Plant Archives Vol. 19 No. 2, 2019 pp. 4062-4066 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF CITRIC AND MALIC ACIDS CONCENTRATION ON SOME GROWTH AND YIELD PARAMETERS OF *VICIA FABA* L. PLANT

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Abstract

An experiment was conducted in the plastic house of the Botanical Garden in the Department of Biology, College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad during one growth season. The experiment included the study of the effect of three concentrations of citric acid (0, 75, 150) mg.L^{-1} and four concentrations of malic acid (0, 50, 100, 150) mg.L^{-1} and their interaction in some of the growth and yield parameters of the broad bean plant, plant height, dry weight, number of leaves, total chlorophyll content, the number of flowers and pods and the weight of the pod.

The experiment was carried out in full random design (4×3) and with three duplicates, the results showed a significant effect of citric and malic acid in the measured growth parameters. The interaction between them was also significantly with a concentration of over 150 mg.L^{-1} for both acids to give the best values to the parameters studied compared to other concentrations.

Key words : Citric acit, Malic acid, *Vicia faba*, growth and yield parameters.

Introduction

Vicia faba L. is a winter erect annual herbal plant belonging to Fabaceae family (Al-Kateb, 1988). Many uses are cultivated as a green crop for the consumption of its pods or as a field crop to obtain its dry seeds for human nutrition or as a green feed crop (Shafiqfiq and Al-Dababi, 2008). The broad bean seeds contain high protein and as other legumes crops that have the potential to fixation atmospheric nitrogen by the root nodes symbiosis with *Rhizobium* bacteria, which increases soil fertility (Shalgham and Shuweileh, 2001). In some countries, crop production has recently begun to decline, and in Iraq, the area under cultivation decreased with the production of the unit of the area during the same period (Ali *et al.*, 1990, Al-Younes, 1993).

Citric acid is one of the acids in the Krebs cycle (TAC) and is the most efficient and effective in releasing energy and occurs in mitochondrial with oxygen (Devli and Witham, 1991). Citric acid contributes to the formation of all compounds and components that contribute to the synthesis of plant tissues and composition its parts, such as proteins, carbohydrates and fats, as well as cytoplasm, cytochrome, phytochrome and photosynthesis pigments (Verma and Verma, 2008). It has found that the treatment of plants in organic acids as citric acid plays an important role in the physiological properties of plants and increases their production as in the okra plant (Shaheen *et al.*, 2006).

Malic acid is one of the important organic acids in Krebs cycle, which is an effective osmotically and enters the lively interactions of the cell (Devlin and Witham, 1991a), consisting in the guards cells of organic acids illuminated leaves such as malic and malate, as the head of the malic acid as a proton (H) moving from the guard cells to subsidiary cells. Offset anions are often malic (malate) within the cells that guard the entry of potassium.

Therefore, the osmotic stresses becomes very negative in guard cells because of the presence of K, CL and potassium mallet or dipotassium malate or both of them (Devlin and Witham, 1991b). The other importance of malic acid that it may be a major source of proton needed for ion exchange between H^-, K^+ during the opening of the stomata. Obviously, if the ABA acid is prepared for the flow of malic acid, the absence or loss of the source of protons will stimulate the closure of the stomata. Although CO_2 appears to interfere with the ABA

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in stimulating the closure of the stomata (Devlin and Witham, 1991a). Because of the lack of studies on these two acids in Iraq and their effect on the growth of the board beans, this experiment was conducted to see the effect of the application of citric acid, malic and different concentrations on some of the growth and yield parameters of the plant.

Materials and Methods

Experience in the plastic house of the Department of Biology, College of Education for Pure Sciences "Ibn Al-Haitham", Baghdad University, for one growth season using 7 kg soil pots for each pot, the soil was taken from the Botanical garden, prepared and initialized and the soil weight above was placed in each pot.

The experiment was designed as a working experience within the complete random design (CRD) (4×3) and with three replicates, which included 36 pots (experimental unit). Ten seeds per pots were planted on 26/11, with water irrigated 75% of the raw field capacity and after two weeks of germination, the plants were reduced to six plants per pot with all the agricultural operations of irrigation and the removal of the bushes.

The leaf application was carried out for two acids and at concentration (0, 75, 150) $mg.L^{-1}$ for citric acid (0, 50, 100, 150) $mg.L^{-1}$ for malic acid on the date of 15/1 in the early morning using a hand grenade. After two weeks, the first workshop and on 19/2 the second workshop and the same concentrations. Plant samples were taken from the vegetative part of the plant on the date of 16/3.

Parameters studied

1. Plant height (cm): the height of the plant was measured by a plastic ruler from the plant's area of contact with the soil to the apical end of the developing stem on 16/3 and then calculated the average of the repeaters.

2. Dry weight of the vegetative total (gm): The dry weight was measured after the vegetative part was placed in an electric dryer at 65ºC for 72 hours until the weight was stable.

3. The number of leaves per plant on the date of 16/3.

4. Total chlorophyll content in the field: The chlorophyll content was measured using a chlorophyll meter (Spad) device for three leaves of the plant by placing part of the leaf under the arm of the device and pressing it where the reading appears on the device screen, the average was calculated on the date of 16/3.

5. The number of flowers per plant on the date of 16/3.

6. Pods number of each plant on the date of 16/3.

7. Dry weight of the pod was calculated on the date of 18/4. The plants were harvested at 16/4.

The results were statistically analyzed by statistical program Little and Hill, 1978) and the averages were compared with the lowest significant difference at the 0.05 probability level.

Results and Discussion

1. Plant height (cm): The results of table 1 showed a significant effect of citric acid and malic and the interaction between them in plant height. The concentration 150 $mg.L^{-1}$ of citric acid achieved the highest rate of the plant height 39.00 cm compared with the treatment of control which gave the lowest rate of plant height reached 27.25 cm/plant with an increase rate of 43.12%. The concentration 150 $mg.L^{-1}$ of malic acid also gave the highest rate of plant height was 39.00 cm/plant compared to the control treatment which gave the lowest rate of plant height 29.00 cm/plant. As for the duel interaction between the two study parameters, found that 150 $mg.L^{-1}$ for both acids have given the highest value of the plant height 46.50 and an increase rate 89.80% compared with the non-acid application treatment which gave the lowest value 24.50 cm.

2. Dry weight (gm): The results shown in table 2 indicated a significant increase in average dry weight when the citric acid concentration was raised from 0 to 150 $mg.L^{-1}$. The dry weight increased from 50.58 to 63.54 gm with a higher concentration of 150 $mg.L^{-1}$ in giving it the highest increase of the rate of the characteristic and an increase rate 25.62% compared to the non-acid application treatment. As well as there is a significant increase in the average characteristic by increasing the concentration of malic acid from 0 to 150 $mg.L^{-1}$ from 51.09 to 63.83 and an increase rate of 24.94% compared to the treatment of control. A for the effect of the

Table 1: The effect of citric and malic acids concentration in height (cm) of *V. faba*.

Concentration of Malic acid ($mg.L^{-1}$)	Concentration of Citric acid ($mg.L^{-1}$)			Effect of malic acid average
	0	75	150	
0	24.50	30.50	32.00	29.00
50	25.50	33.50	37.50	32.17
100	28.00	35.50	40.00	34.50
150	31.00	39.50	46.50	39.00
Effect of Citric acid average	27.25	34.75	39.50	
LSD (0.05)	Malic acid= 0.89^{**}, Citric acid= 0.77^{**} Interaction= 1.54^{**}			

Table 2: The effect of citric and malic acids concentration in dry weight (gm) of *V. faba*.

Concentration of Malic acid ($mg.L^{-1}$)	Concentration of Citric acid ($mg.L^{-1}$)			Effect of malic acid average
	0	75	150	
0	48.77	51.31	53.18	51.09
50	51.35	56.07	58.72	55.38
100	53.04	60.45	64.85	59.45
150	49.15	64.90	77.43	63.83
Effect of Citric acid average	50.58	58.18	63.54	
LSD (0.05)	Malic acid= 5.38^{**}, Citric acid= 4.66^{**} Interaction= 9.31^{*}			

interaction between citric and malic acids, that found the value of this characteristic, with the highest value at the concentration of 150 $mg.L^{-1}$ for both citric and malic acids 77.43 gm and an increase rate 58.77% compared to the non-acid application treatment which gave the lowest values of dry weight 0.05 gm.

3. Number of leaves ($leaf.plant^{-1}$): The results of table 3 indicated that there were significant differences in the number of leaves when the concentration of citric acid increased. The concentration 150 $mg.L^{-1}$ recorded the highest value 20.00 for the number of leaves ($leaf.plant^{-1}$) compared with control plants which gave the lowest number of leaves 12.75 with an increase rate 56.86%. The results of the table also showed that there is significant differences in the number of leaves when application of malic acid at concentration 150 $mg.L^{-1}$ which recorded the highest value for the number of leaves 21.67 $leaf.Plant^{-1}$ with an increase rate 103.09% compared to non-acid application plants. As for the duel interaction between the concentration of citric and malic acids, the concentration 150 $mg.L^{-1}$ for both citric and malic acid has given the highest value to this characteristic where it was 24.50 sheets. Plant-1 with an estimated increase of 226.67% compared to non-application plants with two acids, additionally this concentration of both acids may

Table 3: The effect of citric and malic acids concentration in number of leaves (leaf. $Plant^{-1}$) of *V. faba*.

Concentration of Malic acid ($mg.L^{-1}$)	Concentration of Citric acid ($mg.L^{-1}$)			Effect of malic acid average
	0	75	150	
0	7.50	9.50	15.00	10.67
50	10.50	15.50	18.00	14.67
100	14.50	20.50	22.50	19.17
150	18.50	22.00	24.50	21.67
Effect of Citric acid average	12.75	16.88	20.00	
LSD (0.05)	Malic acid= 0.93^{**}, Citric acid= 0.80^{**} Interaction= 1.60^{**}			

Table 4: The effect of citric and malic acids concentration in total chlorophyll content (Spad unit) of *V. faba*.

Concentration of Malic acid ($mg.L^{-1}$)	Concentration of Citric acid ($mg.L^{-1}$)			Effect of malic acid average
	0	75	150	
0	41.00	41.75	44.05	42.27
50	41.35	43.39	45.90	43.55
100	43.85	45.64	50.00	46.50
150	46.05	51.80	53.95	50.60
Effect of Citric acid average	43.06	45.65	48.48	
LSD (0.05)	Malic acid= 0.86^{**}, Citric acid= 0.74^{**} Interaction= 1.49^{**}			

be exceed significantly to the rest concentration of the two acids in this characteristic.

4. Total chlorophyll content (Spad unit): The results of table 4 indicated that there were significant differences in the average total chlorophyll content under citric acid concentrations. The concentration 150 $mg.L^{-1}$ recorded the highest average of the total chlorophyll content 48.48 and an increase rate 12.59% compared to the treatment of control. As for the results of the table same noted that there are significant differences in the total chlorophyll content by increasing the concentrations of malic acid and the concentration 150 $mg.L^{-1}$ recorded the highest average of chlorophyll content in the plant with an increase rate 19.71% compared to the total chlorophyll content at zero concentration of acid. The duel interaction between citric and malic acids, the results of the table showed that the concentration 150 $mg.L^{-1}$ for both acids was the best, it was given the highest value for this characteristic as the record 53.95 with an increase rate 31.59% compared with the treatment of non-acid application which gave the lowest value 41.00.

5. Number of flowers ($flower.plant^{-1}$): The results of table 5, showed presence of significant difference in the average number of flowers in plant by increasing the

Table 5: The effect of citric and malic acids concentration in the flowers number ($flower.plant^{-1}$) of *V. faba*.

Concentration of Malic acid ($mg.L^{-1}$)	Concentration of Citric acid ($mg.L^{-1}$)			Effect of malic acid average
	0	75	150	
0	5.00	7.00	9.00	7.00
50	6.50	8.50	10.50	8.50
100	7.50	11.50	12.50	10.50
150	8.50	12.00	14.00	11.50
Effect of Citric acid average	6.88	9.75	11.50	
LSD (0.05)	Malic acid= 0.85^{**}, Citric acid= 0.74^{**} Interaction= N.S.			

Table 6: The effect of citric and malic acids concentration in the pods number (pod.plant^{-1}) of *V. faba*.

Concentration of Malic acid (mg.L^{-1})	Concentration of Citric acid (mg.L^{-1})			Effect of malic acid average
	0	75	150	
0	5.50	7.00	8.50	7.00
50	6.50	9.50	10.50	8.83
100	8.00	11.50	12.50	10.67
150	9.50	13.50	14.50	12.50
Effect of Citric acid average	7.38	10.38	11.50	
LSD (0.05)	Malic acid= 0.81**, Citric acid= 0.70** Interaction= N.S.			

Table 7: The effect of citric and malic acids concentration in the pod weight (gm) of *V. faba*.

Concentration of Malic acid (mg.L^{-1})	Concentration of Citric acid (mg.L^{-1})			Effect of malic acid average
	0	75	150	
0	3.85	4.15	4.55	4.18
50	4.35	4.45	5.05	4.62
100	4.65	6.00	6.70	5.78
150	4.45	6.70	7.65	6.27
Effect of Citric acid average	4.33	5.33	5.99	
LSD (0.05)	Malic acid= 0.29**, Citric acid= 0.25** Interaction= 0.51**			

concentration of citric acid, especially at concentration 150 mg.L^{-1} which gave the highest average of this characteristic 11.50 (flower.plant^{-1}) with an increase rate 67.15% compared with control plants. While the results in the same table showed that there were also significant differences in the average of this characteristic by increasing the concentrations of malic acid and the concentration 150 mg.L^{-1} was the best given the average 11.50 with an increase rate 64.29% compared to control plants. As for the duel interaction between citric and malic acids, there was no significant effect on the value of this characteristic.

6. Number of pods (pod.plant^{-1}): The results of table 6 indicated that there were significant differences in the number of pods with the increase in the concentration of citric acid and the concentration 150 mg.L^{-1} was the best at giving the highest average number 11.50 (pod.plant^{-1}) with an increase rate 55.83% compared to the treatment of non-acid application. It was also significantly exceed on the concentration 75 mg.L^{-1} of the above acid. As well as the results of the table 6 recorded significant differences in the average of this characteristic by increasing the concentration of malic acid and the concentration 150 mg.L^{-1} recorded the highest value of the plant's pods number 12.50 pod.plant^{-1} with an increase rate 78.57% compared to non-acid application plants. As for the duel interaction between citric and malic acid, it did not give significant interaction to the values of this characteristic.

7. The weight of the pod (gm): The results of table 7 indicated that there we as significant differences in the average weight of the pod with increased concentration of citric acid and the concentration 150 mg.L^{-1} was the best concentration that gave the highest average of this characteristic 5.99 gm with an increase rate 38.34% compared to the non-application treatment and significantly exceed 75 mg.L^{-1} with an increase rate 12.38%. Among the results of the table, it also noted that there were significant differences in this characteristic between the concentrations of malic acid and the concentration 150 mg. L^{-1} the highest average of the pod weight 6.27 grams with an increase rate 50.00% compared to non-application plants with acid. As well as this concentration, significantly exceed on the concentrations 50 and 100 mg.L^{-1} for this acid in the average weight of the pod. The duel interaction between the concentration of citric and malic acids had a significant effect on the value of this characteristic. The concentration 150 mg.L^{-1} for both acids was best in giving the highest value of the pod weight 7.65 gm with an increase rate 98.70% compared to the treatment of non-application with acid. This treatment (Non-application treatment) has given the lowest values of the pod weight 3.85 grams and the concentration 150 mg.L^{-1} for both acids may be significantly exceed to the value of this characteristic compared to other concentrations of acid.

Citric acid is a buffer solution in the plant cell that prevents sudden change in pH, which affects the metabolic activities of plants and the presence of these solutions resist the sudden change in pH (Al-Saadi and Al-Moussawi, 1980). From the results and parameters found in the above table, the role of increasing the concentration of citric acid in increasing the values of studied parameters is shown and this is consistent with the finding of Al-Qaisi *et al.* that showed of application of the citric acid on barley improved the physiological qualities of barley plant (Al-Qaisi *et al.*, 2015).

The effect of the malic acid had a positive effect on the parameters studied and the concentration 150 mg.L^{-1} effective role in increasing the values of studied parameters and this is consistent with Talebi *et al.,* (2014) finding of spraying *Gazania rigens* with malic acid by all its concentrations showed significant increased in dry weight of plant. Therefore, these results confirmed the role of the positive malic acid.

The malic acid had a significant effect in the chlorophilic content and this corresponds to the results of Darandeh and Hadavi, (2012) on *Lilium* plant and Kazemi, (2013) study found that using malic acid resulted an increase of phenolics, Flavonoids and non-flavonoids of *Fragaria* plant.

We concluded from this study that the application with concentration 150 $mg.L^{-1}$ for both acids increased the values of morphological and physiological qualities such as plant height, dry weight, number of leaves, total chlorophyll concentration, number of flowers, number of pods and the weight of the pod compared to plants control. Depending on the results of the study we recommend conducting research using many different concentrations to get the best concentration of the acid for the purpose of reaching the best productivity and for different plants.

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