# INFLUENCE OF PLANT GROWTH PROMOTING BACTERIA (PGPB) ON CORIANDER (Coriandrum sativum L.) AND DILL (Anethum graveolens L.) PLANTS

Hegazi, M. A. \*; M. M. S. Metwaly \*\* and E. B. Belal\*\*\*

\*Department of Horticulture, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt.

\*\*Agricultural Botany Dep., (Agricultural Botany) Faculty of Agriculture, Kafrelsheikh Univ., 33516, Kafr El-Sheikh, Egypt.

\*\*\*Agricultural Botany Dep., (Agricultural Microbiology) Faculty of Agriculture, Kafrelsheikh Univ., 33516, Kafr El-Sheikh, Egypt.

#### **ABSTRACT**

Field experiments were conducted at the experimental Farm of the Faculty of Agriculture, Kafrelsheikh University during 2013/2014 and 2014/2015 seasons to study the effect of *Azotobacter chrocooccum* and *Pseudomonas* sp. on coriander (*Coriandrum sativum*, L.) and dill (*Anethum graveolens*, L.) plants. Indole-3-acetic acid in the supernatant of a culture *A. chrocooccum* and *Pseudomonas* sp. was detected. It was shown that Indole-3-acetic acid was induced by the presence of tryptophan. The highest concentration of Indole-3-acetic acid was produced by both strains at the end of the logarithmic phase.

The bacterial strains were applied at the time of sowing as seed treatment. Data showed that *A. chrocooccum* surpassed *Pseudomonas* sp. in most vegetative growth and flowering characters (plant height, number of leaves/ plant, herb fresh and dry weights/ plant and umbels and umbellts number/plant), while *Pseudomonas* sp. gave the highest weight of 100 fruits, fruit yield per plant and per Feddan for both plants, respectively. The highest total chlorophyll contents were obtained when coriander and dill were treated with *Pseudomonas* sp. and *A. chrocooccum*, respectively. *Pseudomonas* sp. gave the best results for total carbohydrates, vitamin C, fresh herb and dry fruit oil % and fruits oil yield/ plant compared to *A. chrocooccum* or control treatments.

Coriander fruit treatments with *A. chrocooccum* and *Pseudomonas* sp. increased anatomical parameters such as xylem vessel diameter, thickness of phloem tissue and vascular bundle as well as vascular bundle width comparing with the control treatment. The obtained results exhibited that, these bacterial strains could be used to increase coriander and dill productivity under field conditions.

**Keywords:** Coriander and dill plants, Plant growth promoting bacteria, Indole-3-acetic acid, vegetative growth, anatomical parameters.

#### INTRODUCTION

Plant growth-promoting bacteria (PGPB) include both free living and symbiotic bacteria, typically found in the soil, that facilitate the growth and development of plants (Glick et al., 1999). This can directly promote plant growth by facilitating the uptake of nutrients from the soil. Thus, (PGPB) can directly facilitate the proliferation of plants by fixing atmospheric nitrogen, producing siderophores which can mineral solubilize and provide it to plants, synthesizing phytohormones which can enhance various stages of plant growth, solubilizing minerals such as phosphorus and synthesizing enzymes that can modulate plant growth and development (Glick, 2007).

Nitrogen fixation and plant growth promotion by plant growth promoting bacteria are important criteria for an effective bio-fertilizer. Inoculation of associative and free living N<sub>2</sub>-fixing bacteria has been shown to produce beneficial effects on plant growth, (Bashan and Holguin, 1998). Azotobacter sp besides fixing nitrogen it is also secreting certain growth hormones such as IAA, GA and Cytokinin (Coppola, et al., 1971) which promote vegetative growth and root development.

The ability to produce the plant hormone indole-3-acetic acid (IAA) is widespread among soil, epiphytic, and tissue-colonizing bacteria (Costacurta and Vanderleyden 1995; Patten and Glick 1996; Barazani and Friedman. 1999). These genera from bacteria comprise Azospirillum, Azotobacter, Rhizobium, Bradyrhizobium. Enterobacter. Xanthomonas. Serratia, Pseudomonas, cyanobacteria and sulfur oxidizing bacteria. These bacteria have shown to encourage plant growth, by promoting the out-break of secondary roots, acting as protectors against phytopathogenic microorganisms via plant hormones release and siderophores (Tien et al., 1979; Fett et al., 1987; Zimmer and Bothe 1988; Sekine et al., 1989, Minamisawa and Fukai, 1991; Gamliel and Katan 1992; Amstroen, et al., 1993; Bar and Okon, 1993; Glick, 1995; Patten and Glick, 1996). Auxins are known to affect much process in plant including cell elongation and adventitious root formation (Trigiano and Grav. 1996).

Coriander (*Coriandrum sativum*, L.) and dill (*Anethum graveolens*, L.) belong to family Apiaceae (Umbelliferae) and believed to have their beginnings in the Mediterranean region. The plants have a long and ancient history in many countries as culinary and medicinal herbs. The earliest known record of dill as a medicinal herb was found in Egypt 5,000 years ago when the plant was referred to as a "soothing medicine." Gladiators were fed meals covered with dill because it was hoped that the herb would grant them valor and courage (The Herb Society of America 2009).

Green coriander and dill are two of the most important aromatic crops in the entire world. There are two products of both plants that are used for human nutrition: fresh green herb and seeds (fruits). The two herbs are consumed fresh in soups and salads, food dressing and as flavoring ingredient. The green herbs are also employed for stem-distilled essential oil, which can be used in flavoring and aroma industries like perfumes, sops and creams. Herbs leaves are good source of vitamin "A" and "C" as well as minerals i.e Ca, K, P, Fe and Mn. As medical plants, herbs have been used to treat stomach disorders, intestinal complaints, colic, fatigue and indigestion (Guenther, 1961 and Fenaroli, 1971).

The present study was designed to produce bio-auxin (IAA) from Azotobacter chrocooccum and Pseudomonas sp. as well as asses their influence on growth, chemical constituents, essential oil yield and anatomical characteristics of coriander (Coriandrum sativum, L.) and dill (Anethum graveolens, L.).

# MATERIALS AND METHODS

# Source of microorganisms:

Two bacterial strains (*Azotobacter chrocooccum* and *Pseudomonas* sp. were isolated and identified in previous study (unpublished data) and were used in improvement of wheat plants under drought-stressed conditions (El-Afry et al., 2012 a and b)

## Indole-3-acetic acid production:

One hundred ml of Jensen's or king's B liquid medium supplemented with tryptophan (0.1g/l) with 1ml of a cell suspension of Azotobacter chrocooccum and Pseudomonas sp. (Jensen's broth medium, 10<sup>7</sup>cfu/ml, incubated at 30°C and 150 rpm for 3days for Azotobacter chrocooccum or king's B liquid medium10<sup>7</sup>cfu/ml, incubated at 30°C and 150 rpm for 3days for Ps eudomonas sp. respectively. The culture was incubated at 30°C and 150 rpm for 5days. The production of indole-3-acetic acid was determined daily by colorimetric analysis method as described below. The growth representing in cell number of the bacterial strain was determined by plating appropriate dilutions of liquid medium onto YMA medium.

# Colorimetric analysis:

After centrifugation (6000 rpm.30 min), the liquid portion of an aliquot of liquid medium was used to determine of Indole-3-acetic acid (IAA) by the method described by Glickman and Dessaux (1995); Ahmad, et al., (2005); El-Mahrouk and Belal, (2007) and the developed (30min) color was measured by spectrophotometer at 530nm. Concentrations were calculated from an adjusted calibration curve.

Effect of seed treatment with both bacterial strains on growth, chemical constituents, essential oil yield and histological parameters of coriander and dill plants under field conditions. Concentration of total chlorophyll pigment µg/g fresh weight was determined according to (Moran, 1982).

#### Cultivation of microorganisms:

Azotobacter chrocooccum and Pseudomonas sp. were cultivated in nutrient liquid medium. 200 ml nutrient liquid medium and inoculated with 2 ml of a cell suspension of (Azotobacter chrocooccum or Pseudomonas sp. (nutrient broth medium, 10<sup>8</sup> cfu / ml) was incubated at 30°C and 150 rpm for 3 days. The cultures were incubated at 30°C and 150 rpm for 5 days. Thereafter, two bacterial strains were applied on wheat as follows:

Field experiments were conducted at the experimental farm of the Faculty of Agriculture, Kafrelsheikh University during 2012 -13 and 2013-14 seasons to study the effect of two bacterial strains (*Azotobacter chrocooccum* and *Pseudomonas* sp.) on growth of coriander (*Coriandrum sativum*, L.) and dill (*Anethum graveolens*, L.) plants.

#### Seeds treatments:

Two bacterial strains were applied at the time of sowing as seeds treatment. Seeds were witted with 10 % sugar syrup, and thoroughly mixed with an amount of bacterial suspension (10<sup>8</sup> cfu / ml) for 30 min. enough to obtain 10<sup>8</sup> cfu / per gram of seeds and then dried.

Coriander and dill fruits were sown on 25<sup>th</sup> October in plots (5 \* 3 meters). Each treatment represented by three plots and each plot consisting of three rows. Twenty five plants in each row (20 cm apart) were planted. All the plants received natural agricultural practices whenever needed. Two months after sowing, plants were harvested by cutting 4-5 mm above soil surface, and then plants reharvested 2 additional harvests at monthly intervals. At each harvesting time, fresh and dry weights of herb were recorded according to Gabal *et al.*, (1984).

Essential oil percentage in both fresh herb and dry seeds were determined by hydro-distillation in Clevenger's apparatus for 3 and 5h, respectively according to the Egyptian Pharmacopoeia (1984) then, the essential oil yield was calculated. Both total chlorophyll and Carbohydrates and vitamin "C" were determined. The obtained data were statistically analyzed according to Snedecor and Conchran, (1982).

Histological parameters:

The stems specimens were taken from the middle of the third internode of the plant stem base. Specimens were taken on day 30<sup>th</sup> after sowing. Specimens were fixed in formalin, alcohol and acetic acid mixture (FAA, 1: 18: 1; v/v), washed and dehydrated in alcohol series. The dehydrated specimens were infiltrated and embedded in paraffin wax (52-54 °C in. p.). The embedded specimens were sectioned using a rotary microtome (Leica RM 2125) at a thickness of 8 – 10 µm. Sections were mounted on slides and deparaffinized. Staining was accomplished with safranin and light green combination, cleared in xylene and mounted in Canada balsam (Ruzin, 1999). Ten readings from 3 slides were examined with electric microscope (Lieca DM LS) with digital camera (Lieca DC 300), and then photographed. The investigated histological features of the stem were thickness of either vascular bundle or phloem tissue as well as diameter of xylem vessels.

## RESULTS AND DISCUSSION

By using specific medium supplemented with tryptophan (0.1 g/l) for Azotobacter chrocooccum and Pseudomonas sp. in a culture medium containing tryptophan as inducer for IAA, produced IAA, as detected by Glickmann and Dessaux, (1995) and El-Mahrouk and Belal, (2007), while the same strains did not produce IAA in the same medium without tryptophan. Most species use tryptophan to produce indole-3-acetic acid (IAA), mainly through indole-3-pyruvic acid and tryptamine pathways (Bar and Okon, 1993). The highest accumulation of IAA exhibited in the 48th hours of cultivation in the supernatant of A. chrocooccum and Pseudomonas sp. (Fig 1 and Fig. 2). The maximum accumulation of IAA occurred at the end of logarithmic phase, and after that the accumulation of IAA decreased at the beginning of the stationary growth phase. IAA accumulation coincided with increase in the specific growth rates of the cultures.

Figs. 1 and 2. Illustrate that IAA formation started when A. chrocooccum and Pseudomonas sp. grew on the medium supplemented with tryptophan. The highest accumulation of IAA exhibited in the 48th hours of

cultivation. The maximum accumulation of IAA occurred at the end of logarithmic phase, and after that the accumulation of IAA decreased at the beginning of the stationary growth phase. IAA accumulation coincided with increase in the specific growth rates of the cultures. Most species use tryptophan to produce indole-3-acetic acid (IAA), mainly through the indole-3-pyruvic acid and tryptamine pathways (Bar and Okon, 1993). The results obtained in this work provide useful information about the production behavior of IAA which is of importance for instance for the application in production of coriander and dill plants.

Our results are in agreement with previous findings reported by (Ernsten et al., 1987, Fukuhara, et al., 1994. Glickmann and Dessaux, 1995; Torres-rubio et al., 2000), who found that addition of tryptophan in the growth medium led to production of IAA by many bacterial strains. Several different IAA biosynthetic pathways are used by prokaryotes, and a single bacterial strain can contain more than one pathway. Manulis et al., (1998) reported that indole -3-acetic acid (IAA) biosynthetic pathways detected in Erwinia herbicola pv. gypsophilae. iaaM, iaaH, and ipdC are genes encoding tryptophan-2-monoxygenase, indole-3-acetamide hydrolase, and indole-3-pyruvate decarboxylase, respectively.

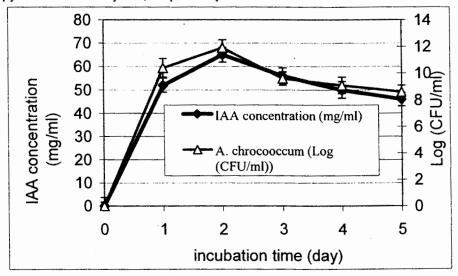


Fig. (1). IAA production by *Azotobacter chrocooccum* in submerged culture with tryptophan

Effect of seed treatment with the both bacterial strains on growth, chemical constituents, essential oil yield and histological parameters of coriander (*Coriandrum sativum*, L.) and dill (*Anethum graveolens*, L.) plants under field conditions.

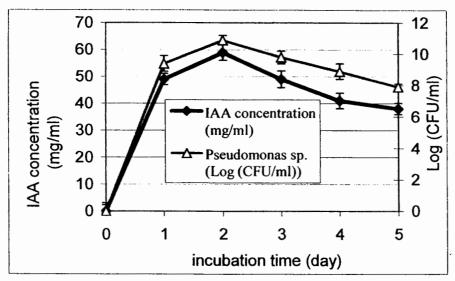


Fig. (2). IAA production by *Pseudomonas* sp. in submerged culture with tryptophan.

# Vegetative growth and herb yield:

Data presented in Tables (1 and 2) show that growth parameters (plant height, number of leaves per plant) of coriander and dill plants increased gradually from the first to third cut, both treatments Azotobacter chrocooccum and Pseudomonas sp surpassed control whereas, Azotobacter chrocooccum surpassed Pseudomonas sp in the three cuts for the two plants. As for herb fresh and dry weights, data show that both treatments Azotobacter chrocooccum and Pseudomonas sp surpassed control for the two plants. Azotobacter chrocooccum treatment surpassed Pseudomonas sp treatment and second cut surpassed both first and second one.

Table (1): Effect of plant growth promoting bacteria on plant height and leaves number of coriander (*Coriandrum sativum*, L.) and dill (*Anethum graveolens*, L.) (Combined analysis of two seasons).

Treatments		Pla	ınt heig	ght		Le		aves No.	
			(cm)				/ plan	t	Mean
		1 <sup>st</sup> cut	2cut	3 <sup>nd</sup> cut		1 <sup>st</sup> cut	2cut	3 <sup>nd</sup> cut	
	Control	9.45i	10.33h	11.50g	10.43c	6.31i	6.86h	7.10g	6.75c
Coriander	A. chrocooccum	15.82e	18.11c	20.64a	18.19a	8.38c	8.75b	8.80a	8.64a
(Coriandrum	Pseudomonas sp.	14.50f	16.77d	19.43b	16.90b	7.52f	7.79e	8.23d	7.85b
sativum L.)	Mean	13.26c	15.07b	17.19a		7.40c	7.80b	8.04a	
	Control	26.88i	31.76h	47.53e	35.39c	3.22i	4.06h	4.53g	3.94c
Dill	A. chrocooccum	35,11f	60.29c	63.23b	52.88a	5.60e	6.03d	6.85b	6.16b
(Anethum graveolens L.)	Pseudomonas sp.	33.25g	57.84d	66.14a	52.41b	5.32f	6.75c	7.40a	6.49a
	Mean	31.75c						6.26a	

Means within each column followed by the same letter are not statistically different at 0.05 level (Duncan's range test)

Table (2): Effect of plant growth promoting bacteria on Herb fresh and dry weights of coriander (*Coriandrum sativum*, L.) and dill (*Anethum graveolens*, L.) (Combined analysis of two seasons).

Treatments						Herb	Herb dry weight		
					Mean				Mean
		1 <sup>st</sup> cut	2cut	3 <sup>nd</sup> cut		1 <sup>st</sup> cut	2cut	3 <sup>nd</sup> cut	
	Control	4.05i	5.21g	4.36h	4.54c	1.09h	1.25g	1.03i	1.12c
Coriander	A. chrocooccum	8.11e	9.52a	9.23b	8.95a	1.65d	1.96a	1.89b	1.83a
(C. sativum L.)	Pseudomonas sp.	7.52f	8.42c	8.22d	8.06b	1.51f	1.77c	1.64e	1.64b
	Mean	6.56b	7.72a	7.27c		1.42c	1.66a	1.52b	
	Control	3.86h	5.22g	3.85i	4.31c	0.68i	0.77g	0.73h	0.73c
Dill (A. graveolens L.)	A. chrocooccum	10.43 c	13.49a	8.69d	10.87a	2.16c	2.57a	2.00d	2.24a
	Pseudomonas sp.	8.38e	10.55b	6.33f	8.42b	1.88e	2. <b>4</b> 2b	1.71f	2.01b
	Mean	7.55b	9.75a	6.29c		1.57b	1.92a	1.48c	

Means within each column followed by the same letter are not statistically different at 0.05 level (Duncan's range test)

#### Flowering and fruit characters and yield:

Data in Table (3) revealed that, flowering characters (umbels and umbellts numbers) were the highest when *Azotobacter chrocooccum* used compared to *Pseudomonas* sp and control for both coriander and dill as recorded 33.20 and 6.41, respectively for coriander and 33.26 and 23.40, respectively for dill. The heaviest weight of 100 fruit (1.43g for coriander and 2.62g for dill) were recorded with *Pseudomonas* sp. As for plant fruit yield, the obtained data varied in the case of dill from that obtained from coriander plant. *Pseudomonas* sp gave the highest coriander fruit yield (6.25g/ plant) whereas *Azotobacter chrocooccum* gave the highest dill fruit yield/ plant (5.60 g/ plant). The highest fruit yield /feddan for both coriander (237.52kg/fed) and dill (490.07kg/fed) resulted from *Pseudomonas* sp. treatment.

Table (3): Effect of plant growth promoting bacteria on umbels number, umbllets number/umbel, weight of 100 fruit and fruit yield per plant and per fadden of coriander (Coriandrum sativum, L.) and dill (Anethum graveolens, L.) (Combined analysis of two seasons)

CAA	u stasulisj.					
Treatments		Umbels No. /plant	Umbelits No. / umbel	Weight of 100 fruit (g)	Fruit yield /plant (g)	Fruit yield (kg/fed.)
	Control	22.88c	5.44c	0.86c	1.26c	137.61c
Coriander	A. chrocooccum	34.63a	7.29a	1.36b	5.78b	235.10b
(C. sativum L.)	Pseudomonas sp.	33.20b	6.41b	1.43a	6.25a	237.52a
Dill	Control	21.00c	13.55c	1.25c	1.55c	283.65c
(A. graveolens L.)	A. chrocooccum	33.26a	23.40a	2.58b	5.60a	487.53b
	Pseudomonas sp.	30.05b	20.81b	2.62a	4.89b	490.07a

Means within each column followed by the same letter are not statistically different at 0.05 level (Duncan's range test)

#### Chemical constituents:

The results in the Table (4) revealed that, chemical components of both plants greatly influenced with both Azotobacter chrocooccum and Pseudomonas sp.compared to control. Second cut of Pseudomonas sp treatment of coriander plant exceeded all in total chlorophyll contents as gave 1.33  $\mu$ g/g fresh weight whereas, in the case of dill plants the third cut of Azotobacter chrocooccum treatment was the best as recorded 1.51  $\mu$ g/g fresh weight.

Table (4): Effect of plant growth promoting bacteria on total chlorophyll μg/g of coriander (Coriandrum sativum, L.) and dill (Anethum graveolens, L.) (Combined analysis of two seasons).

Gedeenej.		T.4	1 - 1-1	- h11	
Treatme	Tota (µg/g	Mean			
	1 <sup>st</sup> cut	2cut	3 <sup>nd</sup> cut	1	
	Control	1.22i	1.25h	1.29f	1.25c
Cariandar	A. chrocooccum	1.26g	1.34c	1.33d	1.31b
Coriander (coriandrum sativum L.)	Pseudomonas sp.	1.32e	1.40a	1.37b	1.36a
(Conandidin Salivum L.)	Mean	1.27b	1.33a	1.33a	
	Control	1.25i	1.29h	1.33g	1.29b
Dill	A. chrocooccum	1.42e	1.55c	1.62a	1.20c
(Anethum graveolens L.)	Pseudomonas sp.	1.38f	1.43d	1.58b	1.46a
	Mean	1.35c	1.42b	1.51a	

Means within each column followed by the same letter are not statistically different at 0.05 level (Duncan's range test)

Table (5): Effect of plant growth promoting bacteria on total carbohydrates and vitamin "C" percentages of coriander (Coriandrum sativum, L.) and dill (Anethum graveolens, L.) (Combined analysis of two seasons).

(Combined analysis of two scasons).									
Treatments		Total carbohydrates			Vitamin "C" Mean %			Mean	
		1 <sup>st</sup> cut	2cut	3 <sup>nd</sup> cut		1stcut 2cut 3ndcut			
	Control	13.88i	14.56h	15.11f	14.52c	33.29i	34.77h	36.21g	34.76c
Cariandar	A. chrocooccum	14.79g	15.22d	16.03b	15.36b	38.55e	43.38d	44.60b	42.18a
Coriander (C. sativum	Pseudomonas sp.	15.13e	15.66c	16.14a	15.64a	36.81f	44.59c	44.82a	<b>4</b> 2.07b
E.,)	Mean	14.60c	15.15b	15.76a		36.22c	40.91b	41.88a	
5:11	Control	12.75d	13.73b	13.35c	13.28b	32.70i	35.30g	35.70f	34.57c
Dill (A. graveolens	A. chrocooccum	13.64bc	13.81ab	13.99ab	13.81a	35.24h	39.18d	40.03b	38.15b
	Pseudomonas sp.	13.80ab	13.92ab	14.12a	13.95a	36.33e	39.78c	41. <b>16a</b>	39.09a
F,	Mean	13.40b	13.82a	13.82a		34.76c	38.09b	38.96a	

Means within each column followed by the same letter are not statistically different at 0.05 level (Duncan's range test)

As for total carbohydrates, data in Table (5) show that third cut of coriander plant treated with *Pseudomonas* sp gave the highest percentage (16.14%), whereas, there was no significant difference among *Azotobacter chrocooccum* and *Pseudomonas* sp. through out the three cuts. The lowest percentage was in recorded control treatment. The third cut of *Pseudomonas* 

sp. treatment gave the highest vitamin C percentage for both studied plants as recorded 44.82 % for coriander and 41.16% for dill plant compared to *Azotobacter chrocooccum* or control.

#### **Essential oil contents:**

Data in Table (6) revealed that, fresh herb oil percentage of both coriander and dill was the highest during the second cut of *Pseudomonas* sp treatment as gave 0.247 and 0.278%, respectively compared to *Azotobacter chrocooccum* or control. The highest dry fruits oil and fruits oil yield per plant resulted from *Pseudomonas* sp treatment for both studied plants as gave 0.371% and 0.232ml for coriander and 0.542% and 0.248ml for dill plant.

Table (6): Effect of plant growth promoting bacteria on fresh herb and dry fruits oil percentage and fruits oil yield of coriander (Coriandrum sativum, L.) and dill (Anethum graveolens, L.) (Combined analysis of two seasons).

Treatments			Fresh herb oil %		Mean	Dry fruits oil %	Fruits oil Yield/plant (ml)
		1 <sup>st</sup> cut	2cut	3 <sup>nd</sup> cut			
	Control	0.095i	0.110g	0.107h	0.104c	0.236c	0.102c
Coriander	A. chrocooccum	0.175f	0.236b	0.215d	0.209b	0.364b	0.225b
(C. sativum L.)	Pseudomonas sp.	0.189e	0.247a	0.223c	0.219a	0.371a	0.232a
(C. Sallvulli L.)	Mean	0.153c	0.197a	0.182b			
	Control	0.129i	0.143g	0.132h	0.135c	0.388c	0.185c
Dill	A. chrocooccum	0.188f	0.245c	0.229d	0.221b	0.526b	0.221b
(A. graveolens L.)	Pseudomonas sp.	0.212e	0.278a	0.256b	0.249a	0.542a	0.248a
(A. graveoleris L.)	Mean	0.176c	0.222	0.206b			

Means within each column followed by the same letter are not statistically different at 0.05 level (Duncan's range test)

# **Anatomical study:**

Data presented in Table (7) and Fig. (3) illustrated that, coriander seed treatments with *A. chrocooccum* and *Pseudomonas* sp. individually have a positive impact on coriander plant stem anatomical features in the present investigation. Where, led to an increase in xylem vessel diameter and thickness of phloem tissue, vascular bundle and vascular bundle width, as compared with control treatment. These results are in agreement with Metwaly, (2012) and El-Afry *et al.*, (2012 a and b) on wheat plants.

Table (7): Effect of plant growth promoting bacteria on anatomical features of coriander (*Coriandrum sativum*, L.) plant stem.

Treatments		Xylem vessel diameter µm	Phloem tissue thickness µm	Vascular bundle width µm	Vascular bundle thickness µm	
	Control	35.00 <sup>B</sup>	31.67 <sup>8</sup>	93.33 <sup>C</sup>	183.30 <sup>c</sup>	
Coriander (C. sativum L.)	A. chrocooccum	56.67 <sup>A</sup>	46.67 <sup>A</sup>	133.33 <sup>A</sup>	245.00 <sup>8</sup>	
	Pseudomonas sp.	48.33 <sup>A</sup>	45.00 <sup>A</sup>	115.OO <sup>B</sup>	231.67 <sup>B</sup>	

Means within each column followed by the same letter are not statistically different at 0.05 level (Duncan's range test)

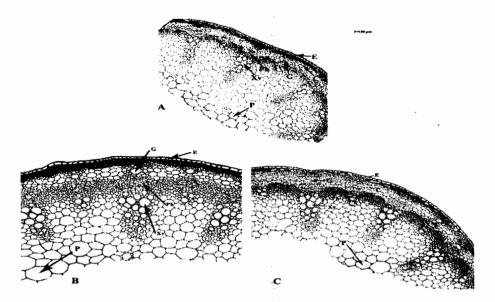


Fig. (3) Transverse sections through the coriander plant stem as affected by fruits soaking application with plant growth promoting bacteria.

Where:-

A: Control (untreated plant).

B: Azotobacter chrocooccum.

C: Pseudomonas sp.

E: Epidermis

G: Gland

Xv: Xylem vessels

Ph: Phloem tissue

P: Pith

C: Cortex

Microbial inoculants that can promote plant growth and productivity is internationally accepted as an alternative source of N-fertilizer. It is environmental friendly and can be used to ensure a sustainable coriander and dill production. In this bio-fertilizer technology new systems are being developed to increase the biological  $N_2$  fixation (BNF) with cereals and other non-legumes by establishing  $N_2$ -fixing bacteria within the roots (Cocking, 2000). Nitrogen fixation and plant growth promotion by plant growth promoting bacteria are important criteria for an effective bio-fertilizer.

Inoculation of associative and free living N<sub>2</sub>-fixing bacteria have been shown to produce beneficial effects on plant growth, thus they are termed plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1980; Bashan and Holguin, 1998). Significant increases in crop yields following application of PGPR have been documented under diverse field conditions (Bashan, 1998). They have been widely reported to fix atmospheric nitrogen with grasses and cereals (Dobereiner, 1997) and enhance nutrient uptake (Lin et al., 1983; Murty and Ladha, 1988; Bashan and Holguin, 1998).

Azotobacter sp besides fixing nitrogen also secrete certain growth hormones such as IAA, GA and Cytokinins (Coppola *et al.*, 1971) which promote vegetative growth and root development.

## CONCLUSION

From the foregoing results, it may be concluded that inoculation coriander (Coriandrum sativum, L.) and dill (Anethum graveolens, L.) plants with A. chrocooccum and Pseudomonas sp. under field conditions improved the growth parameters vegetative growth and flowering characters (plant height, number of leaves/ plant, herb fresh and dry weights/ plant and umbels and umbellts number/plant), while Pseudomonas sp. gave the heaviest weight of 100 fruits, fruit yield per plant and per Feddan for both plants, respectively. The highest total chlorophyll contents were obtained when coriander and dill were treated with Pseudomonas sp. and A. chrocooccum, respectively. Pseudomonas sp. gave the best results for total carbohydrates, vitamin C, fresh herb and dry fruit oil % and fruit oil yield/ plant as compared to A. chrocooccum or control treatments. Coriander seed treatments with A. chrocooccum and Pseudomonas sp. improved anatomical parameters such as xylem vessel diameter, thickness of phloem tissue, vascular bundle and vascular bundle width comparing with the control treatment. The obtained results exhibited that these bacterial strains could be used to increase coriander and dill productivity under field conditions.

# REFERENCES

- Ahmad, F.; I. Ahmad and M. S. Khan (2005). Indole Acetic Acid Production by the Indigenous Isolates of Azotobacter and Fluorescent Pseudomonas in the Presence and Absence of Tryptophan. Turk. J. Biol. 29: 29 34.
- Amstroen, B; A. Gustafsson and B. Gerhardson (1993). Characteristics of a plant deleterious rhizosphere pseudomonad and its inhibitory metabolite (s). J. Appl. Bac-teriol.74:20 28.
- Bar, T and Y. Okon (1993). Tryptophan conversion to indole-3-acetic acid via indole-3-acetamide in Azospirillum brasilense Sp7. Can. J. Microbiol. 39:81 - 86.
- Barazani, O. and J. Friedman (1999). Is IAA the major root growth factor secreted from plant-growth-mediating bacteria. J. Chem. Ecol. 25:2406.
- Bashan, Y. (1998). Inoculants of plant growth promoting rhizobacteria for use in agriculture. Biotechnol. Adv., 16: 729 770.
- Bashan Y. and G. Holguin (1998). Proposal for the division of plant growth promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. Soil Biol. Biochem., 30: 1225 1228.
- Cocking, E. C. (2000). Helping plants get more nitrogen from air. European Rev., 8: 193 200.
- Coppola, S; G. Percuoco; A. Zoina and G. Picci (1971). Citochinine in germi terricoli e relativo significato nei rapporti piante-microrganismi. Ann. Microbiol. Enzimol. 21, 45 53.
- Costacurta, A. and J. Vanderleyden (1995). Synthesis of phytohormones by plant associated bacteria. Crit. Rev. Microbiol. 21:1 18.

- Dobereiner, J. (1997). Biological nitrogen fixation in the tropics: social and economic contributions. Soil Biol. Biochem., 29: 771 774.
- Egyptian Pharmacopeia (1984). General Organization for Governmental Printing Affairs, Cairo.p.31.
- El-Afry, M. M.; M. F. El-Nady; E. A. Belal and M. M. S. Metwaly (2012a). Anatomical studies on drought-stressed wheat plants (*Triticum aestivum* L.) treated with some bacterial strains. Acta Biologica Szegediensis, 56(2):165 174.
- El-Afry, M. M.; M. F. El-Nady; E. A. Belal and M. M. S. Metwaly (2012b). Physiological responses of drought stressed wheat plants (Triticum aestivum L.) treating with some bacterial endophytes. J. plant protection, Mansoura Univ., 3 (7): 2069 2089.
- El-Mahrouk, M.E. and E. B. A. Belal (2007). Production of Indole Acetic Acid (bioauxin) from *Azotobacter* sp. isolate and effect it on Callus induction of *Dieffenbachia maculata* cv. Marianne. Acta Biol. Szeged. 51(1):.53 59.
- Ernsten A.; G. Sandberg; A. Crozier and C. T. Wheeler (1987). Endogenous indoles and the biosynthesis and metaboism of indole-3-acetic acid in cultures of *Rhizobium phaseoli*. Planta 171:42 428.
- Fenaroli, G. (1971). Fenaroli's hand-book of Flavour ingredients. 136 138.
- Fett, W. F.; S. F. Osman and M. F. Dunn (1987). Auxin production by plant pathogenic pseudomonads and xanthomonas. Appl. Environ. Microbiol. 53:1839 1845.
- Fukuhara, H.; Y. Minakawa; S. Akao and K. Minamisawa (1994). The involvement of indole-3-acetic acid produced by *Bradyrhizobium elkanii* in nodule formation. Plant Cell Physiol. 35:1261 1265.
- Gabal, M. R.; I. M. Abd-Allah; F. M. Hass and S. Hassannen (1984). Evaluation of some American tomato cultivars grown for early summer production in Egypt, Annals of Agriculture Science Moshtohor, 22: 487 - 500.
- Gamliel, A. and J. Katan (1992). Chemotaxis of fluorescent *Pseudomonas* towards seed exudates and germinating seeds in solarized soil. Ecol. Epidemiol. 82:328 332.
- Glick, B. R.; L. Changping; G. Sibdas and E. B. Dumbroff (1999). Early development of canola seedlings in the presence of the plant growth promoting rhizobacterium Pseudomonas putida GR12 2. Soil Biol. Biochem., Vol.29, pp. 1233 1239.
- Glick, B. R. (1995). The enhancement of plant growth by free living bacteria. Can. J. Microbiol. 41:109 114.
- Glic, B. R. (2007). Promotion of plant growth by soil bacteria that regulate plant ethylene levels. Proceedings 33rd PGRSA Annual Meeting.15 – 21.
- Glickmann, F. and Y. Dessaux (1995). A critical Examination of the Specificity of the Salkowski Reagent for Indolic Compounds Produced by Phytopatogenic Bacteria. Appl. Eniron. Microbiol. 61(2), 793 796.
- Guenther, E. S. (1961). The essential oils. Vol. 1, 3 and 4, D. Van Nostr and Princeton, N. J., New York, 18 178.

- Kloepper, J. W.; J. Leong; M. Teintze and M. N. Schorth (1980). Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature, 286: 885–886.
- Lin, W.; Y. Okon and R. W. Hardy (1983). Enhanced mineral uptake by Zea mays and Sorghum bicolor roots inoculated with Azospirillum brasilense. Appl. Environ. Microbiol. 45: 1775 1779.
- Manulis, S.; A. Haviv-Chesner; M. T. Brandl; S. E. Lindow and I. Barash (1998). Differential Involvement of Indole-3-Acetic Acid Biosynthetic Pathways in pathogenicity and Epipytic Fitness of *Erwinia herbicola pv. Gypsophilae*. Mol. Plant-Microbe Interact. 11(7): 634 642.
- Metwaly, M. M. (2012). Ecophysiological and anatomical responses of drought stressed wheat plants (*Triticum aestivum* L.) treating with some bacterial endophytes. Ph.D.thesis, Faculty of Agriculture, Kafr-El Sheikh University, Kafrelsheikh, Egypt.
- Minamisawa, K. and K. Fukai (1991). Production of Indole-3-Acetic Acid by *Bradyrhizobium japonicum*: A Correlation with Genotype Grouping and Rhizobitoxine Production. Plant and Cell Physiol., Vol 32, No. 1:1 9.
- Moran, R. (1982). Formulae for determination of chlorophllous pigments extracted with N, N-Dimetheylformamide. Plant Physiology, 69: 1376 1381.
- Murty, M. G. and J. K. Ladha (1988). Influence of *Azospinllum* inoculation on the mineral uptake and growth of rice under hydroponics conditions. Plant Soils, 108: 281–285.
- Patten, C. L. and B. R. Glick (1996). Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol. 42:207 220.
- Ruzin, S. E. (1999). Plant microtechniques and microscopy. First Ed. Oxford University press, USA.
- Sekine, M.; K. Watanable and K. Syono (1989). Molecular cloning of a gene for indole-3-acetamide hydrolase from *Bradyrhizobium japonicu*m. J. Bacteriol. 171:1718 1724.
- Snedecor, G.W. and W.G. Cochran (1982). Statistical Methods. 6th Edn., Iowa State University Press, Ames, USA., p. 593.
- The Herb Society of America ©2009/Text taken from The Herb Society of America's Essential Guide to Dill.
- Tien, T.; M. Gaskins and D. Hubbel (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of Pearl Millet (*Pennisetum americanum* L.). Appl. Environ. Microbiol. 37:1016 1024.
- Torres-Rubio, M. G.; S. A. Valencia-Plata; J. Bernal-Ccastillo and P. Martinez-Nieto (2000). Isolation of Enterobacteria, *Azotobacter* sp. and *Pseudomonas* sp., Producers of Indole-3-Acetic Acid and Siderophores, from Colombian Rice Rhizosphere. Revista Latinoamericana de Microbiologia.42:171 176.
- Trigiano, R. N. and D. J. Gray (1996). Plant Tissue Culture Concepts and Laboratory Exercises. CRC press. Inc., pp.11 71.
- Zimmer, W. and H. Bothe (1988). The phytohormonal interaction between *Azospirillum* sp. and wheat. Plant Soil 110:239 247.

ت أثير البكتيريا المشجعة لنمو النبات (PGPB) على نباتات الكزبرة (Anethum graveolens L.) والشبت (Anethum graveolens L.) محمدود عبد النبسي حجدازي\* ، متدولي محفدوظ سسالم متدولي السيد بلال عبد المنطلب بلال\*\*\*

\* قسم البساتين ، كلية الزراعة ، جامعة كفر الشيخ، ٣٣٥١٦ - كفر الشيخ، مصر.

\*\* النبات الزراعي ، قسم النبات الزراعي ، كليةً الزراعة ، جامعة كفر الشيخ، ١٦ ٥ ٣٣- كفر الشيخ، ١٦ ٥ ٣٣- كفر الشيخ، مصر.

\*\*\*الميكروبيولوجيسا الزراعية ، قسم النبات الزراعي ، كلية الزراعة ، جامعة كفر الشيخ، ٢٥٥٦ - كفر الشيخ،

أجريت تجارب حقلية في المزرعة التجريبية بكلية الزراعة - جامعة كفر الشيخ خلال موسمي نمو ٢٠١٤/٢٠١٢ و ٢٠١٥/٢٠١٤ لدراسة تأثير كل من بكتيريا الأزتوباكتر كروكووكم موسمي نمو Azotobacter chrocooccum وبكتيريا بسيدوموناس. Azotobacter chrocooccum على كل من نبات الكزبرة والشبت. وقد أظهرت النتائج أنه قد تم تحديد الاندول أسيتيك أسيد في معلق لمزرعة لكل من بكتيريا الأزتوباكتر كروكووكم Azotobacter chrocooccum وبكتيريا بسيدوموناس Pseudomonas sp. وقد أوضحت النتائج أنه يتم تحفيز إنتاج الاندول أسيتيك أسيد في وجود النربتوفان. وقد وجد أيضا أن أعلى تركيز من الأندول أسيتيك أسيد للسلالتين كان عند نهابة الطور اللوغاريةمي.

وقد تم معاملة البنور بكل من السلالات البكتيرية أثناء وقت الزراعة، وأظهرت النتائج أن بكتيريا الأزتوباكتر كروكوكم A. chrocooccum تفوقت على بكتيريا بسيدوموناس As والزوريا الأزتوباكتر كروكوكم Pseudomonas sp. في معظم الصفات الخضرية والزهرية (طول النبات، عدد الأوراق / نبات، الأوزان الطازجة والجافة للعشب / نبات، عدد النورات والنويرات / نبات) ، في حين أن بكتيريا بسيدوموناس Pseudomonas sp. أعطت أتقل وزنا لمحصول مائة ثمرة والثمار لكل نبات ولكل فدان لكل النباتين ، على التوالى.

قد تم الحصول على أعلى محتوى للكلوروفيل الكلي كل من الكزبرة والشبت عند معاملتها ببكتيريا بسيدوموناس .pseudomonas sp وبكتيريا الأزتوباكتر كروكووكم . A chrocooccum على التوالي. وقد أعطت بكتيريا بسيدوموناس .pseudomonas sp افضل النتانج للكربو هيدرات الكلية، فيتامين س، النسبة المنوية لزيت الثمار الجافة والعشب الأخضر ومحصول زيت الثمار لكل نبات مقارنة بمعاملات بكتيريا الأزتوباكتر كروكووكم . A chrocooccum أو الكونترول.

A. وقد وجد أن معاملة بنور الكزبرة بكل من بكتيريا الأزتوباكتر كروكووكم A. المتعدد وجد أن معاملة بنور الكزبرة بكل من بكتيريا الأزتوباكتر كروكووكم pseudomonas sp. التشريحية مثل قطر أوعية الخشب، سمك نسيج اللحاء، سمك الحزمة الوعائية بالاضافة الى عرض الحزمة الوعائية مقارنة بمعاملة الكونترول. كما أظهرت النتائج التي تم الحصول عليها أنه يمكن إستخدام هذه السلالات البكتيرية لزيادة إنتاجية كل من الكزبرة والشبت تحت ظروف الحقل.