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ROLE OF SOME RHIZOSPHERE MICROORGANISMS ON THE GROWTH AND NITROGEN CONTENT OF FABA BEAN CULTIVARS

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ABSTRACT: ***Under green house conditions; Rhizobium leguminosarum var. viciae, Bacillus megaterium var. phosphaticum and Trichoderma harzianum were applied to nonsterilized soil; individually or in combination; at the rate of 3% of soil weight. Surface sterilizede seeds of faba bean cultivars Sakha 1, Giza 3 Mohassan and Giza 716 were planted.***

Tested treatments minimized the required time for seed germination and increased the percentage both of germinated seeds and survived plants. Growth characters; i. e., plant height, number of leaves, plant length, plant fresh and dry weight of all tested cultivars were increased in response to the soil microorganisms application. Number of emerged flowers and formed bacrterial nodules were positively responded by variable biofertilizer treatments. Significant increase of total nitrogen was observed in faba bean plants sown in treated pots in comparison with control.

From the results of total protein analysis of Sakha 1 cv. showed that R. leguminosarum + T. harzianum treatment gave the best result which showed 13 bands whereas B. megaterium gave the worst result which showed 8 bands. Giza 3 Mohassan showed that R. leguminosarum + B. megaterium + T. harzianum gave the best result and showed 12 bands whereas B. megaterium + T. harzianum gave the worst result and gave 7 bands. Giza 716 R. leguminosarum gave the best result and showed 11 bands whereas B. megaterium, R. leguminosarum + B. megaterium + T. harzianum and control gave the worst result and showed 7 bands for each of them.

key words: ***Faba bean, Rhizobium leguminosarum, Trichoderma harzianum, Bacillus megaterium, biocontrol agents.***

INTRODUCTION

Man began to cultivate land for food around 8000 B. C. Very soon, he learned that the same land cannot support plant growth endlessely. This led him to think about the means of improving soil fertillty (Subba Rao, 1981).

The experiments of Bousingault,1834 revealed the important chemical constituents of both plant and soil. He also proposed that legume plants fix nitrogen in the soil which substantiated by Beijernick in 1888 who discovered the root nodules bacteria (*Rhizobium*) which fix atmospheric nitrogen. Microbiologists of USSR introduced "phosphobacterin"; a product contains cells of *Bacillus megaterium* var. *phosphaticum* to the soil in order to improve seed germination (Subba Roa, 1981). Bachman (1997) mentioned that biological nitrogen fixation (BNF) has been assured place in agriculture as a source of nitrogen for legumes. Abd-allah (1994) showed that strain TAL 1236 of *Rhizobium leguminosarum* var. *viciae* contributed significantly to the release of phosphorus from organic compounds through the action of acid and alkaline phosphates. El-Sheikh and El- Zaidany (1997) cleared that *Rhizobium* inoculation significantly increased yield, 100 seeds weight and cookability of faba bean. Their results indicate that *Rhizobium* inoculation is a promising fertilizer where it is cheap, easy to handle and improves plant growth and seed quality. Ballard *et al.*, (2004) proved that inoculation of pea cultivar Parafield with *Rhizobium* strain SU 303 produced as much shoot dry weight as the mineral nitrogen treatment and more than 2-9 times the shoot dry weight of the uninoculated treatment. Gary *et al.*, (2006) showed that gram-negative bacteria (rhizobia) within the Rhizoblaceae phylogenetic family (α-protobacteria) have the unique ability to infect and establish a nitrogen fixing symbiosis on the roots of leguminous plants. This symbiosis is of agronomic importance where it reduces the need for nitrogen fertilizer of legumes. Siser and Basu (2006) cleared that the mature root nodules of *Phaseolus mungo* (L.) contain higher amount of indole acetic acid (IAA) than non-nodulated roots.

Bacillus megaterium is a gram positive rod shape, spore producing eubacteria and found in the soil. It is a very important in the biotechnological industry because of its size, enzymes and cloning ability. However, phosphate solubilizing bacteria are known to increase phosphorus uptake resulting in better grown and higher yield of crop plants (Algawadi an Gaur, 1988). Pal *et al.*, (2001) found that bacillus spp. MRF produced an organic acid and IAA, solubilized tricalcium phosphate and fixed nitrogen from the atmosphere. El-Komy (2005) reported that *Bacillus megaterium* and *Azospirillum lipoferum* 137 as mixed inocula exhibited wheat high shoot dry weight, total nitrogen, yield and the shoot phosphorus content. Results demonstrate the beneficial influence of co-inoculation of both microbes for providing balanced N and P nutrition of wheat plants.

Trichoderma spp. are well known as biocontrol agent of certain soil- and air-borne plant diseases. However; Naseby *et al.*, (2000) reported that several strains of *T. harzianum* positively affected plant growth through either shoot or root fresh weight and increased root length and number of lateral roots. Naseby *et al.*, (2000) found that pea seedlings treated with *Trichoderma harzianum* strains Th1 and N47 exhibited an increase in the number of

nodules per root system. Zouxing and Kalidas (2000) decleared that preinoculation of pea seeds with *Trichoderma viride*, *T. harzianum* and *T. pseudokoningii* extracts, increased the germination rate in potting soil by 15, 15 and 20 % respectively compared with water treatment (control). After 5 days, the average plant height increased by 27, 39 and 31 % respectively and the average fresh weight increased by 33, 46 and 36 %, in the same respect. Rudresh *et al.*, (2005-b) reported that the all nine tested *Trichoderma* spp. isolates solubilized insoluble tricalcium phosphate to various exlint. They also mentioned that inoculation of *Trichoderma* spp. increased growth and yield parameters of chickpea compared with the uninoculated controls under both glass-house and field conditions.

Combined applications of the biocontrol agents may have positive or negative effects on growth parameters. Anusuya and Sullia (1985) noted that mixing *Trichoderma viride* and *Rhizobium* spp. caused significant reduction in the number and weight of nodules formed on peanut roots. They mentioned that this may be due to the abillty of *T. viride* to grow quickly in the soil and colonize plant surfaces, thus preventing the subsequent invasion of roots by the bacteria. Jain *et al.*,(1999) and Khurana and Sharma (2000) showed that the combined inoculation of *Rhizobium* and phosphate solubilizing bacteria increased nodulation, growth and yield parameters of chickpea. Saini *et al.*, (2004) found that microbial biomass, C, N and P under sorghum and chickpea were significantly higher when a combination of *Azospirillum brasilense* or *Rhizobium* or *Bacillus megaterium* and *Glomus fasciculatum* were used and was maximum with 50 % recommended fertilizers (40 kg N ha^{-1} and 8.73 kg P ha^{-1} for sorghum; 10 kg N ha^{-1} and 8.73 kg P ha^{-1} for chickpea) along with bioinoculants. Rudresh *et al.*, (2005-a) reported that combined inoculation of *Rhizobium*, a phosphate solubilizing Bacillus megaterium var. phosphaticum strain PB and a biocontrol fungus *Trichoderma* sp. increased germination, number of branches, nodulation and yield of pea and total biomass of chickpea compared with either individual inoculations or the uninoculated control. Wu *et al.*, (2005) identified the biofertilizer as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming.

The objective of this greenhouse study was to evaluate the role of single and combined soil infestation with *Rhizobium leguminosarum* var. *viciae*, *Bacillus megaterium* var. *phosphaticum* and *Trichoderma harzianum* on the growth and nitrogen content of faba bean (*Vicia faba* L.).

MATERIALS AND METHODS:

This work has been carried out at the Environmental Biotechnology Dept., GEBRI, Sadat City, Minoufiya, Egypt.

1- Rhizosphere Microorganisms Tested:

Rhizobium leguminosarum var. *fabae* "Okkadeen biofertilizer" was obtained from Legume Crops Dept. Field Crops Research Institute, ARC, Giza, Egypt. *Bacillus megaterium* var.*phosphaticum* isolate was obtained from MERCIN, Fac. of Agric., Ain Shams Univ. An identified isolate of *Trichoderma harzianum* was achieved from Agricultural Botany Dept., Fac. of Agric., Minuf. Univ.

2- Pot Experiment:

Pots (20 cm in diameter) were sterilized by immersing them into 5 % Sodium hypochlorite solution for 15 min. and then left to dry in open air. Non-sterilized sandy-loam soil of Sadat City mixed thoroughly with peat moth at the rate of 1:1 were left for a week in the open air before using in this experiment.

Bacillus isolate was grown on Nutrient Broth medium for 48 hr. on a rotary shaker at 25 °C. The bacterial inoculum was applied as a soil treatment at the rate of 5 ml bacterial suspension per plant (1×10^8 cfu/ml).

However; application of fungal isolates was carried out on Barley medium at the rate of 3% of soil weight.

3- Chemical Analysis:

3- 1-Total nitrogen content:

Total nitrogen content was estimated using the modified semi-micro kjeldahl method according to Hassouna, 1962 and Rahal, 1978.

3-2- Protein electrophoresis:

For most proteins that are not secreted, expression levels are generally such that protein can be easily visualized by Comassie blue staining of total protein on SDS-polyacrylamide gels. Denaturing polyacrylamide gel electrophoresis is simple and powerful method for proteins separation according to their size. In the most commonly used procedure, the protein samples are first denatured by heating in the presence of (Sodium dodycyl sulphate) SDS as reducing agent and strong anionic detergent. The treatment dissociates virtually all protein complexes. The denatured proteins bind SDS and acquire negative charge. The amount of SDS bound is proportional to the molecular eeight of SDS-polyacrylamide complex during electrophoresis is denaturing on the size of the polypeptide.

SDS-polyacrylamide Gel Electrophoresis (SDS-Page) was carried out by using a discontinuous buffer system described by (Laemmli, 1970).

4- Statistical Analysis:

The data were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, inc, 1988).

RESULTS AND DISCUSSION:

A- Effect of the Rhizosphere Microorganisms on Growth Characters:

A-1- Effect of the rhizosphere microorganisms on seed germination and survival:

Results present in table (1) indicate that the rhizosphere microorganisms encouraged the emergence of faba bean seedlings both in time and numbers. As for time; conrol seedlings of the three tested cultivars started their emergence after 15 days from seedlings. While application of *Rhizobium leguminosarum* to the soil minimized this time to be 10,11and 14 days; respectively for Sakha 1, Giza 3 Mohassan and Giza 716 cultivars. Abd-Allah (1994) cleared that *Rhizobium leguminosarum* var. *viciae* contributed significantly to the release of phosphorus from organic compounds through the action of acid and alkaline phosphates.

On the same respect; *Bacillus megatherium* resulted the emergence after 12,12 and 14 days. These were 12,12 and 15 days when *T. harzianum.* was applied. However; Subba Rao (1981) introduced *Bacillus megaterium* var. *phosphaticum* to the soil in order to improve seed germination.

Combination of *Rhizobium* with either or both *Bacillus megatherium* and *Trichoderma harizianum*; in general; gave the same effect. This result was also observed by Rudresh *et al.,* (2005-b).

On the other hand, number of emerged and survived seedlings didn't severely affected by the various tested rhizosphere microorganisms applied to the soil. *Rhizobium leguminosarum* + *Trichoderma harizianum* was the only treatment that increased Sakha 1 seedlings emergence than control. However, *Rhizobium leguminosarum* application showed the worst results. As for Giza 3 Mohassan cultivars, all except *Rhizobium leguminosarum* treatment resulted either equal or higher seedlings emergence percentages than the control. While faba bean Giza 716 cultivar showed much better response, where all treatments caused more emergence percentages except for the application of the three tested microorganisms which gave the same result as control. This last case could be attributed to the competition among the three microorganisms which minimize their competitive saprophytic ability in the soil (Ammar, 2003).

Table (1): Effect of some rhizosphere microorganisms on Faba bean seedling emergence and survival .

Treatments	Faba bean cultivars								
	Sakha 1			Giza 3 Mohassan			Giza 716		
	Start emergence days	Number of Plants	EM . %	Start emcr. days	No. of plants	EM . %	Start emcr. days	No. of plants	EM . %
Rhizobium leguminosarum	10	16	80	11	18	90	14	17	85
Bacillus megaterium	12	18	90	12	20	100	14	19	95
Trichoderma harzianum	12	18	90	12	19	95	15	17	85
R. leguminosarum + B. megaterium	11	18	90	11	19	95	13	20	100
R. leguminosarum +T. harzianum	10	20	100	11	20	100	13	19	95
B.megaterium +T. harzianum	14	17	85	14	20	100	14	17	85
R. leguminosarum +B.megaterium+ T. harzianum	13	19	95	14	19	95	15	16	80
control	15	19	95	15	19	95	15	16	80

Number of tested seeds = 20 EM: emergence

A-2- Effect of the rhizosphere microorganisms on plant height:

Faba bean cultivars showed different responses in plant height as for microorganisms application in comparison with the untreated control ones (Table 2-A).

Plant height of Sakha 1 cultivar was significantly increased in response to the applications up to 50 days after seeding; except the application of the three microorganisms which showed insignificant increase. The best plant height (31.6 cm) was achieved when *B. megatherium* and *T. harizianum* were applied to the soil.

Giza 3 Mohassan faba bean cultivar plant height showed high response to the microorganisms application. All the individual and combined treatments significantly increased plant height than control 20,30,40 and 50 days after seeding.

However; Giza 716 cultivar showed insignificant increase of plant height than control in most cases.

Results shown in Table 2-B clear that the tested microorganisms significantly increased faba bean plant heigt than control (20-50 days from seeding). The best effect,however, was noticed when *B. megatherium* and *T. harizianum* were applied in combination to the soil.

In the mean time; Sakha 1 faba bean cultivar showed the most response of plant height to the treatments. While Giza 716 cultivar was the worst one. However,Giza 3 Mohassan showed moderate respons (Table 2-C).

Table 2 (A): Effect of some rhizosphere microorganisms on the average height (cm) of some Faba bean cultivars after 20, 30, 40 and 50 days from seeding.

Treatments	Faba bean cultivars											
	Sakha 1				Giza 3 Mohassan				Giza 716			
	20	30	40	50	20	30	40	50	20	30	40	50
Rhizobium leguminosarum	14.7	22.8	23.0	30.3	13.4	18.0	25.4	30.2	9.8	13.4	17.3	25.6
Bacillus megaterium	15.6	22.6	25.5	31.3	12.2	19.9	23.5	29.3	11.0	16.7	22.1	25.1
Trichoderma harzianum	13.8	21.3	22.9	26.5	14.6	20.5	21.9	30.1	10.9	15.1	19.6	27.0
R.leguminosarum + B. megaterium	13.9	22.2	25.0	30.1	12.7	20.0	24.6	29.7	13.3	17.0	20.2	25.6
R. leguminosarum +T. harzianum	13.6	20.9	22.8	31.1	14.7	21.6	25.4	29.9	12.1	15.8	20.5	27.9
B.megaterium +T. harzianum	13.4	23.4	25.5	31.6	13.4	19.6	23.9	28.2	12.6	16.1	20.2	27.3
R. leguminosarum +B.megaterium+ T. harzianum	14.7	21.9	24.2	29.4	12.1	18.4	22.2	26.1	11.4	15.6	19.3	24.2
Control	12.5	19.6	21.5	27.7	7.9	12.9	17.6	23.4	10.4	15.5	19.8	25.4
$L.S.D_{0.05}$	1.3	1.7	1.8	1.9	1.3	1.7	1.8	1.9	1.3	1.7	1.8	1.9

Table 2-(B) :The main effect of treatments:

Treatments	Height (cm)							
	20 days		30 days		40 days		50 days	
Rhizobium leguminosarum	11.950	b	18.108	a	21.9	a	28.800	ab
Bacillus megaterium	12.883	ab	19.750	a	22.700	a	28.775	ab
Trichoderma harzianum	13.096	ab	19.008	a	21.467	a	27.917	ab
R. leguminosarum + B. megaterium	12.975	ab	19.783	a	23.267	a	28.467	ab
R. leguminosarum +T. harzianum	13.467	a	19.467	a	22.933	a	29.642	a
B.megaterium +T. harzianum	12.783	ab	19.900	a	23.250	a	29.050	a
R. leguminosarum +B.megaterium+ T. harzianum	12.708	ab	18.692	a	21.958	a	26.608	bc
control	10.250	c	16.025	b	19.675	b	25.525	c

Table 2-(C): The main effect of cultivars:

Cultivars	Height (cm)							
	20 days		30 days		40 days		50 days	
Sakha-1	14.025	a	21.881	a	23.756	a	29.769	a
Giza3 Mohassan	12.230	b	18.900	b	22.775	a	28.416	b
Giza 716	11.288	c	15.744	c	19.909	b	26.109	c

A-3- Effect of the rhizosphere microorganisms on leaves number:

Results present in (Table 3-A) clear that number of faba bean leaves of the three tested cultivars were nearly the same (around 4 leaves/plant) after 20 days from seedling. However, the separate application of *Rhizobium leguminosarum* caused significant reduction in number of leaves than control (20 days around).

Number of leaves was significantly increased than control in response to the beneficial rhizosphere microorganisms application 30,40 and 50 days after planting except few cases of Sakha 1 cultivar where insignificant increases were noticed.

Dunken's statistical analysis (Table 3-B) showed the above mentioned results.

However, statistical analysis of main cultivars response to leaves number were insignificant all over the experimentation period (20-50 days of seeding); Table 3-C.

Table 3-(A): Effect of some rhizosphere microorganisms on the average leaves number of some Faba bean cultivars after 20,30,40 and 50 days from seeding .

Treatments	Faba bean cultivars											
	Sakha 1				Giza 3 Mohassan				Giza 716			
	20	30	40	50	20	30	40	50	20	30	40	50
Rhizobim leguminosarum	3.8	6.0	7.2	9.3	3.8	5.7	7.0	9.1	4.0	5.8	6.8	9.7
Bacillus megaterium	4.1	5.7	7.2	9.1	4.1	6.0	6.9	9.1	4.0	6.3	7.1	9.5
Trichoderma harzianum	4.1	5.7	6.7	8.3	4.1	5.9	6.9	9.2	4.0	5.8	6.5	8.2
R. leguminosarum +B. megaterium	4.0	6.2	7.2	8.7	4.1	6.0	7.1	8.7	4.0	6.2	6.8	9.1
R. leguminosarum+ T.harzianum	4.0	5.9	7.0	8.5	4.0	6.8	7.2	9.0	4.0	6.0	6.6	9.1
B. megaterium + T.harzianum	4.0	6.0	7.1	9.0	4.0	6.1	6.9	8.3	4.0	5.9	6.9	8.8
R. leguminosarum + B. megaterium + T.harzianum	4.0	5.8	6.8	8.2	4.0	6.0	6.9	8.6	4.0	6.1	6.7	9.0
Control	4.0	5.4	6.5	8.2	4.0	5.5	6.1	8.1	4.0	5.7	6.4	8.0
$L.S.D_{0.05}$	0.1	0.4	0.4	0.5	0.1	0.4	0.4	0.5	0.1	0.4	0.4	0.5

Table 3-(B) :The main effect of treatments:

Treatments	Leaves number							
	20 days		30 days		40 days		50 days	
Rhizobium leguminosarum	3.850	B	5.858	abc	7.033	a	9.375	a
Bacillus megaterium	4.054	a	6.33	ab	7.067	a	9.250	ab
Trichoderma harzianum	4.54	a	5.842	bc	6.750	a	8.908	abc
R. leguminosarum + B. megaterium	4.000	a	6.167	ab	7.042	a	8.842	bc
R. leguminosarum +T. harzianum	4.000	a	6.258	a	6.958	a	8.717	c
B.megaterium +T. harzianum	3.983	a	6.017	ab	6.975	a	8.625	c
R. leguminosarum +B.megaterium+ T. harzianum	4.000	a	5.992	ab	6.817	a	8.572	cd
control	4.000	a	5.517	c	6.342	b	8.125	d

Table 3-(C) : The main effect of cultivars:

Cultivars	Leaves number							
	20 days		30 days		40 days		50 days	
Sakha-1	3.981	a	5.866	a	6.969	a	8.944	a
Giza3 Mohassan	3.995	a	6.006	a	6.897	a	8.775	a
Giza 716	4.000	a	6.009	a	6.753	a	8.944	a

A-4- Effect of the rhizosphere microorganisms on plant length:

Results present in Table 4-A,B and C indicate that application of the beneficial rhizosphere microorganisms; either separate or in combination, increased shoot and root length of faba bean plant cultivars. Significant differences could be noticed; in most cases; between the treated and untreated control plants. The best length of shoot system was opserved when *B. megaterium* was applied to the soil (Table 4-B). *R. leguminosarum* stimulated the growth of root system.

Response of the tested faba bean cultivars was variable,where Sakha 1 was more longer than both of Giza 3 Mohassan and Giza 716 cultivars.. Significant variations were obtained between the total length of the three tested cultivars (Table 4-C).

Table 4-(A): Effect of some rhizosphere microorganisms on the length (cm) of some Faba bean cultivars at the pod production stage.

Treatments	Length of Faba bean cultivars (cm)								
	Sakha 1			Giza 3 Mohassan			Giza 716		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Rhizobim leguminosarum	36.5	42.2	78.7	34.4	36.4	70.8	27.9	31.2	59.1
Bacillus megaterium	38.1	37.0	75.1	32.0	37.4	69.4	30.6	33.7	64.3
Trichoderma harzianum	31.3	34.0	56.3	30.7	39.3	70.0	29.3	32.3	61.6
R. leguminosarum +B. megaterium	33.8	34.9	68.7	29.5	33.3	62.8	30.9	31.3	62.2
R.leguminosarum+ T.harzianum	31.2	27.1	58.3	30.5	32.2	62.7	28.2	32.8	61.0
B. megaterium + T.harzianum	36.7	33.9	70.6	30.1	33.2	63.3	29.2	36.0	65.2
R.leguminosarum+ B.megaterium+ T.harzianum	36.9	33.1	70.0	30.2	33.1	63.3	27.0	33.2	60.2
Control	30.3	30.6	60.3	25.8	31.7	57.5	27.9	29.7	57.6
L.S.D$_{0.05}$	2.3	3.3	4.5	2.3	3.3	4.5	2.3	3.3	4.5

Table 4-(B): The main effect of treatments:

Treatments	Length (cm)					
	Shoot		Root		Total	
Rhizobium leguminosarum	32.93	ab	36.65	a	66.34	ab
Bacillus megaterium	33.62	a	36.10	ab	70.34	a
Trichoderma harzianum	30.47	bcd	35.24	ab	65.07	bc
R. leguminosarum + B. megaterium	31.48	abcd	33.23	abc	64.17	bcd
R. leguminosarum +T. harzianum	30.00	cd	30.72	c	60.87	cd
B.megaterium +T. harzianum	32.00	abc	34.43	ab	66.46	ab
R. leguminosarum +B.megaterium+ T. harzianum	31.39	abcd	33.16	abc	65.79	abc
control	29.02	d	32.83	bc	59.80	d

Table 4-(C) : The main effect of cultivars:

Cultivars	Length (cm)					
	Shoots		Roots		Total	
Sakha-1	34.75	a	34.95	a	69.55	a
Giza3 Mohassan	30.44	b	34.63	a	64.05	b
Giza 716	28.90	c	32.55	b	61.19	c

Generally; increasing plant height and number of leaves (shoot system) in response to the tested microorganisms; individually or in combination; was also noticed by El-Sheikh and El-Zaydani (1997), Naseby *et al.*, (2000), Ballard *et al.*, (2004), Rudresh *et al.*, (2005) and Gary *et al.*, (2006). However; Sakha 1 cultivar was the best responded for the tested microorganisms. In addition; it is of logic that plant height of faba bean seedlings emerged earlier were significantly more than those raised later (control). In the mean time, significant increase of leaves number; 30-50 days age; in response to microorganisms application improved photosynthation process which consequently increased faba bean plants growth.

A-5- Effect of the rhizosphere microorganisms on plant fresh weight:

All tested rhizosphere microorganisms increased the fresh weight of faba bean plant cultivars than the uninoculated ones. Total fresh weight of Sakha 1 cultivar plants increased significantly than control in response to *Rhizobium leguminosarum*, *Bacillus megaterium*, both of them and *B. megaterium* + *Trichoderma harzianum* applications (Table 5-A). However, insignificant increases of fresh weight were recorded when *T. harzianum*, *R.leguminosarum* +*T. harzianum* and all the three microorganisms in combination were applied to the soil.

Total fresh weight of Giza 3 Mohassan cultivar plants was significantly higher than control ones in all treated plants with the rhizosphere microorganisms, except those of the three tested microorganisms application. As for Giza 716 cultivar, all microorganisms application led to significant increases of fresh weight with the exception of *R. leguminosarum* + *T. harzianum* application.

stastistical analysis using Dunkan's method (Table 5-B) clear that the best treatment for increasing faba bean fresh weight was *B. megaterium* + *T. harzianum* followed by *R. leguminosarum* one. The untreated control pots followed by application the three tested microorganisms together resulted the worst fresh weight.

On the other hand, Giza 716 cultivar gave the highest fresh weight, while Giza 3 Mohassan cultivar was and Sakha 1 cultivar gave the least fresh weight (Table-C).

Table 5-(A): Effect of some rhizosphere microorganisms on the fresh weight (gm) of some Faba bean cultivars at the pod production stage.

Treatments	Length of Faba bean cultivars (cm)								
	Sakha 1			Giza 3 Mohassan			Giza 716		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Rhizobim leguminosarum	13.5	17.4	30.9	14.4	22.4	36.8	13.5	24.8	38.3
Bacillus megaterium	13.3	19.3	32.6	12.5	20.0	32.5	11.4	27.9	39.3
Trichoderma harzianum	9.8	14.3	24.1	13.9	20.1	34.0	11.6	27.1	38.7
R.leguminosarum +B. megaterium	10.9	17.3	28.2	15.7	15.5	31.2	10.8	25.0	35.8
R.leguminosarum+ T.harzianum	10.1	17.0	27.1	14.1	22.5	36.6	11.3	22.5	33.8
B. megaterium + T.harzianum	12.8	21.1	33.9	12.1	24.2	36.4	13.7	28.9	42.6
R.leguminosarum+ B.megaterium+ T.harzianum	10.8	12.4	23.2	10.1	17.1	27.2	11.8	25.5	37.3
Control	11.1	11.9	23.0	13.2	12.1	25.3	12.2	18.3	30.5
$L.S.D_{0.05}$	1.8	4.1	4.9	1.8	4.1	4.9	1.8	4.1	4.9

Table 5-(B): The main effect of treatments:

Treatments	Fresh weight (gm)					
	Shoot		Root		Total	
Rhizobium leguminosarum	13.92	a	21.55	ab	37.10	ab
Bacillus megaterium	12.31	abc	22.43	ab	34.77	abc
Trichoderma harzianum	11.80	bc	20.55	ab	31.98	bc
R. leguminosarum + B. megaterium	12.50	abc	19.30	b	31.81	bc
R. leguminosarum +T. harzianum	11.85	bc	20.69	ab	32.04	bc
B.megaterium +T. harzianum	13.58	ab	24.75	a	38.70	a
R. leguminosarum +B.megaterium+ T. harzianum	10.93	c	18.36	b	29.71	c
Control	12.20	abc	14.14	c	26.30	c

Table 5-(C) : The main effect of cultivars:

Cultivars	Fresh weight (gm)					
	Shoots		Roots		Total	
Sakha-1	11.56	b	16.30	c	27.86	c
Giza3 Mohassan	13.54	a	19.20	b	32.74	b
Giza 716	11.56	b	25.00	a	36.56	a

A-6- Effect of the rhizosphere microorganisms on plant dry weight:

Results shown in Table 6-A clear that the dry weight of faba bean shoots and roots was higher than control because of rhizosphere microorganisms application. Total dry weight of Sakha 1 cultivar plants increased significantly than control in response to the microorganisms treatments except those of *Trichoderma harzianum* and *Rhizobium leguminosarum* +*T. harzianum* ones. The same result was noticed for Giza 3 Mohassan cultivar but significant increases of total dry weight were noticed when *R leguminosarum* + *Bacillus megaterium* and all the three microorganisms were applied to the soil. Significant increase of dry weight was noticed in all treatments of Giza 716 cv. With the exception of *R leguminosarum* + *T. harzianum* one, which gave insignificant dry weight increase.

Dunkan statistical analysis (Table 6-B) clear that *R leguminosarum* treatment was the best to increase plants dry weight, the untreated control plants resulted the worst dry weight and all the other treatments showed insignificant increase of dry weight among themselves.

As for faba bean cultivars, Giza 716 gave the best dry weight followed by Giza 3 Mohassan and Sakha 1 cultivar was the worst. Significant variations could be noticed between the tested three cultivars in plants dry weight (Table 6-C).

Table 6-(A): Effect of some rhizosphere microorganisms on the dry weight (gm) of some Faba bean cultivars at the pod production stage.

Treatments	Length of Faba bean cultivars (cm)								
	Sakha 1			Giza 3 Mohassan			Giza 716		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Rhizobim leguminosarum	1.1	1.4	2.5	1.6	1.9	3.5	0.9	1.9	2.8
Bacillus megaterium	1.2	1.7	2.9	1.2	1.7	2.9	1.2	1.9	3.1
Trichoderma harzianum	0.8	1.1	1.9	1.3	1.9	3.2	0.9	1.9	2.8
R.leguminosarum +B. megaterium	1.1	1.3	2.4	1.1	1.7	2.8	1.3	1.5	2.8
R.leguminosarum+ T.harzianum	0.9	1.0	1.9	1.4	2.1	3.5	0.9	1.5	2.4
B. megaterium + T.harzianum	1.1	1.2	2.3	1.2	1.7	2.9	1.0	1.6	2.6
R.leguminosarum+ B.megaterium+ T.harzianum	1.1	1.2	2.3	1.0	1.7	2.7	1.0	1.5	2.5
Control	0.9	0.9	2.0	1.0	1.5	2.5	1.1	1.0	2.1
L.S.D$_{0.05}$	0.2	0.3	0.4	0.2	0.3	0.4	0.2	0.3	0.4

Table 6-(B): The main effect of treatments:

Treatments	Dry weight (gm)					
	Shoot		Root		Total	
Rhizobium leguminosarum	1.192	a	1.742	a	2.900	a
Bacillus megaterium	1.158	a	1.758	a	2.808	ab
Trichoderma harzianum	0.983	a	1.617	ab	2.658	abc
R. leguminosarum + B. megaterium	1.158	a	1.533	ab	2.633	abc
R. leguminosarum +T. harzianum	1.033	a	1.500	ab	2.575	abc
B.megaterium +T. harzianum	1.107	a	1.483	ab	2.567	abc
R. leguminosarum +B.megaterium+ T. harzianum	1.008	a	1.450	ab	2.450	bc
Control	0.992	a	1.308	ab	2.342	c

Table 6-(C) : The main effect of cultivars:

Cultivars	Dry weight (gm)					
	Shoots		Roots		Total	
Sakha-1	1.016	b	1.228	b	2.238	c
Giza3 Mohassan	1.016	b	1.659	a	2.684	b
Giza 716	1.194	a	1.759	a	2.722	a

Fresh dry weight of faba bean plants were positively responded by the soil microorganisms applied to the soil. Such microorganisms could be used as biofertilizers as reported also by Abd-allah (1994), Elsheikh and El-zaidany (1997), Rudresh (2005-a,b) and Gary *et al.*, (2006).

B- Effect of the Rhizosphere Microorganisms on Flowering:

Results of flowering date and the average number of flowers per plant as affected by the beneficial microorganisms application to the soil are shown in Tables (7 A, B and C).These results clear that starting flowering of Sakha 1 cultivar was noticed 45 days after seeding in control, *Rhizobium leguminosarum* and *Bacillus megatherium + Trichoderma harizianum* treatments. The rest applications significantly minimized the required time for flowering beginning; up to 40 days from seeding; in the cases of *B. megatherium*, *R. leguminosarum + B. megatherium* and *R. leguminosarum + T. harizianum* applications.

On the other hand; the average number of flowers per plant (50 days age) of Sakha 1 faba bean cultivar significantly increased in response to all the benifecial microorganisms application to the soil; except that of *Rhizobium leguminosarum + Trichoderma harizianum* one which showed significantly less flowers than control.

As for Giza 3 Mohassan cultivar; all treatments except *T. harzianum* one significantly reduced the time of first flower appearance. However; the average number of flowers per plant of this cultivar (50 days age) were either

equal or significantly less than control in most treatments. Insignificant increase in flowers number was recorded when *R. leguminosarum* + *T. harizianum* were applied to the soil. Significant flowers increase was only noticed when the three microorganisms were added to the soil.

Single application of any rhizosphere microorganisms didn't affect flowering date of Giza 716 faba bean cultivar. While any other combined application significantly reduced the starting flowering date of this cultivar (Table 7-A). However; number of flowers per plant was superior when the three microorganisms were applied to the soil (3.1). Significant increase of flowers number was also noticed when *B. megaherium* and *R. leguminosarum* + *T. harizianum* were the treatments.

Table 7-B indicates that the best and lonely significant treatment that increased flowers of all tested cultivars was the application of the three microorganisms to faba bean soil.

As for the tested genotypes; flowers number of Giza 716 was the best and significantly more than Giza 3 Mohassn one (the worst cultivar).

However; Sakha 1 cultivar showed moderately flowering rate in comparison with the other two cultivars.

The above results; in general; clear that application of the beneficial microorganisms to the soil increased the number of flowers which consequently increase faba bean yields. This was also observrd by Abd-Allah (1994), El-Sheikh and El-Zaidany (1997), El-Komy (2005) and Rudresh *et al.*, (2005).

Table 7- (A): Effect of some rhizosphere microorganisms on the flowering date and averag flowers number of some faba bean cultivars.

Treatments	Faba bean cultivars					
	Sakha 1		Giza 3 Mohassan		Giza 716	
	Days	Number	Days	Number	Days	Number
Rhizobim leguminosarum	45	1.5	44	1.4	44	1.3
Bacillus megaterium	40	1.9	44	1.3	44	2.5
Trichoderma harzianum	44	1.5	45	1.8	44	1.8
R. leguminosarum +*B. megaterium*	40	2.5	41	1.3	41	2.4
R. leguminosarum+ *T.harzianum*	40	1.1	41	2.3	38	2.5
B. megaterium + *T.harzianum*	45	2.4	41	1.0	38	2.0
R. leguminosarum+ *B. megaterium*+ *T.harzianum*	44	2.0	44	2.6	41	3.1
Control	45	1.4	45	1.8	44	1.8

L.S.D$_{0.05}$: 0.772 " for number of flowers"

Table7- (B): The main effect of treatments:

Treatments	Flowers number	
Rhizobium leguminosarum	1.375	b
Bacillus megaterium	1.875	ab
Trichoderma harzianum	1.667	b
R. leguminosarum + B. megaterium	2.042	ab
R. leguminosarum +T. harzianum	1.958	ab
B.megaterium +T. harzianum	1.800	ab
R. leguminosarum +B.megaterium+ T. harzianum	2.583	a
control	1.633	b

Table 7-(C) : The main effect of cultivars:

Cultivars	Flowers number	
Sakha-1	1.785	ab
Giza3 Mohassan	1.656	b
Giza 716	2.156	a

C-Effect of the Rhizosphere Microorganisms on Bacterial Nodules Formation:

Results present in Table 8 clear that the best nodules number (116.40) was achieved when *Rhizobium leguminosarum* alone was applied. The least nodules number was noticed on control plants grown in non-sterilized soil (average 14.15). Insignificant increase of nodules number was observed in response to soil infestation with different tested rhizosphere microorganisms. However, the highst nodule numbers were achieved when *Trichoderma harzianum*, *Bacillus megaterium* and *R. leguminosarum + B. megaterium + T. harzianum* were treated. These average numbers were 50.85, 49.43 and 44.89 respectively.

Increasing of nodules number in response to *Trichoderma* spp. was also observed by Naseby *et al.*, (2000), Zouxing and kalidas (2000) and Rudresh *et al.*, (2005-a). However; reduction in the number of nodules due to combined application of *Trichoderma viride* and *Rhizobium* spp. was noticed by Anusuya and Sullia (1985). They attributed this reduction to quick colonization of plant surfaces by the faster organism (*Trichoderma viride*).

On the other hand, faba bean cultivars had no effect on bacterial nodule formation. Insignificant differences were noticed between Sakha 1 cultivar (40.44 nodules), Giza 3 Mohassan cv. (49.74) and Giza 716 one (53.48).

Table 8-(A): Effect of some rhizosphere microorganisms on nodules average number/plant of some Faba bean cultivars at pod production stage.

Treatments	Nodules number of faba bean cultivars		
	Sakha 1	Giza 3 Mohassan	Giza 716
Rhizobim leguminosarum	66.3	166.5	116.2
Bacillus megaterium	36.6	79.4	32.2
Trichoderma harzianum	50.0	39.8	62.7
R. leguminosarum + *B. megaterium*	62.2	19.3	44.3
R.leguminosarum+ *T.harzianum*	37.5	16.2	41.8
B.megaterium+ *T.harzianum*	18.2	27.0	55.2
R. leguminosarum+ *B.megaterium*+ *T.harzianum*	52.5	34.5	67.6
Control	00.0	14.9	27.5
$L.S.D_{0.05}$	24.3		

Table 8-(B): the main effect of treatments

Treatments	Nodules number	
Rhizobim leguminosarum	116.40	a
Bacillus megaterium	49.43	b
Trichoderma harzianum	50.85	b
R. leguminosarum +B. megaterium	42.00	b
R. leguminosarum+ T.harzianum	31.86	bc
B. megaterium + T.harzianum	33.50	bc
R. leguminosarum+ B. megaterium+ T.harzianum	44.89	b
Control	14.15	c

Table 8-(c): The main effect of cultivars

Cultivars	Nodules number	
Sakha-1	40.44	a
Giza3 Mohassan	49.74	a
Giza 716	53.48	a

D- Effect of the Rhizosphere Microorganisms on Plant Total Nitrogen Content:

Results present in Table 9 indicate that total nitrogen content of Sakha 1 faba bean cultivar highly increased than control in response to the application of the three tested microorganisms, either single or in combination. Total nitrogen levels was more than twice of the control, as for the microorganisms application. These nitrogen levels were about 1.5 folds of control, as for Giza 3 Mohassan cultivar. However, variable increase were recorded in total nitrogen levels than control of Giza 716 cultivar.

Generally, the best results of nitrogen contents were noticed when *Rhizobium leguminosarum* was applied to the soil. This is agreement with Abd-Allah (1994), El-sheikh and El-Zaidany (1997) and Gary *et al.*, (2006).

Table (9): Effect of some rhizosphere microorganisms on total nitrogen content of some faba bean cultivars.

Treatments	Total nitrogen content (mg/g)								
	Sakha 1			Giza 3 Mohassan			Giza 716		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Rhizobim leguminosarum	2.0	1.52	3.52	2.21	1.66	3.87	2.08	1.39	3.47
Bacillus megaterium	1.61	1.18	2.79	2.12	1.20	3.32	1.71	1.16	2.87
Trichoderma harzianum	1.39	1.25	2.64	1.48	1.29	2.77	1.57	1.06	2.63
R.leguminosarum +B. megaterium	1.61	1.25	2.86	1.84	1.66	3.50	1.62	1.25	2.87
R.leguminosarum+ T.harzianum	1.61	1.38	2.99	2.03	1.20	3.23	1.62	1.39	3.01
B. megaterium + T.harzianum	1.61	1.02	2.99	1.84	1.76	3.60	1.62	1.39	3.01
R.leguminosarum+ B.megaterium+ T.harzianum	2.08	1.14	3.10	2.31	1.11	3.42	2.08	1.80	3.88
Control	1.25		1.39	1.39	1.02	2.41	1.52	0.97	2.49

E- Effect of the Rhizosphere Microorganisms on PlantProtein Analysis:

Electrophoresis analysis was carried out using SDS-PAGE for water soluble protein fraction and was stained to detect the whole protein banding pattern.

SDS-PAGE were achieved to screen the water soluble leaf protein extracted from faba bean plants under the treatments of the rhizosphere microorganisms after 50 days.

From the results of Sakha 1 cv. plants total protein analysis Fig. (1) and Table (10) it could be concluded that *R. leguminosarum + T. harzianum* treatment gave the best result which showed 13 bands whereas *B. megaterium* treatment gave the worst result which showed 8 bands. *R. leguminosarum*, *T. harzianum*, *R. leguminosarum + B. megaterium*, *B. megaterium + T. harzianum* and *R. leguminosarum + B. megaterium + T. harzianum* and control showed 9, 10, 11, 9, 9 and 9 bands respectively.

From the results of Giza 3 Mohassan total protein analysis in Fig. (2) and Table (11) it could be concluded that *R. leguminosarum + B. megaterium + T. harzianum* treatment gave the best result and showed 12 bands. *B. megaterium + T. harzianum* gave the worst result and showed 7 bands. *R. leguminosarum*, *B. megaterium*, *T. harzianum*, *R. leguminosarum + B.*

megaterium*, *R. leguminosarum* + *T. harzianum **and control showed 8, 10, 11, 9, 8 and 11 bands respectively.**

From the results of Giza 716 plants total protein analysis in Fig. (3) and Table (12) itcould be cocluded that *R. leguminosarum* treatment gave the best result and showed 11 bands whereas *B. megaterium*, *R. leguminosarum* + *B. megaterium* + *T. harzianum* **and control gave the worst result and showed 7 bands for each of them. *T. harzianum*, *R. leguminosarum* + *B. megaterium*** **and *R. leguminosarum* + *T. harzianum*** **showed 9,8,8 and 8 bands respectively.**

Table (10): Presence (1) versus absence (0) of SDS-PAGE protein bands of soluble protein extracted from faba bean plants (Sakha 1 cv.,):

No	MW(kd)	*Rhi.,*	*B.,*	*Tri.,*	*Rhi., +B.,*	*Rhi.,+Tri.,*	*Rhi.,+B.,*	*Rhi.,+B.,+Tri.,*	Control
1	92.742	1	1	1	1	1	1	1	1
2	84.012	0	0	1	0	0	0	0	1
3	81.141	1	1	1	1	1	1	1	1
4	78.102	1	1	0	1	1	1	1	0
5	73.231	1	1	1	1	1	1	0	1
6	69.665	0	1	1	1	1	1	1	1
7	67.255	1	0	0	0	1	0	1	0
8	63.814	0	0	1	1	1	0	1	0
9	61.638	0	0	1	0	0	1	0	1
10	59.489	1	1	1	1	1	0	1	1
11	56.257	0	0	0	1	1	1	0	0
12	49.487	1	1	1	1	1	1	1	1
13	46.899	0	0	0	0	0	1	0	0
14	44.267	0	0	0	0	1	0	0	1
15	41.168	1	1	1	1	1	0	0	1
16	35.533	1	0	0	0	0	0	0	0
17	29.649	0	0	0	1	1	0	0	0

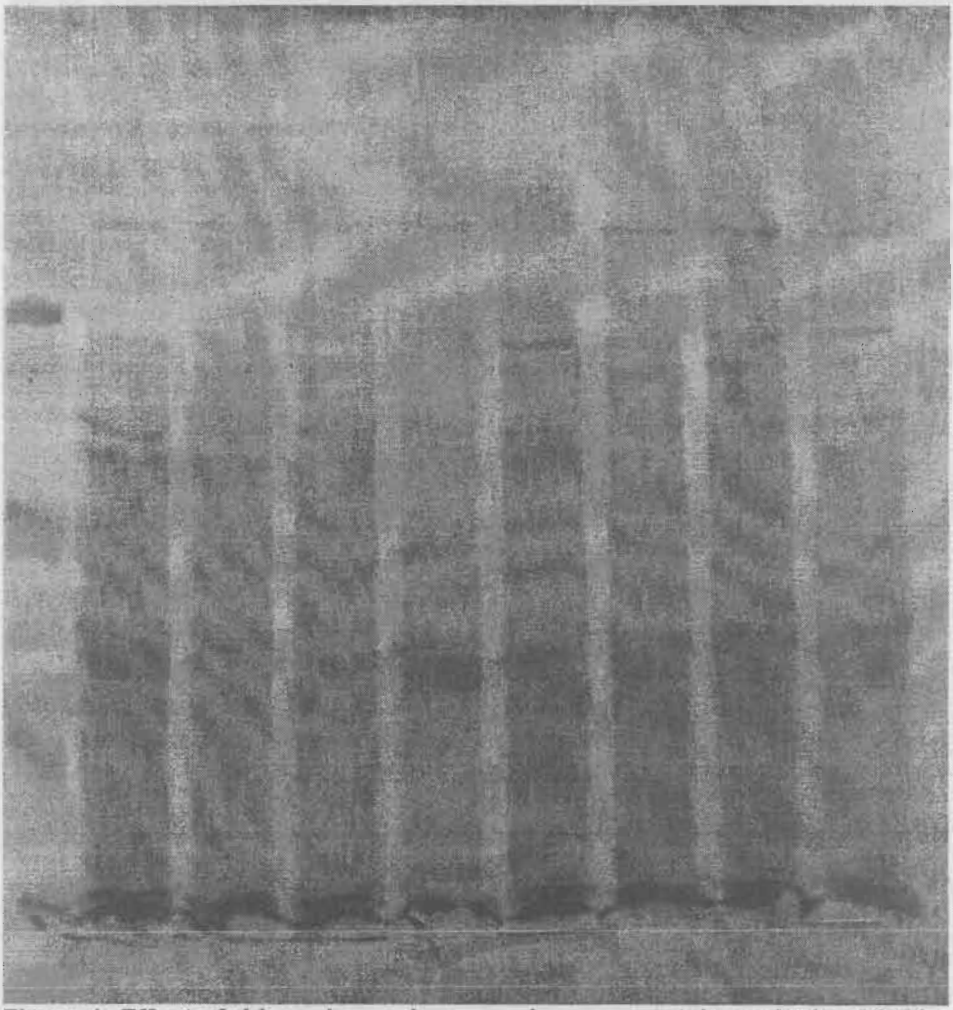

Figure 1: Effect of rhizosphere microorganisms on protein analysis of Sakha 1 faba bean plants (1- *Rhi.*, 2-*B.*, 3-*Tri.*, 4-*Rhi.*,+*B.*, 5- *Rhi.*, +*Tri.*, 6-*B.*, +*Tri.*, 7-*Rhi.*,+*B.*,+*Tri.*, 8-control)

Table (11): Presence (1) versus absence (0) of SDS-PAGE protein bands of soluble protein extracted from faba bean plants (Giza 3 Mohassan cv.,):

No	MW(kd)	*Rhi.,*	*B.,*	*Tri.,*	*Rhi.,* +*B.,*	*Rhi.,*+*Tri.,*	*Rhi.,*+*B.,*	*Rhi.,*+*B.,*+*Tri.,*	Control
1	165.600	0	1	0	0	0	1	0	0
2	160.581	1	1	1	1	1	0	1	1
3	106.847	0	0	1	1	1	1	1	1
4	086.444	1	1	1	1	1	1	1	1
5	064.710	1	0	1	0	0	0	0	0
6	056.965	0	1	0	1	1	1	1	1
7	049.666	0	1	1	1	0	1	1	1
8	036.950	0	0	1	0	0	0	0	0
9	035.464	0	1	1	1	1	1	1	1
10	032.048	1	1	1	1	1	1	0	0
11	031.100	1	0	0	0	0	0	1	1
12	028.531	1	1	1	1	0	0	1	0
13	026.559	1	1	1	1	0	0	0	1
14	024.935	0	0	0	0	0	0	1	0
15	022.015	1	0	0	0	1	0	1	1
16	019.854	0	0	0	0	0	0	1	1
17	018.180	0	1	0	0	0	0	0	0
18	016.053	0	0	1	0	1	0	1	0
19	014.000	0	0	0	0	0	0	0	0
20	012.680	0	0	0	0	0	0	0	1

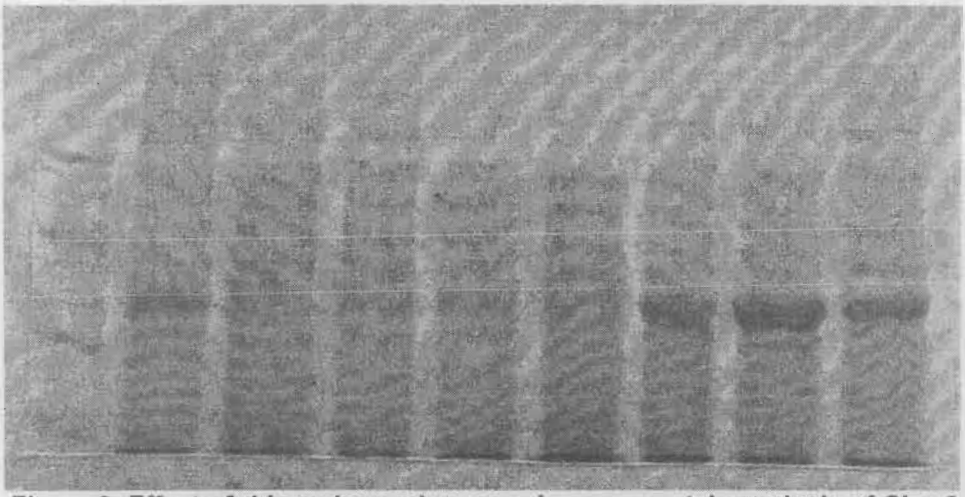

Figure 2: Effect of rhizosphere microorganisms on protein analysis of Giza 3 Mohassan faba bean plants plants (1- *Rhi.*, 2-*B.*, 3-*Tri.*, 4-*Rhi.*,+*B.*, 5- *Rhi.*, +*Tri.*, 6-*B.*, +*Tri.*, 7-*Rhi.*,+*B.*,+*Tri.*, 8-control).

Table (12): Presence (1) versus absence (0) of SDS-PAGE protein bands of soluble protein extracted from faba bean plants (Giza 716 cv.,):

No	MW(kd)	*Rhi.*,	*B.*,	*Tri.*,	*Rhi.*, +*B.*,	*Rhi.*,+*Tri.*,	*Rhi.*,+*B.*,	*Rhi.*,+*B.*,+*Tri.*,	Control
1	183.890	0	0	0	1	0	0	0	1
2	180.990	0	0	0	0	1	1	0	0
3	172.570	0	0	0	0	0	0	1	0
4	169.850	0	0	1	0	0	0	1	0
5	160.665	1	1	0	0	0	0	0	0
6	127.600	0	0	0	0	0	0	0	1
7	117.240	0	0	0	1	1	1	0	0
8	112.370	1	0	0	0	0	0	0	0
9	108.860	0	0	1	0	0	0	1	0
10	104.620	1	0	0	1	0	0	0	0
11	100.540	0	0	0	0	1	0	0	0
12	097.400	0	0	0	0	0	1	0	0
13	087.198	1	1	1	0	0	0	1	0
14	063.737	0	0	1	1	1	0	0	1
15	056.236	0	1	0	0	0	1	0	0
16	051.549	0	0	1	1	0	0	0	0
17	037.421	0	0	0	0	0	1	0	0
18	035.505	1	1	0	0	0	0	0	0
19	032.842	0	0	1	1	1	1	1	1
20	031.408	1	1	0	0	1	0	0	0
21	030.182	0	0	1	0	0	0	0	0
22	028.820	1	0	0	0	0	0	0	1
23	025.310	1	0	0	0	0	1	1	0
24	022.640	1	1	1	1	1	0	0	1
25	018.584	1	0	0	0	0	0	0	0
26	012.401	0	0	0	0	1	1	0	1
27	010.966	0	1	1	1	0	0	1	0
28	009.292	1	0	0	0	0	0	0	0

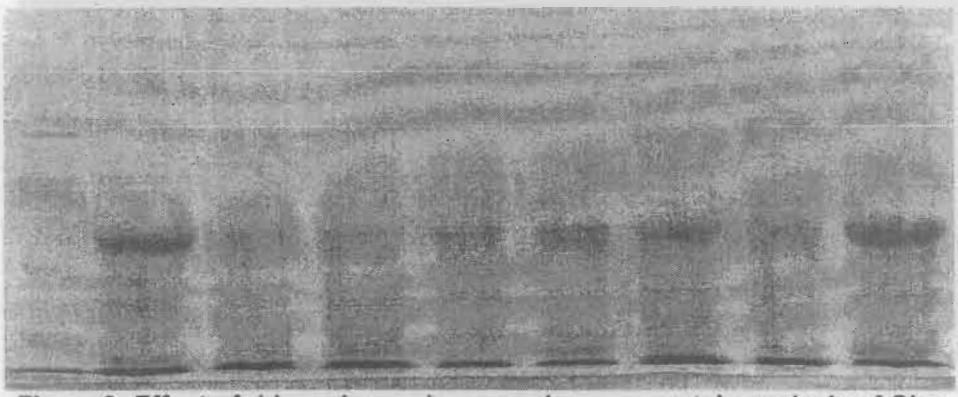

Figure 3: Effect of rhizosphere microorganisms on protein analysis of Giza 716 faba bean plants (1- *Rhi.*, 2-*B.*, 3-*Tri.*, 4-*Rhi.*,+*B.*, 5- *Rhi.*, +*Tri.*, 6-*B.*, +*Tri.*, 7-*Rhi.*,+*B.*,+*Tri.*, 8-control).

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تأثير بعض كائنات التربة الدقيقة على النمو و المحتوى النيتروچينى لأصناف الفول البلدى

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الملخص العربى

أجريت عدوى التربة الغير معقمة ببكائنات رايزوبيم لجيومينوزارم, باسيلس ميجاتيريم فوسفاتيكم بتركيز ١×١٠^٨ وحدة /مل والفطر ترايكودرما هارزيانم من وزن التربة بمعدل ٣% منفردة أو مشتركة. زرعت بذور الفول البلدى أصناف سخا ١ , جيزة ٣ محسن, جيزة ٧١٦ بعد إجراء التعقيم السطحى لها. قللت المعاملات المختلفة الفترة اللازمة لإنبات البذور فى حين زادت النسبة المئوية للإنبات و كذلك النباتات الحية و بقياس صفات النمو الخضرى لنبات الفول مثل إرتفاع النبات, عدد الأوراق, طول النبات, الوزن الغض و الوزن الجاف لجميع الأصناف المنزرعة؛ وجد أنها إزدادت كإستجابة لإضافة الكائنات الحية الدقيقة للتربة. و قد لوحظ زيادة عدد الأزهار و كذلك العقد البكتيرية المتكونة على نباتات الفول نتيجة لمعاملات التسميد الحيوى. و قد سجلت زيادة معنوية للمحتوى الكلى للنيتروچين فى نباتات الفول المنزرعة فى تربة الأصص المعاملة مقارنة بغير المعاملة.

عند تحليل البروتين وجد أنه فى الصنف سخا ١ أدت المعاملة ببكتيريا رايزوبيم ليجيومينوزارم مع الفطر ترايكودرما هارزيانم غلى إنتاج ١٣ بروتين بأوزان جزيئية مختلفة بينما أدت المعاملة ببكتيريا باسيلس ميجاتيريم إلى أقل نتيجة حيث أنتجت ٨ بروتينات فقط. فى الصنف جيزة ٣ محسن أدت المعاملة بالثلاثة ميكروبات مجتمعة إلى أفضل نتيجة حيث أنتجت ١٢ بروتين بينما أدت المعاملة ببكتيريا باسيلس ميجاتيريم مع فطر ترايكودرما هارزيانم إلى أقل نتيجة حيث أدت إلى إنتاج ٧ بروتينات فقط. فى الصنف جيزة ٧١٦ أدت المعاملة ببكتيريا رايزوبيم ليجيومينوزارم إلى أحسن نتيجة حيث أنتجت ١١ بروتين بينما أدت كل من المعاملات ببكتيريا باسيلس ميجاتيريم منفردة و الثلاثة ميكروبات مجتمعة و كذلك الكنترول إلى أقل نتيجة حيث انتجت ٧ بروتينات فقط لكل منها.