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COMBINATION EFFECTS OF BIOFERTILIZATION AND BIOSTIMULANTS FOLIAR APPLICATION ON YIELD, QUALITY AND MARKETABILITY OF WASHINGTON NAVEL ORANGE FRUITS .

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ABSTRACT

Two field experiments were conducted at Brembal , Motobus District, Kafr El-Sheikh Governorate during 2013-2014 and 2014-2015 seasons to study the influence of bio-fertilization with a mixed inoculum containing *Azospirillum* sp., *Bacillus megatherium* and *Bacillus circulans* as well as spray with Jisemar (commercial bio-stimulant) and/ or *Azospirillum* sp. on productivity and fruit quality of oranges [fruits drop % , fruits set % , fruits number tree^{-1}, fruit weight (g)] , fruit yield (kg tree^{-1}) , as well as some quality traits [firmness, acidity % , vitamin C content , soluble solids content (SSC%) , SSC / acid ratio of fruits and total chlorophyll, chlorophyll A&B and carotene of fruit rind and NPK % , in leaves].

Regarding spray with bio-stimulants of *Azospirillum* sp. + Jisemar or *Azospirillum* sp. individual treatments attained high records over those of not sprayed control, and the differences were mostly significant for fruits set , fruits number / tree and yield (kg / tree), chlorophyll , firmness , SSC% and vitamin C content , except other components fruit drop, carotene, and SSC/acid ratio which displayed decreases compared to water spray - 100 % NPK fertilized control.

Data revealed that treatments with biofertilization + 75% NPK and spray with biostemulants spray with *Azospirillum* sp. with Jisemar or *Azospirillum* sp. individual attained the best . As the first one resulted in a high productivity of orange trees , improved fruit quality and most marketing characteristics at room temperature compared to control and other treatments , thanks to the effects of continuous supplement of plant nutrients and phytochemical compounds, decline rots infection and retarded peel senescence causing longevity for life .

Keywords : Biofertilization , Biostimulants , Orange fruits , *Azospirillum* sp. , microbial pathogens, Phosphate dissolving bacteria and Potassium dissolving bacteria.

INTRODUCTION

Citrus occupies a large percentage of horticulture area in Egypt . Orange is superior between all citrus types. The agriculture area of orange increased continuously because the expansion of interior markets demand and exportation . The annual cultivated area in Egypt reached 112000 feddans with an average production about 434000 tons (El-Khawaga and Makald, 2013).

Biofertilization is the addition of beneficial microorganisms to plant rhizosphere in order to provide nutrients to the plant, through nitrogen fixation, solubilization of unavailable phosphatase and potassium

compounds.It also induced plant growth by the producing plant phytohormones (Sivakumer, 2014). Thus, it led to the improvement of productivity and quality of the trees (Shamseldin *et al*., 2010) these products has many important influences in plant rhizosphere, whereas, they fix atmospheric nitrogen, solubilize rock phosphates and potassium, release siderophres which chelate Fe (III), in addition to producing organic and inorganic acids as well as plant hormones. Finally they grant plants more tolerant to biotic and abiotic stresses (Sivakumer, 2014 and Jaha and Saraf, 2015). Therefore, biofertilizers save a lot of chemical fertilizers annually and greatly contribute in decreasing environmental pollution in addition to it is fruitful role in improving plant yield and quality.

Biostimulants are biological substances and microorganisms containing bioactive compounds as mineral nutrients, humic substances, vitamins, free amino acids, chitin, polysaccharides and oligosaccharides (Bulgari *et al*., 2015). Biostimulants increased plant use efficiency of nutrients and induced plant tolerance to biotic stresses which reflected an increase of plant yield.

Microbial biostemulants treatments can lower fruit decay percentage due to the influence of antibiotics production (Esitken, 2011) on (Anna) apple fruits; resist plant pathogens (Van Loon, 2007) . This is may be the direct reason to the decline in rots infection and decayed fruits during shelf life. Microbial spray with EM leads to improve most marketing characteristics of apple fruits, PGPB had multi-mechanisms for increasing fruit quality and storability (Sahain *et al*., 2007) .

For these reasons, the current investigation aimed to study the influence of biofertilization in the form of a mixed inoculum (*Azospirillum* sp., *B.megatherium* and *B. circulans*) with biostimulants (Jisemar and *Azospirillum* sp.) on improving yield and quality and marketability of orange fruits.

MATERIALS AND METHODS

Materials

Media used :

Medium 1 used for growing of *Azospirillum* sp.:

Nitrogen-free semisolid malate (NFb) Baldani and Dofbereiner.(1980) ((g.l^{-1} of distilled water): K_2HPO_4, 0.5;$MgSO_4$ – $7H_2O$, 0.2; NaCl, 0.1; yeast extract,0.5; $FeCl_3$. $6H_2O$, 0.015; DL-malic acid, 5.0; KOH, 4.8; and agar, 1.75. The pH was adjusted to 7.0 with 0.1 N KOH, and the medium was autoclaved at 121°C for 20 minutes.

Medium 2 used for growing phosphate dissolving bacteria (PDB)

Nutrient solution (g.l^{-1}): composed of Glucose, 5.0 g, K_2SO_4, o.485; $MgSO_4$. $7H_2O$, 0.2; $FeCl_2$ 0.01; $CaCl_{2,}$, 0.376; H_3PO_4 , 0.018; $ZnSO_4.7H_2O$, 0.0028 (Shrdeta *et al*., 1984). All these contents were dissolved in 1 liter distilled water and the pH of solution was adjusted to pH 6.9 using KOH.

Medium 3 used for growing Potassium dissolving bacteria (KDB) (g.l^{-1})

Aleksandrov medium (Hu *et al*., 2006).Glucose, 5.0 g; $MgSO_4$. $7H_2O$, 0.005 g; $FeCl_3$, 0.1 g; $CaCO_3$, 2.0 g; potassium mineral, 2.0 g, calcium phosphate, 2.0 g and distilled water 1 liter.

Biostimulants:

1-Jisemar: is a commercial biostimulants which contains seaweed extract (20.5%), free amino acids (6.5%), total nitrogen (which 5.8%), phosphorus (3%), Boron (0.17%) and potassium (4.6%).

2-*Azospirillum* sp. : brought about from Bacteriological Lab., Sakha Agricultural Research Station, Egypt.

3-PDB and KDB brought from Bacteriological Lab, Soil , Water, and Environmental Research Institute ARC, Giza , Egypt .

Methods

This study has been carried out on twenty years old (Washington navel) orange trees (*Citrussinensis*, Osbeck) grafted on sour orange rootstock and spaced at five meters a part at a private orchard located in Brembal, Motobus District, Kafr-Elsheikh governorate, Egypt. The Orchard soil is loamy with a good drainage. Twelve treatments were carried out during the two successive season of 2013, 2014 and 2014, 2015. Trees were selected in a good health condition and nearly uniform in both vegetative growth and fruit load, 36 trees were selected in this study and divided in randomly order. Single plot with three replicates for each treatment was arranged in random complete block design.

Treatments were randomly arranged on the selected trees, each treatment contained three trees arranged in random complete design. All studied trees received the same agricultural practices except for the studied materials, the treatments arranged as the following:

I0B0 : 100% NPK not inoculation and not sprayed(traditional treatment) (control)

I0BJ: Fertitized with 100% NPK , not inoculation and sprayed with Jisemar

I0BA:Fertitized with 100% NPK, not inoculation and sprayed with *Azospirillum* sp.

I0BJ+A: Fertitized with 100% NPK , not inoculation and sprayed with Jisemar + *Azospirillum* sp.

I1B0:Inoculation with the mixed inoculum + 50% NPK and not sprayed

I1BJ: Inoculation with the mixed inoculum + 50% NPK +sprayed with Jisemar

I1BA:Inoculation with the mixed inoculum + 50% NPK + sprayed with *Azospirillum* sp.

I1BJ+A:Inoculation with the mixed inoculum + 50% NPK + Jisema + *Azospirillum* sp.

I2B0: Inoculation with the mixed inoculum + 75% NPK and not spreyed

I2BJ: Inoculation with the mixed inoculum + 75% NPK +sprayed with Jisemar

I2BA: Inoculation with the mixed inoculum + 75% NPK + *Azospirillum* sp.

I2BJ+A:Inoculation with the mixed inoculum+75% NPK+ Jisemar + *Azospirillum* sp

Four branches around all sides of each experimental tree were chosen randomly and labeled before the beginning of this study. During both

experimental seasons, data used to determine the yield of each selected branch are as follows :

1- Fruits set % were calculated seasonally according to the following formula

$$\textbf{Fruits set \%} = \frac{Total\ number\ of\ set\ fruits\ x\ 100}{Ttotal\ number\ of\ flowers\ of\ full\ bloom}$$

2-Pre- harvest fruits drop% was calculated 3 days intervals starting from the first August till harvest time during the two seasons and calculated as the following **.**

$$\textbf{Preharvest fruit drop \%} = \frac{Total\ number\ of\ fruits\ x\ 100}{Total\ number\ of\ set\ fruits}$$

Fruits were picked when SSC/acid ratio reached to 12-16 % nearly at mid January during the two successive years and counted and weighted , fruit yield of each tree were calculated as fruit number /tree or fruit weight and total yield kg tree^{-1}.

For the determination of fruit characters, selected fruits of nearly uniform caliber were packed in plastic boxes and transported to the laboratory of Sakha Horticulture Research Station to estimate the parameters

Fruit physical parameters

a-Fruit weight (g)

Ten fruits from each box (replicate) were taken randomly and their weight was recorded and the average of each treatment was calculated .

b-Fruit firmness (g.mlm^{-2})

The results were expressed as the resistance force of the fruit to the penetration tester according to Harold (1985) by using Lfra texture analyzer instrument.

c-Fruit soluble solids content /acidity ratio was calculated

Fruit chemical parameters:

a-Soluble solids content (SSC) percentage: was determined by using a hand refractometer.

b-Total acidity percentage as (citric acid): was estimated in filtered juice according to A.O.A.C (1990).

c-Vitamin C content: was determined in filtered juice sample and expressed as mg.100 ml^{-1} juice as described by A.O.A.C(1990).

d-Chlorophyll and carotene pigments for fruit rind: the method of Wensttein (1957) was used to determine the percentage of total chlorophyll, chlorophyll a, b and carotene. One gram from the skin of three fruits was extracted for five minutes with 10 ml 85% acetone in a warring blender. Chloropast pigments were determined in the filerated extract . Chlorophyll a, b and carotene were determined by measuring the optical density at wave lengths of 662, 644 and 440 nm, respectively, using the 85% acetone as reference. The quantity of the pigments present was calculated as mg/ 100 g of fresh weight.

Samples of leaves were oven dried at 70 C˚then milled and kept for chemical analysis. Nitrogen was determined using the micro-kjeldahl method as described by Jackson, (1967). Potassium was determined using a flame photometer method. Phosphorus was determined calorimetrically according to Snell and Snell (1967).

Determination of soil dehydrogenase activity

Dehydrogenase activity was determined using the method described by Tabatabai (1982).

To determined the effect of these treatments on fruit marketability : Twenty fruits of each replicate were washed , drayed and held at room temperature (18 +_ 2^0 C) and 65-75 % reative humidity for the determination of fruit decay , weight loss percentages and the changes of the above fruit parameters during shelf life period .

$$\textbf{Fruit decay \%} = \frac{Number\ of decayed\ fruits\ x\ 100}{Ttotal\ number\ of\ stored\ fruits}$$

$$\textbf{Weight loss \%} = \frac{fruit\ \ weight\ now\ x\ 100}{fruit\ weight\ \text{at initial time of storage}}$$

Statiscal analysis: data of the present study were subjected to analysis of variance.

The treatments means were compared using L.S.D. method according to Steel and Torrie (1980). Duncan^s s multiple rang test at 5% level of significance (p :0.05) was used for means comparisons according to Snedecor and Cocharn (1980) .

The chemical properties of the studied soil were determined before cultivation process according to Black *et al*. (1965) and Jackson (1967)

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Table 1 : Some physical and chemical properties of the soil experimental site

Parameter	Value
Some physical properties	
Particle size distribution %	
Clay	51.36
Silt	38.31
Sand	10.33
Texture grade	Clayey
F. C %	43.00
PWP	23.37
Some chemical properties	
pH (1: 2.5 Water suspension)	8.2
E C (ds. m in soil paste)	1.68
O. M %	1.31
Soluble Cations (meq. L)	
Ca^{++}	3.71
Mg^{++}	2.94
Na^{+}	9.5
K^{+}	0.15
Solube Anions (meq. L)	
So_4^{--}	5.3
Cl^{-}	9.00
HCo_3^{-}	2.00
Co_3^{--}	00
Available macro element (ppm)	
N	42.6
P	15.28
K	231.7

Jisemar was sprayed twice, the first before flower emerge and the second was before fruit set . The *Azospirillum* culture was also sprayed at the same time by a concentration of 20 L. per Faddan, each ml of the culture contains $1x10^9$

RESULTS AND DISCUSSION

1 : Influense of Biofertilization and Biostimulants Foliar Application on : A : dehydrogenase , µ g tpf.g^{-1}soil . 96 h $^{-1}$.

Data in Table 2 showed that , Dehydrogenase activity of the soil increased obviously in two seasons due to the inoculation, spray with biostimulants and their combination compared to control.

Table 2:Influense of Biofertilization and Biostimulants Foliar Application on dehydrogenase , µ g tpf.g^{-1}soil . 96 h $^{-1}$.

Treatment		Dehydrogenase (µ g tpf.g^{-1}soil . 96 h $^{-1}$)	
		Season 1	Season 2
Main	I0	10.62 c	10.91 c
	I1	16.14 b	15.22 b
	I2	24 39 a	24.51 a
F test		**	**
L.S.D. 0.05		1.76	1.02
Sub main	B0	14.12 b	13.73 b
	BJ	14.85 b	14.51 b
	BA	19.32 a	19.23 a
	BJ+A	19.91 a	19 .78 a
F test		**	**
L.S.D. 0.05		1.62	1.44
Interaction			
I0B0(control)		7.91 f	8.03 f
I0BJ		10.59 ef	10.19ef
I0BA		11 54 e	12.28de
I0BJ+A		12.45 de	12.35cde
I1B0		14.36 d	13.06cd
I1BJ		14.57 d	14.67cd
I1BA		15.13 d	14.82 c
I1BJ+A		20.49 c	18.32 b
I2B0		20.08 c	20.11 b
I2BJ		19.40 c	18 67 b
I2BA		31.30 a	30.60 a
I2BJ+A		26.80 b	27.67 a
F test		**	**
L.S.D. 0.05		2.81	2.5

The data in a column followed by the same symbol are not significant at p= 0.05

C:control, fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum*

Dehydrogenase activity of the soil increased obviously due to the inoculation. The highest increase were attained by the treatment of inoculation + 75% NPK (I2) giving 24.39 and 24.51 µ g tpf.g^{-1}soil . 96 h^{-1} compared to non inoculated treatment (I0) which reached 10.62 and 10.91 µ g tpf.g^{-1}soil . 96 h^{-1} for 2013 and 2014 seasons , respectively. The spray of biostimulants resulted in significant increases in dehydrogenase activity if compared to non bio-stimulants in both seasons . The best treatment was spray with Jisemar +*Azospirillum* sp.(BJ+A) which recorded 19.91 and 19.78 compared to 14.12 and 13.73 µ g tpf.g^{-1}soil . 96 h^{-1} for (B0) at 2013 and 2014 seasons, respectively. In this respect, the highest remarkable increase of dehydrogenase activity records were attained by the application of the combination treatment of inoculation + 75% NPK + *Azospirillum* sp. spray which gave 31.30 and 30.60 µ g tpf.g^{-1}soil . 96 h^{-1} as well as treatment of inoculation + 75 % NPK + Jisemar and *Azospirillum* sp. spray which recorded 26.8 and 28.67 µ g tpf.g^{-1}soil . 96 h^{-1} compared to 7.91 and 8.03 µ g tpf.g^{-1}soil . 96 h^{-1} for control treatment at 2013 and 2014 , respectively

Dehydrogenase activity in rhizosphere of orange trees, in the current study, increased notably due to both inoculation and bio-stimulant spray and their combination .This is explained by the role of these treatments in increasing plant root surface, which led to an increase of roots production of organic substance like sugars and organic acids, induceding microbial biomass and activity in the soil (Steenhoudt and Vanderleyden, 2006) , there fore dehydrogenase activity increased too

B : NPK in leaves .

Data presented in Table 3 showed that, inoculation of orange trees with the mixed inoculum led to considerable increase in NPK in leaves. The treatment of inoculation + 75 % mineral NPK (I2) was superior over those of not inoculated (I0), recording 1.76, 1.79 ; 0.44 , 0.47% and 1.13, 1.03 % for N, P and K through the 2013 and 2014 seasons , respectively and the differences were significant compared to non inoculated trearment .

Similarly, spray with bio-stimulants raised the concentration of N, P and K in orange leaves over those of water sprayed (B0). The treatment of *Azospirillum* sp. (BA) spray attained the highest values in both 2013 and 2014 seasons.The percentage of N, P and K reached 1.67, 1.72 ; 0.47, 0.50 and 1.04, 1.05% , respectively, unsprayed (B0) displayed 1.56,1.55 ; 0.43,0.44 and 0.79, 0.76%% , respectively.

The interaction between inoculation and bio-stimulants spray resulted in most cases a considerable increase over untreated control (I0B0), the best treatment in this respect was(I2BA).

The improving action of inoculation with the mixed inoculum in the present study on leaves mineral concentration (NPK) may be related to many factors like: (1) nitrogen fixation, phosphate and potassium solubilizing bacteria contained in the studied inoculum. Gaber and Nour El-Din (2005) found similar results due to inoculation of apple trees with an inoculum contains *Azospirillum* sp. and *B. megatherium*; (2)the increase of plant root surface which enhanced plant acquisition of macro- and micro-elements from soil; (3) the release of plant hormones, like cytokinins and auxins which enhance root cell division and size (Jaha and Saraf, 2015); (4) the release of

siderofores that chelate iron , making it available for plant absorption(Jaha and Saraf, 2015); (5) the release of polysaccharides and organic acids in soil leading to decrease of soil pH (Steenhodt and Vanderleyden, 2006).

At the current study, the treatment of inoculation + 75 % NPK gave higher response in this regard than that had 50 % NPK, this may be due to the of microorganisms in the inoculums to offer the inability amount of plant needs of NPK, therefore we must supply the rest of plant requirements as a mineral and organic fertilizers. In addition, microorganisms itself need elements to meet their requirements of nutrition for their proliferation and growth and consequency enabling planst to be more vigorous and healthy.The highest positive effects on NPK % in leaves were due to the treatment of combination of inoculation + 75% NPK and spray with the bio-stimulants. This may be return to the synergistic effect between inoculation and bio-stimulants spray which enhanced elements acquisition and plant performance.

Table 3:Influense of Biofertilization and Biostimulants Foliar Application onNPK % in orange leaves .

Treatment		N %		P %		K%	
		Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Main	I0	1.52 b	1.54 b	0.40 b	0.41 b	0.69 c	0.73 c
	I1	1.54 b	1.56 b	0.45 a	0.47 a	0.88 b	0.89 b
	I2	1.76 a	1.79 a	0.44 a	0.47 a	1.13 a	1.03 a
F test		**	**	*	*	**	**
L.S.D. 0.05		0.11	0.09	0.03	0.04	0.15	0.10
Sub main	B0	1.56 b	1.55 c	0.43 b	0.44 b	0.79 b	0.76 c
	BJ	1.64 b	1.64 ab	0.42 b	0.44 b	0.79 b	0.83 bc
	BA	1.67 a	1.72 a	0.47 a	0.50 a	1.04 a	1.05 a
	BJ+A	1.56 ab	1.60 bc	0.40 b	0.42 b	1.00 a	0.89 b
F test		*	**	**	**	**	**
L.S.D. 0.05		0.098	0.091	0.03	0.03	0.08	0.11
Interaction							
I0B0(Control)		1.57 cd	1.48 c	0.39	0.40 c	0.61 f	0.74 b
I0BJ		1.60 cd	1.57 c	0.42	0.42 bc	0.67 ef	0.70 b
I0BA		1.41 d	1.53 c	0.42	0.43 bc	0.74 def	0.75 b
I0BJ+A		1.52 cd	1.58 c	0.38	0.38 c	0.76 de	0.74 b
I1B0		1.58 cd	1.63 bc	0.44	0.47 b	0.79 de	0.73 b
I1BJ		1.53 cd	1.59 c	0.42	0.42 bc	0.81 de	0.89 b
I1BA		1.59 cd	1.56 c	0.51	0.54 a	1.08 b	1.12 a
I1BJ+A		1.46 d	1.45 c	0.44	0.46 b	0.85 cd	0.82 b
I2B0		1.52 cd	1.54 c	0.46	0.46 b	0.96 bc	0.80 b
I2BJ		1.79 b	1.77 b	0.44	0.47 b	0.89 cd	0.89 b
I2BA		2.03 a	2.07 a	0.47	0.54 a	1.30 a	1.30 a
I2BJ+A		1.70 bc	1.77 b	0.39	0.41 bc	1.38 a	1.12 a
F test		**	**	n.s	**	**	**
L.S.D. 0.05		0.17	0.16	--	0.05	0.14	0.18

The data in a column followed by the same symbol are not significant at p= 0.05

C:control,fertilizedwith 100%of recommended NPK
I0 : not inoculated by bio-fertitizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum* sp.

Regarding the effect of bio-stimulant spray on orange NPK % in leaves, their stimulation can be explained by the Jisemar content of different nutrients, free amino acids and sea weed extract as well as nitrogen fixation by *Azospirillum* sp. spray , Steenhodt and Vanderleyden, (2006). Otherwise, Amanda *et al*. (2008)found that, these bio-stimulants improve chlorophyll content and net photosynthesis, which led to enhancement of plant growth and nutrient uptake efficiency. Whereas, the high content of sugars in leaves usually accelerate incorporation of nitrogen through nitrate assimilation pathway.The highest effects of NPK% in leaves were due to the synergistic effect of combination of inoculation + 75% NPK and spray with the bio-stemulants.

C : Fruit set % and fruit drop % :

Data presented in Table 4 illustrated that, the inoculation + 75% NPK (I2) gave an increase in fruit set % with a highly significant differences over those of 100% NPK (I0) due to this treatment. The resulted observations giving of 7.95% with (I2) compared to 6.71% for non inoculated plants(I0) at the first season , respectively, data of the second season followed the same trend

Table 4:Influense of Biofertilization and Biostimulants Foliar Application on fruit set% and fruit drop % .

Treatment		Fruit set %		Fruit drop %	
		Season 1	Season 2	Season 1	Season 2
Main	I0	6.71 b	6.86 b	14.17 b	10.51 b
	I1	6.49 b	6.56 c	15.90 a	11.83 a
	I2	7.95 a	8.06 a	12.51 c	8.65 c
F test		**	**	**	**
L.S.D. 0.05		0.29	0.19	0.29	0.36
Sub main	B0	6.29 d	6.32 d	16.42 a	12.18 a
	BJ	6.90 c	7.01 c	13.95 b	10.33 b
	BA	7.38 b	7.39 b	13.40 c	9.66 c
	BJ+A	7.64 a	7.93 a	13.00 d	9.15 d
F test		**	**	**	**
L.S.D. 0.05		0.26	0.28	0.35	0.27
Interaction					
I0B0(control)		5.36 g	5.84 f	18.19 a	13.40 a
I0BJ		6.67 ef	6.89 e	13.17 f	10.13 e
I0BA		7.49 cd	7.12 de	12.83 fg	9.63 f
I0BJ+A		7.34 cd	7.5 cd	12.50 gh	8.90 g
I1B0		6.38 f	6.28 f	17.18 b	12.87 b
I1BJ		6.38 f	6.13 f	16.30 c	12.28 c
I1BA		6.51 f	6.81 e	15.32 d	11.24 d
I1bJ+A		6.70 ef	7.01 e	14.79 d	10.91 d
I2B0		7.13 de	6.83 e	13.90 e	10.27 e
I2BJ		7.66 c	8.01 bc	12.38 gh	8.59 g
I2BA		8.14 b	8.23 b	12.06 hi	8.11 h
I2BJ+A		8.88 a	9.20 a	11.70 i	7.65 h
F test		**	**	**	**
L.S.D. 0.05		0.45	0.48	0.61	0.48

The data in a column followed by the same symbol are not significant at p= 0.05

C:control,fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum* sp.

In contrast fruits drop was increased significant with the inoculation+ 50% NPK (I1) copared to the other inoculated one (I2) and non inoculated (I0) , it records 15.90 and 11.83% drop at the two seasons .

The spray with Jisemar and/or *Azospirillum* sp. gave better results (P= 0.01) than those of water sprayed treatment in fruit set %, in contrast, drop % of fruits decreased for the two seasons . The spray with the mixture of Jisemar and *Azospirillum* sp. (BJ+A) attained the highest fruit set % (7.64 %) and lowest drop % (13.00 %) compared to those of water sprayed (B0) which gave 6.29% and 16.42 %, respectively for the two parameters during the first season .

The combination of inoculation and bio-stimulants spray led to varied responses, in this respect, whereas, the highest fruit set % was attained by the treatment of inoculation + 75% NPK and spray with Jisemar plus *Azospirillum* sp. (8.88 and 9.20 %) and the lowest drop% (11.70 and 7.65 %) through 2013 and 2014 growth seasons , respectively

The dropping of small fruits is a natural physiological process, aiming at improving quality of ripened fruits (Kolaric, 2010). But when dropping increased because of any reason like low level of nutrients, for example, it became dangerous phenomena, for the yield of the tree (Lahav and Zamet, 1999). Gomez-Cadenas *et al*.(2014) reported that , the addition of nutrients after hormonal activity of fruit set improved fruit growth and decreased falling fruit percentage, also reported that , competition between fruits on the photosynthesis products is the reason of falling.

d : Fruit number tree $^{-1}$, fruit weight (g. fruit^{-1}) and yield (kg tree^{-1}).

Data illustrated in Table 5 showed that , there was a general trend of increase in fruit number per tree, fruit weight (g. fruit $^{-1}$) and fruit yield (kg. tree $^{-1}$) due to inoculation with the NPK mixed with biofertilizer compared to (I0) treatment (fertilized with the full dose of chemical fertilizers N, P, and K). The differences were mostly significant especially with the inoculation with the biofertilizer and with 75% of recommended NPK chemical fertilizers(I2), which surpassed in its beneficial effects than the other applied treatments .

Bio-stimulants types (Jisemar, *Azospirillum* sp., or mixture of them) spray, significantly increased orange fruits number per tree and fruits yield but did not significantly affect on fruit weight in the two seasons .

The combination of biofertilization with the mixed inoculum and biostimulants spray gave the higher records than non inoculated non sprayed control. The treatment of I2BJ+A exhibited the highest values, which gave 385.33 and 480.00 compared to 247.33 and 397.67 (fruits number/tree) for control treatment at the 2013 and 2014 seasons . Similarly, it gave fruit weight about 234.73 and 223.04 (g. fruit $^{-1}$) compared to 252.48 and 190.37 (g. fruit $^{-1}$) for control treatment at the same sequence. High increases were displayed fruits yield, which had 90.47and 107.06 (kg. tree $^{-1}$) compared to 62.5 and 75.71 (kg. tree $^{-1}$) for control at season 1 and season 2 , respectively. The most increases over control were significant at p= 0.05.

The positive effect of inoculation on orange yield per tree may regard to:

(1) Nitrogen fixation by *Azospirillum* sp. which providing the tree with continuous flow of nitrogen, supported by, Malik *et al*., (2002) were

confirm that *Azospirillum brsilense* and *Azospirillum lipoferum* fix nitrogen from7-12 % satisfying of wheat total nitrogen , by use of N^{15} technique. They also claimed that, *A.diazotrophicus* fix from 60-80% of total nitrogen content of sugar beet needs .

(2) Solubilization of tricalcium and dicalcium phosphate was confirmed also through the action of phosphate solubilizing bacteria like *B. megatherium* which was used in the present study. These type of microbes release many of organic acids causing solubilization of the unavailable phosphate through carboxylic and hydroxide groups founded in these acids (Rodriguez and Fraga 1999). Also, phosphate solubilizing bacteria take the mineral phosphorus from organic phosphatic compounds like phytatas by using phosphatase and phytase enzymes (Richardson *et al*., 2009).

(3) Organic acids released by potassium solubilizing bacteria had beneficial role in solubilizing unavailable potassium which linked with potassium rock minerals like mika and illite Sivakumar, (2014)

(4) Sidrophores produced by these bio-fertilizers chelate Fe^{+3} and change it to available form, and many of these compounds had been isolated and identified from these bacterial types (Park *et al*., 2006).

(5) The bio-fertilizers produce phytohormones which enhance plant growth (Idris *et al*., 2007), and the most important hormones is IAA and GAs (Ali *et al*., 2009) , cytokinins and ethylene (Spaepen *et al*., 2009) . Gibberilines contribute in regulating plant cell division and size (Spaepen *et al*., 2009) .

(6) In addition, these bio-fertilizers give the plant tolerance against drought, salinity and chilling (Hanaoui *et al*., 2001) through different mechanisms like enhancing prolin production in plant. Many studies confirmed the role of inoculation with these bio-fertilizers in bio-remediation of different soil pollutants (Zein *et al*., 2013). Therefore, all these vital roles contribute in to increasing of plant yield and improvement of quality.

On the other hand, the beneficial effect of bio-stimulants were reported by many authors . Bulgari *et al*. (2015) claimed that, these compounds increased nutrient use efficiency and enhanced plant tolerance to biotic and a biotic stresses. They also found that bio-stimulants application increase sugar biosynthesis as a result of the increase of chlorophyll content, and by consequence the increase of sugar accumulation lead to an increase of protein biosynthesis. Additionally they cited that, the accumulation of reactive oxygen species in the cell produced from the high photo excitation through photosynthesis process causes cell damage and decreases plant yield, but fortunately, biostimulants mostly contains antioxidants which had the potentiality to decrease these reactive oxygen species, thus protect the cell from the danger of these free radicals.

Table 5:Influense of Biofertilization and Biostimulants Foliar Application on fruit number tree $^{-1}$,fruit weight (g. fruit^{-1}) and yield (kg tree^{-}).

Treatment		Fruit number tree $^{-1}$		Fruit weight g^{-1}		Yield Kg tree^{-1}	
		Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Main	I0	308 92 b	439.79 b	222.79 a	208.63 b	68.23 a	92.00 b
	I1	278.96 c	371.88 c	183.00 b	181.90 c	50.92 b	67.61 c
	I2	357.17 a	466.67 a	211.02 a	221.90 a	76.28 a	103.44 a
F test		**	**	*	**	**	**
L.S.D. 0.05		3.32	3.03	25.49	8.43	8.27	3.97
Sub main	B0	277.78 b	402.17 d	202.79	200.61	55.61 b	81.51 c
	BJ	317.17 b	435.11 b	208.66	199.54	66.55 a	87.49 b
	BA	328.89 a	426.78 c	210.15	205.30	69.70 a	88.13 b
	BJ+A	336.22 a	440 39 a	200.81	211.11	68.72 a	93.60 a
F test		**	**	n.s	n.s	*	**
L.S.D. 0.05		9.41	4.62	--	--	10.4	4.42
Interaction							
I0B0(control)		247.33 f	397.67 e	252.48	190.37 d	62.50	75.71 c
I0BJ		332.33 c	454.00 c	208.24	209.02bc	69.22	94.91 b
I0BA		331.67 c	456.67 c	215.31	210.68 b	71.43	96.21 b
I0BJ+A		324.33 c	450.83 c	215.13	224.45 b	69.76	101.18ab
I1B0		269. 00 e	366.17 g	193.68	174.23 d	52.10	63.80 e
I1BJ		267.17 e	380.33 f	195.72	175.47 d	52.28	66.71 de
I1BA		280.67 e	350.67 h	190.04	192.04cd	53.37	67.35 de
I1BJ+A		299.00 d	390.33 e	152.56	185.85d	45.92	72.57 cd
I2B0		317.00 c	442.67 d	162.21	237.23 a	52.24	105.02 a
I2BJ		352.00 b	471.00 b	222.04	214.14 b	78.14	100.85ab
I2BA		374.33 a	473.00ab	225.11	213.19 b	84.29	100.84ab
I2BJ+A		385.33 a	480.00 a	234.73	223.04ab	90.47	107.06 a
F test		**	**	n.s	**	n.s	**
L.S.D. 0.05		16. 3	8.00	--	17.38	--	7.66

The data in a column followed by the same symbol are not significant at p= 0.05

C:control,fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum* sp.

E : Fruit Parameters :

1 : SSC% , acidity % and SSC/acid ratio.

Reults of SSC% , acidity, and SSC/acid ratio presented in Table 6 indicated that , inoculation with the mixed inoculum significantly increased SSC% and acidity of orange juice more than those of not inoculated 100% NPK fertilized at the second season . The highest values of acidity was found with the treatment of inoculation + 75% of recommended NPK +spray with Jisemar plus *Azospirillum* sp.(I2BJ+A) which gave 1.11% and 1.12% compared to 0.98 and 0.99 % of control in the two seasons, respectively . Ratio SSC /acid decreased also with the inoculation treatments and the differences were significant compared to control, that may be due to the increase in acidity % in the two seasons .The reason of the increasing acidity may related to the effect of these stimulants in inducing continuous supplement of elements by the action of these bio-stimulants in the longevity of vegetative growth time, and hence prolonging maturity stage and delayed picking date of the fruits .

Regardless inoculation, the spray with the biostimulants had a positive effect on SSC% and acidity which increased at both study seasons more than water sprayed control plants. Many treatments decreased SSC% like treatment of inoculation+ 50% NPK + water spray (I1B0) which gave 11.73 and 11.73% compared to 13.13 and 13.00% for control at 2013 and 2014 seasons , respectively. Similarly, the treatment of inoculation + 75% NPK + *Azospirillum* sp.(I2BA) spray and inoculation + 75% NPK + spray with the mixture of Jisemar and *Azospirillum* sp .(I2BJ+A) increased SSC% significantly more than control which gave 13.80 and 13.75% compared to 13.13 and 13.00% SSC in control treatment during the two study seasons . While, SSC/acid ratio character showed an obvious variation due to the treatments of combination at both seasons. The treatment of inoculation + 75% NPK plus Jisemar and *Azospirillum* sp. spray exhibited noticeable decrease in SSC/ acid ratio than those of control giving 12.43 % and 12.28% compared to 13.40% and 13.13% for control plants during 2013 and 2014 seasons , respectively.

Table 6:Influense of Biofertilization and Biostimulants Foliar Application on SSC% , acidity % and SSC/acid ratio.

Treatment		SSC (%)		Acidity %		SSC/acid (ratio)	
		Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Main	I0	13.37 a	13.30 b	0.99 b	0.99 b	13.54 a	13.43 a
	I1	12.38c	12.23c	0.97 b	0.97 b	12.74 b	12.61 c
	I2	13.23b	13. 31 a	1.02 a	1.02 a	12.97 b	13.05 b
F test		**	**	**	**	**	**
L.S.D. 0.05		0.11	0.06	0.02	0.02	0.25	0.29
Sub main	B0	12.62 c	12.60 c	0.98 c	0.98 b	12.88 ab	12.86 b
	BJ	12.71 c	12.73 c	0.99 b	0.98 b	12.82 b	12.98 ab
	BA	13.06 b	13.02 b	1.00 b	0.98 b	13.03 ab	13.29 a
	BJ+A	13.61 a	13.40 a	1.03 a	1.03 a	13.21 a	13.00 ab
F test		**	**	**	**	*	*
L.S.D. 0.05		0.13	0.10	0.02	0.02	0.33	0.31
Interaction							
I0B0(control)		13.13 de	13.00 cde	0.98 cd	0.99 b	13.40 ab	13.13 bc
I0BJ		13.20 cde	13.30 bc	0.99 cd	0.98 b	13.40 ab	13.57ab
I0BA		13.26 cd	13.20 bc	1.00 cd	0.98 b	13.33 b	13.47 abc
I0BJ+A		13.87 a	13.73 a	0.99 cd	0.99 b	13.97 a	13.84 a
I1B0		11.73 h	11.73 h	0.97 d	0.96 b	12.16 e	12.19 d
I1BJ		12.20 g	11.93 g	0.98 cd	0.97 b	12.51 de	12.33 d
I1BA		12.53 f	12.53 f	0.96 d	0.97 b	13. 02bcd	12.95 c
I1BJ+A		13.07 de	12.73 e	0.99 cd	0.98 b	13.27 bc	13.03 c
I2B0		13.00 e	13.17 cd	1.00 cd	0.99 b	13.07bcd	13.30 bc
I2BJ		12.73 f	12.97 ef	1.02 bc	0.99 b	12.55 de	13.10 c
I2BA		13.38b	13.33 bc	1.05 b	1.00 b	12.73cde	13.33 abc
I2BJ+A		13.80a	13.75 a	1.11 a	1.12 a	12.43de	12.28d
F test		**	**	**	**	**	**
L.S.D. 0.05		0.22	0.18	0.04	0.05	0.58	0.54

The data in a column followed by the same symbol are not significant at p= 0.05

C: control, fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with Azospirillum sp.
BJ+A: sprayed with Jisemar plus Azospirillums

The application on orange trees by the mixed biofertilizer and biostimulants +75% NPK caused an increase in SSC% and decreased SSC/ acid ratio in the two seasons these are two important parameters for determination of maturity of the fruits as well as their quality . Nour El-Din *et al*., (2012) indicated that , spray of apple trees with *Azospirillum* sp. and *Pseudomonas flurescence* increased fruit acidity. At this context , El-Khayate and Abdel Rehiem (2013) found that , biofertilization of lemon plants with phosphate dissolving increased acidity and vitamin C content of the fruits

The inoculation with the mixed inoculum, in the present study, SSC/ acid ratio . Gaber and Nour El-Din, (2005) confirmed the same decreased phenomena in Anna apple fruits when the trees were inoculated with an inoculum composed of *Azospirillum sp*., *B. megatherium* and mycorrhizal fungi. Likewise,Nour El-Din *et a*l.(2012) reported that , spray with different PGPR decreased SSC and SSC/ acid but increased acidity and firmness of apple trees. This may be related to the role of biological fertilization and /or biostimulants spray in increasing vegetative stage period of the tree as a result of continuous supply of nutrients due to action of bio-fertilizers microorganisms as well as improving nutrient use efficiency resulted from bio-stimulant action (Calvo *et al*., 2014). In this respect (Pirlak and Kose ,(2009) claimed that, spray of strawberry with PGPR improved the quality characteristics of the fruits especially TSS .

2 :**Chlorophyll A&B and total chlorophyll** (mg $100g^{-1}$fresh weight**) on fruit peel .**

Data presented in Table 7 indicated that, chlorophylls A , B and total chlorophyll significant increased over not inoculated through the two study seasons due to the application of the treatment of inoculation + 75% NPK (I2) .On the other hand, the spray with biostimulants enhanced chlorophylls A and total through the two seasons, especially by the spray with the mixture of Jisemar and *Azospirillum* sp. (BJ+A) and the differences were significant at the first season. Likewise, chlorophyll B exhibited significant increases than those of unsorayed treatment (B0) at the second seasons .

The combination of inoculation with the mixture inoculum and the spray with the different biostimulants mostly had an appreciated role on the improvement of chlorophyll A and B as well as total chlorophyll (Table 7). The treatment of inoculation with 75% NPK + spray with Jisemar and *Azospirillum* sp. (I2BJ+A) gave consistent significant increases than those of control (I0B0), recording (15.25, 20.50) ;(23.67, 26.3) and (38.92, 46.80) (mg $100g^{-1}$) compared to control (I0B0)which recorded (13.07, 17.60) ;(19.43, 20.58) and (32.51, 38.18) (mg $100g^{-1}$) for chlorophyll A ; chlorophyll B and total chlorophyll, through seasons of 2013 and 2014, respectively. The increase of chlorophyll A , B and total due to inoculation with the mixed inoculum (*Azospirillum* sp., *B*. *megatherium* and *B. circulans*) may be related to the action of these microorganisms on providing the plant with the necessary elements, especially nitrogen through nitrogen fixation process, phosphorous and potassium by solubilizing rock phosphate and potassium.

Hamza and Suggars, 2001 reported that, *Azospirillum* sp. and others PGPR groups considered as biostimulants. Seaweed extract is a vailable compound in stimulating plant growth, whereas, it contains cytokinins, auxins

and other hormones-like substances. Bulgari *et al*. (2015) mentioned that , biostimulants including PGPR and phytohormones help in increasing the biosynthesis of chlorophyll content and carotenoids and consequently net photosynthesis improved in plant .

Table 7:Influense of Biofertilization and Biostimulants Foliar Application on chlorophyll A&B and total chlorophyll on fruit peel .

Treatment		Chlorophyll A (mg $100g^{-1}$)fresh weight		Chlorophyll B (mg $100g^{-1}$)fresh weight		Total Chlorophyll (mg $100g^{-1}$)fresh weight	
		Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Main	I0	13.28 b	17.54 b	19.40 b	20.42 b	32.69 b	37.96b
	I1	12.41 c	16.93 c	18.58 c	19.49 c	31.00 c	36.42c
	I2	14.64 a	19.72 a	23.52 a	26.17 a	38.17 a	45.89a
F test		**	**	**	**	**	**
L.S.D. 0.05		0.29	0.27	0.08	0.18	0.34	0.39
Sub main	B0	13.21 b	18,30 a	20.30 c	22.01 b	33.51 c	40.31a
	BJ	13.35 b	17.74 b	20.47 b	21.85 c	33.82 bc	39.60b
	BA	13.49 ab	17.89 b	20.65 a	21.98 bc	34.14 ab	39.87b
	BJ+A	13.73 a	18.31 a	20.60 ab	22.27 a	34.33 a	40.58a
F test		**	**	**	**	**	**
L.S.D. 0.05		0.30	0.26	0.14	0.15	0.37	0.33
Interaction							
I0B0(control)		13.07 de	17.60 c	19.43 cd	20.58 b	32.51	38.18 c
I0BJ		13.13 cd	17.48 c	19.24 d	19.93 c	32.37	37.41 f
I0BA		13.30 cd	17.55 c	19.62 c	20.47 b	32.92	38.02 c
I0BJ+A		13.63 c	17.53 c	19.32 d	20.71 b	32.95	38.23c
I1B0		12.23 f	17.83 c	18.29 f	19.35 d	30.52	37.18de
I1BJ		12.51 f	16.35 e	18.64 e	19.47 d	31,.16	35.82f
I1BA		12.60 ef	16.63 de	18.60 e	19.34 d	31.20	35.97f
I1BJ+A		12.31 f	16.90 d	18.80 e	19.80 c	31.44	36.70e
I2B0		14.33 b	19.48 b	23.19 b	26.10 a	37.52	45.58b
I2BJ		14.43 b	19.40 b	23.51 a	26.16 a	37.94	45.56 b
I2BA		14.58 b	19.50 b	23.72 a	26.13 a	38.30	45.63 b
I2BJ+A		15.25 a	20.50 a	23.67 a	26.30 a	38.92	46.80 a
F test		**	**	**	**	n.s	**
L.S.D. 0.05		0.52	0.46	0.24	0.26	--	0.58

The data in a column followed by the same symbol are not significant at p= 0.05

C: control, fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar .
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum*.

3 : Carotene % of fruit peel , vitamin C (mg/ 100 ml juice) and fruit firmness (g / mlm^2) :

Data presented in Table 8 illustrated that, the inoculation + 75% NPK (I2) gave an increase in vitamin C and firmness with a highly significant

differences over those of 100% NPK (I0) due to this treatment . The resulted observations of the two parameters at the first season were 54.28 and 71.43 compared to 51.64 mg/100ml and 69.51 g/mlm^2 for non inoculated plants , respectively, data of the second season followed the same trend . The spray with Jisemar and/or *Azospirillum* sp. gave better results (P= 0.01) than those of water sprayed treatment in V.C and firmness . The spray with the mixture of Jisemar and *Azospirillum* sp. (BJ+A) attained the highest of V.C and firmness compared to the other sprayed and not sprayed treatments, giving (53.20 mg/100ml) V.C and (70.80 g/mlm^2) firmness at first season . In contrast carotene % take a different trend, it as percentages decreased due to the use of these two promising treatments (I2) and (BJ+A) than those of not inoculated (I0) and not biostimulants (B0) treatments.

Table 8:Influense of Biofertilization and Biostimulants Foliar Application on carotene % of fruit peel , vitamin C (mg/ 100 ml juice) and fruit firmness (g / mlm^2) :

Treatment		Carotene %		vitamin C (mg/ 100 ml juice)		Firmness (g/mlm^2)	
		Season1	Season 2	Season 1	Season 2	Season 2	Season 2
Main	I0	73.97 a	81 80 a	51.64 b	51.09 b	69.51 b	67.32 b
	I1	53.42 c	57.34 c	50.72 c	50.13 c	51.80 c	52.36 c
	I2	62.49 b	74.70 b	54.28 a	54.11 a	71.43 a	68.14 a
F test		**	**	**	**	**	**
L.S.D. 0.05		0. 99	1.37	0.51	0.47	1.38	0.73
Sub main	B0	64.15	72.64	51.19 c	50.42 c	59.37 c	59.02 c
	BJ	62.96	70 86	51.92 bc	51.83 b	57.98 d	59.06 c
	BA	63.25	70.33	52.54 ab	52.34 a	68.83 b	65.17 b
	BJ+A	62.81	71.31	53.20 a	52.52 a	70.80 a	67.18 a
F test		n.s	n.s	**	**	**	**
L.S.D. 0.05		--	--	0.79	0.43	0.87	1.22
Interaction							
I0B0(control)		69.22 c	77.72cd	51.04cde	49.91 g	69.52 d	67.22 d
I0BJ		73.21 b	81.70 b	52.08 c	50.46 fg	58.03 g	61.20 f
I0BA		74.98 b	80.32 bc	51.77 cd	52.35 c	73089 c	69.50 c
I0BJ+A		78.46 a	87.44 a	51.68 cd	51.64 cd	76.60 b	71.38 bc
I1B0		56.98 g	63.76 g	50.28 de	48.20 h	46.15 j	50.10 i
I1BJ		53.27 h	56.82 h	49.98 e	50.13 fg	50.34 i	51.68 hi
I1BA		53.01 h	54.95 h	50.76cde	51.40de	53.70 h	52.60 h
I1BJ+A		50.43 i	53.84 h	51.84 c	59.78 ef	57.00g	55.08 g
I2B0		66.25 d	76.43de	52.25 c	53.15 b	62.45 f	59.75 f
I2BJ		62.41 e	74.04ef	53.70 b	54.89 b	65.58 e	64.30 e
I2BA		61.77 e	75.72def	55.10 a	53.27 a	78.90 a	73.42 ab
I2BJ+A		59.52 f	72.63 f	56.07 a	55.12 a	78.80 a	75.10 a
F test		**	**	*	**	**	**
L.S.D. 0.05		2.01	2.97	1.37	0.47	1.51	12.2

The data in a column followed by the same symbol are not significant at p= 0.05

C: control, fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum*

Concerning the combination of inoculation + 75% NPK and spray with Jisemar plus *Azospirillum* sp., there was a general trend to increase of V.C (56.07 and 55.12 mg/100 ml) and firmness (78.80 and 75.10 g/mlm^2) compared to (51.04 and 49.91 mg/100ml) and (69.52 and 67.22 g/mlm^2) in control (I0B0) in the two seasons . Carotene % take a different trend, treatment of 100% NPK with the sprays of Jisemar + *Azospirillum* sp. (I0BJ+A) recorded the highest carotene % (78.46 and 87.44 %) compared to (69.22 and 77.72 %) in control (I0B0) in the two seasons .

Elshazly and Mostafa, (2015) indicated that , the spray of orange trees with bio-stimulants (amino acids and yeast extract) led to increase of vitamin C in fruits. Likewise, Vernieri *et al* (2005) showed an increase in carotenoid in rocket plant due to application of Actiwave (commercial product contains elements and free amino acids). Nour El-Din *et al*.(2012) found that, spray of apple trees with a diluted culture of *Azospirillum* sp. and /or Jisemar bio-stimulants increased vitamin C and fruit acidity. At this context, El-Khayate and Abdel Rehiem (2013) found that , biofertilization of lemon plants with dissolving phosphate increased acidity and vitamin C content of the fruits .

On the other hand, the spray of orange trees with the bio-stimulators (*Azospirillum* sp. and / or Jisemar decreased some parameters and increased others, compared to control . This is related to the effect of these bio-fertilizers and / or bio-stimulants in providing plants with nutrients and phytochemical compounds causing the prolongation life span of plant, which reflected on increasing yield as presented in Table 5 of the present study. Therefore, we recommende to delay the harvest till complete maturation, specially, with biofertilization or biostimulant application which has the ability to increase plant efficiency for nutrient uptake.

2 : Influense of Biofertilization and Biostimulants Foliar Application on fruit parameters during sfelf life at room temperature :

a : Weight loss (%) .

Data presented in Table (9) display the effect of inoculation on weight loss of orange fruit during shelf life . It was clear that, the treatment of inoculation + 75% NPK (I2) reduced the decrease of fruit weight compared to other treatments and control, as it records 21.15and 20.52 % compared to 23.67 and 23.31% in (I0) teatment at the end period for the two seasons with a highly significant .The same trend were found between the sprayed treatments too. The unsprayed treatment (B0) showed the highest values of weight loss compared to the other treatments , but mixture of Jisemar and *Azospirillum sp*. spray (BJ+A) resulted in the lowest values 21.66 and 21.40% at the end time.The combination of inoculation and bio-stimulants spray plus 75% NPK gave the lowest decrease if compared to control and the other treatments , it records 18.08 and 17.35% compared to 25.77and 25.23% for control and 26.41 and 26.13% on (I1B0) during the two seasons, respectively

Table 9:Influense of Biofertilization and Biostimulants Foliar Application on fruit weight loss % during shelf life at room temperature .

Treatment		weight loss%15 days		weight loss% 30 days	
		Season 1	Season 2	Season 1	Season 2
Main	I0	12.26 b	12.39 b	23.67 b	23.31 b
	I1	13.72 a	13.91a	25.49 a	25.44 a
	I2	10.83 c	10.99 c	21.15 c	20.52 c
F test		**	**	**	**
L.S.D. 0.05		0.49	0.32	0.27	0.89
Sub main	B0	13.36 a	13.07a	25.18 a	24.93 a
	BJ	12.81 b	12.73 a	24.32 b	23.73 b
	BA	11.77 c	11.84 b	22.73 c	22.31 c
	BJ+A	*11.68* d	11.46 b	21.66 d	21.40 d
F test		**	**	**	**
L.S.D. 0.05		0.32	0.53	0.44	0.88
Interaction					
I0B0(control)		13.53	13.08 bc	25.77ab	25.49 a
I0BJ		12.90	12.45 c	24.20 d	23.00 bc
I0BA		11.43	11.63 e	22.61 fg	23.30 b
I0BJ+A		12.04	11.89 e	22.11 g	21.47c
I1B0		14.09	14.38 a	26.41a	26.13 a
I1BJ		14.02	13.96 b	25.45 bc	25.18 a
I1BA		14.12	14.73 a	25.31 bc	25.09 a
I1BJ+A		12.77	12.90 bc	24.78 vd	25.37 a
I2B0		12.45	11.83 d	23.3 e	23.18 b
I2BJ		11.48	11.77d	23.32 ef	23.03 b
I2BA		9.77	10.16 f	20.29 h	18.54 d
I2BJ+A		9.76	9.57 f	18.08 i	17.35 d
F test		n.s	**	**	**
L.S.D. 0.05		--	0.55	0.76	1.53

The data in a column followed by the same symbol are not significant at p= 0.05

C:control,fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum* sp.

Microbial spray caused the highest reduction in weight loss on Washington navel orange fruits during storage . Zaghloul (2004) cited that, the loss of fruit weight of Washington navel orange is mainly due to water loss as a result of evaporation and transpiration , plus the amount of dry matter loss by fruit respiration.

b : Fruit decay (%) .

The effect of synthetic and microbial biostemlants on fruit decay percentage of navel orange during shelf life period as displayed in Table (10) data cleared an increase of decay percentage with time . The lowest percentage was found with inculation plus 75% NPK (I2) and the differences were highly significant , it gave 17.16 and 19.60% compared to 20.87 and 22.22% for non inoculated one (I0) in the two seasons . Similarly the same trend was found with biostemulant treatments, as they minimized the increase of fruit decay percentage than those of unsprayed treatment , the lowest values were recorded with the spray of the mixture Jisemar and

Azospirillum sp. , it recorded 16.29 and 18.43 % against 27.57 and 28.55 % decayed fruits of unsprayed one, in both seasons, respectively .

Table10:Influense of Biofertilization and Biostimulants FoliarApplication on fruit decay % during shelf life at room temperature .

Treatment		decay % 15 days		decay % 30 days	
		Season 1	Season 2	Season 1	Season 2
Main	I0	12.88 a	16.07 a	20.87b	22.22 b
	I1	13.53 a	16.54 a	27.22 a	26.64 a
	I2	7.22 b	8.62 b	17.16 c	19.90 c
F test		**	**	**	**
L.S.D. 0.05		1.09	1.03	1.4	2.46
Sub main	B0	13.57 a	16.79 a	27.57 a	28.55 a
	BJ	11.34 b	13.59 b	23.21 b	24.93 b
	BA	11.05 b	13.51 b	19.92 c	19.79 c
	BJ+A	8.88 c	11.08 c	16.29 d	18.43 c
F test		**	**	**	**
L.S.D. 0.05		0.76	1.06	1.91	1.87
Interaction					
I0B0(control)		19.07 a	23.86a	28.89	30.56 a
I0BJ		12.50 c	15.40 b	22.22	23.97 c
I0BA		12.50 c	15.54 b	19.03	17.70 d
I0BJ+A		7.44 d	9.47 c	13.33	16.67 d
I1B0		14.18 b	17.04 b	31.61	30.56 a
I1BJ		14.07 b	17.04 b	28.39	27.78ab
I1BA		13.33bc	16.67 b	26.67	25.00bc
I1BJ+A		12.53 c	15.43 b	22.22	23.22c
I2B0		7.46 d	9.47 c	22.22	24.53 c
I2BJ		7.44 d	8.33 c	19.03	23.03 d
I2BA		7.32 d	8.33 c	14.05	16.67 d
I2BJ+A		6.67 d	8.33 c	13.33	15.39 d
F test		**	**	n.s	*
L.S.D. 0.05		1.31	1.83	--	3.24

The data in a column followed by the same symbol are not significant at p= 0.05

C: control, fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum*

Regarding the combination of inoculation and spray with biostimlants , decay increase with time . The highest values was showed by the inoculation plus 50% NPK (I1B0) as 31.61 and 30.56% , in contrast with 13.33 and 15.39 % in mixed inoculated and sprayed + 75% NPK treatment (I2BJ+A) with highly significant differences after 30 days at first and second seasons , respectively .

In this respect EL- Hammady *et al*.(2000) on Balady mandarin and Zaghloul (2004) on navel orange fruits recorded that, GA3caused a decline in fruit decay and rot pathogens and reduced rind susceptibility to decay and enhancing shelf life of fruits . Microbial biostimulant treatments decline fruit decay percentage on Anna apple fruits which means an enhanced control fo fruit pathogens . This is may be the direct reason of decline in rots infection and the decrease of decayed fruits during shelf life (Esitken, 2011); and resistance to plant pathogens (Van Loon , 2007) .

c : Vitamin C(mg/100ml juice) .

Data of synthetic and microbial biostimulants influence on V.C content of navel orange fruits during shelf life were presented in Table (11) . V.C decreased during that period . Values of V.C varied significantly between the inoculated or sprayed treatments during the two seasons . All treatments reduced the decrease in V.C than that of control . The highest values of V.C was 42.11 and 43.04 mg /100 ml fruit juice with inoculation with 75% NPK treatment (I2) compared to 34.39 and 34.56 mg/100ml with inoculation + 50% NPK (I1) , and 40.44 and 40.26 mg/100ml juice with mixed biostimulants spray (BJ+A) compared to 36.59 and 35.89 mg/100ml with unsprayed treatment (B0) during the two study seasons .

The combination effects on V.C content indicated that, inoculated and mixture sprayed + 75% NPK treatment was the most effective in showing the loss of V.C compared to other treatments and control, as it recorded 46.16 and 44.94 mg/ 100ml juice in contrast to 32.78 and 33.62 mg/100ml juice with inoculation plus 50% NPK(I1B0) in both seasons

Table11:Influense of Biofertilization and Biostimulants Foliar Application on: vitamin C(mg/100ml juice) during shelf life at room temperature .

Treatment		V.C zero time		V.C 15 days		V.C 30 days	
		Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Main	I0	51.64 b	51.09 b	42.15 b	41.90 b	38.03b	38.11 b
	I1	50.70 c	50.13 c	39.38 c	38.60 c	34.39c	34.56 c
	I2	54.28 a	54.13 a	44.40 a	44.53 a	42.11a	43.04 a
F test		**	**	**	**	**	**
L.S.D. 0.05		0.51	0.45	0.59	0.68	0.84	0.65
Sub main	B0	51.19 c	50.42 c	41.57 c	40.91 c	36.59 d	35.89 d
	BJ	51.92 bc	51.83 b	*39.96* d	40.68 c	37.90 c	37.79 c
	BA	52.54 ab	52.34 a	*42.37 b*	41.46 b	39.36 b	38.77 b
	BJ+A	53.20 a	52.54 a	*44.00 a*	43.65 a	40.44 a	40.26 a
F test		**	**	**	**	**	**
L.S.D. 0.05		0.79	0.42	0.65	0.31	0.61	0.47
Interaction							
I0B0(control)		51.04c de	49.92 g	42.17 c	40.24 e	36.00 f	34.20 hi
I0BJ		52.08 cd	50.46 fg	40.49 e	39.51 f	38.34 e	38.68 e
I0BA		51.77 cd	52.35 c	41.72 cb	40.34 e	38.34 e	37.85 f
I0BJ+A		51.68 cd	51.64 cd	44.20 b	47.49 a	39.77d	41.38 c
I1B0		50.28 cd	48.20 h	37.92 f	39.37 f	32.78 h	33.62 i
I1BJ		49.98 e	50.13 fg	38.64 f	37.29 h	34.26 g	34.10 hi
I1BA		50.76 de	51.40 de	40.11e	38.71 g	35.80 f	35.38 g
I1BJ+A		51.84 cd	50.78 ef	40.84 de	39.03 fg	35.38 f	34.45 h
I2B0		52.25 c	53.15 b	44.63 b	43.13 d	40.98 c	39.86 d
I2BJ		53.70 b	54.89 a	40.75 de	45.23 b	41.10 c	40.58 cd
I2BA		55.10 a	53.27 b	45.27 b	45.34 c	43.93 b	43.07 b
I2BJ+A		56.07a	55.12 a	46.97 a	44.42 c	46.16 a	44.94 a
F test		**	**	**	**	**	**
L.S.D. 0.05		1.37	0.73	1.13	0.54	1.05	0.81

The data in a column followed by the same symbol are not significant at p= 0.05

C: control, fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum*

These results were in agreement with EL- Hmmady *et al*.(2000) and Zaghloul (2004) who reported that, spray with GA3 increased vit.C and reduced its loss during storage period

d : Soluble solids content (SSC %) .

Results of SSC were noted in Table (12) indicating that, inoculation treatments had a different significantly during shelf life . Inoculation with 75% NPK (I2) and (I0) showed the high values of SSC at the end time, these values were 13.01 ; 12.92 % and 12.96 ; 12.82 % compared to 12.07 and 11.78 % with the inoculation plus 50% NPK .

Table12:Influense of Biofertilization and Biostimulants FoliarApplication on SSC % during shelf life at room temperature .

Treatment		SSC zero time		SSC 15 days		SSC 30 days	
		Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Main	I0	13.37 a	13.30 b	13.4 a	13.10 a	12.96 a	12.82 b
	I1	12.38 c	12.23 c	12.24 c	11.98 b	12.07 b	11.78 c
	I2	13.23 b	13.31 a	12.88 b	13.01 a	13.01 a	12.92 a
F test		**	**	**	**	**	**
L.S.D. 0.05		0.11	0.06	0.12	0.25	0.15	0.02
Sub main	B0	12.62 c	12.60c	12.72 b	12.54 b	12.38c	12.20 c
	BJ	12.71 c	12.73c	12.84 b	12.58 b	12.67 b	12.45 b
	BA	13.06 b	13.02 b	12.68 b	12.60 b	12.71 b	12.58 b
	BJ+A	13.61 a	13.40 a	13.13 a	13.07 a	12.96 a	12.80 a
F test		**	**	**	**	**	**
L.S.D. 0.05		0.13	0.10	0.19	0.15	0.14	0.14
Interaction							
I0B0(control)		13.13 de	13.00cde	13.37 bc	13.07bc	12.87 b	12.80 bcd
I0BJ		13.20 cde	13.30 bc	13.47 ab	13.07bc	12.93 b	12.80 bcd
I0BA		13.26 cd	13.20 bc	13.20 c	12.87 cd	12.93 b	12.67 d
I0BJ+A		13.87a	13.73 a	13.60 a	13.40 a	13.10 ab	13.00 ab
I1B0		11.73 h	11.73 h	11.60 h	11.60 f	11.40 e	11.07 g
I1BJ		12.20 g	11.93 g	12.13 g	11.67 f	12.21 d	11.67 f
I1BA		12.53 f	12.53 f	12.23 g	12.02 e	12.20 d	12.13 e
I1BJ+A		13.07de	12.73 e	13.00 d	12.63 d	12.47c	12.27 e
I2B0		13.00 e	13.17 cd	13.20 c	12.95bc	12.87 b	12.73 cd
I2BJ		12.73 f	12.97 ef	12.93 de	13.00bc	12.87 b	12.87 bcd
I2BA		13.38 c	13.33 bc	12.60 f	12.92bc	13.00 b	12.93 abc
I2BJ+A		13.80 b	13.75 a	12.80 e	13.18ab	13.30 a	13.13 a
F test		**	**	**	**	**	**
L.S.D. 0.05		0.22	0.18	0.19	0.26	0.25	0.25

The data in a column followed by the same symbol are not significant at p= 0.05

C: control, fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum*

In this respect spraying with microbial plus synthetic biostimulants had the highest of SSC content compared to other treatments in both seasons and maintaining SSC content with highly significant differences , recording 12.96 and 12.80 % compared to 12.38 and 12.20 in unsprayed treatment during study seasons .

Referring to combination of inoculation and biostimulants , the inoculation with 75% NPK plus microbial biostemlants, they recorded the highest SSC % (13.30 and 13.13%), in contrary to control treatment (I0B0) with 12.87 and 12.80 % of SSC content against the other interacted treatments in both seasons at the end time .

Results showed that , inoculation with 75% NPK treatment and the combination of this treatment with the mixture of two biostimulants had the highest value of SSC at the end time against the least values of the other treatments .

EL-Hammady *et al*. (2000) and Zaghloul (2004) claimed that, applied GA3on Balady mandarin and navel orange, decreased the loss of SSC at the end of storage . Govindasamy et al. (2008) found that, the application of PGPR regulates fruit production and delay fruit senescence . This explain the reason of the increase of SSC with inoculation of 75% NPK treatment and its compination with spray of the two biostimulators especially the effect of these treatments in increasing vegetative stage resulting from continuous supply of nutrients

e : **Fruit firmness (g/mlm$^{2)}$.**

Results in Table (13) indicated that, fruit firmness was decreased with the progress in time with high values for the inoculation plus 75% NPK (I2) or biostimulation by the two biostimulator treatments (BJ+A) and with their combination (I2BJ+A) in two seasons with high significant differences . Inoculation treatments recorded 40.89 and 40.19 while non inoculated (I0) values 34.76 and 33.81 g/mlm^2, biostimulation(BJ+A) recorded 38.43 and 37.92 compared to 30.77 and 28.95 g/mlm^2 for unsprayed one (B0) and their combination showed 46.75 and 45.82 againest 32.59 and 30.91g/mlm^2 for control at the end of the period during the two seasons .

The rate of decrease in firmness with time was lower for treatments compared to untreated , these values were 42.75,45.71and 40.66% compared to 50.00 , 48.18 and 53.11% for (I0), (B0) and (I0B0) at the second season .

Table13:Influense of Biofertilization and Biostimulants Foliar Application on fruit firmness (g/mlm$^{2)}$ during shelf life at room temperature .

Treatment		firmness zero time		firmness 15 days		firmness 30 days	
		Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Main	I0	69.51 b	67.32 b	54.16 b	55.33 a	34.76 b	33.81 b
	I1	51.79 c	52.36 c	39.71 c	39.12 b	25.85 c	26.55 c
	I2	71.43 a	68.14 a	56.45 a	55.90 a	40.89 a	40.19 a
F test		**	**	**	**	**	**
L.S.D. 0.05		1.38	1.66	0.82	0.92	1.33	1.85
Sub main	B0	59.38 c	59.02 c	48.87 bc	48.12 c	30.77 c	28.95 d
	BJ	57.97d	59.06 c	48.45 c	47.32 d	31.52 c	30.66 c
	BA	68.85 b	65.17 b	50.21 b	51.44 b	34.60 b	36.54 b
	BJ+A	70.79 a	67.18 a	52.90a	53.59 a	38.43 a	37.92 a
F test		**	**	**	**	**	**
L.S.D. 0.05		0.85	3.35	1.65	0.79	0.83	0.57
Interaction							
I0B0(control)		69.52 d	67.23 d	55.74 bc	54.42 d	32.59 e	30.91 f
I0BJ		58.02 g	61.20 f	50.40 e	52.31 e	29.28 f	26.96 h
I0BA		73.89 c	69.58 c	52.61d e	56.80 c	35.95 d	37.52 d
I0BJ+A		76.59 b	71.39 bc	57.91ab	57.81 bc	41.22 b	39.85 c
I1B0		46.15 j	50.10 i	37.10 g	39.10 h	24.19 i	21.78 i
I1BJ		50.33 i	51.68 hi	39.79fg	36.66 i	25.77 h	27.76 gh
I1BA		53.71 h	52.60 h	41.40 f	38.79 h	26.10 gh	28.56 g
I1BJ+A		57.00 g	55.08 g	40.54 f	41.94 g	27.32 g	28.09 g
I2B0		62.48 f	59.74 f	53.76 cd	50.84 f	35.52 d	34.17 e
I2BJ		65.58 e	64.30 cde	55.16 bcd	53.00 e	39.52 c	37.25 d
I2BA		78.90 a	73.42 ab	56.63 b	58.73 b	41.76 b	43.53 b
I2BJ+A		78.78 a	75.11 a	60.26 a	61.02 a	46.75 a	45.82 a
F test		**	*	**	**	**	**
L.S.D. 0.05		1.47	5.8	2.85	1.37	1.43	0.99

The data in a column followed by the same symbol are not significant at p= 0.05

C: control, fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum*.

GA3 reduced pectin methyl esterase activity (Chen and Zhang, 1988 and Zaghloul , 2004), these findings could axplain the influence on slowing the fruit respiration rate and slowed the rate of rind softening during storage . These results are in harmony with (Farage,2001 and Zaghloul, 2004))on navel orange, that ,GA3 sprays reduced the increase respiration of fruit rind and retarding the progress of peel senescence during storage . The applications of PGPR regulate of fruit ethylene production and fruit senescence .

f : Total Chlorophyll (mg 100g^{-1} fresh weight).

Data of Table (14) illustrated the values of total chlorophyll for the inoculation and or without spraying with Jisemar and *Azospirillum* .Results showed that, there was a gradually decrease in total chlorophyll with advance in time for the inoculation treatment, inoculation with 75% NPK had the highest values of total chlorophyll than non inoculated, which recorded 26.03

and 32.86 compared to 22.90 and 24.76 mg/100 gm fresh weight in the last treatment with the highest significant in the two seasons .

Table14:Influense of Biofertilization and Biostimulants FoliarApplication on total Chlorophyll (mg 100g^{-1})fresh weight during shelf life at room temperature .

Treatment		total chlorophyll zero time		total chlorophyll 15 days		total chlorophyll 30 days	
		Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Main	I0	32.96	37.96 b	25.48 b	27.19 b	22.90 b	24.67 b
	I1	37.31	36.42 c	23.34 c	24.87 c	18.34 c	19.26 c
	I2	38.17	45.89 a	27.68 a	32.92 a	26.03 a	32.86 a
F test		ns	**	**	**	**	**
L.S.D. 0.05		--	0.39	0.74	0.89	0.11	2.46
Sub main	B0	33.51	40.31 a	24.27c	27.10 b	20.10 c	23.31 b
	BJ	33.82	39.60 b	25.93 b	27.71 b	22.80 b	26.23 a
	BA	34.14	39.87 b	25.64 b	28.92 a	22.75 b	25.45ab
	BJ+A	42.75	40.58 a	26.62 a	29.59 a	24.05 a	27.40 a
F test		ns	**	**	**	**	*
L.S.D. 0.05		--	0.33	0.52	0.67	0.44	2.38
Interaction							
I0B0(control)		32.51	38.18 c	24.81 d	26.80 ef	19.05 e	21.39
I0BJ		32.37	37.41 f	26.19 c	26.21 fg	23.82 d	25.33
I0BA		32.92	38.02 c	25.02 d	27.77 de	23.43 d	25.52
I0BJ+A		32.95	38.23 c	27.32 b	27.97 d	25.31 bc	26.46
I1B0		30.52	37.18 de	21.67g	24.95 hi	16.41 f	19.26
I1BJ		31.16	35.82 f	23.74 ef	24.08 i	18.77 e	19.83
I1BA		31.20	35.97 f	23.48 f	25.25 gh	19.06 e	16.91
I1BJ+A		31.44	36.70 e	24.47de	25.21 ghi	19.11 e	21.03
I2B0		37.52	45.58 b	26.34 c	29.54 c	24.85 c	29.27
I2BJ		37.94	45.56 b	27.87 ab	32.83 b	25.81 b	33.52
I2BA		38.30	45.63 b	28.43 a	33.73 b	25.75 b	33.92
I2BJ+A		38.92	46.80 a	28.08 ab	35.58 a	27.72 a	34.72
F test		n.s	**	**	**	**	n.s
L.S.D. 0.05		--	0.58	0.9	1.16	0.76	--

The data in a column followed by the same symbol are not significant at p= 0.05

C: control, fertilized with 100% of recommended NPK.
I0 : not inoculated with bio- fertilizers
I1: inoculated with 50% recommended NPK.
I2: inoculated with 75% recommended NPK.
B0: not sprayed by bio-stimulants.
BJ: sprayed with Jisemar.
BA: sprayed with *Azospirillum* sp.
BJ+A: sprayed with Jisemar plus *Azospirillum*

This trend was found with the spraying treatments , thus the mixture of spraying with the two biostimulators indicated a higher level of total chlorophyll (24.05 and 27.40 mg/100gm fresh weight) than the other sprayed treatments and unsprayed one having the values (20.10 and 23.31 mg/100gm fresh weight) at the end period with high significant differences.

The highest total chlorophyll value was observed with combination of inoculation + 75% NPK spray with Jisemar plus *Azospirillum* sp. (27.72 and 34.72 mg/100gm) against the lowest value(16.41mg/100gm) of inoculation with50% NPK (I1B0) with a high significant difference at the first season , results nearly followed the same trend in the second season but with no

significant difference between all treatments at the end of shelf life period at room temperature .

The previous results are in agreement with those of (Farage ,2001 and Zaghloul, 2004) who noticed that, GA3 treatments retarding fruit rind colour development and led to greener fruits during storage , this was be found associated the lowest level of ABA , with a higher significant of chlorophyll (a) content in the flavedo and retarding the progress of peel senescence after storage .

CONCLUSION

The inoculation with the mixed inoculum contained nitrogen fixer, phosphate and potassium dissolving bacteria notably increased productivity of orange trees especially, fruit set, fruit number and fruit yield. On the other hand, quality parameters like chlorophyll, firmness, vitamin C content and SSC% were increased . Likewise, spray with Jisemar (commercial biostimulants), *Azospirillum* sp. and the their mixture enhanced fruit number, fruit yield and the other yield parameters as well as improved quality of fruits as chlorophyll, firmness , vitamin C content and SSC% .

Thus, it is recommended that , inoculation of orange trees with the mixture inoculum plus adding 75% only of recommended NPK with spraying with liquid culture of *Azospirillum* sp. surpassed Jisemar in this respect for obtaining high productivity, improved fruit quality , retarded the peel senescence , decline rots infection and enhancing fruit pathogens control and good quality of most marketing characteristics .

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التأثيرات المشتركة للتسميد الحيوى و الرش بمحفزات النموالحيوية على إنتاجية وجودة والقدرة التسويقيه لثمار البرتقال أبو سره

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أجريت تجربتين حقليتين بمنطقة برمبال - مطوبس - محافظة كفر الشيخ خلال عامى ٢٠١٣-٢٠١٤ و ٢٠١٤- ٢٠١٥ بغرض دراسة تأثير التسميد الحيوي بمخلوط من ميكروبات الأسمدة الحيوية (ازوسبيريلم – باسيلس ميجاثيرم – و باسيلس سيركيولنس) و/ أو الرش بمحفزات النمو الحيوية {المركب التجاري (الجيسيمار) و الأزوسبيريلم } علي اشجار البرتقال صنف أبوسرة عمر عشرون عاما والمطعوم على أصل النارنج لمعرفة تأثير ذلك على بعض الصفات الإنتاجية لأشجار البرتقال وهي نسبة عقد الثمار ونسبة الثمار المتساقطة وإنتاجية الشجرة من الثمار (كجم / شجرة) و عدد الثمار / شجرة ووزن الثمرة (جم) بالإضافة إلى بعض الصفات النوعية مثل درجة صلابة الثمار و درجة الحموضة ومحتوي الثمرة من فيتامين ج ونسبة المواد الصلبة الذائبة الكلية فى العصير ونسبة المواد الصلبة / الحموضة ومحتوى القشرة من الكلوروفيل الكلي وكلوروفيل ا و ب و الكاروتين ومحتوي الأوراق من عناصر النيتروجين والفوسفور والبوتاسيوم .

أظهرت معاملات الرش بمحفزات النمو الحيوية (الرش بالأزوسبيريلم مع الجيسيمار أو الرش بالأزوسبيرليم بمفرده) مستويات مرتفعة فى أغلب الصفات المدروسة مقارنة بمعاملة الكنترول وكانت معظم الفروق معنوية فى حالة نسبة العقد و عدد الثمار / شجرة والمحصول كجم /شجرة و الكلوروفيل ومعظم صفات الجودة من الصلابة و المحتوى من فيتامين ج و المواد الصلبة الذائبة الكلية فيما عدا بقية الصفات المدروسة مثل نسبة التساقط والكاروتين و نسبة المواد الصلبة الذائبة الكلية / الحموضة والتي سجلت فيها هذه المعاملة إنخفاضا بالمقارنة بمعاملة الكنترول (١٠٠ % تسميد معدنى مع الرش بالماء) .

أوضحت الدراسة أن أفضل النتائج كانت مع معاملات التسميد الحيوى مضافا إلي ٧٥% تسميد معدنى مع الرش بمحفزات النمو الحيوية (الآزوسبيريلم مع الجيسيمارأو الآزوسبيريلم بمفرده) . وكانت المعاملة الأولى هى الأفضل حيث أعطت أعلى الإنتاجية /شجرة وحسنت من جودة الثمار ومعظم الصفات التسويقية على درجة حرارة الغرفة مقارنة بالكنترول والمعاملات الأخرى نتيجة لتأثيرها في إستمرارية إمداد النبات بالعناصر و الموا د الغذائية وزيادة مقاومة الثمار للمسببات المرضية الميكروبية وتأخير شيخوخة قشرة الثمار مما يزيد من القدرة التسويقية وإطالة فترة حياة الثمار.