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Effect of Humic Acid and Amino Acids on Pomegranate Trees under Deficit Irrigation. I: Growth, Flowering and Fruiting

¹Magda M. Khattab, ¹Ayman E. Shaban, ²Arafa H. El-Shrief and ²Ahmed S. El-Deen Mohamed

¹Department of Pomology, Faculty of Agriculture, Cairo University, Giza, Egypt ²Horticulture Research Institute, Agriculture Research Center, Giza, Egypt

Abstract: This experiment was carried out during 2007 and 2008 seasons on 20 years old pomegranate trees of Manfalouty cultivar. Trees under investigation were grown in a sandy soil at El-Kassasien Research Station, Ismailia Governorate. The trees received humic acid (32- 48gm / tree/season) or amino acids (8- 16 gm/ tree/ season) incorporated with irrigation levels 7 and 9m³/tree/year in comparison to farm control (11m³). The results showed that, shoot length, number of leaves per shoot, leaf area, number of flowers per shoot, fruit set percentage, fruit retention percentage, number of fruits per tree and yield (kg/tree) significantly increased by increasing water level from 7 and 9 to 11m³. On the other side increasing irrigation water amount from 7 and 9 to 11m³ decreased fruit drop percentage significantly. Increasing humic acid doses from 32 to 48g and amino acids from 8 to 16g/tree enhanced vegetative growth and fruiting. When the lower water levels 7 or 9m³/tree/ season were supplemented by the higher doses of either humic acid (48g) or amino acids (16g) all studied parameters were improved.

Key words: Pomegranate • Irrigation • Humic acid • Amino acids • Growth • Flowering • Fruiting

INTRODUCTION

The amount and the quality of available irrigation water of the arid and semi- arid regions of the world such as Egypt, are limiting for the extension agriculture [1]. Plant growth and development retarded when water supply was restricted [2]. Pomegranate trees are considered as a crop tolerant to soil water deficit [3]. However, very little is known about Pome granatum orchard water management. Water use for this crop is for instance not listed in FAO water use book by Allen et al. [4]. Humic acid (polymeric polyhydroxy acid) was reported as the most significant component of organic substances in aquatic systems. Humic acid is highly beneficial to both plant and soil; it is important for increasing microbial activity, it is considered as a plant growth bio-stimulant, an effective soil enhancer; it promotes nutrient uptake as chelating agent and improves vegetative characteristics, nutritional status and leaf pigments [5, 6]. Humic acid are complex substances derived from organic matter decomposition. Humic substances have indirect effects involve improvements of soil properties such as

aggregation, aeration, permeability, water holding capacity, micronutrient transport and availability [7]. Proline and other amino acids help in osmotic adjustment and crucial to sustain cellular functions under drought conditions [8]. Moreover, Aseri *et al.* [9] enhanced growth of pomegranate by using biofertilizers.

The present study aimed at assessing the effect of deficit irrigation on growth, flowering and fruiting also, testing some soil conditioners that help in improving the soil water holding capacity to enable the growers to lessen the amount of water used.

MATERIALS AND METHODS

This experiment was conducted during two successive seasons of 2007 and 2008 on 20 year old mature pomegranate trees (*Punica granatum* L.) Manfalouty cultivar. Trees under investigation were grown in a sandy soil at El- Kassasien Research Station, Ismailia Governorate. Trees distances were of 5 meters between trees and between lines and drip irrigation system was applied. Trees received the recommended

Treatments	Month (2007 and 2008)									
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	
7m ³ /tree/season	4	10	28	50	50	50	28	10	4	
9m3/tree/season	6	15	40	60	60	60	40	15	6	
11m ³ /tree/season	8	21	50	70	70	70	50	22	8	

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Table 1: Distribution of the irrigation water (L/day/tree) through the two seasons of study (2007 and 2008).

horticulture management of the Horticultural Research Institute (H.R.I.). The experiment was designed to study the effect of adding humic acid or amino acids (as a soil water holding capacity) to the lower water levels (7 or $9m^3$ /tree/season) than the farm control ($11m^3$ /tree/season) on growth, flowering and fruiting of the trees. Uniform thirty three trees were selected randomly for this experiment which included eleven treatments each treatment was replicated three times with one tree for each replicate. The randomized complete blocks design was used. The treatments were arranged as follows.

- 7m³ water (tree/season)
- 7m³ water plus 32g humic acid (tree/season)
- 7m³ water plus 48g humic acid (tree/season)
- 7m³ water plus 8g amino acids (tree/season)
- 7m³ water plus 16g amino acids (tree/season)
- 9m³ water (tree/season)
- 9m³ water plus 32g humic acid (tree/season)
- 9m³ water plus 48g humic acid (tree/season)
- 9m³ water plus 8g amino acids (tree/season)
- 9m³ water plus 16g amino acids (tree/season)
- 11m³ water (tree/season) (control)

The daily amount of irrigation water as liters per tree for each treatment in both seasons are shown in Table 1.

The dose of humic acid or amino acids for each treatment was divided into 16 equal doses and were added from February till September (two doses/ month).

Humic acid (85% potassium humates) or amino acids (commercial name pepton) were added to tree by dissolving the previously mentioned doses in one liter of water then added to the soil in the area of drippers and these doses applied through growing season every two weeks intervals from February till September. The following parameters were determined:

Length of the New Developed Shoots (cm): Ten shoots of one year old on each tree were tagged for measuring new developed shoots length at the end of growing season in September. **Number of Leaves per Shoot:** Leaves developed on the new shoots were counted at the end of growing season in September.

Leaf Area (cm²): Was determined by using the leaf area meter CL203.

Number of Flowers per Shoot: Was counted at balloon stage.

Fruit Set (%): Pomegranate has two types of flowers (perfect and male flower). Male flower dropped after opening immediately. At balloon stage the total number of flowers was counted then the number of set fruits was counted two weeks after full bloom. Fruit set% was calculated according to the formula: Fruit set% = number of set fruits / total number of flowers (balloon stage) X 100

Fruit Drop (%): Was calculated by the following equation:

Fruit drop% = total number of fruit set - total number of fruits at harvest time / total number of fruit set X 100.

Fruit Retention (%): Was determined by counting the number of fruits at harvest time / initial number of fruit set X 100.

Number of Fruits per Tree: Fruits were picked at September 15th in both seasons and number of fruits per tree was counted.

Yield per Tree (kg): At harvest time, fruits per tree for each treatment were weighted and then average yield/tree as kg was estimated.

Statistical Analysis: The obtained data were tabulated and statistically analyzed according to Snedecor and Cochran [10]. Differences between means were compared by Duncan's multiple range test at 5% level of probability according to Duncan [11].

Table 2: Effect of irrigation levels, humic acid and amino acids on shoot length, number of leaves per shoot and leaf area of pomegranate cv. Manfalouty in 2007 and 2008 seasons

Treatments							
		Shoot length	(cm)	Number of le	aves per shoot	Leaf area (cn	1 ²)
Irrigation Levels	Soil conditioner						
(m ³ /tree/season)	(g/tree/season)	2007	2008	2007	2008	2007	2008
7m ³		20.77 h	18.92 g	20.00 e	18.61 d	4.91 g	4.45 g
7m ³	32g humic acid	22.37 fg	20.18 f	20.17 de	20.04 c	5.15 f	4.82 f
7m ³	48g humic acid	23.00 de	21.32cde	21.40 bc	20.29bc	5.63 bc	5.26 de
7m ³	8g amino acids	22.17 g	20.59 ef	21.03 c	18.90 d	5.07 f	4.98 ef
7m ³	16g amino acids	23.30 cd	21.67 bc	20.77 cd	19.74 c	5.58 c	5.22 de
9m ³		22.70 ef	20.89 def	21.03 c	20.09 c	5.36 e	5.38 cd
9m ³	32g humic acid	23.00 de	20.88 def	21.27 bc	20.29 bc	5.51 cd	5.64 bc
9m ³	48g humic acid	23.73 bc	21.74 bc	22.73 a	22.03 a	5.83 a	6.02 a
9m ³	8g amino acids	23.23 d	21.36 cd	21.90 b	20.99 b	5.39 de	5.52 cd
9m ³	16g amino acids	23.90 b	22.31 b	22.60 a	21.80 a	5.73 ab	5.84 ab
11m ³ (control)		25.07 a	23.96 a	22.80 a	22.09 a	5.77 a	5.60 bc

Number followed by the same letter (s) in the same column are not significantly different at 0.05 level of probability

Table 3: Effect of irrigation levels, humic acid and amino acids on number of flowers per shoot, fruit set and fruit drop of pomegranate cv. Manfalouty in 2007 and 2008 seasons

		Number of f	lowers/shoot	Fruit set (%)		Fruit drop (%)
Irrigation Levels	Soil conditioner						
(m3/tree/season)	(g/tree/season)	2007	2008	2007	2008	2007	2008
7m ³		2.70f	2.77h	23.27e	21.81e	17.15a	17.88a
7m ³	32g humic acid	2.80f	2.83gh	23.82de	22.45de	16.81ab	17.65a
7m ³	48g humic acid	3.23cd	2.97de	24.58d	22.76d	16.34c	16.93b
7m ³	8g amino acids	2.80f	2.87fg	23.82de	21.91e	16.93ab	17.74a
7m ³	16g amino acids	3.00e	2.93 ef	24.43d	22.44de	16.57bc	17.41ab
9m ³		3.20d	2.97de	26.96c	25.94c	14.77d	15.82c
9m ³	32g humic acid	3.30cd	3.03cd	27.12bc	26.40bc	14.44de	15.66c
9m ³	48g humic acid	3.47a	3.20a	27.87b	27.13ab	14.33e	15.11d
9m ³	8g amino acids	3.27cd	3.07bc	27.13bc	26.13c	14.52de	15.71c
9m ³	16g amino acids	3.43ab	3.13ab	27.41bc	26.61abc	14.55de	15.37cd
11m ³ (control)		3.33bc	3.17a	28.98a	27.36a	13.69f	14.52e

Values followed by the same letter (s) in the same column are not significantly different at 0.05 level of probability

RESULTS AND DISCUSSION

Shoot length: Data in Table 2 showed that, shoot length significantly increased by increasing water level. Regarding the level 7m³ of water incorporated with humic acid, results indicated that shoot length significantly increased by increasing the doses of humic acid from 32 to 48 g/tree/season. Among 7m³ plus amino acids treatments, the highest significant shoot length was obtained by using 7m³ plus16g amino acids in both seasons. Concerning using the higher level 9m³ combined with humic acid or amino acids shoot length significantly increased by increasing humic acid or amino acids doses. Regarding humic acid treatments, results showed that, the longer significant shoot length was detected with using 9m³ plus 48g humic acid. Also, a higher shoot length was recorded by increasing water level to 9m³ plus 16g amino acids. The lower irrigation levels 7m³ and 9 m³ gave lower

values of shoot length when compared with farm control (11m³). Referring to the values of shoot length in the Table 3, it is obvious that we can obtain reasonable shoot length almost similar to those achieved by the highest level (the control farm) by reducing the level of water irrigation to 9m³ incorporation with each of humic or amino acids. Also, we can lower the level to 7m³ with the help of both amino acids and humic at higher doses and realize a good shoot growth so water save could be realized by using 7or 9m³ levels instead of 11m³ as lessening the vegetative growth to a certain limit is required to reach the balance between vegetative, flowering and fruiting of the trees.

Number of Leaves: Number of leaves significantly increased by increasing the irrigation water levels (Table 2). Regarding 7m³ water plus humic acid or amino acids treatments, the average number of leaves was

significantly increased by increasing the quantity of both additives. Using 9m3 water plus humic acid, improved average number of leaves significantly with increasing humic acid quantity from 32 to 48g. The addition of amino acids at higher dose (16g amino acids) to 9m³ water level, resulted in the highest significant value. Moreover, in both seasons, the addition of humic acid at 48g/tree/season to 9m³ water level, indicated the highest significant average number of leaves. Comparing 7m³ water treatments with farm control data showed that, farm control recorded the highest significant value of number of leaves. The highest significant number of leaves was recorded by using the irrigation level of (11m³), followed by 9m³ plus 48g humic acid and 9m³ plus 16g amino acids in both seasons. About 2m³ water/ tree/season could be saved by using 9m³ treatments with 48g humic acid or 16g amino acids.

Leaf Area: Leaf area increased significantly by increasing water irrigation level (Table 2). The addition of some soil conditioner to improve the soil water holding capacity increased significantly leaf area. In both tested additives the higher doses were better. No significant difference was detected between the lower doses in both additives. Also no significant difference was recorded between the two higher tested concentrations of the two additives in this regard. The best treatment in this concern was that of 9m³ plus 48g humic acid. Therefore the choice will be determined according to the safety and economic measures. It is worthy to mention that the highest leaf area was recorded by using the highest water irrigation level (11m³), data in the Table 3 indicated that a similar leaf area was actually recorded by using the lower irrigation level (9m³) with the help of any of humic acid at 48g or amino acids at 16g with respect to the economic and safety bases. In this concern, when the lower water level (9m³) was supplemented by the higher doses of either humic acid (48g) or amino acids (16g) produced the highest number of leaves as well as best records of leaf area which were similar to those obtained by irrigation with the adopted farm irrigation levels (11m³). Enhancing vegetative growth parameters (shoot length, number of leaves per shoot and leaf area) of pomegranate by adding humic acid and amino acid were obtained by Shaddad et al. [12] on apricot, Omar and Abdelall [13] and Abbas et al. [14] on grape, Eissa et al. [15] on Anna apple, Ismail et al. [16] on pear they clearly showed a gradual increase in shoot diameter, average shoot length, number of leaves/shoot and leaf area parallel to increasing humic acid application.

Number of Flowers per Shoot: Data in Table 3 showed that, number of flowers significantly increased by increasing water irrigation levels. Regarding the level 7m³ subjoined with humic acid and amino acids, data evidenced that, number of flowers significantly increased by raising amount of humic acid from 32g to 48g in both seasons. Also, the higher doses of amino acids induced number of flowers but it was significant only in the first season. Concerning the level 9m³ treatments, number of flowers increased significantly in both seasons by adding 48g humic acid. Moreover, the same results were obtained with amino acids treatments whereas 9m³ plus 16g amino acids gave a higher value, but proved significant only in the first season. Addition humic acid to 7 or 9m³ water levels, data indicated the highest average number of flowers from using 9m³ plus 48g humic acid. Comparison between adding amino acids treatments to levels 7 or 9m³, revealed that using 9m³ plus 16g amino acids was superior. Referring to 7m³ and 9m³ water treatments results revealed that, the treatment of 7m³ water either solely or with lower doses of both additives resulted in the lowest average number of flowers. Where there were no significant differences between them especially in the first season. All 7m³ water treatments showed the lowest values when compared to the farm control 11m³. In the case of 9m³ water treatments when compared to farm control (11m³), in the first season, 9m³ plus 48g humic acid and 9m³ plus 16g amino acids gave the highest significant number of flowers. The same treatments and farm control showed the highest significant values in the second season. No significant difference was detected between the lower doses also between the higher doses in both substances.

Fruit Set: Fruit set percentage increased significantly by increasing amount of water from 7 and 9 to 11m³ (Table 3). Regarding the 7m³ water level associated with humic and amino acids, data revealed no significant differences between both concentrations of humic acid in both seasons. Also, the same trend was noticed with both concentrations of amino acids. Same results were observed with 9m³ treatments, whereas raising quantity of humic acid or amino acids increased fruit set but this increment was non-significant. Comparing between the addition of humic acid to 7 or 9m³ water proved that, the highest fruit set resulted from using 9m³ plus 48g humic acid. Also, in case of adding amino acids treatments to 7 or 9m³ water proved that, using 9m³ plus 8 or 16g amino acids increased fruit set significantly and was the highest in this concern. Differences between 7m³ water

treatments and 9m³ revealed that, all 7m³ treatments were lower significantly than 9m³. No significant differences were detected between all 7m³ water treatments in the first season. In the second season, no significant differences were detected between the treatments of 7m³ plus 32g humic, 7m³ plus 48g humic and 7m³ plus 16g amino acids also between 7m³water and 7m³ plus 8g amino acids. 7m³ water gave the lowest fruit set. When comparing among farm control (11m³) and 7m³ water treatments, all 7m³ treatments showed the lowest significant values.

Concerning 9m³ water treatments when compared with farm control (11m³) it is obvious that, in the first season, the differences among all 9m3 treatments were non-significant, while farm control gave the highest significant fruit set percentage. In the 2nd season, the highest significant fruit set was observed by the three treatments of 9m³ plus 48g humic, 9m³ plus 16g amino acids and the farm control. So fruit set percentage was the highest by using the level of 11m³, but the obtained data recorded also high percentage by lowering the water level consumption to 9m³ with the soil conditioner that increase water holding capacity, both tested material were effective in this regard. A similar effect was reported by Abbas et al. [14] on grape furthermore, with Ismail et al. [16] on Le-Conte pear. They clearly showed a gradual increase in fruit set percentage by increasing amount of humic acid.

Fruit Drop: By increasing amount of irrigation water from 7 and 9 to 11m³ fruit drop percentage significantly decreased (Table 3). Increasing humic acid doses from 32 to 48g, fruit drop significantly decreased in both seasons. But with increasing amino acids doses no effect was observed. Considering 9m³ water treatments, no significant differences showed among both doses of

humic acid in the first season, but in the second season fruit drop decreased significantly by raising humic acid doses. While, fruit drop with both amino acids concentrations did not show any significant differences in both seasons.

Table 3 showed the effect of adding of humic acid to 7 and 9m³ water, in this sphere the lower fruit drop was 14.33% and 15.11% in both seasons respectively resulted from using 9m³ plus 48g humic acid. Also, in the treatments of amino acids with 7 or 9m³ water, the lower significant fruit drop in both seasons were recorded with the two treatments 9m³ plus 8g amino acids and 9m³ plus 16g amino acids. Comparison between 7m³ water treatments or 9m³ revealed that, in both seasons all 7m³ treatments resulted in the highest significant fruit drop than 9m³. No significant differences appeared between the lower concentrations also among the higher concentrations of both substances in both seasons. Farm control (11m³) gave the lowest significant fruit drop compared to 7m³ water treatments. Differences among 9m³ water treatments and farm control (11m³), farm control gave the lowest significant fruit drop percentage and no significant differences illustrated among all 9m3 treatments in both seasons. It can be abbreviated that, the lowest significant fruit drop was obtained by farm control then by both treatments 9m³ plus 48g humic acid and 9m³ plus 16g amino acids.

Fruit Retention: Fruit retention percentage was affected significantly by increasing amount of irrigation water from 7 and 9 to 11m³ in both seasons (Table 4). Regarding 7m³ water integrated with humic and amino acids treatments, fruit retention significantly increased by increasing humic acid doses from 32 to 48g in both

Table 4: Effect of irrigation levels, humic acid and amino acids on fruit retention (%), number of fruits per tree and yield (kg/tree) of pomegranate cv.
Manfalouty in 2007 and 2008 seasons.
Therefore

		Fruit retention (%)		Number of fruits/tree		Yield (kg/tree)	
Irrigation Levels	Soil conditioner						
(m ³ /tree/season)	(g/tree/season)	2007	2008	2007	2008	2007	2008
7m ³		82.85 f	82.12 e	88.67 ef	81.00 e	20.47 gh	17.41f
7m ³	32g humic acid	83.19 ef	82.35 e	86.00 fg	80.00 e	21.19 gh	18.08 f
7m ³	48g humic acid	83.66 d	83.07 d	89.33 e	86.00 d	23.48 f	20.44 e
7m ³	8g amino acids	83.07 ef	82.26 e	83.67 g	81.00 e	20.35 h	17.90f
7m ³	16g amino acids	83.43 de	82.59 de	86.00 fg	83.00 e	21.91 g	19.54 e
9m ³		85.23 c	84.18 c	102.0 d	93.00 c	25.61 e	22.87 d
9m ³	32g humic acid	85.56 bc	84.34 c	108.7 bc	97.00 b	28.79 cd	25.36 c
9m ³	48g humic acid	85.67 b	84.89 b	113.7 a	103.0 a	31.74 b	28.50 a
9m ³	8g amino acids	85.48 bc	84.29 c	106.0 c	93.00 c	27.75 d	23.97 d
9m ³	16g amino acids	85.45 bc	84.63 bc	109.7 b	101.0 a	30.15 c	26.64 b
11m ³ (control)		86.31 a	85.48 a	113.3 a	101.7 a	33.87 a	29.20 a

Number followed by the same letter (s) in the same column are not significantly different at 0.05 level of probability

seasons. On the contrast, increasing amino acids doses gave non-significant effect on fruit retention in both seasons. Concerning 9m³ water treatments, no significant differences were shown among both doses of humic acid in the first season. But in the second season fruit retention improved significantly by raising humic acid doses. Meanwhile, fruit retention was not affected significantly in both seasons with both amino acids concentrations. Comparing the merging of humic acid with 7 and 9m³ water data revealed that, the highest fruit retention was recorded with using 9m³ plus 48g humic acid in both seasons. Also, in case of using amino acids treatments with the levels of 7 or 9m³ water, both 9m³ plus 8g amino acids and 9m³ plus 16g amino acids recorded the highest significant fruit retention in both seasons. Differences between 7m³ water treatments and 9m³ revealed that, all 7m³ treatments recorded lower significant values than 9m³. No significant differences appeared between the lower concentrations also among the higher concentration of both substances in both seasons. Farm control (11m³) recorded the highest significant fruit retention in both seasons. The farm control resulted in the highest significant fruit retention percentage and the differences among all 9m3 treatments were non-significant in both seasons. It can be summarized that, the best fruit retention resulted by farm control then by both treatments 9m³ plus 48g humic acid and 9m ³plus 16g amino acids. Our results are in line with Shaddad et al. [12] who noticed that applying humate to soil weekly from fruit set till harvest increased retained fruit of apricot per tree.

Number of Fruits/tree: Number of fruits was significantly increased by increasing the irrigation water levels (Table 4). Number of fruits per tree for the control farm (11m³) recorded the highest significant values compared to the two lower tested levels. In regard to 7m³ water with humic acid treatments, the average number of fruits per tree significantly increased by increasing humic acid doses. While, 7m³ water with amino acids treatments did not have any significant differences between both doses in both seasons. Using 9m³ water append to humic acid increased average number of fruits significantly by increasing humic acid quantity. Also, the merging of amino acids to 9m³ level had the positive effect whereas, the higher significant number of fruits were observed by using 9m³ plus 16g amino acids in both seasons.

The addition of humic acid to 7 or $9m^3$ water levels, indicated the higher significant average number of fruits resulted by using $9m^3$ plus 48g humic acid. Concerning the effect of adding amino acids to the lower levels of 7 and 9m³ water, the higher significant average number of fruits resulted from using 9m³ plus 16g amino acids in both seasons. All 7m³ treatments had the lowest significant number of fruits compared to 9m³. Generally, the highest significant number of fruits was recorded by the two treatments farm control (11m³) or 9m³ plus 48g humic acid in both seasons. About 2m³ water/ tree/season could be saved by using the lower water level 9m³ with 48g humic acid. In this sphere, Omar and Abdelall [13] on grape, furthermore, with Ismail *et al.* [16] on Le-Conte pear showed that, a gradual increase in number of fruits per tree parallel to increasing humic acid application.

Yield (kg/tree): Yield was significantly increased by increasing the irrigation water levels (Table 4). Appending humic acid to 7m³ water increased yield significantly by increasing humic acid concentration. The same trend was noticed with 7m³ water with amino acids treatments. Using humic acid with 9m³ water level have a positive effect on yield. The addition of humic acid to 7 or 9m³ water levels, the higher significant yield obtained by using 9m³ plus 48g humic acid. Also, adding amino acids to 9m³ water resulted in the higher significant yield by using16g amino acids in both seasons. Comparing 7m³ water treatments with farm control revealed that, farm control recorded the highest significant yield whilst, 7m³ recorded the lowest one. Data resulted from farm control and 9m³ water treatments revealed in the first season that, there were significant differences between all 9m³ treatments except within the lower quantity of the two used substances and the highest yield was taken from farm control. In the second season, the highest yield was recorded with the two treatments 9m³ plus 48g humic acid and the farm control. In brief, the highest significant yield was recorded by the highest irrigation level (11m³) followed by 9m³ plus high concentration of humic acid which gave a good but less significant yield, finally adding 16g amino acids to 9m³ water level recorded the 3rd rank of yield significance in both seasons. Generally, the lowest irrigation level (7m³) with the help of all additives resulted in the least yield so it cannot be recommended with these treatments. The results agreed with Fathi et al. [17] on peach, Eissa et al. [18] and Shaddad et al. [12] on apricot, Omar and Abdelall [13] and Abbas et al. [14] on grape, Ismail et al. [16] on Le-Conte pear. They clearly showed a gradual increase in yield parallel to increasing humic acid application. Also, El-Shenawi et al. [19] on Grandnain banana they reported that increasing amount of humic acid markedly increased yield per feddan.

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