Effect of Humic acid, Atonic and Salicylic acid spraying on some chemical and vegetative traits of sweet orange transplants cv.Mahali and Blood

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Abstract
The experiment was conducted in the nursery of Diyala Agriculture Directorate during the growing seasons 2021 from March to November 2021 to study the effect of Humic acid, Atonic and Salicylic acid spraying on some chemical and vegetative parameters of two years old Mahali and blood orange transplants budded on sour orange rootstock. and included two factors, the first one was two orange cultivars (Mahali and blood), and the second was the spray of The second factor included six treatments of biostimulants spray of (humic acid at1%, salicylic acid at 300 mg l^{-1}, atonic at 1 ml l^{-1} ,humic acid 1%+ salicylic acid at 300 mg l^{-1}, and salicylic acid at 300 mg l^{-1} + atonic at 1 ml l^{-1}) . The experiment was conducted using RCBD with three replicates, and 3 transplants per experimental unit. Results means analyzed using Duncan's multiple range test at 0.05 propability level.
Results revealed the superiority of the blood cultivar compared to Mahali one in most studied characteristics(branch number , leaves number,the fresh and dry weight of the shoots, leaves carbohydrates content, and decreasing leaves proline content). The combination of salicylic + atonic showed a significant increase in branches number and length,leaves number,the fresh and dry weight of the shoots, leaves carbohydrates content , and decreasing leaves proline content compared to untreated transplants.
Keywords: Blood and Mahali orange, Atonic,Humic acid, Salicylic acid.

Introduction

The orange (*Citrus sinensis* L) belongs to the family Rutaceae, it is one of the most widespread citrus fruits in the world. Its fruits are characterized by high nutritional value due to the vitamins it contains and some mineral elements such as potassium, calcium, phosphorous and others. The Mahali orange is characterized by the spread of its cultivation in Iraqi orchards, as its cultivation is old-fashioned among palm trees or by the open fields, and its trees are characterized by the presence of differences in the strength of vegetative growth and the abundance of yield and its fruits are often round to oval and its peel is soft, the pulp is juicy and taste sweet tinged with sourness (8). As for the blood orange, it is one of the orange Cultivars with high economic value in the world, and its cultivation has begun to spread in the Iraqi orchards in recent years. It is characterized by the fact that its fruits have a crimson-coloured pulp, and as a result of the juice vesicles containing anthocyanin pigment, which gives the fruits a red or pink colour (7). Bio-stimulants are a class of relatively new products with diverse formulations that positively affect plant biological processes, usually more pronounced under stress conditions, by increasing plant tolerance and repairing damage caused by unsuitable conditions. Humic acid is one of these catalysts, and it is one of the environmentally friendly materials, as it does not cause pollution to the soil, water or the atmosphere. It also positively affects plant growth because it contains nutrients in good proportions (17). Salicylic acid has aroused the interest of researchers recently, for its importance in plant tolerance to water stress, heat stress, salt stress and stress caused by excess elements (13). Salicylic acid regulates ion uptake, hormonal balance, and stomata movement. It has a role in inhibiting ethylene synthesis and has an opposite effect to abscisic acid (ABA) growth inhibitor. Responsible for defoliation,

as it accelerates the process of formation of carotene and chlorophyll pigments, photosynthesis and increasing enzyme activity (11). Atonic, awater solution of phenolic compounds: sodium para-nitrophenolatePNP(0.3%), sodium ortho-nitrophenolate ONP (0.2%), and sodium 5-nitroguaiacolate 5NG (0.1%) used as a synthetic bio stimulant which have many other trade names likeChap-perone or Asahi SL(16), and classified among the modern growth regulators that were produced and put on the market under a trade name by the company AsahichemicalMfg, It is a nitrogenous aromatic compound that causes an increase in biological activities in plants without causing any distortion or toxicity to plants treated with it (14),

Materials and methods

The experiment was carried out in the nursery of the Diyala Agriculture Directorate during the 2021 agricultural season for the period from 9/3/2022 to the end of November to study the effect of spraying with growth stimulants humic acid, atonic and salicylic acid on some chemical and vegetative parameters of transplantsMahali and raspberry (blood) orange.

Two years old Orange transplants(mahali and Blood) budded on sour orange rootstock were brought from a private nursery in Al-Krayat area - Baghdad, and transferred to10 kg plastic containers filled with a growing medium composed of loamy sand soil mixed with peat moss in a ratio of 3 soil : 1 peat moss.

The plants were sprayed with humic and atonic acids six times during the growing season, starting from 4-4-2021 at 21 days intervals for three times, and three other times started from 6/9 at the same intervals. As for salicylic acid, plants were sprayed three times at one month interval starting from 5/27, and liquid soap (0.1%) was added instead of Tween 20 and the spraying process was carried out in the evening a day after the irrigation process.

A factorial experiment was carried out according to the Randomized Complete Block Design, with two factors the first one was two orange cultivars(Mahali and Blood orange), and the second was six growth stimulants treatments), (humic acid at1%, salicylic acid at 300 mg l^{-1}, atonic at 1 ml l^{-1},humic acid 1%+ salicylic acid at 300 mg l^{-1}, and salicylic acid at 300 mg l^{-1} + atonic at 1 ml l^{-1}). so that the experimental treatments was 12 with three replications, with 3 transplants per experimental unit, so that the number of transplants involved in the experiment was 108, resulting from the two factors of the experiment and their interactions. The data were collected and analysed statistically according to the design used in the experiment using the SAS program (2003), and the averages were compared according to Duncan's Multiple Range Test, at a probability level of 0.05 (9).The following traits were measured through the experiment

1- Average increase in the number of branches (branch plant^{-1})

2- length of branches (cm)

3- number of leaves (leaf plant^{-1})

4-The fresh weight of the vegetative system (gm plant^{-1})

5-The dry weight of the shoot (gm plant^{-1})

The vegetative parts (vegetative branches, stem and leaves) were placed in perforated paper bags for each experimental unit and were air-dried and then placed in the oven at a temperature of 65° C for 72 hours until the weight was stable, and then weighed with a sensitive scale.

6- Determination of Total Carbohydrates:

The total carbohydrate content of leaves was estimated according to(12).

7- Determination of leaves proline content (mg g^{-1} dry weight)

The method of (10) was used to estimate the proline content of leaves.

Results and discussion

1-The average increase in the number of branches (plant branch^{-1})

It is evident from the results presented in Table 1 that blood orange was significantly superior to the Mahali orange by giving the highest average increase in the number of branches(4.644 branch), while the Mahali orange gave the lowest average (4.155 branch). With regard to growth stimulants, it is

noticeable that all treatments were significantly superior to the untreated treatment, and the spraying with salicylic acid + atonic acid gave the highest value(5,500 branch per plant), while the control treatment gave the lowest average of 2.333 branch.

The interaction between treatments and cultivars differed in the average increase in the number of branches, and it is noted from the table that the interaction between blood orange and salicylic + atonic gave the highest value of 5.833 branch per plant, whereas the untreatedMahali orange transplants gave the lowest average (2.267 branch per plant .

Table 1: Effect of Humic acid, Atonic and Salicylic acid spray on the mean increase in the number of branches of Mahali and Blood orange transplants(branch plant^{-1})

Orange Cultivars	**growth promoters**						**Cultivars**
	Control	**Humic 1%**	**Salicylic 300 mg L^{-1}**	**Atonic 1ml L^{-1}**	**Salicylic + Humic**	**Salicylic + Atonic**	
Mahali	2.267 f	4.167 de	4.633 b-e	4.367 c-e	4.333 c-e	5.167 a-c	4.155 B
Blood	2.400 f	3.700 e	4.967 a-d	5.600 a	5.367 ab	5.833 a	4.644 A
Growth promoters	2.333 D	3.933 C	4.800 B	4.983 AB	4.850 B	5.500 A	

2 - length of the branches (cm):

It is clear from the results presented in Table 2, that there is no significant difference between blood and Mahali orange transplants in the average length of branches. As for growth stimulants, it is noted that all treatments were significantly superior to the control treatment, and spraying with atonic + salicylic gave the highest average (30.62 cm), while the control treatment gave the lowest mean branch lengths(19.68 cm).

The effect of the interaction between treatments and cultivars differed in the average branch lengths, and it is noted from the table that the interaction between blood orange and the spray treatment with atonic + salicylic growth stimulator gave the highest average branch lengths of 31.80 cm, while the control treatment of blood oranges gave the lowest length of 18.52 cm.

Table 2:: Effect of Humic acid, Atonic and Salicylic acid spray on branches length (cm) of Mahali and Blood orange transplants

Orange Cultivars	growth promoters						Cultivars
	control	Humic 1%	Salicylic 300 mg L^{-1}	Atonic 1ml L^{-1}	Salicylic + Humic	Salicylic + Atonic	
Mahali	20.85 d	24.99 c	27.50 cb	26.84 cb	29.17 ab	29.45 ab	26.46 A
Blood	18.52 d	27.11 cb	27.61 cb	27.18 cb	26.21 cb	31.80 a	26.40 A
Growth promoters	19.68 C	26.05 B	27.55 B	27.01 B	27.69 B	30.62 A	

3- number of leaves per plant :

From observing the results presented in Table 3, we find that blood orange transplants significantly outperformed the Mahali orange by giving it an average of 123.9 leaf per plant, compared to the Mahali orange, which gave the lowest average of 120.9 leaf, As for growth stimulants, it is noted that all treatments were significantly superior to the untreated plants, and the spraying with salicylic + atonic gave the highest average (139.1 leaf per plant), it was followed without significant difference by the spraying with atonic treatment by giving 136.4 leaf per plant, while the control treatment gave the lowest average of 84.26 leaf per plant. The interaction between blood orange and the growth stimulator Salicylic + Atonic gave the highest value of 141.0 leaf, while the control treatment for Mahali orange gave the lowest average and reached 79.20 leaf.

Table 3::Effect of Humic acid, Atonic, and Salicylic acid spray on the number of leaves per plant of Mahali and Blood orange transplants

Orange Cultivars	growth promoters						Cultivars
	Control	Humic 1%	Salicylic 300 mg L^{-1}	Atonic 1ml L^{-1}	Salicylic + Humic	Salicylic + Atonic	
Mahali	79.20 f	120.5 d	123.6 cd	136.1 a	128.8 bc	137.2 a	120.9 B
Blood	89.33 e	122.5 d	124.3 b-d	136.7 a	129.7 b	141.0 a	123.9 A
Growth promoters	84.26 D	121.5 C	123.9 C	136.4 A	129.3 B	139.1 A	

4- The fresh weight of vegetative system (gm plant-1)

Results presented in Table 4 showed that blood orange was significantly superior to the Mahali orange by giving the highest fresh weight of the plant (147.2 g plant^{-1}), compared to the Mahali orange, which gave the lowest average of 136.8 g plant^{-1}. As for growth stimulants, it was noted that all treatments were significantly superior to the Control treatment, and the salicylic + atonic spray treatment gave the highest average of 167.3 g plant^{-1}, while the Control treatment gave the lowest average of 80.50 g plant^{-1}. The interaction treatment between blood orange and salicylic + atonic gave the highest value of 180.0 gm plant^{-1}, whereas the untreated plants of blood orange gave the lowest average of 80.00 gm plant $^{-1}$.

Table 4: Effect of Humic acid, Atonic and Salicylic acid spray on the fresh weight of the vegetative system (g plant^{-1})of Mahali and Blood orange transplants.

Orange Cultivars	growth promoters						Cultivars
	Control	Humic 1%	Salicylic 300 mg L^{-1}	Atonic 1ml L^{-1}	Salicylic + Humic	Salicylic + Atonic	
Mahali	81.00 f	151.7 cd	153.0 cd	145.7 de	135.3 e	154.7 cd	136.8 B
Blood	80.00 f	136.7 e	154.0 cd	161.3 bc	171.7 ab	180.0 a	147.2 A
Growth promoters	80.50 C	144.1 B	153.5 B	153.5 B	153.5 B	167.3 A	

5- The dry weight of vegetative system (gm plant^{-1})

The results presented in Table 5 indicate that there was no significant difference between blood and Mahali orange in the vegetative system dry weight.Spraying with salicylic + atonic treatmentgave the highest weight (72.00 g plant^{-1}), whereas the control treatment gave the lowest average (35.50 g plant^{-1}). All interaction treatments between cultivars and growth stimulants were significantly superior to the control treatment of both cultivars, The interaction treatment between blood orange and spraying with salicylic acid + atonic was characterized by giving it the highest dry weight (76.33 g plant^{-1}), while untreated transplants of Mahali orange gave the lowest average (35.33 g plant^{-1}).

Table 5Effect of Humic acid, Atonic and Salicylic acid spray on the dry weight of the vegetative system (g plant^{-1}) of Mahali and Blood orange transplants.

Orange Cultivars	growth promoters						Cultivars
	Control	Humic 1%	Salicylic 300 mg L^{-1}	Atonic 1ml L^{-1}	Salicylic + Humic	Salicylic + Atonic	
Mahali	35.33 d	70.67 ab	63.33 bc	72.67 ab	54.33 c	67.67 ab	60.67 A
Blood	35.67 d	54.67 c	76.33 a	68.67 ab	72.67 ab	76.33 a	64.05 A
Growth promoters	35.50 D	62.67 C	69.83 A-B-C	70.67 AB	63.50 BC	72.00 A	

6- Leaves carbohydrate content (%):

The results in Table 6 show that the carbohydrate content of the leaves was significantly affected by the research treatments. It is clear from the results that the blood orange was significantly superior to the Mahali orange by giving it the highest average in the carbohydrate content of the leaves and amounted to 10.74% compared to the Mahali orange, which gave the lowest average and reached 10.19%. With regard to growth stimulants, it is noted from the same table that all treatments were significantly superior to the Control treatment, and the salicylic + atonic spray treatment gave the highest average of 11.21%, while the control treatment gave the lowest average of 8.348%. The effect of the interaction between treatments and cultivars differed on the average carbohydrate content of the leaves, as it is noted from the table that the interaction treatment between blood orange and atonic growth stimulator with salicylic gave the highest percentage of 11.81%, while the control treatment for Mahali orange gave the lowest percentage and amounted to 8.257%.

Table 6: Effect of Humic acid, Atonic and Salicylic acid spray on leaves carbohydrate content (%)of Mahali and Blood orange transplants.

Orange Cultivars	growth promoters						Cultivars
	Control	Humic 1%	Salicylic 300 mgL^{-1}	Atonic 1ml L^{-1}	Salicylic + Humic	Salicylic + Atonic	
Mahali	8.257 g	10.92 cd	10.56 ef	10.28 f	10.51 ef	10.62 de	10.19 B
Blood	8.440 g	11.03 c	10.72 de	10.90 cd	11.50 b	11.81 a	10.74 A
Growth promoters	8.348 D	10.98 B	10.64 C	10.59 C	11.00 B	11.21 A	

7- Proline content of the leaves (mmol g^{-1}):
Results in Table 7 showed that blood orange leaves contained the lowest value of proline, reaching 26.05 mmol g^{-1}, Compared with the Mahali orange, which gave the highest value(30.00 mmol g^{-1}), as for growth stimulants, significant differences were noticed between the treatments and the control, as the control treatment gave the highest leaf content of proline, which amounted to 43.89 mmol g^{-1}, Whereas, spraying with salicylic + atonic led to the lowest leaf content of proline, which was 18.75 mmol g^{-1}. The interaction between treatments and cultivars differed in its effect on the average content of proline in leaves. It is noted from the table that the interaction treatment between blood orange and atonic + salicylic growth stimulator gave the lowest average content of proline leaves, as it reached 17.28 mmol g^{-1}. Whereas, the control treatment of Mahali orange gave the highest proline content of 45.27 mmol g^{-1}.

Table 7: Effect of Humic acid, Atonic and Salicylic acid spray on leaves Proline content (mmol g^{-1}) of Mahali and Blood orange transplants

Orange Cultivars	growth promoters						Cultivars
	Control	Humic 1%	Salicylic 300 mg L^{-1}	Atonic 1ml L^{-1}	Salicylic + Humic	Salicylic + Atonic	
Mahali	45.27 a	36.31 c	22.63 f	30.19 d	25.39 e	20.22 g	30.00 A
Blood	42.50 b	30.15 d	21.18 g	24.83 e	20.38 g	17.28 h	26.05 B
Growth promoters	43.89 A	33.23 B	21.90 E	27.51 C	22.89 D	18.75 F	

2 - Discussion
It is noted from the results that blood orange is superior to Mahali orange in most of the studied traits (Tables 1, 3, 4, 7, 6). The reason for this is due to the genetic difference between them, as well as the difference in the extent to which they are affected by the environmental conditions surrounding them (2).
It is noted from the above-mentioned results that the treatment of spraying with atonic + salicylic acid has given the best results for most of the studied traits (Tables 1, 2, 3, 4, 5, 6, 7), This is due to the positive role of salicylic acid in increasing the plant's tolerance to various stress conditions, and thus improved the physiological processesof plant such as the photosynthesis which caused an increase in carbohydrate content(table 6), nitrogen content which is reflected positively on most of the studied growth characteristics (11), The atonic also increases the efficiency of the roots in the absorption of nutrients, including nitrogen, which leads to meeting the demand of the plant for these elements in the process of cell division and expansion, especially since N is included in the construction of nucleic acids and works to increase the efficiency of the photosynthesis process, Thus, carbohydrates and proteins are increased. These results are in agreement with the findings of(1), (5), The decrease in the leaf content of proline as a result of this treatment

(Table 7) is consistent with many studies that showed that the external addition of salicylic acid leads to a decrease in proline and the increase in other amino acids that used in protein synthesis, encouraging growth and reducing growth inhibition resulting from abiotic environmental stress in many of plants. This is consistent with the findings of (15), (6), and (3), as well as the findings of (4).

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