

EVALUATION OF SELECTED *Azospirillum* SP. ISOLATES FOR IAA PRODUCTION AND THEIR POTENTIAL IMPACT ON IMPROVING GROWTH, YIELD AND FRUIT QUALITY OF 'ANNA' APPLE TREES

Gabr, M. A.* and M. Nour El-Din**

* Hort. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt

** Soils, Water and Environ. Res. Inst., ARC, Giza, Egypt

ABSTRACT

Foliar spray with the diluted PGPR (plant growth promoting rhizobacteria) cultures had utmost importance due to its content and release of stimulants, nutrients, antibiotics, biocides and siderophores. Potentiality of these microorganisms, in plant rhizosphere, in activation and improving of plant growth as well as increasing plant tolerance to different plant biotic and abiotic stresses have been proved. However, microbial types and even microbial strains varied for their potentiality to adapt, inhabit and release of stimulants and phytochemicals.

A number of *Azospirillum* sp. isolates were isolated from phyllosphere of apple, orange, lemon, mango and guava trees, grown at El-Bostan area, Egypt. These isolates were used in spray of apple trees with the dose of 20 and 40 L/feddan compared to water spray control. Foliar spray of apple trees with all *Azospirillum* isolates notably induced the plant growth and increased fruit yield, but did not significantly affected fruit quality. However, the used isolates largely varied in their efficiency and potentiality. A3 isolate was the superior followed by A7 which attained the highest increases in shoot length, shoot diameter, leaf area, leaf dry weight leaf content of chlorophyll a, b and total chlorophyll as well as fruit yield over those of control treatment. But, the quality parameters, i. e., SSC (soluble solids content), acidity, firmness and color did not show consistent significant variations. The treatments of spray with *Azospirillum* isolates attained high increase in net return (L E/fed), the spray with the treatments of A7D (isolated from mango phyllosphere and used with the rate of 40 L/fed.) and A7R, which isolated from mango phyllosphere and used with the rate of 20L / fed.) were the superior treatments which achieved increases in net return over that of control (water spray) by 6120 and 6200 L E/fed respectively, followed by A3R (isolated from phyllosphere of apple and applied with 20 L/fed.) which gave 5595 L E/fed. It is noted that the microbial culture dose of 40 L/fed had no significant differences than the application of 20 L/fed. Therefore, we recommend the spray with an efficient *Azospirillum* isolate like A3 or A7 with the dose of 20 L/fed, and it is also of importance to condense studies on isolation and evaluation of these microorganisms to select the most efficient strains for use, as inoculants, in spray of apple trees.

INTRODUCTION

The Egyptian economy needs to be improved; this requires us to find out practical and applicable solutions for improving plant yield. The foliar spray with PGPR bacteria had been proved efficiency for enhancing plant growth and yield of different crops (Esitken *et al.*, 2009; Sekar and Kandavel, 2010 and Ryu *et al.*, 2011). Saharan and Nehra (2011) attributed the enhancement effect of PGPR to their direct effect in releasing plant growth hormones, nitrogen fixation, increase of plant potentiality to absorb nutrient elements and release of siderophores, which chelate Fe and making it

available for plant use. Moreover, the PGPR containing ACC (1-Aminocyclopropane-1-Carboxylate) deaminase are present in various soils and offer promise as a bacterial inoculum for improvement of plant growth, particularly under unfavorable environmental conditions such as flooding, heavy metals, phytopathogens, drought and high salt (Belimov *et al.*, 2001). Also, production of biotoxins and antibiotics by these PGPR gave the plant high potentiality to resist pathogens (Anith and Momol, 2004), in addition spray with PGPR filling phyllosphere area at the expense of harmful microbes.

It was found that the potentiality of PGPR types in inducing plant growth varied from type to another and from strain to another in the same species (Dursun *et al.*, 2010). The ability of the IAA production, antibiotic synthesis and N₂-fixation are variable (Fernando *et al.*, 2005; Aslantas *et al.*, 2007 and Saharan and Nehra, 2011). PGPR was found also to modify the plant hormones status (Dodd *et al.*, 2010). Therefore, it is of importance to select high effective strains had potential influence in increasing plant growth which ,consequently, will reflects on the agricultural economics and national income.

Therefore, the present investigation aims to study the potentiality of different *Azospirillum* isolates, which isolated from the phyllosphere of different fruit trees on producing IAA and enhancing apple tree growth, productivity and fruit quality.

MATERIALS AND METHODS

Materials:

Microbial media:

Azospirillum: Semi solid Döbereiner medium (Döbereiner *et al.*,1976) contained (g/liter of distilled water): Malic acid, 5.0; KH₂PO₄ , 0.4; K₂HPO₄, 0.1; MgSO₄. 7H₂O, 0.2; NaCl, 0.1; CaCl₂.7H₂O, 0.02; FeCl₃.6H₂O, 0.01; Na₂MoO₄. 2H₂O, 0.002; agar, 1.75.

This study has been carried out on eight years old "Anna" apple trees budded on Malus rootstock during 2009 and 2010. Trees were grown at Elbostan region of Elbehira Governorate, where drip fertigation system was applied and soil texture analysis was shown in Table 1.

Table 1: Some chemical and physical analysis characteristics of the experimental soil

Sand %	Silt %	Clay %	Texture	O.M %	pH	EC (ds m ⁻¹)			
80.2	8.5	11.3	Sandy clay loam	0.63	8.1	1.19			
Cations (mg./L)			Anions (mg./L)			Macro-nutrient (mg/kg)			
Na ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ⁻⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻⁻	N	P	K
5.89	10.42	3.91	-	1.37	12.41	6.44	139	7	86

Methods:

The *Azospirillum* bacteria were isolated from phyllosphere of different fruit trees (lemon, guava, apple, orange and mango). The liquid culture of the different isolates was used for spray the experimental apple trees and the densities ranged between 4 to 5.5 X 10⁸ cfu/ml culture. Treatments were

arranged in a random order on the selected trees. Single tree plot with 3 replicates for each treatment was arranged in random complete blocks design.

Bacterial strains were tested for their capability to produce indole acetic acid (IAA) (Bric *et al.*, 1991)

All trees were subjected with common regional horticultural practices, while treatments were applied as follows in Table (2):

Treatment	Description
A1R (Lemon)	Spray with <i>Azospirillum</i> isolated from lemon phyllosphere, 20L / feddan.
A 1D (Lemo)	Spray with <i>Azospirillum</i> isolated from lemon phyllosphere, 40L / feddan.
A 2R (Guava)	Spray with <i>Azospirillum</i> isolated from guava phyllosphere, 20L / feddan.
A 2D (Guava)	Spray with <i>Azospirillum</i> isolated from guava phyllosphere, 40L / feddan.
A 3R (apple)	Spray with <i>Azospirillum</i> isolated from apple phyllosphere, 20L / feddan.
A 3D (apple)	Spray with <i>Azospirillum</i> isolated from apple phyllosphere, 40L / feddan.
A 4R (Orange)	Spray with <i>Azospirillum</i> isolated from orange phyllosphere, 20L / feddan.
A 4D (Orange)	Spray with <i>Azospirillum</i> isolated from orange phyllosphere, 40L / feddan.
A 7R(Mango)	Spray with <i>Azospirillum</i> isolated from mango phyllosphere, 20L / feddan.
A 7D (Mango)	Spray with <i>Azospirillum</i> isolated from mango phyllosphere, 40L / feddan.
Control	Spray with water.

The microbial inoculants were prepared in Soil Bacteriology Laboratory of Sakha Agricultural Research Station, ARC. Bacterial suspension was diluted by mixing 20 L or 40 L of bacterial stocks with 600 L of water per feddan.

Three branches, five years old, in different directions on each tree were selected and labeled to estimate growth parameters. All current shoots developed on these branches were measured to get shoot length (cm). Li-Core-3100 Areameter was used to measure detached leaves of nine shoots (three shoots per branch) to get area per leaf (cm²). Leaves were dried at 70°C and weighed to get dry weight (mg) and then specific leaf weight (SLW) was calculated as (mg cm⁻²).

Spectrophotometer was used to estimate chlorophyll a and chlorophyll b, which extracted from fresh leaves with di-methyl formamide (DMF) as described by Rami and Porath (1980). The concentration of chlorophyll a and chlorophyll b and its total value were calculated by Rami's formulas as (µg / ml) (Rami, 1982). The results were presented as (mg.cm⁻²).

Fruits were picked at maturity stage, weighed and counted. Fruit pulp texture (firmness) was recorded by using Lfra texture analyzer instrument. The results were expressed as a resistance force of the fruit to the penetrating tester (g/cm²) according to Harold (1985). Fruit skin color measurements (a*, b*, L* & H°) were determined using Minolta colorimeter (Minolta Co. Ltd., Japan). The instrument estimated skin color of fruits with color metric CIE Lab method where L* measure lightness scale readings and the two coordinates a* and b* included. Positive values of a* is a measure of redness and becomes greenish measure when values changed into negative, while b* of yellowness and blueness (- b*) on the Hue circle. The Hue angle [H° = arc tan (b*/a*)] describe the relative amounts of redness and yellowness where point at 0°/360° is defined for red/magenta, 90° yellow, 180° for green and 270° for blue color (McGuire, 1992 and Voss, 1992).

Soluble solids content (SSC) was determined by using a hand refractometer and total acidity percentage was estimated in filtered juice according to A.O.A.C. (1990).

Statistical analysis:

Data obtained were subjected to the analysis of variance and treatment means were compared using the L.S.D. methods according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Carefully considering readings of Table 3, spraying with different *Azospirillum* isolates that have been isolated from phyllosphere of different tree species i.e. apple, orange, lemon, mango and guava, the foliar spray of apple trees with these isolates positively affected shoot length with varied degrees, and this trend was consistent throughout both seasons of study, but the differences than water spray control treatment were significant at the second season, only. The spray with A3D isolate gave the highest difference throughout both studied seasons.

Table 3: Response of vegetative growth of "Anna" apple trees to spray with phyllosphere *Azospirillum* isolates.

Treatments	Shoot length (cm)		Shoot diameter (cm)		Leaf dry weight (g)		Leaf area (cm ²)		SLW (mg cm ⁻²)	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
A1R (Lemon)	57.8 c	54.7 a	1.6 a	1.5 def	0.36 cde	0.33 c-g	34.5 b	32.5 a	10.6 ab	10.1 c
A1D (Lemon)	59.5 bc	55.7 a	1.7 a	1.5 cd	0.38 bcd	0.35 b-e	36.6 ab	34.4 a	10.2 b	10.1 bc
A2R (Guava)	56.8 c	54.1 a	1.6 a	1.4 f	0.33 efg	0.30 efg	26.0 cd	24.3 bc	12.6 a	12.2 abc
A2D (Guava)	58.3 c	54.5 a	1.6 a	1.5 def	0.35 def	0.32 d-g	27.7 cd	25.3 b c	12.5 ab	12.6 abc
A3R (apple)	67.4 a	63.2 a	1.7 a	1.6 bc	0.41 ab	0.38 ab	38.4 ab	35.9 a	10.6 ab	10.6 abc
A3D (apple)	76.9 a	64.5 a	1.7 a	1.6 ab	0.42 a	0.39 a	39.3 a	36.8 a	10.7 ab	10.2 bc
A4R (Orange)	57.0 c	55.1 a	1.6 a	1.5 def	0.36 cde	0.33 e-g	29.7 c	25.9 bc	12.8 a	12.7 ab
A4D (Orange)	57.8 c	54.9 a	1.6 a	1.5 de	0.36 cde	0.34 b-f	28.7 cd	27.4 b	12.4 ab	12.2 abc
A7R (Mango)	61.9 bc	59.1 a	1.7 a	1.6 bc	0.39 a-d	0.36 a-d	36.4 ab	33.9 a	10.6 ab	10.5 abc
A7D (Mango)	64.9 ab	61.0 a	1.7 a	1.7 a	0.39 bc	0.37 abc	37.4 ab	34.9 a	10.5 ab	10.5 abc
C (water spray)	56.4 c	54.0 b	1.5 b	1.4 b	0.30 g	0.28 d	24.4 d	22.3 g	12.5 ab	12.5 a-d

AR: *Azospirillum* spray with 20 L / feddan; AD: *Azospirillum* spray with 40 L / feddan, SLW : Specific leaf weight.

Means with different letters within the same column differ significantly at P < 0.05.

Data of shoot diameter pursued the same approach of shoot length which similarly increased due to the foliar application of *Azospirillum* isolates. The increases over control mostly were significant at the second season. Likewise, the spray treatments attained big and consistent increments in leaf dry weight and leaf area at both seasons, the differences than control, mostly, were significant. It is also noted that the application of A3 isolate achieved the highest values, i. e., 0.42 and 0.39 g/leaf, and 39.3 and 36.8 cm² for leaf area, respectively. While, the spray treatments did not affect the SLW values, whereas, all the differences than control, at both seasons, were not significant.

The spray of apple trees with the different bacterial strains had an effective role in increasing shoot length and diameter, leaf area and leaf dry weight compared to control. These results are in harmony with those of Eissa *et al.* (2007) who indicated that the spray of pear trees with *Saccharomyces cerevisia* had a stimulated effect on tree growth. While, Aslantas *et al.* (2007) reported that applications with different types of PGPR lead to significant increases in shoot length and diameter and they attributed this effect to the potentiality of the bacteria for releasing cytokinins and IAA. They found also that plant growth responses were variable and dependent on bacterial strain. These results agreed also with the results of the present study which revealed that the different isolates of *Azospirillum* had varied stimulation potentiality to the plant growth and the best isolate was that isolated from phyllosphere of apple (A3).

The readings of Table 4 showed that foliar spray with *Azospirillum* isolates increased leaf chlorophyll content. Whereas, chlorophyll a showed high and significant increases over control, the influence at the first season higher than those of the second season. Similarly, the spray treatments attained remarkable increases in chlorophyll b content, the increases were obvious at first season and the most differences than control were significant. The total chlorophyll content exhibited the same trend.

Table 4: Response of chlorophyll content of "Anna" apple leaves to spray with phyllosphere *Azospirillum* isolates.

Treatments	Chlorophyll a (mg cm ⁻²)		Chlorophyll b (mg cm ⁻²)		Total chlorophyll (mg cm ⁻²)	
	2009	2010	2009	2010	2009	2010
A1R (Lemon)	4.89 a	4.93 ab	2.40 a	2.30 cde	7.29 a	7.07 bc
A1D (Lemon)	4.57a	4.68 bc	2.50 a	2.35 bcd	7.37 b	7.04 c
A2R (Guava)	4.36 a	4.69 bc	2.20 a	2.08 ef	6.57 d	6.20 e
A2D (Guava)	4.30 a	4.12 ef	2.20 a	2.06 ef	6.60 d	6.24 e
A3R (apple)	4.95 a	4.18 ef	2.08 a	2.58 ab	7.71 a	7.36 ab
A3D (apple)	4.90 a	4.78 ab	2.78 a	2.61 a	7.69 a	7.33 abc
A4R (Orange)	4.60 a	4.72 abc	2.37 a	2.21 def	6.98 c	6.59 d
A4D (Orange)	4.66 a	4.38 de	2.37 a	2.28 def	7.03 c	6.71 d
A7R (Mango)	5.12 a	4.21 ef	2.62 a	2.47 abc	7.87 a	7.43 a
A7D (Mango)	5.15 a	4.96 a	2.62 a	2.48 abc	7.78 a	7.41 a
C (water spray)	2.96 b	4.09 f	1.56 b	2.14 def	6.52 d	6.23 e

AR: *Azospirillum* spray with 20 L / feddan; AD: *Azospirillum* spray with 40 L / feddan.
Means with different letters within the same column differ significantly at P < 0.05.

Whereas, leaf total chlorophyll content increased with high and significant values due to spray with the different *Azospirillum* isolates. The application of isolates used in treatments A3 and A7 attained the highest values of total chlorophyll content.

Spray of apple trees with *Azospirillum* isolates significantly increased the contents of chlorophyll a, b, and total chlorophyll of leaves, these results are harmony with those of Eissa (2003) who found that spray of apricot with dry yeast extract increased chlorophyll contents of leaves. This may attributed to the release of beneficial compounds as polyamines (Babalola, 2010), which were found to increase chlorophyll a, b and carotenoids (Taha and Eid, 2011).

The beneficial effect of spray with *Azospirillum* isolates reflected, also, on the fruit yield (Table 5) whereas, number of fruits/tree notably increased. The fruits weight (kg/tree), at the both seasons was increased too, with high consistent and significant values compared to control treatment. The highest productivity (45.93 and 43.23 kg/tree) were recorded for A3D treatment and 45.30 and 41.96 kg/tree for A3R treatment followed by A7D and A7R treatments, which attained 45.20 and 41.93 kg/tree, and 44.43 and 41.06 kg/tree at seasons 2009 and 2010, respectively. The results of IAA values of the studied *Azospirillum* isolates followed the same trend of fruit yield, whereas, the highest IAA values were achieved by application with A3 treatment (3.45 µg/ml of culture) followed by A1 and A7 which exhibited, to some extent, similar values i. e. 2.85 and 2.76 µg/ml respectively. The treatment of A4 was exhibited the lowest IAA release that estimated 1.81 µg/ml of culture.

The present study showed that spray with all *Azospirillum* isolates significantly increased yield of apple fruits per tree, and the spray with the isolate A3 ,which isolated from apple leaves, were absolutely the best followed by the isolate which isolated from mango leaves. These results are completely agreed with those of Esitken *et al.* (2004) who reported that spray of apricot with *Bacillus* OSU-142 increased fruit yield. Similarly, Eissa (2003) reported that the spray with EM resulted in an increase in number and weight of "Kelsey" plum fruits/tree. Also, Eissa *et al.* (2007) indicated that the spray of pear trees with *Saccharomyces cerevecia* had a stimulated effect and increased number and weight of fruits. Martinez-Viveros *et al.* (2010) summarized the mechanisms of PGPR action on plant growth as follow: the plant growth stimulation by PGPR is the net result of multiple mechanisms of action:

- 1- Microorganisms having mechanisms that facilitate nutrient uptake or increase nutrient availability as fix of nitrogen or solubilizing phosphates and mineralize organic compounds.
- 2- Production of phytohormones is now considered to be one of the most important mechanisms by which many rhizobacteria enhance plant growth, like IAA.
- 3- Regulate plant ethylene levels, the high accumulation of ethylene leads to poor roots growth leads a diminished ability to acquire water and nutrients.

4- Can provide biocontrol of diseases or insect pests (biopesticides) via production of antibiotics, siderophores, HCN, hydrolytic enzymes (chinases, proteases, lipases... etc.).

The present study (Table 5) indicated that the different isolates released IAA differently and A3 isolate had the highest level of IAA production and also tree fruit yield, followed by isolate A7.

Table 5: Response of "Anna" apple yield to spray with *Azospirillum* isolates and the concentration of IAA in culture

Treatments	Fruit weight / tree (kg)		Fruit number / tree		IAA (µg/ml)
	2009	2010	2009	2010	
A1R (Lemon)	44.00 ab	40.73abc	340a-d	312ab	2.85
A1D (Lemon)	44.06ab	40.96abc	313def	314ab	
A2R (Guava)	40.60b	37.36c	298def	265de	1.98
A2D (Guava)	41.36ab	37.80bc	301def	270de	
A3R (apple)	45.30a	41.96ab	367ab	335a	3.45
A3D (apple)	45.93a	43.23a	374a	336a	
A4R (Orange)	43.10ab	39.43abc	323c-f	255def	1.81
A4D (Orange)	43.46ab	40.20abc	327b-e	286de	
A7R(Mango)	44.43ab	41.06abc	368ab	275de	2.76
A7D (Mango)	45.20a	41.93ab	362abc	301de	
C (water spray)	33.73c	32.13d	292ef	291de	-

AR: *Azospirillum* spray with 20 L / feddan; AD: *Azospirillum* spray with 40 L / feddan. Means with different letters within the same column differ significantly at P < 0.05.

Data presented in Table 6 showed the effect of foliar spray with *Azospirillum* isolates on some determinations of apple fruit quality. The different treatments ,in general, did not exhibit significant influence on juice soluble solids content (SSC) percentage and firmness. While, the effect of different isolates on fruit acidity percentage was varied. In spite of incidence of decrease of fruit acidity due to the spray with *Azospirillum*, A1 isolates attained significant increase, whilst not reached significance in case of A3 isolate.

Table 6: Response of "Anna" apple fruit quality to spray with phyllosphere *Azospirillum* isolates

Treatments	SSC %		Acidity %		Firmness (g/cm ²)		Color	
	2009	2010	2009	2010	2009	2010	2009	2010
A1R (Lemon)	12.30d	12.63d	0.90 a	0.86 a	237.0abc	274.3a	89.16ab	82.85ab
A 1D (Lemon)	12.43cd	12.76cd	0.89a	0.86 a	236.0abc	269.3a	88.25abc	83.090ab
A 2R (Guava)	12.43cd	12.80bcd	0.887a	0.853ab	225.3bc	234.3bc	92.103a	86.52a
A 2D (Guava)	12.53bcd	12.73d	0.87 ab	0.84 abc	236.0abc	241.3abc	90.17ab	86.57a
A 3R (apple)	12.80abc	13.16abc	0.81cd	0.78 b-e	243.3abc	256.6ab	83.17bcd	78.25bc
A 3D (apple)	12.80abc	13.16abc	0.82bc	0.79 bcd	246.3abc	258.3ab	82.18b-e	77.81bc
A 4R (Orange)	12.90ab	13.20ab	0.74 e	0.71 ef	243.3abc	295.3ab	80.23c-f	71.57cd
A 4D (Orange)	12.93ab	13.26a	0.75 cde	0.71 def	246.6abc	258.6ab	73.9 ef	69.68d
A 7R(Mango)	12.93ab	13.33a	0.73 e	0.69 f	262.0a	274.3a	71.67 f	66.70d
A 7D (Mango)	13.00a	13.40a	0.723e	0.68 f	262.6a	274.3a	72.26 f	67.52d
C (water spray)	13.03a	13.03a-d	0.77 cde	0.77 cde	220.6c	220.6c	90.34ab	90.34a

AR: *Azospirillum* spray with 20 L / feddan; AD: *Azospirillum* spray with 40 L / feddan, SSC: soluble solids content.

Means with different letters within the same column differ significantly at P < 0.05.

On the other hand, the spray with *Azospirillum* isolates, generally, decreased color degree of fruits, and the differences than control were significant. Sahain *et al.* (2007) mentioned that the microbial spray with EM (Japanese inoculant) leads to improvement of most marketing characteristics of apple fruits except for firmness decrease. Pirlak and Köse (2009) claimed that spray of strawberry with PGPR (*Pseudomonas* BA-8) and *Bacillus* OSU-142 improved the quality characteristics of the fruits especially TSS.

Data of Table 7 illustrated the economical evaluation for the spray with different *Azospirillum* isolates, the treatments brought high increases in the net return per feddan. The highest net return was achieved by the application of the treatments of A3R and A3D that gave net return about 21875 and 21950 L E/fed. with an increase over net return of the control treatment by 6120 and 6195 L E/fed. respectively, followed by the spray with the treatments A7R and A7D which gave increase in net return over control by 5595 and 5485 L E/fed. respectively. The spray with bacterial biostimulants (*Azospirillum* isolates) resulted in considerable net return (L E/feddan), whereas, the application with the treatment A3 increased the net return over control by 6120 L E/feddan. These results are in harmony with the results of Nour El-Din (2006) as the spray of peanut plants with liquid culture of *Azospirillum* lead to increase of the net return (L E/feddan).

Table 7: Response of "Anna" apple crop economics to spray with phyllosphere *Azospirillum* isolates

Treatments	Fixed costs (LE/ fed.)	Changed costs (LE / fed.)	Total costs (LE / fed.)	Total yield (Ton/ fed)	Crop value (LE /fed.)	Net return (LE /fed.)	Increase in return over control (LE)
A1R (Lemone)	5000	600	5600	7.63	26705	21105	5350
A 1D (Lemone)	5000	1200	6200	7.65	26755	20377	4622
A 2R (Guava)	5000	600	5600	7.02	24570	18970	3215
A 2D (Guava)	5000	1200	6200	7.13	24955	18755	3000
A 3R (apple)	5000	600	5600	7.85	27475	21875	6120
A 3D (apple)	5000	1200	6200	8.03	28105	21950	6195
A 4R (Orange)	5000	600	5600	7.43	26005	20405	4650
A 4D (Orange)	5000	1200	6200	7.53	26355	20155	4400
A 7R(Mango)	5000	600	5600	7.70	26950	21350	5595
A 7D (Mango)	5000	1200	6200	7.84	27440	21240	5485
C (water spray)	5000	0.00	5000	5.93	20755	15755	-

AR: *Azospirillum* spray with 20 L / feddan; AD: *Azospirillum* spray with 40 L / feddan.

CONCLUSION

The foliar spray with bacterial biostimulants (*Azospirillum* isolates) increased apple growth and fruit yield, but the marketing quality of the fruits not significantly affected. The treatments were economically valuable. The used *Azospirillum* isolates were isolated from phyllosphere of different types of fruit trees (lemon, guava, apple, orange and mango), whereas the best efficient isolates was that isolated from apple leaves. The foliar application with PGPR biostimulants may become, in the near future, an effective tool for inducing growth and productivity of the plants.

REFERENCES

- A.O.A.C. (1990). Official methods of analyses. 15th Ed., Association of Official Analytical Chemists. Washington, DC, USA.
- Anith, K. N. and Momol, M. T. (2004). Efficacy of plant growth-promoting rhizobacteria, acibenzolar-s-methyl, and soil amendment for integrated management of bacterial wilt on tomato. *Plant Disease*, 88: 669-673.
- Aslantaş, R.; Çakmakçı, R. and Şahin, F. (2007). Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Scientia Horticulturae*. 111: 371-377.
- Babalola, O. O. (2010). Beneficial bacteria of agricultural importance. *Biotechnol. Lett.*, 32:1559–1570.
- Belimov, A. A., Safranov, V. I., Sergeyeva, T. A., Egorova, T. N., Matveyeva, V. A., Tsyganov, V. E., Borisov, A. Y, Tikhonovich, I. A., Kluge, C, Preisfeld, A., Dietz, K. J., and V. V. Stepanok (2001). Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane -1- carboxylate deaminase. *Can. J. Microbiol.*, 47: 642-652.
- Bric, J. M.; R. M. Bostock and S. E. Silverstone (1991). Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Appl. Environ. Microbiol. Biotechnol.*, 35: 646-650.
- Döbereiner, J.; Marriall, L. E. and Nery, M. (1976). Ecological distribution of *Spirillum lipoferum*. Beijerinck. *Can. J. Microbiol.*, 22: 1464-1473.
- Dodd, I.C. ; Zinovkina, N.Y. and Safronova, V. I. (2010). Rhizobacterial mediation of plant hormone status. *Annal. Appl. Biol.*, 157: 361–379.
- Dursun, A.; Ekinci, M. and Donmez, M.F (2010). Effect of foliar application of plant growth promoting bacteria on chemical contents, yield and growth of tomato (*Lycopersicon esculenum* L.) and cucumber (*Cucumis sativus* L.). *Pak. J. Bot.*, 42: 3349-3356.
- Eissa Fawzia, M. (2003). Effect of some biostimulants on vegetative growth, yield and fruit quality of "Kelsey" Plum. *Egypt. J. Appl. Sci.*, 18:716-735.
- Eissa Fawzia, M.; Fathi, M. A. and Kandil Eman, A. (2007). Response of "Leconte" pear (*Pyrus communis* L.) trees to foliar application with some biostimulants. *Minufiya J. Agric. Res.*, 32: 1143-1154.
- Esitken, A.; Karlidag, H.; Ercisli, S.; Turan, M. and Sahin, F. (2004). The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus persica* L. cv. Hacihaliloglu) *Aust. J. Agric. Res.*, 54: 377–380.
- Esitken, A.; Pirlak, L.; Ipek, M.; Donmez, M. F.; Cakmakci R. and Sahin, F. (2009). Fruit bio-thinning by plant growth promoting bacteria (PGPB) in apple cvs. Golden delicious and Braeburn. *Biol. Agric. Horti.*, 26: 379–390.
- Fernando, W. G.; Nakkeeran, S. and Zhang, Y. (2005). Biosynthesis of antibiotics by PGPR and its relation in biocontrol of plant diseases. In Siddiqui Z.A. (ed.), *PGPR: Biocontrol and Biofertilization*, 67-109, Springer, Dordrecht, The Netherlands.

- Harold, E. P. (1985) Evaluation of quality of fruits and vegetables. AVI publications – West Port. Comm., USA.
- McGuire, R. G. (1992). Reporting of objective color measurements. Hort. Sci., 27 :1254 - 1260.
- Martínez-Viveros, O.; Jorquera, M. A.; Crowley, D. E.; Gajardo, G. and Mora, M.L. (2010). Mechanisms and practical consideration involved in plant growth promotion by rhizobacteria. Soil Sci. Plant Nutr. 10: 293 – 319.
- Nour El-Din, M. (2006). Influence of foliar application with some micronutrients and some microorganisms on growth of peanut plant. Alex. J. Agric. Res.,51: 113-119.
- Pirlak, L. and Köse, M. (2009). Effects of plant growth promoting rhizobacteria on yield and some fruit properties of strawberry. Journal of Plant Nutrition, 32: 1173-1184.
- Rami, M. (1982). Formulae for determination of chlorophyllous pigments extracted with *N, N*- dimethyl formamide. Plant Physiol., 69: 1376-1381.
- Rami, M. and Porath, D. (1980). Chlorophyll determination in intact tissues using *N, N*- dimethyl formamide. Plant Physiol. 65: 478-479.
- Ryu, C.; Shin, J.; Qi, W.; Ruhong, M.; Kim, E. J. and Jae Gu Pan, J. G. (2011). Potential for augmentation of fruit quality by foliar application of bacilli spores on apple tree. Plant Pathol. J., 27: 164-169.
- Sahain, M. F. M.; Elham , Z. Abd El Motty, El- Shiekh, M. H. and Laila, F. Hagagg (2007). Effect of some biostimulant on growth and fruiting of Anna apple trees in newly reclaimed areas. Res. J. Agric. Biol. Sci., 3: 422-429.
- Saharan, B.S. and Nehra, V. (2011). Plant Growth Promoting Rhizobacteria: A Critical Review. Life Sci. and Med. Res.,: LSMR-21.
- Sekar, S. and Kandavel, D. (2010). Interaction of plant growth promoting rhizobacteria (PGPR) and endophytes with medicinal plants – new avenues for phytochemicals. J. Phyt., 2: 91-100.
- Steel, R. G. and Torrie, J. H. (1980). Principles and procedures of statistics. Ambometrical approach. MC Grow hill, New York, USA.
- Taha, L.S. and Eid, R.A. (2011). Stimulation effect of some bioregulators on flowering, chemical constituents, essential oil and phytohormones of tuberose (*Polianthes tuberos* L.). J. Amer. Sci., 7: 165 -171.
- Voss, H. D. (1992). Relating colormeter measurements of plant color to the Royal Horticultural Society Color Chart. Hort. Sci., 27 :1256-1260.

تقييم بعض عزلات الأروسبيرليم المنتخبة لإنتاج اندول حامض ألكليك وتأثيرها على
تحسين نمو أشجار التفاح "صنف آنا" و محصول وجودة الثمار
محمد عبد السلام جبر** و محمد نورالدين*
* معهد بحوث الاراضي والمياه والبيئة- مركز البحوث الزراعية- الجيزة- مصر.
** معهد بحوث البساتين- مركز البحوث الزراعية- الجيزة- مصر.

الرش الورقي بمزارع PGPR المخففة له أهمية قصوى لما تحتويه و تفرزه من مغذيات
و منشطات و مضادات ميكروبية, فقدرة هذه الميكروبات في منطقة الفيلوسفير على تنشيط و تحسين
نمو النبات بالإضافة إلى زيادة تحمل النبات للممرضات كان واضحا و مؤثرا. ولكن الأنواع
الميكروبية بل و السلالات المختلفة تتباين في قدرتها على التأقلم و التعايش و إفراز المنشطات و
المغذيات.

تم عزل ميكروبات الازوسبيرليم من أشجار مختلفة و هي التفاح و البرتقال و الليمون و
الجوافة و المانجو و تم عمل مزارع نقية من هذه العزلاتو استخدمت في رش أشجار تفاح بالجرعات
20 و 40 لتر للفدان في مقابل الرش بالماء كعمالة مقارنة. أظهرت النتائج ان الرش الورقي
للأشجار بعزلات الازوسبيرليم قد زادت و بدرجة ملحوظة من نمو الأشجار و زادت إنتاجية الثمار
و لم تؤثر معنويا على نوعية الثمار. ومع ذلك فقد اختلف تأثير و قدرة العزلات بدرجة معنوية, فقد
كان التفوق في التأثير للعزلة A3 تبعها A7 واللذان حققا أعلى زيادة في طول المجموع الخضري
وقطره ومساحة الورقة ووزنها الجاف و محتوى كلوروفيل أ و ب و المحتوى الكلي وكذلك إنتاجية
الثمار في مقابل معاملة المقارنة, ولكن الخصائص النوعية للثمار (SSC و الحموضة و الصلابة
واللون) لم تظهر اختلافات معنوية ثابتة. وقد حققت معاملات الرش بعزلات الازوسبيرليم زيادة
كبيرة في صافي العائد (بالجنيه المصري), وكان في الصدارة معاملتنا الرش بالعزلة A3 R و D
A3 واللذان حققنا زيادة في صافي العائد عن معاملة المقارنة (الرش بالماء) بمقدار 6120 و 6195
جنيها على التوالي وتبعهما المعاملة A7R (5595 جنيها/ فدان). وقد لوحظ أن الرش بتركيز 40
لتر للفدان لم يعطي فروق معنوية عن الرش بتركيز 20 لتر للفدان, وبالتالي فإننا نوصي بالرش
بالعزلات الفعالة من الازوسبيرليم مثل العزلة A3 و A7 بالجرعة 20 لترا للفدان, كذلك من المهم
أن نركز الأبحاث على عزل و تقييم الميكروبات الفعالة لاستخدامها في التنمية الزراعية حيث أن لها
عائد اقتصادي مجدي.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
مركز البحوث الزراعية

أ.د / فتحي اسماعيل على حوقه
أ.د / فكرى محمد غزال