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Impact of Nano Manganese and Siapton on the Growth of Fenugreek

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> Abstract. A field study was conducted in a nursery in the Euphrates region, AL Qadisiyah Governorate of Iraq, to study the impact of the nano manganese and siapton fertilizer on the growth of Trigonella foenum-graecum L., The experiment was designed with randomized complete blocks, by factorial organization, and with three replications. It included 4 concentrations of nano manganese (0, 1, 2, and 3) g L⁻¹ and 3 concentrations of Siapton (0, 2, and 4) ml L-1. After (90) days from the date of seed germination, the vegetative growth indicators were measured, while the properties of the oil were measured after 6 months from the date of sowing the seeds. The least significant difference (LSD) was used at the 0.05 probability level to test the differences between the means of the treatments. Results showed that the concentration of 2 g L⁻¹ of nano Manganese showed superiority in increasing most of the studied parameters, which included (plant height, number of leaves, leaf area, leaf content of chlorophyll, and the percentage of Linoleic acid), and the impact of nano manganese was negative on the percentage of Palmitic acid. The concentration 2 m L⁻¹ of Siapton fertilizer outperformed increasing plant height, chlorophyll content of leaves, and the percentage of unsaturated fatty acid Linoleic acid, and distinguishing a concentration of 4 m L⁻¹ in achieving the largest number of leaves and the highest leaf area, and the impact of Siapton organic fertilizer was negative on the percentage of saturated fatty acid Palmitic acid. The interaction between the two factors showed the superiority of the combination of 2 g L⁻¹ of nano manganese and 4 m L⁻¹ of organic fertilizer Siapton in achieving the highest plant height, while the highest numeral of leaves, leaf area, chlorophyll content and percentage of Linoleic acid with the combination was 2 g L⁻¹ of nano manganese and 2 m L⁻¹ l. of organic fertilizer Siapton. The impact of the two interactions caused a significant decrease in the percentage of Palmitic acid.

Keywords. Nano Manganese, Siapton, Fenugreek, Linoleic acid, Palmitic acid.

1. Introduction

Trigonella foenum-graecum is an annual plant of the family Fabaceae and is one of the oldest known medicinal plants its widespread medical importance characterizes it because it contains many effective compounds, especially in the leaves and seeds, it is digestive stimulant action, antioxidant anti-diabetic, anti-pyretic, anti-inflammatory, antiradical, antibacterial activity[1]. [2] mentioned that the leaves have a significant role in stabilizing insulin, blood sugar and hemoglobin levels, as well as the part of the seeds, as giving 25-50 grams of fenugreek seeds to diabetic patients daily in the diet significantly reduced blood sugar, and that it has a role in the prevention of atherosclerosis and

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coronary heart disease. However, it should be avoided during pregnancy because its excess causes miscarriage [3].

Micronutrient deficiency is manifested in the abnormal growth of plant parts; It is necessary to explore strategies to improve the quality of crops and their essential nutrients, and that nanotechnology is an alternative due to the unique properties of nano-fertilizers, which are characterized by their fine size, large surface area and low toxicity compared to other compounds and can significantly improve environmental sustainability, especially in developing countries [4]. Nano-fertilizers are more soluble or reactive with controlled release and targeted delivery [5]. Nanotechnology is a promising field with broad applications in biotechnology and other areas of research, its impacts can be positive or negative, so it is necessary to conduct more research on nanoparticles in particular about interaction with plants and their impacts Physiological, biochemical and molecular [6]. Manganese is an essential micronutrient and has many functional roles in plant metabolism; it acts as an activator and cofactor for hundreds of metallic enzymes in plants due to its ability to easily alter the oxidation state in biological systems and plays a vital role in a wide variety of enzyme-catalyzed reactions, including oxidation reactions reduction, phosphorylation, decarboxylation, and hydrolysis show rapid bonding exchange movement Mn can often be replaced by other metal ions, such as Mg, which have similar ionic properties and requirements for the bond [7]. It is one of the nutrients needed by the plant at concentrations less than 100 mg kg⁻¹ of plant dry matter [8].

Amino acids are stimulants that promote plant growth [9]. Use amino acids regulates the absorption and representation of nitrogen, and this phenomenon is mediated by enzymes involved in the process of nitrogen metabolism; in addition, it was found that the use of amino acids increases potassium K+ ions in the presence or absence of salt stress [10]. Among the amino acids that are included in the composition of Siapton organic fertilizer are the amino acid proline, hydroxyproline and glycine, which are ready for absorption; the impact of proline depends on its concentration because the excess amount of free proline has adverse or side impacts on cell growth or protein functions and the impactiveness of the applied proline depends As a foliar spray depends on the plant growth stage, type and concentration, it is, therefore, necessary to determine the optimum concentrations of externally applied proline that can provide beneficial impacts in crop plants when exposed to abiotic stress [11]. Since the use of nano-fertilizers is one of the modern techniques that ensure the direct and rapid delivery of nutrients to the plant, and considering that manganese and amino acids in Siapton organic fertilizer play an essential role in plant growth and development, given the medical and importance of the active substances in the fenugreek, as well as its economic importance, this study aimed to find the impact of nano-manganese and Siapton organic fertilizer on the growth of fenugreek plants and to obtain a combination that makes increasing the indicators of vegetative growth and yields economically feasible.

2. Material and Methods

A field study was conducted in a nursery in the Euphrates region, Qadisiyah with coordinates (longitude 44.9060 and latitude 32.0112), during the 2019-2020 agricultural season. The field soil was analyzed, and Table (1) shows its chemical and physical properties. The experiment was designed with a completely randomized block with a factorial organization; it included four concentrations (0, 1, 2 and 3) g L⁻¹ of nano-manganese, and Siapton with 3 concentrations (0, 2 and 4) ml L⁻¹ and in three replicates with an area of a square meter to each unit is separated by half a meter. The seeds were sown on 20/10/2019; nano chelated Manganese, imported from Al-Khadra Company for Nano Fertilizers in Iran, was used as a fertilizer sprayed on the leaves after the plants reached the stage (4-8) real leaves on 20/11/2019 (which is a powder consisting of 12% chelated manganese is entirely soluble in water at pH 3-11), by weighing each concentration individually and placing it in a 1-liter hand sprayer and completing the volume by adding distilled water. On the second day, on 21/11/2019, Siapton organic fertilizer (composed of balanced amino acids, free amino acids 10% and amino acids such as proline, hydroxyproline and glycine at a rate of 55% ready for absorption) as a fertilizer

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sprayed on the leaves, measuring each concentration separately put it in a 1-liter hand sprayer and complete the volume by adding distilled water.

The vegetative characteristics were studied after 90 days of seed germination on 20/1/2020 by taking the average of 5 random plants from each experimental unit for each of the following traits: plant height (cm), number of leaves (leaf plant⁻¹) and leaf area (cm2 plant⁻¹), and the chlorophyll content of the leaves (SPAD) was measured using a Japanese-origin chlorophyll meter SPAD-502 Plus by taking ten random readings and then extracting the average from each treatment for each replicate [12]. On 20 April 2020, the oil was extracted from the seeds using the method of [13] with some modifications by taking 100 g of ground seeds and 600 ml of n-hexane for 3 hours at a temperature of (65-70) C ° with a Soxhlet extractor device and then filtering the mixture using a filter with nozzles diameter (0.45) Hispanic micrometer attached to a 10 ml medical syringe to speed up the filtration process, and two microliters of injected in to the Gas Chromatography-Mass Spectrometry device (GCMS-QP2010 Ultra), which includes an automatic identification unit for compounds based on mass spectra, oven temperature was programmed automatically to obtain a temperature gradient, starting from 50°C (equal temperature for 3 minutes) It increases 15 °C every one minute up to 180 °C. It then increases 10 °C every 3 minutes up to 300°C, and then the temperature stabilizes at 300 °C, the separation column consists of 100% dual-polysilocane with dimensions nm30×0.25nm×1µm and the helium gas carrier with a flow rate of 1 ml min⁻¹, the injector temperature 250°C and the ionic source temperature 200°C, and the. Components were identified using the National Institute of Standards and Technology (NIST) database by comparing the resulting spectrum of the unknown component with the known component stored in the NIST library. Based on the importance and the highest percentage, two impactive fatty acids were selected: Linoleic acid and Palmitic acid.

Table 1. Some chemical and physical properties of field soil.

properties	Value
The degree of reaction of the soil pH	8.21
Electrical Conductivity EC (1:1) Ds m ⁻¹	3.60
Soil organic matter SOM	2.93
Available Nitrogen mg Kg ⁻¹	12.44
Available Potassium mg Kg ⁻¹	176.94
Available Phosphorous mg Kg ⁻¹	6.77
Sand %	29.8
Silt %	51.9
Clay %	18.3
Soil texture	Silty Loam

3. Result

3.1. Plant Height (cm)

The Table (2) indicate the concentration of 2g L⁻¹ of nano manganese caused a significant increase in plant height, which reached 47.74 cm, which significantly different from all concentrations and the control treatment, which amounted to 36.89 cm.

Also, Siapton organic fertilizer concentration coefficients indicate that the highest significant increase of 46.91 cm was with 4 ml L⁻¹ concentration did not differ significantly from 45.11 cm obtained using a concentration of 2 ml L⁻¹ compared to the control treatment, which amounted to 35.41 cm. The binary interactions between the two factors of the study had a significant impact on increasing plant height, as the combination of 2g L⁻¹ of nano manganese and 4ml L⁻¹ of Siapton organic fertilizer achieved the highest height of 56.42 cm, which differed significantly from all combinations and control treatment, which amounted to 32.32 cm.

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Table 2. Impact of nano manganese and Siapton and their interactions on height (cm) of *T. foenum-graecum* plant.

concentration of nano	Concentrations of Siapton organic fertilizer ml L ⁻¹			Average impact of
manganese g L ⁻¹	0	2	4	nano manganese
0	32.12	38.23	40.31	36.89
1	34.26	42.96	43.99	40.40
2	38.30	48.49	56.42	47.74
3	36.97	50.75	46.91	44.88
Average impact of organic fertilizer Siapton	35.41	45.11	46.91	
L.S.D 0.05		2.11		2.44
two interactions between nano manganese and organic fertilizer			4.22	
Siapton				

3.2. B. Number of Leaves (leaf plant-1)

The table (3) show the significant impact of the study factors and their interactions in increasing the average number of leaves of the plant. The concentrations of (2 and 3) g L⁻¹ of nano-manganese were significantly superior by giving the most number of leaves which reached (90.89 and 89.11) leaf plant⁻¹, respectively, which did not differ significantly from each other compared to the concentration of 1 g L⁻¹ which reached 81.00 leaf plant⁻¹ and the comparison treatment that gave 73.11 leaf plant⁻¹ indicate the economic feasibility of using the concentration of 2 g L⁻¹. Also, under the influence of Siapton organic fertilizer, the average number of leaves increased significantly from 74.33 leaves plant⁻¹ for the control plants to 86.58 leaf plant⁻¹ for plants treated with a concentration of 2 ml L⁻¹, up to 89.67 leaf plant⁻¹ for plants treated with the highest concentration of 4 ml L⁻¹. The binary interactions between nano manganese and Siapton organic fertilizer showed a significant impact in increasing the number of leaves so that the use of nano manganese at a concentration of 2 g L⁻¹ with a concentration of 4 ml L⁻¹ of Siapton organic fertilizer recorded the highest number of leaves, which was 97.67 leaf plant⁻¹ Which did not differ significantly from the two mixtures containing 2 and 3 g L⁻¹ with 2ml L⁻¹ of Siapton organic fertilizer, which gave (95.33 and 94.67) leaf plant⁻¹, respectively compared to all other mixtures and with the control treatment that it gave 66.33 leaf plant⁻¹.

Table 3. Impact of nano manganese and Siapton and their interactions on number of leaves (leaf plant of *T. foenum-graecum* plant

concentration of nano	Concentrations of Siapton organic fertilizer ml L ⁻¹			Average impact of
manganese g L ⁻¹	0	2	4	nano manganese
0	66.33	72.67	80.33	73.11
1	71.67	83.67	87.67	81.00
2	79.67	95.33	97.67	90.89
3	79.67	94.67	93.00	89.11
Average impact of organic fertilizer Siapton	74.33	86.58	89.67	
L.S.D 0.05		1.98		2.29
two interactions between nano manganese and organic fertilizer			3.97	
Siapton				



3.3. Total leaf area (cm² plant¹)

Table (4) shows that the use of nano manganese at concentrations of 2 and 3 g L⁻¹ led to a significant increase in the total leaf area of plants, as the highest total leaf area for them reached (211.76 and 209.01) cm² plant⁻¹, respectively, which did not differ significantly from each other compared to the concentration 1 g L⁻¹, which gave 190.64 cm² plant⁻¹ and the control treatment, which gave 171.47 cm² plant⁻¹. Siapton organic fertilizer caused a significant increase in the total leaf area, which reached a maximum of 211.48 cm² plant⁻¹ at a concentration of 4ml L⁻¹ compared to the control treatment and at a concentration of 2ml L⁻¹, which gave (172.00 and 203.68) cm² plant⁻¹ on straight. The significant binary interaction between nano manganese and organic fertilizer showed that the addition of organic fertilizer caused a significant increase in leaf area compared to the control treatment at each concentration of nano manganese. The combination achieved 2 g L⁻¹ for nano manganese and 4 ml L⁻¹ for organic fertilizer, the highest leaf area. It reached 226.60 cm² plant⁻¹, which did not differ significantly from (224.95 and 224.68) cm² plant⁻¹ for plants treated with the combination of 2 and 3 g L⁻¹ of nano manganese with 2ml L⁻¹ of Siapton organic fertilizer on respectively, compared to all other combinations and the control treatment which gave 153.89 cm² plant⁻¹.

TABLE 4. Impact of nano manganese and Siapton and their interactions on total leaf area (cm2 plant⁻¹) of *T. foenum-graecum* plant

concentration of nano	Concentrations of Siapton organic fertilizer ml L ⁻¹			Average impact of
manganese g L ⁻¹	0	2	4	nano manganese
0	153.89	169.31	191.22	171.47
1	165.55	195.78	210.58	190.64
2	183.73	224.95	226.60	211.76
3	184.83	224.68	217.50	209.01
Average impact of organic fertilizer Siapton	172.00	203.68	211.48	
L.S.D 0.05		4.23		4.88
two interactions between nano)			
manganese and organic fertiliz	er		8.45	
Siapton				

3.4. Total Chlorophyll Content of Leaves (SPAD)

Table (5) shows that all concentrations of nano manganese had a significant impact on increasing the chlorophyll content of leaves, which reached a maximum of (70.20 and 70.61) SPAD for concentrations (2 and 3) g L⁻¹, respectively, which did not differ significantly from each other compared to the comparison treatment that gave 48.81 SPAD. The application of Siapton organic fertilizer at concentrations of 2 and 4 ml L⁻¹ led to a significant increase in the chlorophyll content of leaves, which amounted to (66.86 and 66.34) SPAD, respectively, compared to 53.64 SPAD resulting from the control treatment. Binary interaction between nano manganese and Siapton organic fertilizer indicates that increasing the fertilizer concentrations from 0 to 4 ml L⁻¹ for each concentration of nano manganese concentrations led to a significant increase in the chlorophyll content of leaves compared to the control treatment if the combination achieved 2 g L⁻¹ for nano manganese and 2 ml L⁻¹ for Siapton organic fertilizer, the highest content was 78.47 SPAD, which did not differ significantly from 76.17 SPAD for plants treated with the combination of 3 g L⁻¹ of nano manganese with 2 ml L⁻¹ of Siapton organic fertilizer compared to the rest The combinations and the comparison treatment which gave 44.40 SPAD.



Table 5. Impact of nano manganese and Siapton and their interactions on total leaf content of chlorophyll (SPAD) of *T. foenum-graecum* plant.

concentration of nano	Concentrations of Siapton organic fertilizer ml L ⁻¹			Average impact of
manganese g L ⁻¹	0	2	4	nano manganese
0	44.40	50.33	51.70	48.81
1	50.27	62.47	65.77	59.50
2	56.67	78.47	75.47	70.20
3	63.23	76.17	72.43	70.61
Average impact of organic fertilizer Siapton	53.64	66.86	66.34	
L.S.D 0.05		1.35		1.56
two interactions between nano				
manganese and organic fertilizer			2.70	
Siapton				

3.5. Percentage (%) of Linoleic Acid

Table (6) shows the significant impact of nano-manganese on increasing the percentage of Linoleic acid, as the highest percentage reached 43.19% at a concentration of 2g L⁻¹, which did not differ significantly from 42.99% resulting from the use of 3g L⁻¹ of nano copper and compared with 41.71%.resulting from the comparison treatment. The use of Siapton fertilizer with concentrations (2 and 4) ml L⁻¹ was significant in the percentage increase, which amounted to 42.76% and 42.90%, respectively, which did not differ significantly compared to the control treatment given 41.86%. The interaction between the two factors of the study showed a significant effect, and the highest percentage of Linoleic acid was 43.87% with the combination of 3 g L⁻¹ of nano manganese with 2 ml L⁻¹ of Siapton, which did not differ significantly from 43.70% and 43.46% resulting from the use of the two combinations 2 g L⁻¹ of nano manganese with (2 and 4) ml L⁻¹ of Siapton, respectively, indicating the possibility of using alternative combinations according to the availability of fertilizers or economic feasibility.

Table 6. Impact of nano manganese and Siapton and their interactions on Percentage (%) of Linoleic acid of *T. foenum-graecum* plant

concentration of nano manganese g L ⁻¹	Concentrations of Siapton organic fertilizer ml L ⁻¹			Average impact of
	0	2	4	nano manganese
0	40.97	41.36	42.80	41.71
1	41.75	42.11	42.55	42.14
2	42.41	43.70	43.46	43.19
3	42.32	43.87	42.78	42.99
Average impact of organic fertilizer Siapton	41.86	42.76	42.90	
L.S.D 0.05	0.49			0.57
two interactions between nano				
manganese and organic fertilizer			0.98	
Siapton				

3.6. Percentage (%) of Palmitic Acid

The table (7) show the negative impact of nano-manganese on the percentage of palmitic acid and the variation in the impact of different concentrations; the lowest percentage was 8.66% at the concentration 2 g L⁻¹, which did not differ significantly from 9.06% resulting from the use of the concentration 3 g L⁻¹ compared to With a comparison treatment of 9.63%.

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Table 7. Impact of nano manganese and Siapton and their interactions on Percentage (%) of Palmitic acid of *T. foenum-graecum* plant.

concentration of nano manganese g L^{-1}	Concentrations of Siapton organic fertilizer ml L ⁻¹			Average impact of
	0	2	4	nano manganese
0	10.11	9.75	9.04	9.63
1	10.07	9.16	8.25	9.16
2	9.14	8.75	8.10	8.66
3	8.97	8.45	9.77	9.06
Average impact of organic fertilizer Siapton	9.57	9.03	8.79	
L.S.D 0.05		0.38		0.43
two interactions between nano mangan and organic fertilizer Siapton	ese		0.75	

The Siapton fertilizer also had a significant impact on the decrease in the percentage of this fatty acid for both concentrations (2 and 4) ml L⁻¹, which gave 9.03% and 8.79%, respectively, which did not differ from each other significantly and compared to the control treatment. The two interactions of the study factors caused a significant decrease in the percentage of most of the combinations. The combination achieved 2 g L⁻¹ of nano manganese with 4 ml L⁻¹ of Siapton organic fertilizer; the lowest percentage was 8.10%, which did not differ significantly from (8.25%, 8.75% and 8.45%) obtained from using the combination of 1 g L⁻¹ of nano manganese with 4 ml L⁻¹ of Siapton organic fertilizer and the combination of (2 and 3) g L⁻¹ of nano manganese with 2 ml L⁻¹ of Siapton organic fertilizer, on respectively, and compared to the comparison treatment, which gave 10.11%.

4. Discussion

The results showed that the concentrations of nano manganese and Siapton organic fertilizer and their interactions significantly affected vegetative growth characteristics. The increase in plant height, number of leaves, total leaf area and leaf content of chlorophyll (Tables - 2, 3, 4 and 5) by increasing the concentrations of nano manganese from (0 to 3 g. L⁻¹) is because of nano-fertilizers with a high surface area and small particles led to increase its absorption by the nano-pores of the leaves and stomata holes and its penetration deep into the leaves and the ease of delivery of nutrients between cells through plasmodesmata links that form nano-channels 50-60 nm [14]. Manganese is of great importance in plant growth, it is found in many enzymes of plant cells, including the enzymes oxidoreductases, hydrolases, lyases and ligases. Its presence in the nitrite, hydroxylamine and manganese reductase enzymes is a cofactor for the first enzyme of the shikimate pathway that prepares the precursors for the biosynthesis of the aromatic amino acids tyrosine, phenylalanine and tryptophan [15]. The amino acid tryptophan is a primary source of building phytohormone Indole Acid Acetic and the latter is responsible for cell elongation and apical dominance and thus increases plant height [16]. This caused an increase in the number of leaves and leaf area, the findings of [17] in their study on fenugreek are consistent with the current results. The role of nano-manganese in the photosynthesis process led to the rise in the construction of chlorophyll pigment, this result is consistent with the findings of [18] in their study on the snap bean plant *Phaseolus vulgaris* L. The reason for the use of nano manganese is increasing the percentage of unsaturated fatty acid Linoleic acid (Table -6), and this is in agreement with [19] Who indicated that the use of nano manganese leads to an increase in the activity of the electron transport chain of the second photosystem and thus increasing the overall efficiency of the building process The plants fertilized with nano-manganese showed a high rate of nitrogen uptake, metabolism and fatty acid biosynthesis compared to their counterparts. Whereas the increase in nano-manganese caused a significant decrease in the percentage of saturated palmitic acid (Table -7), and this is consistent with the findings of [20] in their study on wheat Triticm aestivum L., Where they indicated that saturated fatty acids are negatively associated with an increase in manganese. The impact of using Siapton at a concentration of 2 ml L⁻¹ on increasing plant height,

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number of leaves, total leaf area, and leaf content of chlorophyll (Tables- 2, 3, 4 and 5) is due to the role of its components of proline and hydroxyproline in building the cell wall of cells, a component of cell wall proteins, and plays a pivotal role in plant growth and development; cell walls also contain glycoproteins rich in hydroxyproline as complex macromolecules [21]. As well as the role of glycine and other amino acids as growth stimulants in plants [22]. This led to an increase in the size of cells, stimulating them to divide and an increase in their number, and consequently an increase in the indicators of vegetative growth from plant height, number of leaves and leaf area. This result is consistent with the findings of [23] in their study on the cucumber plant Cucumis sativus L. Suppose they found that the use of Siapton organic fertilizer has a positive impact on most growth characteristics. The significant increase in the percentage of Linoleic acid using organic fertilizer Siapton (Table - 6) and the significant decrease in the percentage of Palmitic acid (Table - 7) agree with [24] reached in their study on Nigella sativa Linn, as they indicated The use of organic materials in fertilization works to bring about biochemical changes and affect the formation of fatty acids, thus increasing the proportion of unsaturated fatty acids at the expense of saturated fatty acids. The interaction of the study factors, nano manganese and Siapton organic fertilizer, had a significant impact on increasing the vegetative growth and yield indicators. The use of nano-manganese helped to withdraw nutrients and the role of Siapton organic fertilizer, which contains amino acids, which is a source of some plant hormones. And their synthesis role in improving growth and absorption of nutrients from the soil led as a result to an increase in the content of chlorophyll in leaves and in improving most of the vegetative traits and oil of the plant. As for the combinations with equal influence from the study factors, they indicate the possibility of using them as alternative combinations according to the available ones and their economic return.

Conclusion

The use of nano-manganese and Siapton organic fertilizer stimulated the growth of the vegetative plant and the binary interactions between the two factors of the study had a significant impact on increasing plant height, as the combination of 2g L⁻¹ of nano manganese and 4ml L⁻¹ of Siapton organic fertilizer achieved the highest height, while, using the combination of 2 g L⁻¹ of nano manganese with 2 ml L⁻¹ of Siapton organic fertilizer led to incrase of the number of leaves, the total leaf area of the plant, and an increase in the formation of chlorophyll, it also led to an improvement in the quality of the oil due to chemical changes that affected the formation of fatty acids, increasing the percentage of unsaturated fatty acid Linoleic acid and decreasing the percentage of saturated fatty acid Palmitic acid. This opens the way to study the impact of these fertilizers on other medicinal and economic plants to see their impact on the active substances.

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