0.20	0.31	0.29
Cu (mg kg ⁻¹)	0.04	0.03	0.04	0.04

* DTPA: Diethylene triamine Penta acetic acid

The following treatments were applied

1-Uninoculated plants (control)

2-Inoculation with *Bradyrhizobium* sp.

3-Inoculation with *Bradyrhizobium* sp. and PGPR (*Pseudomonas fluorescens*).

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The above biofertilizer treatments were carried out in the presence of two levels of compost, *i.e.*, 8 and 15-ton/feddā applied 15 days before sowing with or without the organic liquid humex.

Groundnut seeds were inoculated with gamma irradiated vermiculite-based inoculant of each bacterium at a rate of 300g/40kg seeds using Arabic gum solution (16%) as sticking agent.

The experimental design was split-split plot design with three replicates. The main plots included compost levels and the organic liquid humex represents the sub plots, whereas biofertilization treatments were assigned to the sub sub plots. Plot size was 10.5m² (1/400 fed).

All plots received the recommended rates of superphosphate (15.5% P₂O₅) at a rate of 200 kg/fed and potassium sulfate (48% K₂O) at a rate of 50 kg/fed. Nitrogen fertilizer was applied at a rate of 20 kg N/fed in the form of ammonium sulphate (20.5%N) after 15 days from planting as starter dose.

After 75 days from sowing, samples were taken to study the following growth traits:

- 1- Number and dry weight of nodules/plant (mg).
- 2- Plant height (cm), number of branches/plant and number of leaves/plant.
- 3- Shoot fresh and dry weights (g/plant) as well as shoot N,P and K contents (mg/plant).

Methods of analysis

Soil and compost properties were determined according to Piper (1950) and Page *et al* (1982).

Total nitrogen, phosphorus and potassium in groundnut shoot were assumed according to Page *et al.*(1982).

Statistical analysis

All obtained data were subjected to Analysis of Variance (ANOVA) and L.S.D test was used to compare the treatment means according to Snedecor & Cochran (1980) using MSTAT Statistical software.

Results and Discussion

Peanut nodulation status

Data in Table 3 showed that uninoculated plants failed to form nodules, which indicated that the soil under investigation was free from native peanut rhizobia. These results are in accordance with those obtained by Ghobrial *et al.* (2002) and Saleh *et al.* (2010) on soybean. Inoculating peanut seeds with *Bradyrhizobium* alone showed significant increases in number and dry weight of nodules as compared with the uninoculated seeds. (El-Sawy *et al.*, 2006 and Kandil *et al.*, 2008). Peanut plants inoculated with *Bradyrhizobium* and *Pseudomonas* recorded significant increases in

nodules number by 60.19% and 68.79% and nodules dry weight by 28.26 and 34.01 compared with the single inoculation treatment of *Bradyrhizobium* after 75 days from sowing in both seasons, respectively. The promotive effect of co-inoculation with *Pseudomonas* on boosting the nodulation status of peanut roots may be attributed to their ability to increase the infection site for *Bradyrhizobium* and to the enhancement of symbiotic performance between macro and microsybiont via the action of growth promoting substances such as auxin, vitamins B group and flavonide like-substances (Vessey & Buss, 2002 and Verma *et al.*, 2010).

Regarding the effect of compost levels results revealed that increasing compost levels from 8 to 15-ton/fed led to significant increases in number and dry weight of nodules in both seasons. The increases in nodules number were 21.14% and 18.59% and in nodules dry weight were 14.67 and 14.89% at 75 days from sowing, respectively. These results reflected the prominent role of organic matter for enhancing the nodulation pattern originated on groundnut roots in sandy soil through the effect of organic substances in survival of rhizobia in the rhizosphere as well as improving the peanut vegetative growth leading to establishing intact nodulation pattern. The promotive effect of organic materials could be triggered as an end result of improvement of physical, chemical and biological features of sandy soil. These results are in agreement with those reported by El-Tahlawy (2006) and Abdel-Hafez & Abo El-Soud (2007).

In respect to the effect of humex, data in Table 3 illustrated that supplementary foliar nutrition of peanut plants with humex significantly increased number and dry weight of nodules compared to unsprayed control after 75 days from sowing in both seasons. These increases amounted 14.76% and 11.94 in nodules number and 8.22% and 5.99 in nodules dry weight at 75 days from sowing in both seasons, respectively. The effect of humex activity in promoting plant growth may have several proposed explanations, *i.e.* increasing cell membrane permeability, which is important for the transport and availability of micronutrients, nutrient uptake, oxygen uptake, respiration (especially in roots) and photosynthesis, phosphate uptake and root cell elongation (Serenella *et al.*, 2002 and Shehata & El-Helaly, 2010).

Concerning the interaction effect between biofertilization and compost, data in Table 3 showed that, inoculation of peanut seeds with *Bradyrhizobium* either solely or in combination with *Pseudomonas* combined with any level of compost caused significant increases in number and dry weight of nodules. Co-inoculation with *Bradyrhizobium*+ *Pseudomonas* combined with either 8 or 15- ton compost/fed gave significant increases in number and dry weight of nodules as compared with plants inoculated with *Bradyrhizobium* alone and received the same compost level at 75 days from sowing in both seasons. These increases amounted 65.11% and 65.52 in nodules number and 28.30% and 31.19% in nodules dry weight with 15-ton compost at 75 days from sowing in both seasons, respectively. The number and dry weight of nodules obtained in plants inoculated with *Bradyrhizobium* + *Pseudomonas* combined with 15-ton compost/fed were significantly higher than those observed with *Bradyrhizobium* + *Pseudomonas* combined with 8-ton compost/fed. This significant superiority hold fairly true in both seasons at 75 days from sowing. These results revealed that the response of number and dry weight of nodules to

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biofertilization treatments were not the same under the two levels of compost. The promotive effect of rhizobacteria on boosting the nodulation status may be attributed to the increase of infection sites for *Bradyrhizobium* and to the enhancement of symbiotic performance between macro and via the action of growth promoting substances microsybiont (Verma *et al.*, 2010 and Badawi *et al.*, 2011). In addition, the combined application of rhizobacteria with compost tended to magnify the promotive effect of nodulation on groundnut roots. This may be elucidated by the effect of decomposable organic substances, which enhance the root growth traits of groundnut and encourage the PGPR activity in the rhizosphere, particularly under sandy soil conditions. Similar trends were obtained by Abdel-Wahab & Said (2004) and Abdel-Wahab *et al.* (2006).

Concerning respect the interaction effect between compost and humex, results in Table 3 showed significant effects of the above interaction on number and dry weight of nodules/plant after 75 days from sowing in both growing seasons. The response of these two traits to humex treatments was not the same under the two rates of compost. The increases in number and dry weight of nodules/plant due to humex treatment were greatly higher with the higher rate of compost compared with the low rate. This finding hold fairly true at the two growing seasons. These results are in agreement with those obtained by Lawn Care Academy (2010) who found that organic matter is one of the most important issues of agriculture and it contains three very important components humic acids, fulvic and humein. They added that plant and microorganisms in soil benefit from applications of humic acid in several ways. Also humic acid stimulate root growth, increase carbohydrate production, have a hormone-like effect within the plant, and increase soil microorganisms.

Also data in Table 3 showed that the interaction effect between biofertilizers inoculation and humex led to significant increases in number and dry weight of nodules/plant compared with the control at 75 days from sowing in both seasons. The highest values of nodules number (131.83 and 147.17 nodules/plant) and nodules dry weight (478.33 and 506.33 mg/plant) were obtained by using the treatment of mixture strains + foliar spray with humex (2.0 L/fed) at 75 days in both seasons, respectively. Similar trends were obtained by Modukwe *et al.* (2008) who reported that addition of organic manure in the soil enhance the symbiotic relationship between microorganisms in the soil. Results in Table 3 revealed that the interaction effect between compost, biofertilizer and humex was significant on number and dry weight of nodules after 75 days from sowing in both years (2008 and 2009). The highest values of nodules number (147.33 and 155.67 nodules/plant) and nodules dry weight (508.00 and 539.67 mg/plant) after 75 days from sowing in both seasons were attained by using 15-ton compost/fed + mixture of rhizobacteria strains + humex. This significant effect of the above inter action means that the different levels of the three studied factors did not behave the same among themselves. This finding was the same with the double inoculations and hold fairly true to a great extent in both seasons from sowing.

TABLE 3. Nodulation status of peanut roots as affected by dual inoculation with *Bradyrhizobium* and rhizobacteria under different levels of compost after 75 days from sowing during 2008 and 2009 seasons.

During 2005 and 2007 seasons.																	
Compost (ton/fed) and humex treatments	Number of nodules/plant						Dry weight of nodules(mg/plant)										
	8 (ton/fed)			15 (ton/fed)			8 (ton/fed)			15 (ton/fed)							
	Without Humex	With humex	Mean	Without humex	With humex	Mean	Without humex	With humex	Mean	Without humex	With humex	Mean					
	First season (2008)						Second season (2009)										
Biofertilization																	
Control	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	000.00					
<i>BradyHizobium</i> (Br)	65.67	75.67	70.67	78.67	85.67	82.17	76.42	315.00	354.00	334.50	374.33	392.00	383.33				
Br. + <i>Pseudomonas</i>	102.00	116.33	109.17	124.00	147.33	135.67	122.42	408.67	448.67	428.67	475.67	508.00	491.83				
Mean	55.89	64.00	59.94	67.55	77.66	72.61	66.28	241.22	267.56	254.39	283.00	300.00	291.72				
L.SD at 0.05	H: 1.65						C x B x H: 4.05			C : 15.79		B: 8.27		H: 8.18		C x B x H: 20.03	
C: 3.79																	
Control	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	000.00				
<i>BradyHizobium</i> (Br)	70.00	80.67	75.33	84.00	99.67	91.83	83.59	328.00	339.67	333.83	388.67	398.00	393.33				
Br. + <i>Pseudomonas</i>	121.67	138.67	130.17	148.33	155.67	152.00	141.09	442.33	473.00	457.67	494.00	539.67	516.00				
Mean	63.92	72.90	68.50	77.44	85.11	81.28	74.89	256.78	270.90	263.83	294.22	312.56	303.11				
L.SD at 0.05	H: 2.92						C x B x H: 7.16			C : 7.18		B: 6.99		H: 7.74		C x B x H: 18.97	
C: 5.16																	
B: Biofertilization																	
H: Humex																	

The growth response of groundnut to the inoculation with *Bradyrhizobium* either solely or combined with rizobacteria under different levels of compost is presented in Table 4. Results revealed that uninoculated plants recorded the lowest values of plant height (37.34 and 40.08) and number of branches/plant (6.83 and 7.92) after 75 days from sowing in both seasons, respectively. Data in Table 4 also revealed that the highest values for plant height (42.33 and 44.08) and number of branches (9.33 and 9.91) were obtained for co-inoculation with *Bradyrhizobium* + *Pseudomonas* in both seasons, respectively. This improvement in peanut growth could be attributed to N₂-fixation and/or certain growth promoting substances such as indole acetic acid (IAA) and gibberellic acid, which positively affect plant growth. These results stand in accordance with those early reported by Saubidet *et al.* (2000), Bai *et al.* (2003) and Mekhemar *et al.* (2007) who reported that *Bradyrhizobium* inoculation or combined with rhizobacteria caused significant increases in nodulation status, plant growth and nitrogen content.

Irrespective of inoculation, results in Table 4 revealed that the application of compost at a rate of 15 ton/fed resulted in highly significant increases in plant height and number of branches per plant compared with the treatment received compost at a rate of 8- ton/fed. These increases were 17.03% and 14.10 in plant height, 25.10% and 18.12% in number of branches/plant at 75 days from sowing in both seasons, respectively. The improvement in the previous growth traits, *i.e.* plant height and number of branches due to the application of compost could be attributed to either its direct effects, by increasing the availability and supplying of nutrients or to its indirect effects by modifying soil physical properties that can improve the root environment, increase plant uptake of nutrients and consequently stimulate plant growth (Rizk *et al.* 2000 and Mohamed, 2005).

Also data presented in Table 4 revealed that plant height and number of branches per peanut plant were increased consistently and significantly by the supplementary foliar nutrition of peanut plants with humex compared with the unsprayed control. The increases in plant height were 5.66% and 4.12 and 8.60 and 10.98% in number of branches/plant in both seasons, respectively. The stimulative effect of humex on plant growth includes the assimilation of major and minor elements, biochemical effects *i.e.* enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production (Bidegain *et al.*, 2000).

With regard to the interaction effect between biofertilizer and compost results in Table 4 revealed that this interaction effect on some vegetative traits, *i.e.*, plant height (cm) and number of branches/plant after 75 days from sowing in both seasons was significant. The highest values of plant height (44.83 and 47.66 cm) and number of branches (10.33 and 10.67/plant) were obtained for plants inoculated with mixture strains combined with 15-ton compost/fed after 75 days from sowing in both seasons, respectively. The values obtained with plants inoculated with double strains combined with 15 tons/fed were significantly higher than those obtained with single inoculation combined with either 15 tons or 8 tons/fed. The superiority of dual inoculations was more pronounced when combined with the higher rate of compost.

TABLE 4. Effect of co-inoculation with *Bradyrhizobium* and rhizobacteria under different levels of compost on plant height and number of branches after 75 days from sowing during 2008 and 2009 seasons.

Compost (ton/fed) and humex treatments	Plant height (cm)						Number of branches/plant					
	8 (ton/fed)			15(ton/fed)			Mean			15 (ton/fed)		
	Without humex	With humex	Mean	Without humex	With humex	Mean	Without humex	With humex	Mean	Without humex	With humex	Mean
	First season (2008)						Second season (2009)					
Control	32.00	34.67	33.50	39.67	43.00	41.33	37.34	5.33	6.33	7.33	8.33	6.83
<i>Bradyrhizobium</i> (Br)	37.67	38.67	38.17	43.67	45.00	44.33	41.25	7.33	8.33	8.33	10.33	8.58
Br + <i>Pseudomonas</i>	38.60	41.00	39.83	43.33	46.33	44.83	42.33	8.00	8.67	9.67	11.00	9.33
Mean	36.22	38.11	37.17	42.22	44.78	43.50	40.33	6.89	7.78	7.33	9.89	8.25
LSD at 0.05 C: 5.14	B: 1.58 H: 1.47 C x B x H: 3.59						C: 0.41 B: 0.43 H: 0.04 C x B x H: 0.98					
Control	36.67	38.00	37.33	42.00	43.67	42.83	40.08	6.67	7.33	7.00	9.33	7.92
<i>Bradyrhizobium</i> (Br)	41.33	41.67	41.50	44.67	46.67	45.67	43.58	8.00	9.33	8.67	10.33	9.25
Br + <i>Pseudomonas</i>	39.67	42.33	40.50	46.00	49.33	47.67	44.08	8.67	9.67	9.17	11.00	9.91
Mean	39.22	40.33	39.78	44.22	46.56	45.39	42.58	7.78	8.78	8.28	10.33	9.03
LSD at 0.05 C: 1.95	B: 1.76 H: 0.88 C x B x H: 2.45						C: 0.41 B: 0.55 H: 0.40 C x B x H: 0.89					
C: Compost	B: Biofertilization H: Humex											

Data presented in Table 4 revealed that the response of these two vegetative growth traits, *i.e.*, plant height (cm) and number of branches/plant to the interaction effect between compost and humex after 75 days from sowing were very similar. The effect of humex treatment was more pronounced with the higher rate 15-ton compost/fed. In other words, the effect of humex on these two traits did not behave the same with the applied rates (8 and 15-ton) of compost. This finding hold fairly true with the above three mentioned traits in both seasons. These results confirmed with those obtained by Pettit (2004) who found that humic substances have very strong influence on the growth of plant roots, when humic and fulvic acids were applied to the soil, enhancement of root initiation and increased root growth.

Results in Table 4 revealed that the effect of the interaction between biofertilizers and humex foliar spray on plant height and number of branches/plant was significant at 75 days from sowing in both seasons. The highest values of plant height, and number of branches were obtained by the treatment of strains mixture (*Bradyrhizobium* + *Pseudomonas*) + foliar spray with humex (2.0 L/fed). These results are in agreements with those of Graves *et al.* (2004) and Kaya *et al.* (2005), who found that humic substances can produce materials that may affect plant growth such as substances acting as plant hormone analogues or growth regulators. Also, increasing microbial populations resulting from humic acid activity in the soil may have influenced plant growth indirectly. Also, Abdel-Wahab *et al.* (2007) found that the highest value of some vegetative growth was obtained by foliar application of the enriched compost tea in combination with co- inoculation with *Rhizobium* and the mixture of rhizobacteria (*Serratia* sp., *Bacillus megaterium* and *Pseudomonas fluorescens*).

Also results in Table 4 demonstrated that the effect of the above interaction on plant height (cm) and number of branches/plant was significant. The effect of this interaction on these traits was very similar to a great extent in both seasons . The highest values in growth studied traits were obtained by groundnut plants fertilized with 15-ton compost/fed, inoculated with the mixture of rhizobacteria strains and sprayed with humex. The significant increases in plant height (cm) and number of branches/plant due to compost treatments was more pronounced than that of humex treatment particularly with the treatment of dual inoculation. This finding revealed that the response of groundnut plants, to the levels of the studied factors was not the same.

The response of fresh and dry weights groundnut of shoots to inoculation with *Bradyrhizobium* either solely or combined with *Pseudomonas* are given in Table 5. Results revealed that inoculation with *Bradyrhizobium* either alone or in combination with *Pseudomonas* gave significant increases in fresh and dry weights of shoots compared with the uninoculated plants (control). *Bradyrhizobium* inoculation alone increased shoots fresh weight by 25.21 and 31.23% and shoot dry weight by 41.38 and 25.99% over the uninoculated plants at 75 days from sowing in both seasons, respectively. These results agreed with those obtained by Abdel-Wahab *et al.* (2008) and Badawi *et al.* (2011) who reported that co-inoculation with *Rhizobium* and PGPR gave the highest values of shoots dry weight.

TABLE 5. Effect of co-inoculation with *Bradyrhizobium* and rhizobacteria under different levels of compost on fresh and dry weights of shoot after 75 days from sowing during 2008 and 2009 seasons.

Compost (ton/fed) and humex treatments	Fresh weight (g/plant)				Dry weight (g/plant)				Mean					
	8(ton/fed)		15(ton/fed)		8(ton/fed)		15(ton/fed)							
	Without humex	With humex	Without humex	With humex	Without humex	With humex	Without humex	With humex						
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean						
First season (2008)														
Biofertilization														
Control	144.97	174.53	258.33	286.57	272.45	216.10	22.53	27.77	25.15	32.77	43.00	37.83	31.52	
<i>Bradyrhizobium</i> (Br)	203.67	216.90	312.60	349.17	330.88	270.58	34.63	37.97	36.30	50.47	53.80	52.13	44.18	
Br. + <i>Pseudomonas</i>	303.53	328.87	316.20	420.07	477.17	448.62	382.41	42.53	51.37	46.95	58.93	65.30	54.53	
Mean	217.39	240.10	228.74	330.33	370.97	289.70	33.23	39.03	36.13	47.39	54.03	50.71	43.42	
L.S.D at 0.05	C : 21.62	B: 17.09	H: 14.99	C x B x H :36.71				C : 3.38		B: 2.12	H: 1.19			C x B x H : 2.90
Second season (2009)														
Control	179.60	200.60	190.10	286.73	315.50	301.12	245.61	25.27	33.73	29.50	54.03	61.47	43.63	
<i>Bradyrhizobium</i> (Br)	229.07	255.63	242.35	398.90	405.63	402.27	322.31	40.47	44.30	42.38	65.90	69.20	54.97	
Br. + <i>Pseudomonas</i>	287.27	350.83	319.05	422.03	442.20	432.12	375.52	47.80	55.93	51.87	73.63	76.77	63.53	
Mean	231.98	269.02	250.50	369.22	387.78	378.50	314.50	37.84	44.66	41.25	64.52	69.14	54.04	
L.S.D at 0.05:	C: 9.35	B: 16.35	H: 6.25	C x B x H: 15.29				C: 11.62		B: 2.78	H: 1.47			C x B x H :3.60
Compost	B: Biofertilization													
	H:Humex													

Regarding the main effect of compost levels data given in Table 5 revealed that increasing compost rate to the highest level (15-ton/fed) increased plant fresh weight by 53.29 and 51.08% and 40.38 and 61.01% in plant dry weight in both seasons, respectively. The increase in dry matter could be attributed in part to the effect of organic materials on increasing the water holding capacity of the soil which led to increment in the availability of nutrients in the soil. These results are in agreement with those obtained by Badawi (2003) and Mohammed (2005). They found that using higher rates of compost organic materials such as city refuse compost, chicken manure and compost of plant residues led to a marked increases in the dry matter of plants grown in clay and sandy soils. They attributed this to the beneficial effect of such compost levels on production of humus substances which improves soil properties as well as increasing nutrients release and hence increase their availability to the growing plants.

Results of Table 5 indicated that foliar spray of humex increased fresh and dry weights of shoots compared with non-sprayed plants in both seasons. These increases were 11.56 and 9.25% in fresh weight and 15.43 and 11.18% in dry weight in both seasons, respectively. These results are in consistent with those obtained by Ulukan (2008) and El-Ghamry *et al.* (2009) who reported that humic acid stimulate plant growth through assimilation nutrients and in turn the increase the biomass.

The interaction effect between inoculation and rates of compost fertilizer on fresh and dry weights of groundnut shoots are presented in Table 5. Peanut plants inoculated with *Bradyrhizobium*+ *Pseudomonas* conjugated with the addition of 15-ton compost/fed gave significant increases in fresh weight and dry weight over plants inoculated with *Bradyrhizobium* alone. This significant interaction effect revealed that the response of fresh and dry weights to biofertilizer treatments were not the same under the two different levels (8 and 15 ton compost/fed) of organic compost. Abdel-Wahab *et al.* (2005) and Abd El-Hafez & Abo-El-Soud (2007) explained the favorable effects of the combination between compost and biofertilizers on the basis of the beneficial effect of bacteria on the nutrients availability, vital enzymes, hormonal stimulating effects on plant growth as well as increasing of photosynthetic activity.

Results in Table 5 revealed that the effect of compost and humex treatments interaction on fresh and dry weights of groundnut plants after 75 days from sowing in both seasons was significant. The highest values of fresh weight (370.97 and 396.22 g/plant) and dry weight of shoots (54.03 and 69.14 g/plant) were recorded at the rate of 15-ton compost + humex after 75-day from sowing in both seasons, respectively. The increase in dry matter may be attributed in part to the effect of organic material used on the production of hums substances which improve the physical and chemical properties of the soil as well as increasing the water holding capacity and increment in the availability of nutrients of the soil, which leading to establish suitable growth media for growing plants, (Saruhan *et al.*, 2011).

The interaction effect between *Bradyrhizobium* inoculation, compost and humex on fresh and dry weights of shoot are shown in Table 5. The obtained data showed that the fresh and dry weights of shoots were significantly affected by the above interaction. The highest values of fresh and dry weights of shoot after 75 days from sowing in both seasons were obtained by groundnut plants inoculated with *Bradyrhizobium* plus *Pseudomonas* combined with 15-ton compost/fed and foliar sprayed with humex. Many researchers illustrated the positive effect of combination between inoculation and organic fertilizer for promotion growth and productivity. These results are in accordance with those obtained by Abd El-Wahab *et al.* (2005) and El-Egami (2011). The significant effect of this interaction means that the levels of the three factors under investigation did not behave the same.

Shoot N, P and K –contents as affected by the co-inoculation with *Bradyrhizobium* and rhizobacteria under different levels of organic fertilization are presented in Tables 6 and 7. Results elicited that inoculation of peanut with *Bradyrhizobium* significantly increased the total N-content of shoots by 76.50 and 43.35%, P-content by 66.58 and 79.94% and K-content by 46.95 and 39.81% over the uninoculated plants in both seasons, respectively. Double inoculation with *Bradyrhizobium* and *Pseudomonas* gave increases in shoots N-content by 133.73 and 74.86%, P-content by 125.19 and 79.94% and K-content by 98.53 and 67.33% over the uninoculated control in both seasons, respectively. Also, data in Tables 6 and 7 revealed that double inoculation with *Bradyrhizobium* and *Pseudomonas* showed significant increases in shoots N-content by 32.43 and 17.49%, P-content by 125.23 and 79.94% and K-content by 98.53 and 67.33% as compared to the single inoculation treatment of *Bradyrhizobium* in both seasons, respectively. The improvement in nutrient uptake may be attributed to several mechanisms such as biological nitrogen fixation (Bai *et al.*, 2002) synthesis of siderophores, compounds that chelate iron from soil, making it available to the plant (Kloepper, 2003), solubilization of minerals, or synthesis of plant hormones, such as auxins or gibberellins, (Probanza *et al.*, 2001) or other plant growth regulators, such as 1-aminocycloperphane-1-carboxylate deaminase (ACC) enzyme that decrease endogenous concentrations of ethylene and disease suppression and their coordinated expression were responsible in enhancing plant growth, and nutrients uptake of groundnut (Dey *et al.*, 2004 and Tilak *et al.*, 2005).

Also results in Tables 6 and 7 indicated that foliar spray with humex significantly increased shoots N-content by 16.15 and 14.46%, P-content by 24.75 and 21.33% and K -content by 24.56 and 17.49% compared with the non-sprayed plants in both years (2008 and 2009), respectively. These results are mostly coincided with those obtained by Ulukan (2008), who found that humic acid did not only increase macro-nutrient contents, but also enhanced micro-nutrient contents of the plant organs. Also, reported that humic acid plays a major role in plant nutrients uptake and growth parameters in plants in both vegetative and generative stages.

Data presented in Tables 6 and 7 demonstrated that nitrogen, phosphorus and potassium contents in groundnut shoots were significantly affected by the
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combined effect between compost levels and bacterial inoculation. The highest values in shoots N, P and contents were obtained in groundnut plants inoculated with the mixture of strains + 15- ton compost/fed. These results reflect the vital role of organic materials which supplied with biofertilizers that accelerate the decomposition of organic materials in soil and increased the availability of most nutrients for plant growth and uptake. Abdel-Wahab *et al.* (2005) reported that composted crop residues enriched with rock phosphates and inoculated with rhizobacteria led to improve the availability of N,P and K as well as humus content which enhanced plant growth and increased their uptake.

The effect of the interaction between compost and humex on N, P and K contents of groundnut shoots was significant after 75 days from sowing in both seasons as shown in Tables 6 and 7. This significant effect means that the above mineral contents did not behave the same under the different levels of the studied two factors. The effect of humex on the increases of N, P and K contents was more pronounced with the high rate of compost (15-ton/fed) particularly after 75 days from sowing. These results are in agreement with those obtained by Saruhan *et al.* (2011) who found that the stimulatory effect of humic substances have been directly correlated with the enhanced uptake of macronutrients, such as nitrogen, phosphorus and sulfur and micronutrients.

Results in Tables 6 and 7 demonstrated that the interaction effect between biofertilizer and foliar spray with humex on N, P and K-contents of groundnut shoot was significant at 75 days from sowing in both seasons. Maximum contents of N, P and K in shoot were recorded with the inoculation treatment of mixture of bacterial strains + foliar spray with humex. These results are similar to those obtained by Abdel-Wahab *et al.* (2007). Who demonstrated that the highest values of shoot nitrogen and phosphorus contents of chickpea was obtained by foliar application of the enriched compost tea in combination with co-inoculation with the mixture of tested rhizobacteria (*Serratia* sp., *Bacillus megaterium* and *Pseudomonas fluorescens*) and *Rhizobium*. Also Paksoy *et al.* (2011), found that humic substances played a major role in plant nutrients uptake and growth parameters in plant seedlings.

Data in Tables 6 and 7 showed that the interaction effect between biofertilizer inoculation, compost and foliar spray with humex had positive significant effects on N, P and K-contents. The highest values of N, P and K-contents were obtained, when compost were applied at a rate of 15-ton/fed and inoculation with *Bradyrhizobium* and *Pseudomonas* and foliar application with humex. These results reflect the vital role of organic materials which supplied with biofertilizers that accelerate the decomposition of organic materials in soil and increased the availability of most nutrients for plant growth and uptake. Abdel Wahab *et al.* (2005) and El-Egami (2011) reported that application of bio-organic conditioner and inoculation with rhizobacteria led to improve the availability of N, P and K as well as humus content which enhanced plant growth and increased their uptake. Also, Ulukan (2008) reported that humic acid did not only increase macro-nutrients, but also enhanced micro-nutrient contents of the plant oranges.

TABLE 6. Shoot N, P- content as affected by inoculation with *Bradyrhizobium* associated with rhizobacteria under different levels of compost after 75 days from sowing during 2008 and 2009 season.

Compost(ton/fed) and humex treatments	Shoots chemical content (mg/plant)									
	N					P				
	8 (ton/fed)		Mean			8 (ton/fed)		Mean		
	Without humex	With humex	Without humex	With humex	Mean	Without humex	With humex	Without humex	With humex	Mean
First season (2008)										
Control	415.37	528.50	830.73	1112.23	971.48	82.61	114.90	126.80	169.17	147.98
<i>Bradyrhizobium</i> (Br)	964.63	1066.20	1483.87	1580.57	1532.22	161.70	192.47	220.33	247.57	233.95
Br. + <i>Pseudomonas</i>	1269.40	1543.33	1849.10	2082.60	1966.06	215.40	272.37	273.03	350.67	311.85
Mean	883.13	1046.01	1387.90	1591.80	1489.91	153.24	193.24	206.72	255.80	231.26
LSD at 0.05	C: 151.00		B: 67.73	H: 34.19	C x B x H: 83.75		C: 9.58	B: 11.56	H: 8.30	C x B x H: 20.52
Second season (2009)										
Control	687.67	955.27	821.47	1563.40	1819.43	87.14	149.30	118.22	219.73	280.77
<i>Bradyrhizobium</i> (Br)	1214.10	1429.33	1321.72	2196.73	2364.33	161.80	193.23	177.52	301.60	350.57
Br. + <i>Pseudomonas</i>	1559.67	1879.13	1719.40	2574.70	2765.63	216.77	271.43	244.10	400.13	437.70
Mean	1153.81	1421.24	1287.53	2111.61	2316.47	155.23	204.66	179.95	307.16	356.34
LSD at 0.05	C: 45.08	B: 78.23	H: 46.30	C x B x H: 112.41			C: 27.03	B: 13.40	H: 8.45	C x B x H: 28.69
C:compost B:Biofertilization H: Humex										

TABLE 7. Shoot K- content as affected by inoculation with *Bradyrhizobium* associated with rhizobacteria under different levels of compost after 75 days from sowing during 2008 and 2009 season.

Compost (ton/fed) and humex treatments	Shoots chemical content (mg/plant)						
	K						Mean
	8 (ton/fed)			15(ton/fed)			
	Without humex	With humex	Mean	Without humex	With humex	Mean	
Biofertilization	First season (2008)						
Control	463.33	642.23	552.78	715.40	1027.60	871.50	712.14
<i>Bradyrhizobium</i> (Br)	779.50	898.50	839.00	1180.77	1327.10	1253.93	1046.47
<i>Br.</i> + <i>Pseudomonas</i>	991.43	1253.87	1122.65	1520.47	1889.40	1704.93	1413.79
Mean	744.76	931.53	838.14	1138.88	1414.70	1276.79	1057.47
LSD at 0.05	C: 83.35		B: 55.64		C x B x H: 71.22		
	Second season (2009)						
Control	542.96	806.90	674.93	1273.97	1488.30	1381.13	1028.03
<i>Bradyrhizobium</i> (Br)	924.07	1127.80	1025.93	1753.13	1944.33	1848.73	1437.33
<i>Br.</i> + <i>Pseudomonas</i>	1161.73	1476.53	1319.13	2042.20	2200.50	2121.35	1720.24
Mean	876.25	1137.08	1006.67	1689.77	18771.71	1783.74	1395.20
LSD at 0.05	C: 109.01		B: 74.33		C x B x H: 156.21		
C: Compost	B: Biofertilization		H: Humex				

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إستجابة نمو الفول السوداني للتلقيح بالبرادى ريزوبيوم بالمشاركة مع الريزوباكترىا تحت مستويات مختلفة من التسميد العضوى فى الأراضى الرملية

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أجريت تجربتان حقليتان فى المزرعة البحثية الخاصة بالمعهد العالى للتعاون الزراعى خلال الموسمين الصيفيين 2008، 2009 وذلك لدراسة استجابة النمو الخضرى وحاصل البذور وبعض مكونات الحاصل لنباتات الفول السودانى للتلقيح البكتيرى بالبرادى ريزوبيوم *Bradyrhizobium* إما بصورة منفردة أو بالأشتراك مع البكتريا المشجعة للنمو *سيدوموناس فلوريسنس Pseudomonas fluorescens* متداخلة مع مستويات مختلفة من التسميد العضوى تحت نظام الري بالتنقيط .

- أظهرت نباتات الفول السودانى إستجابة للتلقيح البكتيرى حيث اشارت النتائج خلال الموسمين أن هناك زيادة معنوية فى جميع صفات النمو نتيجة التلقيح باى من نوعى البكتريا. ولكن أظهرت المعاملة المشتركة (*Bradyrhizobium* مع *Pseudomonas fluorescens*) تفوقاً ملحوظاً على باقى المعاملات فى جميع الصفات تحت الدراسة.
- أدى التسميد العضوى (الكمبوست) إلى حدوث زيادة معنوية فى قياسات صفات النمو الخضرى حيث أعطى إستخدام المعدل العالى من السماد العضوى كمبوست (15 طن/فدان) أعلى قيم لجميع الصفات تحت الدراسة فى كلا الموسمين.
- أشارت النتائج ايضا أن رش نباتات الفول السودانى بمركب الهيومكس (2 لتر/فدان) اعطى زيادة معنوية فى جميع الصفات تحت الدراسة فى كلا الموسمين.
- أشارت النتائج الخاصة بالتأثير المشترك لكل من التلقيح البكتيرى ومستويات الكمبوست إلى الحصول على أفضل نتائج للنمو الخضرى لنباتات الفول السودانى لا سيما بإستخدام معاملة التلقيح الخليط (*Bradyrhizobium* مع *Pseudomonas fluorescens*) فى وجود 15 طن كمبوست/فدان خلال موسمى الزراعة تحت الدراسة.
- أظهر التفاعل بين الكمبوست والرش بالهيومكس وكذلك التفاعل بين التسميد الحيوى والهيومكس الى حدوث زيادة معنوية فى بعض صفات النمو كما كان للتفاعل الثلاثى بين الكمبوست ، التسميد الحيوى والرش بالهيومكس نفس التأثيرات.
- أدى إستخدام المعدل العالى من الكمبوست (15 طن/فدان) والتلقيح الخليط بالبرادى ريزوبيوم و *السيدوموناس* والرش بالهيومكس إلى حدوث زيادة معنوية فى بعض صفات النمو خلال موسمى الزراعة تحت الدراسة .