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BIOFERTILIZERS AND THEIR IMPORTANCE IN ENVIRONMENTAL AND SUSTAINABLE AGRICULTURE

Abdallah, A. R.; M. S. A. Safwat; T.M. Moharram; Samia F. M. Ahmed and Heba S. Soliman
Agric. Microbiology Dept., Fac. of Agric., Minia Univ.,El – Minia, Egypt

ABSTRACT

The use of biofertilizers has become a great hope for Egyptian agriculture, particularly in the field of production of medicinal plants, especially when the economic and environmental points of view are considered, since they reduce the environmental pollution and production costs, in addition to improving the quality. Therefore, the influence of using locally isolated nitrogen fixing bacteria as biofertilizers were studied. The isolates used were three representative strains of either *Azotobacter* and *Azospirillum* (two isolates of *Azotobacter* out of 60 isolates, these isolates were *Az.*NP7- *Az.*PR1, and one isolate of *Azospirillum* out of 60 isolates, this isolate was *As.*Pp1) which were screened for their activity in nitrogen fixing and stimulation effect by used root exudates of black-cumin plants and used for further inoculation studies as biofertilizers. These isolates were isolated at random from rhizosphere and rhizoplane of black-cumin, parsley and fenugreek cultivars at the different stages of growth. The effect of inoculation with N_2-fixing bacteria (biofertilizer) on growth plant characteristics of tested medicinal plants (*Nigella sativa*, *Trigonella foenum- graecum* and *Petroselinum sativum*) plants were studied. Inoculation was conjugated with the application of four doses of mineral N, i.e. 0, 50%, 75% and one full dose (100%) from the recommended doses. Results obtained showed that the application of biofertilizers (N_2-fixers) produced better growth and reduced the N requirement in many medicinal plants such as *Nigella sativa*, *Trigonella foenum* and *Petroselinum sativum* compared with untreated plants.

INTRODUCTION

The use of biofertilizers in medicinal plants production, particularly in Egypt, becomes unavoidable to minimize the nonstop addition of high doses of chemical fertilizers in which enormous amounts of deleterious heavy metals and other environmental pollutants might be present, as well as to lower their production costs. Biofertilization is generally based on altering the rhizosphere flora, by seed or soil inoculation with certain organisms (microbial inoculants), capable of inducing beneficial effects on a compatible host (El-Haddad *et al.* 1993).

Nowadays, the evidence on hand prominently indicates the beneficial impacts of microbial inoculants to field crops (Gomaa, 1995). On the other hand, little attention was directed to the nature of the relationship between biofertilizers and medicinal plants (Sharaf, 1995).

In short, the use of biofertilizers has become a great hope for the Egyptian agriculture, particularly in the field of production of medicinal plants, especially when the economical and environmental points of view are considered, since they reduce the environmental pollution and production costs, in addition to improving the quality.

The aim of this research was to study the biological activities of nitrogen fixing bacteria (*Azotobacter* and *Azospirillum*) as biofertilizers in the field of production of some medicinal plants (Black-cumin, Fenugreek and Parsley).

MATERIALS AND METHODS

I- Microorganisms and media:

I-1. Bacterial isolation:

The bacteria used in this work were isolated from rhizosphere soil and rhizoplane of some medicinal plants. Sixty *Azotobacter* and 60 *Azospirillum* isolates were picked up from the suitable two media used for *Azotobacter* and *Azospirillum* counting. All *Azotobacter* and *Azospirillum* isolates were purified by successive streaking on nitrogen deficient modified Ashby and Döbereiner media respectively, using the techniques adopted by Abd El-Malek and Ishac (1968) for *Azotobacter* and Döbereiner *et al* (1976) for *Azospirillum.*

I-2 . Selection of isolates:

Six isolates of *Azotobacter* and 6 isolates of *Azospirillum* were screened for their activity in nitrogen fixation according to the method described by Postage (1972). The root exudates were studied for their effect on these isolates of both *Azotobacter* and *Azospirillum* according to the method described by (Thornberry, 1950 and Jain and Kar, 1971). The most active in N_2-fixation and stimulation effect by used root exudates of tested medicinal plants were selected.

I-3 Preparation of root exudates:

Seeds of the three tested plants, *Nigella, Petroselinum* and *Trigonella* were surface sterilized by 0.1% mercuric chloride for 3 minutes; then washed three times with sterilized distilled water (Prikryl and Vancura, 1980). Tubes (25x 3.5 cm) contained 50 g glass beads of 0.5 cm diameter to reach 6 cm high, as well as 30 ml of distilled water were sterilized by autoclaving at 121 ºC for 1 hr. Ten sterilized seeds of each of *Nigella, Petroselinum* and *Trigonella* were aseptically emerged in each tube. The tubes were covered with aluminum foil paper 6 cm from bottom to avoid light effect. Seeds of each plant were emerged in 9 tubes (3 tubes for each interval) and were incubated at room temperature (28-30 ºC) exposed to normal daylight.

Water was brought back to 30 ml by daily addition of sterilized distilled water. Root exudates were collected at intervals of 1, 2 and 3 weeks from emergence. The 30-ml of root exudates at the defined time were considered as crude root exudates of the medicinal plants under investigation. The crude root exudates were diluted with sterilized distilled water to give 1:1 and 1: 2 dilutions.

The effect of crude and diluted root exudates on twelve active strains of either Azotobacter or Azospirillum was studied.

Filter paper disks (Whatman No. 1.9 mm diameter) containing aliquots of 50 µl of the plant exudates solution were applied to the surface of agar plates which were previously inoculated with standard amount of 48 hr old culture of the tested microorganisms (Thornberry, 1950 and Jain and Kar, 1971). The plates were kept in a refrigerator at 4ºC for 4 hr. to permit the

diffusion of the exudates in the agar, before organisms were sufficiently dense to allow for accurate measurement of the inhibition zone. These plates were then incubated at 28 ºC and the diameter of the inhibition or stimulation zone (mm) was recorded after 24-48 hr. Sterilized distilled water was used instead of exudates of medicinal plant as a control.

II- Fertilizers used:

II-1 Organic fertilization:-

The compost used in this study was provided from the Egyptian Company for Agricultural Residues (ECAR) in Minia .

II-2 Mineral fertilization:-

Nitrogen source was provided as a commercial urea fertilizer (46 % N).

III- Cultivars:

The seeds of tested plants**;** *Nigella sativa* (Giza 1), *Trigonella foenum-graecum* (Giza 2), and *Petroselinum sativum* (cv. Balady) were obtained from Fac. of Agric., Minia University.

IV- Effect of inoculation with biofertilizers and/or chemical fertilizers on growth of tested medicinal plants:

A field experiment was conducted on a clay loam soil in the experimental farm of the Faculty of Agriculture, Minia University. This experiment was designed to study the effect of inoculation with the efficient strains of *Azotobacter* and *Azospirillum* (Biofertilizer) on growth plant characteristics of tested medicinal plants. Inoculation was conjugated with the application of four doses of mineral N, i.e. 0, 50%, 75% and full dose (100%) from the recommended dose. The treatments were arranged in complete randomized design with three replications (the whole area was 42m². for each) . Each of the above mentioned dose of mineral nitrogen (urea fertilizer, 46% N) was applied in two equal parts after 15 and 45 days of seed cultivation, except, control soil (untreated with biofertilizer). Also, all plots treated or untreated with mineral fertilizer were supplemented with organic matter (at the rate of 10 ton/fed.). Each of the two isolates of *Azotobacter* and one isolate of *Azospirillum* was separately grown in modified Ashby's medium for *Azotobacter* and semi-solid medium for *Azospirillum* respectively, for 7 days at 30°C, with mild shaking (200 rpm). At cultivation, *Nigella, Trigonella* and *Petroselinum* seeds were successively washed and then inoculated with biofertilizer for 30 minutes. The treated or untreated seeds were sowed in the soil (treated or untreated with mineral fertilizer). The plots were irrigated constantly through the growth period of the plants.Morphological characteristics of medicinal plants were determined after (90 days) of seed cultivation.

The treatments were as follows:

1- Uninoculated + full dose of mineral N-fertilizer (100 kg N/fed).
2- Inoculated with *Azotobacter* and *Azospirillum* either singly or in mixture without mineral N-fertilizer.
3- Inoculated with *Azotobacter* and *Azospirillum* either singly or in mixture + half dose of mineral N-fertilizer (50 kg N/fed).
4- Inoculated with *Azotobacter* and *Azospirillum* either singly or in mixture + three quarter dose of mineral N-fertilizer (75 kg N/fed).
5- Inoculated with *Azotobacter* and *Azospirillum* either singly or in mixture + full dose of mineral N-fertilizer (100 kg N/fed).

Chemical determinations: Total nitrogen content of plant parts was performed according to Jackson (1958).

IV-1 Plant measurements:

(a) Shoot length (cm/ plant). (b) Fresh weight (g/ plant).

(c) Dry weight of whole plant (g/ plant) was recorded after oven drying at 70ºC.until reaching a constant weight, Black *et al.* (1965).

(d) Weight of seeds per plant (g/ plant).

Data of field experiments were statistically analyzed according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

I- Selection of the most efficient N_2-fixing bacterial isolates:

Evaluation of the biological activity of nitrogen fixing bacteria towards their efficiency for nitrogen fixation was performed by estimating the nitrogenase activity using the acetylene reduction technique which is a good indicator reflecting the activity of nitrogen assimilation. Results shown in Table (1) indicate that the production of C_2H_4 (ethylene) by the sixty *Azotobacter* isolates ranged from 1.57 to 92.14 nanomoles C_2H_4 ml^{-1} hr^{-1}, while the sixty isolates of *Azospirillum* produced 3.14 to 53.21 nanomoles C_2H_4 $ml^{-1}hr^{-1}$. The most efficient six isolates of *Azotobacter* were found to be *Az.*NR5, *Az*.NP7, *Az*.TR5, *Az*.TP3, *Az*.PR1 and *Az*.PP3, while those of *Azospirillum* were *As*.NR2, *As*.NP6, *As*.TR6, *As*.TP8, *As*.PR5 and *As.*Pp1. The most active strains in N_2-fixation were selected. The importance of *Azospirillum* as an active nitrogen fixer was reported by many investigators (Döbereiner and Day, 1976; Stewart, 1976; Youshida, 1976 and El- Tayeb**,** 2000).

II- Effect of medicinal plants root exudates on *Azotobacter* and *Azospirillum* isolates :

The results in Table (2) indicated that the influence of root exudates varied according to the dilution factor and the age of plant. Regardless, the dilution factor and the age of plant, *Nigella sativa* and *Trigonella foenum* root exudates stimulated the growth of *Azotobacter* isolate (*Az.*NP7). The stimulative effect of *Nigella* root exudates was more pronounced than that of *Trigonella*. However, *Trigonella* and *Petroselinum* stimulated the growth of *Azotobacter* isolate(*Az.*PR1).On the other hand, *Petroselinum* root exudates inhibited the growth of all the tested *Azotobacter* isolates except, *Azotobacter* isolate (*Az.*PR1) which was stimulated by *Petroselinum* root exudates. These results indicated that, *Petroselinum* root exudates might content inhibitory compounds depressing the growth of *Azotobacter* isolates, while, *Trigonella* root exudates stimulated the growth of most tested isolates. The stimulative effect of *Trigonella* root exudates was more pronounced in case *Azotobacter* isolate (*Az.*PR1) and such effect was related to the nutrient contents of *Trigonella* root exudates. Moreover**,** *Nigella, Trigonella* and *Petroselinum* root exudates stimulated the growth of the tested *Azospirillum* isolate (*As*.PP_1). The stimulative effect of *Petroselinum* root exudates on *Azospirillum* isolate (*As*.PP_1) was more pronounced than that of *Nigella* and *Trigonella*.

Table (1): N-ase activity (nanomol C_2H_4 $ml^{-1}hr^{-1}$) of *Azotobacter* and *Azospirillum* isolates.

Isolates No.	N-ase activity	Isolates No.	N-ase activity	Isolates No.	N-ase activity	Isolates No.	N-ase activity
Strains of *Azotobacter* isolated from				Strains of *Azospirillum* isolated from			
Nigella sativa							
Rhizosphere		Rhizoplane		Rhizosphere		Rhizoplane	
Az.NR1	9.29	*Az*.NP1	3.93	*As*.NR1	12.14	*As*.NP1	22.14
Az.NR2	3.93	*Az*.NP2	4.07	*As*.NR2	30.78*	*As*.NP2	8.21
Az.NR3	11.54	*Az*.NP3	2.71	*As*.NR3	7.92	*As*.NP3	20.0
Az.NR4	4.79	*Az*.NP4	5.18	*As*.NR4	6.07	*As*.NP4	15.21
Az.NR5	23.17*	*Az*.NP5	2.68	*As*.NR5	4.71	*As*.NP5	15.64
Az.NR6	6.21	*Az*.NP6	2.0	*As*.NR6	11.78	*As*.NP6	53.21*
Az.NR7	4.18	*Az*.NP7	27.80*	*As*.NR7	3.85	*As*.NP7	8.14
Az.NR8	4.29	*Az*.NP8	10.0	*As*.NR8	21.0	*As*.NP8	6.71
Az.NR9	12.86	*Az*.NP9	15.93	*As*.NR9	7.35	*As*.NP9	3.78
Az.NR10	18.57	*Az*.NP10	14.64	*As*.NR10	5.42	*As*.NP10	24.92
Trigonella foenum							
Az.TR1	5.43	*Az*.TP1	1.57	*As*.TR1	7.85	*As*.TP1	8.21
Az.TR2	8.25	*Az*.TP2	2.64	*As*.TR2	10.85	*As*.TP2	4.78
Az.TR3	2.68	*Az*.TP3	92.14*	*As*.TR3	10.35	*As*.TP3	15.21
Az.TR4	13.57	*Az*.TP4	18.32	*As*.TR4	21.35	*As*.TP4	8.28
Az.TR5	42.5*	*Az*.TP5	7.32	*As*.TR5	27.85	*As*.TP5	9.07
Az.TR6	19.0	*Az*.TP6	4.18	*As*.TR6	30.28*	*As*.TP6	5.07
Az.TR7	1.86	*Az*.TP7	2.68	*As*.TR7	21.0	*As*.TP7	3.5
Az.TR8	3.71	*Az*.TP8	4.07	*As*.TR8	11.78	*As*.TP8	23.64*
Az.TR9	14.86	*Az*.TP9	21.5	*As*.TR9	3.28	*As*.TP9	15.64
Az.TR10	21.5	*Az*.TP10	4.29	*As*.TR10	4.07	*As*.TP10	8.42
Petroselinum sativum							
Az.PR1	60.86*	*Az*.PP1	5.43	*As*.PR1	11.78	*As*.PP1	47.21*
Az.PR2	3.71	*Az*.PP2	4.79	*As*.PR2	7.35	*As*.PP2	14.5
Az.PR3	3.93	*Az*.PP3	22.54*	*As*.PR3	4.18	*As*.PP3	5.42
Az.PR4	10.35	*Az*.PP4	7.96	*As*.PR4	3.14	*As*.PP4	3.28
Az.PR5	4.71	*Az*.PP5	1.93	*As*.PR5	24.29*	*As*.PP5	8.57
Az.PR6	6.07	*Az*.PP6	4.18	*As*.PR6	18.57	*As*.PP6	20.0
Az.PR7	3.78	*Az*.PP7	4.64	*As*.PR7	14.64	*As*.PP7	12.14
Az.PR8	15.64	*Az*.PP8	12.5	*As*.PR8	5.18	*As*.PP8	7.92
Az.PR9	8.28	*Az*.PP9	4.71	*As*.PR9	4.00	*As*.PP9	22.14
Az.PR10	12.14	*Az*.PP10	1.57	*As*.PR10	15.79	*As*.PP10	20.71

***= Most efficient isolates.**

Results indicated that, *Trigonella* root exudates stimulated the growth of most tested *Azospirillum* isolates. The stimulative effect of *Trigonella* root exudates was more pronounced than that of inhibitive effect on *Azospirillum* isolates. Such effect was related to the nutrient contents of *Trigonella* root exudates.These results are in agreement with the findings of El-sharouny (2007) who observed that, high density of *Azotobacter* and *Azospirillum* in the rhizosphere soil plants could be attributed to the stimulative effect of root exudates on the studied bacteria. Moreover, Hob-Allah (1999) studied the influence of medicinal plants root exudates on *Mycobacterium phelii* (acid-fast organism) and he found that, *M. phelii* was stimulated by *Nigella* and *Trigonella* root exudates except few cases in the first and second growth week of *Nigella* and *Trigonella* respectively, crude and 1:1 dilution fold of root exudates inhibited the growth of *M. phelii*. The inhibitory effect at these ages

of plant growth was due to the phenolic compounds content in the exudates and the dilution factor.

III- Effect of mineral nitrogen and biofertilizers as well as combination between them, on growth plant characteristic of tested medicinal plants:-

Data presented in Table (3), show the influence of *Azotobacter* and/or *Azospirillum* on the vegetative growth, yield and N% or content of tested medicinal plants.

Results indicated that a positive effect of inoculation by *Azotobacter* and/or *Azospirillum* isolates (*Az.*NP7– *Az.*PR1- *As*.Pp1) on the plant height, fresh weight, dry weight and N % or content of tested medicinal plants compared with uninoculated control. The increase in plant height of *Nigella sativa* was more pronounced at the *Azospirillum* isolate used in inoculation of the tested plants. Regardless of the type of isolate, inoculation with *Azotobacter* and/or *Azospirillum* increased plant growth and N% or content. Data also showed that the highest densities of plant growth and N% or content were found in *Nigella sativa* plants inoculated with the active isolates of *Azotobacter* and/or *Azospirillum* (*Az.*NP7-*As.*Pp1), respectively in the presence of one half of the normal dose (50 kg/fed) of N-fertilizer. These data show that one half of the normal field rate of added mineral N-fertilizer (50 kg/fed) can be saved by seed inoculation with asymbiotic N_2-fixers. Moreover; the results indicated that, inoculation of *Trigonella foenum* seeds with *Azotobacter* and/or *Azospirillum* isolates (*Az*.PR1-*As*.Pp1) led to an improvement in the growth parameters and N% or content, respectively without mineral N-fertilizers as compared with the other fertilizer treatments. On the other hand, the results indicated that inoculation of *Petroselinum sativum* seeds with *Azotobacter* and/or *Azospirillum* isolates (*Az*.PR1-*As*.Pp1) in the presence of full dose of mineral N-fertilizer led to an improvement in the plant growth and N% or content. Data also showed that the highest densities of plant growth were found in plant inoculated with a mixture of *Azotobacter* and *Azospirillum* combined with a full dose of recommended mineral N-fertilizer as compared with the other treatments. These results are in agreement with the finding of Kandeel *et al.,* (2002) who found that dual inoculation with *Azotobacter* and *Azospirillum* combined with a half or a full dose of recommended mineral N-fertilizer significantly led to increments in plant height, branch number per plant, as well as fresh and dry weight of herb of *Ocimum basilicum* plants. In addition, Surendra *et al.*, (2002) found that total N uptake of *Coriandrum sativum* plants was promoted due to inoculation of the seeds with *Azotobacter* and *Azospirillum* either singly or in combination compared with uninoculated plants. While, Abdou and El-Sayed (2002) observed that N% in the leaves of *Carum carvi* plants was higher due to inoculation with *Azotobacter* and *Azospirillum* than those of untreated plants. Moreover, Sayed (2004) reported that biofertilization with *Azotobacter chroococcum* and *Azospirillum brasilense* bacteria resulted in higher percent and content of N in the leaves and corms of *Gladiolus grandiflorus* cv. Eurovision plants in comparison with uninoculated treatments.

Table(2): Effect of medicinal plants root exudates on growth of the selected isolates of *Azotobacter* and *Azospirillum*(zone in mm)

Experimental plants	Root exudates dilution	*Azotobacter* isolates																	
		Az.N_{P7}			*Az.*N_{R5}			*Az.*T_{P3}			*Az.*T_{R5}			*Az.*P_{P3}			*Az.*P_{R1}		
		Weeks			Weeks			Weeks			Weeks			Weeks			Weeks		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Nigella sativa	Crude	+9	+11	+8	-14	-22	-15	-7	-8	-9	+10	-10	-9	-15	-16	-19	-7	-19	-14
	1:1	+8	+11	+8	-13	-15	-14	0	-8	-7	+8	-10	-9	-11	-13	-15	0	-9	-14
	1:2	+7	+11	+7	-9	-14	-10	0	0	-7	0	-7	-7	-9	-11	-10	0	0	-12
Trigonella foenum	Crude	+8	+8	+7	-7	-10	-8	+8	+7	+7	+7	+8	+8	-7	-7	-9	+11	+10	+9
	1:1	+8	+7	0	0	-8	0	+8	+7	0	+7	+7	+7	-7	0	-7	+9	+8	+8
	1:2	0	0	0	0	-8	0	0	0	0	0	+7	0	0	0	-7	+8	+8	+8
Petroselinum sativum	Crude	-10	-10	-10	-22	-21	-37	-9	-8	-8	-11	-11	-10	-15	-22	-25	+12	+9	+11
	1:1	-10	-7	-7	-20	-21	-36	-9	-7	-7	-10	-11	-7	-15	-22	-19	+12	+7	+10
	1:2	-8	-7	0	-19	-20	-34	-7	0	0	-7	-9	0	-11	-21	-18	+8	+7	+10
		Azospirillum isolates																	
		As.N_{P6}			*As.*N_{R2}			*As.*T_{P8}			*As.*T_{R6}			*As.*P_{P1}			*As.*P_{R5}		
Nigella sativa	Crude	+8	+7	+7	-7	-7	-8	-10	-15	-15	+7	-7	0	+9	+8	+8	-20	-26	-25
	1:1	+7	0	0	0	-7	-8	-10	-14	-10	+7	0	0	+8	+8	+8	-18	-25	-24
	1:2	0	0	0	0	0	-7	-9	-11	-9	+7	0	0	+8	+8	+7	-13	-25	-24
Trigonella foenum	Crude	+8	+7	+7	+8	+8	+7	+7	-9	-10	+7	+8	+7	+9	+9	+8	-20	-15	-9
	1:1	+7	+7	0	+8	+8	0	0	-9	-7	+7	+7	+7	+8	+8	+7	-12	-9	-8
	1:2	+7	0	0	+7	0	0	0	0	-7	0	0	0	+8	+8	0	-8	-8	0
Petroselinum sativum	Crude	-8	-7	-7	+8	+7	0	-17	-19	-16	-8	-7	-7	+12	+9	+15	+7	0	0
	1:1	-7	0	0	0	0	0	-10	-8	-8	-7	0	0	+11	+8	+12	0	0	0
	1:2	0	0	0	0	0	0	-9	0	-8	-7	0	0	+9	+7	+9	0	0	0

+ = Stimulation zone diameter (mm).
- = Inhibition zone diameter (mm).

Table (3): Effect of mineral nitrogen and biofertilizers as well as combination between them, on plant heights, fresh and dry weight, N% and uptake (mg/plant) in tested medicinal plants after 90 days of seed cultivation.

Fertilization treatments	*Nigella sativa*					*Trigonella foenum*					*Petroselinum sativum*				
	Determination														
	P.h. (cm.)	P.F.W (g/p)	PDW (g/p)	N%	N-uptake (mg/p)	P.h. (cm.)	P.F.W (g/p)	PDW (g/p)	N%	N-uptake (mg/p)	P.h. (cm.)	P.F.W (g/p)	PDW (g/p)	N%	N-uptake (mg/p)
Control (Uninoculate)	**27.00**	**1.07**	**0.17**	**3.03**	**5.15**	**26.33**	**0.94**	**0.10**	**3.73**	**3.73**	**34.67**	**3.06**	**0.52**	**3.17**	**16.48**
Plant inoculated with															
Azotobacter	28.00	1.67	0.28	3.50	9.80	32.50	1.17	0.17	6.07	10.32	22.17	0.89	0.18	2.99	5.38
Az + 50% N	30.80	2.35	0.47	4.43	20.82	30.30	1.12	0.16	4.20	6.72	27.67	1.44	0.31	2.61	8.09
Az.+ 75% N	30.60	1.37	0.26	3.03	7.88	29.33	1.06	0.13	3.97	5.16	26.67	2.26	0.49	2.99	14.65
Az.+ 100% N	31.00	1.55	0.27	3.03	8.18	29.17	1.00	0.12	3.73	4.48	35.50	3.62	0.61	3.17	19.34
Azospirillum	30.67	1.62	0.28	3.73	10.44	34.70	1.31	0.18	7.47	13.45	23.33	1.03	0.19	3.08	5.85
As.+ 50% N	32.67	2.27	0.46	4.20	19.32	32.67	1.11	0.17	5.60	9.52	23.67	1.23	0.22	2.8	6.16
As.+ 75% N	32.07	1.81	0.32	3.73	11.94	30.17	1.08	0.15	3.97	5.96	27.50	2.51	0.46	3.08	14.17
As.+ 100% N	32.00	1.54	0.29	3.27	9.48	28.00	1.00	0.13	3.03	3.94	35.17	3.13	0.55	3.55	19.53
Mixture	28.00	0.96	0.16	3.27	5.23	30.33	1.23	0.20	6.07	12.14	22.33	0.88	0.15	2.70	4.05
Mix.+ 50% N	31.67	1.88	0.38	4.20	15.96	27.67	1.17	0.20	4.90	9.8	21.50	1.19	0.27	2.94	7.94
Mix.+ 75% N	30.00	1.38	0.23	3.50	8.05	29.50	1.06	0.18	4.20	7.56	29.83	3.08	0.54	2.89	15.61
Mix+100%N	29.67	1.13	0.21	3.27	6.87	29.83	1.07	0.17	4.20	7.14	36.17	4.37	0.78	3.89	30.34
$LSD_{0.05}$	3.772	0.649	0.12	0.83	4.64	4.52	0.272	0.053	0.91	2.77	6.005	1.436	0.261	0.31	8.37

P.h. = Plant height. PFW = Plant fresh weight. PDW = Plant dry weight. Control = Uninoculated + full dose of mineral N-fertilizer. Mixture = *Azotobacter* +*Azospirillum* (1:1).

The effect of inoculation on numbers and weight of nodules recorded in *Trigonella foenum* plants are presented in Table (4).The results indicated that the highest number of nodules/plant generally was found on the root of plants inoculated with *Azotobacter* and/or *Azospirillum,* respectively without mineral N-fertilizer as compared with the other fertilizer treatments. The increase in application of mineral N-fertilizer in the soil, resulted in a decrease in the number of the formed nodules.

The observed decrease may be due to the inhibitory effect of the large amount of applied mineral nitrogen on the root-hair infection by rhizobia, Mohamed (1985) found that nitrogenase activity of nodules was stimulated by low and inhibited by high rates of ammonium nitrate.

These results are in accordance with those obtained by Cowie *et al.*, (1990), Nicoloso and Santos(1990) and Rai (1992) who reported that the number and dry weight of nodules decreased by increasing nitrogen rates, applied on mung bean.

Table (4): Effect of mineral nitrogen and biofertilizers as well as combination between them, on nodules number, fresh weight and nodules dry weight (g/plant) in fenugreek plants after 90 days of seed cultivation.

Fertilization treatments	Determination		
	NN/P	N.F.W/P (g)	NDW/P (g)
Control (Uninoculate)	48.6	0.69	0.08
Plant inoculated with			
Azotobacter	63.2	0.86	0.14
Az + 50% N	59.9	0.85	0.11
Az.+ 75% N	53.6	0.82	0.11
Az.+ 100% N	51.5	0.78	0.11
Azospirillum	61.9	0.94	0.15
As.+ 50% N	58.7	0.92	0.13
As.+ 75% N	57.8	0.91	0.13
As.+ 100% N	56.5	0.89	0.12
Mixture	64.5	1.12	0.17
Mix.+ 50% N	62.9	0.97	0.15
Mix.+ 75% N	60.7	0.91	0.14
Mix+100%N	58.2	0.90	0.11
$LSD_{0.05}$	3.8	0.119	0.02

NN/P = Nodules No. /plant. NFW/P = Nodules fresh weight/plant.
NDW/P = Nodules dry weight/plant.
Control = Uninoculated + full dose of mineral N-fertilizer.
Mixture = *Azotobacter* +*Azospirillum* (1:1).

Generally, inoculation of *Trigonella* and *Nigella* seeds with *Azotobacter* and/or *Azospirillum* led to an improvement in the yield. The increased yield after inoculation may be due to increasing the biological N_2-fixation or by production of phytohormones or both. These results are in agreement with the findings of Ghosh and Mohiuddin (2000) obtained an improvement in yield components i.e. number of capsules/plant, number of seed/capsule, 1000-seeds weight and seed yield of *Sesamum indicum* plant due to biofertilizers. Moreover, Kenawy (2005) found that, inoculation with *Azotobacter chroococcum* and *Azospirillum lipoferum* increased yield

components including number and fresh weight of fruits/ plant, sepals fresh weight/plant, as well as sepals dry weight per plant and per feddan and seed yield/plant and per feddan of *Hibiscus sabdriffa* plants in comparison with uninoculated plants.

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الأسمدة الحيوية وأهميتها للبيئة وتدعيم الزراعة

أحمد رفعت عبد الله ، محمد سعيد علي صفوت، طريف محمد محرم ، سامية فرحات وهبه سليمان

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنيا – المنيا – مصر

أجريت هذه التجربة بهدف دراسة إمكانية التطبيق العملي لاستخدام البكتريا المثبتة للنتروجين كأسمدة حيوية وإمكان إحلالها محل الأسمدة النتروجينيه المعدنية أو تقليل معدل إضافتها وتأثيرها على معدلات النمو النباتية للنباتات المختبرة والمشتملة على كل من نبات حبة البركة والحلبة والبقدونس. وقد تم عزل البكتريا من رايزوسفير ورايزوبلان بعض النباتات الطبية. وبينت النتائج أن عزلات البكتريا المثبتة للنتروجين والممثلة في (الأزوتوباكتر والأزوسبيريللام) قد أظهرت نشاطا في قدرتها علي تثبيت النتروجين معمليا ولكن بدرجات مختلفة. وقد تم انتخاب العزلات وفقا لكفاءتها من حيث تثبيت النتروجين وكذلك حدوث تنشيط لها بواسطة استخدام إفرازات جذور كلا من نبات حبة البركة والحلبة والبقدونس معمليا. وقد تم استخدام العزلات المنتخبة والممثلة في (عزلتين من الازوتوباكتر وهى *Az*.NP7 خاصة لتلقيح نبات حبة البركة، *Az*.PR1 خاصة لتلقيح كلا من نبات الحلبة والبقدونس وعزلة أزوسبيريللام وهى *As*.PP1) كسماد حيوي. اقترن التلقيح بالأسمدة الحيوية بأربع مستويات من السماد المعدني النتروجينى وتم تقدير العينات بعد ٩٠ يوما من الزراعة. وقد أوضحت النتائج أن تلقيح نبات حبة البركة بمثبتات النتروجين في وجود نصف كمية السماد الأزوتى الكيماوي أدت إلى زيادة طول النبات ، والوزن الطازج والجاف ، والنسبة المئوية للنتروجين ، والنتروجين الكلى ، علاوة علي ذلك أدى تلقيح نبات الحلبة بمثبتات النتروجين إلي زيادة معدلات النمو النباتية خاصة في حالة عدم وجود أسمدة كيماوية علي الجانب الأخر أثبتت النتائج أن تلقيح نبات البقدونس بمثبتات النتروجين أدى إلي زيادة معدلات النمو النباتية ولكن في وجود إضافة كاملة للسماد المعدني .

لذا تقترح هذه الدراسة استخدام هذه البكتريا كأسمدة حيوية في إنتاج النباتات الطبية.