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The Effects of some Micronutrients and Putrescine on Growth and Fruiting of Navel Orange in New Land

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THE PRESENT investigation was carried out during two successive seasons 2006 and 2007at a private orchard in Kafr-Dawood, Behera Governorate on 12 years old trees of Washington navel orange budded on sour orange rootstock, grown in a sandy soil. The work aimed to study the effectiveness of foliar application with chelated (Fe + Zn +Mn) at the rate of 0.5 g of each element / L of water, as well as putrescine (Amino acid 10 micro molar / L) solution for improving vegetative growth, fruit yield and fruit quality. The obtained results showed a pronounced increase in some vegetative growth characteristics (tree canopy and leaf area) and leaf chlorophyll (A & B) content, due to spraying either with biostimulant putrescine or micronutrients. The applied treatments corrected the imbalanced plant nutrition and caused significant increase in fruit quality and leaf mineral contents. The highest number of fruits / tree 234.67, 238.33were obtained from using micronutrients plus putrescine treatments in both seasons respectively, followed by putrescine then micronutrients spray. From the economical point of view. We can say that foliar application of micronutrients plus putrescine (as amino acid) increased the yield and gave net income of 1857 in the first season and 2989 Egyptian pounds / fed. in the second season.

Keywords: Putrescine, Micronutrients, Amino acid, Citrus, Vegetative growth, Fruit quality, Leaf chlorophyll and Mineral content.

In Egypt, Navel orange is a popular fresh fruit due to seedlessness and its high content of total soluble solids. It is considered, as reported by many workers, the first crop cv. among the different of citrus in Egypt, leading local market and exports (El-Shobaky and Mohamed, 2000). So, the improvement of fruit yield and quality are very important objective to be achieved by different management practices, especially in poor sandy soils. nutrients imbalance or deficiency of micronutrients (micro), particularly Fe, Zn and Mn considered one of the major limiting factors of plant growth and production (El-Fouly and Fawzi, 1982). In arid, or semi - arid Zones, characterized by low organic matter

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content and high pH, which caused nutrition imbalance and in availability. Soil or foliar application of micronutrients (Fe, Zn and Mn) either individually or together improved mandarin yield and both physical and chemical fruit quality (El-Kassas *et al.*, 1987).Three Sprays from micronutrients (chelated or non chelated) on Balady mandarin trees increased yield, leaf mineral and chlorophyll (A & B) content and improved fruit quality (Sayed,1998). Foliar application of micronutrients or biostimulant solution has an effective role in fruit trees nutrition, especially in sandy soils (Awad, 1988, Sweitlik & Laduke, 1991 and Mansour, 2004). Nathan *et al.* (1984) noticed that there are relation between polyamine metabolism and citrus fruit set and development. Increasing level of Polyamine, Putrescine, Arginine and proline showed all parts of the tree from autumn to winter improved flowering and fruiting (Kato *et al.*, 1984). Putrescine or Arginine supplied exogenously increased flower formation in apple (Edwards, 1986). In this concern, Ali and Lovatt, (1995) noticed that spray of arginine, putrescine and spermidine at low –temperatures enhance the flowering response of cv. Washington navel orange. These and the following observations strongly suggest that the synthesis and tissue content of polyamines might also be elevated during early flower development. Moreover early flower, especially ovary development and increase of polyamine biosynthesis in rapidly dividing cells has been reported in plant tissues (Speranza and Bagni, 1977). In addition Abu El-Fotoh *et al.* (2007) reported that the positive role of applied putrescine as foliar spray was improve of many plants yield. The present study was carried out to evaluate the effect of foliar application of putrescine and micronutrients (Fe +Zn +Mn) individually or as combined treatments, on the vegetative gfowth, leaf mineral and chlorophyll content, yield and fruit quality of Washington navel orange trees under sandy soil conditions.

Material and Methods

This investigation was implemented during two consecutive experimental seasons of 2006 and 2007 on healthy trees about 12 years old Washington navel orange (*Citrus sinensis L.Osbeck*) budded on sour orange rootstock (*Citrus aurantium.L.*).Trees were planted at 5×5 m and grown in sandy soil, located in a private orchard Kafr Dawood, El-Behera Governorate. All the studied trees were subjected to the common horticultural practices.

TABLE 1. Physical and chemical prqperties of the soil used.

Depth (cm)	Texture	PH	E.C.	CaCo3	Micronutrients (mg/Kg $^{-1}$)			
	grade	(1 -2.5)	(ds/m)	%	Fe	Zn	Mn	Cu
0 - 30	Sandy	7.90	0.35	2.40	3.36	0.25	1.64	0.24
30 - 60	Sandy	8.20	0.29	1.30	2.42	0.28	1.50	0.24
60 - 90	Sandy	8.10	0.30	1.40	1.25	0.30	1.80	0.20

The chemical analysis of soil was done according to Black *et al.* (1965). Some properties of the studied soil are illustrated in Table 1.

Drip irrigation system was followed with about 50 Cm (GR) drippert, with two laterals along the row of trees. Four treatments (Table 2) were carried out as foliar application, three times in the year (the first application was applied at late February to early Marsh during flower bud differentiation, the second was at late May or early June (for decrease fruitlet drop), the third application was at late July to early August (to achieve the swelling growth of the fruitlet). salient-film at 0.3% was used as a wetting agent.

TABLE 2. Layout of foliar application in two seasons.

No. of Treat.	Treatment
1	Control (spray with water)
2	Foliar spray with Micronutrentes (used at 0.5 gm /litter)
3	Foliar spray with Putrescine (used at 10 Micromolar concentration)
4	Foliar spray with Micronutrentes + Putrescine

The obtained data were arranged in a complete randomized blocks design. Each treatment was replicated three times and each replicate was represented by one tree.

1 - Canopy volume (CV) was determined using the following Formula (Turrell 1946).

$$C V. - 0.5238 H D^2$$

where H = tree height

D = canopy diameter.

2 - Leaf area

Leaf area was measured (using mature leaf at the second week of September) by laser leaf area meter (model CI-203CA from CID. Inc. company).

3 -Leaf chlorophyll

Chlorophyll (A & B) contents as mg /gm f.w. of mature leaf tissue were estimated according to Arnon (1945).

4 - Yield and fruit quality

At harvest time 15 Des.of each experimental season fruit yield was recorded as a number of fruits per tree. A sample of ten fruits were picked from each replicate to determine fruit weight (gm), fruit size (Cm^3), peel thickness (Cm), juice percentage, acidity percentage , total soluble solids (TSS) , ascorbic acid (V.C) as mg /100 Cm^3 juice . according to A.O.A.C. methods (1977).

5 -Leaf sampling and mineral analysis

Twenty spring shoots were labeled at early March of each experimental season, all shoots were well distributed periphery around each devoted tree. In the second week of September 60 leaves representing the 3^{rd} to the 5^{th} leaf from shoot apex. The collected leaf Samples from non fruiting labeled shoots used to measure leaf area, chlorophyll (A & B) and leaf mineral contents, of each Treatment then washed several times with tap water and then distilled water, oven dried at 60 -70^0 C .The dried ground samples were digested with sulphuric acid and hydrogen peroxide according to Evenhuis and Waard (1962). N was determined by micro–kjeldahl method as out Lined by Pregl (1945). Phosphorus was calorimetrically determined according to Evenhuis (1971) and Murphy and Riley (1962). K, Mg, and Ca were determined using flame photometer as mentioned by Brown and Lilleland (1946), Fe, Zn, and Mn were determined by using Perkin Elemer Atomic Absorption spectrophotometer.

6- Statistical analysis

The data were statistically analysed according to Snedecor and Cochran (1972)

Results and Discussion

Vegetative growth

Regarding the effect of Putrescine (put) micronutrients (micro) and put. plus micro. on Washington navel orange trees it was quite evident from Table 3 that those treatments had statistically higher values of Canopy volume and leaf area compared to control in both 2005 – 06 and 2006 – 07 experimental seasons. As the highest tree canopy volume was recorded by put.+micro. treatment followed by put. alone (27.91 & 28.10 m^3) then micro alone (27.19 & 28.28. m^3) during the first and second seasons respectively. Moreover, leaf area gave the same trend The obtained results goes in the line with finding of El-Kassas *et al.* (1987), Mohsen *et al.* (1992) and Sayed (1998) on Balady mandarin and orange, they found that the stimulation effect of using Micro. increasing tree growth , as well as Abd El Dayem (2001) reported that spraying of put. cause to regulation of plant growth, stimulate development cell division, stabilize cell membrane, retard senescence, inhibition protease peroxidase and RNase and inhibite ethylene biosynthesis in plants. In addition Smith (1985) noted that Put. and Polyamines stimulate the growth of several higher plants.

Leaf Chlorophyll (A & B) content

Regarding the effect of applied treatments on leaf chlorophyll (chl) A & B content Table 3, revealed that leaf chlorophyll content increased with all treatments compared to control in both seasons. The highest values were (1.43 and 1.52 mg for chl A & 0.59 and 0.69 mg for chl B) under in both seasons put. + micro treatment, respectively. Moreover, chlorophyll content in the second season was higher than in the first season, this establishes that there are residual effects with all treatments. The obtained results are in agreeme with Cakmak *et al.* (1989) who suggested that

50% reduction In IAA synthesis due to Zn –deficient might be result of inhibited synthesis or enhanced degradation of IAA rather than vice versa, as well as Nakhlla (1998) found that foliar Zn and IAA increased chlorophyll content in leaves of Navel orange. In addition Abd El Dayem (2001) reported that in leaves of all plant species, the major symptom of iron deficiency is inhibition of chlorophyll development rather than vice versa.

Dry weight

It is quite clear as shown from Table 3 that the highest dry weight percentage was significantly in closed relationship to put +micro. followed by put. and then micro. compared to control in both seasons. The highest values were (46.99 & 49.98, 43.73 & 49.52 and 40.86 & 48.62) for the above mentioned treatments in the two seasons, respectively. It is clear that the values in the first season was lower than those in the second season this revealed that there are residual effects with all treatments. These results are in agreement with those obtained by Singh and Ganguar (1973) who mentioned that zinc participate in the production of IAA which resulted in an increase in growth and sugar production, followed that increase in dry matter, as well as El-Hagah *et al.*, (1983) on Balady mandarin who reported that the number of spring and summer shoots were highest when the trees received soil and foliar N.P, K, Mn, Zn, Cu, Fe, S and B. Moreover, Nathan *et al.* (1984) noticed that there is a possible linke between polyamine metabolism and citrus fruit set and growth and development which is due to increased dry weight.

TABLE 3 . Effect of putrescine and micronutrients spray on Tree canopy , leaf area, chlorophyll (A & B) and dry weight percentage of Navel orange (2006-2007).

Treatments	Tree canopy (m^3)		Leaf area (cm^2)		Chlorophyll A (mg/g F.w.)		Chlorophyll B(mg/gF.w.)		Dry weight (%)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Control	26.99	27.48	10.22	11.25	1.31	1.29	0.44	0.47	40.46	47.87
Micronutrients*	27.19	28.28	14.48	14.46	1.34	1.38	0.48	0.54	40.86	48.62
Putrescine**	27.91	28.10	14.18	14.81	1.37	1.45	0.53	0.52	43.73	49.52
Micro. + Put.	27.93	28.17	15.50	16.65	1.43	1.52	0.59	0.69	46.99	49.98
L.S.D. 0.5 %	0.04	0.17	0.32	0.78	0.02	0.03	0.02	0.02	0.75	0.39

Micronutrients* = Micro Putrescine** = Put

Fruit yield

Concerning the fruit yield expressed as number of fruits per tree, data in Table 4 showed that all the applied treatments were superior over control in both seasons. The fruit yield was (234.67, 238.33 & 227.00, 230.33 & 220.00, 225.00) fruits per tree for put. + micro and put. then micro. treatments in the two seasons, respectively. It is clear that the values in the first season was lower than from the values in the second season this establishes that there are residual effects with all treatments .These results are in accordance with those reported by Khera el al ., (1985) on Blood orange as the highest yield was obtained from trees receiving Zn treatment . As well as Costa and Bagni (1986) as they found that exogenous application of putrescine to apple flowers increased flower retention, fruit set, and yield per tree. As well as Putrescine increased growth during the cell division phase of apple fruit growth. Moreover, finding of El-Kassas et al., (1987), and Sayed (1998) on Balady mandarin took the same trend.

Fruit quality

Fruit Physical properties

In this regard the average fruit weight (g); fruit size (cm^3); peel thickness (cm) and fruit juice % (by weight) were responsed to foliar spray of micro.; put.; micro. + put. treatments. Data presented in Table 4 showed clearly that all treatments gave the highest values compared to control in both seasons. The highest values recorded with micro. + put. followed by put then micro. treatment. The present results regarding the fruit physical properties are in general agreement with findings of Nathan et al. (1984) on Citrus trees as he reported that putrescine, arginin and spermidine induced Citrus fruit set and development and improved fruit quality. Moreover, El-Kassas et al. (1987) and Sayed (1998) on Balady mandarin, they demonstrated that spraying micro. improved fruit physical properties, in addition Oded and Lovatt (1991) who noticed that putrescine spray on Washington navel orange significantly increased ovary development , fruit growth and fruit quality.

TABLE 4. The effect of putrescine and micronutrients spray on yield and fruit physical properties of Washington navel orange trees (2006 - 2007).

Treatments	Yield No./ fruit tree		Fruit weight (g)		Fruit size (cm^3)		Peel thickness (cm)		Juice (%)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Control	215.00	213.33	236.00	236.67	246.67	248.33	0.57	0.68	45.07	44.08
Micronutrients*	225.00	220.00	258.67	266.00	310.00	291.33	0.79	0.77	45.44	45.40
Putrescine**	230.00	227.33	305.33	262.67	323.33	295.33	0.82	0.84	45.85	46.02
Micro. + Put.	238.67	234.33	302.00	328.00	333.11	335.00	0.87	0.87	46.77	48.75
L.S.D. 0.5 %	56.62	93.46	15.41	38.76	13.02	23.74	0.02	0.22	0.75	0.64

Micronutrients* = Micro Putrescine** = Put

2-Fruit chemical properties

In this regard fruit juice total soluble solids (TSS); total acidity percentage; TSS/acid ratio and vitamin C (ascorbic acid) contents in response to specific effect of foliar spray micro. put. + micro. , put. and micro. treatments, presented in Table 5. the obtained data showed obviously that all treatments had significant incrementeffect if compared to control, with highest values in micro. + put. followed by put. and then micro. treatments in both seasons, except total acidity which was the lowest values with all treatments compared to control. The obtained data are in agreement with those obtained by Kato *et al.* (1984) and Nathan *et al.* (1984) on mandarin who noticed that putrescine spray on citrus trees improved fruit quality. Furthermore, El-Kassas *et al.* (1987) and Sayed (1998) they reported that micronutrients spray on mandarin increased fruit TSS %, ascorbic acid and reduced total acidity, Moreover, Abd El Dayem (2001) reported that putrescine spray increased the transport of K and enhanced its translocation into the growing points which due to better fruit chemical properties.

TABLE 5. Effect of putrescine and micronutrients spray on fruit chemical properties of Washington navel orange trees (2006-2007)

Treatments	T.S.S (%)		Total acidity (%)		T.S.S/acid ratio		V.C. mg/100cm juice	
	2006	2007	2006	2007	2006	2007	2006	2007
Control	10.50	11.17	0.83	0.85	18.65	17.30	47.61	51.30
Micronutrients*	11.67	11.50	0.78	0.75	19.02	16.75	50.75	53.50
Putrescine**	11.83	12.08	0.61	0.69	15.12	16.13	51.73	52.86
Micro. + Put.	12.33	12.33	0.56	0.64	14.92	14.92	51.84	52.90
L.S.D. 0.5%	0.37	0.40	0.03	0.03	0.69	1.20	0.63	0.133

Micronutrients* = Micro Putrescine** = Put

Leaf proline and mineral content

It is quite clear as shown from Tables (6 & 7) that leaf proline content decreased with micro. + put. followed by put. treatment, but N; P; K; Fe; Zn; and Mn, increased with all treatments compared to control in both seasons. These results are in agreement with those obtained Kato *et al.*, (1984) on Satsumas mandarin found a marked decrease in proline with a marked increase in putrescine, as well as Smith (1985) who reported that putrescine and proline are analogous in their effect in plant cell against stress condition. Also Ali and Lovatt (1995) on Washington navel orange noticed that trees receiving putrescine had significantly greater leaf concentrations of putrescine, which lead to regulation of plant growth and development, stimulate cell division. Regarding the leaf mineral content Sharma and Naggar (1990) on mandarin found that trees spray with CuSo4 and ZnSo4 had the highest leaf Cu and Zn content Moreover, Sayed (1998) working on Balady mandarin found that the trees which received foliar sprays of Fe, Zn and Mn chelated or non chelated 3 times each year increased all elements in leaves.

TABLE 6. Effect of putrescine and Micronutrients spray on leaf Proline and some mineral contents of Washington navel orange season (2006).

Treatment	Proline (mg/100g)	Macronutrients (%)			Micronutrients (mg/kg^{-1})		
		N	P	K	Fe	Zn	Mn
Control	3.97	1.42	0.102	1.14	88	37	29
Micronutrient*	3. 79	1.48	0.134	1.35	122	43	44
Putrescine**	3.20	1.50	0.161	1.41	135	57	50
Micro. + Put.	3.14	1.57	0.168	1.48	140	62	54

Micronutrients* = Micro Putrescine** = Put

TABLE 7. Effect of putrescine and micronutrients spray on leaf Proline and some mineral contents of Washington navel orange season (2007).

Treatment	Proline (mg/100g)	Macronutrients (%)			Micronutrients (mg/kg^{-1})		
		N	P	K	Fe	Zn	Mn
Control	3. 77	1.51	0.123	1.24	100	41	32
Micronutrient*	3.67	1.88	0.144	1.26	132	54	49
Putrescine**	3.10	2.12	0.163	1.35	145	65	54
Micro. + Put.	3.00	2.46	0.169	1.53	164	73	58

Micronutrients* = Micro Putrescine** = Put

In Table 8 The different between control and put. plus micro. treatment
In the first season = 11307 – 8055 = 3252 Kg = 3252 Egyptian pounds (EP)
In the second season =12525 – 8141 =4384 Kg = 4364 EP
Net income in the first season = 3252 – 1395 (spray cost) = 1857 EP
Net income in the second season =4384 – 1395(spray cost) = 2989 EP

TABLE 8. The different between control and put. plus micro. treatment.

Treatment	Fruit weight (g)		Fruit No./ tree		Yield /tree		Yield ton / feddan	
	2006	2007	2006	2007	2006	2007	2006	2007
Control	236.00	236.67	213.33	215.55	503.46	508.84	8.055	8.141
Put. + micro	302.00	328.00	234.00	238.67	706.68	782.84	11.307	12.525

Economic analysis

Feddan = (160 trees)

Spray costs

Components needed for one application	Cost of one application	Total cost per feddan / year
Labor	40 X 3	360
Sprayer rent	35 X 3	315
Chemicals	240 X 3	720
Total		1395

- Each application need Three full tanks sprayers.
- The application repeated three times every year.

As application were done three times per year, each application need three full motors (600 L of water), the total costs were

Conclusion : Under the condition of this investigation and the resembling condition, concerning all treatments (micro. plus put.; micro. alone and putrescine alone) it can be concluded that micro. plus put. treatment gave the better results of regulation plant growth and development, increment shoot flowering, fruit yield and fruit quality. Consequently it could be concluded that spray Citrus tree with micronutrients Fe, Zn and Mn as chelated at 0.5g /L of water in combination with putrescine at 10 Micromolar / L of water for three times each year. to get the best vegetative growth, yield and fruit quality.

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تأثير اضافة بعض العناصر الصغرى والبتروسين على النمو والاثمار فى البرتقال بسرة فى الاراضى الجديدة

رمضان أبوسريع سيد* ، أميرة محمد حسن** ، محمد عبد المحسن العقباوى** و نادية عمر منجد**
*معهد بحوث البساتين و **معهد بحوث الأراضى والمياه والبيئة - مركز البحوث الزراعية – القاهرة – مصر.

أجرى هذا البحث لمدة موسمين متتاليين ٢٠٠٥-٢٠٠٦ و ٢٠٠٦ -٢٠٠٧ فى مزرعة خاصة بكفر داود محافظة البحيرة على اشجار عمر ١٢ سنة لصنف برتقال بسرة واشنجطن مطعومة على اصل النارنج نامية فى تربة رملية على مسافة ٥ × ٥ م وتهدف هذه الدراسة الى معرفة تأثير رش العناصر الصغرى فى الصورة المخلبية لكل من (الحديد والزنك والمنجنيز) بتركيز ٥ .٠جم لكل لتر ماء بالاضافة الى تأثير الحمض الامينى البتروسين بتركيز ١٠ ميكرومولر / لتر ماء على تحسين النمو الخضرى والمحصول وجودة الثمار .

واوضحت النتائج المتحصل عليها أن هناك زيادة واضحة فى بعض الصفات الخضرية (حجم الشجرة و مساحة الاوراق) ومحتوى الاوراق من الكلورفيل (أ ، ب) راجعة الى تأثير الرش اما بالعناصر أو حمض البتروسين . وأدت المعاملات الى تصحيح عدم التوازن العنصرى داخل النبات كما ادت الى زيادة معنوية فى جودة الثمار ومحتوى الاوراق من العناصر المعدنية وكان اعلى عدد للثمار٦٧ و٢٣٤ & ٣٣ و٢٣٨ ثمرة /شجرة نتيجة استعمال رش البتروسين مضاف الية العناصر الصغرى فى كلا الموسمين ويتبعها معاملة البتروسين فقط ثم معاملة الرش بالعناصر الصغرى . ومن الناحية الاقتصادية نستطيع ان نوصى بان معاملة رش العناصر الصغرى + التروسين (كحمض امينى) ادت الى زيادة المحصول وكان صافى الربح ١٨٥٧ جنيه مصرى فى الموسم الاول ٢٩٨٩ جنيه فى الموسم الثانى.