

RESPONSE OF *Ocimum basilicum* PLANT TO SOME PLANT GROWTH PROMOTING RHIZOBACTERIA

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ABSTRACT

Two field experiments were conducted during the two successive growing seasons of 2011 and 2012 to evaluate the effect of ten inoculation treatments with mixtures of some plant growth promoting rhizobacteria (PGPR); i.e., Paenibacillus polymyxa (P), Azospirillum brasilense (A) and some isolates of growth promoting rhizobacteria; i.e., Pseudomonas synxantha (P1) and Bacillus subtilis 2 (B2) on Ocimum basilicum plant growth and herb yield, and to assess to what extent inoculation treatments reduce the demand of basil plant to nitrogen chemical fertilization. Inoculated plants were received only 75% of the recommended N fertilizer dose (45 kg N/ fad), while control plants were received 100% of the recommended N fertilizer dose (60 kg N/ fad) without PGPR inoculation.

Results indicated that inoculation basil plants with bacterial inoculants mixture containing (A+P1+B2) or (A+P1) under the effect of 75% N dose resulted in the tallest plants bearing the highest number of branches/ plant and increased herb fresh and dry weights/ plant and/ fad comparing to control plants and all other inoculation treatments. Also, plants treated with these two the abovementioned inoculation treatments resulted in essential oil %, essential oil yield/ plant and/ fad statistically exceed or equal to that produced from control plants.

Respecting essential oil components, 75% N+ (A+P1) treatment increased β -pinene and camphor percentages, while 75% N+ (A+P1+B2) treatment increased linalool % comparing to their percentages in oil extracted from control plants. In addition, Methyl-chavicol %, which is described as a carcinogen and limits the application of basil oil in therapy, was decreased under the effect of these two treatments.

The caused improvement in plant growth, herb yield and essential oil yield and its properties in plants received 75% N and inoculated with inoculant mixture containing (A+P1) or (A+P1+B2) was associated with increases in percentages of total carbohydrates, N, P and K in their tissues. Also, inoculation with all PGPR under this study significantly reduced dehydrogenase enzyme activity, but significantly increased

nitrogenase enzyme activity in the soil rhizosphere of inoculated basil plants.

*Conclusively, it could be conclude that inoculation of *Ocimum basilicum* plant with inoculant bacterial mixture containing equal portions of (*Azospirillum brasilense* + *Pseudomonas synxantha*) or (*Azospirillum brasilense*+ *Pseudomonas synxantha* + *Bacillus subtilis* 2) can be compensate the demand of nitrogen mineral fertilization and can improve plant growth, herb yield, essential oil yield and medicinal properties of herb and its essential oil as compare to herb and oil of 100% N fertilized plants without PGPR inoculation.*

Key words: Basil, growth, essential oil, nutrient uptake, PGPR, *Pseudomonas sp*, *Bacillus subtiles*, *Paenibacillus polymyxa*, *Azospirillum brasilense*.

INTRODUCTION

Nowadays, medicinal and aromatic plants gained great importance because the safety and effectiveness of their medicinal components. The world now getting far from chemicals as pollution factors because their harmful effects and turned toward the natural products.

Basil belongs to the genus *Ocimum*, having strong odor referred to its essential oil (McIntosh, 1953). Basil is a widely grown as aromatic crop cultivated for production of essential oil, dry leaves for fresh market, or as an ornamental plant (Simon *et al.*, 1990). Basil has been used as a folk remedy for an enormous number of ailments including: boredom, cancer, convulsion, deafness, diarrhea, epilepsy, gout, impotency, nausea, sore throat, toothaches, and whooping cough. Also, basil has been reported in herbal publications as an insect repellent (Duke, 1985). Recently, the potential uses of *Ocimum basilicum* essential oil as antimicrobial and antioxidant agents have also been investigated (Politeo *et al.*, 2007). *Ocimum basilicum* essential oil exhibited a wide and varying array of chemical compounds depending on variations in chemo types, leaf and flower colors, aroma and origin of the plant (Sajjadi 2006). It has been reported that the leafy parts of basil have tonic, antiseptic (Kosekia *et al.*, 2002) and insecticidal properties (Umerie *et al.*, 1998). It is also known the leaves of basil are suitable for the treatment of pain (Basilico and Basilico, 1999).

Bio-fertilizers including plant growth promoting rhizobacteria (PGPR) has become a feasible practice. PGPR are root associated bacteria representing many different genera and species that colonize the rhizosphere and improve plant growth. PGPR may improve plant growth and yield by direct and/ or indirect mechanisms (Noel *et al.*, 1996). Indirect mechanisms

of plant growth stimulation may include variety of mechanisms by which the bacteria prevent phyto-pathogens from inhibiting plant growth and development (Glick and Bashan, 1997). While, direct mechanisms may act on the plant itself and affect growth by providing plants with fixed nitrogen, phyto-hormones, iron and soluble phosphate (Kloepper and Schroth, 1978). Also, PGPR can protect plants against the deleterious effects of some environmental stresses including flooding (Grichko and Glick, 2001), drought (Mayak *et al.*, 2004a), salt (Mayak *et al.*, 2004b) and phytopathogens (Harman and Björkman, 1998). They can increase the availability of nutrients to the host plant, lowering of the ethylene level within the plant and/ or enhance production of stimulatory compounds, such as some plant growth regulators (Phippen and Simon, 1998).

Different PGPR including associative bacteria such as *Bacillus*, *Azospirillum*, *Pseudomonas* and *Enterobacter* have been used for their beneficial effects on plant growth (Grayer *et al.*, 1996). Many marketable biofertilizers are mainly based on PGPR that exert beneficial effects on plant development often related to the increment of nutrient availability to host plant (Bozin *et al.*, 2006).

Paenibacillus polymyxa, also known as *Bacillus polymyxa*, is one of the most important and often reported as PGPR (Timmusk *et al.*, 1999) with a wide range of properties including: phosphorus solubilization (de Freitas *et al.*, 1997); nitrogen fixation (Coelho *et al.*, 2003); produced cytokinins (Timmusk *et al.*, 1999), antibiotics (Rosado and Seldin, 1993), and hydrolytic enzymes (Nielson and Sorenson, 1997); enhance colonization of hair and cortical cells (Shishido *et al.*, 1999); and increased root and shoot growth of crops (Sudha *et al.*, 1999). According to Ramadan *et al.* (2007), *Bacillus polymyxa* produces active products including indole acetic acid and gibberellins as high as 167 and 584 mg/L, respectively. Another effect includes an increase in mobilization of insoluble nutrients followed by enhancement of uptake by the plants (Lifshutz *et al.*, 1987). Moharram *et al.* (1997) reported that inoculation of wheat with *Bacillus polymyxa* together with organic or inorganic nitrogen applications increased plant dry weight and N₂-fixation. Also, Abou-Zeid *et al.* (2003) on wheat plant reported that bacterial inoculation with *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus polymyxa* led to significant increases in grain yield, grain protein and 1000 grain weight.

In the last decade, distinct microbial communities have been found in various plant organs such as roots, stem and leaves, flowers as well as fruits and seeds or even during plant development (Okunishi *et al.*, 2005). Many of them have been reported to improve plant growth and health (Hardoim *et al.*, 2008).

Therefore, such work aimed to evaluate to what extent inoculation of *Ocimum basilicum* plant with some plant growth promoting rhizobacteria (PGPR) reduce the demand of N mineral fertilization, and to assess effects of bacterial inoculation on plant growth, volatile oil production and its properties, and some chemical constituents of *Ocimum basilicum* plant.

MATERIALS AND METHODS

Presented work was carried out during the two successive growing seasons of 2011 and 2012 at the Experimental Farm of medicinal and Aromatic plants Research section, Agricultural research center, Ministry of Agriculture El Kanater ElKhairia, Egypt to investigate the effect of inoculation with some plant growth promoting rhizobacteria (PGPR) under 75% of the recommended mineral nitrogen fertilizer dose on growth, volatile oil % and yield and nutrient uptake of basil cultivar Grandvert.

The used bacterial inoculants:

Paenibacillus polymyxa which also known *Bacillus polymyxa* (referred P) and *Azospirillum brasilense* (referred A) were kindly supplied from Microbiology Dept., Soil, Water and Environment Res. Inst., Agric. Res. Center, Ministry Agric., Giza, Egypt. Also, samples from some rhizosphere of sugar beet, bean and maize plants were collected from different locations of Sahl Eltina and Ismailia. Then, isolation of the rhizosphere bacteria was done; the used method for isolation can be summarized as follows:

Ten grams of rhizosphere soil were suspended in 90 ml of sterilized water in conical flask (250 ml), thoroughly shaken for 10 minutes, and dilution series up to 10^7 were prepared. Dilutions from each soil sample were placed in plates on King's Media (King *et al.*, 1954). Plates were incubated at $28^{\circ}\text{C} \pm 2$ for 1-3 days, and then the individual colonies were picked up, purified and microscopically examined for morphological characteristics. Isolates were maintained on nutrient agar at 4°C and subcultures of the purified isolates were done monthly.

Quantitative capability for indole acetic acid (IAA) and gibberellins (GA) production in culture media of *Paenibacillus polymyxa* (P), *Azospirillum brasilense* (A) and all rhizobacteria isolates was determined (Table 1). Procedures of Bric *et al.*, (1991) and Udagwa and Kinosheta (1961) were used for IAA and GA determinations, respectively. However, it can be obtained four rhizobacteria isolates. Plant growth promoting substances determinations in culture media of these isolates showed that *Pseudomonas synxantha* (P1) excreted the maximum amount (89.2 mg/l) of IAA, while the highest GA production (358.6 mg/L) was resulted by *Bacillus subtilis* 2 (B2). So, P1 and

Table 1: Production of indole acetic acid (IAA) and gibberellins (GA) by used bacterial strains and isolates

Inoculant	Growth promoting substance	
	IAA	GA
Bacterial strain		
<i>Paenibacillus polymyxa</i> (P)	157.8	456.3
<i>Azospirillum brasilense</i> (A)	101.4	604.7
Rhizobacteria isolate		
<i>Pseudomonas fluorescence</i>	75.33	184.20
<i>Pseudomonas synxantha</i>	89.20	247.43
<i>Bacillus subtilis</i> 1	22.11	224.93
<i>Bacillus subtilis</i> 2 (B2)	67.70	358.60

B2 bacteria were chosen as inoculants to test their effects on basil plant in this study, while the other two isolates; *i.e.*, *Pseudomonas fluorescence* and *Bacillus subtilis* 1 were canceled.

Identification of bacterial isolates:

Active isolates of P1 and B2 bacteria which produced more IAA and GA were selected and identified by Bio-log technique in Plant Path. Res. Inst., Agric. Res. Center, Ministry Agric., Giza, Egypt.

The experimental layout:

Such experiment was implicated 10 treatments. They were set as a simple experiment in a completely randomized block design with three replicates. Each replicate contained 24 plants. The 10 experimental treatments were as follows:

- 1- Control: plants were received 60 kg N/ *fad* (100% of the mineral recommended Nitrogen fertilizer dose).
- 2- Plants were received 45 kg N/ *fad* (75% of the recommended Nitrogen fertilizer dose). It was referred as: 75% N treatment.
- 3- Plants were inoculated with *Paenibacillus polymyxa* (P) bacteria and received 45 kg N/ *fad*. It was referred as: 75% N+ (P) treatment.
- 4- Plants were inoculated with mixture containing *Paenibacillus polymyxa* (P) + *Pseudomonas synxantha* (P1) bacteria and received 45 kg N/ *fad*. It was referred as: 75% N+ (P+P1) treatment.
- 5- Plants were inoculated with mixture containing *Paenibacillus polymyxa* (P) + *Bacillus subtilis* 2 (B2) bacteria and received 45 kg N/ *fad*. It was referred as: 75% N+ (P+B2) treatment.

- 6- Plants were inoculated with mixture containing *Paenibacillus polymyxa* (P) + *Pseudomonas synxantha* (P1) + *Bacillus subtilis* 2 (B2) bacteria and received 45 kg N/ *fad*. It was referred as: 75% N+ (P+P1+B2) treatment.
- 7- Plants were inoculated with *Azospirillum brasilense* (A) and received 45 kg N/ *fad*. It was referred as: 75% N+ (A) treatment.
- 8- Plants were inoculated with mixture containing *Azospirillum brasilense* (A) + *Pseudomonas synxantha* (P1) bacteria and received 45 kg N/ *fad*. It was referred as: 75% N+ (A+P1) treatment.
- 9- Plants were inoculated with mixture containing *Azospirillum brasilense* (A) + *Bacillus subtilis* 2 (B2) bacteria and received 45 kg N/ *fad*. It was referred as: 75% N+ (A+B2) treatment.
- 10- Plants were inoculated with mixture containing *Azospirillum brasilense* (A) + *Pseudomonas synxantha* (P1) + *Bacillus subtilis* 2 (B2) bacteria and received 45 kg N/ *fad*. It was referred as: 75% N+ (A+P1+B2) treatment.

The Experimental procedures

Sweet basil cultivar Grandvert was used in this study. Seeds were obtained from the Agric. Res. Center, Giza, Egypt. Seeds were sown on 15th February during the two tested seasons of 2011 and 2012 in peat moss medium in nursery beds; germination was occurred during 7-10 days. Two months after sowing for the two tested seasons, when seedling were about 15-17 cm in height and bearing 6-8 leaves, they were transplanted in plots (1.8 X 2.4 m), each had 3 rows with distance of 60 cm between rows and 30cm between plants within the row, 8 plants/ row. Chemical properties of the used experimental soil are shown in Table 2.

Table 2: Chemical properties of the experimental soil

Season	.S.S	H	E.C Mhos/ Cm	Anions (mg/ 100 g soil)				Cations (mg/ 100 soil)				Ppm	
				o ₃	co ₃	l	o ₄	a ⁺⁺	g ⁺⁺	a ⁺	+		
011	0.128	7.3	0.44	01	.65	.67	.88	.86	.46	.48	.81	2.0	2.5
012	0.200	7.8	0.65		.78	.85	.70	.23	.83	.21	.93	8.0	2.8

Fertilization treatments

Ammonium sulfate (20.5% N) was used as a source of nitrogen fertilization. Plants were received the calculated N amount according to the treatment; *i.e.*, 60 or 45 kg N/ *fad* (100% or 75% of the recommended N fertilizer dose, respectively) in two equal portions, the first was on 30 days after transplanting and the second portion was 20 days after the first one.

Additionally, all of the experimental plots were received phosphorus and potassium fertilization for encourage plant growth. Phosphorus fertilizer in the form of superphosphate (15.5% P₂O₅) at a rate of 100 kg/ *fad* was incorporated with the experimental soil of all plots during preparation, while potassium fertilizer was added in the form of potassium sulfate (48% K₂O) at the rate of 150 kg/ *fad* equally to all experimental plots before the first irrigation.

Inoculation treatments

The tested bacterial inoculants (bacterial strains and isolated bacteria) were grown to the maximum density (10⁹ cells ml⁻¹) in Difco nutrient broth medium (Difco Manual, 1984) for 24 *h*. Then, basil seeds were inoculated just before planting by dipping the seeds in bacterial cultures according to the designed treatments; each seed was received about 10⁶ cells ml⁻¹. Arabic Gum was used as adhesive agent. Seeds were left overnight for air drying before sowing in the nursery beds. After transplanting, plants were subjected to additional inoculation doses by spraying the bacterial inoculant suspensions, according to the designed treatments, thrice at 30, 60 and 90 days from transplanting. Each 100 ml of bacterial culture (10⁹ cells ml⁻¹) was diluted with tap water up to 1 liter for preparing the bacterial suspensions for spray. In designed treatments that plants inoculated with more than one bacterial inoculant, the inoculant cultures were well mixed in equal portions just prior to use them in inoculation.

Data recorded

Growing basil plants were harvested two times by cutting the vegetative parts 10-15 cm above the soil surface. The first cut was done on 15th June while the second one was on 30th July during the two tested seasons. Response of basil plants to the tested bacterial inoculations were noticed at the two cuts by recording the following data:

I. Vegetative growth:

Vegetative growth responses were recorded as plant height (cm), branches number/ plant, and herb fresh and dry weights per plant (g) and per *Faddan* (ton).

II. Essential oil determinations:

Essential oil percentage was determined in fresh herb at two cuts of the two tested seasons according to the method described by British Pharmacopoeia (1963), then oil yield/ plant (ml) and/ *Faddan* (L) were calculated. In addition, essential oil samples of the 2nd cut during the 2nd season were subjected to GLC for constituents as percentages according to the methods of Hoftman (1967) and Bunzen *et al.* (1969).

III. Chemical analysis:

Random leaf samples were taken for the two cuts in both experimental seasons and were dried at 70 °C for 72 hours to determine the leaves chemical constituents. Total nitrogen was determined using semi-micro Kjeldahl method which described by Black *et al.* (1965), total phosphorus was determined using spectrophotometer according to methods of Jackson (1973) and K was determined by using flame photometer according to Yamagnchi and Minges (1956). Also, total carbohydrates were estimated using the method described by Michel *et al.*, (1956). Generally, all N, P and K nutrients as well as total carbohydrate contents were determined as percentages in dry weight.

IV. Rhizosphere enzyme activities

Activity of Nitrogenase (N₂-ase) and dehydrogenase (DHA) enzymes were estimated in the basil rhizosphere soil at 1st and 2nd cuts for the two tested seasons. N₂-ase enzyme activity in n mole C₂H₄/ g dry rhizosphere soil/ h was determined using acetylene reduction assay (Hardy *et al.*, 1973). While, DHA enzyme activity in rhizosphere soil, in µg TPF/ g dry soil /day, was also determined using the method described by Thalmann (1967).

Statistical Analysis

Recorded data were subjected to statistical analysis and means separation was performed using the least signification difference (LSD) test at 5% level as described by little and hills (1978).

RESULTS AND DISCUSSION

Effect of some plant growth promoting rhizobacteria:

I. Vegetative growth:

Basil plants received 75% of the recommended N fertilizer dose (45 Kg N /*fad*) and inoculated with bacterial inoculants mixture containing *Azospirillum brasilense* + *Pseudomonas synxantha* + *Bacillus subtiles* 2 or *Azospirillum brasilense* + *Pseudomonas synxantha* [75% N+ (A+P1+B2) treatment or 75% N+ (A+P1) treatment] resulted in the tallest plants bearing the highest number of branches/ plant comparing to all other inoculation treatments (Table 3). These increments were significant as compare to plants received 100% of the recommended N fertilizer (60 Kg N/ *fad*) without bacterial inoculation (control). However, 75% N+ (A+P1+B2) treatment was the superior in this respect. All other inoculation treatments under 75% N fertilizer did not exceed the control treatment, also fertilized plants with 75% N and uninoculated resulted in the shortest plants with the least number of branches/

Table 3: Effect of the some plant growth promoting rhizobacteria (PGPR) on plant height and branches number of basil plant

Treatments		Plant height (cm)		Branches number/ plant	
		1 st cut	2 nd cut	1 st cut	2 nd cut
N fertilization	Bacterial inoculant*				
Season 2011					
60	Without	56.3	50.7	14.3	18.7
45	Without	33.3	30.3	10.0	12.0
45	P	42.7	40.7	10.7	13.7
45	P1	48.7	43.7	13.0	17.0
45	B2	43.3	41.0	11.7	14.0
45	P+P1+B2	52.3	50.7	14.0	17.3
45	A	42.7	40.0	11.3	14.3
45	A+P1	64.7	59.7	17.3	21.0
45	A+B2	45.7	45.0	12.0	15.3
45	A+P1+B2	68.7	66.3	18.3	27.0
LSD 0.05		2.62	3.51	1.03	2.01
Season 2012					
60	Without	49.7	48.3	13.0	16.7
45	Without	30.0	28.7	8.00	10.7
45	P	38.3	38.7	8.67	11.0
45	P1	46.7	42.7	11.7	14.3
45	B2	39.0	39.0	9.67	12.0
45	P+P1+B2	49.0	47.0	12.0	15.0
45	A	40.7	39.3	11.0	12.7
45	A+P1	61.0	58.0	14.3	19.3
45	A+B2	44.0	42.3	11.3	13.3
45	A+P1+B2	65.0	63.0	16.7	23.0
LSD 0.05		1.77	2.62	1.00	1.11

60 kg N/ *fad* = the recommended N fertilizer dose as control and 45 kg N/ *fad* = 75% of the recommended N fertilizer dose.

* P = *Paenibacillus polymyxa*, P1 = *Pseudomonas synxantha*, B2 = *Bacillus subtiles* 2 and A = *Azospirillum brasilense*.

plant. Such results were confirmed during the two cuts of the two tested seasons.

Regarding herb fresh and dry weights (Table 4), the noticed increments in plant height and branching of treated plants with 75% N+(A+P1+B2) or 75% N+(A+P1) treatments were, generally, reflected as significant increases in herb fresh and dry weights/ plant and/ *fad* as compare to other inoculation treatments or to fertilized plants with 100% N (control). This was true during the two cuts of the two tested seasons.

These results are in harmony with those obtained by Abou-Zeid (2011) on soybean and Awad alla *et al* (2013) on coriander. The obtained results in uninoculated plants were acceptable even they were lower than the other inoculated plants, this behavior may be due to the positive role of plant growth promoting rhizobacteria (PGPR). The increments in plant growth under the effect of inoculation with mixtures of more than one PGPR [(A+P1+B2) or (A+P1)] may be attributed to the vital role of such bacteria in production and accumulation of IAA and gibberellins in the plant rhizosphere, as previously mentioned in this research (Table 1). However, according to Galic (1995) and Antoun *et al.*, (1998), PGPR may be improving plant growth and increase yield productivity through different mechanisms including:

- 1- Production of secondary metabolites such as antibiotic, hydrogen cyanide and plant hormones like substances.
- 2- The production of siderophors.
- 3- Antagonism to soil borne root pathogens,
- 4- Phosphate solubilization and/ or 5- dinitrogen fixation.

As for N fertilizer effect, Sifola and Barbieri (2006) reported that an increase in the rate of applied nitrogen up to 300 kg/ *ha* contributed to a rise in the above-ground yield and fresh leaf biomass of basil plant.

II. Essential oil determinations:

1. Essential oil percentage and yield

Results in Table 5 show significant reductions in essential oil % in herb of plants received 75% N fertilizer dose without PGPR inoculation comparing to control plants which received 100% of N recommended dose. Also, data of the same Table 5, exhibit that all inoculation treatments with different tested PGPR under 75% N did not compensate the caused reduction in oil%, with exception of the triple inoculation treatment of A + P1 + B2 under 75% N or the dual inoculation treatment of A + P1 under 75% N. Since, plants treated with these two inoculation treatments resulted oil % statistically exceed or equal to that in plants fertilized with 100% N fertilizer. This was confirmed in the two cuts of the two tested seasons.

As for essential oil yield/ plant and/ *fad* (Table 5) the same previously mentioned trend in oil % was repeated in oil yield. Since, triple inoculation treatment of A + P1 + B2 under 75% N, generally, resulted significant increases in oil yield/ plant and/ *fad*. While, dual inoculation treatment of A + P1 under 75%, generally, resulted in oil yield/ plant and/ *fad* equal to as in fertilized plants with 100% N. However, the increases in oil yield under these two inoculation treatments were expected as a result of the caused increases in oil percentages and in plant growth and herb yield/ plant and / *fad* under these inoculation treatments (Tables 3&4).

Table 5: Effect of some plant growth promoting rhizobacteria (PGPR) on essential oil % and essential oil yield per plant and per *fad* of basil plant during 2011 and 2012 seasons

Treatments		Essential oil (%)		Essential oil yield/ plant (ml)		Essential oil yield/ <i>fad</i> (L)	
N fertilization	Bacterial inoculant*	1 st cut	2 nd cut	1 st Cut	2 nd Cut	1 st Cut	2 nd cut
Season 2011							
60	Without	0.293	0.313	0.592	0.705	14.2	16.9
45	Without	0.193	0.207	0.196	0.241	4.71	5.78
45	P	0.213	0.227	0.220	0.295	5.29	7.07
45	P1	0.253	0.280	0.395	0.518	9.48	12.4
45	B2	0.200	0.227	0.236	0.318	5.67	7.62
45	P+P1+B2	0.267	0.300	0.460	0.575	11.1	13.8
45	A	0.227	0.240	0.274	0.348	6.58	8.34
45	A+P1	0.327	0.340	0.703	0.781	16.9	18.8
45	A+B2	0.233	0.253	0.297	0.393	7.14	9.43
45	A+P1+B2	0.347	0.360	0.794	0.859	19.1	20.6
LSD 0.05		0.074	0.018	0.104	0.212	2.11	3.08
Season 2012							
60	Without	0.280	0.307	0.520	0.636	12.5	15.3
45	Without	0.167	0.187	0.154	0.210	3.69	5.05
45	P	0.187	0.207	0.181	0.236	4.34	5.67
45	P1	0.247	0.273	0.352	0.474	8.44	11.4
45	B2	0.193	0.213	0.216	0.272	5.18	6.53
45	P+P1+B2	0.260	0.293	0.412	0.527	9.88	12.6
45	A	0.207	0.227	0.233	0.294	5.58	7.06
45	A+P1	0.287	0.333	0.538	0.715	12.9	17.2
45	A+B2	0.227	0.247	0.258	0.350	6.19	8.39
45	A+P1+B2	0.313	0.347	0.690	0.795	16.6	19.1
LSD 0.05		0.052	0.193	0.087	0.208	1.64	2.53

60 kg N/ *fad* = the recommended N fertilizer dose as control and 45 kg N/ *fad* = 75% of the recommended N fertilizer dose.

* P = *Paenibacillus polymyxa*, P1 = *Pseudomonas synxantha*, B2 = *Bacillus subtilis* 2 and A = *Azospirillum brasilense*.

The results regarding N effects on essential oil are in accordance with those reported by Sifola and Barbieri (2006) and Nurzynska-Wierdak *et al.*, (2013). They noticed that an increases in nitrogen fertilizer amount up to 300 kg *ha* resulted in more accumulation in essential oil in sweet basil herb.

Abd-El Kader and Ghaly (2003) on coriander as well as Badran and Safwat (2004) and Badran *et al.* (2007) on fennel plants recorded similar results by applying PGPR. Banchio *et al.* (2008) reported that essential oil yield of *Origanum majorana* L. was significantly increased by inoculation treatments of *P. fluorescence* and *Bradyrhizobium sp* comparing to non-inoculated plants, without alteration in oil composition.

2-Essential oil components

Essential oil analysis of the extracted samples from basil plants of different treatments (Table 6) showed that, generally, plants received 75% N fertilizer resulted essential oil had percentages of main components lesser than that in essential oil of plants received 100% N with exceptions of Geranyl-acetate and Camphore, since they were increased with decreasing N fertilization. Such result clear that there is an indefinite role for N-fertilizer where, increasing N fertilizer gave high percentages of most essential oil components but, gave low values of Camphor and Geranyl-acetate components.

As for effect of PGPR inoculation on essential oil components, results of the same Table 6 showed that linalool, the main component of basil plant oil, was increased in plants received 75% N by inoculation with B2 or A + P1+B2 inoculants comparing to plants treated with 100% N without PGPR inoculation (control). These two inoculation treatments resulted in the highest linalool percentages (39.0 and 37.4%, respectively) comparing to the all other inoculation treatments. The highest camphor percentages (25.8 and 33.9%) were recorded in oil samples of plants received 75% N under effect of the inoculation treatments of P1 or A.

Methyl chavicol component% (Table 6), which is described as a probable carcinogen (Kaledin *et al.* 2009) and limits the application of basil oil in therapy, reached to the maximum value (5.20%) in essential oil of plants received 100% N-fertilizer, while it decreased by decreasing the N fertilizer to 75% under all inoculation treatments. The lowest percentages of Methyl chavicol (0.388% or 0.511%) were recorded in essential oil extracted from plants received 75% N and inoculated with A or P+P1+B2 inoculants, respectively. Such result was opposite to that reported by Nurzyńska-Wierdak *et al.* (2013) on sweet basil; they found that an increase in rate of nitrogen fertilizer caused a decrease in the proportion of methyl chavicol in essential oil.

Respecting the effect of the two inoculation treatments of 75% N+(A+P1) and 75% N+(A+P1+B2), which enhanced plant growth and increased herb yield and essential oil% and its yield as previously mentioned in this research (Tables 3, 4 and 5), on essential oil components, 75% N+(A+P1) treatment increased β -Pinene and Camphor percentages, while 75% N+(A+p1+B2) increased Linalool% comparing to their percentages in oil extracted from control plants (Table 6). Also, Methyl-chavicol % was decreased under the effect of these two treatments. So, under this study inoculation with some PGPR can increase basil essential production and improve its properties.

Table 6: Effect of some plant growth promoting rhizobacteria (PGPR) on basil essential oil components (%) of the 2nd cut during the 2nd season 2012

Treatments N fret. (kg/fe d [#]	Bacterial inoculan t [*]	α -Pinene	β -Pinene	1,8-cinole	Linatool	Campho r	Terpeneo l	Geranyl- acetate	Methyl- chavicol	β -Caryo- phyllene
60	without	0.65	1.85	9.48	36.20	0.20	31.70	.612	5.20	7.08
45	without	3.46	10.1	21.57	30.30	1.05	15.30	3.79	4.56	5.70
45	P	4.88	2.06	10.47	34.30	14.98	18.86	1.37	3.99	5.52
45	P1	3.70	1.95	27.20	16.44	25.80	11.52	5.03	3.34	0..23
45	B2	1.32	8.53	36.30	39.00	0.51	0.19	3.32	2.57	3.25
45	P+P1+ B2	4.87	1.96	13.64	33.00	7.12	33.00	1.25	0.51	0.21
45	A	2.84	1.71	5.63	23.62	33.90	20.22	1.32	1.39	4.97
45	A+P1	1.73	9.94	20.96	26.70	25.10	5.11	4.38	1.46	0.24
45	A+B2	5.99	10,68	28.69	36,99	10.46	0.27	0.31	2.12	0.40
45	A+P1+ B2	0.99	5.64	18.89	37.40	0.27	30.20	0.18	1.03	0.61

[#] 60 kg N/ *fad* = the recommended N fertilizer dose as control and 45 kg N/ *fad* = 75% of the recommended N fertilizer dose.

^{*} P = *Paenibacillus polymyxa*, P1 = *Pseudomonas synxantha*, B2 = *Bacillus subtilize* 2 and A = *Azospirillum brasilense*.

III. Chemical analysis:

Data represented chemical analysis (Table 7) clear that reduction N fertilization level from 100% (60kg N/ *fad*) to 75% (45kg N/ *fad*) of the recommended dose significantly reduced total carbohydrates, N, P and K percentages in basil leaf tissues. Simultaneously, applying the all tested PGPR inoculation treatments to plants which received the low N fertilizer level (75% N) resulted in increases in the abovementioned percentages comparing to plants fertilized 75% N without inoculation. The highest increases in total carbohydrates, N, P and K percentages were recorded in fertilized plants with 75% N and inoculated with inoculant mixture containing (A+P1) or (A+P1+B2). Such percentages were statistically more than (significant) or equal to (not significant) that in leaf tissues of control plants (fertilized plants with 100% N). This was confirmed in the two cuts of the two tested seasons.

The increments in total carbohydrates percentage in fertilized plants may be attributed to the structural role of N in synthesis of porphyrine molecules, which are found in many metabolically active compounds including chlorophylls and cytochrome enzymes. These increases in chlorophylls

Table 7: Effect of the some plant growth promoting rhizobacteria (PGPR) on basil leaf chemical constituents during the 2011 and 2012 seasons

Treatments		Total carbohydrates (%)		N (%)		P (%)		K (%)	
N fertilization X	Bacterial inoculant*	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
Season 2011									
60	Without	22.1	20.6	2.58	2.8	0.37	0.43	2.2	2.7
45	Without	13.2	14.3	1.68	2.1	0.22	0.23	1.2	2.0
45	P	16.8	16.1	1.93	2.4	0.31	0.31	1.6	2.2
45	P1	18.2	18.8	2.06	2.6	0.33	0.37	1.9	2.3
45	B2	17.8	17.8	2.04	2.5	0.34	0.36	1.8	2.3
45	P+P1+B	21.8	20.7	2.23	2.8	0.36	0.39	2.1	2.4
45	A	18.6	16.4	1.88	2.3	0.23	0.26	1.5	2.0
45	A+P1	23.8	22.7	2.63	2.8	0.38	0.45	2.3	2.7
45	A+B2	16.4	17.3	2.17	2.7	0.35	0.38	2.0	2.3
45	A+P1+B	25.5	28.4	2.81	2.9	0.39	0.46	2.4	2.8
LSD 0.05		1.71	4.02	0.22	0.0	0.01	0.04	0.2	0.1
Season 2012									
60	Without	22.8	23.6	2.71	2.8	0.38	0.48	2.2	2.9
45	Without	13.0	14.8	1.78	2.1	0.22	0.24	1.2	2.3
45	P	15.1	16.2	1.95	2.6	0.32	0.32	1.7	2.4
45	P1	19.5	18.8	2.18	2.7	0.34	0.38	1.9	2.5
45	B2	17.1	18.1	2.05	2.6	0.36	0.38	1.9	2.5
45	P+P1+B	23.6	20.1	2.84	2.8	0.37	0.40	2.3	2.6
45	A	18.5	17.1	1.87	2.2	0.34	0.28	2.2	2.2
45	A+P1	25.2	28.3	2.81	2.9	0.39	0.49	2.5	2.8
45	A+B2	18.2	17.0	2.21	2.8	0.35	0.39	2.4	2.6
45	A+P1+B	26.5	28.1	2.79	2.9	0.40	0.49	2.6	2.9
LSD 0.05		1.50	1.73	0.09	0.4	0.01	0.01	0.1	0.0

60 kg N/ fad = the recommended N fertilizer dose as control and 45 kg N/ fad = 75% of the recommended N fertilizer dose.

* P = *Paenibacillus polymyxa*, P1 = *Pseudomonas synxantha*, B2 = *Bacillus subtilis* 2 and A = *Azospirillum brasilense*.

and cytochrome enzymes might be in turn results in an increase in photosynthesis rate and promote carbohydrates synthesis and accumulation (Devlin, 1975).

As for PGPR effects on carbohydrates accumulation, Abou-zeid (1993) found that total soluble carbohydrate % was decreased in inoculated wheat, but increased in rice grain with *A. brasilense*.

Increases N, P and K percentages after PGPR inoculation treatments are in harmony with the results obtained by Fallik *et al.*, (1994) and (Soudi *et al.*, 2008). The enhancing effect of PGPR treatments on N, P and K absorption may be referred to their influence on increasing the availability of such nutrients via production of plant growth regulators at the root interface, which may stimulate root development and resulted in better absorption of water and nutrients from soil and increased plant growth which enhance the absorption of nutrients from soil (Ordookhani *et al.*, 2010). However, there is some controversy regarding the mechanisms that PGPR employs for uptake of minerals. Many investigators suggested that phytohormones promote uptake of minerals by plant roots due to increase of root surface area, thickness and length (Biswas *et al.*, 2000). Whitelaw *et al.* (1997) stated that phosphate-solubilizing *Bacillus spp* stimulates plant growth through enhancing P absorption.

IV. Rhizosphere enzyme activities

Nitrogenase enzyme (N_2 -ase) activity in basil rhizosphere in n mole C_2H_4 /g dry rhizosphere soil / h and dehydrogenase (DHA) enzyme activity in rhizosphere soil in μg TPF/g dry soil /day in the 1st and 2nd cuts for two tested seasons are presented in Table 8.

With respect to DHA activity, results indicate that the inoculation with all PGPRs under this study significantly reduced DHA enzyme activity, where the maximum values were obtained when plants were fertilized with 75% of N without inoculation followed by 100% N. Inoculation with *Azospirillum* + 75% of N led to the minimum values of DHA. However, opposite results were found by Zaghoul *et al.*, (2007) on tomato and Abou-zeid (2011) on soybean. They stated that seeds inoculation with both of *Bacillus polymyxa* and *Bacillus megatherium* led to significant increases in DHA activity in the rhizosphere soil.

Concerning N_2 -ase activity, data of the same Table 8 indicate that N_2 -ase activity was significantly increased by inoculation with the most tested PGPR. The highest values of N_2 -ase activity were recorded in the rhizosphere soil of plants inoculated with *Azospirillum* + 75% N in the first season and that inoculated with P1 under 75% N in the second season. The lowest values of N_2 -ase activity were found when plants inoculated with A + P1+ B2 with 75% N during the two tested seasons.

Obtained results of uninoculated treatments were acceptable since, they were lower than the other inoculated treatments, this behavior could be

Table 8: Effect of the some plant growth promoting rhizobacteria (PGPR) on activity of nitrogenase and dehydrogenase enzymes in basil soil rhizosphere during 2011 and 2012 seasons

Treatments		Dehydrogenase enzyme activity (n mole C ₂ H ₄ /g dry soil/h)		Nitrogenase enzyme activity (µg TPF/g dry soil/day)	
N fertilization n kg/ fed [#]	X Bacterial inoculant [*]	1 st Cut	2 nd Cut	1 st Cut	2 nd Cut
		Season 2011			
60	Without	163.6	243.6	55.29	21.94
45	Without	217.2	262.7	34.90	43.86
45	P	55.27	116.4	79.05	48.86
45	P1	96.35	150.4	130.87	174.64
45	B2	48.08	98.0	30.28	26.90
45	P+P1+B2	74.46	124.1	18.34	54.29
45	A	46.45	67.70	434.43	55.20
45	A+P1	72.81	100.1	198.12	51.90
45	A+B2	120.4	226.8	59.46	30.13
45	A+P1+B2	101.9	156.8	18.86	19.87
LSD 0.05		25.74	51.29	15.01	24.46
Season 2012					
60	without	187.54	343.2	53.32	27.18
45	without	226.89	343.0	42.12	30.91
45	P	65.45	130.1	85.10	39.81
45	P1	107.31	169.1	163.24	222.65
45	B2	92.52	126.1	28.78	21.21
45	P+P1+B2	138.32	194.5	12.46	56.47
45	A	45.99	73.6	210.53	71.31
45	A+P1	63.98	139.5	180.21	55.21
45	A+B2	115.53	188.2	72.32	43.51
45	A+P1+B2	121.15	176.8	22.71	28.91
LSD 0.05		26.16	26.73	21.17	15.34

[#] 60 kg N/ fed = the recommended N fertilizer dose as control and 45 kg N/ fed = 75% of the recommended N fertilizer dose.

^{*} P = *Paenibacillus polymyxa*, P1 = *Pseudomonas synxantha*, B2 = *Bacillus subtilis* 2 and A = *Azospirillum brasilense*.

due to the positive role of native bacteria inhabiting soil among several decades ago (Kloepper and Schorth, 1981), also the presence of active nitrogenase enzyme and DHA enzyme for uninoculated plants is owing to the indigenous bacteria in soil.

Conclusively, from results of such research, it could be conclude that inoculation of *Ocimum basilicum* plant with inoculant bacterial mixture containing equal portions of *Azospirillum brasilence* + *Pseudomonas synxantha* or *Azospirillum brasilence* + *Pseudomonas synxantha* + *Bacillus subtilis* 2 can be reduce the demand of nitrogen mineral fertilization up to 75% of the recommended dose (45 kg N/ fed instead of 60 kg N/ fed). Since, these two inoculation treatments compensated the mineral N fertilizer and increased plant growth, herb yield and essential oil yield. Also, inoculation treatments improved medicinal properties of basil herb and its essential oil as compare to herb and oil of 100% N fertilized plants without PGPR inoculation.

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استجابة نمو نبات الريحان والمحصول والمكونات الكيميائية لبكتيريا الريزوسفير المشجعة لنمو النبات

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الزراعية – القاهرة – مصر .

أجريت تجربتان حقليتان خلال الموسمين المتتاليين من عام ٢٠١١ و ٢٠١٢
لدراسة تأثير التلقيح ببعض سلالات بكتيريا الريزوسفير المشجعة لنمو النبات
(PGPR) و هي *Paenibacillus polymyxa* و *Azospirillum brasilence*

وبعض عازلات من الـ *Pseudomonas sp* , *Bacillus subtilis* تحت تركيز ٧٥ و ١٠٠٪ من سماد النيتروجين المعدني على النمو ونسبة ومحصول الزيت وامتصاص العناصر الغذائية من نبات الريحان الحلو cultivar Grandvert. تم تلقيح البذور بهذه البكتيريا ثم تم رش النباتات بالمعلق البكتيري ثلاث مرات بعد الزراعة.

أشارت النتائج أن التلقيح مع هذه PGPR تسبب زيادة كبيرة في طول النبات، والأوزان الرطبة والجافة، وعدد الأفرع / نبات، ونسبة ومحصول الزيت وكذلك الكربوهيدرات الكلية (%). ومحتويات P،N وK في أوراق نباتات صنف الريحان الحلو تحت الدراسة. أعلى زيادة في هذه المقاييس التي تم الحصول عليها عند النباتات الملقحة بـ *Azospirillum brasillense* (A) + *Pseudomonas synxantha* (B2) + *Bacillus subtilis* 2 (B2) في وجود ٧٥٪ من N المعدني، تليها معاملة A + P1 مع التسميد بـ ٧٥٪ N ثم الكنترول الغير معامل. تم الحصول على أدنى متوسط لجميع المقاييس للخالعات خلال موسمي تحت هذه الدراسة عندما سممت النباتات فقط ٧٥٪ من N. بينما تلقيح النباتات بـ *Azospirillum brasillense* + *Pseudomonas synxantha* (P1) + *Bacillus subtilis* (B2) (A) في وجود ٧٥٪ من السماد N أعطى أعلى نسبة ارتفاع في هذه المقاييس.

وفيما يتعلق بنشاط انزيم النيتروجينيز والديهيدروجينيز في ريزوسفير نباتات الريحان، تشير النتائج إلى أن التلقيح مع هذه PGPR تقلل بشكل ملحوظ نشاط انزيم DHA، حيث تم الحصول على أقصى قدر من DHA عندما تم تلقيح النباتات مع ٧٥٪ من N بدون تلقيح تليها ١٠٠٪ N في حين نشاط انزيم النيتروجينيز زاد معنوياً بـ التلقيح مع هذه PGPR.

وبالنسبة لمكونات الزيت الأساسية (%). أظهرت البيانات ان مكون اللينالول (Linalool) زاد بتلقيح النباتات بـ P + B2 أو A + P1 + B2 في وجود ٧٥٪ من السماد N مقارنة بالنباتات المعاملة بـ ١٠٠٪ N. مكون الميثيل شافيكول (Methyl chavicol) وصل إلى أدنى قيمة (0.388%) عندما تم تلقيح النباتات مع *Azospirillum* ثم زاد إلى ٥١١٪ مع *P. polymyxa* + *Pseudomonas synxantha* + *Bacillus subtilis* 2 تحت ٧٥٪ من سماد N.

التوصية: يمكن القول أن تطبيق PGPR يوصى به بديلاً لاستخدام الأسمدة الكيميائية لزيادة الإنتاجية والجودة لنبات الريحان وتحسين خصائص الدواء لمواجهة الاستهلاك المحلي والتصدير.