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EFFECT OF SEEDLING INOCULATION WITH SOME ASYMBIOTIC N_2-FIXERS ON .0THE GROWTH OF BASIL PLANT (*Ocimum basilicum*) AND ITS ACTIVE CONSTITUENTS.

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ABSTRACT

The effect of seedling inoculation after 2 months of cultivation with asymbiotic N_2-fixers only, or asymbiotic N_2-fixers and either organic fertilizer, or with half normal dose of inorganic N-fertilizer on the growth of Basil plant (*Ocimum basilicum*) and active constituents (volatile oils) were investigated. The experiment was done during the season in the field of experiments in sandy farm soil of Applied Research Center of Medicinal Plants (ARCMP) related to the National Organization for Drug Control and Research (NODCAR). .

The asymbiotic N_2-fixing bacteria used were *Azotobacter chroococcum* and *Azospirillum lipoferum* which were isolated from the rhizosphere of Basil plants. The highest densities of *Azotobacter chroococcum* and *Azospirillum lipoferum* were found in the rhizosphere of Basil plants, which were inoculated with the active local strains of *Azotobacter* and *Azospirillum* respectively in the presence organic fertilizer after four months of cultivation. .

Data also showed that the growth of Basil plants and their active constituents were positively influenced by seedling inoculation with the asymbiotic N_2-fixers with organic fertilizer, the highest growth of fresh weight of plant were 2039.5g plant $^{-1}$ and the highest amount of volatile oil being 0.73 ml/100g fresh weight of plant. On the other hand, the un-inoculated controls gave 1417.0g fresh weight plant^{-1} and 0.38ml volatile oil/100gm fresh weight of plant after 6 months of cultivation

INTRODUCTION

A series of comprehensive experiments for inoculation with asymbiotic N_2-fixers with different plants were carried out by many investigators. It was clearly showed that inoculation with *Azotobacter* (Lavshman, 1982, Ishac *et al*., 1984a, Pareek, *et al*., 1996, and Kandil *et al.*, 2002) or *Azospirillum* (Dobereiner *et al.*, 1976, Okon, 1982; Chezhiyan, *et al.*, 2003; Shaalan, 2005 and Lakshmanan, *et al*., 2005;) led to a considerable improvement in the plant growth and its constituents as well as the reduction in the costs of the agricultural production, by reducing the amount of inorganic nitrogen fertilizers through the enhancement of asymbiotic N_2-fixation.

Therefore, the present investigation was carried out to evaluate the effect of seedling inoculation with the local selected strains of *Azotobacter chroococcum* and/or *Azospirillum lipoferum* on the growth of the medicinal plant [(Basil plant) *Ocimum basilica.*

In addition, the effect of application of the asymbiotic N_2-fixers with organic fertilizer and the asymbiotic N_2-fixers with inorganic N-fertilizer, in sandy soil, was also considered.

MATERIAL AND METHODS

A field experiment was carried out using the sandy soil farm of (ARCMP) related to (NODCAR

Data of the mechanical, physical and chemical analyses of the used sandy soil are given in Table (1) super calcium phosphate (15.5% P_2O_5) was added to the soil before cultivation with rate of 100kg feddan^{-1}

Table (1): Some mechanical and physico-chemical analyses of the soil used in the experiment from (ARCMP)..

Mechanical analyses			Physico-chemical characteristics				
Sand	84.32%		**WHC%***	10.6		**Ca^{++}**	0.31
			pH	8.7		**Mg^{++}**	0.54
Silt	12.51%		**organic carbon**	20.40%		**Na^{+}**	0.25
						K^{+}	0.1
Clay	3.17%		**total nitrogen**	1.80%		**Co_3^{-2}**	0.8
			C/N ratio	11:33		**HCO_3^{-}**	0.2
Soil texture	Sand		**E.C.** mmhos/cm**	0.11		**Cl^{-}**	0.4
						So_4^{-2}	0.5

WHC%* : Water holding capacity.
E.C.mmhos/cm :Electrical conductivity.**

Organic fertilizer (40m^3 feddan^{-1}) was added to a part of field experiment before cultivation. .

Inorganic N-fertilizer (ammonium nitrate, 33.5% N) was added at half normal dose to the field experiment at the rate 50kg feddan^{-1}, after 2 months from cultivation. The experiment contained nine treatments bio-fertilizer only, *Azotobacter* and/or *Azospirillum*+organic and *Azotobacter* and/or *Azospirillum*+ half N dose of inorganic (50kg feddan^{-1}). Each treatment contained three replicates, ten hills were in each replicate. Five seeds of Basil plant, (*Ocimum basilicum*), (kindly supplied from [(ARCMP) related to (NODCAR) Giza, Egypt], were planted in each hill. The plants were thinned two months after sowing and one plant per hill was left. .

Preparation of inocula: Efficient local strains of *Azotobacter chroococcum* or *Azospirillum lipoferum* which had been isolated by Saleh *et. al*. (1986) from the rhizosphere of some medicinal plants, and Karthikeyan, et.al., (2007) were used. Heavy cell suspension of each strain was obtained by growing 5 days at 29^{o}C, on Ashby,s and Dobereiner's media for *Azotobacter* and *Azospirillum* respectively. .

Ten gm (10.2ml) of suspension of *Azotobacter* inoculum which contained 5.5X10^7 cells/ml of medium or ten gm (10.2ml) of suspension of *Azospirillum* inoculum, which contained 5.25X10^7 cells/ml of medium. *Azotobacter* and/or *Azospirillum* suspension was mixed with 90 gm of saw

dust, which is used as a carrier. For each plant, put 5gm of mixture was done as biofertilizer, under soil in the rhizospheric area.

Mechanical, physical and chemical analyses:

Mechanical analyses of soil were determinant according to Piper (1950), moisture content and water holding capacity (Black *et.al.*, 1965a), determination of pH and organic carbon (Jackson, 1958), total nitrogen (Black *et.al.*, 1965b), electrical conductivity and total soluble salts (Richards, 1954).

The mean of plant height, main branch diameter, number of branches, leaf width and leaf length of plant materials were measured after 4 and 6 months of cultivation. While, the mean of fresh weight, roots weight and active of constituents plant (volatile oils) were determined after 6 months of cultivation.

Microbiological determinations: microbiological determinations of rhizospheric and non-rhizosphereic soil were determined after 4 and 6 months from cultivation. The six months period covers the plant growth from seedling stage to complete flowering stage

The most probable number (MPN) of *Azotobacter* and *Azospirilla*, in non-rhizospheric soil and rhizosphere of Basil plant, were determined on modified Ashby's medium (Abdel-Malek and Ishac,1968) and semi-solied malate, medium (Dobereiner, 1978) respectively. Estimates of number of organisms by MPN technique were calculated using cochran's tables (Cochran, 1950).

The data were subjected to two way analysis of variance according to Snedecor and Cochran (1967)..

RESULTS AND DISCUSSION

Data presented in Table (2) show that the total microbial count in rhizosphere of Basil plant which were planted in (ARCMP) farm being 119.33 X 106 cells/g dry soil, *Azotobacter* count was 16.00 X 10^4 cells/g dry soil, and *Azospirillum* count was 3.66 X 10^4 cells/g dry soil.

Table(2): Densities of microbial and asymbiotic N_2-Fixers in non-rhizospheric soil and Rhizosphere of Basil plant.

(Means of counts per g dry soil)									
Medicinal plants	**Total microbial counts10^6**			***Azotobacter* (x10^4)**			***Azospirillum* (x10^4)**		
	R	**S**	**R/S**	**R**	**S**	**R/S**	**R**	**S**	**R/S**
Basil	119.33	33.66	3.50	16.00	3.33	4.80	3.66	1.33	2.80

R=Rhizosphere samples.
S=soil samples from non-rhizospheric area between hills.
R/S= the ratio between counts organisms in rhizosphere to non-rhizospheric soil.

Data presented in Table (3) show that the highest densities of *Azotobacter* were found in the rhizosphere of plants which were inoculated with *Azotobacter chroococcum* and amended with organic fertilizer, being (78.33X10^4 cells g^{-1} dry soil), after 4 months of cultivation.On the other hand,

the plants inoculated by mixture of *Azotobacter* and *Azospirillum's* inoculum with added organic fertilizer, given ($64.67X10^4$ cell g^{-1} dry soil) after 4 months of cultivation. The plants inoculated with *Azospirillum lipoferum* and fertilized with organic fertilizer, given ($36.16X10^4$ cell g^{-1} dry soil). This was followed in descending by the amended treatments of (*Azotobacter* and/or *Azospirillum* with half normal dose of inorganic N-fertilizer and treatments amended by *Azotobacter* and/or *Azospirillum* only. The lower densities of *Azotobacter* and *Azospirillum* found in rhizosphere of plant controls being ($21.98X10^4$, $7.00X10^4$ cell g^{-1} dry soil) after 4 months of cultivation, respectively.

Table (3): Effect of bioinoculation, organic and inorganic N-fertilizers on asymbiotic N-fixers in rhizospheric and non rhizospheric regions of Basil plant.

Treatments		Microbial count (X 10^4 cells g^{-1} dry soil)											
		Times						Control					
Inoculation	Fertilizer	4			6			4			6		
		R	S	R/S %	R	S	R/S %	R	S	R/S %	R	S	R/S %
Azotobacter+	**Without**	51.50	8.50	6.06	38.67	6.50	5.95	21.98	5.67	3.88	16.83	3.16	5.33
	Organic	78.33	11.17	7.01	58.50	7.83	7.47						
	Inorganic	67.83	10.16	6.68	50.50	6.50	7.77						
Azospirillum+	**Without**	36.33	3.15	11.53	20.67	2.17	9.53	7.00	2.17	3.23	3.00	1.00	3.00
	Organic	36.16	2.67	13.54	28.83	2.00	14.42						
	Inorganic	32.50	2.83	11.48	26.33	2.33	11.30						
Mixture+	**Without**	48.16	5.49	8.77	32.16	3.16	10.18	26.02	6.66	3.91	17.67	4.16	4.25
	Organic	64.67	8.99	7.19	50.66	6.16	8.22						
	Inorganic	59.16	8.00	7.40	38.33	4.67	8.21						

Control: Without nitrogen supplementation or inoculation.
R=Rhizosphere samples of planted hills.
S=soil samples non-rhizospheric soil two between hills.
R/S= the ratio between counts organisms in rhizosphere to soil .
Mixture=Mixed culture of *Azotobacter* and *Azospirillum* strains.

The use of mixture of both *Azotobacter* and *Azospirillum* strains for seedling inoculation gave lower *Azotobacter* and *Azospirillum* densities in rhizosphere of tested plants when compared with the use of *Azotobacter* only.

It was also found that maximum densities of *Azotobacter* noticed after 4 months of cultivation then a decrease in its densities was observed there after. .

As for R/S ratio, it was found that R/S ratios calculated for both organisms were more than 1.0. The maximum R/S ratios were found for *Azotobacters* in sandy soil amended with organic N-fertilizer ($40m^3$ $feddan^{-1}$) and half normal dose of inorganic fertilizer (50kg $feddan^{-1}$) after 6 months of cultivation.

It is clear from the obtained results that inoculation with the selected efficient strains of asymbiotic N_2-fixers increased the densities of *Azotobacters* and *Azospirillum* in non-rhizospheric soil and rhizosphere of Basil plants. The increase continued till after 4 months from cultivation. Such findings confirm those obtained by Hegazi, *et.a*l. (1979). They reported that inoculation of maize plants with *Azospirilla* as well as *Azotobacters*, resulted

in a transitional increase in their densities at early stages of growth and maximal N_2-ase activities were observed during flowering and grain filling.

Supplementing the soil with carbonic organic materials having a wide C/N ratio, resulted also in a marked increase in densities of *Azotobacter* and *Azospirillum* when compared with untreated treatments. This may be due to that organic fertilizer amendment has a fundamental effect on biological, physical and chemical properties of soil which enhanced the free living N_2-fixing bacteria. In this respect, a significant correlation between densities of N_2-fixers and the amount of organic fertilizer added was reported by several investigators (Dobereiner & Day, 1976, and Ishac, *et.al.*,1985

Obtained data also revealed that half normal dose of inorganic N-fertilizer added to the soil with *Azotobacter* and/or *Azospirillum* resulted in a considerable effect on the densities of asymbiotic N_2-fixers in non-rhizospheric soil and rhizosphere of the growing plants. This is in accordance with several reports which showed that concentration of N-fertilizers may be limiting factors exhibits a negative effect on the development of N_2-fixers in various ecosystems (Abrantes, *et.al*.,1975, Dobereiner, 1978 and Reynders & Vlassak, 1979). .

Results in Tables (4, 5, 6, 7, 8, 9, 10 and 11) clearly showed that the plants amended with a mixture of *Azotobacter* and *Azospirillum* inoculum , *Azotobacter* inoculum and *Azospirillum* inoculum by arrangement with organic fertilizer gave the highest data. Results in Table (4)for mean of (plant height, being 76.34, 74.50 and 66.67cm after 6 months of cultivation. The plants amended with inoculation, with the same arrangement before with half normal dose of inorganic N-fertilizer being 66.00, 65.17 and 59.50cm after 6 months of cultivation. The plant treated with inoculation only with the same arrangement before being 63.33, 61.84 and 57.17cm after 6 months of cultivation.

Table (4) : Effect of bacterial inoculation, organic fertilizer and half normal dose of inorganic N-fertilizer on plant height(cm) of Basil plant.

Inoculation	Time in months after cultivation	Control plants	Inoculated plants		
			n1	n2	n3
Azotobacter	4	34.00	46.34	54.00	47.00
	6	40.84	61.84	74.50	65.17
Azospirillum	4	34.00	45.84	49.17	44.50
	6	40.84	57.17	66.67	59.50
Mixture	4	34.00	47.17	55.83	47.50
	6	40.84	63.33	76.34	66.00

L.S.D

	4 months		6 months	
	5%	**1%**	**5%**	**1%**
Inoculation	0.3	0.43	0.32	0.46
Fertilizer	0.66	0.95	0.72	1.04
Interaction	0.93	1.35	1.02	1.47

Control: Without nitrogen supplementation or inoculation.
n1 :Plants amended with asymbiotic N_2-fixers only.
n2:Plants amended with asymbiotic N_2-fixers and organic fertilizer.
n3:Plants amended with asymbiotic N_2-fixers and half normal dose of inorganic N-fertilizer.
Mixture: A mixture of *Azotobacter* and *Azospirillum* strain.

Table (5): Effect of bacterial inoculation, organic fertilizer and half normal dose of inorganic N-fertilizer on main branch diameter (mm) of Basil plant.

Inoculation	Time in months after cultivation	Control plants	Inoculated plants		
			n1	n2	n3
Azotobacter	4	13.50	17.50	21.50	20.50
	6	16.00	23.17	31.00	29.34
Azospirillum	4	13.50	17.34	20.00	19.17
	6	16.00	22.84	28.00	27.00
Mixture	4	13.50	17.84	22.84	20.67
	6	16.00	25.34	35.17	25.50

L.S.D	4 months		6 months	
	5%	1%	5%	1%
Inoculation	0.34	0.5	0.31	0.45
Fertilizer	0.77	1.11	0.69	1
Interaction	1.09	ns	0.98	1.41

Control: Without nitrogen supplementation or inoculation.
n1 :Plants amended with asymbiotic N_2-fixers only.
n2:Plants amended with asymbiotic N_2-fixers and organic fertilizer.
n3:Plants amended with asymbiotic N_2-fixers and half normal dose of inorganic N-fertilizer.
Mixture: A mixture of *Azotobacter* and *Azospirillum* strain.

Table (6): Effect of bacterial inoculation ,organic fertilizer and half normal dose of inorganic N-fertilizer amendment on number of branches of Basil plant.

Inoculation	Time in months after cultivation	Control Plants	Inoculated plants		
			n1	n2	n3
Azotobacter	4	32.00	55.00	65.50	63.50
	6	47.50	75.17	84.34	83.67
Azospirillum	4	32.00	52.50	61.00	59.50
	6	47.50	73.84	81.84	80.17
Mixture	4	32.00	56.50	67.50	64.50
	6	47.50	78.67	85.67	84.50

L.S.D	4 months		6 months	
	5%	1%	5%	1%
Inoculation	0.45	0.64	0.36	0.53
Fertilizer	1	1.44	0.82	1.17
Interaction	Ns	ns	ns	ns

Control: Without nitrogen supplementation or inoculation.
n1 :Plants amended with asymbiotic N_2-fixers only.
n2:Plants amended with asymbiotic N_2-fixers and organic fertilizer.
n3:Plants amended with asymbiotic N_2-fixers and half normal dose of inorganic N-fertilizer.
Mixture: A mixture of *Azotobacter* and *Azospirillum* strain.

Table (7): Effect of bacterial inoculation , organic fertilizer and half normal dose of inorganic N-fertilizer amendment of leaf length (cm) of Basil plant.

Inoculation	Time in months after cultivation	Control Plants	Inoculated plants		
			n1	n2	n3
Azotobacter	4	3.17	5.00	7.33	7.00
	6	6.17	7.50	9.33	9.00
Azospirillum	4	3.17	4.67	6.17	6.00
	6	6.17	7.17	8.17	8.00
Mixture	4	3.17	5.50	7.33	7.33
	6	6.17	7.67	9.67	9.50

L.S.D	4 months		6 months	
	5%	1%	5%	1%
Inoculation	0.21	ns	0.29	ns
Fertilizer	0.47	0.68	0.64	0.93
Interaction	0.67	ns	ns	ns

Control: Without nitrogen supplementation or inoculation.
n1 :Plants amended with asymbiotic N_2-fixers only.
n2:Plants amended with asymbiotic N_2-fixers and organic fertilizer.
n3:Plants amended with asymbiotic N_2-fixers and half normal dose of inorganic N-fertilizer.
Mixture: A mixture of *Azotobacter* and *Azospirillum* strain.

Table (8): Effect of bacterial inoculation, organic fertilizer and half normal dose of inorganic N-fertilizer amendment on leaf width (cm) of Basil plant.

Inoculation	Time in months after cultivation	Control plants	Inoculated plants		
			n1	n2	n3
Azotobacter	4	3.50	3.67	4.33	4.00
	6	4.84	4.33	5.50	5.50
Azospirillum	4	3.50	3.33	4.00	3.67
	6	4.84	4.17	5.33	4.50
Mixture	4	3.50	3.67	4.50	4.34
	6	4.84	4.67	6.33	5.50

	L.S.D			
	4 months		6 months	
	5%	1%	5%	1%
Inoculation	ns	ns	ns	ns
Fertilizer	0.54	0.78	0.54	0.77
Interaction	ns	ns	ns	ns

Control: Without nitrogen supplementation or inoculation.
n1 :Plants amended with asymbiotic N_2-fixers only.
n2:Plants amended with asymbiotic N_2-fixers and organic fertilizer.
n3:Plants amended with asymbiotic N_2-fixers and half normal dose of inorganic N-fertilizer.
Mixture: A mixture of *Azotobacter* and *Azospirillum* strain.

Table (9): Effect of bactereial inoculation, organic fertilizer and half normal dose of inorganic N-fertilizer amendment on fresh weight (g) of Basil plant after 6 months of cultivation.

Inoculation	Control plants	Inoculated plants		
		n1	n2	n3
Azotobacter	1416.17	1841.00	2031.50	2000.67
Azospirillum	1416.17	1818.00	2009.34	1915.33
Mixture	1416.17	1845.00	2039.50	2011.33

	L.S.D	
	5%	1%
Inoculation	1.2	1.72
Fertilizer	2.68	3.85
Interaction	3.79	5.45

Control: Without nitrogen supplementation or inoculation.
n1 :Plants amended with asymbiotic N_2-fixers only.
n2:Plants amended with asymbiotic N_2-fixers and organic fertilizer.
n3:Plants amended with asymbiotic N_2-fixers and half normal dose of inorganic N-fertilizer.
Mixture: A mixture of *Azotobacter* and *Azospirillum* strain.

Table (10): Effect of bacterial inoculation, organic fertilizer and half normal dose of inorganic N-fertilizer amendment on roots weight (g) of Basil plant after 6 months of cultivation.

Inoculation	Control plants	Inoculated plants		
		n1	n2	n3
Azotobacter	252.50	316.83	327.67	308.50
Azospirillum	252.50	304.67	314.50	306.84
Mixture	252.50	323.50	334.84	325.17

	L.S.D	
	5%	1%
Inoculation	1.26	1.81
Fertilizer	2.81	4.05
Interaction	3.98	5.73

Control: Without nitrogen supplementation or inoculation.
n1 :Plants amended with asymbiotic N_2-fixers only.
n2:Plants amended with asymbiotic N_2-fixers and organic fertilizer.
n3:Plants amended with asymbiotic N_2-fixers and half normal dose of inorganic N-fertilizer.
Mixture: A mixture of *Azotobacter* and *Azospirillum* strain.

The lower mean plant height after 6 months observed in the plant control, being 40.84cm..

The highest data presented in Table (5), for the mean of main branch diameter in the plants treated by inoculation on the same arrangement before with organic fertilizer, being 35.17, 31.00 and 28.00mm after 6 months of cultivation, but the lower mean of branch diameter obtained in the plants control, being 16.00mm after the same times.

Table (11): Effect of bactereial inoculation, organic fertilizer and half normal dose of inorganic N-fertilizer amendment on volatile oil of Basil plant after 6 months of cultivation

Inoculation	Control plants	Inoculated plants		
		n1	n2	n3
Azotobacter	0.38	0.63	0.68	0.59
Azospirillum	0.38	0.51	0.63	0.54
Mixture	0.38	0.63	0.73	0.61

L.S.D		
	5%	1%
Inoculation	0.02	ns
Fertilizer	0.05	0.07
Interaction	Ns	ns

Control: Without nitrogen supplementation or inoculation.
n1 :Plants amended with asymbiotic N_2-fixers only.
n2:Plants amended with asymbiotic N_2-fixers and organic fertilizer.
n3:Plants amended with asymbiotic N_2-fixers and half normal dose of inorganic N-ertilizer.
Mixture: A mixture of *Azotobacter* and *Azospirillum* strain.

The highest data showed in Table (6) for the mean of number of branches in the plants amended with inoculation on the same arrangement before with organic fertilizer, being, 85.67, 84.34 and 81.84 branches plant^{-1} after 6 months of cultivation, but the lower mean of branch's number, was plants control, being 47.50 branch after 6 months of cultivation.

The highest data presented in Table (7) for the mean of leaf length in the plants treated with inoculation on the same arrangement before with organic fertilizer being 9.67, 9.33 and 8.17cm after 6 months of cultivation, but the lower mean of leaf length was in plants control, being 6.17cm after 6 months of cultivation. .

The highest data showed in Table (8) for the mean of leaf width in the plants treated by inoculation on the same arrangement before with organic fertilizer, being 6.33, 5.50 and 5.33cm after 6 months of cultivation.

The lower mean of leaf width was in plants control, being 4.84cm after the same time. .

The highest data presented in Table (9) for the mean of fresh weight in the plants amended by inoculation on the same arrangement before with organic fertilizer, being 2039.50 , 2031.50 and 2009.34g plant^{-1} after 6 months of cultivation. The lower data was in plants control, being 1416.17g after the same time. .

The highest data presented in Table (10) for the mean of roots weight in the plants treated with inoculation on the same arrangement before with organic fertilizer, being 334.84, 327.67 and 314.50g plant-1after 6 months of cultivation. The lower data was in plants control, being 252.50g plant^{-1} after 6 months of cultivation

The plant height in Table (4), main branch diameter in Table (5), number of branches in Table (6), leaf length in Table (7), leaf width in Table

(8), fresh weight in Table (9) and roots weight in Table (10) were significantly increased in all treatments under investigation. These results are in accordance with those obtained by Ishac, *et al*., (1984), Kandeel, *et al.,* (2002) and Migahed, *et al*.,(2004).

The highest data showed in Table (11) for the volatile oil in the plants treated with inoculation on the same arrangement before with organic fertilizer, being 0.73, 0.68 and 0.63ml/100g of fresh weight plant^{-1} after 6 months of cultivation.

The lower data obtained in plants control, being 0.38ml/100g of fresh weight plant^{-1} after 6 months of cultivation.

These results are in accordance with those obtained by Kandeel, *et al.,* (2002) and Migahed, *et al*., (2004).

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تأثير تلقيح البادرات بمثبتات النيتروجين الجوى اللاتكافلية على نمو نبات الريحان (أوسيمم بزاليكم) وعلى مكوناته الفعاله

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يهدف البحث لدراسة تأثير تلقيح بادرات نباتات الريحان بمثبتات النيتروجين الجوى اللاتكافلية فقط كمعاملة أولى، او تلقيحها بمثبتات النيتروجين الجوى اللاتكافلية بالاضافة الى الاسمدة العضوية كمعاملة ثانية، أو تلقيحها بمثبتات النيتروجين الجوى بالاضافة الى نصف السماد الكيماوى المقرر لكل فدان كمعاملة ثالثة على نمو النباتات وانتاجها من المادة الفعالة (الزيت العطرى).وهذه الدراسة عبارة عن تجارب حقلية اجريت فى ارض رملية بمرزعة مركز الدراسات التطبيقية لبحوث النباتات الطبية التابع للهيئة القومية للرقابة والبحوث الدوائية وذلك خلال موسم ٢٠٠٢، ٢٠٠٣ .

مثبتات النيتروجين الجوى اللاتكافلية كانت عبارة عن الازوتوباكتر والازوسبيريللم والتى عزلت من ريزوسفير نباتات الريحان ، ثم بعد ذلك لقحت بادرات نباتات الريحان بالسلالات النشطة من الازوتوباكتر و الازوسبيريللم معا بالاضافه الى السماد العضوى (البلدى) بعد الزراعة بشهرين وأوضحت البيانات التى اخذت ان النباتات التى سمدت باللقاح الحيوى بالاضافة الى السماد العضوى أعطت أعلى كثافة عددية من الازوتوباكتر والازوسبيريللم فى ريزوسفير تلك النباتات بعد أربعة أشهر من الزراعة ٠

وقد اعطت اكبركمية من النمو حيث كانت ٢٠٣٩,٥ جرام/ نبات أخضر وكذلك انتاج اكبر كمية من الزيت العطرى حيث كانت٠,٧٣ مل/١٠٠ جرام نبات أخضر اذا ما قورنت بالنباتات التى لم تعامل نهائياً والتى كانت ١٤١٧,٠ جرام/ نبات أخضر، ٠,٣٨ مل/١٠٠ جرام نبات أخضر على التوالى وذلك بعد ٦ أشهر من الزراعة٠