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Effect of Moringa Leaves Extract as a Natural Product Compared with Other Synthetic Compounds on Yield Production and Fruit Quality of Navel Orange Trees

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THIS STUDY was conducted during two successive seasons 2013 and 2014 in private orchard at El-Behiera governorate (Latitude: 30.9833 Longitude: 30.2000), Egypt, to study the effect of spraying Moringa leaf extract, as a safe natural product compared to other substances on yield and fruit quality of 15-years-old Navel orange trees (*Citrus sinensis*) budded on Sour Orange (*Citrus aurantium*, L.Osbeck). Treatments used were; control (spraying with tap water), moringa leaf extract (MLE) at 3, gibberellic acid (GA_3) at 30 ppm, chelated calcium (Ca) at 0.5%, and potassium nitrate (KNO_3) at 1%. They were sprayed at full bloom stage, fruit set, at last week of June and at complete mature stage. The obtained results revealed that, treatments in general improved significantly Navel orange fruit set, where GA_3 and MLE treatments gave the highest value. The highest yield was induced by MLE followed by other treatments. While, MLE treatment gave the lowest fruit peel firmness compared to other ones. In addition, MLE and GA_3 treatments increased juice percentage and gave the highest TSS %. On the other hand, MLE treatment increase significantly juice acidity compared to the other treatments. Moreover, KNO_3 treatment gave the highest values of nitrite and nitrate in fruit juice, while GA3 and MLE treatments were the lowest. KNO_3 and MLE significantly increased leaf N and K contents. Whereas, treatments in this study had insignificant effect on leaf P% content in both seasons. Thus, overall, based on the current results, moringa leaves extract can be used at rates of 3% to stimulate the biomass production of Washington navel orange trees yield, enhance leaf mineral content and some physical and chemical characteristics.

Keyword: Navel Orange, Moringa extract, GA_3, KNO_3, Ca, Yield, Fruit quality.

Citrus are widely distributed for its high nutritive value and economic importance. It takes the first in Egypt and the second after grapes in the world. Navel orange is a popular fresh fruit due to its seedless, large size, flavor and aroma characteristic (Wardowski *et al*., 1985).

Synthetic compounds used to enhance plant productivity and fruit quality are highly polluting, unsafe and much more costly. Consequently, there is

continuous need to search for safe natural sources alternative of plant nutrients and natural growth regulators Phiri (2010).

Moringa oleifera (family: Moringaceae) is one of such alternatives, being investigated to ascertain its effect on growth and yield of crops and thus can be promoted among farmers as a possible supplement or substitute to inorganic fertilizers Phiri (2010). Fresh *Moringa oleifera* leaves have been shown to have high zeatin content. Moringa leaves collect ted from various parts of the world were found to have high zeatin concentrations (up to 200 mcg/g) of leaves (Fuglie 2000). Cytokinins are naturally occurring plant hormones known to be key regulators of various aspects of plant growth and development, including cell division, leaf senescence, apical dominance, lateral root formation, stress tolerance, and nutritional signaling Sakakibara (2006) and Argueso *et al*., (2009). In this concern Iqbal (2014) affirmed that Moringa leaf extract is rich with numerous growth hormones, particularly zeatin and micronutrients in sufficient quantities and suitable proportions that increase the growth and yield components. Moreover, Azra (2011) found that spraying wheat, peas and tomato with *M. oleifera* extract at 3.5% improved crop characteristics. In addition, Mona (2013) found that spraying rocket (*Eruca vesicaria* subsp. sativa) plants with the aqueous extracts of leaves and twigs of *M. oleifera* at rates of 1, 2 and 3% increased all measured growth criteria (plant height), the amounts of each of chlorophyll a and b, total sugars, ascorbic acid and N, P and K.

This trial aimed to study the effect of moringa leaf extracts, as a natural product compared to other parallel synthetic substances on fruit set, yield and fruit quality of Washington navel orange trees. Hence, to identify the best treatments to crop safe fruits and achieve the highest return for the growers.

Material and Methods

This study was conducted during 2013 and 2014 seasons on 15- years old Washington navel orange (*Citrus sinensis* Osbeck) trees budded on Sour orange rootstock (*Citrus aurantium*, L.Osbeck) planted at 5×5 m spacing in sandy soil under drip irrigation system at El-Behiera Governorate, Egypt.

Experimental design and treatments

A complete randomized block design was used and each treatment was replicated three times with 2 trees for each replicate (total of 30 trees).
Five treatments were established as follows:

- Control treatment (spraying with tap water).
- Moringa leaves extract (MLE) at 3%.
- Gibberellic acid (GA_3) at 30 ppm.
- Chelated calcuim (Ca) at 0.5 %.
- Potassium nitrate (KNO_3) at 1 %.

They were sprayed on full bloom stage, fruit set, at last week of June and at complete mature stage.

The 3% moringa aqueous leaf extract was prepared by blending 30 g of young moringa leaves with 675 ml of 80 % ethanol as suggested by Makkar and Becker, (1996). The obtained suspension homogenized and filtered by wringing using a mutton cloth. Finally, the solution re-filtered using no. 2 Whatman filter paper and completed to one liter Fuglie (2000). Commercial product (containing 92% GA_3 and 8% of other gibberellins) were used in the trials at 30 ppm spraying dose. Wetting agent at 0.1% was used for all spraying solutions. In addition foliar sprays of Chelated Calcium 5%, potassium as KNO_3 98% were applied. All trees generally received adequate organic and inorganic fertilization under drip irrigation system according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. However, a balanced foliar fertilization of all microelements was adopted three times yearly (February, May and August).

The following parameters were measured for both seasons:

Fruit set

Four branches were chosen on each tree, one for each direction. During the current spring growth cycle they were labeled to carry out the following flowering and fruiting measurements**:**

-Initial and final fruit set calculated as percentage by the following equations

$$\text{Initial fruit set \% (I.F.S.)} = \frac{\text{Total No. of setted fruit}}{\text{Total No. of flowers}} x100$$

$$\text{Final fruit set\% (F.F.S)} = = \frac{\text{No. of fruits at end of June}}{\text{Total No. of setted fruit}} x100$$

Fruit yield

At the harvesting time (December 20th for 2013 and 2014 seasons) total yield per tree, ton per Fadden, number and weight of mature fruits per tree were determined in both seasons.

Fruit quality

A sample of 10 mature fruits of each tree was taken at the harvest time to determine the physical and chemical properties, fruit weight (g) were measured and peel thickness (mm) were measured by using a digital vernier caliper. Juice weight percentage was calculated and recorded .Total soluble solids (TSS %) was determined by using hand refractometer. Total acidity (%) were determined in fruit juice according to A.O.A.C. (1995).

Chemical constituents

leaf mineral contents: At the end of the experimental season (the end of September), thirty leaves/tree were collected representing the four main directions. The samples were washed, dried, grounded and digested according to Chapman and Pratt (1978).

N, P, and K were determined in the digested solution as follows:

- Total nitrogen was determined as percentage using the micro-Kjeldahl method as described by Pregl (1945).
- Phosphorus was determined colourimetrically methods as described by Murphy and Riley (1962).
- Potassium content was determined by Flame photometer as percentage according to method of Jackson (1958).

Nitrite and nitrate contents in fruit juice were determine according to the methods outlined by Sen and Donaldson (1978).

Zeatin determined in *Moringa oleifera* leave as micro-g/100 g leave dry weight and the amount recorded was 489.88 micro-g/100 g. Samples were analyzed in Arid Land Agricultural Research and Services Center Facultuy of Agric. Ain Shames Univ.

Statistical analysis

The obtained data were statistically analyzed as randomized complete block design according to Snedecor and Cochran, (1980) and treatment means were compared using Duncan Multiple Range Test (DMRT) Duncan (1955).

Results and Discussions

Fruit set

Obtained data (Fig. 1) showed that MLE or GA_3 treatments significantly increase initial and final fruit set for both seasons as compared to other treatments. The increase in fruit set by MLE spraying may be due reduction in flower abscission by the effect of zeatin as zeatin enhanced conversion of abscisic acid to phaseic acid and caused a reduction in the amount of mevalonolactone into abscisic acid Cowan *et al.* (1999). In this concern Guirguis *et al.* (2003) reported that Sitofex (a cytokinin like substance) treatment increased the percentage of fruit set and fruiting when applied at full bloom on "Costata" persimmon trees Furthermore, effect of GA3 may be due to that exogenous GAs suppress completely both post-anthesis abscisic acid increases and fruit abscission as mentioned by Iglesias *et al.* (2007). Whom assumed that reductions of GA levels over the anthesis period, result in subsequent ovary and fruitlet drop. These results were in agreement with Abd El-Rahman *et al*. (2012) on Navel orange and Baghdady *et al.* (2014) on Valencia orange as they found that, spraying orange trees with GA_3 increased fruit set percentage. Moreover, Abd El–Hamied and El-Amary (2015) found that spraying pear trees with moringa extract at (2 and 4 %) under North Sinai conditions increased fruit set and yield. While, control had nearly the lowest values for the initial and final fruit set in both seasons.

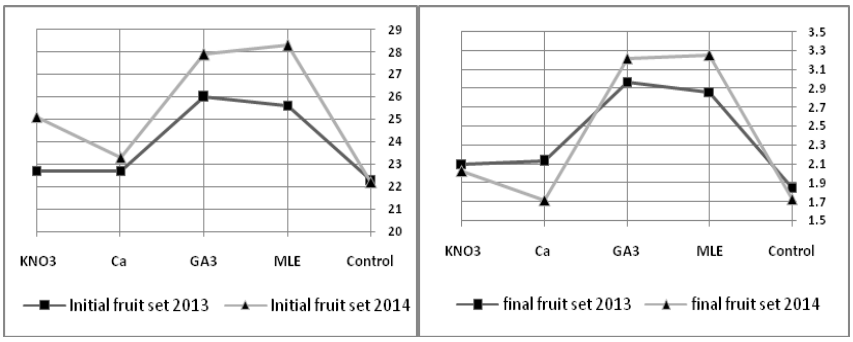

Fig. 1. Fruit set percentage of Washington navel orange trees as affected by MLE, GA_3, KNO_3 and Ca foliar applications during 2013 and 2014 seasons.

Fruit yield

Table 1 illustrate the effect of MLE, GA_3, KNO_3 and Ca foliar application on fruit weight, number/tree, yield/tree and ton/fed during 2013 and 2014seasons. Concerning the number of fruit /tree, data showed that, the highest fruit number/tree was that of the trees sprayed with MLE and GA_3 compared to the other treatments. Such figures achieved by these two treatments were a result of their supremacy in fruit set since fruit number is a function of flower intensity and fruit set. Bons *et al*. (2015). As for fruit weight, MLE treatment followed by KNO_3 had similar results of control without significant differences between them. While, Ca or GA_3 treatments got less values comparing to control ones in both seasons. In spite of MLE treatment achieved the highest fruit number, and since the number of fruit that set and persist to harvest influences fruit size (Bons *et al.*, 2015), it keeps up fruit weight similar to control one. Such consequence may be due to cytokinin activation of cell division Riou-Khamlichi (1999). Where, fruit size is a function of cell division and cell enlargement processes Bons *et al.* (2015).

Moreover, Van Staden *et al.* (2008) mentioned that Cytokinins, together with auxins, take part in the regulation of the cell cycle in plant cells and thus stimulate the cell cycle progression.

Consequently, regarding yield/tree and ton/fed (Table 1) MLE treatment attained the highest yield in both seasons, owing to the increment in fruit number (lower flower abscission) and the augmentation in fruit weight relating to the earlier parameter. These results are in the same line with those obtained by Abo El-Enin (2005) on Navel orange, Shinde *et al*. (2008) on lime, Abd El-Rahman (2010) and Baghdady *et al*. (2014) on Valencia orange. They showed that spraying Ca or GA_3 on citrus trees gave the significant maximum yield (weight and number). Meanwhile, El-Shafey *et al*. (2002) on Valencia orange and Rattanpal *et al*. (2008) on Kinnow mandarin found that spraying Ca or KNO_3 significantly increase yield tree as compared to control.

TABLE 1. Yield of Washington navel orange trees as affected by the different treatments during 2013 and 2014 seasons.

Treatments	No. of fruit. / tree		Average fruit weight (g)		Yield / tree (Kg)		Yield/feddan (ton)	
	2013	2014	2013	2014	2013	2014	2013	2014
Control	246.72c	247.18c	281.69a	287.04a	69.61c	71.44c	11.54c	12.12c
MLE at 3%	304.78a	332.10a	274.36a	277.14a	83.59a	91.84a	14.04a	15.42 a
GA_3 at 30 ppm	304.88a	325.46ab	219.59d	266.39c	66.87c	87.83b	11.49c	14.61b
Ca at 0.5 %	289.12b	299.67b	260.55b	275.50b	75.83b	82.60b	13.11b	13.79b
KNO_3 at 1 %	265.30b	300.50b	274.10ab	278.23a	72.63bc	84.31b	12.34b	14.23b

Mean separation within columns by Duncan's multiple range test, 5% level. Values that don't share the same letter are significantly different

Physical properties of fruits

Concerning fruit size the statistical analysis for data in Table 2 showed that, control, MLE and KNO_3 treatments produced the heights fruit size. The effect of MLE is in confirm with the fact that fruit size is a function of cell division and cell enlargement processes and may be attributed to that cytokinins stimulate cell division Bons *et al.* (2015). In addition, cytokinins appear to be necessary for plant cell division as in their absence, metaphase, is considerably prolonged, and it has been suggested that cytokinins might be required to regulate the synthesis of proteins involved in the formation and function of the mitotic spindle apparatus Van Staden *et al.* (2008). Concerning KNO_3 treatment, its results are in line with the finding of Mongi and Tom (2012) as they metioned that the maximum effect of foliar K is achieved by making this potassium available during bloom and post-bloom when it can be used during both cell division and rapid cell enlargement phases. While, trees treated by GA_3 and Ca were in the second rank in both seasons. These results are in agreement with the findings of Mongi (2011) on tangerine and grapefruit, Saraswathi *et al*. (2002) in kinnow mandarin and Thirugnanavel *et al*. (2007) and Lakshmi *et al*. (2014) in acid lime as they mentioned that, fruit size increased as K fertilization increased, Also Abd El-Rahman (2005) found that spraying GA_3 at 50 ppm increased fruit size on navel orange fruits, also Fuglie (2000) who found that the leaf extract of moringa oleifera accelerated growth of young plants and produced larger fruits. Furthermore, Azra (2011), Abd El–Hamied and El-Amary (2015) found that spraying pear trees with moringa oleifera extract increased crop characteristics.

Furthermore, tabulated data showed that spraying trees with GA_3 increased juice weight % followed by MLE treatment in both seasons. Similar results of increased levels of juice were obtained by Baghdady *et al.* (2014) on Valencia orange Lakshmi *et al.* (2014) in acid lime.

TABLE 2. Yield of Washington navel orange trees as affected by the different treatments during 2013 and 2014 seasons.

Treatments	Fruit size (ml)		Peel thickness (mm)		Fruit firmness (kg/cm^2)		Juice weight %	
	2013	2014	2013	2014	2013	2014	2013	2014
Control	315.0a	278.65a	3.75c	4.40 b	8.61b	7.33c	27.16 b	29.40 c
MLE at 3%	317.03a	273.43a	4.46ab	4.83 a	8.73b	8.35b	34.43 a	33.28 b
GA_3 at 30 ppm	241.20b	271.85b	4.15bc	4.50 ab	9.36 a	8.57b	34.56 a	37.28 a
Ca at 0.5 %	265.40b	248.80b	4.60a	4.60 ab	9.29a	9.26a	28.46 b	31.92bc
KNO_3 at 1 %	317.03a	252.36b	4.86a	4.66 ab	8.80b	8.96a	28.54 b	30.16 c

Mean separation within columns by Duncan's multiple range test, 5% level. Values that don't share the same letter are significantly different.

Chemical properties of fruits

Obtained data (Table 3) showed that spraying trees with GA_3 or MLE gave the highest TSS% compared to the other treatments in both seasons. A similar trend was obtained by Kaur *et al*. (1997) on Kinow mandarin, Saleem *et al.* (2008) on Balady orange and Lakshmi *et al*. (2014). They showed that, spraying GA_3 in citrus trees improved chemical fruit quality, also Abd El–Hamied and El-Amary (2015) found that spraying pear trees with moringa extract at 4% increased TSS%.

Concerning fruit acidity Ca treatment showed significantly the lowest value in both seasons. While, MLE had the highest percentages in this concern. Pertaining to TSS/ acid ratio, data reveled that there was no clear trend for all treatments except for MLE treatment which had the lowest values owing to its high acidity values.

TABLE 3. some Chemical properties of Washington navel orange trees as affected by the different treatments during 2013 and 2014 seasons.

Treatments	T.S.S%		Acidity%		T.S.S/ Acid ratio	
	2013	2014	2013	2014	2013	2014
Control	10.37b	10.20c	1.11bc	0.96c	9.44b	10.63a
MLE	10.80a	10.53b	1.21ab	1.11a	8.85c	9.42bc
GA_3	10.83a	11.02a	1.27 a	1.03ab	8.92c	10.73a
Ca at	9.80c	9.83d	1.040c	0.99bc	8.97c	9.99b
KNO_3	9.84c	9.80d	0.93d	1.06ab	10.01a	9.25c

Mean separation within columns by Duncan's multiple range test, 5% level. Values that don't share the same letter are significantly different.

Regarding nitrite and nitrate content in fruit juice data in Fig. 2 cleared that, trees sprayed with KNO_3 tended to increase nitrite and nitrate in their fruit juice especially in the second season followed by control treatment. While, MLE, GA_3 and Ca treatments gave the lowest nitrite and nitrate values in fruit juice. In this

respect, Ibrahim *et al*. (1994) and Abd El-Migeed, *et al*. (2007) mentioned that, mineral nitrogen fertilization easily forms nitrate in fruit juice as compare with using organic fertilizers, also Abd El-Rahman *et al*. (2012) on navel orange found that trees sprayed with KNO_3 gave the highest values for nitrite and nitrate in their fruit juice.

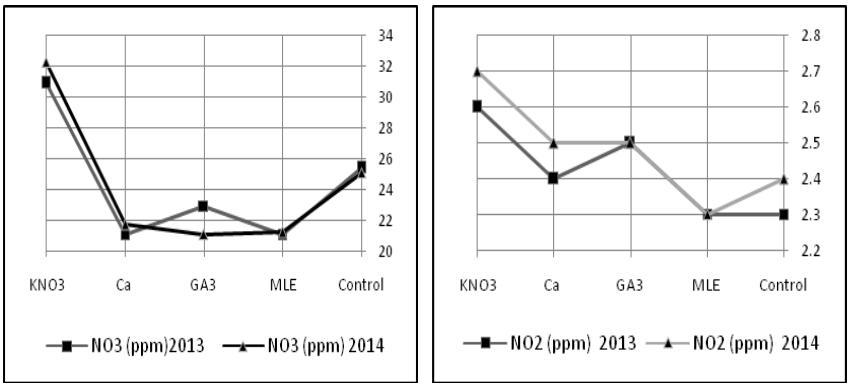

Fig. 2. Nitrite and nitrate in fruit juice of Washington navel orange fruit as affected by moringa leaves extract, GA_3, KNO_3 and Ca foliar applications during 2013 and2014 seasons

Chemical constituents

Leaf mineral content

Data in Table 4 showed that spraying trees with KNO_3 or MLE increased leaf N and K contents compared to the other treatments, while leaf P content did not affected by treatments in both seasons. Concerning the effect of MLE, the obtained data confirmed with that obtained by Abd El–Hamied and El-Amary (2015) on Le-Conte "pear". The obtained results are in agreement with those reported by Mostafa and Saleh (2006) on Balady mandarin, Abd-Allah (2006) on Washington Navel orange and Sarrwy *et al.* (2012) on balady mandrine, since spraying potassium from several forms *i.e.* $KHPO_2$ and KNO_3 raised N, P and K levels in the leaves.

TABLE 4. Yield of Washington navel orange trees as affected by the different treatments during 2013 and 2014 seasons.

Treatments	N%		P%		K %	
	2013	2014	2013	2014	2013	2014
Control	2.3c	2.3b	0.25	0.24	1.02b	1.06c
MLE at 3%	2.5b	2.4b	0.27	0.28	1.44a	1.52a
GA_3 at 30 ppm	2.5b	2.5b	0.28	0.24	1.21b	1.08c
Ca at 0.5 %	2.4bc	2.3b	0.27	0.26	1.13b	1.18b
KNO_3 at 1 %	2.7a	2.8a	0.25	0.26	1.53a	1.58a

Mean separation within columns by Duncan's multiple range test, 5% level. Values that don't share the same letter are significantly different.

Moreover, moringia leaf extract has the potential to promote plant growth; hence, it is used as a natural plant growth enhancer and increased yield by 20 and 35% (Fuglie, 2000).

Economical viable

Economics is the major consideration for the farmers while taking a decision regarding the acceptance of a new technology, hence the cost of treatments, crop value and net income were computed for different treatments. In the present experiment, the highest economical yield with the highest net return was recorded with the treatment of MLE (3%) as it is illustrated in Table 5.

TABLE 5. Yield and economic analysis related to foliar application treatments for Washington navel orange trees (as average of two seasons).

Treatment	Treatment cost L.E/fed.	Yield Ton/fed.	Crops value/fed.	Change in income over the control L.E/fed.
Control	----	11.83	13367.9	----
MLE	258	14.73	16644.7	3276.8
GA_3	402	13.05	14746.5	1378.6
Ca	390	13.45	15198.5	1830.6
KNO_3	420	13.29	15012.1	1644.2

The average price of one kg fruits was 1.13 L.E. in two seasons according to Ministry of agriculture. Egypt. Treatment spray cost =[Material cost +Spraying cost fuel +oil +machine)+labor cost] *Spraying times

Conclusion

Generally, it could be figure out that MLE treatment proved to have a great potential in stimulating Washington navel orange trees biomass production, yield and most of eminent physical and chemical characteristics except for acidity high percentage. Hence, there would be a need to make more investigation to reduce fruit juice acidity to attain the optimum TSS/acid.

Overall, it could be recommended that *M. oleifera* leaf extract (as a natural and safe material) at rates of 3% may be used effectively by farmers owing to its easy preparation, economical and environmentally friendly impact

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تأثير مستخلص أوراق المورينجا كمنتج طبيعى مقارنة ببعض المركبات التخليقية على انتاجية وجودة ثمار اشجار البرتقال ابوسرة

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أجريت هذه الدراسة خلال موسمى ٢٠١٣ و ٢٠١٤ فى مزرعة خاصة بمحافظة البحيرة بهدف دراسة تأثير الرش بمستخلص اوراق المورينجا كمركب طبيعى آمن مع مقارنته بمركبات اخرى على محصول وجودة ثمار أشجار البرتقال أبوسرة عمر ١٥ سنة المطعومة على أصل النارنج. وقد اشتملت الدراسة على خمسة معاملات على النحو التالي : معاملة الكنترول (الرش بالماء) – الرش بمستخلص اوراق المورينجا بمعدل ٣٪، الرش بالجبرالين بتركيز ٣٠ جزء فى المليون، الرش بالكالسيوم المخلبى بمعدل ٠.٥ ٪، الرش بنترات البوتاسيوم بمعدل ١٪. وقد تم رش هذه المعاملات عند اكتمال التفتح ،عند العقد ،وفى الأسبوع الأخير يونيووفى مرحلة إكتمال النمو. أوضحت النتائج ان كل المعاملات وخاصة معاملة الرش بالجبرالين ومستخلص المورينجا ادت الى زيادة نسبة العقد. سجلت معاملة الرش بمستخلص المورينجا تليها المعاملات الاخرى اعلى محصول كلى للفدان. اظهر الرش بمستخلص المورينجا انخفاض فى صلابة القشرة مقارنة بالمعاملات الاخرى ، ايضا اظهرت معاملة الرش بالجبرالين او بمستخلص المورينجا أعلى نسبة مئوية للعصير وأعلى نسبة مواد صلبة ذائبة بالمقارنة بالمعاملات الاخرى فى كلا موسمى الدراسة ومن جهة أخرى فقد أدى الرش بمستخلص المورينجا الى زيادة معنوية فى الحموضة. وقد سجلت المعاملة بنترات البوتاسيوم أعلى محتوى للنترات والنتريت فى العصير بينما اقل قيم سجلت لمعاملة الاشجار بمستخلص المورينجا او الرش بالجبرالين. اظهرت النتائج ان معاملة الرش بمستخلص المورينجا اوبنترات البوتاسيوم ادى الى زيادة محتوى الاوراق من النيتروجين والبوتاسيوم فى حين لم يكن هناك اى اختلافات معنوية بين المعاملات على محتوى الاوراق من الفوسفور. يمكن لمستخلص المورينجا بمعدل ٣٪ زيادة انتاج البرتقال ابو سرة واشنجطن وزيادة محتوى الاوراق من العناصر وكذلك الكثير من الصفات الطبيعية والكيميائية للثمار.