

RESPONSE OF FRENCH BASIL PLANTS (*Ocimum basilicum*) TO NITROGEN FIXED BACTERIA

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ABSTRACT

The experiment was carried out in the Experimental Farm of Medicinal and Aromatic Plants Research Station, Horticultural Research Institute, in El-Kanater El-Khayria, Kalyobia Governorate, Egypt, during the two successive seasons of 2007 and 2008, to study the effect of nitrogen fixed bacteria (*Azotobacter*, *Azospirillum* and *Rhizobium*) and different levels (zero of NPK, half and full recommended doses) on growth, herb yield and volatile oil yield of *Ocimum basilicum* plants. The tallest plants obtained from plants that treated with *Azospirillum* + *Rhizobium* + full recommended dose of NPK followed by *Azotobacter* + *Azospirillum* alone. While number of branches recorded the highest value from the treatment of *Rhizobium* + half recommended dose of NPK in the first cut and *Rhizobium* + full recommended dose of NPK in the second cut. The highest values of fresh and dry weights of herb/plant and total production of fresh and dry weights of herb/plant/season were obtained from *Azotobacter* + *Rhizobium* + half recommended dose of NPK in the two cuts of both seasons. The volatile oil percentages based on fresh and dry weights of herb and volatile oil yield of plant per cut and per season recorded the highest values when plants treated with *Azospirillum* alone. The high concentration from the main component of linalool in the volatile oil obtained from the treatment of *Azospirillum* alone.

INTRODUCTION

French basil plants (*Ocimum basilicum* L.), Fam. Lamiaceae (Labiataea), should be grown in full sun and well drained soil. It acts principally on digestive and nervous system, stomach cramps, colic and indigestion. It can be used to prevent nausea and vomiting and help to kill intestinal worms. it has mild sedative action. The essential oil is found in glands in the plant leaves extracted commercially by steam or water distillation method, (Stary and Jirasek, 1975). The oil was employed quite extensively in many kinds of flavours including confectioners, backed foods and condimentary, (Gunther, 1961).

Recently, a great attention was taken place in the production of the chemical free products from the medicinal and aromatic plants. These chemical free products are safe in use, superior in marketing and exportation, with highest income and least outcome.

Bio-fertilization is one of the important factors used to produce products free from mineral contamination. On the other hand, the intensive use of chemical fertilizers causes environmental pollution problems and high rates of it led to decrease the potential activity of micro flora and the stability of organic matter. Hence, the attention has been focused on the researches of bio-fertilizers to substitute chemical fertilizers. Bio-fertilizers are increasing the number of micro-organisms and accelerate certain microbial process in the rhizosphere of inoculated soils or plants which can change the

unavailable forms of nutrients into available ones, (Subb Roa, 1981 and Alaa El-Din, 1982).

In this concern, the use of biofertilizers (Nitrogen fixed bacteria) with or without chemical fertilizers was experimenting by more authors, Hegazi *et al* (1979) and Saleh *et al* (1986) mentioned that, N concentration may be considered as a limiting factor and exhibits a negative effect on the development of N fixers in various ecosystems.

In the same manner, Neyra and Dobereiner (1977) indicated that, heavy dose of N fertilizers inhibited N₂ fixation. While, Dobereiner and Day (1976) found that, low N concentration promotes the response of plant to inoculation and the establishment of well functional N₂ fixing bacteria. In the same way, Hussna and Mervat (2000) reported that, the application of NPK either individual or in combination with bio-fertilizer improved CO₂ evaluation.

On medicinal and aromatic plants trials were conducted by many researchers, Maheshwari *et al* (1995) recorded an increase in herb and oil of palmarosa plants by *Azotobacter chroococum* than control (40 or 80 kg N/ha). Sankoranayanan *et al* (1995) stated that, *Azospirillum* + 50% (the recommended N) produced the greatest plant height of bhendi plant. Also, Wange (1996) obtained a 50% increment of carrot yield over the recommended N by the use of 45 kg N/ha combined with *Azotobacter* and *Azospirillum*. Amin (1997) concluded that, the highest values of plant height and number of branches in coriander, fennel and caraway plants were obtained by using the full dose of inorganic fertilizer, which was par with biofertilizer (*Azotobacter* and *Azospirillum*) + half dose of the inorganic fertilizer. A similar trend was obtained for oil percentages in seeds of all three crops. Harridy and Mervat (1998) working on roselle plants showed that, the density of the bacteria was varied according to N level and date of sampling. A positive response was found on growth, sepals yield and anthocyanin content due to biofertilizer especially with *Rhizobium* and *Azotobacter* with 0 to 40 kg N/fed. Harridy *et al* (2001) found that, *Azotobacter* solely without NPK treatment produced the highest values of tillers number/plant, highest yield of fresh weight of herb (gm/plant per cut and per season) and the highest volatile oil yield (ml/plant per cut and per season) on lemongrass. Kandeel *et al* (2002) on *Ocimum bacilicum* L. plants showed that, dual inoculation with symbiotic N₂-fixers (*Azotobacter* + *Azospirillum*) supplemented with half or full dose of inorganic – N (50 or 100 kg N/fed.) fertilizer significantly increased plant height, number of branches/plant, fresh and dry weights of herb and yearly oil yield/plot compared with control. Shaalan (2005) indicated that, the biofertilizers treatments (*Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus*) combined with poultry manure resulted in the most increment of volatile oil compositions in black cumin seeds (Thymoquinone, β-cymene and geraniol). Hend *et al* (2007) on peppermint found that, the most effective treatments for increasing total herb fresh weight and highest percentage of oxygenated compounds in the volatile oil was the application of 50% recommended rate of (N and P fertilizers) in combination with *Azotobacter* sp. Mahfouz and Shams-Eldin (2007) reported that, application of biofertilizer which was mixture of *Azotobacter chroococum*, *Azospirillum liboferum* and *Bacillus megatherium* plus a half

dose of chemical fertilizer (recommended dosage of NPK) increased plant height, number of branches, herb fresh and dry weights/plant and volatile oil yield/plant in fennel.

MATERIALS AND METHODS

This experiment was carried out in the Experimental Farm of Medicinal and Aromatic Plants Research Station, Horticultural Research Institute, in El-Kanater El-Khayria, Kalyobia Governorate, Egypt, during the two successive seasons of 2007 and 2008.

This work was designed to study the effect of nitrogen fixed bacteria and the different rates of recommended NPK fertilizers on growth, herb yield, volatile oil yield and chemical composition of volatile oil of *Ocimum basilicum* plants.

The seeds of basil were obtained from Medicinal and Aromatic Plants Research Station in El-Kanater El-Khayria.

The seeds were sown in the nursery bed at 10th April. Meanwhile, seedlings were transplanted in pots at 25th May in the two seasons. Each plant of basil was cultivated in plastic pot (30 cm in diameter) filled with 8.25 kg soil. The experimental soil was sterilized with formalin 5% and covered with plastic film during 10 days, and arid to one week. The soil used was a mixture of clay and sand (1:1), respectively, and 250 gm of compost was added to every pot before cultivation.

Basil seedlings were $\frac{1}{4}$ inoculated with different bacteria solution namely *Azotobacter*, ⁴ *Azospirillum* and *Rhizobium* solely and their combination by soaking the seedling roots in the bacteria solution for hour. Plants were sprayed with bacteria solution two times: the first spraying at 18th July and the second one at 9th February in the two seasons to maximize the benefit of inoculation.

The recommended chemical fertilizers used were 240 P₂O₄/fed. as calcium superphosphate (15.5%), 120 N/fed. as ammonium sulphate (20.5%) and 75 K₂O/fed. as potassium sulphate (48%). The phosphorus fertilizer was adding during soil preparation before seedlings transplanting. While nitrogen fertilizer was divided into five equal doses and added as follows: the first at 29th June, the second at 8th July, the third at 25th July, the fourth at 29th August and the fifth at 12th September. And potassium fertilizer was divided into two equal doses and added as follows: the first dose at 8th July, while the second one was after the first cut at 29th August.

Twenty two treatments with four replicates distributed in complete randomized blocks in the two seasons. The treatments were as follows:

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|--|--|
| 1- Control (Recommended NPK) | 12- <i>Azotobacter</i> + <i>Azospirillum</i> + half NPK |
| 2- <i>Azotobacter</i> + 0 | 13- <i>Azotobacter</i> + <i>Azospirillum</i> + Full NPK |
| 3- <i>Azotobacter</i> + half NPK | 14- <i>Azotobacter</i> + <i>Rhizobium</i> + 0 |
| 4- <i>Azotobacter</i> + Full NPK | 15- <i>Azotobacter</i> + <i>Rhizobium</i> + half NPK |
| 5- <i>Azospirillum</i> + 0 | 16- <i>Azotobacter</i> + <i>Rhizobium</i> + Full NPK |
| 6- <i>Azospirillum</i> + half NPK | 17- <i>Azospirillum</i> + <i>Rhizobium</i> + 0 |
| 7- <i>Azospirillum</i> + Full NPK | 18- <i>Azospirillum</i> + <i>Rhizobium</i> + half NPK |
| 8- <i>Rhizobium</i> + 0 | 19- <i>Azospirillum</i> + <i>Rhizobium</i> + Full NPK |
| 9- <i>Rhizobium</i> + half NPK | 20- <i>Azotobacter</i> + <i>Azospirillum</i> + <i>Rhizobium</i> + 0 |
| 10- <i>Rhizobium</i> + Full NPK | 21- <i>Azotobacter</i> + <i>Azospirillum</i> + <i>Rhizobium</i> + half NPK |
| 11- <i>Azotobacter</i> + <i>Azospirillum</i> + 0 | 22- <i>Azotobacter</i> + <i>Azospirillum</i> + <i>Rhizobium</i> + Full NPK |

Harvesting

Harvesting was carried out in two cuts every season. The first cut was done in August 17th (after 58 days from transplanting date) and the second one was done on October 12th (after 114 days from transplanting date) by cutting the vegetative parts of plant 10 cm above the soil surface leaving 1 or 2 branches for regrowth.

Recorded data:

Vegetative characters

Random samples of four plants from each replicate are taken in the two cuts during the two seasons. The following data were recorded:

- Plant height (cm).
- Number of branches/plant.
- Fresh and dry weights of herb/plant (gm).
- Total production of fresh and dry weights of herb/plant/season (gm).
- Oil percentage (ml/100 gm) based on fresh and dry weights of herb every cut.
- Oil yield (ml/100 gm) based on fresh weight of herb every cut.
- Oil yield (ml/100 gm) based on fresh weight of herb for two cuts
- Chemical analysis for essential oil was conducted in the second cut during the second season by using Ds 6200 Gas Chromatography.

Detector Temp.	300 °C
Injector Temp.	250 °C
Air Flow	300 ml/min.
Hydrogen Flow	30 ml/min.
N ₂ Flow	1 ml/min.
Initial Temp.	70 °C
Rate ₁ : 70 – 80 °C	1 °C/min.
Rate ₂ : 80 – 120 °C	5 °C/min.
Rate ₃ : 120 – 190 °C	10 °C/min.

Statistical analysis:

The statistical analysis of the present data was carried out according to Steel and Torrie (1980) using new L.S.D. at 5% level for comparison between means of the different treatments.

RESULTS AND DISCUSSION

1. Growth

1.1. Plant height

Data in Table (1) show that, the application of most combinations between *Azotobacter*, *Azospirillum* and *Rhizobium* with or without half or full of recommended dose of NPK fertilizers gave taller basil plants over the control (recommended doses of NPK fertilizers). The treatment of *Azospirillum* + *Rhizobium* with full recommended NPK resulted the best plant height with significant difference compared with control in the first cut during the two seasons. Moreover, in the same cut during both seasons, there was a significant increase in plant height obtained from the treatment of *Azotobacter* + *Rhizobium* with half recommended NPK. The difference between *Azospirillum* + *Rhizobium* + full recommended NPK treatment and

Azotobacter + *Azospirillum* + *Rhizobium* with half recommended NPK was not significant in the first cut during the two seasons.

In the second cut the highest results obtained when using *Azotobacter* + *Azospirillum* only treatment with significant increase over the control, followed by the treatment of *Azotobacter* + *Rhizobium* + half recommended NPK during the two seasons.

1.2. Number of branches

Concerning the effect of bacteria strains on the number of branches, the data illustrated in Table (1) indicated that, there were effects of the half or full recommended dose of NPK with *Rhizobium* bacteria treatments in this regard. The treatment of *Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended dose of NPK in the first cut of the first season gave the highest increase with significant difference over the control, followed by *Rhizobium* + half recommended NPK without significant difference. The last treatment (*Rhizobium* + half recommended NPK) gave the best increase in number of branches in the first cut during the second season. This increase was significant compared to control. On the other hand, the treatment of *Rhizobium* + full recommended NPK affected on number of branches in basil plant and gave the highest results with no significant in the second cut during both seasons when compared with the control.

1.3. Fresh weight of herb/plant

Data dealing with the effect of different strains of nitrogen fixed bacteria and various levels of NPK fertilizers on the fresh weight of herb/basil plant in two cuts during the two seasons are shown in Table (2). It is evident that, the treatment of (*Azotobacter* + *Rhizobium* + half recommended NPK) gave the highest results. The increments were significant when compared with the control. The results hold true in the two cuts during the two seasons. The fresh weight of herb/plant in the first season was increased by more than 27.7 and 13.1% for the 1st and 2nd cuts, respectively, as compared to control. While, the increases in the second season were 30.1 and 12.8% for 1st and 2nd cuts, respectively, when compared with plants treated with full recommended dose of NPK.

Also the same trend was obtained with the treatment of *Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended NPK which gave significant increment in the fresh weight of herb/plant when compared with control. The differences between the treatments of (*Azotobacter* + *Rhizobium* + half recommended NPK) and (*Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended NPK) were significant in the first cut and non significant in the second one. The data in the second season showed the same trend as that in the first one.

These results are in agreement with those obtained by Hegazi *et al* (1979) and Saleh *et al* (1986). They indicated that, N concentration may be considered as a limiting factor and exhibits a negative effect on the development of N fixers in various ecosystems, Maheshwari *et al* (1995) on palmarosa plants, Harridy and Mervat (1998) on roselle plants, stated that, the positive response was found on growth when using *Azotobacter* + *Rhizobium* with 0 to 40 kg N/fed.

Table (1): Effect of nitrogen fixed bacteria and rates of recommended chemical fertilizers on plant height and number of branches in French basil plant during the two seasons of 2007 and 2008.

Nitrogen fixed bacteria	NPK	Plant height				Number of branches			
		First season		Second season		First season		Second season	
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
Recommended NPK (control)		57.75	61.00	56.32	61.50	13.25	18.88	13.13	19.25
Azotobacter (A)	0	54.25	63.00	52.50	63.25	13.00	16.50	12.38	16.38
	Half	51.25	58.38	53.25	59.38	13.00	18.50	13.25	18.38
	Full	54.50	62.00	54.25	62.25	15.00	19.63	15.33	19.75
zospirillum(B)	0	54.13	73.00	53.75	72.75	14.50	15.88	15.38	16.38
	Half	56.63	64.88	56.13	65.38	14.50	17.88	15.00	17.88
	Full	51.00	59.00	52.50	59.20	14.75	18.25	16.00	18.25
Rizobium (C)	0	57.75	71.75	57.25	77.00	15.00	15.38	15.25	16.38
	Half	54.50	61.75	53.13	61.75	16.75	17.63	17.50	17.50
	Full	55.63	58.00	55.13	57.50	16.63	20.13	15.88	20.00
(A) + (B)	0	57.75	76.75	57.75	75.63	13.87	16.13	12.75	15.50
	Half	59.58	70.75	59.13	69.75	14.00	18.62	13.75	19.00
	Full	57.00	67.25	54.38	67.63	16.75	17.25	16.63	17.00
(A) + (C)	0	58.75	67.00	57.75	66.88	12.75	13.25	13.63	13.37
	Half	62.50	74.00	64.25	73.00	14.13	17.38	14.13	17.00
	Full	59.00	62.50	59.32	62.88	14.37	19.75	14.00	19.75
(B) + (C)	0	54.00	69.63	56.13	70.87	15.62	15.13	15.38	16.13
	Half	62.00	69.75	61.63	69.38	15.25	17.00	16.00	17.13
	Full	63.63	67.00	64.75	67.88	16.38	16.62	15.38	17.13
(A) + (B) + (C)	0	59.00	67.13	58.38	67.50	14.13	16.75	14.63	16.63
	Half	59.63	71.88	60.00	71.13	13.63	16.13	12.88	16.13
	Full	61.75	68.83	62.63	69.25	17.87	19.00	17.00	18.75
L.S.D. at 5%		2.44	2.47	2.75	2.61	2.22	2.16	1.98	2.12

1.4. Dry weight of herb/plant

Regarding the data tabulated in Table (2) it may be noticed that, the combination treatments between bacteria strains and different doses of recommended NPK fertilizers affected on dry weight of herb/plant in both seasons. The heaviest significant dry weight recorded (*Azotobacter* + *Rhizobium* + half recommended NPK) followed by (*Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended NPK) treatments compared with the control in the two cuts during the two seasons. affected by the treatment of *Azotobacter* + *Rhizobium* + half recommended NPK treatment increasing the dry weight of herb/plant in the first season by more than 20.9 and 21.9% for first and second cuts, respectively, as compared to control. Also, the increases in the second season were 24.1 and 21.6% for both cuts, respectively, as compared with the control.

When comparison was done between means of (*Azotobacter* + *Rhizobium* + half recommended NPK) and (*Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended NPK) treatments, we found significant differences between them in the two cuts during both seasons.

1.5. Total production of fresh weight of herb/plant/season

From the data presented in Table (2), results clear that, the treatment of (*Azotobacter* + *Rhizobium* + half recommended NPK) gave the highest total production of fresh weight of herb/plant/season which recorded 232.54 and 235.12 gm in the first and second seasons, respectively. These results have significant differences when compared with the control which recorded 197.46 and 198.32 gm for the two seasons, respectively. The difference between the treatment of (*Azotobacter* + *Rhizobium* + half recommended NPK) and (*Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended NPK) which gave (229.03 and 231.67 gm, for 1st and 2nd seasons, respectively) was significant in the first season and non significant in the second one.

1.6. Total production of dry weight of herb/plant/season

Data showed in Table (2) demonstrate that, the effect of bacteria strains and NPK levels on the total production of dry weight of herb/plant/season gave the same manner for the total production of fresh weight of herb/plant/season. The best results were obtained from the treatment of (*Azotobacter* + *Rhizobium* + half recommended NPK), followed by (*Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended NPK) with significant differences between them and between both of them and the control in the two cuts of the two seasons.

2. Essential oil

2.1. Oil percentage (ml/100 gm) based on fresh weight

Data tabulated in Table (3) showed the effect of nitrogen fixed bacteria strains and levels of recommended doses of NPK fertilizers on oil percentage based on fresh weight of herb in basil plant. The data concluded that *Azospirillum* alone gave the highest percentage of volatile oil (0.13 and 0.13 %) in the first cut during both seasons, respectively. While, the combined treatment of (*Azotobacter* + *Azospirillum* + *Rhizobium*) without chemical fertilizer gave the highest percentage (0.14 and 0.15%) of volatile oil in the second cut during the two seasons.

Table (2): Effect of nitrogen fixed bacteria and rates of recommended chemical fertilizers on growth characters (fresh and dry weights of herb per plant in French basil plant during the two seasons of 2007 and 2008.

Nitrogen bacteria	fixed NPK	Fresh weight of herb/plant				Dry weight of herb/plant				Total production of fresh weight of herb/plant		Total production of dry weight of herb/plant	
		First season		Second season		First season		Second season		First season	Second season	First season	Second season
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut				
Recommended control)	NP	66.72	130.24	66.02	132.30	17.90	26.83	17.58	27.26	197.46	198.32	44.73	44.84
Azotobacter (A)	0	58.94	92.89	59.21	91.53	16.27	19.14	15.34	18.86	151.83	150.74	34.40	34.19
	Half	56.62	100.46	57.29	99.72	14.61	22.30	14.91	22.14	157.07	157.51	36.91	37.05
	Full	57.00	120.88	55.93	121.02	14.19	26.48	13.93	26.60	177.87	176.95	40.67	40.43
Azospirillum(B)	0	64.17	119.72	63.27	121.06	15.85	24.75	15.68	25.06	183.74	184.32	40.60	40.69
	Half	61.00	124.50	61.72	124.33	13.57	27.40	13.64	27.33	185.61	185.96	40.97	41.00
	Full	61.26	131.82	61.18	132.02	13.66	25.97	13.64	26.01	193.07	193.60	40.12	39.84
Rhizobium (C)	0	61.16	107.33	61.71	107.90	16.18	27.43	16.32	22.55	168.49	169.60	38.81	38.87
	Half	53.67	120.85	54.50	120.85	13.09	22.84	13.24	22.84	174.71	175.35	36.93	36.08
	Full	48.40	111.03	50.17	113.41	12.10	21.76	12.55	22.11	160.00	183.64	33.86	34.66
(A) + (B)	0	67.55	122.71	65.47	123.17	17.09	23.81	16.57	23.90	190.26	188.64	40.89	40.46
	Half	61.88	133.32	64.23	134.70	14.57	24.13	15.10	24.38	195.30	198.94	38.70	39.48
	Full	55.68	141.25	55.52	140.80	13.03	24.72	12.99	24.64	196.93	196.31	37.76	37.63
(A) + (C)	0	61.06	85.41	62.03	84.59	15.39	18.15	15.63	18.02	146.47	196.63	33.58	33.65
	Half	85.21	147.33	85.87	149.26	21.64	32.71	21.81	33.14	232.54	235.12	54.37	54.95
	Full	71.35	145.23	71.18	144.28	18.41	25.85	18.36	25.68	216.97	215.46	44.26	44.04
(B) + (C)	0	61.39	106.69	61.59	103.26	15.93	21.87	15.99	21.17	168.06	164.85	37.80	37.16
	Half	77.94	138.88	78.08	138.29	20.03	27.78	20.07	27.66	216.82	216.36	47.81	47.73
	Full	67.45	115.88	67.22	115.58	15.72	22.93	15.66	22.88	183.33	182.80	38.65	38.55
(A)+(B)+(C)	0	62.42	97.63	62.37	97.67	17.85	18.75	17.31	18.75	160.16	160.04	36.10	36.06
	Half	78.02	129.42	77.93	129.96	19.97	29.38	19.97	29.51	207.44	207.89	49.35	49.48
	Full	81.83	147.19	82.14	147.95	20.54	29.88	20.62	30.03	229.03	231.67	50.42	50.65
L.S.D. at 5%		2.20	2.82	2.39	3.07	0.55	0.57	0.61	0.62	3.42	3.55	0.78	0.79

Similar results were reported by Dobereiner and Day (1976) which they found that, low N concentration promotes the response of plant to inoculation and the establishment of well functional N₂ fixing bacteria, Amin (1997) on fennel, coriander and caraway oils and Harrayd *et al* (2001) on lemongrass stated that, *Azotobacter* solely without NPK treatment produced the highest volatile oil yield. These results were confirmed by Hend *et al* (2007) on peppermint.

2.2. Oil percentage (ml/100 gm) based on dry weight

It was clearly observed from Table (3) that, there were increases recorded in oil percentage of the dry leaves of basil plant in response to biofertilizer treatments and levels of recommended NPK fertilizers. The highest increase was obtained from *Azospirillum* only which gave 0.53 and 0.53% in the first cut during both seasons, respectively. While, the highest percentage recorded from (*Azotobacter* + *Azospirillum* + *Rhizobium* without NPK) treatment which gave 0.74 and 0.78% in the second cut of the two seasons, respectively.

The least percentages of volatile oil were resulted from the most half & full recommended dose of NPK when combined with biofertilizers treatments in the two cuts during the two seasons.

2.3. Oil yield (ml/plant) based on fresh weight

Concerning the effect of biofertilizers and recommended NPK on oil yield, data in Table (4) revealed that, *Azospirillum* without adding chemical fertilizers gave an increment of oil yield/plant with significant differences compared to control in the first cut of the two seasons. While, the treatment of *Azospirillum* + full dose of recommended NPK gave the highest yield in the second cut in the first season. In the second cut during the second season, the data stated that, (*Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended NPK) treatment gave the highest yield of oil with a significant difference when compared with the control.

2.4. Oil yield of two cuts (ml/plant) based on fresh weight of herb

Data in Table (4) emphasize that, treatment of (*Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended NPK) yielded the highest volatile oil (ml/plant) in the first and second seasons with significant differences over the control.

The differences between treatments of (*Azotobacter* + *Azospirillum* + *Rhizobium* + full recommended NPK) and (*Azospirillum* + full recommended NPK) in the first season or *Azospirillum* alone in the second season were non significant.

3. Chemical composition of volatile oil

Data illustrated in Table (5) stated that, linalool followed by methyl chavicol were the main components of basil oil for all treatments which ranged from (39.98 to 32.97%) for linalool, and (18.48 to 14.24%) for methyl chavicol.

Table (3): Effect of nitrogen fixed bacteria and rates of recommended chemical fertilizers on oil percentage (ml/100 gm) in French basil plant during the two seasons of 2007 and 2008.

Nitrogen fixed bacteria	NPK	Oil percentage (ml/100 gm) based on fresh weight				Oil percentage (ml/100 gm) based on dry weight			
		First season		Second season		First season		Second season	
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
Recommended NPK (control)		0.08	0.11	0.08	0.11	0.28	0.65	0.29	0.62
Azotobacter (A)	0	0.11	0.12	0.11	0.12	0.42	0.58	0.43	0.56
	Half	0.11	0.12	0.11	0.12	0.43	0.54	0.44	0.56
	Full	0.08	0.12	0.08	0.12	0.32	0.54	0.31	0.55
Azospirillum(B)	0	0.13	0.13	0.13	0.13	0.53	0.62	0.53	0.64
	Half	0.11	0.11	0.11	0.11	0.49	0.50	0.50	0.50
	Full	0.10	0.14	0.10	0.13	0.45	0.71	0.45	0.65
Rizobium (C)	0	0.12	0.12	0.12	0.12	0.46	0.56	0.46	0.59
	Half	0.11	0.13	0.11	0.13	0.45	0.67	0.45	0.66
	Full	0.07	0.12	0.07	0.12	0.29	0.62	0.28	0.60
(A) + (B)	0	0.12	0.12	0.12	0.12	0.47	0.61	0.47	0.62
	Half	0.11	0.11	0.11	0.11	0.47	0.59	0.46	0.61
	Full	0.10	0.12	0.10	0.12	0.43	0.73	0.43	0.69
(A) + (C)	0	0.12	0.13	0.12	0.12	0.51	0.59	0.46	0.60
	Half	0.10	0.11	0.10	0.11	0.37	0.50	0.38	0.50
	Full	0.10	0.12	0.10	0.12	0.39	0.66	0.41	0.65
(B) + (C)	0	0.13	0.12	0.13	0.12	0.50	0.60	0.47	0.60
	Half	0.10	0.11	0.10	0.12	0.40	0.56	0.39	0.56
	Full	0.10	0.12	0.10	0.12	0.43	0.62	0.42	0.62
(A) + (B) + (C)	0	0.12	0.14	0.12	0.15	0.42	0.74	0.43	0.78
	Half	0.11	0.12	0.11	0.11	0.44	0.51	0.43	0.50
	Full	0.10	0.12	0.10	0.12	0.40	0.59	0.41	0.59

Table (4): Effect of nitrogen fixed bacteria and rates of recommended chemical fertilizers on oil yield (ml/plant) in French basil plant during the two seasons of 2007 and 2008.

Nitrogen fixed bacteria	NPK	Oil yield (ml/plant) based on fresh weight of herb				Oil yield of two cuts (ml/plant) based on fresh weight of herb	
		First season		Second season		First season	Second season
		1 st cut	2 nd cut	1 st cut	2 nd cut		
Recommended NPK (control)		0.05	0.14	0.05	0.15	0.19	0.20
Azotobacter (A)	0	0.07	0.11	0.07	0.11	0.18	0.17
	Half	0.06	0.12	0.06	0.12	0.18	0.18
	Full	0.05	0.14	0.05	0.14	0.19	0.18
Azospirillum(B)	0	0.09	0.15	0.09	0.16	0.24	0.25
	Half	0.07	0.14	0.07	0.14	0.21	0.21
	Full	0.06	0.19	0.06	0.17	0.25	0.23
Rizobium (C)	0	0.07	0.13	0.08	0.13	0.19	0.21
	Half	0.06	0.15	0.06	0.15	0.21	0.22
	Full	0.04	0.13	0.04	0.14	0.17	0.17
(A) + (B)	0	0.08	0.14	0.08	0.15	0.22	0.23
	Half	0.07	0.14	0.07	0.15	0.21	0.22
	Full	0.06	0.17	0.06	0.17	0.23	0.23
(A) + (C)	0	0.08	0.11	0.08	0.11	0.19	0.18
	Half	0.08	0.16	0.08	0.16	0.24	0.25
	Full	0.08	0.17	0.07	0.17	0.25	0.24
(B) + (C)	0	0.08	0.13	0.08	0.13	0.21	0.20
	Half	0.08	0.16	0.08	0.15	0.24	0.23
	Full	0.07	0.14	0.07	0.14	0.20	0.21
(A) + (B) + (C)	0	0.08	0.15	0.07	0.15	0.22	0.22
	Half	0.08	0.15	0.09	0.15	0.24	0.23
	Full	0.08	0.18	0.08	0.18	0.26	0.26
L.S.D. at 5%		0.02	0.02	0.04	0.02	0.03	0.02

Generally, it could be noticed that, basil plants inoculated with *Azospirillum* alone followed by (*Azotobacter* + *Rhizobium* + half recommended NPK) produced an essential oil with a high percentage of linalool in comparison with oil production from control plants. Also, plants that treated with *Azotobacter* + *Rhizobium* + full recommended NPK followed by *Azospirillum* + full recommended NPK yielded volatile oil with highly content of methyl chavicol.

The pervious results of bio-fertilizers agree with those of Shaalan (2005) on black cumin seeds and Hend *et al* (2007) on peppermint. They found that, application of 50% recommended rate of (N and P fertilizers) in combination with *Azotobacter sp.* gave the highest percentage of oxygenated compounds in the volatile oil.

Table (5): Chemical composition of French basil essential oil as affected by biofertilizers (nitrogen fixed bacteria)

Components %	α -Pinene	β -Pinene	Limonene	Linalool	Cineol	Geraniol	Methyl chavicol	Eugenol	Citral
1-Control	0.83	4.26	10.12	37.56	1.77	2.95	14.24	0.91	5.01
2- B + 0 RD	—	3.68	6.92	39.98	1.08	4.37	18.15	0.75	4.95
3- B + full RD	1.33	3.44	11.96	32.97	3.34	3.60	18.19	0.85	5.98
4- A + B + 0 RD	2.04	3.56	8.97	37.45	3.85	3.37	15.65	1.61	4.77
5- A + C + half RD	2.22	3.45	10.33	38.27	2.24	1.37	17.61	0.88	5.77
6- A + C + full RD	0.39	1.20	8.09	36.17	1.66	2.14	18.48	0.44	5.83
7- B + C + half RD	0.30	4.27	11.14	35.10	3.96	4.91	18.04	0.27	4.17
8- A + B + C + half RD	0.93	3.62	8.92	34.40	3.56	3.74	15.24	0.86	5.48
9- A + B + C + full RD	0.71	4.15	11.18	37.31	1.84	2.99	15.36	0.32	4.31

A = *Azotobacter* B = *Azospirillum* C = *Rhizobium*
RD = Recommended Dose of NPK fertilizers

From these conclusion the following may be recommended:

- * Using the combined treatment *Azotobacter* + *Rhizobium* + half recommended dose of NPK to get highest yield of fresh and dry weight of herb/plant.
- * To get the highest percentages of volatile oil from the fresh or dry weight of basil herb can be using the treatment of *Azospirillum* alone in the first cut and using *Azotobacter*, *Azospirillum* and *Rhizobium* without adding chemical fertilizers treatment in the second cut.
- * When using the treatment of *Azospirillum* alone gave the highest yield of volatile oil based on fresh weight from basil herb in the first cut.

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استجابة نباتات الريحان الفرنسي للبيكتريا المثبتة للنيتروجين
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تم تنفيذ التجربة في المزرعة البحثية لمحطة بحوث النباتات الطبية والعطرية ، معهد بحوث البساتين بالقناطر الخيرية ، محافظة القليوبية ، خلال عامي 2007 – 2008 لدراسة تأثير البكتريا المثبتة للنيتروجين (أزوتوباكتر ، أزوسبيريللم وريزوبيوم) ومستويات مختلفة (صفر ، نصف و كل الجرعة الموصى بها) من الأسمدة الكيماوية (نيتروجين ، فوسفور و بوتاسيوم على النمو ومحصول العشب والزيوت الطيار في نباتات الريحان الفرنسي . أطول نباتات تم الحصول عليها من معاملة أزوسبيريللم + ريزوبيوم + كل الجرعة الموصى بها من الأسمدة الكيماوية متبوعة بالمعاملة أزوتوباكتر + أزوسبيريللم فقط . بينما عدد الأفرع سجل أعلى قيمة من المعاملة ريزوبيوم + نصف الجرعة الموصى بها من الأسمدة الكيماوية في الحشة الثانية. تم الحصول على أعلى القيم من الوزن الطازج والجاف من العشب/نبات والإنتاج الكلي من الوزن الطازج والجاف من العشب/نبات/موسم من المعاملة أزوتوباكتر + ريزوبيوم + نصف الجرعة الموصى بها من الأسمدة الكيماوية في الحشتين خلال موسمي الزراعة. نسبة الزيت العطري بالنسبة للوزن الطازج والجاف ومحصول الزيت العطري منسوبا للوزن الطازج/نبات ومحصول الزيت الطيار في الحشتين/نبات/موسم حققت أعلى القيم عندما تعامل النباتات بالأزوسبيريللم بدون الأسمدة الكيماوية. أعلى تركيز من المركب الرئيسي للزيوت الطيار (اللينالول) تم الحصول عليه من المعاملة بالبكتريا أزوسبيريللم فقط .