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INFLUENCE OF *Rhizobium* INOCULATION COMBINED WITH *Pseudomonas* AND *Herbaspirillum* ON GROWTH, NODULATION AND YIELD OF FABA BEAN UNDER NEWLY RECLAIMED SOILS

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ABSTRACT

Four field experiments were carried out at newly reclaimed soil in Minia and New Valley governorates during winter-growing seasons of 2003/04 and 2004/05 to study the effect of inoculation with *Rhizobium leguminosarum* combined with *Pseudomonas fluorescens* and *Herbaspirillum* on nodulation, growth, nitrogen content and yield of faba bean. The results showed that the inoculation of faba bean plants with *Rhizobium* only or in combination with *Pseudomonas* or *Herbaspirillum* caused significant increases in nodules number and dry weight compared to the uninoculated plants which received 15 kg N/fed in Minia and New Valley at the first and second seasons. Triple inoculation with *Rhizobium*, *Pseudomonas* and *Herbaspirillum* combined with starter dose of nitrogen fertilizer (15 kg N/fed) recorded significant increases in shoot dry weight and its nitrogen content compared to the uninoculated plants which received the same starter dose or received full dose of nitrogen (30 kg N/fed) or rhizobial inoculation only plus 15 kg N/fed.

The maximum seed and straw yields were obtained in plants inoculated with triple inoculation (*Rhizobium*, *Pseudomonas* and *Herbaspirillum*) plus 15 kg N/fed, followed by plants inoculated with rhizobia combined with *Pseudomonas* or *Herbaspirillum* plus 15 kg N/fed in Minia and New Valley at the first and second seasons. Regarding to the two locations, data revealed that there are significant increases in nodulation, growth, nitrogen content, seed and straw yields. New valley recorded the higher mean values of these parameters than Minia. On the other hand, there are no-significant differences between the two seasons.

Keywords: Faba bean (*Vicia faba* L.) nitrogen fixation, *Rhizobium leguminosarum*, *Pseudomonas fluorescens*, *Herbaspirillum*, Sandy soil.

INTRODUCTION

Faba bean (*Vicia faba* L.) is considered the major food legume crop in Egypt. Also, it is considered one of the basic sources of protein for the Egyptian diet with relatively low price. In addition, it has good role in enriching and improving chemical, physical and biological properties of the newly reclaimed soils.

The interaction of diazotrophic bacteria in the rhizosphere of leguminous crop inoculated with mixed cultures of *Azospirillum* or *Azotobacter* and *Rhizobium* spp. has been reported in several publications (Singh and Subba, 1979; Burnus *et al.*, 1981; Iruthayathas *et al.*, 1983). Greenhouse and field trials showed a simultaneous inoculation of *Azospirillum* and *Rhizobium* in different cell ratios or additions of *Azospirillum* to naturally rhizobia- colonized legumes resulted in substantial increase in

nitrogenase activity, greater number of nodules and eventually yield increase (Saring *et al.*, 1986; Yahalom *et al.*, 1987; 1990; Del Gallo and Fabbri, 1991).

The naturally occurring soil bacteria that are capable of stimulating plant growth named as plant- growth- promoting rhizobacteria (PGPR), Kloepper and Schroth, (1981). Fluorescent *Pseudomonas* belongs to the genus recognized as ubiquitous soil microorganisms capable of effectively colonizing the roots of different plants. Many species of *Pseudomonas* promote plant growth and reduce the population of deleterious microorganisms (Davison, 1988). The beneficial effect of *Pseudomonas* and other rhizobacteria on plant growth may result from the production of plant growth regulators, vitamins and enhancement the uptake of plant nutrients as well as suppression of pathogenic and deleterious organisms (Dileep-Kumar and Dube, 1992; Dileep-Kumar *et al.*, 2001).

Rhizobia are also, widely used in agriculture for crop improvement because of their ability fix atmospheric nitrogen. Logically, potentiality for improving plant yield by combining these plant growth promoting organisms with rhizobia have also been reported by many workers (Li and Alexander, 1988; Dashti *et al.*, 1998; Parmar and Dodarwal, 1999; Dileep-Kumar *et al.*, 2001). The present work aims to study the effect of inoculation with *Rhizobium leguminosarum* combined with *Pseudomonas fluorescens* and *Herbaspirillum* on nodulation, growth, nitrogen content and yield of faba bean under newly reclaimed soils.

MATERIALS AND METHODS

Bacterial strains:

Rhizobium leguminosarum bv. *viceae* (ICARDA 441), *Pseudomonas fluorescens* (IFO, 2034) and *Herbaspirillum* strains were kindly supplied by Biofertilizer Production Unit; Soils, Water and Environment Research Institute; ARC; Giza; Egypt.

Preparation of bacterial inocula:

Rhizobium strains were grown on yeast extract mannitol broth medium (Vincent, 1970) at 30 °C for 3 days until early log phase ($5X10^9$ cfu/ml culture) Vermiculite supplemented with 10% Iresh peat was packed in polyethylene bags (300 g carrier per bag), then sealed and sterilized by gamma irradiation ($5.0 X 10^6$ rads). *Rhizobium* culture was injected into the carrier to satisfy 60% of the maximal water holding capacity.

Pseudomonas was grown on King's B medium "KBM" (King *et al.*1954) for 48 hr at 28 °C to obtain population of $3X10^8$ cfu/ml culture and injected in sterilized carrier as mentioned before in preparation of *Rhizobium* inoculant.

Herbaspirillum was grown on nutrient broth medium for 48 hr at 28 °C to obtain population of $4X10^8$ cfu/ml culture and injected in sterilized vermiculite carrier.

Field experiments:

Four field experiments were carried out in sandy soil at newly reclaimed land in the west of Minia, Minia governorate and New-valley governorate during two winter-growing seasons of 2003/04 and 2004/05. Some physical and chemical properties of sandy soils used were determined (Table 1) according to the methods described by Jackson (1973).

Table 1. Physico-chemical properties of soils used in Minia and New Valley during the two seasons of 2003/04 and 2004/05.

Properties	Season of 2003/04		Season of 2004/05	
	Minia	New Valley	Minia	New Valley
Sand %	83.54	86.60	85.2	84.57
Silt %	9.20	8.18	6.12	10.32
Clay %	7.26	5.22	6.68	5.11
Textural grade	Sandy	Sandy	Sandy	Sandy
S.P. %	19.30	18.80	21.9	19.20
pH	7.52	7.82	7.80	7.96
E.C. (dS m^{-1} at 25 °C)	0.92	1.23	1.75	1.04
Organic matter %	0.16	0.23	0.38	0.23
Soluble cations (meq l^{-1})				
Ca^{++}	6.10	7.38	8.36	8.15
Mg^{++}	4.32	5.27	5.21	5.27
Na^{+}	3.49	3.15	3.12	3.26
K^{+}	1.45	2.61	2.00	2.50
Soluble anions (meq l^{-1})				
CO_3^{--}	0.00	0.00	0.00	0.00
HCO_3^{-}	5.34	6.35	4.24	6.24
Cl^{-}	3.35	5.98	3.75	5.86
SO_4^{--}	6.67	7.15	10.67	7.18
Total soluble N (ppm)	21.56	36.51	31.1	40.94
Available P (ppm)	5.95	8.62	4.60	7.18

Faba bean seeds variety Giza 40 supplied by Field Crops Research Institute, ARC were inoculated with gamma irradiated vermiculite-based inoculants containing *Rhizobium*, *Pseudomonas* or *Herbaspirillum*. Each inoculant was applied at the rate of 400 g inoculant per 60 kg seeds using 16% Arabic gum solution as a sticking agent.

The following treatments were studied:

1- Uninoculated + 15 kg N/fed at planting
2- Uninoculated + 15 kg N/fed at planting + 15 kg N/fed, 20 days after planting (DAP)
3- *Rhizobium* + 15 kg N/fed at planting
4- (*Rhizobium* + *Pseudomonas*) + 15 kg N/fed at planting
5- (*Rhizobium* + *Herbaspirillum*) + 15 kg N/fed at planting
6- (*Rhizobium* + *Pseudomonas* + *Herbaspirillum*) + 15 kg N/fed at planting

Ammonium sulphate (20.5% N) was used as a nitrogen fertilizer. All treatments received the recommended dose of phosphorus (100 kg super-phosphate/fed, 15.5% P_2O_5) and potassium (50 kg potassium sulphate/fed,

48% K_2O). Randomized Complete Block design with four replications was used and the plot size was 13.5 m^2. Five faba bean plants were uprooted from each plot after 75 days of sowing and assayed for number and dry weight of nodules as well as shoots dry weight per plant. Nitrogen content of the plant tissues was determined according to Page *et al.* (1982). At harvest, seed and straw yield of faba bean were determined. The data were subjected to statistical combined analysis according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Response of faba bean grown in newly reclaimed soils to inoculation with *Rhizobium leguminosarum* either alone or in combination with asymbiotic N_2-fixers bacteria (*Herbaspirillum*) and plant growth promoting rhizobacteria (*Pseudomonas*) were evaluated during the two winter growing seasons of 2003/04 and 2004/05 in Minia and New Valley. Nodulation and growth parameters of faba bean after 75 days from planting in the two locations were determined.

Nodulation parameters:

Results in Table (2) indicated that uninoculated plants received the starter dose of 15 kg N/fed produced the lowest number and dry weight of nodules to be 36 and 28 nodules per plant as well as 323.8 and 267.3 mg nodules/plant in Minia at the first and the second seasons, respectively. While in New-Valley the same treatment recorded number of nodules ranged between 66 and 52 nodules/plant with dry weight of 460.1 and 314.8 mg/plant at the first and the second seasons in the same order. This indicating occupancy of specific indigeneus *Rhizobium* in the used soils. These results are in agreement with those obtained by Abo El-Soud *et al.* (2004) and Mekhemar *et al.* (2005). The uninoculated plants fertilized with 30 kg N/fed recorded the lowest number and lower dry weight of nodules compared to the uninoculated plants received 15 kg N/fed in Minia and New-Valley during the two growing seasons. Same trends were also obtained by Abdel Wahab and Abd-Alla (1995), Saleh *et al.* (2000) and Mekhemar *et al.* (2005). In Minia, inoculation with *R. leguminosarum* caused significant increases in the number and dry weight of nodules as compared with the uninoculated control by 80.6 and 41.0%, respectively at the first season. These increases at the second season were 96.4 and 25.3% in the same order. The corresponding increases in New vally were 60.6 and 96.0% at the first season and 125.0 and 168.9% at the second season, respectively. These results are in harmony with those obtained by Hussein *et al.* (1997), Abo El-Soud *et al.* (2004) and Mekhemar *et al.* (2005). Co-Inoculation with *Rhizobium* and *Pseudomonnas* caused significant increases in nodules number by 103.8 and 267.9% in Minia at the first and second seasons, respectively. The increases in nodules dry weight were 56.4 and 66.9%, at the same order during the two seasons. While in New-Valley, the increase in nodule number were 130.3 and 207.7%, while the increases in nodules dry weight were 203.9 and 447.3% at the first and second seasons in the same order. Also, dual inoculation with

Rhizobium and *Herbaspirillum* caused significant increases in nodules number and dry weight compared with the uninoculated plants or plants inoculated with *Rhizobium* alone.

Table 2. Nodulation status in faba bean as affected by inoculation with *Rhizobium* combined with *Pseudomonas* and *Herbaspirillum* under newly reclaimed soils during two seasons.

Treatments	Nodules/plant							
	Number				Dry weight (mg)			
	Season 2003/04		Season 2004/05		Season 2003/04		Season 2004/05	
	Minia	New-Valley	Minia	New-Valley	Minia	New-Valley	Minia	New-Valley
Uninoculated + 15 kg N/fed	36	66	28	52	323.8	460.1	267.3	314.8
Uninoculated + 30 kg N/ha	28	55	21	28	237.2	372.5	197.0	213.3
Rhizobium (Rh) inoculation + 15 kg N/fed	65	106	55	117	456.7	901.8	335.0	846.5
(Rh+*Pseudomonas*) inoculation + 15 kg N/fed	73	152	103	160	506.3	1398.2	446.0	1722.8
(Rh+*Herbaspirillum*) inoculation + 15 kg N/fed	66	133	84	142	494.6	1324.0	422.5	1483.8
(Rh+*Pseudomonas*+*Herbaspirillum*) inoculation + 15 kg N/fed	82	150	132	168	585.8	1426.5	512.0	1847.5
L.S.D. 0.05	4.0	5.0	8.2	13.4	27.6	33.0	36.0	42.4

Moreover, triple inoculation (*Rhizobium* + *Pseudomonas* + *Herbaspirillum*) caused significant increases in nodule number and dry weight compared to the alone inoculation with *Rhizobium*. These increases at the first and second seasons were 26.2 and 140.0% in Minia, respectively. The corresponding increases in New vally were 41.5 and 43.6% in the seame order during two seasons. The increases in nodules dry weight for triple inoculation over the alone inoculation with *Rhizobium* in Minia were 28.3, 52.8% during the two seasons. While, these increases were 58.2 and 118.3% in New Valley at first and second seasons, respectively.

The maximum number of nodules and their dry weight were obtained in plants inoculated with *Rhizobium*, *Pseudomonas* plus *Herbaspirillum* followed by those inoculated with *Rhizobium* plus *Pseudomonas* and with *Rhizobium* plus *Herbaspirillum*. This could be due to the effect of *Pseudomonas* and *Herbaspirillum* on plant growth resulting from the production of plant growth regulators, vitamines leading to enhancement the uptake of plant nutrients, supperssion of pathogenic organisms as reported by many worker (Glick, 1995; Chebotar *et al.*, 2001; Zaied *et al.*, 2003 and Kennedy *et al.*, 2004).

Growth and nitrogen content:

Data presented in Table (3) revealed that uninoculated plants received 15 kg N/fed recorded the lowest dry weight of shoots (14.1 and 12.1

g/plant) and the lowest nitrogen content (280 and 384 mg N/plant) at the first and second season in Minia, respectively. Similar trends were obtained in New-Valley which recorded shoots dry weight of 18.5 and 17.6 g/plant and nitrogen content of 467 and 542 mg N/plant during the two seasons in the same order. In Minia, uninoculated plants received the full dose of nitrogen (30 kg N/fed) increased shoot dry weight and nitrogen content by 68.8, 112.4% and 152.1, 184.1% over the control at the first and second season, respectively. While, in New-Valley these increases were 54.6, 65.3% and 157.6, 74.7% in the same order during the two seasons. Inoculation with *R. leguminosarum* either alone or in combination with *Pseudomonas* and/or *Herbaspirillum* gave significant increases in dry weight of shoots and nitrogen content compared with the uninoculated plants received 15 kgN/fed in Minia and New-Valley. Rhizobial inoculation alone increased shoots dry weight and N-content by 45.4 and 75.4% over the uninoculated plant which received the same dose of nitrogen fertilizer, respectively in Minia at the first season. These increases were 49.6 and 133.1% in the second season. While, in New-Valley these increases in dry weight of shoots and N-content were 28.6 and 100.4% in the same order at the first season. In the second season, these increases were 24.4 and 34.5%, respectively. This could be due to the biological role of *Rhizobium* in enhancing plant growth and N_2-fixation as reported by Saleh *et .al.* (2000), Sessitsch *et al.* (2002), Abo El-Soud *et al.* (2004) and Mekhemar *et al.* (2005).

Table 3. Effect of rhizobial inoculation combined with *Pseudomonas* and *Herbaspirillum* on plant growth and nitrogen content of faba bean under newly reclaimed soils during two seasons.

Treatments	Shoot							
	Dry weight (g/plant)				N-content (mg/plant)			
	Season 2003/04		Season 2004/05		Season 2003/04		Season 2004/05	
	Minia	New-Valley	Minia	New-Valley	Minia	New-Valley	Minia	New-Valley
Uninoculated + 15 kg N/fed	14.1	18.5	12.1	17.6	280	467	384	542
Uninoculated + 30 kg N/ha	23.8	28.6	25.7	29.1	706	839	1091	1184
Rhizobium (Rh) inoculation + 15 kg N/fed	20.5	23.8	18.1	21.9	491	628	895	986
(Rh+*Pseudomonas*) inoculation + 15 kg N/fed	23.3	28.6	23.6	28.9	650	918	1021	1132
(Rh+*Herbaspirillum*) inoculation + 15 kg N/fed	23.1	26.2	21.4	25.7	586	728	1057	1128
(Rh+*Pseudomonas*+*Herbaspirillum*) inoculation + 15 kg N/fed	24.8	26.9	28.9	31.5	804	1035	1108	1190
L.S.D. 0.05	1.7	2.3	1.9	2.3	4.5	6.9	5.2	7.6

The data again revealed that the maximum dry weight of shoots and nitrogen content were obtained in plants inoculated with triple inoculation (*Rhizobium* + *Pseudomonas* + *Herbaspirillum*) followed by those inoculated

with double inoculation (*Rhizobium* + *Pseudomonas*) or (*Rhizobium* + *Herbaspirillum*).

These results are in agreement with those obtained by Dileep-Kumar *et al.* (2001) and Hassanein and Mekhemar (2003), who found that seed bacterization with both strains of *Pseudomonas fluorescens* and *Pseudomonas putida* with *B. japonicum* gave enhancment in nodulation, growth and N-content of soybean plants. Also, Parmar and Dadarwal (1999) observed that co-inoculation of *Pseudomonas* with *Rhizobium* strains enhanced the weight of nodules, root length, shoot biomass and N-content in chickpea.

Seed and straw yields:

Seed and straw yields of plants grown in Minia and New Valley are presented in Table (4). The results appeared significant differences in seed and straw yields between plants inoculated with *Rhizobium* alone or combined with *Pseudomonas* or *Herbaspirillum* and uninoculated plants which received 15 kg N/fed. Uninoculated plants received starter dose of nitrogen recorded the lowest values of seed and straw yields in Minia and New-Valley during the two seasons. Plants inoculated with *Rhizobium* increased seed yield by 27.4 and 11.9% and straw yield by 7.4 and 8.0% over the uninoculated plants received the same dose of nitrogen at the first and the second seasons, respectively in Minia. Similar trends were observed in New-Valley which recorded increases of 21.7 and 41.8% in seed yield at the same order in the first and the second seasons. The increases in straw yield were 9.1 and 13.8% during the two seasons, respectively. Many studies indicated that rhizobial inoculation increased seed and straw yields of faba bean (Saleh *et al.*, 2000), Abo El-Soud *et al.*, 2004) and Mekhemar *et al.*, 2005).

Table 4. Effect of *Rhizobium* combined with *Pseudomonas* and *Herbaspirillum* on seed and straw yields under newly reclaimed soils during two growing seasons.

Treatments	Seed yield (ardab/fed)				Straw yield (ton/fed)			
	Season 2003/04		Season 2004/05		Season 2003/04		Season 2004/05	
	Minia	New-Valley	Minia	New-Valley	Minia	New-Valley	Minia	New-Valley
Uninoculated + 15 kg N/fed	8.00	12.33	9.81	10.84	2.70	2.41	2.75	3.91
Uninoculated + 30 kg N/ha	11.82	16.26	13.14	16.04	3.16	3.36	3.42	4.70
Rhizobium (Rh) inoculation + 15 kg N/fed	10.19	15.00	10.98	15.37	2.90	2.63	2.97	4.45
(Rh+*Pseudomonas*) inoculation + 15 kg N/fed	11.27	16.26	12.47	16.12	3.06	3.39	3.34	4.48
(Rh+*Herbaspirillum*) inoculation + 15 kg N/fed	11.30	15.53	13.17	16.07	3.02	2.93	3.09	4.38
(Rh+*Pseudomonas*+*Herbaspirillum*) inoculation + 15 kg N/fed	11.52	16.34	13.36	17.10	3.17	3.50	3.41	4.80
L.S.D. 0.05	0.85	1.65	0.95	1.71	0.23	0.33	0.25	0.37

In Minia, the highest seed and straw yield were obtained in plants inoculated with triple inoculation (*Rhizobium* + *Pseudomonas* + *Herbaspirillum*) followed by those inoculated with dual inoculation (*Rhizobium* + *Herbaspirillum*) and (*Rhizobium* + *Pseudomonas*). While, in New Valley the highest seed and straw yields were observed in plants inoculated with triple inoculation followed by plants inoculated with dual inoculation (*Rhizobium* + *Pseudomonas*) or (*Rhizobium* + *Herbaspirillum*).

The obtained results are in harmony with those obtained by Chebotar *et al.* (2001) and Rashad and Ragab (2003), who found that the enhanced nodulation, dry matter and grain yield due to the colonization of *Pseudomonas fluorescens* on soybean roots could be due to the production of growth-promoting substances that stimulate the growth of *B. japonicum*. Also, Singh and Suba Roa (1979) reported that *B. japonicum* and other rhizobacteria inoculated-soybean increased growth, nodulation and yields.

Regardeless of location and seasons, results in Table (5) indicated that the highest means of nodules number and dry weight, shoot dry weight and its nitrogen content as well as seed and straw yields were obtained in plants treated with triple inoculation (*Rhizobium* + *Pseudomonas* + *Herbaspirillum*) followed by those inoculated with dual inoculation (*Rhizobium* + *Pseudomonas*) and (*Rhizobium* + *Herbaspirillum*).

Table 5. Statistical main effect of *Rhizobium* inoculation combined with *Pseudomonas* and *Herbaspirillum* on nodulation, growth and yield of faba bean under newly reclaimed soils during two growing seasons.

Treatments	Nodules/plant		Shoot		Seed yield (ardab/ fed)	Straw yield (Ton/ fed)
	Number	DW (mg)	DW (g/ plant)	N-content (mg/plant)		
Uninoculated + 15 kg N/fed	46	341.5	15.6	418.3	10.25	2.94
Uninoculated + 30 kg N/ha	33	255.0	26.8	955.0	14.31	3.66
Rhizobium (Rh) inoculation + 15 kg N/fed	86	635.0	21.1	750.0	12.89	3.24
(Rh+*Pseudomonas*) inoculation + 15 kg N/fed	122	1018.3	26.1	930.3	14.03	3.57
(Rh+*Herbaspirillum*) inoculation + 15 kg N/fed	106	931.2	24.1	874.8	14.02	3.36
(Rh+*Pseudomonas*+*Herbaspirillum*) inoculation + 15 kg N/fed	133	1093.0	28.0	1034.3	14.58	3.72
L.S.D. 0.05	7.7	34.8	2.2	34.9	0.48	0.67

The data in Table (6) revealed that there are significant differences in nodulation, growth, nitrogen content, seed and straw yields. As shown herein the effect of location is more important than seasonal effect. The location of New valley recorded the highest number of nodules/plant (111 nodule), dry weighty of nodules/plant (1026.0 mg), dry weight of shoots/plant (25.6 g), nitrogen content/plant (898.0 mg), seed yield (15.27 ardab/fed) and straw yield (3.75 ton/fed). The differences between locations reflecting the

differences of soil type and fertility and other environmental conditions, and suggesting the possibility to raise yield level by improving management practices as soil fertility, irrigation and other agronomic practices. The importance of location effect on the traits of legume crops has been reportedd by several researchers (Hamdi *et al.*, 1991; Hamdi and Rabeia, 1991; Hamdi *et al.*, 1992; Mekhemar *et al.* (2005).

Table 6. Statistical main effect of the two locations on nodulation, growth and yield of faba bean under different inoculation treatments during the two growing seasons.

Location	Nodules/plant		Shoot		Seed yield (ardab/ fed)	Straw yield (Ton/ fed)
	Number	DW (mg)	DW (g/plant)	N-content (mg/plant)		
Minia	64	398.7	21.6	756.0	11.42	3.08
New Valley	111	1026.0	25.6	898.0	15.27	3.75
L.S.D. 0.05	14.1	20.0	4.0	26.1	0.82	0.17

On the other hand, the data in Table (7) revealed that there are no-significant differences between the two seasons. So, season did not affect on a number and dry weight of nodules, dry weight of shoot and N-plant content.

Table 7. Statistical main effect of the two seasons on nodulation, growth and yield of faba bean affected by different inoculants at two locations.

Seasons	Nodules/plant		Shoot		Seed yield (ardab/ fed)	Straw yield (Ton/ fed)
	Number	DW (mg)	DW (g/plant)	N-content (mg/plant)		
First season (2003/04)	84	707.3	23.5	677.7	13.00	3.02
Second season (2004/05)	91	717.4	23.7	976.5	13.71	3.80
L.S.D. 0.05	6.8	35.8	5.1	136.6	0.75	0.21

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تأثير التلقيح بالريزوبيا مع البسيدوموناس والهرباسبيريلليم على تكوين العقد والنمو والمحصول للفول البلدى تحت ظروف الاراضى حديثة الاستصلاح

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تم تنفيذ أربع تجارب حقلية فى الأراضى الجديدة حديثة الاستصلاح بمحافظتى المنيا والوادى الجديد خلال الموسمين الشتويين ٢٠٠٣/٢٠٠٤ ، ٢٠٠٤/٢٠٠٥ وذلك لدراسة تأثير التلقيح بالريزوبيا مشتركة مع البسيدوموناس فلورسنت ، الهرباسبيريلليم كبكتريا مشجعة للنمو ومثبتة للنيتروجين فى الحالة الحرة على نمو وتكوين العقد الجذرية ومحتوى النبات من النيتروجين وكذلك على محصول القول البلدى. وقد أوضحت النتائج أن التلقيح بالريزوبيا فقط أو بالريزوبيا مشتركة مع البسيدوموناس والهرباسبيريلليم قد أدى إلى إحداث زياده معنوية فى عدد العقد الجذرية ووزنها الجاف مقارنة بالمعاملة الغير الملقحة والمضافة لها نفس جرعة السماد النيتروجينى (١٥ وحدة نيتروجين/فدان) فى كلا الموقعين والموسمين. وقد أدى التلقيح الثلاثى بالريزوبيا مع البسيدوموناس والهرباسبيريلليم مع إضافة جرعة تنشيطية من السماد النيتروجينى (١٥ وحدة نيتروجين للفدان) إلى إحداث زيادة معنوية فى الوزن الجاف للمجموع الخضرى لنباتات الفول البلدى ومحتوها من النيتروجين مقارنة بالمعاملة الغير ملقحة والمسمدة بنفس جرعة السماد أو الغير ملقحة والمضافة اليها ٣٠ وحدة نيتروجين للفدان او الملقحة بالريزوبيا فقط والمضافة اليها ١٥ وحدة نيتروجين للفدان.

وقد أظهرت النتائج أن أعلى إنتاجية من محصول البذور والقش للفدان قد تم الحصول عليها فى النباتات المتأثرة بالتلقيح الثلاثى بالريزوبيا مع البسيدوموناس والهرباسبيريلليم والمضاف إليها ١٥ وحدة نيتروجين للفدان ويتبع ذلك النباتات المتأثرة بالتلقيح الثنائى (الريزوبيا مع البسيدوموناس) ثم (الريزوبيا مع الهرباسبيريلليم) والمضاف إليها ١٥ وحدة نيتروجين للفدان وذلك فى كل من المنيا والوادى الجديد خلال موسمى الزراعة.

وقد أوضحت النتائج وجود فروق معنوية فى أعداد وأوزان العقد الجذرية والوزن الجاف للمجموع الخضرى ومحتواه من النيتروجين وكذلك فى محصول البذرة والقش للفول البلدى بين الموقعين تحت الدراسة. حيث سجلت النباتات النامية فى أرض الوادى الجديد قيم أعلى عن تلك التى سجلت فى المنيا. وعلى النحو الأخرلم تسجل مواسم الزراعة أى تأثيرات معنوية علىمعدل تكوين العقد الجذرية والنمو والمحصول للفول البلدى.