Effect of Foliar Application with Nano-Organic Fertilizer and Proline Acid and their Interaction in Leaf Content of Antioxidants and Vitamin D3 for *Moringa oleifera*

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Abstract

A potted pot capacity 15 kg experiment was carried out during the agricultural season 2019 in one of the private nurseries of Al-Ramadi / Anbar Governorate, to study the effect of foliar application of nano-fertilizer and proline acid on the content of antioxidants and vitamin D3 for *Moringa oleifera* leaves using sandy mix soil. The experiment was designed using Completely Randomized Design (CRD) with three replications. The study included three concentrations of nano-organic fertilizer (0, 50, 100) mg-liter^{-1} and three concentrations of the amino acid proline (0, 100, 200) mg^{-1} . after 150 days from the date of seed germination the leaf content of vitamin C was measured and phenols using the least significant difference LSD at 0.05 probability level. Qurcetine flavonoids, Rutine flavonoids and vitamin D3 were measured according to the T test to test the differences between the averages of the treatments. The results showed that the nano-fertilizer at concentration100 mg L1 achieved the highest antioxidant level that included vitamin C, phenol, flavonoid quercetin, flavonoid routine and achieved the highest average vitamin D3 content. And that treatment with proline acid at a concentration of 200 mg / L $^{-1}$ achieved the highest antioxidant content (vitamin C, phenols, Qurcetine and Rutine flavonoids) in addition to vitamin D3. The interaction between the study two factors had a positive effect on most of the rates of the studied traits. The plants which treated with (100 mg-liter-1 nano organic fertilizer and 200 mg-liter-1-proline acid) gave the highest rate of leaf content of vitamin C, phenols, Qurcetine, and Rutine flavonoids, while treatment with (100 mg-liter-1 nano organic fertilizer and 100 mg-1-proline acid) has achieved the highest leaf content level of vitamin D3 .

Keywords: *antioxidant, nano organic fertilizer, Proline acid, Moringa oleifera*

Introduction

Moringa from perennial trees that belong to the Moringaceae family, has high medical and nutritional properties, and called the miracle tree , or the tree of life because of the human aspects it carries to the poor, it represents a complete food source for them.(1)

The original habitat of Moringa in northern India, on the foothills of the Himalayas, as it is widespread in Kenya, Sudan and Ethiopia and in the tropics and subtropics lands(2). Moringa is considered one of the beneficial trees for humans, animals and plants, as it is considered the most promising generation of medicine, food, and animal feed(3). It is also a medical tree that takes advantage of all its parts (leaves, fruits, roots, and twigs), its leaves are the most used for what they contain of Proteins, vitamins, salts, minerals and active substances(4).

Nanotechnology is a science concerned with the study and careful control of the production of a substance at the nano scale level, i.e. production of nano materials whose micro dimensions are a part of a billionth of a meter (1 × 10^{-9} m)(5). Nano scale applications have

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many uses, including their use in the agricultural field for the purpose of reaching modern agricultural methods characterized by the low economic cost resulting from increasing the efficiency of manufactured fertilizers in terms of agricultural product resistance to various agricultural conditions and improving nutrient management and enhancing their absorption by the plant[6], given what These materials are distinguished by physical, chemical, and mechanical properties that distinguish them from materials with large dimensions, since characteristics appear similar to those properties when they are in the traditional case[7]. As an effective concentration with solubility and high effectiveness and are used in small quantities compared to regular fertilizers to avoid repeated application to plants and access on good productivity from the first application .[8]

Foliar application is the addition of nutrients by spraying its solutions with certain concentrations on the vegetative group of plants, so that it can be absorbed by the stomata on the surface of leaves or by the cell walls and membranes and their entry into the biological processes of plants leading to an increase in the vegetative and qualitative characteristics in order to avoid the lack of availability in the component .[9]

Nutrition plays an important role in the growth and development of plants, and in the case of medicinal plants nutrients can significantly increase the active substances of these plants .[10]This nutrition is of great importance to plants that suffer from having a weak or weak branching root system, whose spread is limited to the surface of the soil, which cannot enable the plants to absorb nutrients in sufficient quantities that meet the needs of the plant .[11]

Proline acid is one of the amino acids that the plant produces naturally and have an important role in increase the growth of the plant and increase its response to fertilization and its resistance to diseases[12]. Proline accumulates in different parts of the plant, but it collects high in the leaves [13]It has an important role in capturing free radicals, as its accumulation in the plant is evidence of an increased concentration of antioxidants for these roots.[14]

Antioxidants are any substance that has the ability to prevent oxidative damage and are found in a few concentrations that inhibit or delay the oxidation of molecules[15], as they are necessary to control the harmful effect of free radicals in the human body[16], as it possesses pharmacological activity for many Diseases for being safe with long-term use[17].

The aim of this research is to study the effect of foliar applicationing with nano fertilizer and proline acid and their interaction in the antioxidant and vitamin D3 concentrations in the leaves of the Moringa tree.

Materials and Methods

The experiment was conducted during the agricultural season 2019 in one of the private

nurseries of the city of Ramadi / Al-Anbar Governorate to study the effect of three concentrations of nano-fertilizer (0, 50, 100) mg. Liters $^{-1}$ and three concentrations of the amino acid proline (0, 100, 200) mg $^{-1}$ In antioxidant and vitamin D3 content of Moringa leaves . Random samples were taken from soils and analyzed in the High Euphrates Laboratory which belongs to the University of Anbar for the purpose of revealing the physical and chemical properties of the soil (Table-1).

Completely Randomized Design (CRD) was implemented experimenting with Moringa seeds after soaked for 24 hours with three seeds for each pot, and they were diluted to one plant after germination. The Nano NPK produced by the Indian company (Skofarms) was applied when the plant reached the stage of 4 real leaves and the next day it was applied with proline acid produced from the Indian Green River Company. And repeated the process of application with organic nano fertilizer and proline acid after 30 days from the first application and the transactions were carried out from hoes and weeding and irrigation whenever the need arises.

After 150 days of planting, the leaf content of antioxidants and vitamin D3 was estimated.

1- Estimating the vitamin C content according to the method approved by .[18]

2-The leaf content of the total phenols according to the Arno's method.

3- Estimating the leaf content of Qurcetine, Rutine and Vitamin D3 flavonoids in the laboratories of the

Department of Environment and Water / Ministry of Science and Technology and by method[19], using HPLC (High performance liquid chromatography) with the inverse phase system, which is characterized by its ability to separate many Of the compounds such as phenols, polysaccharides, vitamins, and others, and their quantification and separation, the sample was distributed between two phases, one of which is a fixed phase and is liquid or solid in a column, and a mobile phase is a liquid and the separation depends on the efficiency of the specifications for the column in terms of the diameter of the particles of the material packed inside and whenever the diameter is small it led to Improving the work performance of the column by raising the pressure and obtaining a suitable flow rate for the moving phase within it, which leads to separating the components of the sample through the detector and displaying it in the form of chromatography[20].

Results

1- Vitamin C concentration. Mg.100 g:

(Figure -1) indicates the significant effect of nano-organic fertilizer in increasing vitamin C levels, as treatment T2 gave a rate of 102.31 mg. 100 g compared to a comparison treatment (T0) which gave 95.00 mg. 100 g. Proline acid Application had a significant effect in increasing this trait. Treatments T3 and T4 gave an average of 107.66 and 113.00 mg. 100 g respectively which did not differ significantly from each other but differed from the comparison treatment T0, bilateral interference demonstrated a significant effect in vitamin C, since treatment T8 gave the highest rate of 140.72 mg. 100g.

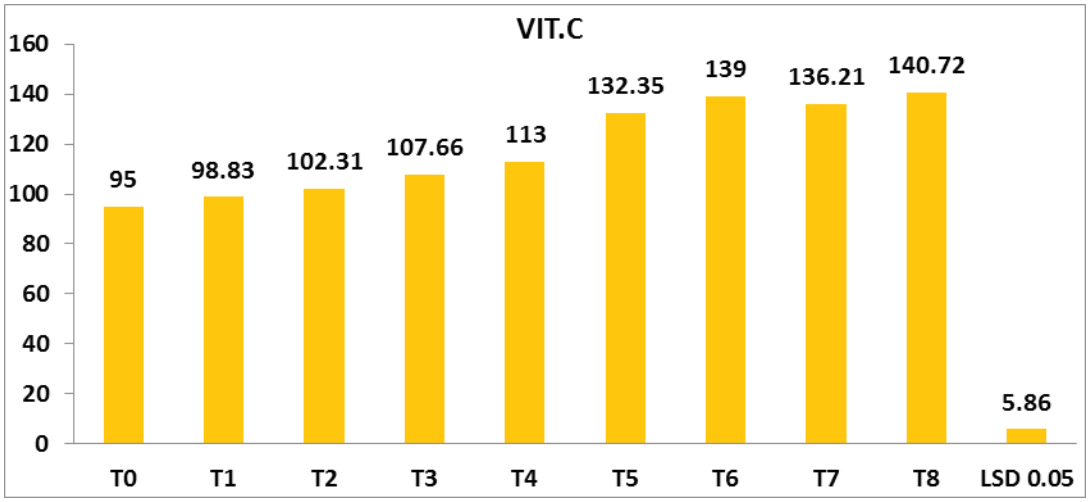

Figure 1 - The effect of foliar application of nanofert. and proline acid and their interactions in vitamin C concentration

2- The total leaf content of phenols mg. 100 g:

The results in (Figure-2) indicate significant differences between the treatments due to the effect of the study factors and their interactions. Whereas, the organic fertilizer treatments T1 and T2 gave a rate of 214.0 and 230.3 mg. 100 g, respectively, which differed significantly with the comparison treatment T0. As for spraying with proline acid, the T3 and T4 treatments showed significant differences at 236.8 and 241.6 mg. 100 respectively, compared to the comparison treatment T0. As for the bilateral interaction between the study factors, it showed significant differences in the rates of this trait, which were highest in T8 and T6 treatments, which did not differ significantly from each other, but they differed significantly from the comparison treatment T0, which gave a rate of 197.0 mg. 100g.

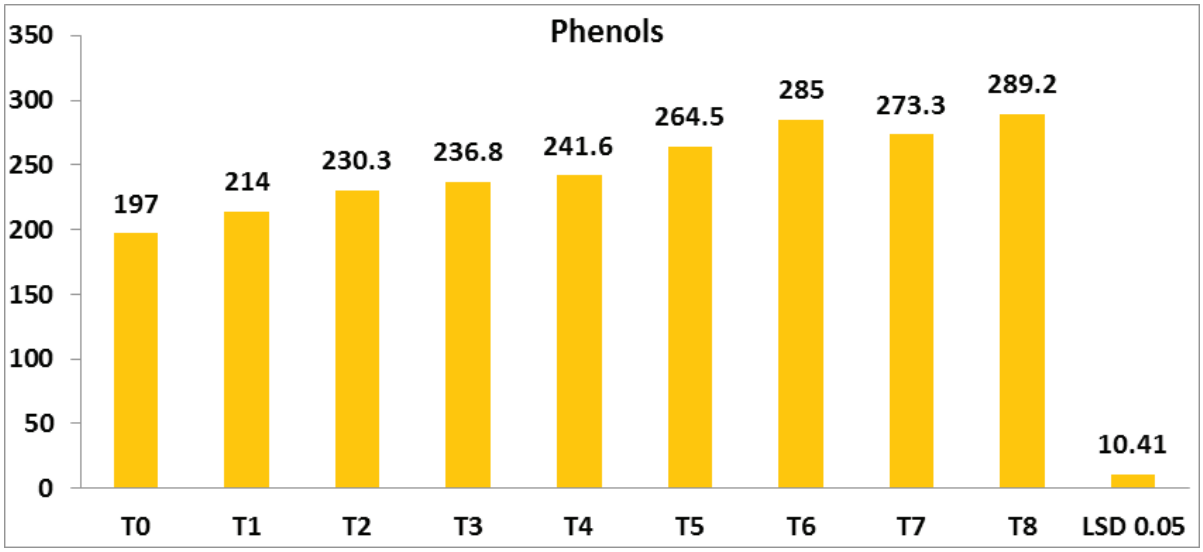

Figure 2 - the effect of foliar application with nano fertilizer and proline acid and their interactions on the phenol content

- Total leaf content of Flavonoids Quercetine mg.L^{-1}3

The results shown in (Figure -3) indicate that there were significant differences between the treatments, as treatment T8 gave the highest rate for this trait of 113.34 mg. L-1, which did not differ significantly from treatment T7, but differed significantly from the rest of the other treatments

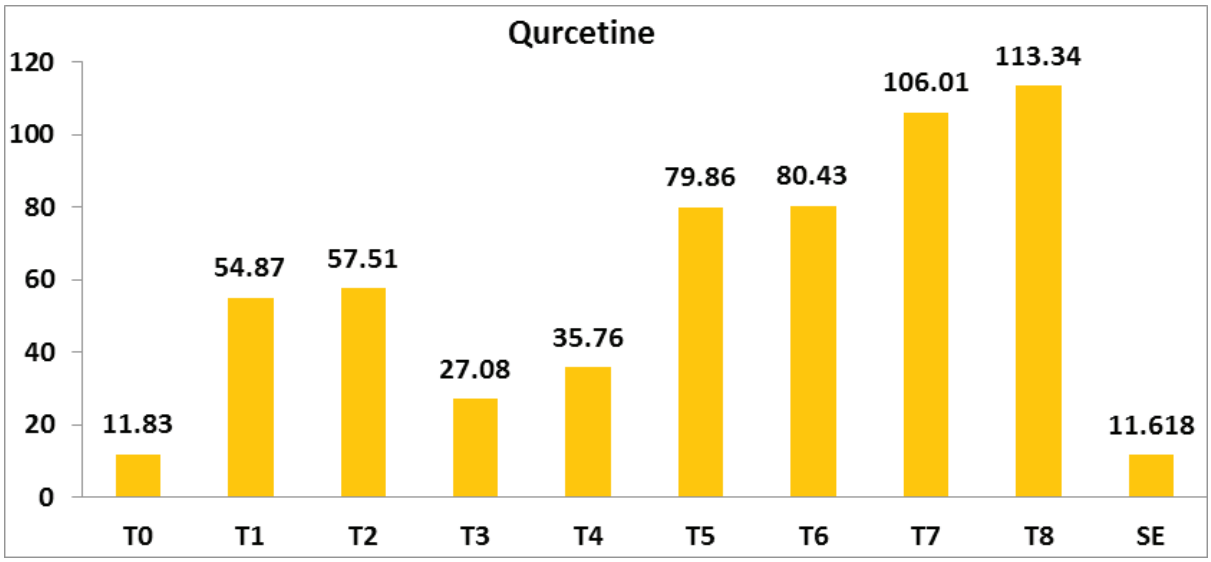

Figure 3 - The effect of foliar spray with nano fertilizer and proline acid and their Interactions on the content of Quercetine

4-Total leaf content of Flavonoids Rutine mg.L^{-1}

The results shown in (Figure -4) indicate that there were significant differences between the treatments, as treatment T8 gave the highest rate for this trait of 22.51 mg. L^{-1}, which did differ significantly from the rest of the other treatments.

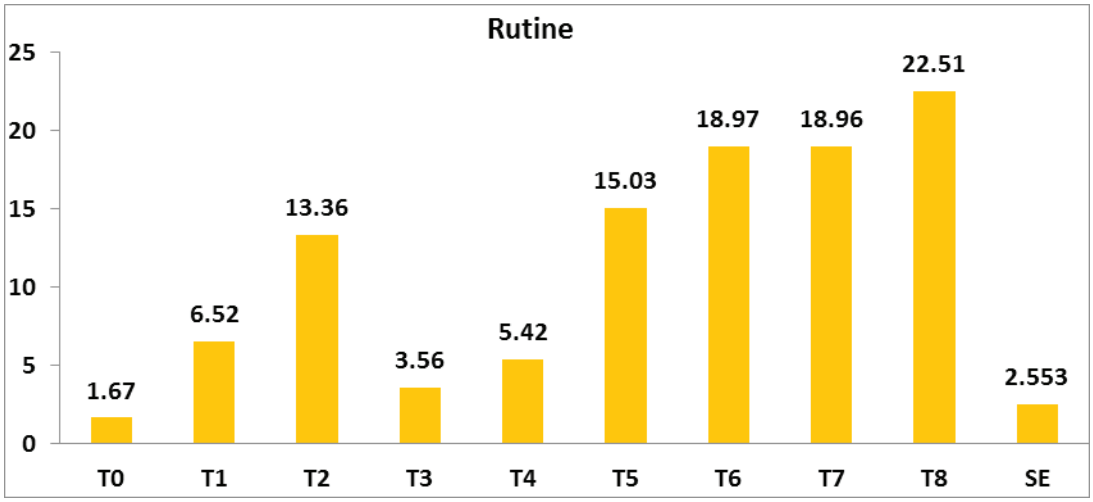

Figure 4 - The effect of foliar spray with nano fertilizer and proline acid and their

Interactions on the content of Rutine

: 5- Estimate the total leaf content of Vitamin D3 mg. L^{-1}

The results in (Figure-5) indicate that there were significant differences between the treatments in the leaf content of the vitamin, as treatment T6 was given the highest rate of 0.838 mg.L^{-1}, which did not differ significantly from treatment T8, but differed from the rest of the other treatments.

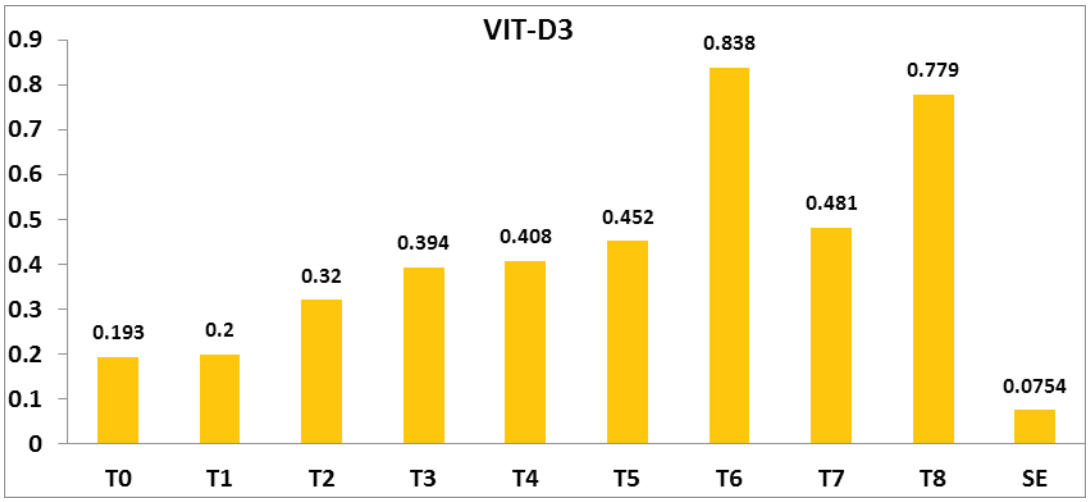

Figure 5 - The effect of foliar spray with nano fertilizer and proline acid and their Interactions on vitamin D3 content

Discussion

The results of the statistical analysis showed that the concentrations of nanoparticles and proline acid fertilizer and their interactions significantly affected the properties of antioxidants and vitamin D3. The increase in the studied characteristics due to the effect of spraying with nano fertilizer is due to its unique and distinctive properties represented by its small minutes and the large effective surface area of these minutes, which led to the speed of its absorption by the plant and then the increase of enzymatic activity and biochemical reactions within it that caused the increase of photosynthesis.[21]The nanoparticles also have a high ability to penetrate plant tissues through stomata due to the size of their ions that are smaller than stomatal slots and in particular that are sprayed onto the vegetative system .[22]This finding was consistent with what[23] in their study on Some medicinal plants, and with what[10] indicated that leafy nutrition is of great importance in plant growth and for medicinal

plants this method leads to increased nutrients and then stimulates plants to increase their growth and increase the antioxidants. As for the increased rates of the studied traits using the proline acid, it is due to the fact that the amino acids represent an important source of carbon and nitrogen, and hence the increase in building most of the byproducts of phenols, flavonoids, and alkaloids[24]. Closely related to metabolism of these acids[25].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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References

1- Quarcoo,Phyllis. *Development of moringa oleifera leaf beverage*. 2008. PhD Thesis.

2- Padayachee, Berushka; BAIJNATH, Himansu. An overview of the medicinal importance of Moringaceae. *Journal of Medicinal Plants Research*, 2012, 6.48: 5831-5839.

3- Santos, Andréa FS, et al. Moringa oleifera: resource management and multiuse life tree. 2015.

4- El- Sohaimy, Sobhy A., et al. Biochemical and functional properties of Moringa oleifera leaves and their potential as a functional food. *Global Advanced Research Journal of Agricultural Science*, 2015, 4.4: 188-199.

5- Saleh, M. M. S. Nanotechnology and a New Scientific Era. *King Fahd National Library. Riyadh: 246pp*, 2015.

6- Mehrotra, A., Nagarwal, R. C., and Pandit, J. K. Fabrication of lomustine loaded chitosan nanoparticles by spray drying and in vitro cytostatic activity on human lung cancer cell line L132. *J Nanomedic Nanotechnolo*, 2010, 1: 103.

7- Phogat, Navneet, et al. Fate of inorganic nanoparticles in agriculture. *Adv. Mater. Lett*, 2016, 7.1: 03-12.

8- Monreal, C. M., et al. Nanotechnologies for increasing the crop use efficiency of fertilizer-micronutrients. *Biology and fertility of soils*, 2016, 52.3: 423-437.

9- Jamal, Z.; Hamayun, M.; Ahmed, N. and Chaudhary, M. F. Effects of soil and foliar application of different concentrations of NPK and foliar application of (NH4) 2SO4 on growth and yield attributes in wheat (Triticum aestivum L.). *Pakistan Journal of Plant Sciences (Pakistan)*, 2007.

10- Aziz, E. E., El-Danasoury, M. M., and Craker, L. E. Impact of sulfur and ammonium sulfate on dragonhead plants grown in newly reclaimed soil. *Journal of herbs, spices & medicinal plants*, 2010, 16.2: 126-135.

11- Mostafavi, K., et al. Grain yield and yield components of soybean upon application of different micronutrient foliar fertilizers at different growth stages. *International Journal of Agriculture: Research and Review*, 2012, 2.4: 389-394.

12- Gardener, Mark C.; GILLMAN, Michael P. The effects of soil fertilizer on amino acids in the floral nectar of corncockle, Agrostemma githago (Caryophyllaceae). *Oikos*, 2001, 92.1: 101-106.

13- Singh, T. N., Paleg, I. G., & Aspinall, D. Stress metabolism I. Nitrogen metabolism and growth in the barley plant during water stress. *Australian Journal of Biological Sciences*, 1973, 26.1: 45-56.

14- Gupta, S. Dutta. Oxidative Burst-mediated ROS Signaling Pathways Regulating Tuberization in Potato. In: *Reactive Oxygen Species and Antioxidants in Higher Plants*. CRC Press, 2010. p. 95-116.

15- Sacca, S. C., Roszkowska, A. M., & Izzotti, A, Alberto. Environmental light and endogenous antioxidants as the main determinants of non-cancer ocular diseases. *Mutation Research/Reviews in Mutation Research*, 2013, 752.2: 153-171.

16- Valko, Marian, et al. Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, 2007, 39.1: 44-84.

17- Raza, A., & Sood, G. K. Hepatocellular carcinoma review: current treatment, and evidence-based medicine. *World journal of gastroenterology: WJG*, 2014, 20.15: 4115.

18- AOAC .Official Methods of Analysis. 13th ed. , Association of Official Analytical Chemists

Washington ,D.C.1980.

19- Suárez, Belén, et al. Liquid chromatographic method for quantifying polyphenols in ciders by direct injection. *Journal of chromatography A*, 2005, 1066.1-2: 105-110.

20- Tomás-Barberán, Francisco A., et al. High performance liquid chromatography, thin layer chromatography and ultraviolet behaviour of flavone aglycones with unsubstituted B rings. *Phytochemical Analysis*, 1990, 1.1: 44-47.

21- Laware, S. L., and Raskar, Shilpa. Influence of zinc oxide nanoparticles on growth, flowering and seed productivity in onion. *International Journal of Current Microbiology Science*, 2014, 3.7: 874-881.

22- Tripathi, Durgesh Kumar, et al. An overview on manufactured nanoparticles in plants: uptake, translocation, accumulation and phytotoxicity. *Plant Physiology and Biochemistry*, 2017, 110: 2-12.

23- Phani,Ch RS, et al. Quantitative analysis of quercetin in natural sources by RP-HPLC. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2010, 1.1: 19-22.

24- Yassin, Bassam Taha. Basics of plant physiology. College of Science. Biological Sciences Department. Qatar University,2001.

25- Fitzpatrick,Teresa B., et al. Vitamin deficiencies in humans: can plant science help?. *The Plant Cell*, 2012, 24.2: 395-414.