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RESPONSE OF GROWTH, YIELD AND FRUIT QUALITY OF "ANNA" APPLE TREES TO FOLIAR SPRAY WITH SOME PLANT GROWTH PROMOTING RHIZOBACTERIA AS A SUBSTITUTE TO SYNTHETIC BIOSTIMULANTS

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ABSTRACT

Many synthetic biostimulants containing amino acids, macro- and micro-elements, humic acids and, sometimes, algae extracts have been used effectively for stimulating and increasing of fruit trees yield. The foliar spray with some PGPR (plant growth promoting rhizobacteria) has proved high potentiality in stimulating and increasing plant yield. The current study is concerned with the use of some PGPR types (*Azospirillum brasilense, Pseudomonas fluorescens and Bacillis polymyxa*) as an alternative to some commercial synthetic biostimulants i. e. Jisamar (J) and Furdos (F) in stimulating and improving yield of "Anna" apple trees.

The study field of apple trees (eight years old) was chosen at El-Bostan area, El-Behira Governorate, Egypt. The trees were sprayed twice with the different stimulators, the first was at fruit set and the second was done after 30 days from fruit set. The control trees were sprayed with water.

The microorganisms which were used for spray were counted in the plant phyllosphere and they showed heavy increase in the phyllosphere of the treated plants. The spray with the synthetic biostimulants showed remarkable improvement to the plant morphological characteristics, e. g. shoot length, shoot diameter, leaf area, leaf dry weight and leaf content of chlorophyll a, b and total chlorophyll. While, specific leaf weight (SLW) did not significantly change. Likewise, the spray with bacterial biostimulants proved similar potentiality as synthetic ones, and even, sometimes, surpassed them. The spray with *Azospirillum brasilense* gave the highest fruit yield; 45.1 and 41.2 kg/tree versus 33.5 and 32.0 kg/tree for control treatment in 2009 and 2010 seasons, respectively. The spray with Jisamar and Furdos gave 37.4 and 33.2 kg/tree and 36.5 and 34.7 kg/tree, respectively. The experimental treatments, raised the net return (£E/feddan) over that of control. *Azospirillum brasilense* spray attained the highest net return i. e. 21595 versus 16660, 16870 and 15650 £E/feddan for treatments of Jisamar, Fordose and control, respectively. Therefore, the substitution of synthetic biostimulant products with biological ones may be suitable for improving the plant growth, increasing productivity and raising the net return per feddan of Anna apple trees.

INTRODUCTION

The overwhelming increase of population and exacerbate of poverty and hungry problems in the world needs hard and continuous work to increase agricultural productivity. For a lot of reasons, horizontal expansion of agriculture represent a difficult equation for many countries of the world, therefore the vertical increase in plant productivity is the suitable solution.

Despite the balanced plant nutrition is of great importance for plant productivity, the application of some synthetic stimulants had the positive and effective influence in increasing plant growth and productivity. Many of these stimulants contain amino acids, vitamins, humic acids, sea weeds extracts, plant phytohormones and sometimes microelements (Ferrini and Nicese, 2002). Spinelli *et al.* (2010) treated the strawberry with "Actiwave" which is a product derived from the algae *Ascophyllum nodosum*. Spray with "Actiwave" enhanced the tree growth and yield and had a significant effect on reducing the negative effect of alternative bearing. They claimed that the biostimulant Actiwave may represent a promising strategy to reduce the use of phytochemicals in agriculture. They indicated that Actiwave application as a spray to the strawberry increased the mineral nutrient uptake, chlorophyll content and the abiotic stress tolerance.

Other authors found significant increase in plant growth and productivity due to spray with different types of PGPR cultures. Karakurt and Aslantas (2010) demonstrated that the spray of apple tree with some PGPR (*Bacillus subtilis* OUS-142 and *Pseudomonas putida* BA-8) enhanced tree growth and nutrient uptake. Erturk *et al.* (2011) observed that spray of hazelnut seedlings with *B. lentus*, *B. atrophaeus* and other types of PGPR significantly increased the plant growth parameters. The foliar spray of apple trees with three *Bacillus* sp. was also found by Ryu *et al*. (2011) to increase tree growth, fruit yield and quality.

The PGPR had multi-mechanisms for enhancing plant growth such as fixing atmospheric nitrogen (Saharan and Nehra, 2011); facilitate nutrient uptake (Martinez-Viveros *et al.,* 2010); solubilizing phosphates (Oliveira *et al.,* 2009); producing plant phytohormones (Spaepen *et al.*, 2008); regulate plant ethylene level (Govidasamy *et al.,* 2008); resist plant pathogens (Van Loon, 2007) and produce antibiotics which reflected on plant growth, yield and quality (Esitken, 2011).

Therefore, the present investigation aimed to study the significance of spray of apple trees with some PGPR bacterial biostimulants as an alternative to synthetic biostimulants i. e. Jisamar and Furdose.

MATERIALS AND METHODS

Materials:

Microbial media:

1- *Azospirillum*: Semi solid Döbereiner medium (Döbereiner *et al*., 1976) contained (g/liter of distilled water): Malic acid, 5.0; KH_2PO_4, 0.4; K_2HPO_4, 0.1; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $CaCl_2.7H_2O$, 0.02; $FeCl_3.6H_2O$, 0.01; $Na_2MoO_4.2H_2O$, 0.002; Agar, 1.75.

2- *Bacillus:* N-deficient medium for Bacilli (Hino and Wilson, 1958) was composed of solution A: It contains (g/500 ml distilled water): $MgSO_4.7H_2O$, 0.5; Sucrose, 20.0; NaCl, 0.01; $FeSO_4$,0.02; $Na_2MoO_4.2H_2O$, 0.005; $CaCO_3$, 0.2 and solution B which contains Para amino benzoic acid, 10 µg; Biotin, 5 µg ; K_2HPO_4, 6.81 g ; KH_2PO_4, 1.43 g ; distilled water 500 ml. The two solutions were mixed after autoclaving at 1.5 atm. for 15 minutes.

3- *Pseudomonas*: King s medium (King et al., 1954) was composed of (g/liter distilled water): sucrose, 20.0 g ; peptone, 5.0 g ; K_2HPO_4, 0.5 g ; Agar , 20.0 g ; distilled water 1000 ml, pH, 7.2 (0.1N KOH).

Jisamar: is a commercial synthetic biostimulant contains seaweed extract (20.5%), free amino acids (6.5%), total nitrogen (5.8%), phosphorus (3%), boron (0.17%) and potassium (4.6%).

Furdose: : is a commercial synthetic biostimulant contains humic and vulvic acids (22%), natural and organic substances (40%), free amino acids (14.6%), N, (4.5%), P (3.8), K (5%), Ca (0.4%), Mg (0.4%), Fe (0.1%), Mn (15 ppm), Zn (20 ppm), Cu (15 ppm).

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. Drip fertigation system was applied for irrigation and fertilization. The experimental soil analysis is shown below 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	1.17

Cations (mg./L)			**Anions (mg./L)**				**Macro-nutrient (ppm)**		
Na^+	**Ca^{++}**	**Mg^{++}**	**CO_3^{--}**	**HCO_3^-**	**Cl^-**	**SO_4^{--}**	**N**	**P**	**K**
5.89	10.42	3.91	-	1.37	12.41	6.72	139	7	86

Methods:

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.

All trees were subjected with common regional horticultural practices, while treatments were applied as follows:

(A): Trees were sprayed with a suspension of *Azospirillum brasilense.*

(P): Trees were sprayed with a suspension of *Pseudomonas fluorescens*.

(B): Trees were sprayed with a suspension of *Bacillus polymyxa*

(J): Trees were sprayed with a suspension of Jisamar.

(F): Trees were sprayed with a suspension of Furdose.

(A+J): Trees were sprayed with a suspension of *Azospirillum brasilense* and Jisamar.

(A+F): Trees were sprayed with a suspension of *Azospirillum brasilense* and Furdose.

(P+J): Trees were sprayed with a suspension of *Pseudomonas fluorescens* and Jisamar

(P+F): Trees were sprayed with a suspension of *Pseudomonas fluorescens* and Furdose.

(B+J): Trees were sprayed with a suspension of *Bacillus polymyxa* and Jisamar.

(B+F): Trees were sprayed with a suspension of *Bacillus polymyxa* and Furdose.

(M): Trees were sprayed with a suspension of the bacterial mixture.

(M+J): Trees were sprayed with a suspension of the bacterial mixture and Jisamar.

(M+F): Trees were sprayed with a suspension of the bacterial mixture and Furdose.

(C): Control trees sprayed with tap water

The Microbial inoculants were prepared and provided by Biofertilizers Production Unit, Soil, Water and Environment Research Institute, ARC, Egypt. Bacterial suspensions were diluted by mixing of 400 ml of bacterial stocks with 9 L of water per 3 trees of each treatment. Each of the used bacterial species was grown on its specific medium. *Bacillus polymyxa* was grown up on liquid medium of Hino and Wilson (1958) for 3 days at 30^{o}C. *Azospirillum brasilense* was grown up for 3 days at 30^{o}C on semi solid Döbereiner medium (Döbereiner *et al*., 1976) and *Pseudomonas fluorescense* was grown up for 3 days at 30^{o}C on King s medium (King *et al.*, 1954). Each tree was sprayed twice, at fruit sitting and 30 days later for both seasons.

Viable counts of *P. fluorescense, A. brasilense* and *B. polymyxa*:

P. fluorescense, A. brasilense and B. polymyxa viable count in the phylosphere of apple tree were determined, 30 days before fruit maturity at the second season, using respective media.

Estimation of plant growth promoting substances by bacteria:

Bacterial strains were tested for their capability to produce indole acetic acid (IAA) (Bric et al., 1991), were grown on Tryptone Soya Agar Medium (TSA) (Difco, 1984). Quantitative determination of IAA was performed according to Glickmann and Dessoux (1995). Total gibberellins were measured by the procedure of Udagwa and Kinoshita (1961).

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 (cm^2). Leaves were dried at 70˚C and weighted to get dry weight (mg.) and then specific leaf weight (SLW) was calculated as (mg /cm^2) according to Ferree and Forshey (1988).

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) while results were presented as (mg /cm^2).

Fruits were picked at maturity stage and weighted 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^2) 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

The synthetic and/or bacterial biostimulants foliar spray treatments had an effective role in increasing shoot length of apple trees (Table 2). At the first season, Jisamar treatment attained the highest and significant increase (64.83 cm) followed by the treatment of *P. fluorescens* which gave, a significant increase of 64.63 cm compared to water spray (control) treatment (56.36 cm). The second season trend was similar to that of the first one. The studied treatments exhibited not significant increments in shoot diameter. The spray with Furdose, Jisamar and bacterial inoculants and their mixtures with PGPR biostimulants had a great effect in increasing leaf area and dry weight. In most cases, the treatments achieved consistent, remarkable and significant increases over control. Specific leaf weight (SLW) data did not show significant differences.

Spray of fruit trees with compounds containing amino acids, plant phytohormones, humate, N,P,K and some microelements contribute in improving tree growth, promote flowering process and fruit setting, which consequently, reflected positively on the fruit yield (Eissa, 2003). Likewise, foliar application with some PGPR types (*Azotobacter chroococcum*) gave results similar to the action of these treatments (Nour El-Din, 2006). The results of the present study showed that foliar spray with some synthetic plant stimulants (Furdose and Jisamar) which contain sea weeds extracts, amino acids, low percentages of N, P and K with microelements had the positive effect on shoot length, stem diameter, leaf area and leaf dry weight. The spray with the varied PGPR types alone or mixed with the synthetic biostimulants, similarly, increased these characteristics of apple trees, in some cases surpassed synthetic ones. This promoting influence is attributed to the plant phytohormones, amino acids, vitamins and macro and microelements contained in bacterial biostimulants. Fathi *et al.* (2002) found that spray with GA_3, K-humate and ascorbic acid significantly increased shoot length, leaf area of "Desert Red" peach and increased leaf area and leaf dry weight of "Anna" apple. Esitken *et al.* (2004), also, reported that spray with

Bacillus OSU142 at full bloom, 30 and 60 days after blooming increased shoot length and growth of apricot tree especially when sprayed at full bloom.

Table 2: Effect of spray with some synthetic and some bacterial biostimulants (PGPR cultures) on "Anna" apple vegetative characteristics

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
A	61.2 abc	58.43 abc	1.59 ab	1.53 ab	0.370 bcd	0.343 abc	33.93 abc	30.63 bcd	10.84 bcd	11.16 b-e
A+J	57.56 bc	55.57c	1.62 ab	1.57 ab	0.363cd	0.333bc	34.8 abc	32.43abc	10.38d	10.37d-e
A+F	60.16 abc	57.17bc	1.69 ab	1.57 ab	0.39 abc	0.377ab	37.06a	34.13ab	10.66cd	10.86be
B	61.93 ab	53.73abc	1.75 a	1.64 a	0.347d	0.320cd	32.23a-d	28.73c-f	10.71cd	11.11b-e
B+J	56.1 c	57.47bc	1.63 ab	1.51 ab	0.347d	0.317cd	31.46a-d	28.73c-f	11.00bcd	10.09e
B+F	61.06 abc	55.43c	1.69 ab	1.56 ab	0.39 abc	0.373ab	31.7 a-d	29.3 b-e	12.04ad	12.88abc
P	64.63 a	60.8 ab	1.66 ab	1.57 ab	0.397abc	0.370ab	35.96a	36.07a	11.24bcd	10.34de
P+J	60.2 abc	56.67bc	1.73 ab	1.60 a	0.370bcd	0.350abc	36.1 a	33.10abc	10.29d	10.56cde
P+F	61.6 abc	55.83c	1.65 ab	1.52 ab	0.410ab	0.387a	35.26ab	35.57a	11.70bcd	10.91b-e
M	58.53 bc	54.2 c	1.58 ab	1.5 ab	0.337de	0.30 cd	26.3 de	24.0 fg	12.84abc	12.95abc
M+J	57.63 bc	55.0 c	1.580ab	1.46 ab	0.343de	0.317cd	26.53de	24.17fg	12.97abc	13.01ab
M+F	58.16 bc	55.8 c	1.63 ab	1.55 ab	0.347d	0.320cd	26.63de	24.37efg	13.10 a b	13.17ab
J	64.83 a	57.3 bc	1.69 ab	1.56 ab	0.417a	0.333bc	29.06cde	26.13d-g	14.37a	12.78abc
F	60.4 abc	61.97a	1.66 ab	1.62 a	0.36 cd	0.38 a	29.2 bc	26.93d-g	12.44 a-d	14.42a
C	56.36 bc	53.97c	1.53 ab	1.40 b	0.303e	0.283d	24.46e	22.3 g	12.05 a-d	12.55a-d

***J: Jisamar; F: Furdos; A: *Azospirillum brasilense*; B: *Bacillus polymyxa*; P: *Pseudomonas fluorescens*; M: microbial mixture, SLW : Specific leaf weight.**
****Means with different letters within the same column differ significantly at $P < 0.05$.**

The results presented in Table 3 showed an increase in chlorophyll content of leaf as a result of treatment with Jisamar and Furdos synthetic biostimulants as will as spray with PGPR biostimulants. The first season results showed significant increments in chlorophyll a resulted from spray with each of *A. brasilense*, *B. polymyxa and P. fluorescen*s alone, while, the mixture of them did not give significant difference. However, a significant increase was observed due to application with Furdose compared to control.

The treatment with *P. fluorescens* + Jisamar recorded 5.04 and 4.89 mg/cm^2 at first and second seasons, respectively. The trend of the second season results was similar to that of the first one. The spray with PGPR bacteria as will as PGPR with F or J potentially increased fruit production per tree, The spray with *Azospirillum brasilense* culture gave the highest results for both studied seasons, which attained 45.16 and 41.20 kg/tree at 2009 and 2010 seasons, respectively, followed by the spray with *Azospirillum brasilense* + Jisamar which achieved production averaged 43.86 and 40.73 kg/tree for both seasons compared to 33.53 and 32.07 kg/tree for control treatment at the seasons 2009 and 2010, respectively. The differences than control were significant. Likewise, number of fruits per tree was positively affected by the different spray treatments, although, the most differences were not significant except the treatment of spray with *Azospirillum brasilense* or *Azospirillum brasilense* with F or J as will as *B. polymyxa or B. polymyxa* with F or J which attained significant differences at both seasons. The treatment of *Azospirillum brasilense* spray recorded the highest fruit number / tree at 2009 and 2010 seasons (346.3 and 314.0, respectively).

The increase of leaves chlorophyll content resulted from spray with synthetic or bacterial biostimulants is due to the stimulation and nutrientive effect on the plant. Gisamar and Furdose contains nutrients and stimulating compounds like short chain amino acids, plant phytohormones, vitamins and some macro- and micro-elements. Maini (2000) reported that the biostimulants (Siapton) which based on short chain amino acids increased the chlorophyll content for wheat plant. Also, Ferrini and Nicese (2002) sprayed Oak plants with two types of biostimulants; WP^{th} which composed of humic acid, seaweeds extract, Yucca extracts, vitamins and nitrogen fixing bacteria, and DP^{TM} compound which composed of indo and ecto mycorrhiza, biostimulant bacteria, humic acids, carbohydrate, vitamins and Yucca extract. They claimed that photosynthesis and chlorophyll content were enhanced. The spray with Furdose and Jisamar, in the present study, stimulated the growth and increased fruit yield, but the spray with bacterial biostimulants had a strong influence than the synthetic biostimulants, and the treatment of *Azospirillum brasilense* foliar spray was the best in this concern. The mechanisms of bacterial biostimulants action may be summarized as follow:

1- Increase of number and size of plant cells due to the function of plant phytohormones like IAA, cytokinens and gebrillins (Iqbal *et al*., 2011).
2- The polyamines contained in the synthetic biostimulants and these released by bacterial biostimulants regulate many growth processes; differentiation, formation of embryo, setting and ripening of fruits (Taha and Eid, 2011).
3- Stimulate production of antioxidants, therefore, decrease free radicals or reactive oxygen molecules leads to improvement of plant growth especially at biotic and abiotic stress like salinity, dryness and high exposure to UV. Most importantly, they provide essential information on cellular redox state, and regulate gene expression

associated with biotic and abiotic stress responses to optimize defense and survival (Shao *et al*., 2008).

4- These stimulants contained some macro and micro-elements which enhance plant growth (Maini, 2000).

5- The bacterial biostimulants release plant phytohormones, amino acids, antioxidants, siderofores and antibiotics in addition to fixing atmospheric nitrogen in the rhizosphere or phyllosphere (Martinez-Viveros *et al*., 2010).

Table 3: Effect of spray with some synthetic and some bacterial biostimulants (PGPR cultures) on chlorophyll content and fruit yield of "Anna" apple trees

Treatments	Chlorophyll a (mg/cm^2)		Chlorophyll b (mg/cm^2)		Total chlorophyll (mg/cm^2)		Fruit weight (Kg/tree)		Fruit number	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
A	4.64 cd	4.44 cd	2.56 ab	2.42 ab	7.20 bc	6.86 cde	45.16 a	41.20 a	346.3a	314.0 a
A+J	4.69 bcd	4.52 bc	2.51 ab	2.39 ab	7.20 bc	6.91 bcd	43.86 ab	40.73 ab	330.0 ab	300.3 abc
A+F	4.56 de	4.13 cde	2.47 abc	2.37 abc	7.03 cd	6.76 def	42.46 abc	39.10 abc	312.0 abc	274.3 cde
B	4.407 d-g	4.23 de	2.32 bcd	2.20 bcd	6.73 def	6.43 fg	42.96 ab	39.40 abc	327.6 ab	298.0 a-d
B+J	4.55 de	4.32 cde	2.29 bcd	2.18 bcd	6.08 c-f	6.50 fg	40.93 a-d	37.53 a-d	318.3 abc	295.3 a-d
B+F	4.44 def	4.25 de	2.39 a-d	2.29 a-d	6.84 c-f	6.54 efg	40.83 a-d	37.23 a-d	328.0 ab	304.6 ab
P	4.91 abc	4.72 ab	2.61 a	2.49 a	7.53 ab	7.21 ab	39.23bcd	36.10b-e	316.6abc	283.3b-e
P+J	5.04 a	4.89 a	2.60 a	2.49 a	7.66 a	7.39 a	37.4 cde	34.97c-f	306.3bc	278.0 b-e
P+F	4.93 ab	4.73 ab	2.53 ab	2.42 ab	7.47 ab	7.15 abc	37.03cde	33.93def	321.0abc	296.0a-d
M	4.15 g	3.96 f	2.16 d	2.05 d	6.31 g	6.01 h	33.43e	30.50f	287.6c	263.3e
M+J	4.41 d-g	4.16 ef	2.20 cd	2.09 d	6.62 efg	6.25 gh	37.23cde	34.37c-f	300.6bc	262.0e
M+F	4.25 fg	4.07 ef	2.32 bcd	2.19 bcd	6.57 fg	6.26 gh	38.5b-e	36.20b-e	303.6bc	269.7de
J	4.89 abc	4.17 ef	2.53 ab	2.41 ab	7.42 ab	6.59d-g	37.4cde	33.27def	315.0abc	285.6a-e
F	4.427d-g	4.71 ab	2.56 ab	2.41 ab	6.93 cde	7.11 abc	36.53de	34.77c-f	313.3abc	287.6a-e
C	4.29 efg	4.09 ef	2.230cd	2.14 cd	6.52 fg	6.23 gh	33.53e	32.07ef	292.0c	266.0e

***J: Jisamar; F: Furdos; A: *Azospirillum brasilense*; B: *Bacillus polymyxa*; P: *Pseudomonas fluorescens*; M: microbial mixture.**
****Means with different letters within the same column differ significantly at $P < 0.05$.**

Data of Table 4, some important quality parameters of the fruits were determined like soluble solid content (SSC), acidity, firmness and color. The values of SSC through the two studying seasons were generally lowered due to spraying with stimulants whether were synthetic or biological, but the differences from the control did not usually reach to significance. While acidity of the fruits, in general, increased by the sprayed treatments especially with bacterial biostimulants in both seasons. The foliar spray with the studied bacterial biostimulants, generally, led to significant increases in firmness values of the fruit, at the second season only. The treatments of synthetic or biological decreased the color degree of the fruits and this effect was clear for results of the second season where all differences than control treatment were significant. The control values recorded 94.84 and 90.34 for seasons 2009 and 2010 compared to values ranged from 65.02 to 83.84 at season 2009 and from 70.63 to 82.25 at season 2010.

Table 4: Effect of spray with some synthetic and some bacterial biostimulants (PGPR cultures) on some fruit quality characters of "Anna" apple trees

Treatments	SSC		Acidity		Firmness		Color	
	2009	2010	2009	2010	2009	2010	2009	2010
A	13.20 a	13.33a	0.82ab	0.86bc	226.0ab	263.3b	79.23a	77.68bcd
A+J	12.93abc	13.16ab	0.85b	0.86bc	260.0a	263.0b	74.17a	75.01bcd
A+F	13.06ab	13.26a	0.84b	0.87c	237.6a	245.3c	76.17a	77.85bcd
B	12.60bcd	12.98abc	0.82bc	0.78ef	267.6a	280.0a	73.02a	75.62bcd
B+J	12.40cd	12.26d	0.94a	0.92ab	215.6ab	285.6a	72.96a	75.37bcd
B+F	12.23d	12.83abc	0.94a	0.93ab	280.0a	283.6a	71.11a	75.26bcd
P	12.43cd	12.63bcd	0.84b	0.86bcd	269.0a	277.6a	72.10a	75.51bcd
P+J	12.40cd	12.66bcd	0.82bc	0.77ef	286.3a	284.6a	65.02a	70.63d
P+F	12.43cd	12.63bcd	0.97a	0.96a	286.3a	286.3a	66.29a	70.26d
M	13.13ab	13.30a	0.80bc	0.77ef	221.0ab	229.0d	71.81a	73.84cd
M+J	12.43cd	12.60cd	0.82bc	0.81cde	221.0ab	227.0d	73.73a	75.32bcd
M+F	12.60bcd	12.86abc	0.81bc	0.79def	220.0ab	229.0d	73.83a	75.29bcd
J	12.66a-d	12.83abc	0.74cd	0.72fg	222.0ab	232.6cd	83.84a	81.54bc
F	12.88abc	13.00abc	0.74cd	0.70g	223.0ab	234.6cd	82.30a	82.25b
C	13.06ab	13.03abc	0.69d	0.77ef	207.0ab	220.6d	94.84a	90.34a

***J: Jisamar; F: Furdos; A: *Azospirillum brasilense*; B: *Bacillus polymyxa*; P: *Pseudomonas fluorescens*; M: microbial mixture.**
****Means with different letters within the same column differ significantly at P < 0.05.**

The spray with gibberellic acid (GA_3) showed improvement for fruit quality of jambu madu (Moneruzzaman *et al*, 2011), apple fruits (Ryu *et al*., 2011). At the same time Pirlak and Köse (2009) claimed that the spray of strawberry with *Pseudomonas* BA-8 and other PGPR bacteria increased TSS and acidity of the fruits. The spray with synthetic or bacterial biostimulants lead to decrease of SSC values and color of fruits but increased acidity and firmness, however, most variations were not significant. Sahain *et al*. (2007) reported different results, whereas, they showed that spray of apple trees with EM biostimulant (composed of fluorescent bacteria and fermentative fungi) increased TSS and decreased acidity and firmness of the fruits, the difference in the influence may be due to the variation in the microbial composition between EM and the present used PGPR bacteria. Results of

Esitken *et al.* (2009) were similar to our findings as they sprayed the trees with some PGPR types.

Viable counts (Table 5) of the used microorganisms as foliar spray notably increased in the phyllosphere of the plant. The counts of *Azospirillum brasilense* appeared very high due to spray with the liquid culture of *Azospirillum brasilense*, this effect clearly appeared after spray with *Azospirillum brasilense* alone or mixing with Jismar or Furdos. The spray with the mixture of *Azospirillum brasilense* and Jismar attained the highest average number of *Azospirillum* evaluated 240 x 10^2 cfu/cm^2 of leaf. Likewise, the spray with *B. polymyxa* culture alone or mixed with Jisamar had potential effect in increasing its numbers in the phyllosphere, which represented 45 x 10^2 and 40 x 10^2 cfu/cm^2 of leaf, respectively. Similar results happened with the spray with *P. fluorescens* which caused clear increase of the applied microbe in the phyllosphere area, the counts were 5 x 10^2, 30 x 10^2 and 25 x 10^2 cfu/cm^2 of leaf due to spray with *P. fluorescens* only, *P. fluorescens* + J and *P. fluorescens* + F, respectively. The spray with the mixture of the three microorganisms only or mixed with J or F attained increments over that of the control, but these increments were lower than these caused by spray with each microbe alone. The counts of the tested baceria of control treatment averaged 0.1 x 10^2, 0.05 x 10^2 and 0.04 x 10^2 cfu/cm^2 of leaf due to the spraying with *Azospirillum brasilense*, *B. polymyxa* and *P. fluorescenc*, respectively.

Table 5: Effect of spray with some synthetic and some bacterial biostimulants (PGPR cultures) on the counts of the used PGPR bacterial cells in the phyllosphere of "Anna" apple trees at 2010 season.

Treatment	*Azospirillum* x10^2	*B. polymyxa* x10^2	*P. fluorescens* x10^2
A	34.0	0.1	0.4
A+J	240.0	0.2	0.3
A+F	4.0	0.1	0.3
B	0.2	45.0	0.3
B+J	0.2	40.0	0.5
B+F	0.2	6.0	0.5
P	0.3	0.2	5.0
P+J	0.3	0.11	30.0
P+F	0.5	0.3	25.0
M	2.0	0.3	0.5
M+J	5.0	2.0	0.3
M+F	7.0	0.4	1.0
J	0.5	0.02	0.05
F	1.0	0.4	1.0
C	0.1	0.05	0.04

***J: Jisamar; F: Furdos; A: *Azospirillum brasilense*; B: *Bacillus polymyxa*; P: *Pseudomonas fluorescens*; M: microbial mixture.**

Spray of plant phyllosphere with PGPR bacteria resulted in increased counts of these bacteria in the phyllosphere, our results confirmed those of Kim *et al.* (2011) as they reported effective colonization of the used PGPR bacteria in the phyllosphere of pepper plants compared to control plants. They, also, noted that most of the fluorescent bacterial cells were localized near the stomata and throughout the blade part of the inoculated leaves.

Readings of Table 6 show the productivity of apple fruits (tons/fed.), costs of production, total and net return (LE/fed). The fixed costs (land rent, labors, fertilizers, pesticides, hoeing and pruning) reached about 5000 **£** E/fed. Changed costs include chemical or biological stimulators, rent of spray machine and spray labor reached 600 **£** E/fed. The price of apple fruits evaluated 3500 **£** E/ton.

Table 6: Economic evaluation of apple trees spray with some synthetic and some bacterial biostimulants (PGPR cultures)

Treatments	Fixed costs (£.E/fed.)	Changed costs (£.E/fed.)	Total costs (£.E/fed.)	Total yield (Ton/ fed.)	Value (£.E/fed.)	Net return (£.E/ fed.)	Increase / decrease in return than control
A	5000	600	5600	7.77	27195	21595	5945
A+J	5000	600	5600	7.61	26635	21035	5385
A+F	5000	600	5600	7.34	25690	20090	4440
B	5000	600	5600	7.41	25935	20335	4685
B+J	5000	600	5600	7.06	24710	19110	3460
B+F	5000	600	5600	7.03	24605	19005	3355
P	5000	600	5600	6.78	23730	18130	2480
P+J	5000	600	5600	6.51	22785	17185	1535
P+F	5000	600	5600	6.39	22365	16765	1115
M	5000	600	5600	5.75	20125	14525	-1125
M+J	5000	600	5600	6.44	22540	16940	1290
M+F	5000	600	5600	6.72	23520	17920	2270
J	5000	600	5600	6.36	22260	16660	1010
F	5000	600	5600	6.42	22470	16870	1220
C	5000	0.0	5000	5.90	20650	15650	-

J: Jisamar; F: Furdos; A: *Azospirillum brasilense*; B: *Bacillus polymyxa*; P: *Pseudomonas fluorescens*; M: microbial mixture.

The calculations of net return per feddan exhibited that the application of treatments of Jisamar and Furdose attained net return over the treatment of control by about 1010 and 1220 **£** E/fed, respectively. The application of bacterial biostimulants alone or mixed with Jisamar or Furdose, however, attained net return much higher than both the control and synthetic stimulators. The highest obtained increase in net return over control treatment was attained by spray with *Azospirillum brasilense* (5945 **£** E/fed) followed by treatment of *Azospirillum brasilense* +J which achieved 5385 **£** E/fed. An increase reached 4685 **£** E/fed due to spray with *B. polymixa*. Otherwise, the only treatment that exhibited decrease than control was the spray with the mixture of the microorganisms.

Finally, from the economic evaluation for the influence of synthetic and bacterial biostimulants, it could be conclude that these compounds increased plant growth and yield without a notable increase in costs because of the lower price of these compounds. Thus, the net return was positive, whereas, the spray with *Azospirillum brasilense* liquid culture gave the highest net return (5945 **£** E/feddan), and the net return of bacterial biostimulants was much larger than those of synthetic biostimulants. Therefore, we recommend the spray of apple trees twice with liquid cultures of effective *Azospirillum brasilense* cultures by the rate of 20 L per feddan as alternative to the application of synthetic biostimulants.

Table (7): Production of indole acetic acid (IAA) and total gibberellins by the used bacterial strains.

Strain	G.P.S*(mg/l)	
	IAA	GA
Pseudomonas fluorescense	160.0	485.0
Bacillus polymyxa	157.0	574.5
Azospirillum brasilense	101.4	604.3

G.P.S.* Growth Promoting Substances

The different bacterial isolates produced different amounts of IAA and GA (Table 7). However, *Azospirillum brasilense* release the highest amounts of GA (604.3 mg/l) compared to 485 and 574.5 mg/l for *Pseudomonas fluorescence* and *Bacillus polymyxa* respectively. *Pseudomonas fluorescence* attained the highest IAA amount (160 mg/l) compared to 157 and 101.4 mg/l for *Bacillus polymyxa* and *Azospirillum brasilense*, respectively.

Yicheng Tu (2000) reviewed that gibberellins(GA) were first discovered by Japanese scientists studying a rice disease characterized by excessive stem elongation named “bakenae” (foolish seedling). The symptom was found to be stimulated by compound(s) released by a fungus *Gibberella fujikuroi*. In 1935, the compound was isolated and named after the fungus as “Gibberellin”. Further experiments showed that GA not only occurred in plants but also played a key role in various aspects of plant growth and development. GAs were found to promote cell expansion and perhaps cell division that led to elongation of shoot tissues and development of a number of other morphological structures such as inflorescences and fruit.

The effects of plant growth promoting rhizobacteria (PGPR) on the rooting and root growth of semi-hardwood and hardwood kiwifruit stem cuttings were investigated by Martínez-Viveros (2010). The PGPR used were *Bacillus* RC23, *Paenibacillus polymyxa* RC05, *Bacillus subtilis* OSU142, *Bacillus* RC03, *Comamonas acidovorans* RC41, *Bacillus megaterium* RC01 and *Bacillus simplex* RC19. All the bacteria showed indole-3-acetic acid (IAA) producing capacity. Among the PGPR used, the highest rooting ratios were obtained at 47.50% for semi-hardwood stem cuttings from Bacillus RC03 and Bacillus simplex RC19 treatments and 42.50% for hardwood stem cuttings from *Bacillus* RC03. As well, *Comamonas acidovorans* RC41 inoculations indicated higher value than control treatments. The results suggest that these PGPR can be used in organic nursery material production and point to the feasibility of synthetic auxin (IBA) replacement by organic management based on PGPR. While Anwar (2007) found that *Azospirillum* K-I produced higher amount of GA (10 ug/ml) which decreased in later growth stages. This decrease in GA might be due to its hydrolysis by bacterial strain. *Azoarcus* K-I and *Azospirillum* ER-2 and ER- 20 also produced indole, acetamide as identified by HPLC. These strains were also useful for increasing rice biomass, N-uptake and fertilizer-N use efficiency. In green house experiments, inoculation of rice with PGPR increased chlorophyll, leaf area, tiller number, plant height, root shoot biomass and grain yield in rice. Soil, root, leaf and stem inoculation methods were equally useful for plant growth improvement

CONCLUSION

The spray of Anna apple trees with synthetic biostimulants (Jisamar and Furdose) resulted in an increase in growth and yield of apple trees and did not significantly affected the quality of the resulted fruits, but leads to increase of fruit weight. The spray with bacterial biostimulants, however, gave best results and the application of *Azospirillum brasilense* surpassed the other bacterial biostimulants. The single spray with bacterial biostimulant showed best results than their mixture with Jisamar or Furdose. Thus, the spray with *Azospirillum brasilense* was the best which may be safely used as an alternative to the chemical synthetic biostimulants.

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إستجابة نمو و محصول و جودة ثمار أشجار التفاح "أنا" للرش الورقي ببعض الميكروبات المنتجة لمنظمات النمو كبديل عن الرش بمنشطات النمو الكيماوية

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تم مقارنة الرش بالمركبات الكيماوية بالرش بالمزارع الميكروبية و الخلط بينهم لتحديد كفاءتها و إمكانية استبدال هذه المركبات الحيوية الطبيعية بتلك الكيماوية.

تم اختيار حقل منزرع بأشجار التفاح صنف "أنا" عمر ثماني سنوات و تم تحديد عدد ثلاثة أشجار لكل معاملة ثم رشت الأشجار بالمعاملات المختلفة مرتين الأولى عند الإزهار و الأخرى بعد عقد الثمار بالمقارنة بالرش بالماء كمعاملة مقارنة.

حققت معاملة الرش بالمنشطات الكيماوية تحسنا ملحوظا في خواص النبات الظاهرية مثل طول الفروع, قطر الفروع, مساحة الورقة, الوزن الجاف للورقة و محتوى الورقة من كلوروفيل أ و ب و الكلوروفيل الكلي, فيما لم يتغير الوزن النوعي للورقة معنويا. أظهرت معاملات الرش بالمنشطات الحيوية تأثيرا مماثلا للمنشطات الكيماوية (الجيسمار و الفردوس) بل تفوقت عليها في بعض الحالات. كما أظهرت أعداد الميكروبات التي تم الرش بها أعدادا كبيرة بمنطقة الفيلوسفير. على الرغم من زيادة إنتاجية ثمار التفاح بسبب الرش بالمحفزات الحيوية و الكيماوية, فان الرش بالمحفزات الحيوية تفوقت و بدرجة عالية على تأثير الرش الكيماوي, حيث حققت معاملة الرش بالازوسبيرليم أعلى إنتاجية و هي45,16 و 41,2 كيلوجرام/شجرة لموسمي 2009 و 2010 في مقابل 33,53 و 32,07 على التوالي لمعاملة الكنترول و أعطى الرش بالجيسمار 37,4 و33,27 و بالفردوس 36,53 و 34,77 كيلوجرام/شجرة على التوالي. كما حققت معاملة الرش بالازوسبيرليم أعلى صافي عائد بالجنيه المصري وهو21595 في مقابل 16660 و 16870 و 15650 لمعاملات الجيسمار والفردوس والكنترول على التوالي. لذلك فمن المناسب استبدال الرش بالمحفزات الحيوية مثل مزارع الازوسبيرليم المخففة لهذه المنشطات الكيماوية لكفاءتها في تحسين نمو الأشجار و زيادة الإنتاجية مع تحسين العائد الاقتصادي للفدان.

قام بتحكيم البحث

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