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Effect of Bio and Nitrogen Fertilizers on Growth Aspect and Leaf Content of Mineral and Pigment of Washington Navel Orange Trees

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ABSTRACT

The field experiment was carried out during 2020/ 2021and 2021/ 2022 seasons on Washington navel orange trees and grown in a private citrus orchard located at Wady Al-Molak, district Al-Ismailia Governorate, Egypt. Used randomized complete block design with three replicates. The results showed that significant between treatments for all studies characters.

The treatment (5 L. Bio. + 70 kg. N/fed) gave the highest leaf length, leaf width and leaf area. The highest values of N, P and K % obtained from the trees fertilized by resulted from the trees fertilized by (5 L. Bio. + 100 kg. N/fed), treatment in first and second seasons, respectively.

In addition, the same treatments gained the highest values of Fe (ppm), Mn (ppm), Zn (ppm , chlorophyll A (mg/100g), chlorophyll B (mg/100g), total chlorophyll (mg/100g) and carotene (mg/100g), in the first and second seasons.

Conclusively, from these results it could be a combination of chemical and bio fertilizer is not only beneficial in improving the properties and environment of soils, but also promotes growth, macro and micro nutrients, chlorophyll contents in orange. Here, our work confirmed that was helpful for citrus growth.

Key words: Washington, Navel orange, bio-fertilizers, Growth aspects, chlorophyll, Mineral content**.**

INTRODUCTION

Citrus fruits are one of the major fruit crops with dietary significance, worldwide accessibility, and recognition (Abouzari and Nezhad 2016; FAO 2021; Rivera *et al.* 2022). Citrus tree nutritional status is a major factor affects growth development (Esteves *et al*. 2021). The use of these materials encourages growth and flowering as well as reflected positively on tree productivity. There are various benefits of bio-fertilizers as they increase supplement of various nutrients, eco Friendly, cost-effective, improve and help plant to tolerate stress conditions (Ortaş and Ustuner 2014). Using organic and bio fertilizers considered a key tool for sustainable horticulture crop production system, it offers improving soil health, increasing crops, and sustains natural resources (Hazarika and

Aheibam, 2019).. Aseri *et al.* (2008) found that the use of bio-fertilizers significantly improved yield and fruit quality of pomegranate in India. In addition, Bio-fertilization considered a positive alternative to chemical fertilization lost the enhancing enhance citrus yield and fruit quality, because it is safe for human, animal, and environment. Using bio-fertilizers in organic food production accompanied with the reduction of environmental pollution. Application of mineral fertilizers with organic or bio-fertilizers proved to be highly effective in improving nutritional status, fruiting and fruit quality of various fruit trees (Abd El-Migeed *et al.* 2006; Hegazi *et al.* 2007). Moreover, the yield of citrus is largely determine by the N concentration of the leaves (Fan *et al.* 2020).

Therefore, the present study aimed to investigation is application of some bio and mineral nutritive compounds on vegetative growth of fruitful Washington navel orange trees.

MATIRIALS AND METHODS

The field experiment was carried out during 2020/ 2021and 2021/ 2022 seasons on Washington navel orange trees and grown in a private citrus orchard located at Wady Al-Molak, district Al-Ismailia Governorate, Egypt. Trees were planted at 5×5 m apart with a total number of 168 trees/Feddan, the trees 10 years old in sandy soil under drip irrigation system.

Experimental design and tested treatments:

The field experimental was randomized complete block design the treatments were ((Cont. 0 Bio.+100 kg N/fed), (3 L. Bio. + 100 kg N/fed), (3 L. Bio. + 90 kg N/fed), (3 L. Bio. + 80 kg N/fed), (3 L. Bio. + 70 kg N/fed), (4 L. Bio. + 100 kg N/fed), (4 L. Bio. + 90 kg N/fed), (4 L. Bio. + 80 fed.), (4 L. Bio. + 70 kg N/fed), (5 L. Bio. + 100 kg N/fed), (5 L. Bio. + 90 kg N/fed), (5 L. Bio. + 80 kg N/fed), (5 L. Bio. + 70 kg N/fed)). With three replicates was used to implement the field experiment, whereas a single tree represented each replicate. Consequently, thirty-nine healthy fruitful Washington navel orange trees were carefully selected, as being healthy, disease free and in the on-year state. physical and chemical characteristics of soil at the experimental site shown in Table 1.

Nitrogen and bio fertilizers treatments:

A- *Nitrogen fertilizers:*

Used four different treatments of nitrogen fertilizers were as follows:

1-100 unit N/fed., as follows: During the first week of March (early spring) of both seasons, trees received 100 kg/feddan of calcium nitrate. Another 50-liter/feddan of nitric acid added set fertilization was repeated again in

Table 1: Physical and chemical properties of the soil at the experimental site.

Constituents	Values	Constituents	Values
Particles size distributions %		Soluble anions (mmolc L^{-1})	
Sand	94.2	Co	-
Silt	2.4	Hco3	0.075
Clay	3.4	CI	0.43
Texture grade	Sandy soil	So4	7.065
Chemical properties		Extractable macronutrients (ppm)	
Ph	8.38	N	60
E.C	0.757	P	2.5
$CaCo_3$	1.69	K	10.3
Soluble captions (mmolc L^{-1})		DTBA extractable micronutrients (ppm)	
Ca	0.14	Fe	0.22
Mg	0.11	Mn	0.24
Na	7.11	Zn	0.2
K	0.21	Cu	0.22

both seasons by adding 230 kg/feddan of ammonium nitrate, at a rate of 595 g N/tree.

2- 90 unit N/ fed., as follows: During the first week of March (early spring) of both seasons, trees received 100 kg/feddan of calcium nitrate. Another 50-liter/feddan of nitric acid added after fruit set fertilization was repeated again of both seasons by adding 200 kg/feddan of ammonium nitrate. At a rate of 535 g N /tree.

3- 80 unit N/fed., as follows: During the first week of March (early spring) of both seasons, trees received 100 kg/feddan of calcium nitrate. Another 50-liter/feddan of nitric acid added after fruit set Fertilization was repeated again of both seasons by adding 170 kg/feddan of ammonium nitrate. At a rate of 476 g. N/tree.

4- 70 unit N/fed., as follows: During the first week of March (early spring) of both seasons, trees received 100 kg/feddan calcium nitrate. Another 50-liter/feddan of nitric acid added after fruit set Fertilization was repeated again of both seasons by adding 140 kg/feddan ammonium nitrate. At a rate of 416 g N/tree.

The treatments were carried out with nitrogen fertilizers in three doses as follows: Calcium nitrate 15.5% during the first week of March (early spring) of both seasons, trees calcium nitrate. Another of nitric acid added in May after fruit set and fertilization was repeated again in July of both seasons by adding ammonium nitrate.

Bio-fertilizers used four treatments of mega plus fertilizer as follows:-

1- Control at zero/tree.
2- At 93cm^3/tree.
3- At 125 cm^3/tree.
4- At 156 cm^3/tree.

They were add on three doses as follows (the first dose before the flowering, the second dose after fruit set and third dose after 30 days fruit set).

Data recorded as follows:

1. Leaf area (cm^2): Six months after the end of spring growth, forty leaves were collected in September during both seasons. Leaves collection started at the fourth leaf on the branch from all four directions of the tree, and leaf area was calculated according to Chou (1966) as follows equation:

   Leaf area = 2/3 leaf length × leaf width.
2. Leaf length (cm): It was calculated using the average length of five leaves after coefficients using centimeters.
3. Leaf width (cm): It was calculated using the average width of five leaves after coefficients using centimeters.
4. Chlorophyll content: By the end of spring growth cycle in September, fresh leaf samples were collected from all four directions of each tree. Leaf collection started at the fourth leaf from the top of non-bearing shoots only. Leaves were washed three times with tap water, and then washed using distilled water to measure total chlorophyll, and both chlorophyll (a) and (b). Values were calculated as $\mu g/cm^2$ according to Moran (1982).

5- Determination of NPK and micronutrients in leaves*:* To determine leaf mineral content, forty leaves were collected in September from non-fruiting shoots that were previously tagged during the spring growth cycle (Jones and Embleton, 1960).

Leaves were washed with tap water followed by distilled water, and leaf fresh weight was calculated. Leaves were oven dried at 70°C until constant weight. Leaf dry weight was calculated, and then dry leaves were finely ground. Samples of 0.1 g from each leaf sample were wet digested using a 5:1 mixture of concentrated H_2SO_4 + perchloric acid ($HCLO_4$) to determine total nitrogen using the micro-kjeldahl method. Nitrogen, phosphorus, and potassium percentage was determined using the same methods used in soil samples. The amount of micronutrients, such as Mn, Zn, and Fe were determined according to the methods described by (Cottenie *et al.*, 1982).

Statistical analysis:

Data were analyzed using M-Stat program in a randomized complete block design (CBD) as described by Snedecor and Cochran (1980).

Means were compared using least significance difference (LSD) at $P \leq 0.05$ (Little and Hills, 1972).

RESULTS AND DISCUSSION

1. *Leaf length, leaf width and leaf area:*

The results presented in Table (2) show the leaf length, leaf width and leaf area were significantly affected by the tested fertilization treatments during 2021and 2022 seasons on Washington navel orange trees.

As such, the treatment (5 L. Bio. + 70 kg. N/fed), gave the highest leaf length in the first and second seasons. Whereas the treatment (5 L. Bio. + 80 kg. N/fed) gave highest leaf width in the first and second seasons. Moreover, the treatments as (5 L. Bio. + 70 kg. N/fed., and 5 L. Bio. + 80 kg. N/fed) gave the highest value of leaf area cm^2 in the first season, but in the second season the treatments (5 L. Bio. + 70 kg. N/fed. and 5 L. Bio. + 80 kg. N/fed) were gave the highest for this treat. Whereas, the 4 L. Bio. + 80 kg. N/fed treatment was recorded the lowest value for this lasted traits in both seasons. The other tested treatments came in between in both lasted seasons.

These results are consistent with the results of Gamal and Ragab (2003) on Balady mandarins, Abd El-Naby *et al*. (2004) indicated that bio-fertilization increased leaf area in 'Washington' navel orange. The effect of different organic and bio- fertilizers on 'Washington' Navel orange growth. Fikry *et al*. (2020) on Murcott tangerine. Shukr and Al Shaheen (2021) on Citrus lemon.

2. *Leaf N, P and K contents %:*

Data presented in Table (3) revealed that the tested fertilization treatments significantly affected leaf N, P and K percentages in both seasons. The highest values of N %.

The highest values P % and the highest values K % obtained from the trees fertilized by resulted from the trees fertilized by (5 L. Bio. + 100 kg. N/fed)., treatment in first and second seasons respectively. Whereas, the Control 0 Bio.+100 kg. N/fed treatment was recorded the lowest value for this lasted traits in both seasons. The other tested treatments came in between in both lasted seasons.

These results agreed with those reported by Attia *et al*. (2002) on Balady mandarin, Abd El-Samad *et al*. (2006) on Valencia' orange, Hegazy *et al*. (2007) on olive, Fawzi *et al*. (2010) on Le-Conte" pear trees, Osman (2010) on olive, Abdel-Sattar *et al*. (2011) on Washington navel orange and mandarin, Rabeh *et al*. (2012) on mango trees, El Khayat and Abdel Rehiem, (2013) on mandarin, Haggag *et al*. (2014) on olive seedlings Manzanillo cv., Xiao *et al*. (2014) on mandarin, Ahmed *et al*. (2017) on Washington navel orange and Kinnow, El-Aidy *et al*. (2018) *on* 'Valencia' orange, Hameed *et al*. (2018) on kinnow mandarin, Ennab *et al*. (2019) on Washington navel orange.

Table 2: leaf characters as affected by bio-fertilizer and nitrogen rates on Washington navel orange trees during two 2021and 2022 seasons.

Characters	Leaf length (cm)		Leaf width (cm)		Leaf area (cm^2)	
Seasons	2021/22	2022/23	2021/22	2022/23	2021/22	2022/23
Treatment groups						
Cont. (0 Bio.+100 kg N/fed.)	9.6333abc	9.26a	4.9333a	3.66de	31.70a	21.63de
3 L. Bio. + 100 kg N/fed	8.8667abcde	8.30cde	5.00a	4.633b	29.7a	25.60bc
3 L. Bio. + 90 kg N/fed	9.8333ab	8.53bcd	5.033a	4.63b	32.967a	26.36bc
3 L. Bio. + 80 kg N/fed	8.80bcde	8.233de	4.833ab	4.46b	28.433ab	24.50cd
3 L. Bio. + 70 kg N/fed	8.30de	7.866de	3.733c	4.06c	20.667cd	21.33de
4 L. Bio. + 100 kg N/fed	8.5333cde	8.30cde	4.366b	4.70b	24.8bc	25.96bc
4 L. Bio. + 90 kg N/fed	9.30abcd	9.33a	4.766ab	5.36a	29.4ab	33.26a
4 L. Bio. + 80 kg N/fed	8.0333e	7.66e	3.466c	3.33e	18.6d	18.80e
4 L. Bio. + 70 kg N/fed	9.4667abc	9.10ab	4.966a	4.03cd	31.26a	24.43cd
5 L. Bio. + 100 kg N/fed	9.20abcd	9.26a	5.10a	5.30a	31.23a	32.66a
5 L. Bio. + 90 kg N/fed	9.2667abcd	8.96abc	5.033a	4.83b	31.06a	28.86b
5 L. Bio. + 80 kg N/fed	9.5667abc	9.63a	5.166a	5.53a	32.96a	34.93a
5 L. Bio. + 70 kg N/fed	9.9667a	9.46a	4.80ab	5.36a	31.83a	34.40a
LSD at 0.05%	1.1508	0.6681	0.4943	0.3994	5.3191	3.3345

Similar letter(s) in the same column are non-significant statistically at 0.05 level of probability.

Table 3: Leaf NPK contents % as affected by bio-fertilizer and nitrogen rates on Washington navel orange trees during two seasons.

Characters	Nitrogen %		Phosphorus %		Potassium %	
Seasons	2021/22	2022/23	2021/22	2022/23	2021/22	2022/23
Treatment groups						
Cont. (0 Bio. +100 kg N/fed.)	1.529j	1.583j	0.311h	0.316h	1.432g	1.428j
3 L. Bio. + 100 kg N/fed	1.981c	1.994c	0.405d	0.4117d	1.888bc	1.901d
3 L. Bio. + 90 kg N/fed	1.798h	1.813fg	0.391ef	0.3950f	1.824de	1.833f
3 L. Bio. + 80 kg N/fed	1.795h	1.774hi	0.384ef	0.3903f	1.751f	1.761h
3 L. Bio. + 70 kg N/fed	1.742i	1.751i	0.373g	0.3713g	1.718f	1.726i
4 L. Bio. + 100 kg N/fed	2.013b	2.044b	0.445b	0.4487b	1.908b	1.921b
4 L. Bio. + 90 kg N/fed	1.914e	1.904d	0.415c	0.4227c	1.864bcd	1.874e
4 L. Bio. + 80 kg N/fed	1.853f	1.870de	0.391ef	0.3933f	1.815e	1.818g
4 L. Bio. + 70 kg N/fed	1.793h	1.804gh	0.383f	0.3937f	1.761f	1.761h
5 L. Bio. + 100 kg N/fed	2.071a	2.145a	0.479a	0.4830a	1.977a	1.982a
5 L. Bio. + 90 kg N/fed	1.985c	2.012bc	0.441b	0.4483b	1.904bc	1.908c
5 L. Bio. + 80 kg N/fed	1.932d	1.981c	0.410cd	0.4137d	1.860cde	1.874e
5 L. Bio. + 70 kg N/fed	1.845g	1.844ef	0.393e	0.4060e	1.869bcd	1.823g
LSD at 5%	0.0176	0.035	0.0236	0.0328	0.0476	0.0061

Similar letter(s) in the same column are non-significant statistically at 0.05 level of probability.

3. *Leaf Fe, Mn and Zn contents /(ppm):*

Data presented in Table (4) obtained that the tested fertilization treatments significantly affected leaf Fe, Mn and Zn (ppm) in both seasons. The highest values of Fe (ppm). The highest values Mn (ppm) and the highest values Zn (ppm) obtained from the trees fertilized by resulted from the trees fertilized by (5 L. Bio. + 100 kg. N/fed.,) treatment in first and second seasons. Whereas, the Control 0 Bio.+100 kg. N/fed treatment was recorded the lowest value for these lasted traits in both seasons. The other tested treatments came in between in both lasted seasons.

These results agreed with those reported by Hegazy *et al.* (2007) on olive, Abdel-Sattar *et al.* (2011) on Washington navel orange and mandarin, Ahmed et al. (2013), Ennab *et al.* (2019) and Maklad (2019)on Washington navel orange trees.

Table 4: Leaf Fe, Mn and Zn contents as affected by bio-fertilizer and nitrogen rates on Washington navel orange trees during two seasons.

Characters	Fe (ppm)		Mn (ppm)		Zn (ppm)	
Seasons	2021/22	2022/23	2021/22	2022/23	2021/22	2022/23
Treatment groups						
Cont. (0 Bio. +100 kg N/fed.)	61.62e	60.41l	31.05m	30.61m	19.84h	19.61h
3 L. Bio. + 100 kg N/fed	77.64b	76.76f	41.85h	41.72h	24.09ef	25.82de
3 L. Bio. + 90 kg N/fed	72.05c	72.20h	39.72j	39.81j	23.08fg	23.14f
3 L. Bio. + 80 kg N/fed	70.63cd	70.51j	38.14k	38.31k	22.71fg	22.84fg
3 L. Bio. + 70 kg N/fed	68.43d	68.80k	37.80l	37.20l	21.66gh	21.72g
4 L. Bio. + 100 kg N/fed	81.05a	81.12b	49.61d	49.43d	30.61b	31.08b
4 L. Bio. + 90 kg N/fed	78.18b	78.67e	45.03f	45.12f	28.54c	28.68c
4 L. Bio. + 80 kg N/fed	72.62c	73.06g	42.66g	42.82g	26.72cd	25.17e
4 L. Bio. + 70 kg N/fed	70.78cd	70.52j	40.11i	40.15i	25.14df	25.21e
5 L. Bio. + 100 kg N/fed	82.34a	85.47a	52.05a	52.17a	33.20a	32.94a
5 L. Bio. + 90 kg N/fed	81.04a	80.51c	51.47b	51.52b	30.61	30.75b
5 L. Bio. + 80 kg N/fed	79.88ab	78.89d	50.63c	50.81c	28.20c	28.28c
5 L. Bio. + 70 kg N/fed	71.61c	71.42i	47.11e	47.34e	23.57ef	26.91d
LSD at 5%	2.679	0.1432	0.0726	0.2786	1.885	1.3366

Similar letter(s) in the same column are non-significant statistically at 0.05 level of probability.

4. *Leaf chlorophyll contents*:

Data presented in Table (5) obtained that the tested fertilization treatments significantly affected leaf chlorophyll A, chlorophyll B, total chlorophyll and Carotene (mg/100g) in both seasons. The highest values of chlorophyll A (mg/100g). The highest values chlorophyll B (mg/100g), and the highest values total chlorophyll (mg/100g) moreover, the highest values of carotene (mg/100g), were obtained from the trees fertilized by resulted from the trees fertilized by (5 L. Bio. + 100 kg. N/fed.), treatment in first and second seasons.

Whereas, the (3 L. Bio. + 70 kg. N/fed), treatment was recorded the lowest values for chlorophyll A, chlorophyll B, total chlorophyll in both seasons. Meanwhile, the lowest values for carotene were recorded by the treatments 3 L. Bio. +80 kg N/fed., in first season and 3 L. Bio.+70 kg. N/fed., treatment in second season. The other tested treatments came in between in both lasted seasons.

The present results agreed with those obtained by Helail *et al*. (2003) in Washington' navel orange. Fayed, (2005) on orange trees. Mohamed and Massoud (2017) proved that biofertilizer combined with mineral fertilizer had more positive effects than both microbial inoculants solely and non-inoculated control. Azotobacter + AM at 75% or 50 % induced significant increment in chlorophyll content of orange trees cv. Washington navel orange. Hameed *et al*. (2018) on *Citrus reticulata* cv. kinnow mandarin. Ashraf and Zagzog (2019) on cv Zaghloul Shukr and Al Shaheen (2021) on leaves content of relative chlorophyll of *Citrus lemon*.

Conclusively, from these results it could be a combination of chemical and bio fertilizer is not only beneficial in improving the properties and environment of soils, but also promotes growth, macro and micro nutrients, chlorophyll contents in orange. Here, our work confirmed that was helpful for citrus growth.

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تأثيرات الأسمد الحيوية على خصائص النمو ومحتوى الاوراق من الكلوروفيل والعناصر الكبرى والصغرى في أوراق البرتقال أبو سرة

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اجريت التجربة البحثية في موسمين متتاليين ٢٠٢١/٢٠٢٠ و ٢٠٢٢/٢٠٢١ على التوالي ، على اشجار البرتقال بسره واشنطن بمزرعة بساتين خاصة بوادي الملاك محافظة الاسماعيلية. استخدم تصميم القطاعات كاملة العشوائية في ثلاث مكررات.

أظهرت النتائج الى ان:

فروق ذات دلالة احصائية معنوية بين جميع المعاملات لجميع الصفات تحت الدراسة. وأشارت النتائج الى أن استتخدام (٥ لتر من السماد الحيوي + ٧٠ كجم نيتروجين\فدان) أعطت أعلى النتائج لصفات عرض الورقة ومساحة الورقة. كما سجلت المعاملة (٥ لتر سماد حيوي+١٠٠ كجم نيتروجين\فدان) أعلى القيم لصفات (النيتروجين ، الفسفور والبوتاسيوم %)، كما أعطت نفس المعاملة أعلى القيم لعناصر الحديد و الزنك و المنجنيز و محتوى الكلوروفيل (أ، ب، و الكلوروفيل الكلي و الكاروتين).

التوصية: أشارت النتائج الى أهمية استخدام الاسمدة الحيوية في تحسين محتوى اوراق اشجار البرتقال صنف برتقال بسرة من العناصر والصبغات.