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Response of Washing on Navel Orange Trees to Foliar Spray with Some Stimulents

1- some vegetative growth measurments and nutritional status

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Abstract

The present study carried out on fruitful Washington Navel orange trees 12 years old, budded on sour orange rootstock grown in clay loamy soils in private farm at Toukh region, El-Kalubeia Governorate to evaluate response the vegetative growth trees to foliar spray with some stimulating compounds at two concentrations for each i.e., yeast extract at 2.50 ml/l & 5.0 ml/l, sea weeds at 0.2 &0.4 ml/l, promalin at 100 & 200 ppm and humic acid at 3.0 & 6.0 g/l, beside control which sprayed with tap water during 2019 and 2020 seasons. The results indicated that, all treatments increased all studied vegetative growth measurements and improved nutritional status compared to control. Promalin at 200ppm or yeast extract at 2.5 m/l+ sea weeds extract at 0.2m/l + promalin at 100 ppm gave highest values of vegetative growth measurements, leaf photosynthetic pigments and mineral content.

Key words: yeast extract, sea weeds extract, promalin, humic acid, citrus vegetative growth, nutritional status.

Introduction

The citrus (*Citrus spp*.) is considered one of the most important fruit cropsgrown in many tropical and subtropical countries. The Egyptian Agriculture statistics in 2019 indicated that, citrus total planted area reached 477510 feddans and total citrus production 420000 tons of fruits, this represents 28.78 % and 37.08% of total fruits orchards area and fruits production, respectively. Total orange cultivated area represents 326484 feddans with total fruits production of 3147545 tons. (**Ministry of Agriculture and land reclamation, Economic Affairs Sector, 201**9).

Nowadays, some bio-stimulating materials such as yeast extract, sea weeds extract and humic acid is a new generation of natural organic fertilizers containing highlyeffective nutritious and promotes faster germination of seeds and increase yield and resistant ability of many crops. Unlike, chemical fertilizers, extracts derived from sea weeds are biodegradable, nontoxic, nonpolluting and non-hazardous to human, animals and birds **(Dhargalkar and Pereira, 2005).**

Yeast extract and sea weeds extract are found to be viable alternatives to fertilizing input for agricultural crops due to its high level of organic matter, micro and macro elements, vitamins, fatty acids, also rich in growth regulators **(Crouch and Van-Staden, 1993).**

Yeast as a natural source of cytokinins stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid and chlorophyll **(Atawia and El-Desouky, 1998).**

Yeast is one of the richest source of high quality protein, namely the essential amino acids like lysine, tryptophan etc., contains the essential minerals and trace elements, namely calcium, cobalt, iron etc. and the best sources of the B-complex vitamins such as B1, B2, B6 and B12. The extract is a valuable source of bio-constituents especially cytokinins (**Amer, 2004).**

The growth promoting effect of liquid extract of sea weeds on germination, vegetative growth and biochemical characteristics are being carried out in some economic vegetables and fruits **(Khan *et al.*, 2009).**

Sea weeds extract application for different crops has a great importance dueto its content with high levels of organic matter, micro elements, vitamins and amino acids and also, is being rich in growth regulators (**Khan *et al.*, 2009**)

Spray orange seedlings with promalin (BA+GA4+7) increased shoot length **(Kiang, 1985)**. The number and weight of fruits /tree and fruit TSS, TSS/Acid ratio and peel thickness were significantly increased when Washington Navel orange trees were sprayed with promalin (BA+GA4+7) **(Ibrahim *et al.*, 1994).** Spray apple trees with promalin just after full bloom, with the second application one week later increased fruit size and length **(Burak *et al.*, 1998)**.

Humic substances have different effects on plants. In this respect, **Vaughan *et al.*, (1985)**, showed evidence of stimulation on plant growth by humic substances by acting on mechanisms involved in: cell respiration, photosynthesis, protein synthesis, water, and nutrient uptake, enzyme activities. From other side, humic substances appear to be beneficial in chelating nutrients, preventing their tie up on plant roots and leaves, also, improving conductivity of nutrients into plant tissue, resulting in more efficient utilization of nutrients **(Beames, 1986).**

Humic acids were reported to increase the uptake of both macro and micronutrients, such as N, P, K, Fe, and Zn thereby improving the nutritional status of the plant **(Mackowiak *et al.*, 2001)**. Humic acids may also reduce plant uptake of certain toxic metal ions, adsorbing them from the soil solution **(Strickland *et al.*, 1979)**.

Hence, this study aimed to improve growth and

nutritional status of Washington Navel orange trees by using some stimulating substances i.e., yeast extract, sea weeds extract, promalin and humic acid.

Materials And Methods

The present study was carried out in private farm at Toukh region, El-Kalubeia Governorate during two successive seasons 2019 and 2020 seasons to study the effect of some bio-stimulatingcompounds on fruitful Washington Navel orange trees (*Citrus sinensis*) budded on sour orange rootstock. The trees were 12 year-old, grown in clay loamy soil, planted at 5x5 meters and subjected to the recommended management of orange orchard. Thirty trees of almost similar as possible in growth vigour, free from diseases were carefully selected and divided into ten treatments each included three replicates. Each replicate was represented by one tree.

The Washington Navel orange (*Citrus sinensis*) trees were sprayed monthly from first March to first July (five times) with bio-stimulating compounds with two concentrations from each (yeast extract at 2.5 ml/l & 5.0 ml/l, sea weeds extract at 0.2 &0.4ml/l, promalin at 100 & 200 ppm and humic acid at 3.0 &6.0 g/l) in addition to the control treatment which was sprayed with tap water only during two experimental 2019 and 2020 seasons.

Each tree was sprayed with 5 liters of spraying solution or water (control) using hand sprayer to the run off. The applied treatments could be summarized as follows:

- Control (trees sprayed with tap water). -yeast extract at 2.5 and 5.0 ml/L. - sea-weeds extract at 0.2 and 0.4 ml/L. - promalin at 100 and 200 ppm. - humic acid at 3.0 and 6.0 g/L. - yeast extract at 2.5 ml/l. + sea-weeds extract at 0.2ml/L. + promalin at 100 ppm. + humic acid at 3.0 g/L.

- Experiment layout:

The complete randomized block design with three replications was employed for arranging the ten investigated treatments, (including control) whereas each replicate was represented by one tree. Consequently, thirty healthy fruitful Washington Navel orange trees were carefully selected, as being healthy, disease free. Chosen trees were divided according to their growth vigour into four categories (blocks) each included ten similar trees for receiving the investigated nine fertilization treatments besides the control (tap water spray). Taking into consideration that, spray treatments were applied covering the whole foliage of each tree canopy, whereas 5 liters found to be sufficient in this concern.

Methodology as has been reported in this study in order to evaluate the response of Washington Navel orange trees under studied to various bio-stimulating treatments were carried out through determining changesin different measurements of the following examined characteristics:

On mid February 2019 and 2020, four main branches well distributed around each tree periphery were carefully selected and tagged during 1st and 2nd seasons. Moreover, 15 newly spring developed shoots were also labeled.

- Vegetative growth measurements:

On mid October 2019 and 2020 years the following vegetative growth parameters were determined during 1st and 2nd experimental seasons.

In this regard, average number of newly developed shoots per one meter of every tagged limb, average (length & thickness) and number of leaves, per each labeled shoot were estimated. Besides, average leaf area (cm^2) and leaf dry weight (g) were also determined.

-Leaf chemical analysis:

-Photosynthetic pigments:

Total chlorophyll and carotenoids were calorimetrically determined in leaves, according to the methods described by **Wettestein (1957)** and calculated as mg/g fresh weight.

- Leaf mineral content:

-Total Nitrogen: Total leaf (N) was determined by the modified micro Kjeldahl method mentioned by **(Pregl, 1945).**

- **Total phosphorus:** Total lea (P) was determined strongly recommended by **(Piper, 1958).**

- Total potassium: Total leaf (K) was determined photo- metrically the method described by **(Brown and Lilliand, 1946).**

- Calcium and Mg percentages as well as Iron, Manganese and Zinc were determined using the Atomic absorption spectrophotometer "Perkin Elmer -3300" after (**Chapman and Pratt, 1975)**

- Statistical analysis:

All obtained data during the two seasons were statistically analyzed using the analysis of variance method according to **Sendecorand Cochran (1990)**. However, means were distinguished by the Duncan's Multiple Range Test (**Duncan, 1955**), which used to compare between them.

Results And Discussions

Data obtained during both 2019 and 2020 experimental seasons could be summarized as follow**:**

-Vegetative growth measurements:

Referring the influence of differential bio-stimulating compounds treatments on some vegetative growth measurements i. e., number of shoots per one meter length limb, shoot length, shoot diameter (cm), number of leaves per an individual shoot, leaf area and leaves dry weigh of Washington Navel orange trees. Data presented in **Tables (1 and 2)** indicated that, all treatments significantly increased all studied vegetative growth measurements compared to control. Promalin at 200ppm or yeast extract at 2.5 ml/L+ sea weeds extract at 0.2ml/L + promalin at 100 ppm + promalin at 100 ppm gave highest values all vegetative

growth measurements during 1st and 2ed seasons. On the other hand, other investigated treatments could be significantly arranged into the following descending order i.e., promalin at 100 ppm, yeast extract at 5.0, sea weeds extract at 0.4 ml/l, yeast extract at 2.5 ml/l, humic acid at 3.0 g/l and 6.0 g/l during 2019 and 2020 seasons.

In this respect, it could be noticed that, the present results are in general accordance with those previously found by **Ahmed *et al*., (2013)** on growth measurements of sea weeds extract treatments. While, **El-Tanany (2018)** concerning the effect of yeast extract bio-stimulants on some vegetative growth measurements, whereas, he revealed that, all studied bio-stimulants at different concentrations resulted significantly increased in growth behaviors.

Table 1. Effect of foliar spray with yeast extract, sea weeds extract, promalin and humic acid on number of shoots, shoot length and shoot diameter of Washington Navel orange trees during 2019 and 2020 seasons.

Parameters	Number of shoots/one meter limb		Shoot length (cm)		Shoot diameter (cm)	
Treatments	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
T1- Control	12.34 g	15.00 g	19.00 h	20.00 f	1.94 d	2.04 c
T2- Yeast extract at 2.5 m/l	16.00 de	18.00 def	26.66 d	31.33 bc	2.54 bcd	2.80 bc
T3-Yeast extract at 5.0 m/l	17.67 bcd	19.67 cd	30.33 c	33.00 ab	3.00 abc	3.24 abc
T4- Sea weeds extract at 0.2m/l	14.34 efg	16.67 fg	23.00 f	24.33 de	2.14 bcd	2.20 c
T5- Sea weeds extract at 0.4m/l	17.00 cd	19.00 de	26.00 de	28.00 cd	2.30 bcd	2.64 bc
T6- Promalin at 100 ppm	18.67 bc	21.67 bc	31.00 bc	34.00 ab	3.00 abc	3.50 ab
T7- Promalin at 200 ppm	22.34 a	24.34 a	33.33 a	35.33 a	4.00 a	4.34 a
T8- Humic acid at 3.0 g/l	15.67 def	17.00 efg	24.66 e	26.66 d	2.27 bcd	2.37 bc
T9- Humic acid at 6.0 g/l	13.67 fg	15.67 g	21.00 g	22.33 ef	2.00 cd	2.10 c
T10= T2 + T4 + T6 + T8	19.67 b	22.00 b	32.33 ab	34.33 ab	3.07 ab	3.54 ab

Mean followed by the same letters within each column are not significantly different at 0.5 level.

Table 2. Effect of foliar spray with yeast extract, sea weeds extract, promalin and humic acid on number of leaves, leaf area and leaf dry weight of Washington Navel orange trees during 2019 and 2020 seasons**.**

Parameters Treatments	Number of Leaves /shoot		Leaf area $(cm)^2$		Leaf dry weight (mg)	
	2019	**2020**	**2019**	**2020**	**2019**	**2020**
T1- Control	18.00 e	17.48 d	17.48 d	18.35 e	256.33 g	267.67 g
T2- Yeast extract at 2.5 m/l	23.00 cd	26.47 a	26.47 a	27.49 a	366.33 cd	389.33 bc
T3-Yeast extract at 5.0 m/l	24.67 bc	27.31 a	27.31 a	28.07 a	369.33 bc	395.33 ab
T4- Sea weeds extract at 0.2m/l	19.00 e	19.67 cd	19.67 cd	21.32 cd	331.33 f	361.00 f
T5- Sea weeds extract at 0.4m/l	21.00 de	23.13 b	23.13 b	23.81 b	359.00cde	383.00 cd
T6- Promalin at 100 ppm	26.00 bc	26.51 a	26.51 a	27.24 a	390.00 ab	399.33 ab
T7- Promalin at 200 ppm	30. 00 a	27.27 a	27.27 a	28.18 a	395.33 a	402.67 a
T8- Humic acid at 3.0 g/l	20. 67 de	20.94 bc	20.94 bc	22.07 bc	346.33def	373.00 de
T9- Humic acid at 6.0 g/l	18.34 e	18.78 d	18.78 d	19.58 de	342.00 ef	367.67 ef
T10= T2 + T4 + T6 + T8	27.00 ab	26.78 a	26.78 a	27.55 a	389.33 ab	399.67 ab

Mean followed by the same letters within each column are not significantly different at 0.5 level.

- Nutritional status:

Concerning the influence of the different applied bio-stimulating compounds treatments on the nutritional status i.e. photosynthetic pigments, leaf N, P, K, Ca, Mg, Fe, Zn and Mn content.

Data tabulated in **Tables (3, 4, 5 and 6)** indicated that, different applied treatments i.e., promalin at 200ppm or yeast extract at 2.5 ml/l+ sea weeds extract at 0.2ml/l + promalin at 100 ppm and promalin at 100ppm significantly increased photosynthetic pigments (total chlorophylls and carotenoids); leaf N, P, K, Ca, Mg content of Washington Navel orange trees during both seasons. The maximum significantly affect was observed with using promalin at 200 ppm during two seasons. In the same context the second effect of photosynthetic pigments, leaf N, P, K, Ca, Mg, Fe, Zn and Mn content were noticed with using promalin at 200ppm or yeast extract at 2.5 m/l+ sea weeds extract at 0.2m/l + promalin at 100 ppm during 2019 & 2020 seasons. Latest increase with using humic acid at 6.0 g/l during 2019 and 2020

seasons.

Generally, the obtained results it could be safely concluded that, all bio-stimulating treatments increased leaf photosynthetic pigments (total chlorophyll and carotenoids), leaf N, P, K, Ca, Mg, Fe, Zn and Mn contents as compared with the control, however promalin at 200 ppm and promalin at 200ppm or yeast extract at 2.5 ml/l+ sea weeds extract at 0.2ml/l + promalin at 100 ppm were the most effective in regards during two seasons. These results are of great interest, because they are lightly considered direct reason for the more dry matter production and distribution in shoots of orange trees as affected by different applied treatments.

The obtained results are agreement with **Bakry (2007) and Ataweia *et al.*, (2017)** who spray these investigated bio-stimulating compounds under study on citrus species and reported that, foliar spray with the studied compounds at different concentrations increased pigments content of leaves.

In this respect, the obtained increase of macro and micro elements could be attributed to those beneficial effects of Promalin, yeast and sea-weed extracts on nutrient availability, vital enzymes and accumulation of essential nutrients in samples and increased N, P, K, Ca, Mg, Fe, Zn and Mn concentrations in leaf. Other studies, also nearly got similar results **Atawia and El-Desouky (1998) and Ahmed e*t al.*, (2013)** on Washington Navel orange trees and different growth substances such as auxins promote growth, increase building metabolites, retard senescence, enhance cell division, chlorophyll accumulation and stimulate dry matter production as a result of higher photosynthetic activity and consequently increased translocation and accumulation of macro and micro elements in plant organs.

Increase of mineral concentration causes an enhancement plant growth and mineral nutrients uptake and translocation or partially due to that sugar acts as an osmoregulation in plant cell; that process that participates in enhancing mineral uptake and translocation in plants and consequently higher concentration of mineral in plant tissues **(Brown *et al.*, 1993)**.

Also, bio-stimulating treatments (yeast extract, sea-weeds extract, promalin and humic acid) is important element in energy storage or structure integrity functions including sugar transport and accumulation in sink (fruit properties) cell wall synthesis signification and cell wall structure, carbohydrate and other bio constituents mobilization **El-Tanany and Mohamed (2016)**.

Table 3. Effect of foliar spray with yeast extract, sea weeds extract, promalin and humic acid on chlorophyll and carotenoids pigments of Washington Navel orange trees during 2019 and 2020 seasons.

Parameters / Treatments	Total chlorophyll (mg/g f. w.)		Total carotenoids (mg/g f. w.)	
	2019	2020	2019	2020
T1- Control	2.30 e	2.37 e	1.22 bc	1.20 f
T2- Yeast extract at 2.5 m/l	2.80 bcd	3.00 bcd	1.42 a	1.50 cd
T3-Yeast extract at 5.0 m/l	2.97 bc	3.17 bc	1.42 a	1.54 bc
T4- Sea weeds extract at 0.2m/l	2.30 e	2.44 e	1.21 c	1.25 f
T5- Sea weeds extract at 0.4m/l	2.64 cde	2.80 cde	1.30 b	1.41 de
T6- Promalin at 100 ppm	3.20 ab	3.37 ab	1.39 a	1.60 ab
T7- Promalin at 200 ppm	3.44 a	3.67 a	1.48 a	1.69 a
T8- Humic acid at 3.0 g/l	2.47 de	2.64 de	1.24 bc	1.38 e
T9- Humic acid at 6.0 g/l	2.27 e	2.40 e	1.19 c	1.21 f
T10= T2 + T4 + T6 + T8	3.24 ab	23.40 ab	1.40 a	1.60 ab

Mean followed by the same letters within each column are not significantly different at 0.5 level.

Table 4. Effect of foliar spray with yeast extract, sea weeds extract, promalin and humic acid on leaves nitrogen, phosphorus and potassium content of Washington Navel orange trees during 2019 and 2020 seasons.

Parameters	N (%)		P (%)		K (%)	
Treatments	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
T1- Control	1.90 h	1.94 h	0.38 g	0.39 f	0.94 g	0.97 h
T2- Yeast extract at 2.5 m/l	2.90 d	3.04 d	0.59 d	0.62 c	1.57 c	`1.70 cd
T3-Yeast extract at 5.0 m/l	3.10 c	3.27 c	0.65 c	0.69 b	1.74 b	1.87 bc
T4- Sea weeds extract at 0.2m/l	2.17 g	2.34 f	0.44 ef	0.47 de	1.10 f	1.24 g
T5- Sea weeds extract at 0.4m/l	2.70 e	2.84 e	0.57 d	0.59 c	1.40 d	1.57 de
T6- Promalin at 100 ppm	3.34 b	3.50 b	0.68 bc	0.71 ab	1.80 b	1.97 ab
T7- Promalin at 200 ppm	3.67 a	3.77 a	0.72 a	0.74 a	1.97 a	2.10 a
T8- Humic acid at 3.0 g/l	2.44 f	2.70 e	0.47 e	0.51 d	1.27 de	1.44 ef
T9- Humic acid at 6.0 g/l	2.00 h	2.14 g	0.42 f	0.46 e	1.17 ef	1.35 fg
T10= T2 + T4 + T6 + T8	3.37 b	3.54 b	0.69 ab	0.71 ab	1.84 ab	2.00 ab

Mean followed by the same letters within each column are not significantly different at 0.5 level.

Table 5. Effect of foliar spray with yeast extract, sea weeds extract, Promalin and humic acid on some leaf calcium and magnesium content of Washington Navel orange trees during 2019 and 2020 seasons.

Parameters	Ca (%)		Mg (%)	
Treatments	2019	2020	2019	2020
T1- Control	1.20 g	1.26 g	0.29 h	0.31 f
T2- Yeast extract at 2.5 m/l	1.52 cd	1.57 cd	0.57 d	0.58 c
T3-Yeast extract at 5.0 m/l	1.58 bc	1.61 bc	0.59 c	0.61 bc
T4- Sea weeds extract at 0.2m/l	1.42 ef	1.46 f	0.47 fg	0.49 e
T5- Sea weeds extract at 0.4m/l	1.48 de	1.53 de	0.52 e	0.53 d
T6- Promalin at 100 ppm	1.63 ab	1.66 ab	0.61 b	0.63 b
T7- Promalin at 200 ppm	1.66 a	1.71 a	0.65 a	0.67 a
T8- Humic acid at 3.0 g/l	1.45 ef	1.49 ef	0.49 f	0.50 e
T9- Humic acid at 6.0 g/l	1.39 f	1.43 f	0.46 g	0.48 e
T10= T2 + T4 + T6 + T8	1.64 a	1.68 a	0.62 b	0.63 b

Mean followed by the same letters within each column are not significantly different at 0.5 level.

Table 6. Effect of foliar spray with yeast extract, sea weeds extract, promalin and humic acid on leaf iron manganese and zinc content of Washington Navel orange trees during 2019 and 2020 seasons**.**

Parameters	Fe (ppm)		Mn (ppm)		Zn (ppm)	
Treatments	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
T1- Control	64.54 i	65.44 i	38.87 g	39.10 g	66.07 h	66.54 h
T2- Yeast extract at 2.5 m/l	79.54 d	80 .57 d	80.97 c	85.64 c	81.74 d	83.80 d
T3-Yeast extract at 5.0 m/l	82.04 c	83. 84 c	86.67 b	89.64 b	85.00 c	87.04 c
T4- Sea weeds extract at 0.2m/l	69.94 g	71.30 g	67.60 f	71.27 f	73.47 g	75.80 g
T5- Sea weeds extract at 0.4m/l	76.70 e	78.37 e	74.00 d	77.10 d	78.67 e	80.30 e
T6- Promalin at 100 ppm	84.54 b	85.44 b	90.10 a	93.50 a	88.64 b	89.94 b
T7- Promalin at 200 ppm	86.04 a	87.74 a	91.77 a	94.74 a	90.00 a	91.87 a
T8- Humic acid at 3.0 g/l	73.17 f	75.27 f	69.97 e	73.84 e	75.47 f	77.30 f
T9- Humic acid at 6.0 g/l	66.30 h	67.54 h	66.97 f	69.77 f	73.24 g	75.00 g
T10= T2 + T4 + T6 + T8	84.57 b	85.47 b	90.14 a	93.54 a	88.67 b	89.97 b

Mean followed by the same letters within each column are not significantly different at 0.5 level.

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استجابة اشجار البرتقال ابوسرة للرش الورقى ببعض المنشطات

1- النمو الخضرى والحالة الغذائية

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تم اجراء هذه الدراسة على أشجار البرتقال أبوسرة عمرها 12 سنة والمطعومة على أصل النارنج والمنزرعة على مسافة 5 × 5 متر فى تربة طينية فى منطقة طوخ بمحافظة القليوبية لتقييم استجابة هذه الاشجار للرش الورقى ببعض المركبات المنشطة مثل مستخلص الخميرة ومستخلص الاعشاب البحرية والبرومالين وحمض الهيوميك. وقد أوضحت النتائج المتحصل عليها ان رش أشجار البرتقال أبوسرة باى من هذه المركبات المنشطة قد حسن من النمو الخضرى والحالة الغذائية مقارنة برش الاشجار بالماء (المقارنة). كما أوضحت النتائج أن المعاملة بالبرومالين بتركيز 200 جزء فى المليون جاءت فى المرتبة الاولى بينما جاءت فى المرتبة الثانية المعاملة بمستخلص الخميرة 2.5 مل/لتر + مستخلص الاعشاب البحرية 2مل/لتر + البرومالين 100 جزء فى المليون + حمض الهيوميك 3جم/ لتر حيث أعطت هذه المعاملات أفضل النتائج فى قياسات النمو الخضرى مثل عدد الافرخ وطولها وسمكها وعدد الاوراق ومساحة الورقة وكذلك محتوى الاوراق من صبغات التمثيل الضوئي والعناصر الكبرى والصغري التى تم تقديرها مقارنة بباقى المعاملات خلال موسمى الدراسة.