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EFFECT OF SEEDLING INOCULATION WITH SOME ASYMBIOTIC N_2-FIXERS ON GROWTH OF ROSEMARY PLANT (*Rosmarinus officinalis*) 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 its as well as organic N_2-fertilizer, or its with half recommended dose of inorganic N_2-fertilizer on the growth of Rosemary plant (*Rosmarinus officinalis*) and its active constituents (volatile oils) were investigated. These were done in the sandy farm of Applied Research Center of Medicinal Plants (ARCMP) related to the National Organization for Drug Control and Research (NODCAR).

Asymbiotic N_2-fixing bacteria (*Azotobacter* and *Azospirillum*) were isolated from the rhizosphere of Rosemary plants. The highest densities of *Azotobacter* and *Azospirillum* were found in the rhizosphere of Rosemary plants, inoculated by the active local strains of *Azotobacter* and *Azospirillum*, respectively, in the presence of organic N_2-fertilizer after four months of cultivation.

Data also showed that the growth of Rosemary plants and their active constituents were positively influenced by seedling inoculation with asymbiotic N_2-fixers along with organic N_2-fertilizer which gave the highest fresh weight (1912.50g plant^{-1}) and the highest amount of volatile oil (0.63ml/100g fresh weight of plant), when compared with the un-inoculated plants (395.00g fresh weight plant^{-1} and 0.34ml 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. They clearly showed that inoculation with *Azotobacter* led to 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 (Lavshman, 1982; Ishac *et al.*, 1984; 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;).

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 Rosemary plant *Rosmarinus officinalis*. .

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

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 this 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 physio-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	20.40%	Na^{+}	0.25
		carbon		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	sand	E.C.**	0.11	Cl^{-}	0.4
texture		mmhos/cm		SO_4^{-2}	0.5

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

Organic fertilizer (40m^3 feddan^{-1}) was added for three treatments. Inorganic N-fertilizer (calcium ammonium nitrate, 33.5% N) was added at half-normal dose to the field experiment for three treatments 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 fertilizer (50kg feddan^{-1}) each treatment contained three replicates. Ten hills in each replicate were prepared, two seedlings of Rosemary plant (*Rosmarinus officinalis*) [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) and Karthikeyan *et al.* (2007) from the rhizosphere of some medicinal plants, were used. Heavy cell suspensions of each strain were obtained by growing for 5 days at 29°C, on Ashby and Dobereiner's media for *Azotobacter* and *Azospirillum*, respectively. .

Ten grams (10.2 ml) of *Azotobacter's* inoculum-suspension (5.5X10^7 cells/ml of medium) or ten grams (10.2ml) of *Azospirillum's* inoculum-suspension (5.25X10^7 cells/ml of medium). *Azotobacter* and/or *Azospirillum* suspensions were mixed with 90 g of sawdust, which was used as a carrier. For each plant, 5g of mixture was amended as sub-soil biofertilizer, in the rhizosphere area.

Soil mechanical, physical and chemical analyses:

Mechanical analyses (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 means of plant materials (plant height, main branch diameter, number of branches, leaf width and leaf length) were determined after 4 and 6 months of cultivation, while the means of fresh weight, roots weight and active constituents (volatile oils) were determined after 6 months of cultivation (Snedecor and Cochran, 1967).

Microbiological determinations:

Microbiological determinations of rhizosphere and unplanted soils were determined after 2, 4 and 6 months of cultivation. The six months period covered the plant growth from seedling stage to complete flowering stage. .

The most probable number (MPN) of *Azotobacter* and *Azospirillum*, in unplanted soil and rhizosphere of Rosemary plant, were determined on modified Ashby's medium (Abdel-Malek and Ishac,1968) and semi-solid malate medium (Dobereiner, 1978), respectively. Estimated number of organisms was calculated using Cochran's tables (Cochran, 1950).

RESULTS AND DISCUSSION

Data presented in Table (2) shows that the highest densities of *Azotobacter* were recorded in the rhizosphere of plants inoculated by *Azotobacter chroococcum* (81.00×10^4 cells g^{-1} dry soil) with added organic N-fertilizer, after 4 months of cultivation. The plants inoculated with a mixture of *Azotobacter* and *Azospirillum*, with added organic N-fertilizer, gave 84.66×10^4 cell g^{-1} dry soil after 4 months of cultivation. The plants inoculated by *Azospirillum lipoferum* inocu-lum with added organic N-fertilizer, gave 40.50×10^4 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 or Azospirillum* were in rhizosphere of plant controls being (24.66×10^4 and 7.99×10^4 cell g^{-1} dry soil) after 4 months of cultivation, respectively.

Use of mixture inocula of *Azotobacter* and *Azospirillum* for seedling inoculation gave lower *Azotobacter* and *Azospirillum* densities in rhizosphere of tested plants when compared with *Azotobacter* only as an inoculant. .

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

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 *Azotobacter* in sandy soil amended with organic N-fertilizer (40 m^3 $feddan^{-1}$) and half normal dose of inorganic N-fertilizer (50 kg $feddan^{-1}$) after 6 months of cultivation. .

It is clear from the obtained results inoculation with the selected efficient strains of asymbiotic N_2-fixers increased the densities of *Azotobacter* and *Azospirillum* in soil and rhizosphere of Rosemary plants. The increase continued till after 4 months of cultivation. Such findings confirm those obtained by Hegazi *et al.* (1979). They reported that inoculation of maize plants with *Azospirillum*, as well as *Azotobacter*, 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 of wide C/N radios, as organic N-fertilizer, also resulted in a marked increase in densities of *Azotobacter* and *Azospirillum* when compared with untreated treatments. This may be due to the fundamental effect of organic N-fertilizer amendment on biological, physical and chemical soil properties that enhanced the free-living N_2-fixing bacteria. In this respect, significant correlation between densities of N_2-fixers and the amount of organic N-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 soil and rhizosphere of the growing plants. This is in accordance with several reports showing that concentration of N-fertilizers may be limiting factors exhibiting a negative effect on the development of N_2-fixers in various ecosystems (Abrantes *et al.*, 1975; Dobereiner, 1978 and Reynders & Vlassak, 1979).

The effect of inoculation with *Azotobacter* and/or *Azospirillum* only, or with organic N-fertilizer and with half-normal dose of inorganic N-fertilizer on the growth [means of (plant height, main branch diameter, number of branches, leaf length, leaf width, fresh weights and root weights)] of Rosemary plant, and its active constituents (volatile oils), were also studied.

Data were statistically analyzed and recorded in Tables (3, 4, 5, 6, 7, 8, 9 and 10). These results clearly showed that the plants amended by a mixture of *Azotobacter* and *Azospirillum*, *Azotobacter* inoculum and *Azospirillum* inoculum by arrangement with organic N-fertilizer gave the highest data results (Table 3) for means of plant height (70.34, 68.50 and 60.00cm) after 6 months of cultivation. The plants amended by inoculation, with the same arrangement before with half normal dose of inorganic N-fertilizer being 62.33, 61.00 and 54.33cm after 6 months of cultivation. Plants treated with inoculation only with the same cultivation. Plants treated with inoculation only with the same arrangement before being 56.17, 55.17 and 51.17cm after 6 months of cultivation.

Table (2): Densities of *Azotobacter* and *Azospirillum* in unplanted soil and rhizosphere of Rosemary plants cultivated in sandy soil as affected by inoculation with the selected asymbiotic N_2-fertilizers, organic fertilizer and half recommended dose of inorganic N-fertilizer (The start of inoculation were after 2 months for the plants cultivated).

Inoculation	soil sample	Counts of *Azotobacter*, *Azospirillum* and Mixture of them counts($x10^4$ cells g^{-1} dry soil)							
		C		I		I*		I**	
		Months after inoculation							
		4	6	4	6	4	6	4	6
Azotobacter	R	24.66	19.16	67.00	47.67	81.00	62.16	71.50	56.00
	S	6.66	2.83	10.17	7.83	13.00	8.83	11.00	6.83
	R/S	4.70	6.77	6.60	6.09	6.23	7.04	6.50	8.20
Azospirillum	R	7.99	3.83	43.33	24.33	40.50	30.67	36.67	28.50
	S	2.83	1.83	4.33	2.83	4.16	2.83	3.83	3.00
	R/S	2.82	2.09	10.01	5.60	9.74	10.84	9.57	9.50
Mixture	R	27.83	20.33	57.33	34.99	84.66	61.65	75.50	54.50
	S	6.16	3.83	7.49	4.00	10.49	6.50	8.33	5.50
	R/S	4.52	5.30	7.66	6.25	8.07	9.48	9.06	9.91

R = Rhizosphere samples of planted hills. C = Control treatment without nitrogen.
S = Soil samples unplanted between hills. I = Plants amended by inoculation only.
R/S = The ratio between counts organisms in rhizosphere to soil.
I* = Plants amended by inoculation and organic N-fertilizer.
Mixture = Mixed culture of *Azotobacter* and *Azospirillum* strains.
I** = Plants amended by inoculation and half-normal dose of inorganic N-fertilizer (50kg/fadans).

Table (3): Effect of inoculation with the selected asymbiotic N_2-fixers, organic N-fertilizer and half-normal dose of inorganic fertilizer amendment on Rosemary plant height (cm).

Inoculation	Time after inoculation (months)	Control plant	Inoculated plants n1	n2	n3
Azotobacter	4	31.50	45.67	58.67	50.84
	6	37.50	55.17	68.50	61.00
Azospirillum	4	31.50	42.00	50.17	45.17
	6	37.50	51.17	60.00	54.33
Mixture	4	31.50	47.00	60.00	53.34
	6	37.50	56.17	70.34	62.33

	4 months		6 months	
L.S.D	**5%**	**1%**	**5%**	**1%**
Inoculation	0.54	0.78	1.38	1.99
Fertilizer	1.21	1.73	3.1	4.46
interaction	1.71	2.45	ns	ns

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

Table (4): Effect of inoculation with the selected asymbiotic N_2-fixers, organic N-fertilizer and half-normal dose of inorganic N-fertilizer amendment on main branch diameter (mm) of Rosemary plant.

Inoculation	Time after inoculation (months)	Control plant	Inoculated plants n1	n2	n3
Azotobacter	4	4.17	5.64	6.80	6.30
	6	5.70	8.50	10.34	9.20
Azospirillum	4	4.17	5.37	6.14	5.47
	6	5.70	7.47	9.14	8.47
Mixture	4	4.17	6.00	7.00	6.67
	6	5.70	8.80	11.03	9.80

	4 months		6 months	
L.S.D	**5%**	**1%**	**5%**	**1%**
Inoculation	0.08	0.11	0.08	0.11
Fertilizer	0.18	0.25	0.17	0.24
interaction	0.25	0.36	0.24	0.34

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

Table (5): Effect of inoculation with the selected asymbiotic N_2-fixers, organic N-fertilizer and half-normal dose of inorganic N-fertilizer amendment on mean number of branches of Rosemary plant.

Inoculation	Time after inoculation (months)	Control plant	Inoculated plants		
			n1	n2	n3
Azotobacter	4	11.00	15.00	19.34	16.67
	6	14.00	20.67	24.84	21.83
Azospirillum	4	11.00	13.84	17.67	14.84
	6	14.00	19.00	22.83	19.84
Mixture	4	11.00	16.34	20.67	17.84
	6	14.00	21.50	26.67	23.00

	4 months		6 months	
L.S.D	**5%**	**1%**	**5%**	**1%**
Inoculation	0.36	0.52	0.64	ns
Fertilizer	0.81	1.17	1.42	2.04
interaction	ns	ns	ns	ns

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

Table (6): Effect of inoculation with the selected asymbiotic N_2-fixers, organic N-fertilizer and half-normal dose of inorganic N-fertilizer amendment on leaf length (cm) of Rosemary plant.

Inoculation	Time after inoculation (months)	Control plant	Inoculated plants		
			n1	n2	n3
Azotobacter	4	1.55	1.95	2.30	2.10
	6	1.80	2.55	2.97	2.80
Azospirillum	4	1.55	1.80	2.00	1.85
	6	1.80	2.22	2.63	2.34
Mixture	4	1.55	2.05	2.70	2.25
	6	1.80	2.72	3.37	2.97

	4 months		6 months	
L.S.D	**5%**	**1%**	**5%**	**1%**
Inoculation	0.05	0.07	0.06	0.09
Fertilizer	0.11	0.16	0.15	0.21
interaction	ns	ns	ns	ns

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

Table (7): Effect of inoculation with the selected asymbiotic N_2-fixers, organic N-fertilizer and half-normal dose of inorganic N-fertilizer amendment on leaf width (cm) of Rosemary plant.

Inoculation	Time after inoculation (months)	Control plant	Inoculated plants n1	n2	n3
Azotobacter	4	1.80	2.00	2.35	2.15
	6	1.95	2.30	2.70	2.55
Azospirillum	4	1.80	1.85	2.05	2.00
	6	1.95	2.10	2.45	2.35
Mixture	4	1.80	2.20	2.70	2.55
	6	1.95	2.55	3.05	2.85

	4 months		6 months	
L.S.D	**5%**	**1%**	**5%**	**1%**
Inoculation	0.04	0.06	0.061	0.088
Fertilizer	0.09	0.13	0.136	0.196
interaction	0.13	0.19	ns	ns

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

Table (8): Effect of inoculation with the selected asymbiotic N_2-fixers, organic N-fertilizer and half-normal dose of inorganic N-fertilizer amendment on fresh weight (g) of Rosemary plant after 6 months.

Inoculation	Control plant	Inoculated plants n1	n2	n3
Azotobacter	395.00	533.00	1829.90	1108.95
Azospirillum	395.00	428.87	1513.37	1050.87
Mixture	395.00	542.53	1912.50	1130.47

L.S.D	**5%**	**1%**
Inoculation	14.599	21.006
Fertilizer	32.646	46.972
interaction	ns	ns

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

Table (9): Effect of inoculation with the selected asymbiotic N_2-fixers, organic N-fertilizer and half-normal dose of inorganic N-fertilizer amendment on roots weight (g) of Rosemary plant after 6 months..

Inoculation	Control	Inoculated plants		
	plant	n1	n2	n3
Azotobacter	84.00	107.67	118.67	115.84
Azospirillum	84.00	102.17	107.83	104.34
Mixture	84.00	112.67	128.83	119.34

L.S.D	5%	1%
Inoculation	0.814	1.171
Fertilizer	1.82	2.619
interaction	2.574	3.704

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

Table (10): Effect of inoculation with the selected a symbiotic N_2-fixers,organic N-fertilizer and half normal dose of inorganic N-fertilizer amendment on volatile oil of Basil plant after 6 months.

Inoculation	Control	Inoculated plants		
	plant	n1	n2	n3
Azotobacter	0.34	0.51	0.55	0.50
Azospirillum	0.34	0.43	0.50	0.47
Mixture	0.34	0.53	0.63	0.56

L.S.D	5%	1%
Inoculation	0.005	0.008
Fertilizer	0.013	0.019
interaction	0.18	0.027

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

The lower mean of plant heights after 6 months was recorded in the control was 37.50cm. .

The highest data, presented in Table (4), for the mean of main branch diameter in the plants treated by inoculation on the same arrangement before with organic N-fertilizer, being 11.03, 10.34 and 9.14mm after 6 months of cultivation, but the lower mean of branch diameter was in control plants, being 5.70mm after the same times. .

The highest data, shown in Table (5), for the mean of number of branches in the plants amended by inoculation on the some arrangement before with organic N-fertilizer, being, 26.67, 24.84 and 22.83 branches plant^{-1} after 6 months of cultivation, but the lower mean of number's branch, was in control plants, being 14.00 branchs^{-1} after 6 months of cultivation. .

The highest data, presented in Table (6), for the mean of leaf length in the plants treated by inoculation on the same

arrangement before with organic N-fertilizer being 3.37, 2.97 and 2.63cm after 6 months of cultivation, but the lower mean of leaf length was in control plants, being 1.80cm after 6 months of cultivation. .

The highest data, shown in Table (7), for the mean of leaf width in the plants treated by inoculation on the same arrangement before with organic N-fertilizer, being 3.05, 2.70 and 2.45cm after 6 months of cultivation.

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The lower mean of leaf width was in control plants, being 1.95cm after the same time. .

The highest data, presented in Table (8), for the mean of fresh weight in the plants amended by inoculation on the same arrangement before with organic N-fertilizer, being 1912.50, 1829.90 and 1513.37g plant^{-1} after 6 months of cultivation. The lower data was in control plants, being 395.00g after the same time. .

The highest data, presented in Table (9), for the mean of roots weight in the plants treated by inoculation on the same arrangement before with organic N-fertilizer, being 128.83, 118.67 and 107.83g plant-1after 6 months of cultivation. The lower data was in control plants, being 84.00g plant-1 after 6 months of cultivation. .

The plant height in Table (3), main branch diameter in Table (4), number of branches in Table (5), leaf length in Table (6), leaf width in Table (7), fresh weight in Table (8) and roots weight in Table (9) 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, shown in Table (10), for the volatile oil content in the plants treated with composite inocula on the same arrangement before with organic N-fertilizer, being 0.63, 0.55 and 0.50ml/100g for fresh weight plant^{-1} after 6 months of cultivation..

The lower data was found in plants control, being 0.34ml/100g 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 الجوي اللاتكافلية على نمو نبات حصالبان (*Rosmarinus officinalis*) وعلى مكوناته الفعاله

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يهدف البحث لدراسة تأثير تلقيح بادرات نباتات حصالبان بمثبتات النيتروجين الجوي اللاتكافلية فقط كمعاملة أولى، أو تلقيحها بمثبتات النيتروجين الجوي اللاتكافلية بالإضافة إلى الأسمدة العضوية كمعاملة ثانية، أو تلقيحها بمثبتات النيتروجين الجوي بالإضافة إلى نصف الجرعة المقررة من السماد الكيماوي لكل فدان كمعاملة ثالثة على نمو النباتات وانتاجها من المادة الفعالة (الزيت العطري). وهذه الدراسة عبارة عن تجارب حقلية أجريت في أرض رملية بمزرعة مركز الدراسات التطبيقية لبحوث النباتات الطبية التابع للهيئة القومية للرقابة والبحوث الدوائية.

مثبتات النيتروجين الجوي اللاتكافلية كانت عبارة عن ميكروبات *Azotobacter & Azospirillum* والتي عزلت من ريزوسفير نباتات حصالبان ، ثم بعد ذلك لقحت بادرات نباتات حصالبان بالسلالات النشطة من *Azotobacter & Azospirillum* معا بالإضافة إلى السماد العضوي (البلدي بمعدل ٤٠م^3/فدان) بعد الزراعة بشهرين وأوضحت البيانات التى أخذت أن النباتات التي سمدت باللقاح الحيوى بالإضافة إلى السماد العضوي أعطت أعلى كثافة عددية من *Azotobacter & Azospirillum* في ريزوسفير تلك النباتات بعد أربعة أشهر من الزراعة ، كما أعطت أكبر كمية من النمو حيث بلغت ١٩١٥,٥٠ جرام/ نبات أخضر وكذلك إنتاج أكبر كمية من الزيت العطري حيث أعطت٠,٦٣ مل/١٠٠ جرام نبات أخضر إذا ما قورنت بالنباتات التي لم تعامل نهائياً والتي أعطت ٣٩٥,٠٠جرام/ نبات أخضر، ٠,٣٤ مل/١٠٠ جرام نبات أخضر على التوالي وذلك بعد ٦ أشهر من الزراعة.