Journal of University of Duhok., Vol. 21, No.1 (Agri. and Vet. Sciences), Pp 67-74, 2018
https://doi.org/10.26682/avuod.2019.21.1.8

INFLUENCE OF SEEDS SOAKING PERIODS WITH DIFFERENT CONCENTRATIONS OF HUMIC ACID IN IMPROVEMENT THE GERMINATION AND GROWTH OF BITTER ORANGE SEEDS *(Citrus aurantium)*

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(Received: June 6, 2018; Accepted for publication: October 2, 2018)

ABSTRACT

This investigation was carried out during the growing season of 2017 in the lath house belonged to the nursery of Horticulture Department, College of Agriculture / University of Duhok, Kurdistan region, Iraq. The study aimed to the assess the positive influence of using humic acid in improving the growth of Bitter orange seeds *(Citrus aurantium*) which soaked for 5, 10, and 15 hours in concentrations 0, 250, 500, 1000 and 1500 mg·L^{-1} humic acid and the interactions between them. The obtained results showed that the soaked the bitter orange seeds were treated with for 15 hours recorded a significant increase stem diameter (mm), root length (cm), leaves number and leaves dry weight%, when seeds soaked 10 hours a significant increase seedling height(cm), leaf area(cm^2), germination%, (leaves, shoot and root dry weight %) while the seeds soaked for 5hours significantly increase leaf area(cm^2), germination% and shoot dry weight% , for the interaction between the soaking periods (hr) and humic acid concentration shows that the seeds soaking in 10 hours with 1500 mg.L^{-1} gave the best results with the germination (%), leaves number, seedling height(cm) and (shoot , root dry weight %). Also the seeds soaking with humic acid at 1500 mg.L^{-1} at 15 hours improvement the chlorophyll (%), leaves dry weight (%) and root length (cm).

KEY WORDS: Bitter orange, Soaking periods, Germination%, Humic acid

1. INTRODUCTION

Citrus *(Citrus aurantium*) is considered as one of the most important fruit crops grown in many tropical and subtropical countries. In the world it is yielded almost 40 million metric tons of oranges, lemons and lime (Anonymous, 2008). Bitter orange belong to Rutaceae family is the latin name for the plant usually referred to as bitter orange, sour orange, or Seville orange, in countries around the world where it is used for food, fragrance and medicinal purposes (Preuss, *et al*,.2002; Tang and Eisenbrand, 1992). Bitter orange peel contains a volatite oil with limonene, coumarins, triterpenes, flavonoids, vitamin C, carotene and pectin. The flavonoids have several useful properties, bing anti- inflammatory, antibacterial and antifungal (Suryawanshi, 2011).

Citrus propagation is usually done by seeds to obtain the rootstocks and then budding or grafting is applied on to production good crops and resistance to virus diseases. The rootstocks utilization is beneficial for solving the problems caused by soil, climates, pests and diseases; achieving higher productivity and quality, earlier and later fruit productions. Bitter orange used as rootstock to the most of citrus, where as a good rootstock to the orange, grapefruit and tangerine (Agha and Daoud, 1991; Celil *et al*., 2008). Citrus seed germination is usually slow and erratic due to presence of growth inhibitors and physical resistance of seed coat to radicle protrusion (Cohen, 1956). Nursery owners are highly interested to get higher and earlier germination percent associated with rapid growth of the obtained seedlings to fulfill maximal benefit that will be reflected on their profit. Thus, such task was addressed by implementing pre sowing seed treatment soaks (Castle, 1982). Alalaf and Shayal-Alalam (2014) mentioned that it is necessary to use safe, cheap and easy to use material characterized by low pollution of the environment and agricultural products such as the using of humic acid and others in citrus propagation.

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Humic acid is a constituent of organic matter (Asik *et al*., 2009; Katkat *et al*., 2009). Humic acid supplies both macro- and micro- nutrients to growing plants, increases soil fertility and productivity, enhances seed germination, humic acid also reduces the other chemical fertilizer requirements, increases aeration of the soil, increases the protein and mineral contents of most crops (Salman *et al*., 2005). Humic acid may increase root growth in a manner similar to auxins (Donnell, 1973).

Hassan *et al*., (2010) indicated that, the olive trees when treated with mineral nitrogen source at 100 % + humic acid for 9 months respectively gave higher value of leaves dry weight per plant. Yousef *et al*., (2011) mentioned that treated Chemlali olive seedlings with humic acid +amino acids+ macro elements+ trace elements this treatment gave the best results concerning plant height, brunch numbers, leaf numbers, also it increased plant diameter and leaves area comparing with control. Abbas *et al*., (2013) reported that the application of humate gave rise enhancement of total chlorophyll contents, stomatal conductance, and net photosynthesis rate has resulted in greater plant growth. The permeability of plant membranes due to humate application resulted in improving growth of various groups of beneficial microorganisms; accelerate cell division, increased root growth and all plant organs for different fruit trees (Nardi *et al*., 2002; Ferrara and Brunetti, 2010). Humic substances can improve nutrient uptake and plant growth (Khaled and Fawy, 2011). Humic acid is highly beneficial to plants and soil, increase microbial activity, a plant growth bio-stimulant, an effective soil enhancer, promote nutrient uptake (Mansour, 2007).

The aim of this research is to study the influence of soaking periods with different concentrations of humic acid on bitter orange seeds germination to improve growth and obtain good rootstock to budding on and to increase the growth vigor of bitter orange seedlings which is the most common citrus rootstock.

2. MATERIALS AND METHODS

The study was conducted in the lath house of Agriculture College, University of Duhok during growing season 2017. Ripe fresh bitter orange fruits (*Citrus aurantium*) were used for this experiment. The seeds were extracted from ripened healthy and free diseases fruits. Seeds were separated from pulp and washed properly in clean water and then soaking in 5, 10 and 15 hours with five concentrations (0, 250, 500, 1000 and 1500 $mg.L^{-1}$) of humic acid, after then the seeds were sown in boxes containing the soil on 25 / 2 / 2017 after 40 days of planting, seeds began to germinate.

During the entire period of study (from 25 February to 25 October 2017) the seedlings were kept moist by timely application of water.

The treatments were arranged in randomized complete block design with three replicates for each treatment and each replicate was represented by five plants. At the end of October, the plants of each treatment were removed genteilly with their root system to estimate and record the following data:

2.1. Percentage of seeds germination%: After 40 days of planted the seeds, the number of seeds germinated in each replication calculated on percentage basis as below (Stephen, 2008).

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

2.2. Seedling height (cm): Height was measured at the end of investigation by using the measuring tape

2.3. Stem diameter (mm): The diameter of stem was measured by hand Vernier just above the ground surface

2.4. Leaves number/seedling: The total number of leaves per seedling was counted and the average was calculated.

2.5. Leaf area (cm^2): Estimated by the leaf area meter (Area meter AM-300).

2.6. Chlorophyll (%): The chlorophyll content index was recorded by using chlorophyll meter (Chlorophyll meter, SPAD- 502, Konica Minolta). The estimated Chlorophyll in leaves represent its ratio to the rest of pigments present in the leaves.

2.7. Leaves, Shoots and Roots Dry Weight (%): After recording the fresh weight of shoots, leaves and roots determined separately. The shoots, leaves and roots were placed in electrical oven at 70˚c until weight fixing (Gobara, 1998). Dry matter was calculated as follows:

$$(\%)\ \text{dry matter} = \frac{Dry\ \ weight}{fresh\ \ weight} \times 100$$

2.8. Root length (cm): The roots length per transplant was calculated at the end of the investigation.

3. RESULTS

3.1. Germination %: Data in Table (1) indicate that the seeds that were soaked for 15 hours showed the lowest value (60.99%), when seeds were soaked at 1500 mg.L^{-1} humic acid gave significant affect when compared with other concentrations. The interaction between soaking periods and humic acid concentrations showed that the seeds soaking for 10 hours and treated with 1500 mg.L^{-1} humic acid gave the highest value (77.48%) and a significant effect when compared with other concentrations.

3.2. Seedling height (cm): Data presented in Table (1) indicate that seedling height gave the highest value (22.8cm) when soaking with humic acid at 10 hours when compared with other concentrations. Noticed that the seedling when treated with humic acid at 1500 mg.L^{-1} gave the highest value (25.11cm) when compare with other concentrations. As for the interaction between soaking period and humic acid concentrations we notice that the seedling when soaking at 10 hours and 1500 mg.L-1 humic acid give the best value (28cm) when compared with other concentrations.

3.3. Stem diameter (mm): In table (1) indicated that the seeds soaking in humic acid at 15 hours have a significant effect when compared with other soaking periods, the seeds treated with 500 and 1000 mg.L^{-1} humic acid gave the significant effect (2.89 mm) when compared with control. On other hand to calculate the best interaction between soaking periods and humic acid concentrations notice all the interaction as the best when compared with the control that soaking 5 and 10 hours. While there are no significant effect when treated with humic acid at 15 hours.

3.4. Leaves number per plant: The results in table (1) showed that the better leaves number indicate in seeds that soaked at 15 hours (19.93 leaf), and best humic acid concentration are 1000 and 1500 mg.L^{-1} (20.11 and 20.44). For the interaction between soaking periods and humic acid concentrations it was observed that the seedling gave the significant when treated with humic acid at 1500 mg.L^{-1} at 15 hours (26 leaf).

Table (1): Effect of seeds soaking periods with different concentrations of humic acid and interaction between them on germination %, and on some vegetative growth characteristic

Treatment		Parameters			
		Germination (%)	Seedling height (cm)	Stem diameter (mm)	Leaves number /seedling
Soaking Periods (hours)	5	65.41a	20.47b	2.60 b	16.13c
	10	65.49a	22.80 a	2.53b	17.87b
	15	60.99b	22.27a	2.87 a	19.93a
Humic acid (mg.L^{-1})	0	56.37d	17.00 d	2.33 c	14.11d
	250	61.58c	23.44 b	2.67 ab	18.67 b
	500	61.13c	19.56 c	2.89 a	16.56c
	1000	67.94b	24.11 ab	2.89 a	20.11 a
	1500	72,81a	25.11 a	2.56 bc	20.44a
		period x humic acid			
5 Hours	0	50.67h	13.67i	2.00c	10.33i
	250	71.41bc	25.67 a-c	2.67ab	20.33b-d
	500	62.34g	20.33 e-g	3.00a	14.67hg
	1000	72.96ab	22.67 ed	3.00a	21.67b
	1500	69.67b-e	20.00fg	2.00c	13.67h
10 Hours	0	66.01d-g	20.00fg	2.00c	15.33gh
	250	64.75e-g	22.00 d-f	2.67ab	16.33 fg
	500	52.73h	18.67gh	3.00a	18.33d-f

	1000	66.50c-g	25.33 bc	3.00a	17.67ef
	1500	77.48a	28.00 a	2.00c	21.67 b
	0	52.44h	17.33h	3.00a	16.67fg
	250	48.58h	22.67 ed	3.00a	19.33c-e
	500	68.31b-f	19.67f-h	2.67ab	16.67fg
15 Hours	1000	64.36fg	24.33cd	2.67ab	21.00bc
	1500	71.29b-d	27.33 ab	3.00a	26.00a

*Means with a column, row followed with same letters are not significantly different from each other according to Duncan's multiple range test at 5% levels

3.5. Leaf area (cm^2); Table (2) shows that that there is no significant effects between seedlings soaked for 5 and 10 hours. The highest value of leaf area (147.51 cm^2) recorded in seedling treated with 1500 $mg.L^{-1}$ humic acid. For the interaction between soaking periods and humic acid, it can be noticed that the control seedlings that soaked for 10 hours (170.47 cm^2) gave the highest value.

3.6. Chlorophyll (%): The results of the current study indicate that the best value of chlorophyll (62.75%) was recorded in seedling that soaked for 15 hours. The best humic acid concentration recorded in seedlings treated with 500 $mg.L^{-1}$. As for the interactions between the soaking periods and humic acid concentrations it can be noticed that the seeds soaking in 500 $mg.L^{-1}$ humic acid for 10 hours and seeds immersion in 1500$mg.L^{-1}$ humic acid for 15 hours have significant effects when compared with other interactions. (Table 2)

3.7. Root length (cm); Table (2) shows that the seeds that were soaked for 15 hours (14.87cm) have significant effects when compared with other soaking periods. For the effect of humic acid concentrations, it can be noticed that the all concentrations significantly affect when compared with control that recorded the lowest value (12.78cm). For the interaction between soaking periods and humic acid concentrations, it can be noticed that the seeds that were soaked for 15 hours treated with humic acid in 0 and 1500 $mg.L^{-1}$ gave the highest values when compared with other treatments and the lowest value (10.67 cm) recorded at seeds only soaking for 10 hours.

3.8. Shoot dry weight (%): In Table (3) it can be noticed that the seeds soaked for 10 hours gave the highest value (65.49%). When the seeds were treated with 1500 $mg.L^{-1}$ they showed significant response when compared with other concentrations. For the interaction between soaking periods and humic acid concentrations, the seeds soaked for 10 hours and treated with 1500 $mg.L^{-1}$ humic acid gave the highest value (77.48%) which was is significantly higher than the other concentrations and the lowest value (48.58%) was recorded in the seeds soaked for 15 hours ,and treated with 250 $mg.L^{-1}$ humic acid

Table (2): Effect of seeds soaking periods with different concentrations of humic acid and interaction between them on leaf area (cm^2), chlorophyll % and root length (cm).

Treatment		Parameters		
		Leaf area(cm^2)	Chlorophyll%	Root length (cm)
Soaking Periods (hours)	5	145.74 a	55.21b	13.40 b
	10	146.35 a	61.43a	13.07b
	15	134.97b	62.75a	14.87a
Humic Acid ($mg.L^{-1}$)	0	143.71 ab	55.84d	12.78b
	250	143.96ab	58.21c	13.78 a
	500	135.04c	62.40a	14.22a
	1000	141.56 b	60.51b	13.89a
	1500	147.51a	62.01ab	14.22a
soaking period x humic acid				
5 Hours	0	127.57gh	50.67i	12.33d
	250	134.37e-g	58.00f-h	14.67ab
	500	148.90c	58.90e-g	13.67b-d

	1000	158.33b	55.83h	13.67b-d
	1500	159.53b	52.63i	12.67d
10 Hours	0	170.47 a	52.27i	10.67e
	250	157.73b	59.90d-f	13.00cd
	500	131.17gh	66.50a	13.33b-d
	1000	132.03gh	62.47cd	13.67b-d
	1500	140.37ed	66.00ab	14.67ab
15 Hours	0	133.10fg	64.60a-c	15.33a
	250	139.77d-f	56.73gh	13.67b-d
	500	125.07h	61.80c-e	15.6a
	1000	134.30e-g	63.23bc	14.33a-c
	1500	142.63cd	67.40a	15.33a

Means within a column, row and their interactions followed with the same letters are not Significantly different from each other according to Duncan's multiple range test at 5% level.

3.9. Root dry weight (%): from the abovementioned results, it is clear that when the seeds were soaked for 10 hours showed significant response when compared with other soaking periods. The seeds treated with humic acid at 500 $mg.L^{-1}$ gave the highest value (72.83%) when compared with other concentrations and the lowest value was recorded (51.48%) in non treated seeds. For the interactions between soaking periods and humic acid, it can be noticed that the seeds soaked for 10 hours and treated with 1500 $mg.L^{-1}$ gave the highest value (84.46%) when compared with other concentrations .(Table 3)

3.10. Leaves dry weight %: Data in Table (2) show that the best soaking period of seeds soaking at 15 hours (55.57%) and for the effect of humic acid, the seeds treated with 1500 $mg.L^{-1}$ gave a significant response when compared with the other concentrations. For the interactions between soaking periods and humic acid concentration show the highest value (80.74cm) recorded at the seedling treated with 1500 $mg.L^{-1}$ humic acid at 15 hours and is considered to have a significant effect with compared with other concentrations.

Table (3): Effect of seeds soaking periods with different concentrations of humic acid and interaction between them on shoot, root and leaves dry weight %.

Treatment		Parameters		
		Shoot dry weight %	Root dry weight %	Leaves dry weight %
Soaking Periods(hours)	5	65.41a	58.76b	51.21b
	10	65.49a	70.10a	54.93a
	15	61.00b	59.94b	55.57a
Humic Acid ($mg.L^{-1}$)	0	56.37d	51.48c	51.88bc
	250	61.58c	68.99ab	52.00bc
	500	61.13c	72.83a	49.67c
	1000	67.94b	54.96c	52.80b
	1500	72.81a	66.41b	63.17a
soaking period x humic acid				
5 Hours	0	50.67h	52.90f	49.77c-e
	250	71.41bc	72.28bc	52.73b-d
	500	62.34g	74.80bc	47.40e
	1000	72.96ab	58.77ef	50.04c-e
	1500	69.67b-e	35.06g	56.10b
10 Hours	0	66.01d-g	62.32 d-f	57.42b
	250	64.75e-g	61.69 d-f	55.88b
	500	52.73h	74.76bc	53.53 bc

	1000	66.50c-g	67.29 c-e	55.15b
	1500	77.48a	84.46a	52.68b-d
15 Hours	0	52.44h	39.22g	48.46c-e
	250	48.58h	73.00bc	47.38e
	500	68.31b-f	68.93dc	48.07de
	1000	64.36fg	38.82g	53.22 bc
	1500	71.29b-d	79.72ab	80.74 a

Means within a column, row and their interactions followed with the same letters are not Significantly different from each other according to Duncan's multiple range test at 5% level

4. DISSCUTION

In this study, the influence of humic acid in motivating seeds germination was tested. The results showed that applying humic acid has led to significant increases in germination rate of the citrus seeds and improved the seedlings growth. This is agree with Pati (2010) who reported an improvement in seed germination and seedling vigour due to humic acid addition to the rooting medium**.** The expedite effect of humic acid on seed swelling was due to the change of the protein lipid structure that causes more intensive absorption of solutions from the environment and improves the transport of water through plant water channels. (Cacco *et al*, 2000). Also the abovementioned results are conformity with those obtained by Fernández-Escobar *et al*., (1999) Who reported that, the foliar application of leonardite extracts (humic substances extracted) encourage plant growth of young olive plant. Hagagg *et al*., (2011) observed that Egazy olive seedlings treated with humic substance gave the best results concering percentage of plant height, leaves number per plant and stem diameter this due to influence on the cell division (Chen *et al.* , 2004), and enhance protein synthesis (El-Ghamry *et al*., 2009 ; Patil, 2010). Humic acid provides growth regulators, regulate and control hormone levels in plant and enhancing total protein content in plants, It also increase enzyme catalysis and enhances respiration and photosynthesis processes (Nardi *et al*., 2002). Humic acid stimulates production of plant enzyme and hormons (Mart, 2007). Also enhances the application of humic acid is promote the root length and stimulate the shoot growth (Chen and Aviad 1990; Fernandez-Escobar *et al*., 1996; Canellas *et al*., 2002). Furthermore, the growth promoting activity of humic substances was found to be caused by plant hormone-like material contained in the humic substances (Zhang and Ervin 2004). Humic materials may also increase root growth in a manner similar to auxins (Tatini *et al*., 1991). Aydin and Turan (2012) suggested that the positive effect of humic acid on plant growth through promotes plant growth by increasing cell membrane permeability, oxygen uptake, photosynthesis, phosphate uptake, and root cell elongation.

5-CONCLUSION: In conclusion, soaking duration of citrus seeds by humic acid in different concentations has the potential to enhancement germination and seedling growth.

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پوختە

ئەڤ ڤەکولینە هاتە ئەنجامدان ل وەرزێ شینبوونێ ل سالا **2017** ل سیهاتوکا داری یابسەر نە مام گە ها پشکا بیستانکاری کولیژا چاندنێ/ زانکویا دهوك, هە ریما کوردستان عیراق. ژبوتاقیکرنا کارتێکرنا ترشێ هیومیك لسەر شینبوونا توڤی برتقالا ترش (**Citrus aurantium**) کو توڤ هاتە شیل کرن بو ماوێ **5, 10** و **15** دم ژمیرا ل ترشێ هیومیك و لریژبین (**0**و **250** و **500** و **1000**و **1500**ملغم/لتر) ترشی هیومیك. ئەنجامان دیارکرکو تۆفی برتقالا ترش ئەوی هاتیە شل کرن بو ماوێ **15** دەم ژمیرا کارتیکرنا باش هەبو لسەر فەرهیا جەقا ,دریژیا قەدی ,ژمارا بەلکا و کیشەی هشك یی بەلکا **%**. .تۆڤی هاتیە شل کرن لترشی هیومیك بوماوێ **10** دەم ژمیرا کارتیکرنا باش هەبوو لەسەر دریژیا نەماما و فرهیا بەلکی وشینبوونا توڤی **%** و کیشا هشك یی(بەلکا , جەقین , قەدی **%**). دەمی توڤ هاتە شل کرن بو ماوی **5** دەم ژمیرا کارتیکرنا باش هەبوو سەر شینبوونا توڤی**%**و فەرهیا بەلکی وکیشەی هشك ی جەقین **%**. .تیکل کرنا دەم ژمیرا شلکرن دەکل ریژیت ترشا هیومیك هوسا هاتیە دیارکرن کو توڤی هاتیە شل کرن بو ماوێ **10**دەم ژمیرا د گە ل ریژی **1500** ملغم.لتر ژ ترشا هیومك باشترین ئەنجام پیکهانی هەمیشەی کلوروفیل **%** وکیشەی هشك یابەلکا**%**ودریژیاهیا قەدی (سم).

الخلاصة

اجري هذا البحث في موسم النمو لعام **2017** في الظلة الخشبية التابعة لمشتل قسم البستنة في كلية الزراعة/جامعة دهوك اقليم كردستان العراق. الدراسة هدفت الى معرفة التاثير الايجابي لاستخدام حامض الهيوميك في تحسين نمو بذور البرتقال المر حيث غمرت البذور لمدة **5**و **10** و**15** ساعة في محلول حامض الهيوميك ذو التراكيز (**0**, **250**, **500**,**1000**و **1500** ملغم / لتر) و التداخل فيما بينهما. اظهرت النتائج ان بذور البرتقال المر المغمورة لمدة **15** ساعة ادت الى زيادة معنوية في قطر الساق (مم), طول الجذور (سم), عدد الاوراق والنسبة المئوية للوزن الجاف للاوراق %. وحين غمرت البذور لمدة **10** ساعات ادت الى زيادة معنوية في النسبة المئوية للانبات , ارتفاع الشتلات , مساحة الورقة سم^2, النسبة المئوية للوزن الجاف للاوراق والافرع و الجذور %. اما بالنسبة للتداخل بين فترات النقع وتراكيز حامض الهيوميك ظهرت النتائج ان البذور المنقوعة لمدة **10** ساعات بالتركيز **1500**ملغم/ لتر حامض هيوميك اعطت افضل النتائج للنسبة المئوية للانبات**%**, عددالاوراق, ارتفاع الشتلات سموالنسبة المئوية للمادة الجافة للافرع والجذور **%** وكذلك البذور المنقوعة بالحامض هيوميك بتركيز **1500** ملغم/لتر ولمدة **15** ساعة حسنت من محتوى الاوراق من الكلوروفيل**%**, النسبة المئويةللمادة الجافة**%**وطول الجذور سم.