Research Article

Role of 5-Aminolevulinic Acid and Stigmasterol on the Oxalate Content of Okra Fruits and Some Active Substances

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Abstract | The study was applied including two separated experiments, first experiment was done at open field cultivation during 2022, and second experiment was done under protected cultivation conditions (Green House) on 2022-2023, at a private field at Tahwila area, Khalis District, Diyala Governorate, Iraq. Three concentrations of 5-aminolevulinic acid and stigmasterol 0, 50 and 100 mg.L^{-1} were done. Each experiment was as a factorial experiment, Randomized Complete Block Design (RCBD) with three replicates used. This study was aimed to reduce oxalates content of okra fruits, and increasing their content of important active substances such as linoleic acid, previous studies proved that conjugated linoleic acid exerts various powerful physiological substances, which have multi functions such as anti-cancer properties, anti-obesity, diabetes, and high blood pressure. Linoleic acid can be effective in preventing diseases or metabolic syndromes. Results showed that the interaction treatment between 5-aminolevulinic acid and stigmasterol concentration of 100 mg.L^{-1} both seasons, significantly increased the content of okra fruits of active substances which include total phenols, beta-carotene and alpha-linoleic acid, and reached 2.010 and 1.615%, 0.947 and 0.536 mg 100 g^{-1} fresh weight, 3.637 and 3.446 µmol.g^{-1} respectively. The two factors also led to reducing oxalate content of the fruits, where reached 89.78 mg.g^{-1} first season, and reached 81.00 mg g^{-1} when treated with the interaction of 5-aminolevulinic acid at 50 mg.L^{-1} and stigmasterol at 100 mg L^{-1} in second season. Control treatment produced fruits with a high content of oxalates and lower values for the rest of the studied traits.

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Introduction

Okra (*Albelmoschus esculentum* L.) belonging Malvaceae family. Okra is important plant rich of high nutritional value, as well medicinal and industrial importance. Okra has a special importance in human nutrition. It containing carbohydrates, minerals and vitamins, every 100 g of Fresh green fruits contain 88.9 g water, 36.00 calories, 2.10 g of protein, 0.20g of fat, 7.20g of carbohydrates, 1.40g of fiber, 82.00mg of calcium, 61mg phosphorus, 1.20mg iron, 185 mg carotene, 47.00mg ascorbic acid, 0.06mg riboflavin, 0.2 mg thiamine and 1 mg niacin (Haytowitz *et al.*, 2019) and oxalate 264 mg (Brinkley *et al.*, 1990).

Several reasons are responsible for low yield per hectare of okra, among which are planting spacing and fruit harvest intervals. It has been observed that suitable planting spacing can lead to optimum okra fruit yield while wrong planting spacing could result in relatively low yield and poor-quality fruits (Maurya *et al*., 2013). Harvest interval is also an important index in crop yield as well as vegetative growth (e.g okra) as it is indeterminate intra-row spacing play an important role (Yadev and Dhankhar, 2005). The okra plant grown cross Iraq to obtain its fruits, which are eaten fresh after cooking, or used frozen or dried during the winter season. Okra contains high pectin and wax-like substances (Islam, 2019). Petra cultivar well-known cultivars and most popular in central of Iraq. Recently, increasing of cultivation okra escalated due to the role of diet in human health, and eating plant products increased more extent than animal products. It is contained many vitamins, minerals and nutrients that stimulate health benefits. It is considered a great source of folic acid, fiber, flavonoids as well high percentage of vitamin C (Zaharuddin *et al*., 2014). Various methods are used to increase the plant's mineral and organic content to reduce fruits oxalate content. Spraying 5-aminolevulinic acid (ALA) which is a key precursor play an effective role in the biosynthesis of all porphyrin compounds, including heme, phytochrome, chlorophyll and vitamin B12. ALA could be used as a biofertilizer as it has a promotive effect on the growth and yield of several crops and vegetables under various environmental factors, salinity, drought, extreme temperatures, as well as its effect on the antioxidant system, photosynthesis (Kosar *et al*., 2015).

Stigmasterol is play an essential role in plant structure, such as growth, components of the basic structure of fats in the cell, and vital genetic factors for many metabolic factors. Estexosterol currently used to improve growth, such as increasing plant height, leaf area, fresh and dry weights, increasing the efficiency of water absorption, stimulating cell division and elongation, and cell expansion (Bakry and Fathipour, 2023).

Bakry and Fathipour (2023) have found that a number of growth regulators, including plant steroids, affect the physiological characteristics of the plant, including stimulating processes within the plant, inhibiting or modifying the physiological processes, stimulating and activating the process of photosynthesis, changing the manufactured compounds, stimulating the absorption of ions and flowering, and increasing the transfer of metabolic materials. The study aim to reduce oxalates content of okra fruits which harms human health, also increasing their content of important active substances by using different levels of 5-aminolevulinic acid (ALA) and Stigmasterol.

Materials and Methods

The study was done by conducting two separated experiments, the first one done under open field cultivation on 2022, second experiment done under protected conditions (Green House) during 2022-2023, at a private field of Tahwila área, Khalis District, Diyala Governorate, Iraq. Study conducted to investigate effects of foliar spraying with 5-aminolevulinic acid (ALA) and Stigmasterol, as well as their interaction, on oxalates content of okra fruits and active substances of Petra cultivar.

5-aminolevulinic acid (ALA) sprayed at three concentration levels 0, 50, and 100 mg. L^{-1}, Stigmasterol and 3 concentration levels 0, 50, and 100 mg. L^{-1} Treatments, separately were sprayed with interval of 30 day after. Okra was planting on 25/3/2022 at first season, ended on 1/7/2022. Second season, Okra planted on 25/11/2022 into (Green house) season ended in 25/3/2023. Two seeds per hole were planted, distance between one plant and another one was 40 cm. The process was applied a week after germination, and all agricultural exercises were carried out uniformly for all experimental units.

Table 1: *Some physical and chemical properties of the experimental field soil for first Season 2022 and Second season 2022-2023.*

Unit	Value		Adjective
	Season 2022-2023	**Season 2022**	
	7.10	7.80	P^H
Desi Siemens. M^2	3.1	3.5	EC
Gm. kg^{-1}	230.6	203.17	$CaCO_3$
%	2.32	1.28	organic matter
Gm. kg^{-1}	208	227	Clay
Gm. kg^{-1}	186	228	Silt
Gm. kg^{-1}	606	545	sand
Study indicators	**Loamy**		**Texture**
Mg. kg^{-1}	9.45	11.40	Nitrogen
Mg. kg^{-1}	7.521	7.311	phosphorous
Mg. kg^{-1}	187.58	195.62	Potassium

Randomly soil samples were taken from the field at a depth of 0-30 cm for seasons, some physical and chemical analyze was conducted at laboratory of Agriculture College, Diyala University, Iraq. Three replications were chosen, Randomized Completely Block Design (R.C.B.D) used. Treatment means were compared according to Duncan's multiple range tests at a probability level of 0.05.

Oxalate content of fruits (ml g^{-1}): It was estimated according to the method Karimi and Irwin (1966). The concentration of oxalic acid was calculated according to the equation:

Oxalic acid (ml g^{-1} = 1.800 × V (Volume of permanganate)

Percentage of total phenols: The method of Khoddami *et al.* (2013) was applied, and the Folin Ciocalteu reagent was used when estimating total phenols, this is done by taking 100 g of sample in a test tube, adding 200 ml of methanol, placing it in an ultrasonic bath for 45 minutes for extraction, then taken 100 (μL) of extract and adding 5 ml of distilled water and 500 (μL) of Folen's reagent. Leave for 30 seconds to 8 minutes, and then add 1.5 sodium carbonate solutions (20%) then leave for 30 minutes at 40°C. taken 5 ml in a glass cell and measure the phenols with a spectrophotometer at a wavelength of 765 nm. The calibration curve is prepared using a series of concentrations of Gallic acid.

Beta-carotene content in fruit (mg 100 g^{-1} fresh weight): The pigment was estimated by taking 1 g of the sample and mashing it then placing in 10 ml of Ethanol. Samples were filtered with filteing- paper and the filtrated samples were taken then read the values by using a spectrophotometer at the following wavelengths:

Pigment wavelength fixed
Beta-carotene 450nm 2592

$$X(mg) = A \times Y(m_1) \times 1000000 \div 2592 \times 100$$

Y(m1) = volume of ethanol used; A = device reading of the sample filtrate

Seed content of alpha-linolenic acid (μmol g^{-1}): The extraction process was carried out by washing the seeds with Ethanol, drying, thenand then grinding. Take 5 gm of ground seed powder, the extraction obtained by using a Soxhlet device and used Ethanol 80%. Extracted solution is passed through the (SPE) column, the material is reserved in the SPE with methanol, and then evaporated using a rotary evaporator until dry (Yuegang *et al.*, 2008).

HPLC analytical conditions
Mobile phase: Acetonitrile: 25μm NH4H2Po4 Ph = 38
80 = 20%
Column: ODSC18 (150×4.6 Id) mm, 5 μm partical size
Flow rate = 1.0 ml/min
Detector = uv – vis at f = 254 n.m
Temperature = 40co
Volume injection = 20 μl

Results and Discussion

Table 2 showed significant differences from spraying with different concentrations of 5-aminolevulinic acid in the oxalic acid content of okra fruits for both seasons. The control treatment excelled with the highest content reaching 121.60 and 102.23 mg.g^{-1}, respectively, while the lowest content reached 90.52 and 82.58 mg g^{-1} respectively, for the spraying treatment with 5-aminolevulinic acid, concentration of 100 mg.L^{-1}. Spraying with different concentrations of Stigmasterol had a significant effect on the same trait, as the highest content was reached in the control treatment for both seasons (Open field and Green house) and reached 101.91 and 89.59 mg.g^{-1} respectively, while the spraying treatment with Stigmasterol gave a concentration of 100 mg.L^{-1} lowest content of oxalates for the two seasons reached 100.63 and 88.30 mg.g^{-1}.

Table 2: *Effect of spraying 5-aminolevulinic acid (ALA), Stigmasterol and their interaction on the Oxalate content of Okra fruits (ml g^{-1}).*

Stigmasterol (mg L^{-1})	5-aminolevulinic acid (mg L^{-1})			Average of stig-masterol
	0	50	100	
First season 2022 (Open field)				
0	122.80 a	92.70 d	90.23 e	101.91 A
50	121.68 b	92.36 d	91.55 d	101.86 B
100	120.33 c	91.80 d	89.78 e	100.63 C
Average of 5 aminolevulinic acid	121.60 A	92.28 B	90.52C	
Second season 2022-2023 (Green houses)				
0	102.70 a	84.40 c	81.67 d	89.59 A
50	101.76 b	82.35 d	84.40 c	89.50 B
100	102.23 a	81.00 e	81.67 d	88.30 C
Average of 5 aminolevulinic acid	102.23 A	82.58 B	82.58B	

*The values of similar letters are not significantly different from each other according to the Duncan test below the 5% **

The results also indicated a significant effect of the dual interaction of 5-aminolevulinic acid and Stigmasterol. The control treatment recorded the highest content of oxalates, which was 122.80 and 102.70 mg.g^{-1}, respectively. As for the lowest content of the oxalic acid content of okra in the dual interaction for both seasons, it was the interaction treatment between 5-aminolevulinic acid and Stigmasterol a concentration of 100 mg.L^{-1} for each, and it reached 89.78 mg.g^{-1} in the first season, and it reached 81.00 mg g^{-1} when the interaction treatment was 5-aminolevulinic acid, a concentration of 50 mg.L^{-1} with Stigmasterol 100 mg.L^{-1} in second season.

The reason for reducing the content of oxalates when spraying with 5-aminolevolic acid and Stigmasterol is that the acid works to improve the process of photosynthesis. Thus increase its products. By improving this process, sufficient energy will be available to reduce nitrates to ammonia and reduce its accumulation, and this in turn reflects positively on the increase in enzyme activity oxalic acid oxidase, Nitrates play a role in inhibiting this enzyme and by reducing them, its activity in reducing oxalic acid will increase. These results are consistent with Al-Mharib *et al*. (2022) in a study of the effect of spraying with acid on the growth of spinach *Spinacia oleracea* L as well as its calcium oxalate content.

As for Stigmasterol, the reason for reducing the concentration of oxalates may be due to its role in influencing the physiological characteristics of the plant, such as stimulating processes within the plant, inhibiting or mutating physiological processes, and changing manufactured materials, and it may have contributed to reducing the build-up of oxalates inside the plant by inhibiting its build-up.

Table 3 showed that the spraying treatment with 5-aminolelevulinic and Stigmasterol, concentration of 100 mg L^{-1} was superior in giving the highest values in the percentage of total phenols in okra fruits. The acidity in both seasons achieved 1.981 and 1.501% respectively, while spraying with Stigmasterol l in the two seasons gave values of 1.955 and 1.515%, respectively; however, the control treatment gave the lowest percentage of total phenols in okra fruits, which amounted to 1.861 and 1.883%, respectively, in the first season and 1.378 and 1.380%, respectively in the second season.

Interaction treatment between 5-aminolevulinic acid and Stigmasterol at a concentration of 100 mg. L^{-1} for both seasons produced the highest percentage of total phenols in okra fruits were 2.010 and 1.615%, respectively, while the control treatment recorded the lowest percentage of phenolic, reaching 1.850% in the first season. The control treatment was 1.330% t with 50 mg.L^{-1} of Stigmasterol in the second season.

Table 3: *Effect of spraying 5-aminolevulinic acid (ALA), Stigmasterol and their interaction on the Percentage of total phenols in Okra fruits.*

Stigmasterol (mg L^{-1})	**5-aminolevulinic acid (mg L^{-1})**			**Average of stig-masterol**
	0	**50**	**100**	
First season 2022 (Open field)				
0	1.850 h	1.850 h	1.950 d	1.883 C
50	1.860 g	1.940 e	1.985 b	1.928 B
100	1.875 f	1.980 c	2.010 a	1.955 A
Average of 5 aminolevulinic acid	1.861C	1.923 B	1.981 A	
Second season 2022-2023 (Green houses)				
0	1.375 e	1.366 e	1.400 d	1.380 C
50	1.330 f	1.430 c	1.490 b	1.416 B
100	1.431 c	1.500 b	1.615 a	1.515 A
Average of 5 aminolevulinic acid	1.378 B	1.43 A	1.501 A	

*The values of similar letters are not significantly different from each other according to the Duncan test below the 5% **

Table 4: *Effect of spraying 5-aminolevulinic acid (ALA), Stigmasterol and their interaction on the Beta-carotene content in Okra fruit (mg 100 g^{-1} fresh weight).*

Stigmasterol (mg L^{-1})	**5-aminolevulinic acid (mg L^{-1})**			**Average of stig-masterol**
	0	**50**	**100**	
First season 2022 (Open field)				
0	0.924 e	0.923 e	0.937 c	0.928 B
50	0.924 e	0.932 d	0.935 c	0.930 B
100	0.931 d	0.941 b	0.947 a	0.939 A
Average of 5 aminolevulinic acid	0.926 B	0.932 AB	0.939 A	
Second season 2022-2023 (Green houses)				
0	0.352 f	0.359 f	0.346 g	0.352 C
50	0.450 c	0.378 e	0.399 d	0.409 B
100	0.352 f	0.536 a	0.499 b	0.462 A
Average of 5 aminolevulinic acid	0.384 B	0.42 A	0.414 A	

*The values of similar letters are not significantly different from each other according to the Duncan test below the 5% *.*

Table 4 showed a significant superiority of spraying with 5-aminolevulinic and Stigmasterol concentration of 100 mg.L^{-1} for each of them and for both seasons, as it gave the highest values in the beta-carotene content of okra fruits, recorded as 0.939 and 0.414 mg 100 g^{-1} fresh weight, respectively, and Stigmasterol gave values of 0.0939 and 0.462 mg 100 g^{-1} fresh weight, respectively. The control treatment gave lowest content of beta-carotene in okra fruits, which amounted to 0.926 and 0.928 mg .100 g^{-1} fresh weight respectively, in the first season and 0.384 and 0.352 mg. 100 g^{-1} fresh weight respectively, in the second season.

5-aminolevulinic acid and Stigmasterol concentration of 100 mg. L^{-1} was significantly superior in the first season and gave 0.947 mg. 100 g^{-1} fresh weight, while the interaction treatment between the control and 5-aminolevulinic acid a concentration of 50 mg L^{-1} gave the lowest content of beta-carotene was 0.923 mg. 100 g^{-1} fresh weight. About the second season, the interaction treatment between 5-aminolevulinic acid concentration of 50 mg. L^{-1} and Stigmasterol concentration of 100 mg L^{-1}, was significantly superior and gave the highest content of beta-carotene in okra fruits, which was 0.536 mg. 100 g^{-1} fresh weight, while the control and 5-aminolevulinic acid concentration of 100 mg. L^{-1} was lowest the content of beta-carotene amounted to 0.346 mg. 100 g^{-1} fresh weight.

Table 5 showed a significant superiority for the spraying treatments with 5-aminolevulinic acid and Stigmasterol a concentration of 100 mg L^{-1} for each of them, as it gave the highest values in the content of alpha-linolenic acid in the seeds of okra fruits and 5-aminolevulinic acid recorded 3.598 and 3.441 µmol. g^{-1}, respectively. As for Stigmasterol, it was given Values are 3.578 and 3.440 µmol. g^{-1} for both seasons, while the control treatment recorded the lowest content of alpha-linolenic acid in the seeds of okra fruits, which amounted to 3.491 and 3.514 µmol. g^{-1}, respectively, in the first season and 3.428 and 3,429 µmol. g^{-1}, respectively in the second season. Regarding the interaction treatments the spraying treatment with 5-aminolevulinic acid and Stigmasterol concentration of 100 mg L^{-1} for each of them, was significantly superior and gave the highest value of alpha-linolenic acid in the seeds of okra fruits, amounting to 3.637 and 3.446 µmol. g^{-1}, respectively in both seasons, while the lowest value was for the two seasons. It reached 3.464 and 3.422 µmol. g^{-1}, respectively, in the control treatment.

Table 5: *Effect of spraying 5-aminolevulinic acid (ALA), Stigmasterol and their interaction on the Okra Seed content of alpha-linolenic acid (µmol g^{-1}).*

Stigmasterol (mg L^{-1})	5-aminolevulinic acid (mg L^{-1})			Average of stig-masterol
	0	50	100	
First season 2022 (Open field)				
0	3.464 i	3.511 g	3.568 d	3.514 C
50	3.482 h	3.539 e	3.591 b	3.537 B
100	3.528 f	3.571 c	3.637 a	3.578 A
Average of 5 aminolevulinic acid	3.491 C	3.540 B	3.598 A	
Second season 2022-2023 (Green houses)				
0	3.422 f	3.431 e	3.436 cd	3.429 B
50	3.430 e	3.437 c	3.443 ab	3.433AB
100	3.433 de	3.441 b	3.446 a	3.440 A
Average of 5 aminolevulinic acid	3.428 B	3.436 A	3.441 A	

*The values of similar letters are not significantly different from each other according to the Duncan test below the 5% **

Tables 3, 4 and 5 indicated an increase in the content of active substances in okra fruits as a result of spraying with 5-aminolevulinic acid. The reason may be due to the fact that this acid, as a result of its containing nitrogen is not only used in building proteins, but can also be considered as the starting materials for products. Natural including carbohydrates, phenols, plant pigments, alkaloids and hormones (Pillai *et al*., 2024), or maybe the reason for the increase in the amount of active compounds when spraying with 5-aminolevulinic acid can also be attributed to the chelating effect contained in the amino acids. The absorption of elements and the transport of macro- and micro-nutrients within the plant becomes easier, as well as its role in increasing the permeability of cellular membranes. This is consistent with Maliha *et al*. (2022). The increase in beta-carotene and alpha-linolenic acid can be attributed to the role of 5-aminolevulinic acid in building auxiliary pigments in the fruit and accumulating nutrients and secondary metabolites from biological processes.

Positive effects of increasing phenolic compounds as a result of spraying with sterols can also be attributed to the fact that Stigmasterol to increase phenolic compounds during the stages of plant growth. It also has a limited effect on the enzymes and pathways responsible for physiological processes (Kumar *et al*., 2010) mentioned that the use of Stigmasterol has a strong effect on growth, as well as increasing the biochemical components of the leaves (phenols and indoles), in addition, to improving the structure of carbohydrates, The positive effects of Stigmasterol in increasing some biochemical components and active substances result from it being a growth regulator that improves photosynthesis activity and increases free amino acids, phenols, protein, chlorophyll, and most of the biochemical components represented by plant pigments and alpha-linolenic acid. These results are consistent with what was found by Hussein *et al.* (2022) regarding about the effect of spraying Stigmasterol on plant dyes and effective plant materials.

Conclusions and Recommendations

The study concludes that the okra plant to spraying with 5-aminolevulinic and Stigmasterol at a concentration of 100 mg L^{-1}, which is the concentration that achieved the best production of the important active substances found in the fruits and seeds of okra. It has been proven that conjugated linoleic acid exerts various powerful physiological functions such as anti-cancer properties, anti-obesity, diabetes, and high blood pressure. This means that linoleic acid can be effective in preventing lifestyle diseases or metabolic syndromes. Spraying also improved the quality of fruits by reducing oxalate acid. Also, cultivation in the open field led to an increase in the active substances in okra more than in greenhouses.

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Novelty Statement

Results of the study show that the nutritional value of okra fruits has been improved which can help the farmers achieve the best production of the important active substances found in the fruits and seeds of okra.

Author's Contribution

Ahlam A. Hussein: Research idea with implementation
Luma B. Hussein: Contributed to data recording and analysis of results
Bassim A. Essa: Contributed to writing the results and translating the research

Conflict of interest
The authors have declared no conflict interests.

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