American-Eurasian J. Agric. & Environ. Sci., 20 (4): 255-262 2020
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2020.255.262

Influence of Nano-Hydroxyapatite (Nano-HAp) on Growth and Quality of Black Monukka Grapevine

[1]Salwa A. Bedrech and [2]Khaled Y. Farroh

[1]Viticulture Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt
[2]Nano-Technology and Advanced Materials Central Lab., Agriculture Research Center, Giza, Egypt

Abstract: Nanoparticles (NPs) are being used in the field of agriculture due to their unique physiochemical properties in order to facilitate site targeted delivery of varied nutrients needed for higher quality and productivity. In the present study the application of foliar spraying Nano-hydroxyapatite (nHAp, $Ca_{10}(PO_4)_6(OH)_2$), with different concentrations (50, 100, 150, 200 ppm) along with the mineral calcium phosphate, $Ca_3(PO_4)_2$ at 5% (the control) post veraison, was studied on Black monukka grapevine in order to compare the efficacy of replacing mineral fertilizers by nano-fertilizers. The experiment was carried out in the two successive seasons 2018 and 2019, comprising 5 treatments to improve the berry quality, firmness and adherence. Results indicated that spaying the vines with Nano-hydroxyapatite at 150-ppm treatment was the most effective and appropriated concentration among all other treatments in stimulating all growth characters except for higher concentration at 200 ppm and the control where there are no significant differences between them.

Key words: Black monukka · Nano-materials · Calcium phosphate · Firmness · Adherence

INTRODUCTION

Berry quality plays a crucial role for marketing table grapes. The utilization of some mineral fertilizers to enhance the berry quality is considered very carefully and expensive. However, many producers attempt to use the nano-fertilizers as alternative fertilizers which are applied in very small quantities which maybe cheaper and more effective. Black Monukka is among the table grape cultivars which holds a significant value for commercial purpose therefore, the problem of producing loose clusters and high berry shattering are negatively reflected on productivity [1].

It was found that nearly half of the calcium seasonal requirements in grapevines, are absorbed in the period from the end of bloom to veraison [2]. Thus, plant Ca requirements must be continually obtained from external sources. It can be said the most effective grapevine treatment, increasing berry Ca concentration is foliar application. Foliar fertilization is an option, that is made more frequently, due to the higher efficacy of nutrients applied on the leaves, where they are absorbed and utilized in processes of metabolism [3, 4]. Supplementing secondary macro-elements (particularly Ca and Mg) and micro elements, or applying other bio active substances on the leaves is extremely effective. Numerous researches have monitored this aspect under different climate conditions [5].

Hocking [6] studied the effects of low and high calcium supply in grapevines. It was found that low calcium showed early berry softening and onset of berry weight loss while high calcium showed delayed and asynchronous berry development. These results indicated that Berry hydration assays in the early onset of berry weight loss in low calcium grown berries was due to higher post-veraison berry transpiration. In opposite, higher calcium grown berries showed lower rates of berry water uptake pre-veraison and transpiration throughout its development.

Therefore, there is a need to investigate the use of alternative fertilizers, among them is the Hydroxyapatite that is very widely used in several purposes, including as a source of calcium phosphate that is totally biocompatible and nontoxic [7]. Nano-Hydroxyapatite (nHAp) is a calcium phosphate similar to the human hard tissues in morphology and composition [8]. The stability of hydroxyapatite is another important characteristic when it is compared to other calcium phosphates under some physiological conditions as pH, temperature and composition of the body fluids [9].

Corresponding Author: Salwa A. Bedrech, Viticulture Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt

With the rapid development of nanotechnology, its production has gained a considerable attention for adsorption, particularly when biomaterials are involved. The particles which have one dimension less than 100 nm are counted as "nanoparticles" [10]. The nano-materials can be more efficient in decreasing pollution and several environmental problems that may occur when using chemical fertilizers [11]. It is likely that the retention time of nHAp was longer in the porous medium than that of the soluble phosphate and thus the former had supplied more P to the plants than the latter [12]. One of the advantages of using nano-fertilizers is that application can be done in smaller amounts than when using common fertilizers [13, 14].

Nanoparticles have high surface area to volume ratio, nanometer regime and unique properties, which makes them highly applicable. The uptake of nanoparticles through the cell wall of plants depends on its pore diameter (5-20 nm). Nanoparticles have a diameter less than the pore size of plant cell wall therefore they could easily enter through the cell walls and reach up to the plasma membrane [15]. The nHAps can increase Puse effciency through mechanisms such as targeted delivery, slow or controlled release and low molecule diameter. Moreover, nHAp had nontoxic effects on our model plant and therefore it could be used both as a P supplier and carrier of other elements and molecules [16].

The aim of the present study was to test the efficacy of spraying Nano-Hydroxyapatite in different concentrations on berry chemical composition, as well as the concomitant effects thereof on yield quality and quantity, in order to improve the berry quality, firmness and adherence besides reducing the rates of mineral fertilizations of calcium phosphate without any negative effects on cluster characteristics as a cheaper alternative method to improve crop yields.

MATERIALS AND METHODS

The present work was performed during two successive seasons 2018 and 2019 in a vineyard located at El-Sad at City, 30° 22' 30" N and 30° 30' 1" E. Ten year-old Black Monukka grapevines grown in a sandy soil under Spanish parron shaped-system were used in this investigation. The vineyard is spaced 2 x 3 m. The vines were pruned during the second week of February with a vine load of 120 buds (8 canes x 15 bud/ cane) and irrigated through drip irrigation system. Seventy-five vines (5 treatments x 3 replicates x 5 vines / replicate) uniform in vigor and receiving common horticultural practices were chosen for this study.

All treatments were applied twice as a foliar application and sprayed once post-véraison and two weeks later as follow:

- Control (calcium phosphate at 5%)
- Nano-hydroxyapatite (50 ppm)
- Nano-hydroxyapatite (100 ppm)
- Nano-hydroxyapatite (150 ppm)
- Nano-hydroxyapatite (200 ppm)

A randomized complete block design was used in this experiment.

Preparation and Characterization of Hydroxyapatite Nanoparticles

Preparation and of Hydroxyapatite Nanoparticles: The hydroxyapatite nanoparticles (HA-NPs) were prepared by chemical precipitation method using calcium hydroxide (99.99% Pure, Sigma-Aldrich, USA) as precursor salt according to Bianco *et al.* [17]. Briefly, a solution of calcium hydroxide (0.25M) in deionized water (Milli-Q, Millipore, USA) was prepared separately. Then the 100 mL of solution of orthophosphoric acid solution (85-87 %, Sigma-Aldrich, USA) (0.15M) was added to the calcium hydroxide solution under continuous stirring. The hydroxyapatite nanoparticles were separated and washed with deionized water by centrifugation. The resulting precipitates were dried at 100°C for 2h.

Characterization of Hydroxyapatite Nanoparticles: The prepared HA nanoparticles actual morphology was imaged by High Resolution Transmission Electron Microscope (HR-TEM) operating at an accelerating voltage of 200 kV (Tecnai G2, FEI, Netherlands). Diluted HA nanoparticles solution was ultra-sonicated for 5 min to reduce the particles aggregation. Three drops from the sonicated solution were deposited on carbon coated-copper grid using a micropipette then left to dry at room temperature. HR-TEM images of the HA nanoparticles that deposited on the grid were captures for morphological evaluation. The chemical structure of as prepared HA nanoparticles was assessed using X- ray Diffraction (XRD) technique. The corresponding XRD pattern was recorded in the scanning mode (X'pert PRO, PAN analytical, Netherlands) operated by Cu K radiation tube (= 1.54 A°) at 40 kV and 30 mA. The standard ICCD library installed in PDF4 software interpreted the diffraction pattern.

The Vegetative and Reproductive Growth Measurements Yield:

- Yield/vine (Kg)
- Average cluster weight (kg).

Morphological Measurements:

- Average berry size (cm^3).
- Average berry weight (g).
- Berry firmness (g/cm^3) and adherence (g) by using PHSH-PULL (Dynamometer Model DT101).
- Leaf area: Samples of 15 leaves were randomly collected from each treatment for leaf area determination at harvest (using leaf area meter, Model CI 203, U.S.A.).

Chemical Characteristics of Berries: Representative samples of clusters were collected randomly according to Badr and Ramming [18] when they reached their full color and TSS% reached about 18-20 %.

The Following Determinations Were Carried Out:

- Total soluble solids (%), titratable acidity (%) (g. of tartaric acid / 100 ml of juice) [19] and TSS / acid ratio.
- Total anthocyanin in berry skin (mg/100g) using spectrocolourimeter at 250 µm according to Yilidz and Dikmen [20].
- Total chlorophyll content (SPAD) were measured in the mature leaves of the sixth and seventh positions from the apex by using the nondestructive Minolta chlorophyll meter model SPAD 502 [21].

The Economic Study: The cost per feddan for each treatment in both seasons (2018-2019) was calculated.

Statistical Analysis: The New L.S.D. method at 0.05 was used to compare the means of the tested treatments according to Snedecor and Cochran [22].

RESULTS AND DISCUSSION

Characterization of Hydroxyapatite Nanoparticles: Fig. 1 shows the physicochemical characterization of the synthesized hydroxyapatite nanoparticles to evaluate its properties using different techniques. In Fig. 1A. Nanoparticle morphology and size determination was clearly observed in HR-TEM electrograph showing the X-Ray powder diffraction patterns of HA NPs and the needle-like morphology of the apatite particles with a width less than 15-20.2 nm and a length about 47.5 nm.

The XRD pattern of the synthesized HA-NPs is shownin Fig. 1B. The peaks at 2θ = 25.88°, 31.74°, 32.18°, 32.87° and 49.46° were assigned to (0 0 2), (2 1 1), (1 1 2), (3 0 0) and (2 1 3), respectively, indicating that the crystalline structure of synthesized HA nanoparticles presented a hexagonal phase structure of the wurtzite (Zincite, JCPDS 01-073-0293).

Data Taken on the Vegetative and Reproductive Growth: Yield

Yield per Vine (kg): At harvest time, all clusters on the vines in each treatment were counted and the total cluster fresh weight per vine was recorded. It is evident from the data in Table (1) that spraying the vines with Nano-hydroxyapatite at 150 ppm treatment was found to be significantly effective in increasing grapevine yield but with no significant differences among the Nano-hydroxyapatite at 200 ppm treatment and the mineral calcium phosphate (control) in comparison with the two other concentrations. These results agree with Colapietra and Alexander [23] who report that Ca as foliar applications increased yield of cv. "Italia". These increases in growth parameters maybe attributed to the beneficial effects of nanoparticles, which have high specific surface area, which increased the reactivity on their particle surfaces. These characteristics facilitate the absorption of fertilizers that was used in nanoscale [24]. In addition using apatite nanoparticles as a new class of P fertilizer can potentially enhance agronomical yield and reduce risks of water eutrophication [12].

Cluster Weight (Kg): In both seasons, data of cluster weight (Table 1) showed that cluster weight was increased significantly by (31.4% and 20.8%) with the Nano-hydroxyapatite at 150 ppm, than the in the two lower concentrations 50 and 100 ppm respectively, followed by the higher concentration 200 ppm then the mineral calcium phosphates insignificantly in both seasons. Similarly, Liu and Lal [12] published a work that nHAp increased the growth and seed yield of soybean compared to those of soybean treated with water-soluble P.

Morphological Measurements

Average Berry Size (cm^3): Data in Table (1) ensures that 150 ppm nHap, was better than the lower concentration but with no significant difference with the higher one and the control. The effect of P in increasing the berry size was stated by Smolarz and Marcik [25] who found that the absence of P resulted in a reduction of berry size, although the deficiency symptoms were not

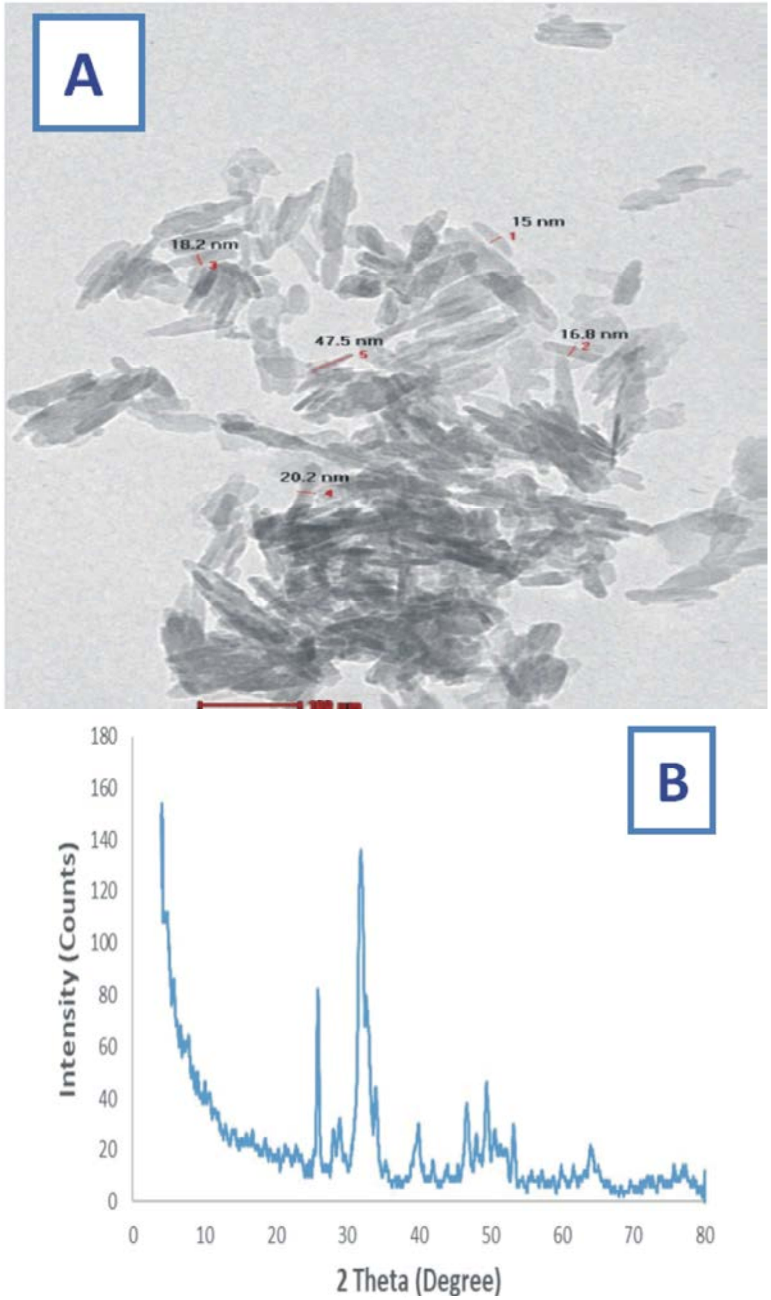

Fig. 1: Characterization of hydroxyapatite nanoparticles (HA-NPs). (A): HRTEM image showing needle-like morphology of the apatite particles with a width less than 15-20.2 nm and a length about 47.5 nm. (B): XRD pattern analysis indicating the formation of HA-NPs

seen indicating the effects of P deficiency can start before symptoms are visible. Moreover, Colapietra and Alexander [23] reported that Ca as foliar applications can increase berry size. In addition, Bala *et al.* [26] mentioned that the increase in activity of growth hormone gibberellins is the possible reason for such beneficial role of nano-HAp on plant growth regulation.

Average Berry Weight (g): Berry weight is an important quality parameter for table grapes and affects yield. The mean values of berry weight are displayed in Table (1). From the results of statistical analysis, there were significant differences in berry weights among nHAp treatments and the control, with a superior effect attributed to using nHAp at 150 ppm, rather than

Table 1: Influence of Nano-hydroxyapatite (nano-HAp) on yield and morphological measurement of Black monukka grapevine during the two successive seasons 2018 and 2019

	Average yield/vine (Kg)		Average cluster weight (Kg)		Average berry weight (g)		Average berry size (cm^3)		Berry firmness (g/cm^2)		Berry adherence (g)		Leaf area (cm^2)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	11.3	11.2	669.0	678.4	3.95	3.99	3.85	3.91	39.9	39.5	206.8	207.5	175.5	191.9
nano-HAp (50 ppm)	9.9	10.0	583.1	592.6	2.55	2.86	2.21	2.74	33.2	33.6	191.0	192. 8	158.5	165.2
nano-HAp (100 ppm)	10.4	10.5	613.8	614.3	2.76	2.94	2.26	2.34	35.4	34.8	198.8	195.0	150.8	176.8
nano-HAp (150 ppm)	12.0	12.1	708.3	711.5	4.28	4.20	3.98	4.11	41.7	40.7	207.7	208.3	185.7	198.4
nano-HAp (200 ppm)	11.5	11.6	676.5	681.3	3.85	4.05	3.75	3.95	39.5	39.2	205.2	205.6	179.3	190.2
New L.S.D. at 0.05	1.1	1.0	44.2	41.8	0.44	0.21	0.23	0.21	2.0	2.0	2.0	2.0	13.2	10.1

the lower concentrations, but with no significant differences between the higher concentration and the mineral calcium phosphates. These results were similar to those obtained by Skinner and Matthews [27] who reported that a reduction in berry weight was observed when they studied the reproductive development of grapes under limited P conditions.

Berry Firmness (g/cm^2) and Adherence (g): As shown in Table (1) the significantly beneficial effect of spraying nHAp at 150 ppm on berry firmness and berry adherence strength is more obvious than the other concentration and the control. From the an unfavorable consequence of low concentrations of Ca that reduces the effectiveness in fruits is stimulating the production of ethylene and increasing the activity of enzymes, by accelerating the ripening process which is responsible for softening of the tissues [28]. It was reported that the Ca content of fruits has a great effect on berry firmness throughout the postharvest period [29]. Spray treatment of Thompson Seedless with $CaCl_2$ at pea-size and veraison resulted in increased berry firmness and berry adherence strength, whilst simultaneously increasing sugar accumulation [30]. However, direct application of calcium at pre- veraison and post-veraison phases were found to increasing berry firmness and breaking force [31].

Leaf Area (cm^2): Leaf area development is an important characteristic affecting yield and fruit quality of grapevines. Table (1) shows the effect of different treatments on the average leaf area of Black Monukka grapevines. It is obvious from the recorded data that there are significant differences among different doses of nHAp treatments with no significant differences compared with the control. The highest values were obtained from the treatment of 150 ppm followed by 200 ppm. These results are in harmony with those of Fageria [32] who found that spraying with nHAp was an effective treatment in improving the vegetative growth parameters and increased the leaf area when compared to control. Moreover, Ehiagiator *et al.* [33] concluded that fixation of the nutrients can be enhanced through higher application of P that expose the plant nutrient to larger surface area. It was reported that nHAp seems to be more effective than that of ordinary phosphorus source (H_3PO_4-P) on growth of the lettuce plants [34].

Chemical Characteristics of Berries

Total Soluble Solids (TSS %), Titratable Acidity % and TSS / Acid Ratio: There are significant differences among treatments in TSS %, titratable acidity and TSS/acid ratio as shown in Table (2). Spraying with 150 ppm nHAp, was better than the lower concentration but with no significant difference with the higher one and the control. Similar results were obtained by Colapietra and Alexander [23] who report that Ca as foliar applications can increase sugar berry accumulation berry size. Besides the effect of P in increasing the TSS% and lowering titratableacidity. These results are in conformity with those obtained by Schreine [35] who stated that supplementary addition of foliar P was associated with higher soluble solids in the fruit reduced berry and must titratable acidity.

Anthocyanin: As shown in Table (2), significantly the best results were obtained from vines treated by 150 ppm nHAp. As stated by Amiri *et al.* [36] Quality components including berry color and appearance were significantly improved by Ca sprays. In addition, nHAp foliar application may be attributed to increase the uptake of phosphorus that affect in direct or indirect ways in increasing a variety of chemicals such as flavonoids, steroids, polyphenolic compounds and carbohydrates [37].

Total Chlorophyll Content (SPAD): The Total chlorophyll content in the leaves was positively affected by the application of nHAp Table (2). Chlorophyll levels reached their maximum value after application of

Table 2: Influence of Nano-hydroxyapatite (nano-HAp) on chemical characteristics of berries of Black monukka grapevine during the two successive seasons 2018 and 2019

	Total soluble solids (TSS %)		Titratable acidity (%)		TSS / acid ratio.		Total anthocyanin (mg/100g)		Chlorophyll (SPAD)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	18.9	18.8	0.6	0.6	31.5	31.3	42.3	41.0	43.2	43.5
nano-HAp (50 ppm)	16.2	15.5	1.1	1.1	14.1	14.0	38.1	37.5	38.7	38.0
nano-HAp (100 ppm)	17.1	16.4	0.9	1.0	19.0	16.4	39.2	38.7	40.0	39.5
nano-HAp (150 ppm)	19.2	19.6	0.5	0.5	38.4	39.2	44.2	43.8	43.9	44.1
nano-HAp (200 ppm)	18.5	19.1	0.7	0.6	30.8	31.8	43.0	42.1	42.9	42.8
New L.S.D. at 0.05	0.8	0.8	0.3	0.2	8.0	8.0	1.0	1.0	2.0	2.0

Table 3: The economic study of the cost per feddan for each treatment in both seasons (2018-2019)

Treatments	Amount in ml/tree	Amount in liters/feddan	Cost of kg/L.E	Cost of feddan/L.E	Cost for two seasons /L.E
Control $Ca_3(PO_4)2$ at 5%	50	35.00	22	770	1540
Nano-hydroxyapatite (50 ppm)	0.5	0.35	22	7.7	15.4
Nano-hydroxyapatite (100 ppm)	1.0	0.70	22	15.4	30.8
Nano-hydroxyapatite (150 ppm)	1.5	1.05	22	23.1	46.2
Nano-hydroxyapatite (200 ppm)	2.0	1.40	22	30.8	61.6

150 ppm and 200 ppm nHAp respectively but with no significant differences between them and the control. It was found by Cherif *et al.* [38] that the supplement of the nHAp can increase the level of chlorophyll, as it protects and restores chlorophyll levels.

Moreover, the beneficial effect of nHAp in increasing total chlorophyll may be due to the increment of nutrients uptake such as nitrogen, magnesium and iron which are involved in chlorophyll formation as mentioned by Fageria [32] who found a consistent increment in total chlorophyll sprayed with nHAp. However, the success of nHAp as foliar fertilizer may be ascribed to a greater density in reactive areas which increase the uptake of phosphorus that led to enhance total chlorophyll [39].

The Economic Study: Data in Table (3) shows the cost per feddan for each treatment in both seasons (2018-2019) in order to calculating the amount and cost saved by using the nano-particle minerals instead of regular ones. It is obvious that all concentration of nHAp are more cost effective than the control where the $Ca_3(PO_4)_{2\ is}$ used in greater amount to give the same results.

CONCLUSION

As a result, we conclude that foliar application of 150 ppm nHAp was an effective treatment in increasing all growth parameters more than the other treatments and the control, as well as enhancing the chemical characteristics of berries, TSS %, TSS/acidity ratio, whereas, acidity% was decreased in berry juice. Besides its beneficial effects on yield, using the calcium and phosphorus in their nano forms led to reducing the amount used and consequently the costs of fertilizers.

REFERENCES

1. Harry, A., J. Fred and P. Elam, 1991.Growing quality table grapes in the home garden. University of California, pp: 1-30.
2. Ollat, N. and J.P. Gaudilliere, 1996. Investigation of assimilates import mechanisms in berries of *Vitis vinifera* var. Cabernet-Sauvignon. Acta Horticulturae, 427: 141-149.
3. Wiens, G. and A. Reynolds, 2008. Efficacy Testing of Organic Nutritional Products for Ontario Canada Vineyards. International Journal of Fruit Science, 8: 125-145.
4. Bratasevec, K., P. Sivilotti and B.M. Vodopivec, 2013. Soil and foliar fertilization affect mineral contents in *Vitis vinifera* L. cv. 'rebula' leaves. J. Soil Sci. Plant Nutr., 13(3): 650-663.
5. Šimanskı, V. And O. Ložek, 2013. Fertilization of Vine By A 5-Aminolevulinic Acid-Based Fertilizer And Its Profitability. Journal of Central European Agriculture, 14(1): 270-283
6. Hocking, B.J., 2015. The Role of Calcium in the Cell Wall of Grape Berries. A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy School of Agriculture, Food & Wine Faculty of Science The University of Adelaide.

7. Wefel, J.S. and M.W.J. Dodds, 1999. Oral Biologic and the Demineralization and Remineralization of Teeth, Appleton & Lange, Stamford, Conn, USA.
8. Wei, G. and P.X. Ma, 2004. "Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering". Biomaterials, 25(19): 4749.
9. Kalita, S.J., A. Bhardwaj and H.A. Bhatt, 2007. "Nanocrystalline calcium phosphate ceramics in biomedical engineering", Materials Science and Engineering: C, 27(3): 441.
10. Thakkar, K.N., S.S. Mhatre and R.Y. Parikh, 2010. Biological Synthesis of Metallic Nanoparticles. Nanomedicine Nanotechnology Biology and Medicine, 6: 257-262.
11. Naderi, M., A.A. Danesh Shahraki and R. Naderi, 2011. Application of nanotechnology in the optimization of formulation of chemical fertilizers. Iran J. Nanotech., 12: 16-23.
12. Liu, R. and R. Lal, 2014. Synthetic apatite nanoparticles as a phosphorus fertilizer for soybean (*Glycine max*). *Scientific Reports* 4, Article Number: 5686.
13. Batsmanova, L.M., L.M. Gonchar, N.Y. Taran and A.A. Okanenko, 2013. Using a colloidal solution of metal nanoparticles as micronutrient fertiliser for cereals. Proceedings of the International Conference Nanomaterials: Applications and Properties.
14. Subramanian, K.S., A. Manikandan, M. Thirunavukkarasu and C. Sharmila Rahale, 2015. Nano-fertilizers for balanced crop nutrition. In: Rai, M., Ribeiro, C., Mattoso, L., Duran, N. (Eds.), Nanotechnologies in Food and Agriculture. Springer International Publishing, Switzerland, pp: 69-80.
15. Solanki, P., A. Bhargava, H. Chhipa, N. Jain and J. Panwar, 2015. Nano-fertilizers and Their Smart Delivery System. 10.1007/978-3-319-14024-7_4.
16. Marchiol, L., A. Filippi, A. Adamiano, L.D. Esposti, M. Iafisco, A. Mattiello, E. Petrussa and Enrico Braidot, 2019. Influence of Hydroxyapatite Nanoparticles on Germination and Plant Metabolism of Tomato (*Solanum lycopersicum* L.): Preliminary Evidence. Agronomy, 9: 161.
17. Bianco, A., I. Cacciotti, M. Lombardi, L. Montanaro and G. Gusmano, 2007. Thermal stability and sintering behavior of hydroxyapatite nanopowders. J. Therm. Anal. Calorim., 88: 237-243.
18. Badr, S.A. and D.W. Ramming, 1994. The development and response of Crimson Seedless cultivar to cultural practices. Proc. of Intern. Symp. On Table Grape Production, California, U.S.A., 29: 219-222.
19. A.O.A.C., 1985. Association of official Agriculture Chemists. Official methods of analysis. Washington D.C., U.S.A.
20. Yilidz, F. and D. Dikem, 1990. The extraction of anthocyanin from black grape skin. Doga Degisi, 14(1): 57-66.
21. Wood, C.W., D.W. Reeves, R.R. Duffield and K.L. Edmisten, 1992. Field chlorophyll measurements for corn nitrogen status. J .Plant Nutr., 15: 487-500.
22. Snedecor, G. and W.G. Cochran, 1980. Statistical methods.7th edition Iowa State Univ. Press, U.S.A.
23. Colapietra, M. and A. Alexander, 2006. Effect of foliar fertilization on yield and quality of table grapes. Acta Hort., 721: 213-218.
24. Anonymous, 2009. Nano technology in agriculture. J. Agric. Technol., 114: 54-65.
25. Smolarz, K. and S. Mercik, 1997. Growth and Yield of Grape in Response to Long Term (since 1923) Different Mineral Fertilization. Acta Horticulturae, 448: 42-432.
26. Bala, N., A. Dey, S. Das, R. Basu and P. Nandy, 2014. Effect of Hydroxyapatite nanorod on chickpea (*Cicer arietinum*) plant growth and its possible use as nano-fertilizer. Iranian Journal of Plant Physiology, 4(3): 1061-1069.
27. Skinner, P.W. and M.A. Matthews, 1989. Reproductive Development in Grape (*Vitis vinifera L.*) under Phosphorous-Limited Conditions. Scientia Horticulturae, 38: 49-60.
28. Poovaiah, B.W., 1979. Role of calcium in ripening and senescence. Commun. Soil Sci. Plant Anal., 10: 83-88.
29. Cicco, N., B. Dichio, C. Xiloyannis, A. Sofo and Lattanzio, 2007. Influence of calcium on the activity of enzymes involved in kiwifruit ripening. Acta Hort., 753: 433-438.
30. Marzouk, H. and H. Kassem, 2011. Improving fruit quality, nutritional value and yield of Zaghloul dates by the application of organic and/or mineral fertilizers. Scientia Horticulturae, 127: 249-254.
31. Ciccarese, A., A. Stellacci, G. Gentilesco and P. Rubino, 2013. Effectiveness of pre- and post-veraison calcium applications to control decay and maintain table grape fruit quality during storage. Postharvest Biology and Technology, 75: 135-141.
32. Fageria, N.K., 2009. The Use of Nutrients in Crop Plants. CRC Press, Boca Raton, FL., USA., pp: 430.
33. Ehiagiator, J.O., A.D. Ariyo and E.E. Imasuen, 2011. Soil fertility and nutritional studies on citrus, fruit and vegetable crops in Nigeria. Proceedings of The 29th Annual National Conference of Horticultural Society of Nigeria, July 24-29, 2011, Horticultural Society of Nigeria.

34. Taskin, M.B., O. Sahin, H. Taskin, O. Atakol, A. Inal and A. Gunes, 2018. Effect of synthetic nano-hydroxyapatite as an alternative phosphorus source on growth and phosphorus nutrition of lettuce (*Lactuca sativa* L.) plant. Journal of Plant Nutrition, pp: 1-7.
35. Schreine, R.P., 2010. Foliar Sprays Containing Phosphorus (P) Have Minimal Impact on 'Pinot noir' Growth and P Status, Mycorrhizal Colonization and Fruit Quality. American Society for Horticultural Science, 45(5): 815-821.
36. Amiri, E.M., E. Fallahi and G. Safari, 2009. Effects of Preharvest Calcium Sprays on Yield, Quality and Mineral Nutrient Concentrations of 'Asgari' Table Grape. Science, 9(3).
37. Amira, Sh. Soliman, M. Hassan, Faten Abou-Elella, A.H. Ahmed and S.A. El-Feky, 2016. Effect of Nano and Molecular Phosphorus Fertilizers on Growth and Chemical Composition of Baobab (*Adansoniadigitata* L.). Journal of Plant Sciences, 11: 52-60.
38. Cherif, J., C. Mediouni, W.B. Ammar and F. Jemal, 2011. Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (Solarium lycopersicum). Journal of Environmental Sciences, 23(5): 837-844.
39. El-Kereti, M.A., S.A. El-feky, M.S. Khater, Y.A. Osman and E.A. El-sherbini, 2013. ZnON anofertilizer and He Ne Laser Irradiation for Promoting Growth and Yield of Sweet Basil Plant. Recent Patents on Food, Nutrition & Agriculture, 5(3): 169-181.