Impact of nano chelated iron, GA3 and organic fertilizer (Acadian) in Moringa leaves content of α-tocopherol and Phytosterols

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Abstract

Pots experiment was conducted during the summer season 2016 in the Department of Biology/College of Education/AL– Qadisiya University to study the effect of nano chelated iron, GA3 and organic fertilizer (Acadian) in the Content of α-tocopherol and Phytosterols (gamma-Sitosterol, Stigmasterol and Campesterol) in Moringa leaves. The experiment was designed as Randomized Complete Blocks (RCBD) in a factorial arrangement with three replicate. These are involved 5 concentrations of nano chelated (0, 1, 2, 3 and 4) $g.l^{-1}$, GA3 concentrations were used (0, 200 and 400) $mg.l^{-1}$ and organic fertilizer Acadian with (0,1) $g.l^{-1}$. After 150 days from the date of germination, Using the GCMS device to measure the percentage α-tocopherol and Phytosterols. Means were compared by using least significant difference (LSD) at 0.05 level probability. Results showed: the concentration of 2 $g.L^{-1}$ of the nano-chelated iron achieved the highest percentage of Stigmasterol and Campesterol. 4 $g.L^{-1}$ of the nano-chelated iron significant decrease in the percentage of α-tocopherol. No significant effect of nano-chelated iron on the percentage of gamma-Sitosterol was found. The concentration of 200 $mg.l^{-1}$ GA3 significantly increased plant sterols (gamma-Sitosterol, Stigmasterol and Campesterol). GA3 had a negative effect on the percentage of α-tocopherol, Acadian had no significant effect on the active substances studied. The three-way interaction of the studied factors showed that the highest percentage of α-tocopherol increased with the use of 2 $g.l^{-1}$ nano-iron with 200 mg. l^{-1} GA3 and 1 $g.l^{-1}$ of Acadian fertilizer. The use of 1 $g.l^{-1}$ nano-iron with 400 mg. l^{-1} GA3 and 1 $g.l^{-1}$ of Acadian fertilizer to increase the percentage of Stigmasterol, while the combination of 1 $g.l^{-1}$ nano-iron with 200 mg. l^{-1} GA3 and 1 $g.l^{-1}$ of Acadian increased the percentage of Campesterol Therefore, we recommend using the combinations above to obtain the highest percentage of active substances α-tocopherol, Stigmasterol and Campesterol due to its economic feasibility.

Key words: *Moringa oleifera*, nano- iron fertilizer, α-tocopherol, Phytosterols

Introduction:

Moringa is a fast-growing tree of angiosperm plants, its scientific name *Moringa oleifera* Lam. In English called the drumstick [1]. It grows in tropical and subtropical regions, originating from India [2]. The Moringa is named as a Miracle Tree because of its nutritional, medical and industrial importance as well as its environmental importance [3]. It contains vitamin E, an antioxidant found in the Moringa plant [4].The most common and active form of vitamin E is the α-tocopherol compound [5]. It has anti-inflammatory and oxidative properties and has a protective and therapeutic role against diabetes caused by oxidative stress, as it accelerates the course of antioxidants to reduce oxidative stress and indirectly reduce the complications of diabetes [6]. Plant sterols have a similar structure to cholesterol and have the ability to lower cholesterol in the blood, which can prevent cholesterol uptake by competitive inhibition [7 and 8].

Nanotechnology is the science that deals with nanoscale material from 10^{-9} meters [9]. Nanoparticles are used because of their unique physical and chemical properties in biotechnology, agriculture and industry, and their application in agriculture leads to sustainable development because they play a crucial role in

increasing production at the quantitative and qualitative level in the production of agricultural materials and crops [10]. [11] noted that nanoparticles possess all the necessary properties for use in agriculture, such as effective concentration with high solubility, good efficiency, small quantities, avoiding repeated application on the plant and thus obtaining a good result from the first application. [12] indicate that the plant's response to nanomaterials varies according to the type of plant and the added quantity, It affects the physiological and biochemical activities of the plant and stimulates the growth of plant species and other inhibition. Some species showed no physiological changes.

Gibberellins are plant-promoting growth hormones, which are found naturally in all plants of the plant kingdom [13]. Gibberellins act to promote stem elongation, cell division and expansion [14]. Gibberellins also regulate the permeability of cellular membranes as well as regulate the growth and development of plants in response to environmental conditions [15].

The use of organic fertilizers is an important and significant factor in plant growth. Organic fertilizers containing seaweed extract are characterized by antimicrobial, yeast and fungal activity. The increase in plant growth is due to the effect of these extracts on cellular metabolism by stimulating the synthesis of antioxidant molecules Which improve plant growth and stress resistance [16] The use of organic fertilizers containing nitrogen, phosphorus and potassium is great importance as they are considred as a key nutrients that play an important role in plant metabolism such as photosynthesis, and improve the quality of many crops [17]. The aim of this study was to determine the effect of nano-chelated iron containing (9%) of nano-iron, GA3 and organic fertilizer in the content of the leaves of moringa of α-tocopherol and Phytosterols.

Materials and Methods:

Pots experiment was carried out during the summer season 2016 - 2017 in the Department of Biology/College of Education/AL– Qadisiya University to study the effect of five concentrations of nano-chelated iron (0, 1, 2, 3 and 4) $g.L^{-1}$ and three concentrations of GA3 (0, 200 and 400) $mg.L^{-1}$ and organic fertilizer (0 and 1) $g.L^{-1}$ In the content of Moringa leaves of α-tocopherol and plant sterols.

The soil was sandy clay mix with pH 7.32 and organic matter content of soil 2.92 g. Soil and EC = 3.6 (DSM^{-1}). Randomized Complete Block Design (RCBD) is a factorial arrangement was used for the experiment in three replicates. Five concentrates of nano-chelated iron and three concentrations of GA3, while organic fertilizer treatment were use and no used. Thus the experiment involved 90 pots with capacity of 20 kg of soil in each.

The seeds were imported from Egypt by one of the agricultural offices. The nano-chelated iron was obtained from Khazra company in Iran (containing 9% iron and zinc and manganese). Gibbiriline tablets (Grofalcs) from Green River India company, contains 10% gibbirellic acid, while the Acadian is organic fertilizer was imported from Canada, Contains 45% marine algae extracts as well as 50% NPK and 4% amino acids and other compounds. The seeds were planted on 20/3/2016 in pots directly. The experimental unit contains to two plant and three replicates per treatment. Nano-chelated iron, GA3 and organic fertilizer were spraye 30 days after germination then organic fertilizer was re-sprayd 30 day after the first application. After 150 days of planting, leaf conteint of sterols and α-tocopherol was determine by modifyning method using by [19]. This involved Taking 1 g of dried moringa leaves from each

treatment and then 5 ml 96% ethanol was added with continuous stirring and filtered. The leachate was taken the precipitate was extracted with 5 mL of chloroform in the same way and collect the second leachate with the first and concentrate at 40 ° C and dry, then dissolve in 5 ml of hexane . 2 microliters of the resulting extract injected into the GCMS-QP2010 Ultra, which includes the AOC-20i auto-determination unit depending on the mass spectra according to the following conditions. The separation column consists of 100% dimethyl polysiloxane and dimensions (nm30 × 0.25nm × 1μm df). The carrier was helium gas at a flow rate of 1 ml. 1 minute. The injector temperature was 250° C and the ion source temperature was 200° C, The temperature of the oven is automatically programmed to obtain a thermal gradient, starting from 40° C and remaining stable for 3 minutes and then increasing 15° C every 1 minute up to 180° C and then increasing 10° C every 3 minutes to 300° C. The total time for each sample is 28 minutes. The mass spectra were taken on a 70 eV a scan interval of 0.5 s and fractional rate of 45 to 450 Da. The components were identified using the National Institute of Standards and Technology (NIST) database by comparing the output spectrum of the anonymous component with the stored components known in the NIST library. The following active compounds were measured (α-Tocopherol, gamma-Sitosterol, Stigmasterol and Campesterol). The analysis was carried out in the laboratory of the Gas Chromatography related to Mass Spectrometry/Food Research and Consumer Protection Unit / College of Agriculture / University of Basrah.

Results:

1- **Percentage of α-tocopherol (vitamin E)**

The results in Table (1) indicate the effect of the study factors and their interactions in the percentage of α-tocopherol. As for the effect of nano-chelated iron concentrations in this parameter, the concentration of 4 $g.L^{-1}$ nano-chelated iron significantly decreased by 4.50% compared to the comparison treatment of 5.41%, which was not significantly different from the concentration (1-3) $g.L^{-1}$ gave 5.47%, 5.71% and 5.62%, respectively. The GA3 effect was significantly reduced from 7.17 to 5.06 for treatment of 200 $mg.l^{-1}$, up to 3.80 for concentration use of 400 $mg.l^{-1}$. Organic fertilizer did not have a significant effect on this parameter.

The interaction between nano-chelated iron and GA3 was significant in this treatment. At each concentration of nano-chelated iron concentrations, the use of the GA3 (0-400) $mg.L^{-1}$ caused a significant decrease in this characteristic for most combinations. The highest percentage was 7.39% when compared to GA3 with 3 $g.L^{1}$ nano-chelated iron, which did not differ significantly from the 1 and 2 $g.L^{-1}$ nano-chelated iron with 0 $mg.L^{-1}$ GA3), which gave 7.26% and 7.33%, respectively, which did not differ significantly from each other and from the comparison treatment which gave 7.30% compared to all other combinations.

Two-way interaction between nano-chelated iron and organic fertilizer caused an increase in the percentage of α-tocopherol for most combinations. The use of organic fertilizer with a concentration of 4 $g.L^{-1}$ nano-chelated iron to significantly increase the percentage of α-tocopherol compared to no-use by giving 4.95% and 4.05%, respectively, while the use of organic fertilizer with nano-chelated iron significantly decreased in concentration 1 $g.L^{-1}$ compared to no-use, giving 4.75% compared to 6.20%, respectively.

The three-way interaction was significant for this character. The highest ratio of interference combinations was 7.45% for the plants resulting from the use of 2 $g.L^{-1}$ nano-chelated iron with 200 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ of organic fertilizer compared to the comparison treatment which gave 5.66%.

Table (1): Impact of nano-chelated iron, GA3 and organic fertilizer and their interactions in α-tocopherol percentage in leaves of *M. oleifera*

Organic Fertilizer ($g.l^{-1}$)	GA3 ($mg.L^{-1}$)	Nano chelated iron ($g.L^{-1}$)					Two-way interaction between GA3 and organic fertilizer
		0	1	2	3	4	
0	0	5.66	9.07	7.34	5.97	5.80	6.77
	200	3.98	4.91	5.36	4.02	3.91	4.44
	400	4.42	4.61	4.00	6.38	2.45	4.37
1	0	8.93	5.44	7.31	8.81	7.35	7.57
	200	6.99	5.77	7.45	4.57	3.60	5.68
	400	2.47	3.03	2.79	3.95	3.91	3.23
Effect of nano chelated iron		5.41	5.47	5.71	5.62	4.50	
L.S.D 0.05		0.48					0.52
three-way interaction		1.17					

Two-way interaction between nano iron and GA3

GA3 ($mg.L^{-1}$)	Nano chelated iron ($g.L^{-1}$)					GA3 Effect
	0	1	2	3	4	
0	7.30	7.26	7.33	7.39	6.58	7.17
200	5.49	5.34	6.41	4.30	3.76	5.06
400	3.45	3.82	3.40	5.17	3.18	3.80
L.S.D 0.05	0.83					0.37

Two-way interaction between nano iron and organic fertilizer

Organic fertilizer ($g.L^{-1}$	Nano chelated iron ($g.L^{-1}$)					Effect of organic fertilizer
	0	1	2	3	4	
0	4.69	6.20	5.57	5.46	4.05	5.19
1	6.13	4.75	5.85	5.78	4.95	5.49
L.S.D 0.05	0.67					N.S

2- Percentage of gamma-Sitosterol:

The results of Table (2) showed the effect of the study factors and their interference in the percentages of gamma-sitosterol. The concentrations of nano-chelated iron did not have a significant effect on this parameter. While the use of GA3 at a concentration of 200 $mg.L^{-1}$ significantly increased this parameter by giving 12.44% compared to 11.65% for comparison treatment. Organic fertilizer did not have a significant effect on this parameter.

Two-way interaction between nano-chelated iron and GA3 and interaction between nano-chelated iron and organic fertilizer had no significant effect on this parameter.

Two-way interaction between GA3 and organic fertilizer was significant in the percentages of gamma-sitosterol in moringa leaves. The use of organic fertilizer with a concentration of 400 $mg.L^{-1}$ GA3 significantly increased the percentage compared to the non-use of organic fertilizer for the same concentration of GA3 given 11.61% compared to 10.53%, while there was no significant difference in the use of compost with the concentration of 200 $mg.L^{-1}$ GA3.

Three-way interaction did not have a significant effect on this parameter.

Table (2): Impact of nano-chelated iron, GA3 and organic fertilizer and their interactions in the percentage of gamma-sitosterol in leaves of *M. oleifera*

Organic Fertilizer ($g.l^{-1}$)	GA3 ($mg.L^{-1}$)	Nano chelated iron ($g.L^{-1}$)					Two-way interaction between GA3 and organic fertilizer
		0	1	2	3	4	
0	0	11.26	10.63	12.07	12.04	11.98	11.60
	200	12.45	12.99	12.24	12.12	12.85	12.53
	400	10.98	10.94	9.94	10.56	10.25	10.53
1	0	10.54	12.32	11.62	11.08	12.98	11.71
	200	11.68	11.99	12.19	12.97	12.88	12.34
	400	11.25	12.34	12.01	10.91	11.53	11.61
Effect of nano chelated iron		11.36	11.87	11.68	11.61	12.08	
L.S.D 0.05		N.S					0.58
three-way interaction		N.S					

Two-way interaction between nano iron and GA3

GA3 ($mg.L^{-1}$)	Nano chelated iron ($g.L^{-1}$)					GA3 Effect
	0	1	2	3	4	
0	10.90	11.48	11.85	11.56	12.48	11.65
200	12.07	12.49	12.22	12.55	12.87	12.44
400	11.12	11.64	10.98	10.74	10.89	11.07
L.S.D 0.05	N.S					0.41

Two-way interaction between nano iron and organic fertilizer

Organic fertilizer ($g.L^{-1}$	Nano chelated iron ($g.L^{-1}$)					Effect of organic fertilizer
	0	1	2	3	4	
0	11.56	11.52	11.42	11.57	11.69	11.55
1	11.16	12.22	11.94	11.65	12.46	11.88
L.S.D 0.05	N.S					N.S

3- Percentage of Stigmasterol

The results of a table (3) indicate the effect of the study factors and their interaction in the percentage of Stigmasterol in Moringa leaves. The effect of nano-chelated iron was significantly increased by 6.83% at the concentration of 2 $g.L^{-1}$, which was not significantly different from 6.59% for the plants treated with 3 $g.L^{-1}$ concentration compared to the comparison treatment which gave 5.94%. GA3 significantly increased the percentage of Stigmasterol by giving 6.58% for both concentrations compared to 5.75% for comparison treatment. Organic fertilizer did not have a significant effect on this parameter.

Two-way interaction between nano-chelated iron and GA3 showed the highest percentage of Stigmasterol 8.27% were obtained from the use of the combination of 2 $g.L^{-1}$ nano-chelated iron with 400 $mg.L^{-1}$ GA3, which was not significantly different from 7.85% resulting from the use of the combination of 3 $g.L^{-1}$ nano-chelated iron with 200 $mg.L^{-1}$ GA3 compared to other combinations and compared to the comparison treatment which gave 5.76%.

The two-way interaction between nano-chelated iron and organic fertilizer indicates the different effect of different combinations when organic fertilizer use. The use of organic fertilizer with concentrations 1 and 2 $g.L^{-1}$ nano-chelated iron was significantly lower than that of organic fertilizers, which gave 5.59% and 6.08% of the above concentrations respectively with 1 $g.L^{-1}$ of organic fertilizer compared to 6.58% and 7% 58%, respectively.

The two-way interaction of the GA3 and the organic fertilizer was significant because the use of organic fertilizer was concentrated at 1 $g.L^{-1}$ with 200 and 400 $mg.L^{-1}$ GA3 was significantly increased by 6.59% and 6.65% respectively, The comparison of GA3, which gave 5.08%, decreased significantly using organic fertilizer compared to the comparison treatment, which gave 6.42%.

Three-way interaction of the study factors was significant in this parameter. The combination of 2 $g.L^{-1}$ nano-chelated iron with 400 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ organic fertilizer and the combination of 3 $g.L^{-1}$ nano-chelated iron with 200 $mg.L^{-1}$ GA3 with 1 $g.L^{-1}$ of organic fertilizer showed a significant decrease in the percentage of Stigmasterol compared to their percentage when not using organic fertilizer, giving 6.90% and 6.66% compared to 9.63% and 9.04%, respectively. The combination of 1 $g.L^{-1}$ nano-chelated iron with 400 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ organic fertilizer gave the highest percentage of Stigmasterol 7.28% compared to the comparison treatment, which gave 5.76%.

Table (3): Impact of nano-chelated iron, GA3 and organic fertilizer and their interactions in the percentage of Stigmasterol in leaves of *M. oleifera*

Organic Fertilizer ($g.l^{-1}$)	GA3 ($mg.L^{-1}$)	Nano chelated iron ($g.L^{-1}$)					Two-way interaction between GA3 and organic fertilizer
		0	1	2	3	4	
0	0	5.76	6.20	7.21	6.50	6.41	6.42
	200	5.39	6.91	5.90	9.04	5.60	6.57
	400	5.19	6.62	9.63	5.32	5.86	6.52
1	0	5.75	3.02	4.41	5.54	6.68	5.08
	200	6.45	6.48	6.94	6.66	6.40	6.59
	400	7.10	7.28	6.90	6.50	5.42	6.65
Effect of nano chelated iron		5.94	6.09	6.83	6.59	6.06	
L.S.D 0.05		0.64					0.70
three-way interaction		1.56					
Two-way interaction between nano iron and GA3							
GA3 ($mg.L^{-1}$)		Nano chelated iron ($g.L^{-1}$)					GA3 Effect
		0	1	2	3	4	
0		5.76	4.61	5.81	6.02	6.54	5.75
200		5.92	6.70	6.42	7.85	6.00	6.58
400		6.15	6.95	8.27	5.91	5.64	6.58
L.S.D 0.05		1.10					0.49
Two-way interaction between nano iron and organic fertilizer							
Organic fertilizer ($g.L^{-1}$		Nano chelated iron ($g.L^{-1}$)					Effect of organic fertilizer
		0	1	2	3	4	
0		5.45	6.58	7.58	6.95	5.96	6.50
1		6.43	5.59	6.08	6.23	6.17	6.10
L.S.D 0.05		0.90					N.S

4-- Percentage of Campesterol

The results in Table (4) indicate the significant effect of the study factors and their interactions in the percentage of Campesterol. The concentration of nano-chelated iron 3 $g.L^{-1}$ was the highest increase of 4.75%, which was not significantly different from the 4.61% obtained from the use of the concentration of 2 $g.L^{-1}$ nano-chelated iron compared to other concentrations and to the comparison treatment which gave 4.35%.

While the use of GA3 was a significant increase of 4.85% at the concentration of 200 mg.L^{-1} compared to the comparison treatment, which gave 3.96%. Organic fertilizer did not have a significant effect on this parameter.

Table (4): Impact of nano-chelated iron, GA3 and organic fertilizer and their interactions in the percentage of Campesterol in leaves of *M. oleifera*

Organic Fertilizer (g.l^{-1})	GA3 (mg.L^{-1})	Nano chelated iron (g.L^{-1})					Two-way interaction between GA3 and organic fertilizer
		0	1	2	3	4	
0	0	3.81	3.70	4.38	4.37	4.30	4.11
	200	4.24	5.11	4.19	5.60	4.54	4.74
	400	4.10	4.02	5.61	4.44	3.71	4.38
1	0	3.99	2.49	3.50	4.55	4.51	3.81
	200	4.83	4.77	4.94	4.82	5.23	4.92
	400	5.11	5.00	5.01	4.69	4.42	4.85
Effect of nano chelated iron		4.35	4.18	4.61	4.75	4.45	
L.S.D 0.05		0.22					0.24
three-way interaction		0.55					

Two-way interaction between nano iron and GA3

GA3 (mg.L^{-1})	Nano chelated iron (g.L^{-1})					GA3 Effect
	0	1	2	3	4	
0	3.90	3.10	3.94	4.46	4.41	3.96
200	4.54	4.94	4.57	5.21	4.89	4.83
400	4.61	4.51	5.31	4.57	4.07	4.61
L.S.D 0.05	0.39					0.17

Two-way interaction between nano iron and organic fertilizer

Organic fertilizer (g.L^{-1}	Nano chelated iron (g.L^{-1})					Effect of organic fertilizer
	0	1	2	3	4	
0	4.05	4.28	4.73	4.80	4.18	4.41
1	4.64	4.09	4.48	4.69	4.72	4.52
L.S.D 0.05	0.32					N.S

Two-way interaction between nano-chelated iron and GA3 indicates a significant increase in most of the combinations used compared with the comparison treatment. With a maximum Percentage 5.31% at the combination of 2 g.L^{-1} nano-chelated iron with 400 mg.L^{-1} GA3, which did not differ significantly from 4.94% and 5.21% for the plants resulting from the use of 1 and 3 g.L^{-1} nano-chelated iron With 200 mg.L^{-1} GA3, respectively, compared with other combinations and with a comparison treatment that gave 3.90%.

The same table shows the significant effect of the interaction between nano-chelated iron and organic fertilizer. The use of organic fertilizer with a concentration of 4 g.L^{-1} nano-chelated iron was significantly increased in the percentage compared to no-use of the same concentration, which gave 4.72% compared to 4.18%. While the use of organic fertilizer with concentration (1-3) g.L^{-1} nano-chelated iron did not cause a significant effect compared to no-use.

Two-way interaction between GA3 and organic fertilizer was significant in increasing the percentage of this parameter. The use of organic fertilizer with a concentration of 400 mg.L^{-1} was significantly increased compared to no-use, which gave 4.85% compared to 4.38%.

Three-way interaction of the study factors was significant in this parameter. The use of organic fertilizer with nano-chelated iron and GA3 significantly increased most of

the combinations compared to the comparison treatment which gave 3.81%. The combination of 4 $g.L^{-1}$ nano-chelated iron with 200 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ of organic fertilizer was the highest Percentage 5.23%, which significantly higher to same combinations when not using organic fertilizer, which gave 4.54%. But did not differ significantly from 4.77%, 4.94%, 4.82%, 5.00% and 5.01% for the three combinations resulting from the use of 1, 2 and 3 $g.L^{-1}$ nano-chelated iron with 200 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ of the organic fertilizer and combinations 1 and 2 $g.L^{-1}$ nano-chelated iron with 400 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ of organic fertilizer. Therefore, recommended using the combination of 1 $g.L^{-1}$ nano-chelated iron with 200 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ of the organic fertilizer for economic feasibility.

Discussion:

The use of nano-chelated iron resulted in a significant decrease in the percentages of α-tocopherol and a significant increase in the percentages of Stigmasterol and Campesterol (Tables 1, 3 and 4). This is due to the fact that nano-chelated iron fertilizer has unique properties due to their large surface area, their small size and high absorption, which causes an increase in photosynthesis [20]. In addition, the compound geranylgeranyl diphosphate (GGDP), a precursor for the synthesis of tocopherol and sterols in the plant, and phosphorus from the nutrients involved in its composition[21]. The phosphorus increases by increasing the nano- chelated iron [22]. Thus due to the fact of the nano-chelated iron compound contains nano-iron by 9% plus zinc and manganese in its composition, and the presence of these elements side by side reduces the stomatal resistance and increases the stomatal conductivity, which provides the plant with enough of the oxide Carbon and water for photosynthesis and pulls the nutrients [23]. This led to an increase in the production of plant sterols at the expense of α-tocopherol, and thus ot affect the percentage of gamma-sitosterol (Table-2). The negative effect of GA3 on the percentage of α-tocopherol (Table 1) and the positive effect of GA3 in the percentages of gamma-Sitosterol, Stigmasterol and Campesterol (Tables 2, 3 and 4) agree with [24] on *Cannabis sativa*, they reported that GA3 has opposite an effect on the diractions pathways of methylerythritol 4-phosphate and Mevalonic acid, which are considare as an intermediate stages of the formation of tocopherol and sterol, respectively [21]. that inhibits the production of the first and stimulates the production of the second and thus decreases the production of tocopherols and the accumulation of stigmasterol, campesterol and gamma-sitosterol in the plant. Organic fertilizer had no significant effect on the percentages of α-tocopherol, gamma-Sitosterol, Stigmasterol, and Campesterol (Tables 1, 2, 3 and 4) because it used in a lower concentration, therefore, we suggest increase its concentration in future experiment.

treatment combinations of the studied factors in the combination of 2 $g.L^{-1}$ nano-chelated iron with 200 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ of organic fertilizer significantly increased the percentage of α-tocopherol. The combination of 1 $g.L^{-1}$ nano-chelated iron with 400 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ organic fertilizer gave the highest percentage of Stigmasterol. and the combination of 1 $g.L^{-1}$ nano-chelated iron with 200 $mg.L^{-1}$ GA3 and 1 $g.L^{-1}$ of the organic fertilizer to increase the percentage of Campesterol, due to their interrelated additive effect. Therefore, we recommended using this combinations treatment to increase the active substances above.

Conclusion

In this study, the low concentrations of nano fertilizer and GA3 have a positive effect on the production of active substances compared to high concentrations. Thus, the behavior of nano fertilizer is close to the behavior of hormones. While the use of organic fertilizer with its low concentrations did not significantly affect on the

production of active substances. So we suggest improving fertilizers using nanotechnology.

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Acceptance Letter

02 October 2017

Dear Author(s)

Dr./ Mr. Abdulameer Ali Yaseen/ University of Al-Qadisiyah, Iraq

Dr./Mis. Akhlass Mery Kadim/ University of Al-Qadisiyah, Iraq

Greetings, with reference (JPT 10) to your article entitled:

Impact of nano chelated iron, GA3 and organic fertilizer (Acadian) in Moringa leaves content of α-tocopherol and Phytosterols

We wish to bring to your kind notice the following

√ **We acknowledge the receipt of the above mentioned article.**

√ **The above mentioned article(s) has been sent to the reviewer of expert comments**

√ **The above mentioned article(s) have been accepted for publication in the** (Research Journal of Pharmacy and Technology). The probable date of publication is; **Vol:10 (No:11-12): 26 December-:2017**