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

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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 siderofores. Potentiality of these microorganisms, in plant rhizosphere, in activation and improving of plant growth as will 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 ٢٠ and ٤٠ 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. A٣ isolate was the superior followed by A٧ 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 will 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 A٧D (isolated from mango phyllosphere and used with the rate of ٤٠ L/fed.) and A٧R, which isolated from mango phyllosphere and used with the rate of ٢٠L / fed.) were the superior treatments which achieved increases in net return over that of control (water spray) by ٦١٢٠ and ٦٢٠٠ L E/fed respectively, followed by A٣R (isolated from phyllosphere of apple and applied with ٢٠ L/fed.) which gave ٥٥٩٥ L E/fed. It is noted that the microbial culture dose of ٤٠ L/fed had no significant differences than the application of ٢٠ L/fed. Therefore, we recommend the spray with an efficient *Azospirillum* isolate like A٣ or A٧ with the dose of ٢٠ 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.*, ٢٠٠٩; Sekar and Kandavel, ٢٠١٠ and Ryu *et al.*, ٢٠١١). Saharan and Nehra (٢٠١١) 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 (١-Aminocyclopropane-١-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.*, ٢٠٠١). Also, production of biotoxins and antibiotics by these PGPR gave the plant high potentiality to resist pathogens (Anith and Momol, ٢٠٠٤), 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.*, ٢٠١٠). The ability of the IAA production, antibiotic synthesis and $N_{٢}$-fixation are variable (Fernando *et al.*, ٢٠٠٥; Aslantas *et al.*, ٢٠٠٧ and Saharan and Nehra, ٢٠١١). PGPR was found also to modify the plant hormones status (Dodd *et al.*, ٢٠١٠). 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.*,١٩٧٦) contained (g/liter of distilled water): Malic acid, ٥,٠; $KH_{٢}PO_{٤}$, ٠,٤; $K_{٢}HPO_{٤}$, ٠,١; $MgSO_{٤}.٧H_{٢}O$, ٠,٢; NaCl, ٠,١; $CaCl_{٢}.٧H_{٢}O$, ٠,٠٢; $FeCl_{٣}.٦H_{٢}O$, ٠,٠١; $Na_{٢}MoO_{٤}.٢H_{٢}O$, ٠,٠٠٢; agar, ١,٧٥.

This study has been carried out on eight years old "Anna" apple trees budded on Malus rootstock during ٢٠٠٩ and ٢٠١٠. Trees were grown at Elbostan region of Elbehira Governorate, where drip fertigation system was applied and soil texture analysis was shown in Table ١.

Table ١: Some chemical and physical analysis characteristics of the experimental soil

Sand %	Silt %	Clay %	Texture	O.M %	pH	EC (ds $m^{-١}$)
٨٠,٢	٨,٥	١١,٣	Sandy clay loam	٠,٦٣	٨,١	١,١٩

Cations (mg./L)			Anions (mg./L)				Macro-nutrient (mg/kg)		
Na^+	Ca^{++}	Mg^{++}	$CO_{٣}^{--}$	$HCO_{٣}^{-}$	Cl^-	$SO_{٤}^{--}$	N	P	K
٥,٨٩	١٠,٤٢	٣,٩١	-	١,٣٧	١٢,٤١	٦,٤٤	١٣٩	٧	٨٦

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 ٤ to ٥,٥ X $١٠^{٨}$ cfu/ml culture. Treatments were arranged in a random order on the selected trees. Single tree plot with ٣ 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.*, ١٩٩١)

All trees were subjected with common regional horticultural practices, while treatments were applied as follows in Table (٢):

Treatment	Description
A١R (Lemon)	Spray with *Azospirillum* isolated from lemon phyllosphere, ٢٠L / feddan.
A ١D (Lemo)	Spray with *Azospirillum* isolated from lemon phyllosphere, ٤٠L / feddan.
A ٢R (Guava)	Spray with *Azospirillum* isolated from guava phyllosphere, ٢٠L / feddan.
A ٢D (Guava)	Spray with *Azospirillum* isolated from guava phyllosphere, ٤٠L / feddan.
A ٣R (apple)	Spray with *Azospirillum* isolated from apple phyllosphere, ٢٠L / feddan.
A ٣D (apple)	Spray with *Azospirillum* isolated from apple phyllosphere, ٤٠L / feddan.
A ٤R (Orange)	Spray with *Azospirillum* isolated from orange phyllosphere, ٢٠L / feddan.
A ٤D (Orange)	Spray with *Azospirillum* isolated from orange phyllosphere, ٤٠L / feddan.
A ٧R(Mango)	Spray with *Azospirillum* isolated from mango phyllosphere, ٢٠L / feddan.
A ٧D (Mango)	Spray with *Azospirillum* isolated from mango phyllosphere, ٤٠L / 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 ٢٠ L or ٤٠ L of bacterial stocks with ٦٠٠ 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-٣١٠٠ Areameter was used to measure detached leaves of nine shoots (three shoots per branch) to get area per leaf (cm^{٢}). Leaves were dried at ٧٠˚C and weighed to get dry weight (mg) and then specific leaf weight (SLW) was calculated as (mg cm$^{-٢}$).

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 (١٩٨٠). The concentration of chlorophyll a and chlorophyll b and its total value were calculated by Rami's formulas as (µg / ml) (Rami, ١٩٨٢). The results were presented as (mg.cm$^{-٢}$).

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/cm^{٢}) according to Harold (١٩٨٥). 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 ٠°/٣٦٠° is defined for red/magenta, ٩٠° yellow, ١٨٠° for green and ٢٧٠° for blue color (McGuire, ١٩٩٢ and Voss, ١٩٩٢).

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. (١٩٩٠).

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 (١٩٨٠).

RESULTS AND DISCUSSION

Carefully considering readings of Table ٣, 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 A٣D isolate gave the highest difference throughout both studied seasons.

Table ٣: 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 ($cm^{٢}$)		SLW (mg $cm^{-٢}$)	
	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠
A١R (Lemon)	٥٧,٨ c	٥٤,٧ a	١,٦ a	١,٥ def	٠,٣٦ cde	٠,٣٣ c-g	٣٤,٥ b	٣٢,٥ a	١٠,٦ ab	١٠,١ c
A١D (Lemon)	٥٩,٥ bc	٥٥,٧ a	١,٧ a	١,٥ cd	٠,٣٨ bcd	٠,٣٥ b-e	٣٦,٦ ab	٣٤,٤a	١٠,٢ b	١٠,١ bc
A٢R (Guava)	٥٦,٨ c	٥٤,١ a	١,٦ a	١,٤ f	٠,٣٣ efg	٠,٣٠ efg	٢٦,٠ cd	٢٤,٣ bc	١٢,٦ a	١٢,٢ abc
A٢D (Guava)	٥٨,٣ c	٥٤,٥ a	١,٦ a	١,٥ def	٠,٣٥ def	٠,٣٢ d-g	٢٧,٧ cd	٢٥,٣bc	١٢,٥ ab	١٢,٦ abc
A٣R (apple)	٦٧,٤ a	٦٣,٢ a	١,٧ a	١,٦ bc	٠,٤١ ab	٠,٣٨ ab	٣٨,٤ ab	٣٥,٩ a	١٠,٦ ab	١٠,٦ abc
A٣D (apple)	٧٦,٩ a	٦٤,٥ a	١,٧ a	١,٦ ab	٠,٤٢ a	٠,٣٩ a	٣٩,٣ a	٣٦,٨ a	١٠,٧ ab	١٠,٢ bc
A٤R (Orange)	٥٧,٠ c	٥٥,١ a	١,٦ a	١,٥ def	٠,٣٦ cde	٠,٣٣ e-g	٢٩,٧ c	٢٥,٩ bc	١٢,٨ a	١٢,٧ ab
A٤D (Orange)	٥٧,٨ c	٥٤,٩ a	١,٦ a	١,٥ de	٠,٣٦ cde	٠,٣٤ b-f	٢٨,٧ cd	٢٧,٤ b	١٢,٤ ab	١٢,٢ abc
A٧R(Mango)	٦١,٩ bc	٥٩,١ a	١,٧ a	١,٦ bc	٠,٣٩ a-d	٠,٣٦ a-d	٣٦,٤ ab	٣٣,٩ a	١٠,٦ ab	١٠,٥ abc
A٧D (Mango)	٦٤,٩ ab	٦١,٠ a	١,٧ a	١,٧ a	٠,٣٩ bc	٠,٣٧ abc	٣٧,٤ ab	٣٤,٩ a	١٠,٥ ab	١٠,٥ abc
C (water spray)	٥٦,٤ c	٥٤,٠b	١,٥ b	١,٤ b	٠,٣٠ g	٠,٢٨ d	٢٤,٤ d	٢٢,٣ g	١٢,٥ ab	١٢,٥ a-d

AR: *Azospirillum* spray with ٢٠ L / feddan; AD: *Azospirillum* spray with ٤٠ L / feddan, SLW : Specific leaf weight.

Means with different letters within the same column differ significantly at P < ٠,٠٥.

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 A٣ isolate achieved the highest values, i. e., ٠,٤٢ and ٠,٣٩ g/leaf, and ٣٩,٣ and ٣٦,٨ cm٢ 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.* (٢٠٠٧) who indicated that the spray of pear trees with *Saccharomyces cervecia* had a stimulated effect on tree growth. While, Aslantas *et al.* (٢٠٠٧) 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 (A٣).

The readings of Table ٤ 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 ٤: Response of chlorophyll content of "Anna" apple leaves to spray with phyllosphere *Azospirillum* isolates.

Treatments	Chlorophyll a (mg cm-٢)		Chlorophyll b (mg cm-٢)		Total chlorophyll (mg cm-٢)	
	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠
A١R (Lemon)	٤,٨٩ a	٤,٩٣ ab	٢,٤٠ a	٢,٣٠ cde	٧,٢٩ a	٧,٠٧ bc
A١D (Lemon)	٤,٥٧a	٤,٦٨ bc	٢,٥٠ a	٢,٣٥ bcd	٧,٣٧ b	٧,٠٤ c
A٢R (Guava)	٤,٣٦ a	٤,٦٩ bc	٢,٢٠ a	٢,٠٨ ef	٦,٥٧ d	٦,٢٠ e
A٢D (Guava)	٤,٣٠ a	٤,١٢ ef	٢,٢٠ a	٢,٠٦ ef	٦,٦٠ d	٦,٢٤ e
A٣R (apple)	٤,٩٥ a	٤,١٨ ef	٢,٠٨ a	٢,٥٨ ab	٧,٧١ a	٧,٣٦ ab
A٣D (apple)	٤,٩٠ a	٤,٧٨ ab	٢,٧٨ a	٢,٦١ a	٧,٦٩ a	٧,٣٣ abc
A٤R (Orange)	٤,٦٠ a	٤,٧٢ abc	٢,٣٧ a	٢,٢١ def	٦,٩٨ c	٦,٥٩ d
A٤D (Orange)	٤,٦٦ a	٤,٣٨ de	٢,٣٧ a	٢,٢٨ def	٧,٠٣ c	٦,٧١ d
A٧R(Mango)	٥,١٢ a	٤,٢١ ef	٢,٦٢ a	٢,٤٧ abc	٧,٨٧ a	٧,٤٣ a
A٧D (Mango)	٥,١٥ a	٤,٩٦ a	٢,٦٢ a	٢,٤٨ abc	٧,٧٨ a	٧,٤١ a
C (water spray)	٢,٩٦ b	٤,٠٩ f	١,٥٦ b	٢,١٤ def	٦,٥٢ d	٦,٢٣ e

AR: *Azospirillum* spray with ٢٠ L / feddan; AD: *Azospirillum* spray with ٤٠ L / feddan.
Means with different letters within the same column differ significantly at P < ٠,٠٥.

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 A٣ and A٧ 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 (٢٠٠٣) 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, ٢٠١٠), which were found to increase chlorophyll a, b and carotenoids (Taha and Eid, ٢٠١١).

The beneficial effect of spray with *Azospirillum* isolates reflected, also, on the fruit yield (Table ٥) 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 (٤٥,٩٣ and ٤٣,٢٣ kg/tree) were recorded for A٣D treatment and ٤٥,٣٠ and ٤١.٩٦ kg/tree for A٣R treatment followed by A٧D and A٧R treatments, which attained ٤٥,٢٠ and ٤١,٩٣ kg/tree, and ٤٤,٤٣ and ٤١,٠٦ kg/tree at seasons ٢٠٠٩ and ٢٠١٠, 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 A٣ treatment (٣،٤٥ µg/ml of culture) followed by A١ and A٧ which exhibited, to some extent, similar values i. e. ٢،٨٥ and ٢،٧٦ µg/ml respectively. The treatment of A٤ was exhibited the lowest IAA release that estimated ١.٨١ 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 A٣ ,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.* (٢٠٠٤) who reported that spray of apricot with *Bacillus* OSU-١٤٢ increased fruit yield. Similarly, Eissa (٢٠٠٣) reported that the spray with EM resulted in an increase in number and weight of "Kelsey" plum fruits/tree. Also, Eissa *et al.* (٢٠٠٧) indicated that the spray of pear trees with *Saccharomyces cervecia* had a stimulated effect and increased number and weight of fruits. Martinez-Viveros *et al.* (٢٠١٠) 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:

١- Microorganisms having mechanisms that facilitate nutrient uptake or increase nutrient availability as fix of nitrogen or solubilizing phosphates and mineralize organic compounds.

٢- Production of phytohormones is now considered to be one of the most important mechanisms by which many rhizobacteria enhance plant growth, like IAA.

٣- Regulate plant ethylene levels, the high accumulation of ethylene leads to poor roots growth leads a diminished ability to acquire water and nutrients.

٤- 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 ٥) indicated that the different isolates released IAA differently and A٣ isolate had the highest level of IAA production and also tree fruit yield, followed by isolate A٧.

Table ٥: 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)
	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠	
A١R (Lemon)	٤٤,٠٠ ab	٤٠,٧٣abc	٣٤٠a-d	٣١٢ab	٢,٨٥
A١D (Lemon)	٤٤,٠٦ab	٤٠,٩٦abc	٣١٣def	٣١٤ab	
A٢R (Guava)	٤٠,٦٠b	٣٧,٣٦c	٢٩٨def	٢٦٥de	١,٩٨
A٢D (Guava)	٤١,٣٦ab	٣٧,٨٠bc	٣٠١def	٢٧٠de	
A٣R (apple)	٤٥,٣٠a	٤١,٩٦ab	٣٦٧ab	٣٣٥a	٣,٤٥
A٣D (apple)	٤٥,٩٣a	٤٣,٢٣a	٣٧٤a	٣٣٦a	
A٤R (Orange)	٤٣,١٠ab	٣٩,٤٣abc	٣٢٣c-f	٢٥٥def	١,٨١
A٤D (Orange)	٤٣,٤٦ab	٤٠,٢٠abc	٣٢٧b-e	٢٨٦de	
A٧R(Mango)	٤٤,٤٣ab	٤١,٠٦abc	٣٦٨ab	٢٧٥de	٢,٧٦
A٧D (Mango)	٤٥,٢٠a	٤١,٩٣ab	٣٦٢abc	٣٠١de	
C (water spray)	٣٣,٧٣c	٣٢,١٣d	٢٩٢ef	٢٩١de	-

AR: *Azospirillum* spray with ٢٠ L / feddan; AD: *Azospirillum* spray with ٤٠ L / feddan.
Means with different letters within the same column differ significantly at P < ٠,٠٥.

Data presented in Table ٦ 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*, A١ isolates attained significant increase, whilst not reached significance in case of A٣ isolate.

Table ٦: Response of "Anna" apple fruit quality to spray with phyllosphere *Azospirillum* isolates

Treatments	SSC %		Acidity %		Firmness (g/cm٢)		Color	
	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠	٢٠٠٩	٢٠١٠
A١R (Lemon)	١٢,٣٠d	١٢,٦٣d	٠,٩٠ a	٠,٨٦ a	٢٣٧,٠abc	٢٧٤,٣a	٨٩,١٦ab	٨٢,٨٥ab
A ١D (Lemon)	١٢,٤٣cd	١٢,٧٦cd	٠,٨٩a	٠,٨٦ a	٢٣٦,٠abc	٢٦٩.٣a	٨٨,٢٥abc	٨٣,٠٩٠ab
A ٢R (Guava)	١٢,٤٣cd	١٢,٨٠bcd	٠,٨٨٧a	٠,٨٥٣ab	٢٢٥,٣bc	٢٣٤,٣bc	٩٢,١٠٣a	٨٦,٥٢a
A ٢D (Guava)	١٢,٥٣bcd	١٢,٧٣d	٠,٨٧ ab	٠,٨٤ abc	٢٣٦,٠abc	٢٤١.٣abc	٩٠,١٧ab	٨٦,٥٧a
A ٣R (apple)	١٢,٨٠abc	١٣,١٦abc	٠,٨١cd	٠,٧٨ b-e	٢٤٣.٣abc	٢٥٦,٦ab	٨٣,١٧bcd	٧٨,٢٥bc
A ٣D (apple)	١٢,٨٠abc	١٣,١٦abc	٠,٨٢bc	٠,٧٩ bcd	٢٤٦,٣abc	٢٥٨.٣ab	٨٢,١٨b-e	٧٧,٨١bc
A ٤R (Orange)	١٢,٩٠ab	١٣,٢٠ab	٠,٧٤ e	٠,٧١ ef	٢٤٣.٣abc	٢٩٥,٣ab	٨٠,٢٣c-f	٧١,٥٧cd
A ٤D (Orange)	١٢,٩٣ab	١٣,٢٦a	٠,٧٥ cde	٠,٧١ def	٢٤٦,٦abc	٢٥٨,٦ab	٧٣,٩ ef	٦٩,٦٨d
A ٧R(Mango)	١٢,٩٣ab	١٣,٣٣a	٠,٧٣ e	٠,٦٩ f	٢٦٢,٠a	٢٧٤.٣a	٧١,٦٧ f	٦٦,٧٠d
A ٧D (Mango)	١٣,٠٠a	١٣,٤٠a	٠,٧٢٣e	٠,٦٨ f	٢٦٢,٦a	٢٧٤,٣a	٧٢,٢٦ f	٦٧,٥٢d
C (water spray)	١٣,٠٣a	١٣,٠٣a-d	٠,٧٧ cde	٠,٧٧ cde	٢٢٠,٦c	٢٢٠,٦c	٩٠,٣٤ab	٩٠,٣٤a

AR: *Azospirillum* spray with ٢٠ L / feddan; AD: *Azospirillum* spray with ٤٠ L / feddan, SSC: soluble solids content.
Means with different letters within the same column differ significantly at P < ٠,٠٥.

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.* (٢٠٠٧) 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 (٢٠٠٩) claimed that spray of strawberry with PGPR (*Pseudomonas* BA-٨) and *Bacillus* OSU-١٤٢ improved the quality characteristics of the fruits especially TSS.

Data of Table ٧ 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 A٣R and A٣D that gave net return about ٢١٨٧٥ and ٢١٩٥٠ L E/fed. with an increase over net return of the control treatment by ٦١٢٠ and ٦١٩٥ L E/fed. respectively, followed by the spray with the treatments A٧R and A٧D which gave increase in net return over control by ٥٥٩٥ and ٥٤٨٥ 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 A٣ increased the net return over control by ٦١٢٠ L E/feddan. These results are in harmony with the results of Nour El-Din (٢٠٠٦) as the spray of peanut plants with liquid culture of *Azospirillum* lead to increase of the net return (L E/feddan**).**

Table ٧: 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)
A١R (Lemone)	٥٠٠٠	٦٠٠	٥٦٠٠	٧,٦٣	٢٦٧٠٥	٢١١٠٥	٥٣٥٠
A ١D (Lemone)	٥٠٠٠	١٢٠٠	٦٢٠٠	٧,٦٥	٢٦٧٥٥	٢٠٣٧٧	٤٦٢٢
A ٢R (Guava)	٥٠٠٠	٦٠٠	٥٦٠٠	٧,٠٢	٢٤٥٧٠	١٨٩٧٠	٣٢١٥
A ٢D (Guava)	٥٠٠٠	١٢٠٠	٦٢٠٠	٧,١٣	٢٤٩٥٥	١٨٧٥٥	٣٠٠٠
A ٣R (apple)	٥٠٠٠	٦٠٠	٥٦٠٠	٧,٨٥	٢٧٤٧٥	٢١٨٧٥	٦١٢٠
A ٣D (apple)	٥٠٠٠	١٢٠٠	٦٢٠٠	٨,٠٣	٢٨١٠٥	٢١٩٥٠	٦١٩٥
A ٤R (Orange)	٥٠٠٠	٦٠٠	٥٦٠٠	٧,٤٣	٢٦٠٠٥	٢٠٤٠٥	٤٦٥٠
A ٤D (Orange)	٥٠٠٠	١٢٠٠	٦٢٠٠	٧,٥٣	٢٦٣٥٥	٢٠١٥٥	٤٤٠٠
A ٧R(Mango)	٥٠٠٠	٦٠٠	٥٦٠٠	٧,٧٠	٢٦٩٥٠	٢١٣٥٠	٥٥٩٥
A ٧D (Mango)	٥٠٠٠	١٢٠٠	٦٢٠٠	٧,٨٤	٢٧٤٤٠	٢١٢٤٠	٥٤٨٥
C (water spray)	٥٠٠٠	٠,٠٠	٥٠٠٠	٥,٩٣	٢٠٧٥٥	١٥٧٥٥	-

AR: *Azospirillum* spray with ٢٠ L / feddan; AD: *Azospirillum* spray with ٤٠ 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.

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تقييم بعض عزلات الأزوسبيرليم المنتخبة لإنتاج اندول حامض ألخليك و تأثيرها على تحسين نمو أشجار التفاح"صنف آنا" و محصول وجودة الثمار

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

تم عزل ميكروبات الازوسبيريلم 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 ٢٠ و ٤٠ لتر للفدان في مقابل الرش بالماء كمعاملة مقارنة.أظهرت النتائج ان الرش الورقي للأشجار بعزلات الازوسبيريلم قد زادت وبدرجة ملحوظة من نمو الأشجار و زادت إنتاجية الثمار ولم تؤثر معنويا علي نوعية الثمار. ومع ذلك فقد اختلف تأثير وقدرة العزلات بدرجة معنوية، فقد كان التفوق في التأثير للعزلة A٣ تبعها A٧ واللتان حققتا أعلي زيادة في طول المجموع الخضري وقطره ومساحة الورقة ووزنها الجاف ومحتوى كلوروفيل أ و ب والمحتوي الكلي وكذلك إنتاجية الثمار في مقابل معاملة المقارنة، ولكن الخصائص النوعية للثمار (SSC والحموضة والصلابة واللون) لم تظهر اختلافات معنوية ثابتة. وقد حققت معاملات الرش بعزلات الازوسبيرليم زيادة كبيرة في صافي العائد (بالجنيه المصري)، وكان في الصدارة معاملتا الرش بالعزلة A٣ R وD A٣ واللتان حققتا زيادة في صافي العائد عن معاملة المقارنة (الرش بالماء) بمقدار ٦١٢٠ و٦١٩٥ جنيها علي التوالي وتبعهما المعاملة A٧R (٥٥٩٥ جنيها/ فدان). وقد لوحظ أن الرش بتركيز ٤٠ لتر للفدان لم يعطي فروق معنوية عن الرش بتركيز ٢٠ لتر للفدان، وبالتالي فإننا نوصي بالرش بالعزلات الفعالة من الازوسبيرليم مثل العزلة A٣ و A٧ بالجرعة ٢٠ لترا للفدان، كذلك من المهم أن نركز الأبحاث علي عزل وتقييم الميكروبات الفعالة لاستخدامها في التنمية الزراعية حيث أن لها عائد اقتصادي مجدي.

قام بتحكيم البحث

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