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COMPARATIVE EFFECT OF ONE SYNTHETIC BIOSTIMULANTS AND MICROBIAL BIOSTIMULANT (*Azospirillum lipoferum*) ON YIELD AND QUALITY OF WASHINGTON NAVEL ORANGE FRUITS.

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ABSTRACT

Foliar sprays with synthetic biostimulants or microbial biostimulants PGPR (plant growth promoting rhizobacteria) were used . They contain amino acids, macro and micro elements, humic acid and vitamins . Also its direct effect in release stimulants, nutrients, antibiotics, biosides and sidrofores or activation of these microorganisms in plant rhizosphere in activation and improving plant growth. This study was carried out during 2013 and 2014 seasons on 10 years old Washington navel orange (*Citrus sinensis* Osbeck) trees budded on sour orange (*Citrus aurantium* L.) rootstock, grown in a private orchard located at Motobus, Kafr El Sheikh Governorate, to study the effect of synthetic stimulants (Furdose) and microbial biostimulant (*Azospirillum lipoferum*)on fruit set, dropping, yield and fruit quality. Furdose as a commercial synthetic biostimulant and microbial biostimulant *Azospirillum lipoferum* were used as foliar application alone or in combination at two stages, before flowering(first mach) or after fruit set(first may) or before flowering and after fruit set. The obtained results revealed that, fruit set and drop percentages, yield and fruit quality were u significantly affected by Furdose and *Azospirillum lipoferum* treatments alone or in combination in both seasons . The data cleared that, both stimulators enhanced fruit set percentage, yield and fruit quality of Washington navel orange trees . *Azospirillum lipoferum* alone or combined with Furdose was more effective on improving the productivity and fruit quality .The T6 (foliar spray of *A. lipoferum* after fruit set) , T7 ((foliar spray of *A. lipoferum* before flowering and after fruit set) and T10 (foliar spray of Furdose plus *A. lipoferum* before flowering and after fruit set) were the most effective treatments on yield and fruit quality. It increased fruit set, yield and fruit quality in terms of fruit number, fruit kg/tree, fruit firmness, soluble solids content, reducing and total sugars and vitamin C. Fruit drop was decreased without significant differences among them in both seasons. Thus spraying with *Azospirillum lipoferum* after fruit set T6 (foliar spray of *A. lipoferum* after fruit set)) gave 112.4 and 115.7 kg/tree compared with T7 (foliar spray with *Azospirillum lipoferum* before flowering and after fruit set) 108.7 and 121.4 kg/tree and T10 ((foliar spray the combination of them before flowering and after fruit set) 114.7 and 130.3 kg/tree during both seasons, respectively . The use of (*Azospirillum lipoferum*) is recommended for increasing fruit yield and quality such as firmness, SSC, V.C and total sugars which may be increase the fruit ability to handling stages and longest shelf life, and gave the highest of net return per feddan and the increase in net return over control. when used alone or with synthetic biostemulants (Furdose) compared with the use of synthetic biostemulants (Furdose) alone .

INTRODUCTION

Washington navel orange (*Citrus sinensis* L.) is an important cultivar in Egypt; due to, vigorous growth and good productivity with high quality fruits. It is considered as the best for local and exporting markets. In order to improve productivity with excellent fruit quality for high exportation potential, the farmers should be tend to the use of agricultural biostimulants practices. Uses of plant biostimulants which include diverse substances (humic substances, seaweed extracts, free amino acids and other N-containing) and microorganisms (free living bacteria, fungi and arbuscular mycorrhizal fungi) are known to improve plant growth, yield and fruit quality (Calvo *et al.,* 2014). *Azospirillum* spp. are considered to be an important plant growth promotive rhizobacteria (PGPR) for many reasons: *Azospirillum brasilense* and *Azospirillum lipoferum* stimulate growth and increase yield in apple, citrus, olive, pomegranate, cherry, strawberry and apricot (Aslantas *et al.,* 2007, Abbas *et al.,* 2013, Mohamed *et al.,* 2009, Hafez *et al.,* 2013, Esitken *et al.,* 2010 and Abd Ella, 2006). In this respect, Malik et al. (2002) found that *Azospirillum brasilense* and *Azospirillum lipoferum* contributed between 7–12 % of the total nitrogen content by using N^{15} tracer techniques on wheat. Also, Boddey *et al.,* (1991) noticed that, about 60 – 80 % of total nitrogen came from nitrogen fixation by *Azospirillum diazotrophicus* on sugarcane plants. The foliar spray with PGPR bacteria had been proved efficience for enhancing plant growth and yield of different fruit crops (Esitken *et al.,* 2004 on apricot, Esitken *et al.,* 2009 on apple and Nour El-Din *et al.,* 2012 on Anna apple). Moreover, phytohormones, like auxins, cytokinins, gibberellins and ethylene, can be synthesized by beneficial microorganisms (Esitken *et al.,* 2006). These plant hormones regulate multiple physiological processes. For example, gibberellins are mainly involved in regulating plant cell division and elongation and hence, they influence almost all stages of plant growth, including seed germination, stem and leaf growth, floral induction, and fruit growth (Spaepen *et al.,* 2009). PGPR was found also to modify the plant hormones statue (Dodd *et al.,* 2010). *Azospirillum brasilense* and *Azospirillum lipoferum* produce different GAs specially GA_1 and GA_3 that are responsible for plant growth promotion that occurs upon inoculation onto plants (Cassan *et al.,* (2001, Mehnaz and Lazarovits, 2006 and Ekine *et al.,* 2014). The use of plant growth promotive rhizobacteria (PGPR) as foliar application mean for producing maximum yield and improving fruit quality like fruit size, fruit firmness, total soluble solids, acidity and vitamin C (El-Shazly and Mustafa, 2013 on Washington navel orange, Esitken, *et al.,* 2002 on apricot, Akea and Ercisli, 2010 on sweet cherry and Arikan *et al.,* 2013 on Quince).

Therefore, the objective of this investigation was to study the effect of synthetic biostimulants Furdose and microbial biostimulant *Azospirillum lipoferum* on fruit set, dropping, yield and fruit quality of Washington navel orange trees..

MATERIALS AND METHODS

The present study was carried out during 2013 and 2014 seasons on 15 years old Washington navel orange (*Citrus sinensis* Osbeck) trees budded on sour orange (*Citrus aurantium* L.) rootstock, spaced a 6×6 meters, grown in a private orchard located at Motobus, Kafr El Sheikh Governorate and subjected to cultural practices usually done in this area. The soil texture was clay (51.91% clay, 39.82% silt and 8.27% sand), 3% total carbonate content, 3.12 ds m^{-1} an electrical conductivity and a pH of 8.15. Thirty trees uniform in vigour were selected to study the effect of synthetic stimulants (Furdose) and microbial biostimulant(*Azospirillum lipoferum*) on fruit set, dropping, yield and fruit quality. The experiment consist of ten treatments arranged in a randomized complete block design, with three replicates for each treatment, one tree in each replicate. *Azospirillum lipoferum* was grown in the semi solid Dobereiner medium (Dobereiner *et al.,* 1976), each liter of distilled water contained 5.0g Malic acid, 0.4g KH_2PO_4, 0.1g K_2HPO_4, 0.2g $MgSO_4$, 0.1g NaCl, 0.02g $CaCl_2$,$7H_2O$, 0.01g $FeCl_3$,$6H_2O$, 0.002g $NaMoO_4$,$2H_2O$ and 1.75g Agar. Solution spray was prepared as 100 ml/100 liter water. Other material Furdose is a commercial synthetic stimulant contained 22% humic and fulvic acids, 40% natural and organic substances, 14.6% free amino acids, 4.5% N, 3.8% P, 5%K, 0.4% Ca,0.4% Mg, 0.1% Fe, 15ppm Mn, 20ppm Zn and 15ppm Cu, and the concentration of solution spray was 5%. Tween-20 (0.1%) as surfactant was added to the solution then the foliar application was applied directly to trees with a handheld sprayer until runoff in the early morning. The following treatments were applied:

T_1 Control, trees sprayed with tap water only.
T_2 Foliar spray of Furdose before flowering (at the beginning of March).
T_3 Foliar spray of Furdose after fruit set (at the beginning of May).
T_4 Foliar spray of Furdose before flowering and after fruit set.
T_5 Foliar spray of *A. lipoferum* before flowering (at the beginning of March).
T_6 Foliar spray of *A. lipoferum* after fruit set (at the beginning of May).
T_7 Foliar spray of *A. lipoferum* before flowering and after fruit set.
T_8 Foliar spray of Furdose plus *A. lipoferum* before flowering.
T_9 Foliar spray of Furdose plus *A. lipoferum* after fruit set.
T_{10} Foliar spray of Furdose plus *A. lipoferum* before flowering and after fruit set.

Four main branches as 2.5 inch in diameter of each tree in different directions were labeled and the following parameters were determined:

1.Final fruit set and preharvest fruit drop percentages:

Final fruit set % : was calculated by dividing the number of fruits before harvesting by the total number of flowers.

Final fruit set % = (No. of fruit set ÷ Total No. of flowers) x 100.

Preharvest drop percentage %: was calculated by recording fruits from August to December).The percentage of preharvest drop was calculated to the equation:

Preharvest fruit drop % = (No. f dropping fruits ÷ No. of fruits at August) x 100

2. Yield:

Yield of each tree was determined as number and weight (kg) /tree.

3. Fruit quality:

To determine fruit quality, 20 fruits were taken at random from each tree at harvest time (first January) SSC between (12-16 %) of both seasons and prepared for determination of physical and chemical fruit characteristics.

Physical character:

Fruit weight (gm) and fruit volume (cm^3) were determined. Fruit firmness(g/cm2) was recorded by using Lfra Texture analyzer instrument. The results were expressed as resistance force of the fruit to the penetrating tester according to Harold (1985).

Chemical character:

Soluble solids content (SSC), Acidity %, SSC/Acid ratio and vitamin C :

Juice samples were prepared for determining, soluble solids content percentage by a hand refractometer, total acidity percentage as citric acid according to (A. O. A. C 1990), ascorbic acid as mg/100 ml/juice by using 2, 6 dichlorophenol indophenol and SSC/acid ratio was estimated.

Sugar contents(Reducing, non reducing and total sugars):

Sugar contents (reducing, non-reducing and total sugars) were extracted from 5 grams of mixed flesh of both fruits sample by using distilled water (Loomis and Stull, 1937). The reducing sugars content were determined as (Shaffer and Hartman, 1921), sugar contents were expressed as gm per 100 gm fresh weight of fruit flesh.

The obtained data were subjected to analysis of variance according to Snedecor and Cochran (1990). Duncan's multiple range test (Duncan,1955) at 5% level was used to compare the mean values.

RESULTS AND DISCUSSION

Final fruit set and preharvest fruit drop percentages:

Data presented in Table 1 revealed that, final fruit set and preharvest fruit drop percentages were significantly affected by foliar application of Furdose and *Azospirillum lipoferum* as biostimulants treatment alone or combined with the other in both seasons. As for final fruit set percentage, it is clear that all treatments significantly increased final fruit set percentage in both seasons as compared with the control (T_1). The treatments of T_5, T_7 and T_{10} gave higher final fruit set percentage than other treatments in both seasons. The difference among T_5, T_7 and T_{10} were not significant in both seasons. On the other hand, the least final fruit set% was found on trees sprayed with tap water(control). The data also cleared that, both stimulators enhanced final fruit set of Washington navel orange trees, but *Azospirillum lipoferum* alone or combined with Furdose was more improved final fruit set as compared with other treatments Table 1. These results are in harmony with those obtained by Esitken *et al.,* (2002) on apricot, Pirlak *et al.,* (2007) and Aslantas *et al.,* (2007) on apple. In this respect, Karakurt *et al.,* (2011) concluded that the combined treatments of four *Azospirillum* spp. bacteria caused highest fruit set rate of sour cherry.

Concerning preharvest fruit drop percentage, data in Table 1 indicated that fruit drop percentage was significantly affected by treatments as compared with control in both seasons. T_1 had the highest preharvest fruit drop followed by T_2 and T_8 respectively in both seasons. The treatments of T_6, T_7 and T_{10} had the lowest values of preharvest fruit drop percentage without significant difference among them in both seasons Table 1. Microbial stimulators (*Azospirillum lipoferum*) decreased preharvest fruit drop% when spray before flowering only or before flowering and after fruit set as compared with the other treatments in the two growing seasons. These findings are confirmed by the results obtained by Omer *et al.,* (2012) on Washington navel orange trees and Abbas *et al.,* (2013) on Kinnow mandarin . In this respect, Taha and Eid (2011) concluded that polyamines contained in biostimulants regulate many growth processes, differentiation, setting and ripening of fruits.

Yield:

Yield as fruit number per tree.

The reading of Table 1 showed that, both of biostimulants alone or combined to other significantly increased fruit number/tree as compared with the T_1 (control) in both seasons. T_7, T_{10} and T_6 had the highest significant values of fruit number/tree during the two seasons, respectively compared with control and other treatments. These values were 459.3, 475.6 and 466.0 number fruit/tree and 510.0, 500.3 and 470.0 number fruit/tree compared with 343.0 and 393.6 number fruit/tree for the control during the two seasons, respectively. These results are in agreement with those obtained by El-Shazly and Mustafa (2013) on Washington navel orange. Also, Shamseldin *et al.,* (2010) reported that inoculation of Washington navel orange trees with *Pseudomonas fluorescence* and *Azospirillum brasilense* resulted in significant increase of the number of fruit per tree. In this line Eissa (2003) who reported that spray with EM (effective microorganisms) resulted in an increase in number of Kelsey plum fruits/tree. Also, Eissa *et al.,* (2007) indicated that the spray of pear trees with *Saccharomyces cervecia* had a stimulated effect and increased number of fruits, Iqbal *et al.,* (2011) with bacterial biostimulants which increased fruit number, Nour El-Din *et al.,* (2012) mentioned, that *Azospirillum brasilense* recorded the highest number fruit/tree than the other treatments during two seasons, Gabr and Nour El-Din (2012) found that, the spray of *Azospirillum* isolates increased fruit number on apple trees .

Yield as kg/tree.

Data in Table 1 cleared that, all sprayed treatments with biostimulants increased yield as kg/tree during the two seasons compared with the control treatment. The highest increment in this respect was found in treatments of T_{10}, T_7 and T_6 with 114.7, 114.6 and 112.4 kg/tree in the first season but, treatments of T_{10}, T_7 and T_9 had 130.3, 128.8 and 121.4 kg/tree in the second season, respectively compared with 92.6 and 97.7kg/tree in control treatment during the two seasons, respectively.

Table1.Effect of plant biostimulators (Furdose and *Azospirillum lipoferum*) on fruit set %, preharvest fruit drop% , fruit number/tree and yield kg/tree of Washington navel orange fruits in 2013 and 2014 seasons

Treatments	Final fruit set %		Preharvest fruit drop %		Yield			
					Number fruit/tree		Kg/tree	
	2013	2014	2013	2014	2013	2014	2013	2014
T_1	6.03e	5.99f	12.05a	11.82a	343.0h	393.6f	92.6f	97.7f
T_2	7.83bc	7.97bc	10.10b	9.77b	369.6g	416.3e	94.8ef	100.0ef
T_3	6.84de	6.17ef	7.63e	7.43de	404.0f	421.6e	98.6de	103.8e
T_4	7.89bc	7.47cd	6.79fg	6.45f	445.0c	459.0bcd	105.9cd	118.7bc
T_5	9.17a	9.39a	8.18d	7.80d	432.0de	449.3d	104.0cd	110.6d
T_6	6.61de	6.91de	6.12h	5.79g	466.0b	470.0b	112.4ab	115.7c
T_7	9.30a	9.28a	6.28h	5.78g	489.3a	510.0a	114.6a	128.8a
T_8	8.48ab	8.55ab	9.40c	8.89c	428.0e	453.3cd	107.4bc	114.4cd
T_9	7.29cd	6.15ef	7.19ef	7.04e	439.3cd	465.3bc	108.7bc	121.4b
T_{10}	9.33a	8.78ab	6.44gh	6.32fg	475.6b	500.3a	114.7a	130.3a

Means followed by different letter are significantly different within columns by Duncan's multiple range test, P ≤ 0.05.

These results agree with Eissa, (2003) who found that, foliar sprays of PGPR increased fruit yield as reflected by promoting of flowering process and fruit setting ; Esitken *et al.,* (2004) reported that, spray of apricot with *Bacillus* OSU-142 increased fruit yield, Abd El-Migeed *et al.,* (2007) noted that inoculation of Washington navel orange trees with *Azospirillum lipoferum* as a source of biofertilizer improved fruit yield (kg/tree), Esitken (2009) showed that, spraying of PGPR bacteria enhanced plant growth and fruit yield of apple trees, Shamseldin *et al.,* (2010) reported that, inoculation of Washington navel orange trees with *Pseudomonas fluorescence* and *Azospirillum brasilense* resulted in significant increase in number of fruit and weight per tree. Similar results were reported by Spinelli et al (2010) who showed that, treated strawberry with Actiwave as a product derived from the algae Ascophyllum modosum enhanced the yield and had significant effect on reducing the native effect of alternative bearing. The PGPR had multi-mechanism for enhancing yield and quality which reflected by producing antibiotics (Esitken 2011). Gabr and Nour El-Din (2012) cleared that, the spray of *Azospirillum* isolates increased fruit weight (kg/tree) of apple during two seasons . Nour El-Din *et al.,* (2012) mentioned that Furdose stimulated the growth and increased fruit yield but the spray with bacterial biostimulants *Azospirillum brasilense* had a strong influence than the synthetic biostimulants in this concern.

Fruit quality:

Physical characters:

The results presented in Table 2 showed the effect of Furdose and *Azospirillum lipoferum* as biostimulants treatments on weight, volume and firmness of Washington navel orange fruits. The results cleared an increase in fruit weight and volume in untreated control in the two seasons

,respectively and T_9 on weight and T_{10} on volume in the second season. There were significant differences between the control and all treatments . The lowest values of fruit weight was noticed with T_7 and T_4 in the first season and T_2 and T_5 in the second season compared with the other treatments.

The lowest values of fruit volume on the data recorded in Table 2 was found with T_5 and T_6 in the first season and T_2 and T_3 in the second season. Similar effects were mentioned by Esitken *et al.,* (2002) on apricot, Akea and Ercisli (2010) on Quince and El-Shazly and Mustafa (2013) on Washington navel orange. Also, Eissa (2003) reported that, the spray with EM (effective microorganisms) resulted in an increase in weight of Kelsey plum fruits/tree, Eissa *et al.,* (2007) noticed that, *Saccharomyces cervecia* had an increase in weight of pear fruits and had stimulate effect. Iqbal *et al.,* (2011) noticed that, bacterial biostimulants increased size of plant cells due to the function of plant phytohormones like IAA, cytokinins and gibberellins.

Also, data in Table 2 indicated that, fruit firmness was significantly increased by all treatments compared with the untreated treatment during the two seasons, respectively. The highest values of fruit firmness was recorded by T_9 and T_{10} compared to the control and the other treatments in both seasons. On the other hand trees sprayed with tap water (control) had the lower fruit firmness than the other treatments in both seasons. Similar results were obtained by Pirlak and Kose (2009) who found that, the spray with synthetic or bacterial biostimulants lead to increase fruit firmness , Abd El-Razek and Saleh (2012) on Florida prince peach and Arikan *et al.,* (2013) on Quince. The data cleared that *Azospirillum lipoferum* alone or combined with Furdose improved firmness , in harmony with the results obtained by Yolcu *et al.,* (2011) and Mosa *et al.,* (2014).

The increase of fruit firmness on T9 and T10 compared with the control and the other treatments may be due to the effect of biostemulators on inducing high potentiatialy of fruit rind resist to pathogens Van Loon , L.C. (2007; so harmful microbes and modify the plant hormones statuis which retarded cell senescence. PGPR regulate plant ethylene level and produce antibiotics Govindasamy *et al.*(2008) which are reflected on fruit quality as firmness .

Table2.Effect of plant biostimulators (Furdose and *Azospirillum lipoferum*) on weight, volume and firmness of Washington navel orange fruits in 2013 and 2014 seasons.

Treatments	Fruit weight (g)		Fruit volume (cm^3)		Fruit firmness (g/cm^2)	
	2013	2014	2013	2014	2013	2014
T_1	270.15a	261.32a	285.66a	281.33a	53.80g	57.80h
T_2	256.48b	240.44d	272.33a	260.66d	54.32g	62.76g
T_3	244.21cde	246.23cd	260.66bc	267.66cd	68.61d	71.36e
T_4	238.70de	258.63ab	265.66bc	280.33a	73.22c	73.94d
T_5	240.86cde	246.20cd	258.00c	271.33bc	58.74f	66.30f
T_6	241.25cde	250.09c	257.33c	268.33cd	75.78b	77.38c
T_7	234.34e	252.68bc	267.66bc	272.66abc	76.30b	79.83b
T_8	250.98bc	252.44bc	266.33bc	270.66bc	62.56e	67.49f
T_9	247.58bcd	261.10a	264.66bc	277.33ab	79.64a	81.28b
T_{10}	240.47cde	260.47a	269.00bc	280.33a	81.77a	83.43a

Means followed by different letter are significantly different within columns by Duncan's multiple range test, $P \leq 0.05$

Chemical characters:

Soluble solids content (SSC) , Acidity , SSC/Acid ratio and vitamin C content:

Data in Table 3 present the effect of biostimulants treatments on soluble solids content %, acidity %, SSC/acid ratio and vitamin C content of Washington navel orange fruits . Concerning soluble solids content % , all treatments significantly affected them in both seasons. T_6, T_7 and T_3 recorded the highest values of soluble solids content % in the first season, but in the second season the highest values came with T_6, T_{10} and T_9 . The T_8 and T_1 (control) gave the lowest soluble solids content % in the first season while T_1 (trees sprayed with tap water only) had the lowest value in the second season. The differences between the highest values and control were highly significant.

Also, the same trend was noticed about acidity that the lowest values belonged with T_1 (Control) during the two seasons. Anyhow, T_7 and T_6 gave the highest values of acidity in both seasons, respectively. There were high significant differences between the control values and all treatment values especially with T7 in both seasons. Similar results were obtained by Abd Ella (2006) on Arabi pomegranate. Pirlak and Kose (2009) claimed that, spray of PGPR bacteria increased SSC and acidity of strawberry fruits, Shamseldin *et al.,* (2010) on Washington navel orange. Nour El-Din et al (2012) noticed that, SSC % of Anna apple fruits were generally lowered due to spraying with stimulants whether were synthetic or biological through two studying seasons, but differences did not usually reached to significance . In general acidity of fruits increased by the spray treatments especially with bacterial biostimulants in the two seasons.

Data in Table 3 showed that, SSC/acid ratio was significantly affected by all treatments in both seasons, but these effects varied from season to other and among treatments in this variable. On the other words, T_1 gave the highest value of SSC/acid ratio in the first season, while T_{10} recorded the highest value of SSC/acid ratio during the second season. T_7 showed the lowest SSC/acid ratio in both seasons,

respectively. However, there was a clear constant trend on the different treatments on SSC/acid ratio in both seasons. These results are in harmony with those reported by Mohamed *et al.,* (2009) on Balady mandarin and Karakurt *et al.,* (2011) on sour cherry.

Reading in Table 3 indicated that, vitamin C content of Washington navel fruits was significantly affected by all treatments as compared with control in both seasons. There was a high significant difference among the control and all treatments during the two seasons. Results showed that T_6, T_7 plus T_{10} had the highest values of vitamin C without significant differences among them in both seasons. These values were 57.89, 57.48 and 57.47 mg/100 ml juice and 58.03, 57.70 and 57.66 mg/100 ml juice compared with 52.59 and 53.24 mg/100 ml juice in the control during the two seasons respectively. Control treatments showed the lowest vitamin C content in both seasons compared to the other treatments. Stimulators (*Azospirillum lipoferum*) increased vitamin C when sprayed only or combined with Furdose after fruit set only or before flowering and after fruit set as compared with the other treatments in both the two studying seasons. These results are in agreement with those obtained by Akea and Ercisli (2010) on Sweet cherry, Arikan *et al.,* (2013) Quince and El-Shazly and Mustafa (2013) on Washington navel orange.

PGPR was more effective on enhancing plant nutrition uptake of mineral and many of these stimulants contains amino acids , vitamins, humycacids, plant phytohormones and sometimes micro elements which improving fruit quality and its contents of SSC and vitamin C .

Table3.Effect of plant biostimulators (Furdose and *Azospirillum lipoferum*) on SSC %, Acidity %, SSC/acid ratio and vitamin C of Washington navel orange fruits in 2013 and 2014 seasons.

Treatments	SSC %		Acidity %		SSC/acid ratio		Vitamin C mg/100 ml juice	
	2013	2014	2013	2014	2013	2014	2013	2014
T_1	12.27d	11.93g	0.99d	0.99f	12.43a	12.01cde	52.59f	53.24f
T_2	12.40d	12.07f	1.08c	1.01e	11.48c-f	11.90de	54.07e	53.77e
T_3	13.67ab	12.93c	1.10c	1.11b	12.39a-d	11.68ef	56.62c	55.99c
T_4	13.47ab	13.07c	1.09c	1.11b	12.40ab	11.77de	57.27b	56.77b
T_5	12.40d	12.60d	1.02d	1.04d	12.20ab	12.08cd	54.28e	54.80d
T_6	13.73a	13.93a	1.17b	1.12b	11.73c-f	12.44b	57.89a	58.03a
T_7	13.67ab	13.47b	1.26a	1.18a	10.82f	11.38f	57.48ab	57.70a
T_8	12.07d	12.33e	1.00d	1.03d	12.07bc	11.93de	55.10d	54.21e
T_9	12.53c	13.47b	1.10c	1.09c	11.36def	12.32bc	56.28c	56.45bc
T_{10}	13.27b	13.80a	1.21b	1.08c	10.98ef	12.78a	57.47ab	57.66a

Means followed by different letter are significantly different within columns by Duncan's multiple range test, P ≤ 0.05

Reducing, non reducing and total sugars content:

Carefully considering readings of Table 4 showed that, reducing and total sugars were significantly affected by all spraying biostimulants treatments as compared with control during the two seasons. T_6, T_{10} and T_7 and T_{10}, T_7 and T_6 recorded the highest values of reducing sugars during the two seasons as 4.85, 4.80 and 4.61 gm/100 gm fresh weight in the first season and 5.06, 5.02 and 5.01 gm/100 gm fresh weight in the second season, respectively. The control treatment gave the lowest value of reducing sugars as 3.43 and 3.86 gm/100 gm fresh weight during the two seasons, respectively with significant differences between it and the other treatments in both seasons. This trend was found with total sugars thus T_{10} and T_7 cleared the highest values in the two seasons, it recorded 8.63 and 8.56 gm/100 gm and 8.90 and 8.91 gm/100 gm with the two seasons respectively while the control treatment recorded the lowest values 7.21 and 7.56 gm/ 100 gm fresh weight in the both seasons, respectively with a high significant differences among the above treatments. Microbial stimulators (*Azospirillum lipoferum*) alone or combined with Furdose increased reducing sugars and total sugars when sprayed before flowering only or before flowering and after fruit set as compared with the other treatments in the two study seasons. There were no clear trend effect for the stimulators on non reducing sugars. The non reducing sugars values varied between the control and all treatments during the study seasons. These results were not significant in most cases. These findings are confirmed by the results obtained by Omer *et al.,* (2012) on Washington navel orange trees and Abbas *et al.,* (2013) on Kinnow mandarin. In this line, El-Shazly and Mustafa (2013) reported that, biostimulants like yeast extract and potassium humate markedly increased total sugars.

Table4.Effect of plant biostimulators (Furdose and *Azospirillum lipoferum*) on reducing, non reducing and total sugars of Washington navel orange fruits in 2013 and 2014 seasons.

Treatments	Sugar content gm per 100 gm fresh weight of fruit flesh.					
	Reducing sugar		Non reducing sugar		Total sugar	
	2013	2014	2013	2014	2013	2014
T_1	3.43e	3.86f	3.78abc	3.70ab	7.21h	7.56g
T_2	3.98d	4.15e	3.43cd	3.66b	7.41gh	7.81f
T_3	4.33c	4.55c	3.20d	3.35c	7.53fg	7.90f
T_4	4.41bc	4.57c	3.47bcd	3.74ab	7.88de	8.05e
T_5	4.06d	4.37d	3.72abc	3.84ab	7.78ef	8.17d
T_6	4.85a	5.01a	3.21d	3.73ab	8.07cd	8.74b
T_7	4.61ab	5.02a	3.95ab	3.90ab	8.56ab	8.91a
T_8	4.03d	4.67bc	3.99a	3.81ab	8.01de	8.48c
T_9	4.52bc	4.83b	3.81abc	3.92a	8.33bc	8.74b
T_{10}	4.80a	5.06a	3.83abc	3.85ab	8.63a	8.90a

Means followed by different letter are significantly different within columns by Duncan's multiple range test, P ≤ 0.05

Data of Table (5) clearly showed the economical evaluation as total productivity/ fed . of Wshington navel fruits, total costs of yield, total return, net return/fed. and the increase in return than control . The fixed costs include (land rent, labors, fertilizers, pruning, hoeing, pesticides and harvest) which reached about 4500 LE/fed. according to the region . Changed costs include synthetic or biological biostemulators, rent of spray machine and spray labor which varied according to the treatment . The price of navel fruits evaluated about 1000 LE according to the region and season .

Calculation of economic evaluation showed that, all treatments gave a high increase in the net return per feddan over control . The application of *Azospirillum liopoferum* alone or in combination with furdose attained net return much higher than furdose biostemulator . The highest obtained increase in productivity and net return /fed. was recorded by the mixture spray before flowering and after fruit set and *Azospirillum liopoferum* before flowering and after fruit set which gave 15.114 ton/ fed. and 10014 LE/ fed and spray with *Azospirillum liopoferum* before flowering and after fruit set alone which achieved 14.940 ton/fed. and 10040 LE / fed. compared with 11.333 ton/fed and 6833 LE net return /fed.in control. The highest increase in net return over control was achieved by the above two treatments that gave 3181, 3207 LE /fed. These biostemulators as shown increased fruit yield, net return /fed. Increased return than control without a notable increase in costs because of the lower price of these compounds . Thus, the net return /fed was positive . Therefore, it is recommended to spray of Washington navel with *Azospirillum liopoferum* alone or plus Furdose twice before flowering and after fruit set at the rate of 20 L per feddan which gave the highest effect on increase net return per feddan and the increase return over control than the other synthetic biostemulants .

Table7:Washington navel orange crop economics resulting from spraying with (Furdose and *Azospirillum lipoferum*) in 2014 season

Treatments	Fixed costs (LE/fed.)	Changed costs (LE/fed.)	Total costs (LE/fed.)	Total yield (Ton/fed.)	Cop value (LE/fed.)	Net return (LE/fed.)	Increase in return over control(LE)
control	4500	------	4500	11.333	11333	6833	________
Furdose B	4500	200	4700	11.600	11600	6900	67
Furdose A	4500	200	4700	12.040	12040	7340	507
Furdose B and A	4500	400	4900	13.769	13769	8869	2036
Azospirillum lep. B	4500	200	4700	12.829	12829	8129	1096
Azospirillum lep. A.	4500	200	4700	13.421	13421	8721	1888
Azospirillum lep. B. and A.	4500	400	4900	14.940	14940	10040	3207
Fu.and*Azo*. B	4500	300	4800	13.270	13270	8470	1637
Fu.and*Azo*.A	4500	300	4800	14.082	14082	9282	2449
Fu.and*Azo*. B and A	4500	600	5100	15.114	15114	10014	3181

A: after fruit set B : before flowering
Fu : Furdose Azo : *Azospirllum lipoferum*

CONCLUSION

The spray of Washington navel orange trees with microbial biostemulants *(Azospirillum lipoferum*) was more effective on enhancing fruit yield and quality as firmness ,V. C , SSC and total sugars which may be increase the fruit ability to handling stages and prolonged its shelf life when used alone or with synthetic biostemulants (Furdose) compared with the use of synthetic biostemulants (Furdose) alone for inducing plant growth and productivity and gave the highest of net return per feddan and the increase in net return over control .

REFERENCES

Abbas, T.; S. Ahmad; M. Ashraf; M. A. Shahid; M. Yasin; R. M. Balal; M. A. Pervez and S. Abbas (2013). Effect of humic acid application at different growth stages of kinnow mandarin (*Citrus reticulate* Blanco) on the basis of physio-biochemical and reproductive responses. Academia J. Biotech,. 1(1):14 - 20.

Abd Ella, E.E.K. (2006). Effect of biofertilization on reducing chemical fertilizers, vegetative growth, nutrition status, yield and fruit quality of Arabi pomegranate trees.J.Agric.&Env.Sci.Alex.Univ.,Egypt 5(3):1– 22.

Abd El-Migeed, M.M.M. and M.M.M. Saleh (2007). The beneficial effect of minimizing mineral nitrogen fertilization on Washington navel orange trees by using organic and biofertilizers. World Journal of Agric. Sci., 3:80 – 85.

Abd El-Razek, E. and M.M.S. Saleh (2012). Improve productivity and fruit quality of florida prince peach trees using foliar and soil application of amino acids. Middle East Journal of Scientific Research, 12(8):1165 – 1172.

Akea, Y. and S. Ercisli (2010). Effect of plant growth promoting rhizobacteria (PGPR) inoculation on fruit quality in sweet cherry (*Prunus avium* L.) cv. Ziraat. *Journal of Food, Agriculture & Environment* 8(2):769 – 771.

Arikan, S.; M. Ipek and L. Pirlak (2013). Effects of plant growth promoting rhizobacteria (PGPR) on yield and fruit quality of Quince. *International Conference on Agriculture and Biotechnology* 60(19):97 – 99.

Aslantas, R.; R. Cakmakci and F. Sahin (2007). Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. Scientia Horticulturae 111(4):371 – 377.

Association of official Agriculture chemists (1990). Official methods of analysis, (A.O.A.C.) 15^{th} ed. Washington, D. C., USA.

Boddey, R.M.; S. Urquiaga; V. Reis and J. Döbereiner (1991). Biological nitrogen fixation associated with sugar cane. Plant Soil 137:111–117.

Calvo, P.; L. Nelson and J.W. Kloepper (2014). Agricultural uses of plant biostimulants. Plant Soil 383:3 – 41.

Cassan, F.; R. Bottini; G. Schneider and P. Piccoli (2001). *Azospirillum brasilense* and *Azospirillum lipoferum* hydrolyze conjugates of GA_{20} and metabolize the resultant aglycones to GA_1 in seedlings of rice. Plant Physiology 125: 2053 – 2058.

Dobereiner, J; L.E. Marrial and M. Nerg (1976). Ecological distribution of *Azospirillum lipoferum*, dejerink. Can. J. Microbiology 22:1464 – 1473.

Dodd, L.C.; N.Y. Zinovkina and V.I. Safronova (2010). Rhizobacteria mediation of plant hormone status. Annal. Appl. Biol., 157:361 – 379.

Duncan,D.B.(1955).Multiple ranges and multiple F-tests.Biometrics,11:1– 42.

Eissa Fawzyia, M. (2003). Effect of some biostimulants on vegetative growth, yield and fruit quality of Kelsey plum. Egypt J. Appl. Sci., 18:716 – 735.

Eissa Fawzyia, M.; M.A. Fathy and Kandil Eman, A. (2007). Response of Le-Conte pear (*Pyrus communis* L.) trees to foliar application with some biostimulants. Minufiya J. Agric. Res., 32:1143 – 1154.

Ekine, M.; M. Turan; E. Yildirim; A. Gunes; R. Kotan and A. Dursun (2014). Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone content of cauliflower (*Brassica oleracea* L. var. botrytis) transplants. *Acta Sci. Pol., Hortorum Cultus* 13(6):71 – 85.

El-Shazly, S.M. and N.S. Mustafa (2013). Enhancement yield, fruit quality and nutritional status of Washington navel orange trees by application of biostimulants. Journal of Applied Sciences Research, 9(8):5030 – 5034..

Esitken, A. (2011). Use of plant growth promoting rhizobacteria in horticulture crops. In Bactria Agrobiology Crop Ecosystems Springer Berlin Heidelberg pp:189 – 235.

Esitken, A.; H. Karlidag; S. Ercisli and F. Sahin (2002). Effects of foliar application of *Bacillus subtilis* Osu-142 on the yield, growth and control of shot-hole disease (*Coryneum blight*) of apricot. Gartenbauwissenschaft, 67(4):139 – 142.

Esitken, A.; H. Karlidag; S. Ercisli; M. Turan and F. Sahin (2004). The effect of spraying a growth promoting bacteria on the yield, growth and nutrient element composition of leaves of apricot (*Prunus persica* L. cv. Hacihaliloglu). Aust. J. Agric. Res., 54:377 – 380.

Esitken, A.; H.E. Yildiz; S. Ercisli; M.F. Donmez; M. Turan and A. Gunes (2010). Effects of plant growth promoting bacteria (PGPR) on yield, growth and nutrient contents in organically grown strawberry. Scientia Horticulturae 124:62 – 66.

Esitken, A.; L. Pirlak; M. Ipek; M.F. Donnez; R. Cakmakci and F. Sahin (2009). Fruit bio-thinning by plant growth promoting bacteria (PGPR) in apple cvs.Golden delicious and Braeburn.Biol.Agric.Hort.,26:379– 390.

Esitken, A.; L. Pirlak; M. Turan; F. Sahin (2006). Effect of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry.Scientia Horticulturae,110:324–327.

Gabr, M.A. and M. Nour El-Din (2012). Evaluation of selected *Azospirillum spp*. isolates for IAA production and fruit potential impact on improving growth, yield and fruit quality of Anna apple trees. J. Agric. Chem. and Biotechn. , Mansoura Univ., 3(3):65 – 75.

Govindasamy V.; M. senthikumar; K. Gaikwad and K. Annapurna (2006). Isolation and characterization of ACC deaminase gene from two plant growth – promoting rhizobacteria . Curr Microbiol. 57 , 312 – 317 .

Hafez, O.M.; M.A. Saleh and S.R. El-Lethy (2013). Response of some seedlings olive cultivars to foliar spray of yeast and garlic extracts with or without vascular arbuscular mycorrhizal fungi. World Applied Sciences Journal 24(9):1119 – 1129. http://astonjournals. com/lsmr

Harold, E.P. (1985). Evaluation of quality of fruits and vegetables. AVI Publication – West Port. Conn. U.S.A.

Iqbal, S.; R. Nazar; R. Iqbal; A. Masoad and N.A. Khan (2011). Role of gibberellins in regulation of source sink relations under optimal and limiting environmental conditions. Current Science 100:998 – 1007.

Karakurt, H.; R. Kotan; F. Dadasoglu; R. Aslantas and F. Sahin (2011). Effects of plant growth promoting rhizobacteria on fruit set, pomological and chemical characteristics, color values and vegetative growth of sour cherry (*Prunus cerasus* cv. Kutahya). Turk. J. Biol., 35:283 – 291.

Loomis, W.E. and C.A. Stull (1937). Method in plant physiology. Mc Grw. Hill Book Company Inc.

Malik, K. A.; M.S. Mirza; U. Hassan; S. Mehnaz; G. Rasul; J. Haurat; R. Bauy and P. Normanel (2002). The role of plant associated beneficial bacteria in rice-
wheat cropping system. In: Kennedy IR, Chaudhry A (eds) Biofertilisers in action. Rural Industries Research and Development Corporation, Canberra, pp 73–83

Mehnaz, S. and G. Lazarovits (2006). Inoculation effects of *Pseudomonas putida, Gluconacetobacter azotocaptans* and *Azospirillum lipoferum* on corn plant growth under greenhouse conditions. Microbial Ecology 51:326 – 335.

Mohamed, A.K.A.; M.M.M. Ahmed and M.M. El-Akkad (2009). Is possible to compensate the annual fertilization in mandarin orchards by using the bio-fertilizers? Assiut J. of Agric. Sci., 40(4):37 – 68.

Mosa, W.F.A.; L.S. Paszt and N.A. Abd El-Megeed (2014). The role of bio-fertilization in improving fruits productivity – a review. Advances in Microbiology, 4(15):1 – 17.

Nour El-Din, M; M.A. Gabr and M.Y. Abou-Zeid (2012). Response of growth, yield and fruit quality of Anna apple trees to foliar spray with some plant growth promoting rhizobacteria substitute to synthetic biostimulants. J. Agric. Chem. and Biotechn. , Mansoura Univ., 3(3):49 – 64.

Omar, A. K.; E. B. Belal and A. A. El-Abd (2012). Effects of foliar application with compost tea and filtrate biogas slurry liquid on yield and fruit quality of Washington navel orange (*Citrus sinensis* Osbeck) trees. *Journal of the Air & Waste Management Association* 62(7): 767 – 772.

Pirlak, L. and M. Kose (2009). Effects of plant growth promoting rhizobacteria on yield and some fruit properties of strawberry. J. Plant Nut., 32:1173 – 1184.

Pirlak, L.; M. Turan; F. Sahin and A. Esitken (2007). Floral and foliar application of plant growth promoting rhizobacteria (PGPR) to apples increases yield, growth and nutrition element contents of leaves. Journal of Sustainable Agriculture, 30:145 – 155.

Shaffer, P.A. and A.F. Hartman (1921). The iodmetric determination of copper and its use in sugar analysis. J. Biol. Chem., 45:390.

Shamseldin, A.; M.H. El-Sheikh; H.S.A. Hassan and S.S. Kabeil (2010). Microbial bio-fertilization approaches to improve yield and quality of Washington navel orange and reducing the survival of nematode in the soil. Journal of American Science 6(12):264 – 271.

Snedecor, W. and W.G. Cochran (1990). Statistical methods. 7^{th} ed. Iowa State Univ., USA. Pp 593.

Spaepen, S.; J. Vanderleyden and Y. Okon (2009). Plant growth promoting actions of rhizobacteria. Advances Bot. Res 51:283–320.

Spinelli, F.; G. Fior; M. Noferini; M. Sprocatti and G. Costa (2010). A novel type of seaweed extract as a natural alternative to the use of iron chelales in strawberry production.Scientia Horticulturae 125:263 – 269.

Taha, L .S. and R.A. Eid (2011). Stimulation effect of some bioregulators on flowering, chemical constituents, essential oil and phytohormones of tuberose (*Polianthes tuberas* L.). j. Amer. Sci., 7:165 – 171.

Van Loon, L . C . (2007). Plant responses to plant growth – promoting rhizobacteria . Eur. J. plant Pathol. 119 , 243 – 254 .

Yolcu, H.; M. Turan; A. Lithourgidis; R. Cakmakci and A. Koc (2011). Effects of plant growth promoting rhizobacteria and manure on yield and quality characteristics of Italian ryegrass under semi arid conditions. Australian Journal of Crop Science 5(13)1730 – 1736.

التـأثير المقـارن لأحـد المنشـطات الحيويـة الصـناعية و المنشـط الحيـوي الميكروبـي ازوسبيرليم ليبوفيرم على إنتاجية وجودة ثمار البرتقال أبو سره.

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لمقارنـة الـرش بالمنشـطات الصـناعية والمنشـط الحيـوي الميكروبـي و التـي يرجـع تأثيرهـا الـى محتواها من الأحماض الامينية و العناصر سواء الصغرى و الكبرى أو لتأثيرها المباشر فى زيادة التنشيط و التغذية و المضـادات الحيويـة و قدرة هذه الميكروبـات علـى تحسين منطقـة الريزوسفير و تنشيط نمو النبـات. اجريـت هـذه الدراسـة خـلال موسـمى 2013 و 2014 علـى اشـجار البرتقـال ابوسـرة عمـر عشـر سـنوات بمزرعـة خاصـة بمنطقـة مطـوبس محافظـة كفـر الشـيخ لدراسـة تـأثير المنشـط الصـناعي الفـردوس و المنشـط الميكروبى ازوسبيرليم ليبوفيرم بالرش المنفرد لكل منهم أو رشـهما معـا و ذلـك فـى مرحلـة مـا قبـل الازهـار او ما بعد العقد منفردين أو مرحلة ما قبل الإزهار و بعد العقد معا.

ولقد اظهرت النتائج ان نسبة العقد و نسبة التسـاقط و المحصـول و جـودة الثمـار قد تـأثرت معنويـا بالرش بالفردوس أو الازوسبيرليم منفردين او مجتمعين معا خلال موسمى الدراسة. هذا و اظهـرت المعـاملات رقم 6 (الرش بالازوسبيرليم بعد العقد) و المعاملة 7 (الرش بالازوسبيرليم قبل الازهـار و بعد العقد) و كـذلك المعاملـة 10 (الـرش بالازوسبيرليم و الفردوس مجتمعين قبل الازهـار و بعـد العقد) زيـادة فـى نسـبة العقد و المحصـول و جـودة الثمـار متمـثلا فـى عـدد الثمـار و المحصـول كجم/شـجرة و الصـلابة و نسـبة المـواد الصـلبة الذائبـة و فيتـامين C و نسـبة السـكريات المختزلـة و الكليـة بينمـا انخفضـت نسـبة تسـاقط الثمـار خـلال موسـمي الدراسة و ذلك دون اختلافات معنوية بين المعاملات الثلاث.

اظهـرت النتـائج ايضـا ان الـرش بكـلا المنشـطين حسـنا مـن نسـبة العقـد و المحصـول وجـودة ثمـار البرتقال ابو سرة و لكن الرش بالازوسبيرليم منفردا او مع الفردوس مجتمعين كـان الاكثـر تـاثيرا فـى زيـادة المحصـول و جـودة الثمـار. هذا وقد أعطت المعاملـة رقـم 6 (الـرش بالازوسـبيرليم بعد العقد) 4و112 & 7و115 كجم/شجرة و ذلك مقارنة بالمعاملة رقم 7 (الرش بالازوسبيرليم قبل الإزهـار و بعـد العقد) 6و114 & 8و128 كجم/شجرة و المعاملة رقم 10 (الرش بالازوسـبيرليم و الفـردوس معـا قبـل الازهـار و بعـد العقد) 7و114 & 3و130 كجم/شجرة و ذلك خلال موسمى الدراسة على التوالى.

وعلـى ذلـك فإنـه ينصـح باسـتخدام المنشـط الحيـوي الميكروبـي الأزوسـبيريليم سـواء منفـردا أو مـع مركب الحيوي الفردوس وذلك من أجل زيادة المحصول وجودة ا لثمار المتمثلـة فـي الصـلابة و المـواد الصـلبة الذائبـة الكليـة وفيتـامين C و السـكريات الكليـة و التـي ربمـا تزيـد مـن زيـادة قـدرة الثمـار علـى تحمـل مراحـل التـداول المختلفـة وإطالـة الفتـرة التسـويقية لهـا وذلـك مقارنـة باسـتخدام المركـب الحيـوي الصـناعي الفـردوس منفردا .

كمـا أن المعاملـة بالمركـب الحيـوى الأزوسـبيريليم سـواء منفـردا أو مـع مركـب الحيـوي الفـردوس رشـا مرتـان أعطت أعلي قيمة نقدية سواء لإنتاج الفدان أو العائد مقارنـة بالكنترول