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Impact of foliar application of humic acid and the measure time on growth and production of roselle *Hibiscus sabdariffa* L.

ABSTRACT

The present study was accomplished to achieve the effect of Humic Acid (HA) spraying on the vegetative and calyxes yield characteristics of *Hibiscus sabdariffa* L. plants, during May ,22th to December, 31st, 2017, at (Girdarasha) open field, Department of Horticulture, Agricultural Engineering Science College, Salahaddin University. Four levels of HA were sprayed (0, 1, 2, 3 g.l^{-1}) at three frequencies (60, 75 and 90 days after sowing). The data were collected after (4, 8 and 12 weeks of last spraying) for vegetative and reproductive parameters: plant height (cm), number of branches, stem diameter (mm), days to 50% of plants flowering, flower diameter (mm), node sequence of 1st flower, number of fruits. plant^{-1} , fruit length (mm), fruit diameter (mm), fresh and dry weight of fruits. plant^{-1} (g) and fresh weight of calyxes. plant^{-1} (g). The obtained results revealed that all treatments of HA increased significantly both vegetative and reproductive parameters and shortened the number of days to flowering as compared with the control. The application of 2g.l^{-1} produced the tallest plants. Maximum numbers of branches were obtained from the treatment 3g.l^{-1} HA. The 2g.l^{-1} and 3g.l^{-1} treatments formed the thickest stems. Regarding the reproductive parameters; the highest values of fruit diameter, fruit length, fresh and dry weights of fruits. plant^{-1}, fresh and dry weights of calyxes.plant^{-1} were recorded with the treatment 3g.l^{-1} after 8 weeks of the last treatment.

1. INTRODUCTION

Hibiscus sabdariffa L. (Roselle) is belonging to the Malvaceae family. It is thought to native Asia (India to Malaysia) or Tropical Africa (Mahadevan *et al*., 2009). It is commonly known as hibiscus or Roselle, Jamaica sorrel, red sorrel, karkade, while in Iraq it is "kujarat". Roselle is a perennial plant when grows in cold climate and becomes annual plant in warm climate. It can be grown as part of multi-cropping systems and can be used as food and fiber and it is a medicinal, adorable and industrial plant (Morton,1987). It can grow in Mediterranean, subtropical, temperate, tropic climate in most continents, especially Africa, South America and Asian as noted by Abu-Zeid (2001). There are direct and indirect effects on plant growth because of the multiple roles of humic acid (Pal and Biswas, 2005). HA is excellent foliar fertilizer carriers and activators, stimulates plant growth by absorbing major and minor elements, activating enzymes, altering membrane permeability, formulating proteins and initiating the production of biomass (Ulukan, 2008). In addition, they are responsible for plant growth patterns such as leaf formation, stem and root elongation, flowering, etc. and are included in

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other biological processes such as plant growth-influencing substances such as free enzymes (Arancon *et al*. 2007). Ahmed *et al.* (2011) noticed that using of humic acid as a foliar application twice monthly on roselle plants grown in the soil mixed with compost or magnetic iron caused increasing in plant height, number of branches. plant^{-1}, stem diameter, fresh and dry weights of leaves and branches. plant^{-1}, number of days to flowering, number of fruits. plant^{-1}, fresh and dry weights of sepals .plant^{-1}and seed yield .plant^{-1} in comparison with the rest of the treatments. Mijwel *et al*. (2012) showed that foliar application of humic acid at the level of 1.5 and 2 ml.l^{-1} on *Hibiscus sabdariffa* L. significantly increased fruit weight and plant height respectively. Ramadan and El Mesairy (2015) showed that Okra plants grown with humic acid at 6 kg.fed^{-1} had the highest plant height fresh and dry weight of fruits. plant^{-1}, number of fruits. plant^{-1} , mean fruit fresh weight, plant yield and total yield.fed^{-1}. Mohammed and Saeid (2017) mentioned to that spraying of Okra plant with 40ml.l^{-1} gave highest leaf area, dry pod and seed weight, while the highest significant seed number was gained with 20 ml.l^{-1} foliar spraying. There are few studies about roselle (*Hibiscus sabdariffa* L.) treated by humic acid, therefore this study was done to consider the effects of humic acid foliar application on the vegetative growth and yield of roselle in different plant growth periods in our condition.

2. MATERIALS AND METHODS

The experiment was carried out during May 22th to December 31th 2017 at Girdarasha open field, Horticulture Department, Agricultural Engineering Science College, Salahaddin University. The experiment coordinated to study the effects of foliar spraying of humic acid on growth and calyxes yield of roselle (*Hibiscus sabdariffa* L.). Some chemical and physical properties of the soil taken from different locations of the field at 0-30 cm depth, the results of soil analysis are shown in the table (1). The metrological data during the experimental period are shown in table (2).

Table (1): Some physical and chemical properties of the soil used in the study

Properties	**Field soil**
pH	7.65
Electro conductivity (EC)	2.36 dS.m^{-1}
Organic matter	1.134%
Total potassium (K_2O)	0.440%
Total iron (Fe)	0.016%
Clay	34.710%
Silt	52.355%
Sand	12.935%
Soil Texture	Silty Clay Loam

*Laboratory of Salahaddin University College of Agriculture/ Soil and Water Department

Table (2): The metrological data during the study periods (2017)

Month	**Average temperature C°**		**Average Relative Humidity %**	**Sum of rain /mm**
	Minimum	**Maximum**		
May	10.24	38.48	27.63	6.7
June	14.21	45.17	17.33	0.8
July	21.42	46.98	12.34	0.0
August	18.29	47.06	13.32	0.0
September	14.51	45.04	15.14	0.0
October	8.04	32.62	25.61	0.0
November	0.38	29.48	52.66	19.4

*Agriculture Research Center Erbil, Ministry of Agriculture /Erbil- Iraq.

2.1 Seed Sowing and cultivation

Seeds of Roselle *Hibiscus sabdariffa* L. were obtained from the Agricultural Research Center, Ministry of Agriculture, Erbil- Iraq.

Roselle seeds were dressed by Raxil fungicide (1.5×kg.ton^{-1}) three days before sowing; the seeds were sown on May, 22th 2017, eighteen seeds per plot were sown in each plot at a rate of 3 seeds per hole at about 2.5 cm depth. After 4 weeks of sowing date thinning was done to 1 plant per hole and 6 plants per plot, leaving healthy and uniform plants (Castro *et al*., 2004; Gebremedin, 2015 and Al-Rawi and Khalaf-Alla, 1980).

2.2 Field Preparation

The land of the field location of study was ploughed 3 times by reversible and disk plow, after that looseness was done. The experiment was considered Randomized Complete Block Design (RCBD) with 3 blocks of 4 experimental units (plots) the plot area was (120×150 cm), 50 cm spacing each in width between two lines of plants (rows) in the same plot, 100 cm between two plots, the space between two plants in the same plot was 40 cm and between two plots was 80 cm. First irrigation was given just after transplanting while subsequent irrigation was applied when needed. Cultural and management practices like weeding, hoeing etc. were done in the three replications throughout the experiment.

2.3 Preparation of humic acid solutions

To make humic acid stock solution, potassium humate powder (humic Total, soluble potassium humate powder, Debbane for modern Agriculture, LTD-Iraq) was used which contain (80%) HA, the pure substance weight of the required concentrations of HA was dissolved in distilled water to reach the required volume for the highest concentration of it, then diluted by distilled water to other concentrations of HA (1, 2 and 3 g.l^{-1}) excepting control. A surfactant (Tween 20) at a concentration of 0.01% was added to all tested solutions including the control which sprayed with distilled water (Ahmed *et al*., 2011). To make a fixed amount of a dilute solution from a stock solution of the above solution preparation method, the formula: $C_1V_1 = C_2V_2$ was used where: V_1= Volume of stock solution needed to make the new solution. C_1= Concentration of stock solution. V_2 = Final volume of new solution.

2.4 Experiment design

This experiment was carried out to study the effect of foliar spraying of humic acid on vegetative and reproductive growth of *H. sabdariffa* L., in this experiment the studied factor was only the humic acid concentrations which four concentrations of humic acid were used (0, 1, 2 and 3 g.l^{-1}) for this reason each treatment sprayed three times until run-off (during the evening hours) at (60, 75 and 90) days after sowing, the control was sprayed with distilled water. The data of vegetative and reproductive growth for studied parameters were collected three times (4, 8 and 12 weeks after the last spraying), from 3 selected plants (Bakheet, 2004 and Apeyuan *et al*., 2017).

2.5 Statistical Analysis

The results were analyzed statistically, the means compared by Duncan's Multiple Range Test (DMRT) at 5% probability level (Al-Rawi and Khalaf-Alla, 1980). The statistical analysis was carried out by using SPSS (Statistical Package for Social Sciences) program (Casanova *et al*., 2004).

2.6 Experimental Parameters

2.6.1 Vegetative growth measurements

1- Plant Height (cm): was measured from the contact point of the stem with the soil to the apical point of the main shoot by using metric ruler.

2- Number of Branches. Plant^{-1}: was counted when they can be seen by naked eye.
3- Stem Diameter (mm): was measured using digital vernier calipers at the height of 5 cm from the soil surface.

2.6.2 Reproductive growth measurements
1- Days to 50% of plants flowering: was measured by counting the number of days from planting till 50% of the plants of each plot bear at least one open flower.
2- Flower Diameter (mm): three flowers at the top, middle and bottom of each three selected plants were measured for the whole plant using the digital vernier calipers, then mean of the three flowers were taken for each of the three selected plants.
3- Node sequence of first flower: was measured on the main shoot according to 50% of plants flowering.
4- Number of fruits. plant^{-1}: number of harvested fruits was counted for the same selected plants in each plot, thereafter the number of fruits. plant^{-1} was measured as follows (Wan Sembok and Norsyuhada, 2015):

Number of fruits. plant^{-1}= Number of fruits. plot^{-1}/ Number of plants. plot^{-1} (Picture 1).

5- Fruit Length (mm): fruit length was measured by digital vernier caliper for six randomly fruits of same selected plants (Wan Sembok and Norsyuhada, 2015).
6- Fruit Diameter (mm): fruit diameter was measured at the middle portion of same fruits by digital vernier caliper (Wan Sembok and Norsyuhada, 2015).
7- Fresh weight of fruits.plant^{-1}(g): fruits of 3 selected plants were collected and putted in plastic bags immediately after harvesting then weighed by electrical balance (Wan Sembok and Norsyuhada, 2015).
8- Dry weight of fruits.plant^{-1}(g): harvested fruits were dried until constant weight at 40C° by oven and calculated as follows:
Dry weight of fruits.plant^{-1}(g)=Total dry weight of fruits.plot^{-1}(g)/ Number of plants.plot^{-1} (Picture 2).
9- Fresh weight of calyxes.plant^{-1}(g): The calyxes were peeled off from the capsules of harvested fruits by hands and weighted immediately and calculated as follows (Timothy and Futuless, 2013):
Fresh weight of calyxes.plant^{-1}(g) = fresh weight of calyxes.plot^{-1}(g)/ Number of plants.plot^{-1} (Picture 3).
10- Dry weight of calyxes.plant^{-1}(g): fresh calyxes were dried until constant weight at 40C° by oven and calculated as follows (Timothy and Futuless, 2013):

Dry weight of calyxes.plant^{-1}(g) =dry weight of calyxes.plot^{-1}(g)/Number of plants.plot^{-1}

Picture 1. Number of fruits of *Hibiscus sabdariffa* L.

Picture 2. Calyxes of *Hibiscus sabdariffa* L. dried in oven

Picture 3. Calyxes peeled off from the capsules of fruits of *Hibiscus sabdariffa* L.

3. RESULTS AND DISCUSSION

3.1 Vegetative growth Parameters:

Spraying of different levels of humic acid caused significant effects on plant height (figure, 1). The highest value (102.00 cm) was recorded from 2g.l^{-1} humic acid foliar application after 12 weeks from the last treatment.

However, the highest number of branches. plant^{-1} (45.00) was obtained from Roselle plants when treated with 3g.l^{-1} after 12 weeks from the last treatment (figure, 2) and the lowest value (21.00) was counted from (2g.l^{-1}) 12 weeks after last treatment.

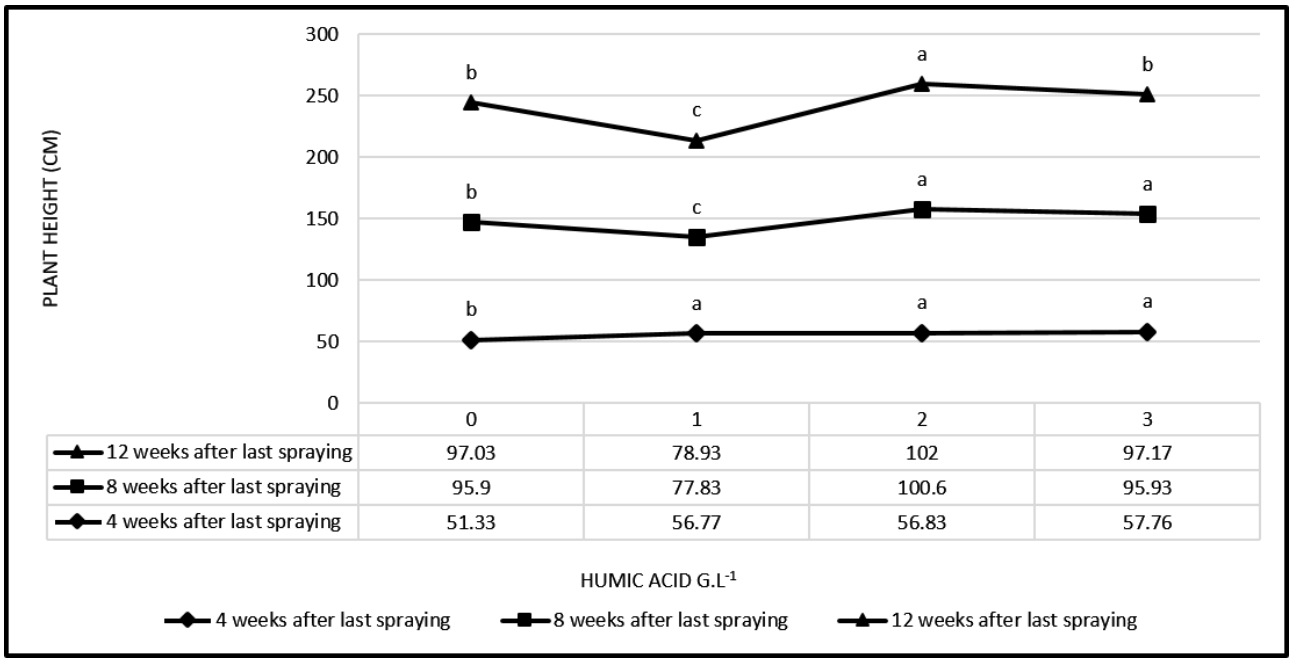


Figure 1. Effect of humic acid sprayed three times on plant height of *Hibiscus sabdariffa* L.
*Horizontal bars with the same letters are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level.

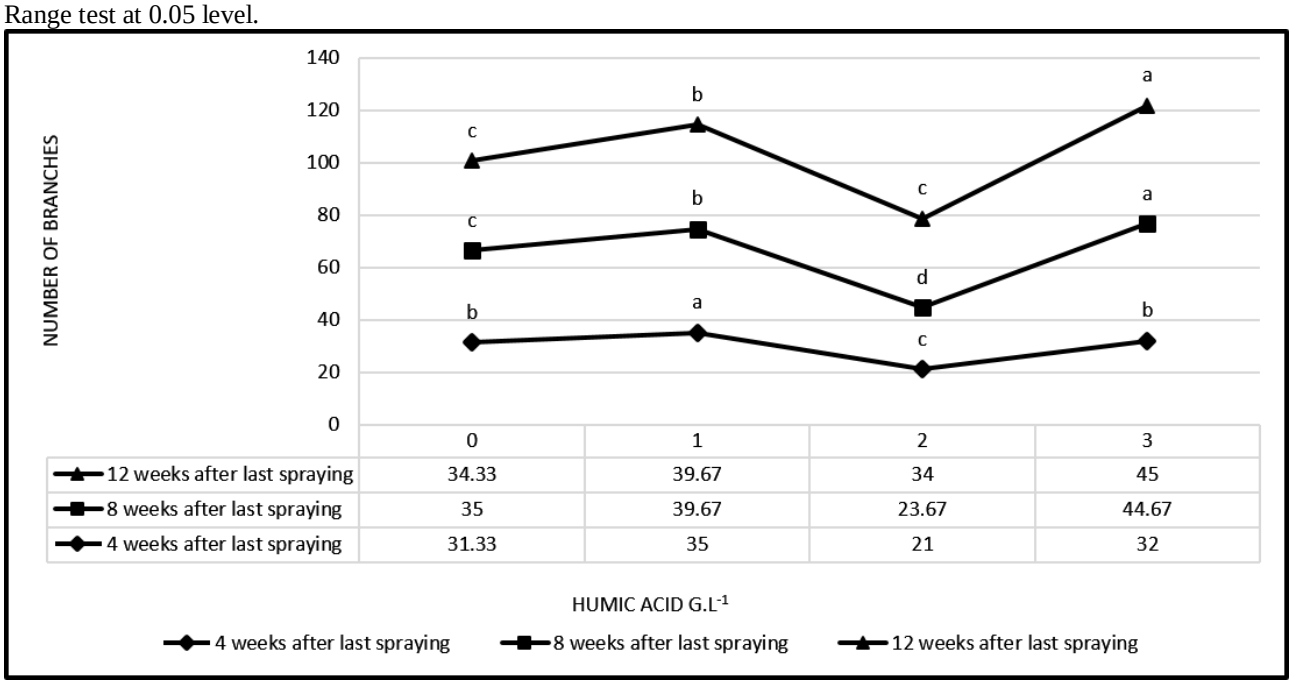
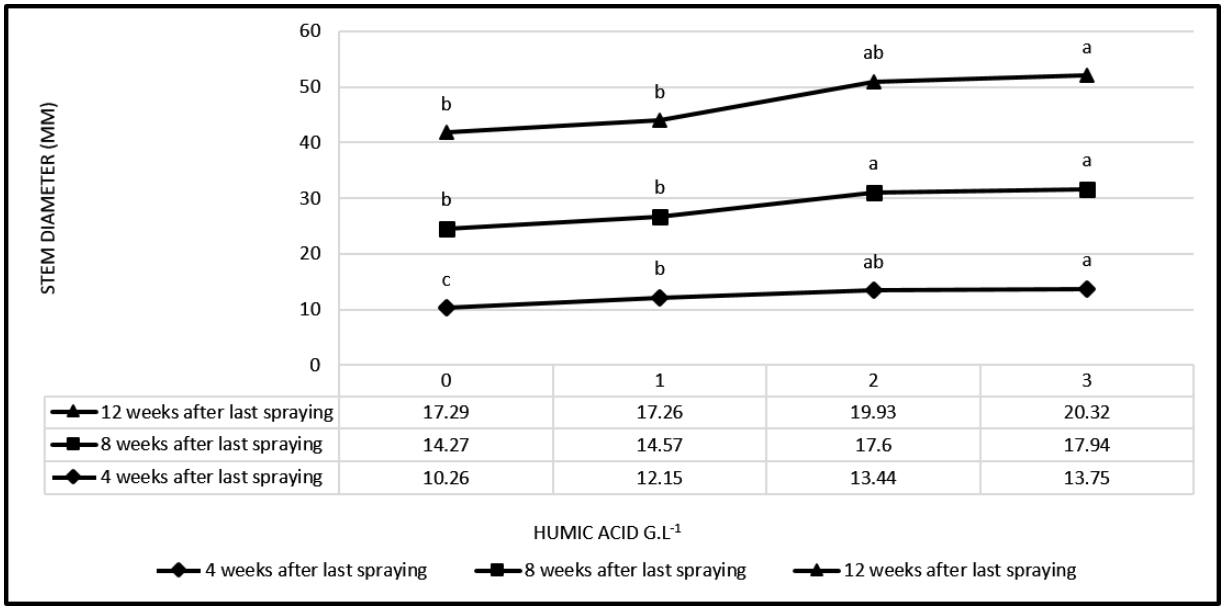


Figure 2. Effect of humic acid sprayed three times on number of branches of *Hibiscus sabdariffa* L.
*Horizontal bars with the same letters are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level.

Figure (3) shows significant differences among different levels of humic acid on stem diameter; the best value was (20.32 mm) measured from spraying of 3 g.l^{-1} humic acid after 12 weeks from the last treatment with no significant differences after 4 and 8 weeks from the last treatment. While, the minimum value was in the control 4weeks after last treatment. These results are partially agreed with those reported by (Ahmed, 2011 and Fathi and Bahamin, 2018) when they found that foliar application of humic acid had significant increase on vegetative growth of *Hibiscus sabdariffa* L. plants. Similar results were discovered by (Ramadan and El Mesairy, 2015 and Mohammed and Saeid 2017) in Okra plants, they observed that spraying of humic acid caused significant increase in plant height and number of branches. The increase in plant height was probably due to synergistic and beneficial effects of humic acid that adequate nutrient availability thus resulted in greater internodal elongation. These growth responses most probably due to hormone-like activity of humic acids could have been due to plant growth hormones adsorbed onto the humates (Atiyeh *et al*., 2002). Increases in vegetative growths of roselle plants as a result of humic acid application may be due to the increase in plants resistance to unfavorable stress factors, which improve a process of nitrogen assimibility, but preventing formation of nitrates, at the same time facilitating synthesis of chlorophyll, sugars, vitamins, essential amino acids and oils (Khalil, 2012).



	0	1	2	3
12 weeks after last spraying	17.29	17.26	19.93	20.32
8 weeks after last spraying	14.27	14.57	17.6	17.94
4 weeks after last spraying	10.26	12.15	13.44	13.75

Figure3. Effect of humic acid on stem diameter of *Hibiscus sabdariffa* L.
*Horizontal bars with the same letters are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level.

3.2. Reproductive Parameters:

Humic acid affected significantly on the number of days required to reach 50% of plants flowering (figure 4). The results revealed that the treatment of 3g. l^{-1} humic acid showed significantly minimum number of days (101.33) to reach 50% of plants flowering followed by 2g. l^{-1} (105.33) then 1g.l^{-1} (110.33) when compared with control treatment which take longest time (116.33 days) to attain 50% of plants flowering. Partially similar results were recorded by (Ahmed, 2011) on number of days to flowering when the plants sown in organic media condition and sprayed with Humic acid.

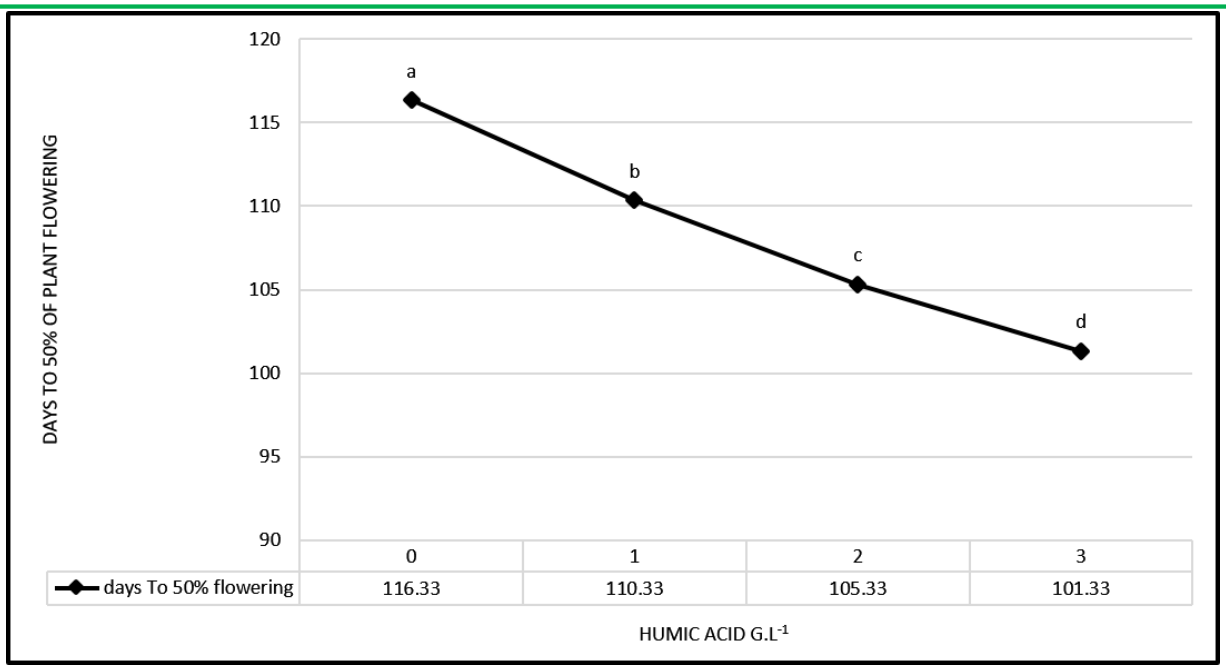


Figure 4. Effect of humic acid sprayed three times on days to 50% of plants flowering of *Hibiscus sabdariffa* L.

*Horizontal bars with the same letters are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level.

These results are partially in congruence with those observed by (Haider *et al*., 2017) on different cultivars of Okra plants when they applied humic acid on the soil.

The results in (figure 5) revealed that there are no significant differences among humic acid levels at 4 weeks after the last treatment but on flower diameter control treatment shows significant differences over the others which recorded the highest flower diameter (27.60 mm) at 8 weeks after the last treatment. humic acid show significant effect on the node sequence of first flower (figure 6), the highest value (44.66) was received from the level 3g.l^{-1}, whereas, the lowest (23.00) was received from the level 2g.l^{-1} and recognized as the best treatment.

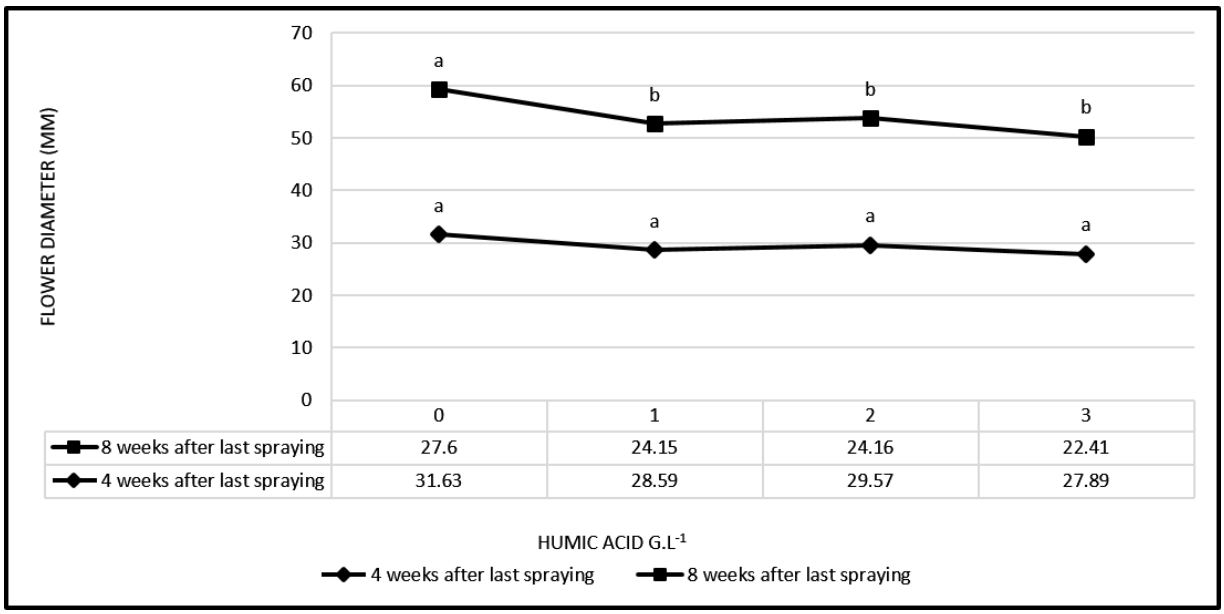


Figure 5. Effect of humic acid sprayed three times on flower diameter of *Hibiscus sabdariffa* L.

*Horizontal bars with the same letters are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level.

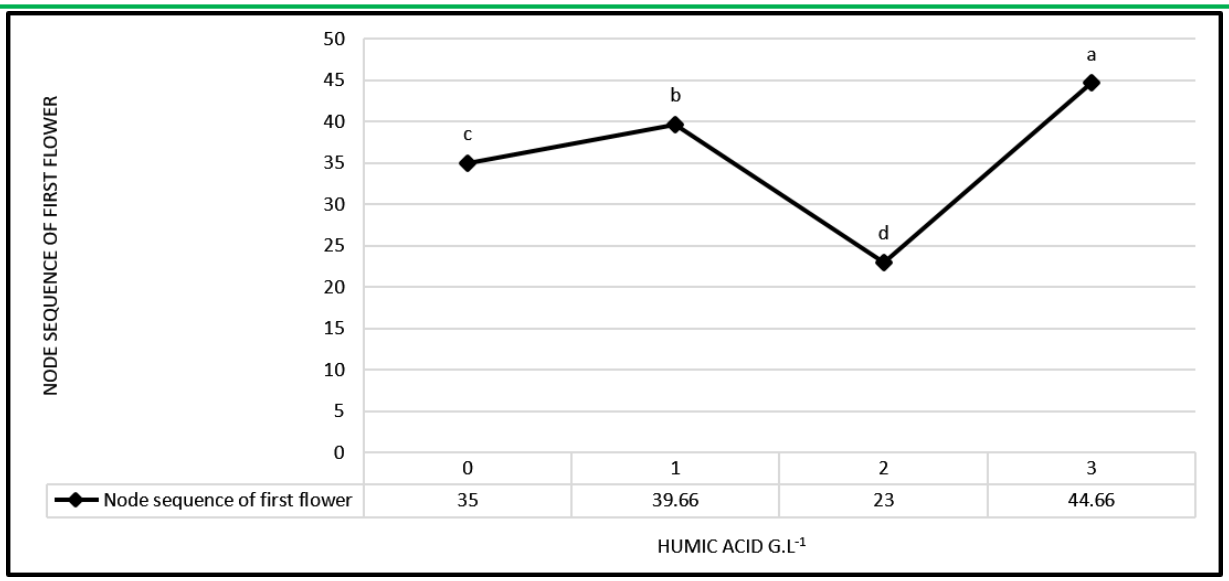


Figure 6. Effect of humic acid sprayed three times on node sequence of first flower of *Hibiscus sabdariffa* L

*Horizontal bars with the same letters are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level.

The positive effects of humic acid on flowering attributes which noticed in our results may be due to that humic acid promote both shoot and root growth improved directly the flowering and yield properties (Ahmed *et al*., 2011 and Stevenson, 1994). Table (3) shows that the levels of humic acid caused significant effects on all studied parameters except fruit diameter at first harvest (8 weeks after the last treatment), however they recorded superior values over the values of second harvest (12 weeks after the last treatment).

The data of 12 weeks after the last treatment was recorded highest number of fruits (72.00) was obtained from 1mg.l^{-1} humic acid and the highest values of fruit length and diameter (39.75 and 20.74 mm respectively) when treated with 3g.l^{-1} humic acid. While, the highest fresh and dry weights of fruits and calyxes. plant^{-1} (185.50, 39.27, 95.26 and 15.32 g respectively) were recorded from 3 g.l^{-1} humic acid 8 weeks after the last treatment. However, the control treatment gave best values of fresh and dry weight of fruits and calyxes (260.48, 47.25, 139.26 and 13.99 g respectively) 8 weeks after the last treatment.

The positive effects of humic acid on flowering attributes which noticed in our results may be due to that humic acid promote both shoot and root growth improved directly the flowering and yield properties (Ahmed *et al*., 2011 and Stevenson, 1994). The obtained results were in harmony with that reported by (Scheuerell and Mahaffee, 2006). Similar results were recorded by (Ahmed *et al*., 2011) about number of fruits and fresh and dry weights of calyces. plant^{-1} of Roselle plants. Also, analogous results were obtained by (Ramadan and El Mesairy, 2015 and Mohammed and Saeid 2017) on Okra plants. The reason of these results may be due to that humic acid stimulates the increasing of cell division, as well as optimized uptake of nutrients and water and it regulates the hormone levels, improvement of plant growth and enhancement of stress tolerance (Piccolo *et al*., 1992).

Table 3. Effect of humic acid sprayed three times on fruiting and calyxes yield at 8 and 12 weeks after last spraying of *Hibiscus sabdariffa* L.

HA g.l^{-1}	No. of fruits. plant^{-1}		Fruit length (mm)		Fruit Diameter (mm)		Fresh weight of fruits. plant^{-1} (g)		Dry weight of fruits. plant^{-1} (g)		Fresh weight of calyxes. plant^{-1} (g)		Dry weight of calyxes. plant^{-1} (g)	
	8 w.a.l.s	12 w.a.l.s	8 w.a.l.s	12 w.a.l.s	8 w.a.l.s	12 w.a.l.s	8 w.a.l.s	12 w.a.l.s	8 w.a.l.s	12 w.a.l.s	8 w.a.l.s	12 w.a.l.s	8 w.a.l.s	12 w.a.l.s
0	19.67 d	68.67 ab	23.42 b	21.55 b	21.53 a*	19.39 b	23.42 b	21.55 b	23.42 b	21.55 b	23.42 b	21.55 b	23.42 b	21.55 b
1	30.67 c	72.00 a	25.47 ab	16.67 c	23.08 a	17.19 c	25.47 ab	16.67 c	25.47 ab	16.67 c	25.47 ab	16.67 c	25.47 ab	16.67 c
2	45.33 a	65.67 b	23.64 b	18.66 c	21.30 a	18.80 b	23.64 b	18.66 c	23.64 b	18.66 c	23.64 b	18.66 c	23.64 b	18.66 c
3	38.00 b	34.00 c	28.43 a	39.75 a	21.69 a	20.74 a	28.43 a	39.75 a	28.43 a	39.75 a	28.43 a	39.75 a	28.43 a	39.75 a

HA= Humic Acid, w.a.l.s.= weeks after last spraying

* Values within each column followed with the same letters are not significantly different from each other according to Duncan's Multiple Range Test at the (0.05) level.

CONCLUSIONS

The effects of humic acid application are safe and as a result, it is effective and easily adopted by farmers. The study assumes that humic acid play a major role in growth and productivity of *Hibiscus sabdariffa* L. plants, this may be due to the role of humic acids aid in correcting plant chlorosis, increase the permeability of the plant membranes and intensify enzyme systems of plants. However, about fruit and calyxes yield the effect of highest level of humic acid 3g. l^{-1} was more effective at 8 weeks after last spraying. It could be suggested that spraying the roselle plants by humic acid is considered a suitable application for improving the vegetative growth and yield production of the plants.

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تأثير الرش الورقي لحامض الهيوميك في نمو وانتاج الكجرات *Hibiscus sabdariffa* L.

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المستخلص

اجريت هذه الدراسة هذه الدراسة لمعرفة تأثير رش حامض الهيوميك (HA) على الخواص الخضرية وخواص انتاجية الاوراق الكأسية لنباتات الكجرات ، خلال الفترة من 22 مايو إلى 31 ديسمبر 2017 في الحقل المفتوح (كردرشه) التابع لقسم البستنة ، كلية علوم الهندسة الزاعية، جامعة صلاح الدين. اربعة مستويات من حامض الهيوميك (1، 2، 3 غم.لتر$^{-1}$) تم رشها بثلاث مواعيد (60 ، 75 ، 90 يوم بعد الزراعة). تم اخذ البيانات بعد (4، 8، 12 اسبوع من الرش) للصفات الخضرية والانتاجية: ارتفاع النبات (سم)، عدد الافرع، قطر الساق (ملم)، عدد الايام لوصول 50% من النباتات للتزهير، قطر الزهرة (ملم) تسلسل العقدة الحامل لأول زهرة، عدد الثمار لكل نبات، طول الثمرة (ملم)، قطر الثمرة (ملم)، الوزن الطري والجاف للثمار. نبات$^{-1}$ والوزن الطري والجاف للأوراق الكأسية. نبات$^{-1}$. أظهرت النتائج التي تم الحصول عليها أن جميع معاملات حامض الهيوميك زادت بشكل كبير كل من الخواص الخضرية وحاصل الاوراق الكأسية وتقصير عدد الأيام إلى الإزهار بالمقارنة مع لباتات الغير معاملة. المعاملة 2 غم. لتر$^{-1}$ انتجت أطول النباتات، وتم الحصول على أكبر عدد من الفروع من المعاملة 3 غ. لتر$^{-1}$ حامض الهيوميك (HA) . والمعاملتان 2 غم. لتر$^{-1}$ و 3 غم. لتر$^{-1}$ انتجت نباتات ذات سيقان اسمك، وفيما يتعلق بالخواص التكاثرية، فقد تم تسجيل أعلى قيم لقطر الثمرة وطول الثمار والأوزان الطرية والجافة للثمار. نبات$^{-1}$ والأوزان الطرية والجافة للأوراق الكأسية. نبات$^{-1}$ قد تم تسجيلها للمعاملة 3 غ. لتر$^{-1}$ بعد 8 أسابيع من آخر رشة.

الكلمات المفتاحية: *Hibiscus sabdariffa* L. ، التطبيق الورقي، حامض الهيوميك، النمو الخضري، إنتاج.