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The Beneficial Effect of Minimizing Mineral Nitrogen Fertilization on Washington Navel Orange Trees by Using Organic and Biofertilizers

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Abstract: Minimizing mineral nitrogen fertilization through using 50% mineral N+50% organic N with *Saccharomyces cerevisae* and/or *Azospirillum lipoferum* as biofertilizer sources were tested on fifteen years old Washington navel orange trees budded on Volkamer lemon rootstock grown on sandy soil under drip irrigation system. Leaf mineral content, yield and fruit quality especially fruit juice nitrate and nitrite contents comparing with the traditional nitrogen fertilization (100% mineral nitrogen) were investigated. The obtained results showed that fertilizing trees with mineral and organic N sources accompanied with both biofertilizers significantly increased yield as number of fruits and weight kg/tree than the other treatments. Moreover, treatments included biofertilizers improved average fruit weight, vitamin C content and peel thickness than that without adding biofertilizers, while TSS and juice acidity were not affected. Nitrate and nitrite in fruit juice were significantly reduced by different treatments especially with adding biofertilizers. So, it seems that yield and fruit quality of Washington navel orange trees could be greatly improved through fertilizing trees with 50% nitrogen as mineral form +50% as organic form with *Saccharomyces cerevisae* + *Azospirillum lipoferum* as biofertilizer source.

Key words: Washington navel orange · organic fertilizer · biofertilizer · yield · fruit quality · nitrate and nitrite content

INTRODUCTION

Citrus is the first fruit crop in Egypt. Washington navel orange cultivar has a great importance either for the local market or export needs. Economically, it ranks the top among orange cvs., since it occupies 124271 feddan (one feddan = 4200 m^2) with fruiting area reached 110050 feddan, producing 1050462 tons according to Ministry of Agriculture and Land Reclamation statistics, 2004. This means that the yield is still low and attained about 9.55 tons per feddan. Increasing productivity and improving fruit quality are main targets of many specialists. There is a general agreement that several factors affect productivity and fruit quality of orange trees. One of the important factors plays a vital role in this concern is nitrogen fertilization which considered as agent of accumulative harmful residues like nitrate and nitrite in fruit juice [1].

Thus, a great attention is focused on minimizing the intensive amounts of mineral nitrogen fertilization especially under sandy soils which are naturally poor either in nutrient elements or organic matter through using alternative organic N fertilization as well as using biofertilizers which had illustrated greater nutrient use efficiencies of crops and in particular fruit crops when such inoculates were added to either organic matter or soil [2].

Accordingly, the present investigation was planned and conducted to evaluate the effect of combined application of mineral N and Farmyard manure (FYM) as organic N fertilizer with or without *Saccharomyces cerevisae* and *Azospirillum lipoferum* as two sources of biofertilizes on leaf mineral content, yield and fruit quality as well as nitrate and nitrite content in fruit juice of Washington navel orange trees grown under sandy soil conditions.

MATERIALS AND METHODS

The present investigation was conducted during two successive seasons 2003 and 2004 in a private citrus orchard located at El-Sadat district, Menofiya Governorate, Egypt on 15 years old Washington navel orange trees. The trees were budded on Volkamer lemon

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Table 1: Analytical properties of the soil at the trail location

a-Mechanical analysis		Sand (%)		Silt (%)			Clay (%)			Texture	
		90		5			5			Sandy	
b-Chemical analysis:				Soluble cations (meq l^{-1})				Soluble anions (meq l^{-1})			
pH (1:2.5)	EC d sm^{-1} (1:1)	$CaCO_3$ (%)	N ppm	K	Na	Ca	Mg	Cl	SO_4	HCO_3	CO_3
8.2	1.5	5.5	traces	0.57	9.18	2.65	2.40	5.3	5.65	3.85	---

Table 2: Some physical and chemical characteristics of tested Farmyard manure

Parameter	Values
Cubic meter weight (kg)	650.00
Moisture (%)	35.00
Organic matter	23.60
Organic carbon	21.40
pH (1:10)	8.70
EC (mmohs cm^{-1})	5.70
C/N ratio	24.00
Total N (%)	0.89
Total P (%)	0.32
Total K (%)	0.92
Total Ca (%)	1.82
Total Mg (%)	0.96
Total Fe (ppm)	1500.00
Total Mn (ppm)	420.00
Total Zn (ppm)	53.00

(*C. volkameriana*) rootstock and planted at 5X5 meters apart under drip irrigation system. The texture of the soil is sandy. The results of soil and farmyard manure analysis according to Wilde *et al.* [3] are given in Table 1 and 2. The selected trees were nearly uniform in vigor as possible.

The experiment included five treatments as follows:

- 100% MNF (control).
- 50% MNF+50% ONF
- 50% MNF+50% ONF+BF_1.
- 50% MNF+50% ONF+BF_2.
- 50% MNF+50% ONF+BF_1+BF_2.
- MNF = Mineral N fertilization.
- ONF = Organic N fertilization.
- BF_1 = Yeast (*Saccharomyces cerevisae*).
- BF_2 = *Azospirillum lipoferum.*

The control trees received the common amount of nitrogen fertilizer (1000 gm N/tree/year) as ammonium sulphate (20.5% N).

Organic N fertilizer was added as Farmyard manure (FYM) (0.89% N) at rate of 56 kg/tree.

Yeast *(Saccharomyces cerevisae)* (BF_1) and *Azospirillum lipoferum* (BF_2) isolated and identified by Gomaa [4] were grown to the late exponential phase in a sterilized medium prepared in Microbiology Department, National Research Centre. The resultant cultures contained 6.2×105 cell ml^{-1} for each biofertilizer (BF_1 and BF_2) which added at rate of one liter of each per tree. Organic and biofertlizers were side dressed in a band of 100 cm wide on both sides of the tree at one meter apart from the tree trunk in the direction of tree row and mixed with the surface of 20 cm of soil in late January of each season while mineral N fertilization was added at three equal doses on March, May and August. Each treatment was replicated tree times on one tree plots and the randomized complete block design was arranged.

The chosen trees received the normal fertilization program including the addition of 100 Kg per feddan calcium super phosphate (15.5% P_2O_5) in late January and 600 g potassium sulphate (48-52% K_2O)/tree/year in March and August. The other horticultural practices were the same for all trees under investigation.

To determine leaf mineral content, about forty leaves were taken in late August in each season from tagged non-fruiting and non-flushing spring growth cycle according to Jones and Embleton [5]. Leaf samples were washed with tap water, then with distilled water and dried at 70°C finally ground and digested. The digested solution was used to determine N, P and K content as percentage on dry weight according to Cottenie *et al.* [6].

At the harvesting time (late December of each season), yield per tree was determined as number and weight of fruits (kg)/tree.

For fruit quality determinations, samples of ten fruits were taken from each replicate to determine the physical and chemical properties as the methods described in A.O.A.C. [7].

A sample of 10 ml of fruit juice was taken from each replicate to determine nitrogen, phosphorus, potassium, nitrate and nitrite content in fruit juice. N, P and K% were determined using the same methods described in leaf mineral content, while NO_3^- and NO_2^- content were determined according to the methods outlined by Sen and Donaldson [8].

The data were subjected to analysis of variance and Duncan's multiple range test was used to differentiate means [9].

RESULTS AND DISCUSSION

Leaf mineral content: Data in Table 3 showed that leaf mineral content was significantly affected by different treatments in both seasons. In this concern, fertilizing trees with 100% mineral N (control) gave the highest leaf N content compared with the other treatments followed descending order by 50% mineral N+50% organic N+BF_1 (treatment 3) in the first season, while in the second season the highest N values were obtained by treatments [3, 4]. On the other hand, the lowest leaf N content was recorded by treatments [2, 4] in the first and second seasons, respectively. It is observed that, treatments included biofertilizers applications gave higher N content in the leaves compared with the analogous treatments without adding biofertilizers. This effect was pronounced with *Saccharomyces cerevisae* rather than *Azospirillum lipoferum*.

Leaf phosphorus content was not affected significantly by different treatments in the two seasons and no particular trend was noticed in this concern. In other word, fertilizing Washington navel orange trees with nitrogen as 100% mineral N or 50% mineral N+50% organic N with or without biofertilizers did not affect significantly leaf P content.

Potassium content in the leaf was significantly affected in the two studied seasons. Results cleared that fertilizing trees with 100% mineral N (control) increased K content in the leaves compared with the other treatments in both seasons. However, treatments included biofertilizers tended to decrease K leaf percentage than that without them. This trend was noticed in both seasons with treatments included BF_1 (treatment 3) alone or both BF_1+BF_2 together (treatment 5), while that included BF_2 solely (treatment 4) did not show a constant trend comparing without adding biofertilizers (treatment 2). In this concern, low K leaf in treatments [3, 5] could be attributing to the depletion of potassium from the leaves into fruit due to high yields obtained by such treatments.

The obtained results are in harmony with the findings by Fayed [10] on apple who reported that chemical fertilizer gave the highest leaf N and K contents compared with organic fertilization with or without biofertilizers. On the other hand, the results obtained due to biofertilzer applications are in agree with those obtained by Fayed [10], Hassan and Abou Raya [11] and El-Naggar [12] who mentioned that application of biofertilizers was favorable in improving nutritional status of the trees in different fruit crops comparing with the organic fertilization alone.

Yield

Number of fruits/tree: The results in Table 4 cleared that number of fruits/tree was significantly affected in the

Table 3: Leaf mineral content of washington navel orange trees as affected by mineral and organic nitrogen fertilization with some biofertilizers during 2004 and 2005 seasons

	N%		P%		K%	
Treatments	2004	2005	2004	2005	2004	2005
100%MNF (control)	1.50a	1.47a	0.13	0.14	0.99a	0.97a
50%MNF+50%ONF	1.33b	1.30c	0.13	0.12	0.91a	0.44c
50%MNF+50%ONF+BF_1	1.40b	1.50a	0.12	0.14	0.40c	0.37d
50%MNF+50%ONF+BF_2	1.20c	1.50a	0.11	0.16	0.44c	0.87b
50%MNF+50%ONF+BF_1+BF_2	1.23c	1.40b	0.12	0.11	0.63b	0.43c
Significance at 5% level	S	S	N.S	N.S	S	S

Means having the same letters within a column are not significantly different at 5% level

Table 4: No. fruits/tree, yield weight/tree and average fruit weight of Washington navel orange trees as affected by mineral and organic nitrogen fertilization with some biofertilizers during 2004 and 2005 seasons

	No. fruits/tree		Yield (kg/tree)		Average fruit weight (g)	
Treatments	2004	2005	2004	2005	2004	2005
100%MNF (control)	173	175c	78.1	40.6b	281a	276a
50%MNF+50%ONF	226	260ab	58.8	53.3b	260ab	205b
50%MNF+50%ONF+BF_1	300	213bc	67.3	56.0b	236b	263a
50%MNF+50%ONF+BF_2	150	205c	41.8	57.0b	281a	281a
50%MNF+50%ONF+BF_1+BF_2	280	303a	75.3	78.7a	278a	260a
Significance at 5% level	N.S	S	N.S	S	S	S

Means having the same letters within a column are not significantly different at 5% level

second season only. The highest value was obtained when trees fertilized with mineral + organic fertilizers + both biofertilizers (treatment 5). Such treatment significantly increased fruit numbers over all other treatments except that treated with mineral and organic fertilizers without adding biofertilizers (treatment 2), since the increment lacked significance.

The positive effect of most treatments on fruit numbers/tree could be attributed to the beneficial effect of the tested materials (organic manure) on improving fruit set. In this respect, El-Kobbia [13], Mostafa [14] and Helail [15] indicated that fertilizing Washington navel orange trees by different organic fertilizing doses caused a significant increase in the fruit set percentage. However, the beneficial effects of biofetilizers are in harmony with the findings of Hassan and Abu Raya [11], Monsour [16] and Fayed [17] on apple and Risk-Alla [18] on grape.

Yield (kg)/tree: Data in Table 4 revealed that yield (kg)/tree followed more or less the same trend obtained in number of fruits/tree. Since yield weight/tree was significantly affected in the second season. In this respect, treatment number [5] gave the highest significant increment than the other treatments. Although the other treatments increased yield weight than the control, no significance differences were detected between them.

From the above results, it is interest to notice that adding the two sources of biofertilizers together (treatment 5) proved to be the superior treatment exerted high positive effect on tree fruiting than adding each of them solely. In other word, adding *Saccharomyces cerevisae* induced higher positive effect on tree fruiting than did *Azospirillum lipoferum* and a synergistic effect was obtained due to adding the two tested biofertilizers together than applying each of them alone in respect with yield (kg/tree). The obtained results are in line with Hassan and Abu Raya [11] and Fayed [17] on apple.

Fruit quality: Data in Table 4 cleared that fruit weight (g) was significantly affected by different treatments in the first season only. In general, the higher values were recorded with mineral+organic N+BF_2 (treatment 4) and 100% mineral N (control). This was true in both seasons.

Data in Table 5 showed that peel thickness and juice weight were significantly affected in the second season only. However, fertilizing with 50% mineral N+50% organic N (treatment 2) gave the highest peel thickness as well as the lowest juice weight. This was true in the second season only. It is noticed that, treatments included biofertilizers [3-5] tended to decrease peel thickness and increase juice content compared with those without adding them (treatment 2).

As for, juice percentage, Total Soluble Solids (TSS) and acidity percentage, they were not significantly affected and no constant trend was detected in both studied seasons although all treatments tended to slightly increase TSS value compared with the control.

Regarding vitamin C, results showed significant differences in the second season only, since adding biofertilizers together with mineral+organic fertilizers raised vitamin C content in the fruit juice than other treatments.

The results of biofertilizers applications go in line with the findings by, Mostafa [14] on Washington navel orange, Akl *et al*., [19] on grapevines and Salama [20] on Balady mandarin.

Juice mineral content: Results in Table 6 show that N, P and K content in fruit juice were affected by different treatments in the two seasons.

As for juice N content, it is clear that fertilizing trees with 100% mineral N (control treatment) gave the highest N values in fruit juice than the other treatments. Generally, treatments included any of the two biofertilizers alone or

Table 5: Physical and chemical properties of Washington navel orange trees as affected by mineral and organic nitrogen fertilization with some biofertilizers during 2004 and 2005 seasons

	Peel thickness (cm)		Juice weight (g)		Juice (%)		TSS (%)		Acidity (%)		Vitamin C mg/100 ml	
Treatments	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
100%MNF (control)	0.55	0.40b	111	100a	52.9	36.2	12.1	12.3	0.68	0.77	44.6	45.7bc
50%MNF+50%ONF	0.50	0.53a	108	77b	41.5	37.6	12.6	13.7	0.73	0.75	47.0	44.0c
50%MNF+50%ONF+BF_1	0.47	0.43ab	121	115a	42.3	43.7	13.1	12.9	0.77	0.76	52.0	51.7abc
50%MNF+50%ONF+BF_2	0.40	0.47ab	117	121a	41.6	43.1	12.3	12.3	0.78	0.82	43.7	54.7ab
50%MNF+50%ONF+BF_1+BF_2	0.43	0.50ab	94	103a	33.8	39.6	12.3	13.0	0.69	0.77	44.0	56.6a
Significance at 5% level	N.S	S	N.S	S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	S

Means having the same letters within a column are not significantly different at 5% level

Table 6: Nitrogen, phosphorus, potassium, nitrate and nitrite in fruit juice of Washington navel orange trees as affected by mineral and organic nitrogen fertilization with some biofertilizers during 2004 and 2005 seasons

	N (ppm)		P (ppm)		K (ppm)		NO_3 (ppm)		NO_2 (ppm)	
Treatments	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
100%MNF (control)	1297a	1624a	237ab	191c	1264	1078ab	46.5a	47.2a	2.50a	2.57a
50%MNF+50%ONF	574c	895b	156b	275a	1487	781b	35.8b	20.7c	2.03b	2.00b
50%MNF+50%ONF+BF_1	872bc	798b	208ab	223bc	1140	1415ab	31.6c	26.2b	1.86b	1.67c
50%MNF+50%ONF+BF_2	1002ab	875b	326a	265ab	1192	1672a	30.5c	28.7b	1.90b	1.30d
50%MNF+50%ONF+BF_1+BF_2	848bc	874b	250ab	220bc	1194	1199ab	33.4bc	26.4b	1.66b	1.67c
Significance at 5% level	S	S	S	S	N.S	S	S	S	S	S

Means having the same letters within a column are not significantly different at 5% level

together gave more or less similar juice N values in both seasons. Such treatments tended to increase juice N content than the analogous treatment without adding biofertilizer in the second season only.

Regarding juice P content, no constant trend in both seasons due to different treatments was noticed. However, no differences were detected between treatments in both studied seasons.

Concerning juice K content, it was significantly affected by treatments in the second season only and it was noticed that adding any of biofertilizers (treatments 3, 4 and 5) tended to increase K content in fruit juice compared without adding them (treatment 2). On the other hand, results cleared that K value recorded by the control treatment did not significantly differ than those obtained by the other treatments.

Nitrate and nitrite content in fruit juice: As shown in Table 6 results revealed that nitrate and nitrite contents in fruit juice were significantly decreased by different treatments in the two studied seasons comparing with 100% mineral N (control). This means that replacing nitrogen fertilization partially through using only 50% N at mineral form and 50% N at organic form (FYM) instead of 100% mineral N form had a beneficial effect on reducing nitrate and nitrite in fruit juice. In this respect, Ibraheem [21] mentioned that mineral nitrogen fertilization easily forms nitrate, whereas organic fertilizers slowly form nitrate.

On the other hand, a particular trend was noticed that, treatments included biofertilizers reduced nitrite content than the analogous treatment without adding them. This was true in both seasons. However, nitrate content in fruit juice did not show a constant trend in the two seasons due to adding biofertizers compared without adding them. This means that the beneficial effect of adding biofertilizers was mainly on reducing nitrite in fruit juice rather than nitrate.

The beneficial effect of organic and biofertilizer on reducing nitrate and nitrite is supported by the results reported by Rizk-Alla [18] and Farag [22] on grapevines.

From the abovementioned results, it could be concluded that yield as number or weight (kg)/tree and fruit quality espacially in respect with reducing nitrate and nitrite in fruit juice of Washington navel orange trees were greatly improved due to replacing the use of nitrogen fertilization (100% mineral N form) partially through using 50% mineral N+50% organic N (Farmyard manure) accompanied with adding biofertilizers i.e. *Saccharomyces cerevisae* and *Azospirillum lipoferum*.

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