Response of "Anna" Apple Storability to Foliar Spray with Some PGPR as A Substitute to Synthetic Biostimulants

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ABSTRACT

Response of fruit quality and storability to spray with PGPR (plant growth promoting rhizobacteria) and synthetic biostimulants were investigated on eight years old "Anna" apple trees budded on Malus rootstock grown in sandy soil at Elbostan region during 2009 & 2010. Fruits were picked at maturity stage and stored for thirty days intervals, at 0° C and 90 - 95% relative humidity up to 120 days. All spraying treatments with *Pseudomonas fluorescens* and *Bacillus polymixa* recorded significant decrease on loss of fruit weight, and enhanced fruit texture against control. All microbial and synthetic biostimulants spray reduced decay of fruit, acidity % and H° value of fruit color, while showed no effect on SSC %. Treatments of *Bacillus polymixa* spray retarded fruit respiration rate, while other treatments did not show a clear trend, when compared with control.

Storage period intervals gradually increased loss of fruit weight, decay fruit percentage and SSC, while decreased fruit texture and acidity % as storage period advanced. Records of hue angle decreased gradually in a significant manner throughout the storage periods while values indicated that fruit color turned to be reddish.

Ps+J treatment after 30 days of storage did not recorded any decayed fruits and after 60 days recorded the lowest decay percentage. The treatment of Ps+J at 120 days of storage significantly increased SSC of fruit juice. Treatments of Ps+J & Ps+F at 0 day of storage had the highest fruit texture, while control treatment (C) after 120 days of storage under 0° C recorded the lowest fruit texture in both seasons. Sprayed fruits with *Pseudomonas* and Jisamar (Ps+J) at 120 days of storage had the most reddish color as recorded by the lowest hue angle values.

INTRODUCTION

'Anna' is the most wide cultivated apple (*Malus domestica*, Borkh) variety in Egypt whereas, apple productivity reached 493119 tons at 2010 (Ministry of Agriculture and Land Reclamation, statistics of Economic Affairs Sector, 2010).

Pre-harvest applications can influence most, if not all, quality parameters of tropical and subtropical fruits (Hofman and Smith, 1993). The application of synthetic stimulants had the positive and effective influence on increasing plant growth and productivity. Moreover, Spinelli *et al.* (2010) claimed that the biostimulant may represent a promising strategy to reduce the use of phytochemicals in agriculture.

All tested nitrogen fixing bacterial strains produced IAA (Pedraza *et. al.*, 2004) especially with tryptophan as precursor. *Azospirillum* strains produced the highest concentrations of indole-3-acetic acid (16.5– 38 µg IAA /mg protein), whereas *Gluconacetobacter* and *Pseudomonas stutzeri* strains produced lower concentrations ranging from 1– 2.9 µg / mg protein in culture medium supplemented with tryptophan. The IAA production may enable bacteria to promote a growth in plants, in addition to their nitrogen fixing ability. Whereas, gram positive bacterium *Bacillus subtilis* is known to positively influence plant growth, vitality and the ability of plant to cope with pathogens often resulting in higher yield (Wotike *et. al.*, 2004). They added that these beneficial effects has been ascribed to abiotic stress release.

In apple, a lot of studies found significant increase in productivity due to spray with different types of PGPR cultures, whereas, Karakurt and Aslantas (2010) demonstrated that the spray of apple tree with some PGPR (*Bacillus subtilis* OUS-142 and *Pseudomonas putida* BA-8) enhanced nutrient uptake. At the same time, foliar spray of apple trees with three *Bacillus* sp. increased fruit yield and quality (Ryu *et al.*, 2011). In apricot, Esitken *et. al.* (2004) determined the effect of floral and foliar application of the bacterial strain *Bacillus* OSU 142 on the yield and nutrient element composition of leaves. They reported that bacterial suspension spraying at full bloom increased leaf content of N, P, K, Ca and Mg and yield of apricot trees. General relationships are noted in temperate and tropical fruits between nitrogen and color, disorders, postharvest disease incidence, fruit size and firmness (Raese and Williams, 1974 and Hofman and Smith, 1993). So, it could be observed that the PGPB had multi-mechanisms for increasing yield and enhancing fruit quality and storability such as facilitating nutrient uptake (Martínez -Viveros *et al,* 2010); producing plant phytohormones (Spaepen *et al.*, 2008); regulate plant ethylene level

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Received February 22, 2012, Accepted March28, 2012

(Govidasamy *et al*, 2008); resist plant pathogens (Van Loon, 2007); produce antibiotics (Esitken, 2011).

For a lake of studies on response of apple storability, this study was carried out to investigate the influences of substituting spraying with chemical stimulants by a suitable PGPB on quality and behavior of fruits during storage period of 'Anna' apple fruits.

MATERIALS AND METHODS

This investigation has been carried out on eight years old 'Anna' apple trees budded on Malus rootstock during 2009 and 2010. Trees were grown at Elbostan district of Elbehira governorate where trees applied of drip fertigation system.

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

(C): Control trees are sprayed with tap water.

(Az): Trees were sprayed with a suspension of *Azotobacter chroococcum.*

(Ba): Trees were sprayed with a suspension of *Bacillus polymixa*

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

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

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

(Az+J): Trees were sprayed with a solution of *Azotobacter chroococcum* and Jisamar.

(Ba+J): Trees were sprayed with a solution of *Bacillus polymixa* and Jisamar.

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

(Az+F): Trees were sprayed with a solution of *Azotobacter chroococcum* and Furdose.

(Ba+F): Trees were sprayed with a solution of *Bacillus polymixa* and Furdose.

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

The Microbial inoculums was prepared and provided from Soil Bacteriology Laboratory of Sakha Agricultural Research Station, ARC, Egypt. Bacterial suspension was diluted by mixing of 400 ml. of bacterial stocks with 9 L. of water per 3 trees of each treatment. At the same time, applied synthetic biostimulants (Jisamar & Furdose) were sprayed according to their recommendations. Jisamar contains seaweed extract (20.5%), free amino acids (6.5%), total nitrogen (5.8%), phosphorus (3%), boron (0.17%) and potassium (4.6%). Furdose 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).

Treatments were arranged in a split plot design where, computerized statistical analysis was done for data by 'Irristat' package. Samples from each replicate were picked at maturity stage in carton packages. These fruits were transported to laboratory of fruit handling research department, HRI, ARC. Sound fruits, nearly uniform in size and color of each replicate were washed with tap water, air dried and then pre-cooled before storage. Fruits of each replicate were packed in carton boxes in one layer. All fruits were stored for thirty days intervals, at 0° C and 90 - 95% relative humidity up to 120 days. At the end of each storage interval, fruits were taken to determine the following estimates:

1) Fruit physical changes and decay disorders:

a. Percentage of weight loss: ten fruits from each replicate were marked for weight loss determination during 30 days of each storage period. Weight loss percentage was calculated according to the initial weight.

b. Fruit decay percentage: decayed fruits characterized with rind breakdown; shriveling or other defects were calculated for each treatment as fruit decay percentage according to McCormack and Brown (1973).

c. Fruit peel puncture resistance: fruit pulp texture was recorded by using Lfra texture analyzer instrument using a penetrating cylinder of 5mm. diameter to a constant distance (1cm.) inside the fruit with a constant speed (3mm./ second). The results were expressed as a resistance force of the fruit to the penetrating tester (gm./ cm.^{2}) according to Harold (1985).

d. Peel color measurements (a*, b*, L* & H° values): 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).

2) Fruit chemical properties: Total soluble solids (TSS) were determined by using a hand refractometer and total acidity percentage was estimated in filtered juice according to (A.O.A.C., 1990).

3) **Fruit respiration rate:** a known weight of fruits was placed in a disicator and connected to a tube contains 25 ml. of KOH (1.0 N). Air free of carbon dioxide was down into the discator through potassium hydroxide for one hour, then KOH was titrated with HCL (1.0 N) using thymol blue indicator. Produced CO_2 was calculated as mg. CO_2 / kg. of fruit / h. according to (A.O.A.C., 1990).

RESULTS

Loss of weight percentage:

Data of microbial and synthetic biostimulants influences on loss of weight percentage of 'Anna' apple fruits were arranged in table (1). Data of table (1) showed that regardless of storage interval, treatment of Ps+J which was sprayed with *Azotobacter chroococcum* and Jisamar recorded the lowest significant loss of weight (3.67 & 3.18) in both studied seasons. On the other hand, control treatment had the worst and highest significant loss of weight percentage as it gave 7.61 & 7.97 %, while all spraying treatments with *Pseudomonas fluorescens* and *Bacillus polymixa* recorded intermediate values with significant differences against control.

As for storage intervals regardless of spraying treatments, data showed that loss of weight percentage of 'Anna' apple fruits significantly increased as storage period advanced in both seasons. It was clear that rate of weight loss of fruits gradually increased from storage period starting (0 day) followed by 30, 60, 90 and then 120 day, differences were permanently significant among intervals of storage period.

Referring to the interaction between spraying of microbial and synthetic biostimulants and storage intervals, data in table (1) concerned with loss of weight percentage of 'Anna' apple fruits showed that control treatment throughout days of storage had the highest significant loss of weight percent followed by Furdose treatment without significant differences in most cases, in both seasons.

Decay percentage:

Concerning the microbial and synthetic biostimulants spray influences on decay percentage of 'Anna' apple fruits, data were arranged in table (2). Data showed that regardless of storage interval, all spraying treatments reduced decayed fruits against control fruits (C) which had the highest significant decay percentage as it gave 9.44 & 10.83 %, in both seasons. On the other hand, Ps+J treatment recorded the lowest decay percentage which gave 3.88 & 2.50, in the studied seasons, respectively.

As respect of storage intervals, data of table (2) showed that decayed 'Anna' apple fruits increased as storage period was advanced in both seasons, while differences were significant among intervals of storage period.

Table 1. Effect of spraying some microbial and synthetic biostimulants on loss of weight % of 'Anna' apple fruits during cold storage at $0^{o}C$.

Treat.	2009						2010					
	Storage intervals per days						Storage intervals per days					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
Az	0.00	3.08	5.99	8.66	12.20	5.98	0.00	3.26	6.50	8.70	11.08	5.90
Az+J	0.00	2.45	5.04	7.24	10.30	5.00	0.00	1.97	4.42	7.20	9.81	4.68
Az+F	0.00	2.08	4.18	6.55	8.68	4.29	0.00	2.07	4.16	6.67	9.30	4.44
Ba	0.00	2.93	5.76	7.93	11.14	5.55	0.00	2.05	5.06	7.20	8.69	6.60
Ba+J	0.00	2.22	4.17	6.70	8.71	4.36	0.00	2.39	4.06	6.41	8.11	4.19
Ba+F	0.00	2.13	4.53	6.58	8.87	4.42	0.00	2.54	3.66	6.25	8.51	4.19
Ps	0.00	2.48	5.31	7.64	10.90	5.26	0.00	2.58	5.44	8.47	11.04	5.50
Ps+J	0.00	1.80	3.66	5.77	7.13	3.67	0.00	1.42	2.85	4.82	6.81	3.18
Ps+F	0.00	2.14	4.08	6.20	8.14	4.12	0.00	2.10	3.93	5.90	7.73	3.51
J	0.00	2.92	6.99	9.30	13.18	6.47	0.00	2.90	6.81	8.95	9.83	5.69
F	0.00	3.63	7.01	10.73	14.19	7.11	0.00	3.26	6.91	9.70	13.59	6.69
C	0.00	3.42	7.92	11.26	15.48	7.61	0.00	3.84	8.88	11.63	15.50	7.97
Mean	0.00	2.60	5.38	7.33	10.74	5.26	0.00	2.53	5.22	7.65	10.00	5.14

L.S.D at 5%	A	B	A x B
2009	1.902	1.501	1.675
2010	2.210	1.342	1.226

*J: Jisamar; F: Furdos; Az: *Azotobacter chroococcum*; Ba: *Bacillus polymixa*; Ps: *Pseudomonas fluorescens*.

**A: Microbial and biostimulants treatments. - B: Storage intervals - A x B: interaction.

Table 2. Effect of spraying some microbial and synthetic biostimulants on decay % of ‘Anna’ apple fruits during cold storage at 0° C.

Treat.	2009						2010					
	Storage intervals per days						Storage intervals per days					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
Az	0.00	2.78	4.17	6.94	10.72	4.92	0.00	4.17	5.55	8.33	12.50	6.11
Az+J	0.00	2.78	4.17	6.94	10.72	4.92	0.00	1.39	5.55	8.33	11.11	5.27
Az+F	0.00	4.17	5.55	8.33	10.72	5.75	0.00	2.78	5.55	9.72	11.11	5.83
Ba	0.00	1.39	6.94	8.33	13.50	6.03	0.00	1.39	4.16	8.33	11.11	4.99
Ba+J	0.00	1.39	4.17	6.94	8.33	4.44	0.00	2.78	4.16	8.33	8.33	4.72
Ba+F	0.00	1.39	5.55	6.94	8.33	4.71	0.00	2.78	4.17	5.55	6.94	3.88
Ps	0.00	4.17	6.94	8.33	11.11	6.11	0.00	2.78	6.94	11.11	11.11	6.38
Ps+J	0.00	0.00	5.55	5.55	8.33	3.88	0.00	0.00	2.78	4.17	5.55	2.50
Ps+F	0.00	0.00	4.17	5.55	8.33	3.61	0.00	1.39	2.78	6.94	9.72	4.16
J	0.00	4.17	5.55	8.33	11.11	5.83	0.00	2.78	4.17	6.94	11.11	5.00
F	0.00	2.78	5.55	9.72	11.11	5.83	0.00	4.17	6.94	8.33	11.11	6.11
C	0.00	6.94	9.72	12.50	18.05	9.44	0.00	8.33	11.11	15.28	19.44	10.83
Mean	0.00	2.54	5.20	7.86	10.86	5.34	0.00	3.13	5.32	8.44	10.76	5.50

L.S.D at 5%	A	B	A x B
2009	3.187	1.954	2.365
2010	3.714	1.677	2.524

*J: Jisamar; F: Furdos; Az: *Azotobacter chroococcum*; Ba: *Bacillus polymixa*; Ps: *Pseudomonas fluorescens*.
**A: Microbial and biostimulants treatments. - B: Storage intervals - A x B: interaction.

Referring to the interaction between microganisms and synthetic biostimulants spray treatments and storage intervals, data in table (2) concerning decay % of ‘Anna’ apple fruits showed that control treatment after 120 days of storage had the highest significant decay percentage as it gave 18.05 & 10.83 % in both seasons, respectively. On the other hand, Ps+J treatment after 30 days of storage did not record any decayed fruits, while after 60 days it recorded the lowest decay percentage.

Fruit texture:

Concerning fruit peel, puncture resistance as a measure for fruit texture of ‘Anna’ apple fruits, data of table (3) cleared that regardless of storage interval, all spraying treatments with *Pseudomonas fluorescens* and *Bacillus polymixa* enhanced fruit texture and recorded the highest values with significant differences against the control. At the same time, other treatments increased fruit texture without significant differences than control.

As for storage intervals, regardless of biostimulants spraying treatments data of table (3) showed that fruit texture significantly decreased with an ascending order with advanced storage period, in both studied seasons. It was clear that rate of fruit texture gradually decreased from 0 day interval followed with 30, 60, 90 and then 120 day. Statistical analyses showed that differences among intervals of storage period were significant.

As respect of interaction between biostimulants spraying treatments and storage intervals, data in table (3) concerning with fruit texture showed that treatments of Ps+J & Ps+F at 0 day of storage had the highest fruit texture. On the other hand, control treatment (C) after 120 day of storage under 0° C recorded the lowest fruit texture (33.0 & 35.6) in both seasons.

Fruit color (H°):

Data of biostimulants spraying effects on fruit color (H°) of ‘Anna’ apple fruits were arranged in table (4). Data showed that regardless of storage interval, both microbial and synthetic biostimulants spraying treatments decreased H° value of fruits. It means that treated fruits were better to be more reddish than control ones which gave hue angle values toward yellowness, especially at first season.

As respect of storage intervals, data showed that records of hue angle were decreased gradually in a significant manner from 0 day (75.92 & 77.27) up to 120 days (66.21 & 64.06) throughout storage period under condition of 0° C, in both seasons.

Referring to interaction between microorganisms and synthetic biostimulants spray and storage intervals, data in table (4) concerning fruit color (H°) of ‘Anna’ apple fruits showed that treatment sprayed with *Pseudomonas fluorescens* and Jisamar at 120 days of storage gave the most reddish fruits as recorded the lowest hue angle value as gave 52.89 & 51.25 in both seasons, respectively. While at picking date (0 interval), control treatment recorded the highest fruit color (H°) and gave 92.80 and 87.20 in studied seasons which indicate a trend toward yellowness.

Table 3. Effect of spraying some microbial and synthetic biostimulants on texture (lp/in^2) of 'Anna' apple fruits during cold storage at 0°C.

Treat.	2009						2010					
	Storage intervals per days						Storage intervals per days					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
Az	226.0	170.0	101.3	70.6	43.3	122.2	263.3	167.6	103.3	66.0	52.3	130.5
Az+J	260.0	182.3	112.3	72.3	49.3	135.2	263.0	178.1	108.3	72.3	58.3	136.0
Az+F	237.6	172.0	101.6	64.6	44.6	124.0	245.3	172.0	102.6	64.6	55.0	127.9
Ba	267.6	185.0	113.3	73.6	56.0	139.1	280.0	183.0	126.3	78.6	69.3	147.4
Ba+J	282.3	204.6	128.6	89.3	57.3	152.4	285.6	218.6	130.6	94.6	70.0	159.8
Ba+F	280.0	199.6	126.0	79.0	53.6	147.6	283.6	228.6	129.3	88.6	74.0	160.8
Ps	269.0	190.0	120.0	76.6	55.5	142.2	277.6	179.0	109.3	73.0	53.6	138.5
Ps+J	286.0	210.6	146.6	101.3	63.0	161.5	284.6	213.3	148.6	106.6	76.6	139.9
Ps+F	286.3	210.6	140.6	96.0	59.3	158.5	286.3	216.0	139.3	99.3	73.3	162.8
J	222.0	162.6	96.0	55.3	37.3	114.6	232.6	159.6	92.3	61.0	41.6	117.4
F	223.0	166.6	95.6	70.3	40.0	119.1	234.6	169.9	102.0	67.0	41.0	122.9
C	207.0	154.0	89.6	50.6	33.0	106.8	220.6	154.3	89.3	51.6	35.6	110.2
Mean	253.9	184.0	114.2	74.9	49.3	135.2	263.0	186.6	106.5	76.9	58.3	138.0

L.S.D at 5%	A	B	A x B
2009	21.439	17.112	7.327
2010	23.138	14.157	11.346

*J: Jisamar; F: Furdos; Az: *Azotobacter chroococcum*; Ba: *Bacillus polymixa*; Ps: *Pseudomonas fluorescens*.
**A: Microbial and biostimulants treatments. - B: Storage intervals - A x B: interaction.

Table 4. Effect of spraying some microbial and synthetic biostimulants on color (H°) of 'Anna' apple fruits during cold storage at 0° C.

Treat.	2009						2010					
	Storage intervals per days						Storage intervals per days					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
Az	79.23	77.06	60.31	72.99	69.32	71.78	77.68	73.56	71.99	68.51	66.16	71.58
Az+J	74.17	74.11	71.38	68.15	62.32	70.02	75.01	69.97	67.31	63.08	62.86	67.64
Az+F	76.17	72.83	70.67	69.41	68.26	71.47	77.85	73.19	69.50	66.97	66.26	70.76
Ba	73.02	71.31	68.21	64.63	62.28	67.89	75.62	69.55	64.18	62.37	59.05	66.16
Ba+J	72.96	66.07	63.82	60.29	64.85	65.59	75.37	65.04	61.94	60.17	57.06	63.91
Ba+F	71.11	67.56	66.51	62.39	59.46	65.40	75.26	67.09	63.16	61.89	59.74	65.42
Ps	72.19	70.66	68.39	64.51	61.87	67.52	75.51	69.71	66.31	63.26	61.99	67.35
Ps+J	65.02	82.82	59.49	55.43	52.89	63.13	70.63	61.37	57.94	54.70	51.25	59.17
Ps+F	66.29	65.23	60.91	57.44	54.95	60.96	70.20	63.03	61.96	58.26	54.64	61.61
J	83.84	83.84	80.33	77.34	75.35	80.14	81.54	78.24	75.35	73.33	71.74	76.04
F	82.30	82.30	80.05	77.76	75.07	79.49	82.25	79.44	76.07	72.72	70.77	76.25
C	94.84	94.83	93.77	93.39	92.80	93.92	90.34	87.60	86.52	87.04	87.20	87.74
Mean	75.92	75.71	70.32	68.64	66.61	71.42	77.27	70.73	68.51	66.02	64.06	69.39

L.S.D at 5%	A	B	A x B
2009	4.415	2.562	5.213
2010	4.718	2.489	4.983

*J: Jisamar; F: Furdos; Az: *Azotobacter chroococcum*; Ba: *Bacillus polymixa*; Ps: *Pseudomonas fluorescens*.
**A: Microbial and biostimulants treatments. - B: Storage intervals - A x B: interaction.

Soluble solids content (SSC %):

Data of table (5) clearly show that regardless of storage interval, all microbial and synthetic biostimulants did not affect soluble solids content (SSC) of 'Anna' apple juice. Some treatments of spraying microbial and synthetic biostimulants increased SSC % in one season while gave decreased values in another, compared with control. Statistical analyses showed that there was no significant differences among treatments or between treatments and control, in most cases.

As for storage intervals, data of table (5) showed that SSC % was increased with an ascending order with advancing storage period. It was clear that soluble solids content percentages were gradually increased from start of storage period (0 day interval) followed with 30, 60, 90 and then 120 days and differences among intervals were significant, in most cases.

Data in table (5) concerned with interaction between microbial and synthetic biostimulants spraying treatments and storage intervals showed that treatment of Ps+J at 120 days of storage had the significant highest SSC as gave 14.73 & 14.70 % in studied seasons, respectively. On the other hand, treatment of Ba+F at 0 day recorded the lowest SSC percent (12.23 %) in season of 2009, while treatment of Ba+F at 0 day gave the same trend in second season when recorded 12.26 %.

Acidity percentage:

Regardless of storage interval, data of table (6) concerned with influences of microbial and synthetic biostimulants spray on acidity percentage of juice illustrated that spray with separate bio-microbial stimulants or synthetic ones increased acidity, significantly. In spite of mixed microbial and synthetic biostimulants treatments increased acidity % without significance compared with control. Whatever, control gave the lowest significant acidity percentages (0.58 & 0.59 %) in both studied seasons.

As respect of storage intervals, data of table (6) cleared that acidity percentages significantly decreased with an ascending order as storage period advanced. It was clear that acidity percentages gradually decreased from storage period start (0.83 %) up to 120 days (0.55 %), while others gave intermediate values with significant differences among intervals, in most cases.

Referring to the interaction between microbial and synthetic biostimulants spraying and storage intervals, data in table (6) concerning acidity % of 'Anna' apple fruits showed that control after 120 days of storage had the lowest significant acidity percentage as it gave 0.41 & 0.42 % in both seasons, respectively. On the other hand at 0 day of storage period, fruits of Ps+F treatment had the highest significant acidity % as it recorded 0.97 & 0.96 % in both seasons of the study.

Table 5. Effect of spraying some microbial and synthetic biostimulants on SSC % of 'Anna' apple fruits during cold storage at 0^{o} C.

Treat.	2009						2010					
	Storage intervals per days						Storage intervals per days					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
Az	13.20	13.16	13.20	13.23	13.76	13.31	13.33	13.20	13.06	13.46	13.66	13.34
Az+J	12.93	12.93	13.23	13.43	13.73	13.25	13.16	13.23	13.50	13.30	13.86	13.41
Az+F	13.06	13.13	13.33	13.36	13.53	13.28	13.26	13.33	13.46	13.66	13.80	13.50
Ba	12.60	13.00	13.23	13.70	13.73	13.25	12.98	13.36	13.16	13.50	14.16	13.43
Ba+J	12.40	12.96	13.30	13.56	14.36	13.31	12.26	12.90	12.90	13.40	14.50	13.19
Ba+F	12.23	12.76	12.80	13.76	14.06	13.12	12.83	13.00	13.06	13.90	14.36	13.43
Ps	12.43	12.66	13.06	13.53	13.93	13.12	12.63	13.20	13.00	13.50	14.10	13.28
Ps+J	12.40	12.60	13.26	13.76	14.73	13.35	12.66	12.80	13.13	13.60	14.70	13.37
Ps+F	12.43	12.43	13.33	13.36	14.46	13.20	12.63	12.83	13.26	14.00	14.56	13.45
J	12.66	12.86	12.93	13.23	13.66	13.06	12.83	13.00	13.16	13.23	13.60	13.16
F	12.88	13.00	13.06	13.40	13.60	13.18	13.00	13.36	13.63	13.63	13.76	13.47
C	13.06	13.06	13.13	13.40	13.50	13.23	13.03	13.20	13.46	13.56	13.70	13.39
Mean	12.69	12.87	13.15	13.47	13.92	13.22	12.88	13.11	13.23	13.56	14.06	13.36

L.S.D at 5%	A	B	A x B
2009	0.2739	0.3274	0.7011
2010	0.3846	0.3669	0.7224

*J: Jisamar; F: Furdos; Az: *Azotobacter chroococcum*; Ba: *Bacillus polymixa*; Ps: *Pseudomonas fluorescens*.
**A: Microbial and biostimulants treatments. - B: Storage intervals - A x B: interaction.

Table 6. Effect of spraying some microbial and synthetic biostimulants on acidity % of 'Anna' apple fruits during cold storage at 0° C.

Treat.	2009						2010					
	Storage intervals per days						Storage intervals per days					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
Az	0.82	0.71	0.69	0.53	0.50	0.65	0.86	0.70	0.60	0.55	0.47	0.63
Az+J	0.85	0.85	0.75	0.65	0.59	0.73	0.86	0.84	0.77	0.65	0.58	0.74
Az+F	0.84	0.84	0.75	0.65	0.64	0.74	0.87	0.81	0.74	0.68	0.56	0.73
Ba	0.82	0.69	0.60	0.56	0.51	0.63	0.78	0.72	0.70	0.60	0.55	0.67
Ba+J	0.94	0.84	0.87	0.67	0.65	0.79	0.92	0.85	0.77	0.69	0.62	0.77
Ba+F	0.94	0.87	0.73	0.72	0.60	0.77	0.93	0.82	0.75	0.69	0.61	0.76
Ps	0.84	0.73	0.60	0.52	0.49	0.63	0.86	0.80	0.69	0.56	0.47	0.67
Ps+J	0.82	0.84	0.87	0.67	0.65	0.77	0.77	0.75	0.73	0.70	0.69	0.72
Ps+F	0.97	0.88	0.81	0.71	0.63	0.80	0.96	0.86	0.84	0.69	0.67	0.80
J	0.74	0.74	0.66	0.68	0.52	0.66	0.72	0.71	0.66	0.60	0.51	0.64
F	0.74	0.74	0.70	0.57	0.50	0.65	0.70	0.69	0.69	0.62	0.56	0.65
C	0.69	0.69	0.62	0.50	0.41	0.58	0.77	0.65	0.61	0.52	0.42	0.59
Mean	0.83	0.78	0.72	0.61	0.55	0.70	0.83	0.76	0.71	0.62	0.55	0.69

L.S.D at 5%	A	B	A x B
2009	0.1476	0.1173	0.2511
2010	0.1352	0.1297	0.2374

*J: Jisamar; F: Furdos; Az: *Azotobacter chroococcum*; Ba: *Bacillus polymixa*; Ps: *Pseudomonas fluorescens*.
**A: Microbial and biostimulants treatments. - B: Storage intervals - A x B: interaction.

Fruit respiration rate:

Response of fruit respiration rate of 'Anna' apples to spray of microbial and synthetic biostimulants is shown in table (7). Data showed that regardless of storage interval, treatments of *Bacillus polymixa* spray retarded the rate of fruit respiration in a significant manner when compared with control. In spite of significant differences presence, the other treatments of microbial and synthetic biostimulants spray did not show a clear trend.

As respect of storage intervals without consideration for treatments, data illustrated that fruit respiration rates increased with an ascending order as storage period advanced. It was clear that rates of respiration gradually increased from start of storage period (0 day interval) followed by 30, 60, 90 and 120 days and differences among intervals were significant.

Data of interaction between microbial and synthetic biostimulants treatments and storage intervals, concerned with fruit respiration rate of 'Anna' apple fruits showed that control apples at the end of storage period recorded the highest significant values (62.63 & 74.20) in both seasons, respectively. On the other hand, apples of Ps+F trees recorded the significant lowest rate as it gave 26.15 at 0 day of storage in the first season, while sprayed fruits with *Bacillus polymixa* (Ba) showed significant lowest rate as it gave 26.51 at start of storage in the second season.

DISCUSSIONS

The present results are firmly coincide with the findings of Karakurt and Aslantas (2010) who 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. Whereas, Ryu *et al*. (2011) reported that foliar spray of apple trees with three *Bacillus* sp. Increased tree growth, fruit yield and quality. At the same line, Spinelli *et al.* (2010) applied the apple trees with a product (Actiwave) 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.

The obtained results are in the same line with those of El-Hammady *et al.* (2000) who recommended that GA_3 application on mandarin increased TSS after 30 days of cold storage and then decreased up to 75 days, while acidity was decreased as storage period advanced. Also, El-Hammady *et al.* (2000) added that GA_3 was effective to decline fruit decay percentage. Meanwhile, we can attribute the superiority of microbial biostimulants treatments in declining fruit decay percentage of 'Anna' apple fruits to the influence of antibiotics production (Esitken, 2011) which consequently reflected on enhancing fruit pathogens control. These strains of bacteria has capability to produce anti-microbial substances in a continuous

Table 7: Effect of spraying some microbial and synthetic biostimulants on respiration rate (mg. CO_2 / kg) of 'Anna' apple fruits during cold storage at 0°C.

Treat.	2009						2010					
	Storage intervals per days						Storage intervals per days					
	0	30	60	90	120	Mean	0	30	60	90	120	Mean
Az	35.37	37.37	42.60	57.34	62.51	47.03	35.08	39.32	46.24	55.58	57.11	46.66
Az+J	41.02	45.99	53.14	58.16	62.30	52.12	39.62	41.57	44.07	54.23	54.97	46.89
Az+F	39.62	44.70	53.90	56.13	59.81	50.83	38.52	43.45	50.55	54.65	57.20	48.87
Ba	26.51	33.53	36.47	43.37	46.26	37.22	26.51	32.42	35.05	38.20	40.25	34.48
Ba+J	28.38	35.57	40.87	47.16	51.79	40.75	28.89	31.80	41.07	42.55	45.90	38.04
Ba+F	28.31	30.02	32.79	37.13	39.36	33.52	30.76	31.63	36.51	39.58	42.47	36.18
Ps	28.49	33.38	42.49	56.02	60.17	44.11	31.87	36.50	42.59	47.67	49.68	41.66
Ps+J	30.27	36.13	40.62	46.51	49.11	40.52	30.50	35.43	40.66	42.43	43.69	38.54
Ps+F	26.15	30.05	36.05	41.69	45.25	35.83	29.38	33.43	34.83	37.78	39.56	34.99
J	39.54	42.51	44.24	54.20	58.18	47.73	36.48	42.61	50.61	56.54	60.52	49.35
F	38.92	46.26	50.50	55.26	60.63	50.31	39.66	42.69	61.40	54.95	56.56	51.05
C	29.78	43.44	50.54	61.79	62.63	49.63	42.60	45.38	51.85	63.47	74.20	55.50
Mean	31.39	38.24	43.68	51.23	54.83	44.04	34.14	38.01	44.61	48.98	51.84	43.51

L.S.D at 5%	A	B	A x B
2009	7.284	4.863	8.593
2010	8.103	3.407	9.631

*J: Jisamar; F: Furdos; Az: *Azotobacter chroococcum*; Ba: *Bacillus polymixa*; Ps: *Pseudomonas fluorescens*.
**A: Microbial and biostimulants treatments. - B: Storage intervals - A x B: interaction.

release manner. This is the direct reason to decline rots infection of fruits and decreased decayed fruits.

At the meantime, Farag (2001) provided that GA_3 retarded the progress of senescence, decreased fruit respiration rate and delayed the decrease of CO_2 conductance resulting in less of an increase in internal CO_2 (El-Otmani *et al.*,1986) of navel orange fruits. Chen and Zhang (1988) concluded that GA_3 reduced pectin methyl esterase activity and protopectin degradation and increase peroxidase levels. So, these findings could explain the influence of *Bacillus polymixa* spray treatments on retarding the fruit respiration rate of "Anna" apple fruits. Additionally, these results found support and explanation with the findings of Govidasamy *et al.* (2008) that applications of PGPR regulate plant ethylene level, causing in turn regulation of fruit ethylene production and delaying fruit senescence.

Although, we can attribute the superiority of microbial biostimulants to multi-mechanisms of PGPR for enhancing plant health such as fixing atmospheric nitrogen (Saharan and Nehra, 2011); facilitate nutrient uptake (Martinez-Viveros *et al.*, 2010); producing plant phytohormones (Spaepen *et al.*, 2008); regulate plant ethylene level (Govidasamy *et al.*, 2008); resist plant pathogens (Van Loon, 2007); produce antibiotics which is reflected on plant growth, yield and quality (Esitken, 2011).

The obtained herein results are in agreement with those concluded by El-Sheikh (2002) in pear and navel orange (Zaghloul, 2004) that weight loss percentage increased with the prolonging the storage period. Zaghloul (2004) showed that the loss in fruit weight is mainly due to water loss as a result of evaporation and transpiration, plus the amount of dry matter loss by fruit respiration. In pear, El-Sheikh (2002) contended that stored fruits had highest SSC after 90 days of cold storage than fruits at harvest time, however acidity was decreased as storage period was advanced due to the consumption of organic acids in fruit respiration.

Roe and Bruemmer (1981) regarded that water soluble and alkali soluble pectin were declined and ammonium oxalate soluble pectin was increased as mature fruits lost its firmness and become soft at ripening. The results of this study are in harmony with Roe and Bruemmer (1981) that loss of firmness was slowly in cool stored ripe fruits as the decline of alkali soluble pectin was slowly. Also, results are in agreement with Abd El-Aziz (2001) who reported that respiration rate of persimmon fruits decreased constantly during cold storage.

According to the results of this study, treatment of *Pseudomonas fluorescens* and Jisamar spray of 'Anna' apple trees was recommended to delay decay of fruits; decrease decay percentage; increase SSC of fruit juice and texture; and enhance fruit color of 'Anna' apple fruits during storage period.

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الملخص العربي

استجابة القدرة التخزينية لثمار التفاح "آنا" للرش الورقي بالبكتيريا المنتجة لمنشطات النمو وبعض المنشطات الحيوية

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استجابة القدرة التخزينية وجودة ثمار التفاح "آنا" لمعاملات الرش بالبكتريا المنتجة لمنظمات النمو والمنشطات الحيوية المخلقة تم دراستها خلال عامي 2009 و2010 على أشجار عمر 8 سنوات ومطعومة على أصل المالص و مزروعة في تربة رملية بمنطقة البستان.

تم قطف الثمار في مرحلة اكتمال النمو, و تم التخزين تحت ظروف درجة حرارة الصفر المئوي ورطوبة نسبية (90– 95%) لمدة 120 يوم, وقد أظهرت النتائج أن كل معاملات الرش ببكتريا البسيدوموناس والباسيلس قد أدت إلى انخفاض النسبة المئوية للفقد في وزن الثمار وتحسن قوام الثمرة, في حين أدت كل معاملات الرش سواء بالبكتريا المنتجة لمنظمات النمو أو المنشطات الحيوية المخلقة إلى تقليل نسبة الثمار التالفة ولون الثمرة وزيادة النسبة المئوية للحموضة في حين لم تؤثر على محتوى الثمار من المواد الصلبة , أما معاملات الرش ببكتريا الباسيلس فقد أدت إلى تخفيض معدل تنفس الثمار أثناء التخزين.

خلال التقدم في مراحل الفترة التخزينية حدثت زيادة تدريجية للنسبة المئوية للفقد في وزن الثمار و نسبة الثمار التالفة ومحتوى الثمار من المواد الصلبة مع حدوث انخفاض للنسبة المئوية للحموضة وتدهور لقوام الثمرة, في حين كان هناك اتجاه واضح لتأثير الفترة التخزينية على لون الثمار بحيث انخفضت تدريجيا قيمة زاوية (هـ) لتعطي زيادة تدريجية في التلوين باللون الأحمر في قشرة الثمرة.

وقد أظهرت النتائج أن معاملة الرش ببكتريا البسيدوموناس والجيسامار معا لم تؤدي لأي تلف للثمار المخزنة بعد 30 يوم من التخزين, كما سجلت أقل نسبة للثمار التالفة بعد 60 يوم من التخزين, وعند الوصول إلى 120يوم من التخزين فقد سجلت أعلى قيمة لكل من محتوى الثمار من المواد الصلبة و أفضل تلوين لقشرة الثمرة. كما أعطت معاملتي الرش ببكتريا البسيدوموناس والجيسامار معا و البسيدوموناس والفردوس معا أفضل قوام للثمار في بداية فترة التخزين في حين أعطت معاملة الكونترول أقل قيمة بعد 120 يوم من التخزين على درجة حرارة الصفر المئوي.