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### Impact of Nitrogen Nano Fertilizer on 'King Ruby Seedless' Grapevines Yield and Berry Qualities

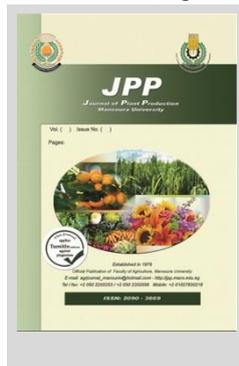
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#### ABSTRACT

The aim of this study is to evaluate the impact of Nano chitosan nitrogen fertilizer as a foliar application at three levels (250, 500, and 1000 ppm) with nitrogen soil application as ammonium sulphate at different doses on vegetative growth, mineral content in leaf petioles, yield and berry quality of 'King Ruby seedless' grapevines grown in a clay loam soil in a private farm existed at Aga near Mansoura city, Dakahlia Governorate, Egypt. Results show that the application of Nano nitrogen foliar fertilizer has a significant impact on shoot diameter, leaf area, N, P and K concentration in leaf petioles, yield and cluster physical and chemical characteristics as compared to control treatment. Furthermore, among all treatments the foliar application of 1000 ppm Nano N fertilizer along with 50% reduction of soil added N fertilizer than the recommended level improved vegetative growth, as well as yield and fruit quality of 'King Ruby seedless' grapevines as it recorded the highest values of the tested parameters.

**Keywords:** Nitrogen, Chitosan, Foliar application, Nano fertilizers, Grapevines.

#### INTRODUCTION

Nutrition is an important factor in the growth and development of grapevines. Among the main nutritional elements, there is nitrogen (N) one of the major nutrients required by plants for sufficient growth. Nitrogen performs significant functions in plant development, fruit yield, and quality; it is needed for chlorophyll and enzyme synthesis and constitutes a component of nucleic acids, proteins, and metabolites (Barker and Pilbeam, 2007; Titus and Kang, 1982). Nitrogen use efficiency in conventional fertilizers is very less, Trenkel (2010) and Solanki *et al.* (2015) pointed out that 40 - 70% of nitrogen of the normal fertilizers is wasted to the environment, and plants cannot absorb it, bringing negative environmental effects such as leached nitrates into marine biological systems and the release of N-oxides into the air (Johnson and Raun, 2003). Accordingly, alternate strategies are needed to face these problems without influencing agricultural productivity and with financial advantages for farmers. For example, nanotechnology is one new methodology that deals with the manufacturing, manipulation, and utilization of different materials to the nanoscale, which is less than 100 nanometers to enhance the use efficiency of nitrogen fertilizers (Dimkpa and Bindraban, 2018).

Nanotechnology has given the achievability of examining nano-structured materials as fertilizer carriers or controlled-release vectors for the building of the so-called smart fertilizers as new facilities to improve the nutrient use efficiency and reduce the cost of ecological contamination (Chinnamuthu and Boopati, 2009). Fertilizer product formed through a nanoscale procedure is called nano fertilizer or nano-enabled fertilizer, based on formulating with synthetic or natural nanoscale minerals (Kottegoda *et al.*, 2017). The reduction of the material size into the nanoscale changing the physico-chemical characteristics as compared with the same

material at bigger-size scales (Peters *et al.*, 2016). Furthermore, surface coatings of nanomaterials on fertilizer particles hold the material much strong due to higher surface tension than the conventional surfaces and thus help in controlled release, which enhanced interaction and efficient uptake of nutrients for crop fertilization (Brady and Weil, 1999; DeRosa *et al.*, 2010).

Plant nutrients are applied directly through the soil or can be spray onto leaves. Colapietra and Alexander (2006) reported that grape leaves and developing green berries absorb nutrients easily from leaf fertilizers. Also, Iacono (1986) revealed that nutrient foliar applications are absorbed by grapevine leaves and clusters. According to Ping *et al.* (2011); Nie *et al.* (2013), the foliar application of N fertilizer had many benefits, consequently, foliar spraying seems to be a technique that includes some benefits like less amount of nitrogen to bring, soil composition and water status independency, assimilation fastness (Gooding and Davies, 1992). Nano fertilizers aimed to make nutrients more available to leaves, thus rising nutrient use effectiveness (Suppan, 2013). Chitosan acts as a carrier enhancing the slow release behavior of fertilizers to prevent leaching and fixation losses due to rapid mobility, high solubility; it can enhance fertilizer's degradation rates to obtain slow-release properties (Wu and Liu 2008 ; Hossain and Iqbal, 2016). It has been used as carrier system to its distinct features such as biocompatibility, biodegradability, and non-toxicity (Campos *et al.*, 2018), and it has the best chelating properties (Kamari *et al.*, 2011).

The aim of this study was to evaluate the impact of the foliar application of different concentrations of Nano chitosan-N fertilizer (250, 500, and 1000 ppm) with different doses of nitrogen soil application (50 and 75% of recommended dose) on growth, yield, physical and chemical properties of cluster and berries, as well as nitrogen,

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potassium and phosphorus % in petioles of 'King Ruby seedless' grapevine leaves.

## MATERIALS AND METHODS

The experiment was carried out in two seasons, 2017 and 2018, in a private farm located at Aga near Mansoura city, Dakahlia Governorate, Egypt on 10-year-old 'King Ruby seedless' vines, planted at 2 × 2.5 meters under flood irrigation system, spur pruned under T- double trellis system, pruning was done in last January in both seasons of study, leaving sixty eyes/vine (based on thirty fruiting spurs/vine × two eyes/spur). The experiment was carried out based on complete randomized blocks design with 8 treatments, each treatment was 3 times replicated with 3 vines per each, seventy-two vines have nearly similar vigor were chosen to do this research, all chosen vines got the same agriculture practices as irrigation, fertilization, pest and diseases control programs that normally done in this location. Soil samples at 40-60 cm depth were taken before the experiment to test the properties of experimental soil. These samples were completely mixed and examined mechanically and chemically, the soil was a clay loam (sand 37%, silt 26%, and clay 37%), with field capacity 27.91%, bulk density 1.06 g/cm<sup>3</sup>, organic matter 2.02%, pH 8.26, an electrical conductivity (EC) of 0.29 dSm<sup>-1</sup>, nitrogen 12.41 mg/kg, phosphorus 14.66 mg/kg, and potassium 260.89 mg/kg. Treatments applied were as follow: 100 % of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> recommended dose. (Control), 75 % of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> recommended dose plus 1000 ppm Nano Chitosan-N, 75 % of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> recommended dose plus 500 ppm Nano Chitosan-N, 75 % of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> recommended dose plus 250 ppm Nano Chitosan-N, 50 % of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> recommended dose plus 1000 ppm Nano Chitosan-N, 50 % of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> recommended dose plus 500 ppm Nano Chitosan-N, 50 % of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> recommended dose plus 250 ppm Nano Chitosan-N, and 1000 ppm Nano Chitosan-N.

Nitrogen mineral source was added as ammonium sulphate form (20.6 % N). Ammonium sulphate was applied as soil application at the rate of 100% (187.5 gm/vine), 75% (140.6 gm/vine) and 50% (93.75 gm/vine) at three equal doses, while Nano chitosan – nitrogen fertilizer was applied to leaves at the rate of 250, 500 as well as 1000 ppm in three equal doses, at the beginning of vegetative growth, after the stage of fruit setting and at the stage of véraison. Nano nitrogen fertilizer from the Department of Genetic Engineering at Ain Shams University.

### Measurements:

#### Vegetative growth Measurements:

Two weeks after the véraison stage, some vegetative growth parameters were estimated, shoot diameter (mm) was measured between the 3<sup>rd</sup> and 4<sup>th</sup> node from the base of shoots using a digital caliper, and average leaf area (cm<sup>2</sup> / leaf) of the 6<sup>th</sup> or 7<sup>th</sup> leaf from the top of the growing shoot was also detected as described by Montero *et al.* (2000): Leaf area (cm<sup>2</sup> / leaf) = 0.587 (L × W), where L= the length of leaf blade. W= the width of leaf blade.

#### Leaf petioles element concentrations:

Two weeks after the véraison stage, nitrogen, phosphorous, and potassium contents in leaf petioles opposite to cluster were measured. Digestion of Nitrogen, Phosphorus, and Potassium by mixed H<sub>2</sub>SO<sub>4</sub>+ HClO<sub>4</sub> method according to the method described by Jackson (1973). Total N content (%) was measured using the micro-Kjeldahl method (Hesse, 1971). The percentage of potassium was determined using a

flame photometer (Jackson, 1973); element concentrations were calculated as percentages on a dry weight basis. Using the method of Schouwenburg and Walinga (1967), P percent was measured colorimetrically spectrophotometer at a wavelength of 700 nm.

#### Yield and Cluster Physical characteristics:

At harvest time, the yield by weight (kg) was determined by multiplying the number of clusters per vine by the mean weight of cluster. The average cluster length (cm) was also measured.

#### Berries chemical properties:

Juice soluble solids content (SSC %) of fresh berries was estimated using Carlsiz hand refractometer. Total acidity (%) was estimated using titration method (AOAC, 1984). The content of soluble solids / acid ratio was also measured. The skin berries (mg/100 g FW) total anthocyanin content was determined (Mazumadar and Majumder, 2003). Total sugars were measured using phenol sulphuric acid method in grape berries (Dubois *et al.*, 1956).

#### Statistical analysis:

Obtained data were analyzed using Analysis of Variance (ANOVA) method in a complete randomized blocks design by CoStat Version 6.0 (CoStat, 2008). Treatment means were compared using the least significant differences (LSD) at 5% of probability (Waller and Duncan, 1969).

## RESULTS AND DISCUSSION

### Results

#### Vegetative growth

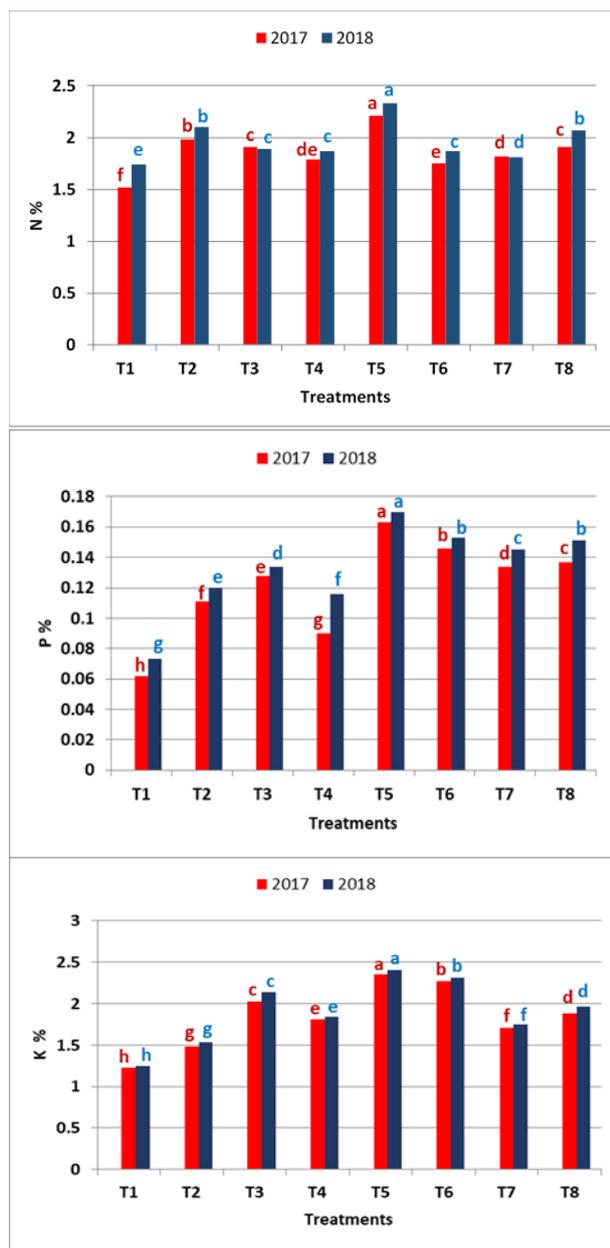
The impact of Nano chitosan – nitrogen application on vegetative growth of 'King Ruby seedless' grapevines is shown in the data presented in Table 1. The data reveal that all Nano nitrogen treatments significantly increased shoot diameter of 'King Ruby seedless' grapevines compared with control vines, the highest values of shoot diameter were determined in vines treated with (T5) 50 % mineral + 1000 ppm Nano N (11.39 & 11.42 mm in 2017 and 2018 seasons, respectively), followed by (T6) 50 % mineral + 500 ppm Nano N and (T8) 1000 ppm Nano N with the values (11.04 & 11.04 mm for T6 and 10.79 & 11.36 mm for T8 in both seasons, respectively). Concerning leaf area, data in the same Table show that the leaf area was greater at high concentration of Nano N (1000 ppm) than low concentrated ones (500 and 250 ppm). The highest values of leaf area 54.40 & 57.20 cm<sup>2</sup> / leaf in both tested seasons, respectively were obtained from (T5) 50 % mineral + 1000 ppm Nano N. Whereas, insignificant differences were found between T5, T8 and T2 in both seasons. On the other hand, the lowest significant values of the above parameters were recorded in control plants, 8.39 & 8.58 mm for shoot diameter and 37.83 & 42.46 cm<sup>2</sup> / leaf for leaf area in 2017 and 2018 seasons, respectively.

#### Leaf petioles element concentrations

Concerning the effect of Nano chitosan – N fertilizer application on N, P and K concentrations in King Ruby leaf petioles, according to the results observed in Figure 1, the Nano N treatments had significant positive effects on the leaf petioles N, P and K content of the grapevines compared with control vines, and their positive effects were more pronounced in the second year.

**Table 1. Impact of Nano chitosan – N fertilizer on shoot diameter (mm) and leaf area (cm<sup>2</sup> / leaf) of ‘King Ruby seedless’ grapevines during the seasons of 2017 and 2018.**

	Shoot diameter (mm)		Area of leaf (cm <sup>2</sup> / leaf)	
	2017	2018	2017	2018
T1 (100% of recommended dose, Control)	8.39	8.58	37.83	42.46
T2 (75% mineral + 1000 ppm Nano N)	10.67	10.60	53.87	56.35
T3 (75% mineral + 500 ppm Nano N)	10.00	9.81	51.91	54.99
T4 (75% mineral + 250 ppm Nano N)	10.28	10.36	52.05	55.11
T5 (50% mineral + 1000 ppm Nano N)	11.39	11.42	54.40	57.20
T6 (50% mineral + 500 ppm Nano N)	11.04	11.04	51.40	53.87
T7 (50% mineral + 250 ppm Nano N)	10.35	10.46	51.66	54.40
T8 (1000 ppm Nano N)	10.79	11.36	53.87	56.74
LSD 5%	0.82	0.74	1.83	2.23



**Fig. 1. Impact of Nano chitosan – N fertilizer on nitrogen, phosphorus and potassium percent in the leaf petioles of ‘King Ruby seedless’ grapes during the seasons of 2017 and 2018.**

T1: 100% of recommended dose, Control, T2: 75% mineral + 1000 ppm Nano N, T3: 75% mineral + 500 ppm Nano N, T4: 75% mineral + 250 ppm Nano N, T5: 50% mineral + 1000 ppm Nano N, T6: 50% mineral + 500 ppm Nano N, T7: 50% mineral + 250 ppm Nano N, and T8: 1000 ppm Nano N.

Foliar application of 1000 ppm Nano N + 50% mineral (T5) significantly gave the highest values of N, P and K % during the seasons of study when compared with other treatments (2.21 & 2.33% for N, 0.163 & 0.170% for P and 2.35 & 2.41% for K in both seasons, respectively). In addition, the least N, P and K contents were determined in leaf petioles of untreated vines (control) which recorded significantly the lowest values (1.52 & 1.74% for N, 0.062 & 0.073% for P and 1.23 & 1.25% for K in 2017 and 2018 seasons, respectively).

**Yield and cluster physical properties**

Regarding the impact of Nano chitosan – N fertilizer application on yield and cluster physical properties of ‘King Ruby’ grapes. As can be seen in Table 2, the foliar Nano-N fertilization significantly increased yield when compared with the untreated control trees. The highest yield per vine was determined in fertilizer treatment T5 (50% mineral + 1000 ppm Nano N), the values were 13.55 and 14.76 Kg/vine for both seasons, respectively. Likewise, the greatest values of cluster weight and length were achieved with the same treatment in the second year. On the other hand, the untreated vines with Nano-N fertilizer significantly had the least yield values 9.32 and 9.42 Kg/vine for the two seasons, respectively. Results of the same Table showed the superiority of Nano-N treatments with 50% of recommended dose of ammonium sulphate soil fertilizer as compared with both 75% and 100% of recommended dose treatments in the case of cluster weight and length in both seasons of study.

**Table 2. Impact of Nano chitosan – N fertilizer on yield/vine (Kg), cluster weight (g) and cluster length (cm) of ‘King Ruby seedless’ grapevines during the seasons of 2017 and 2018.**

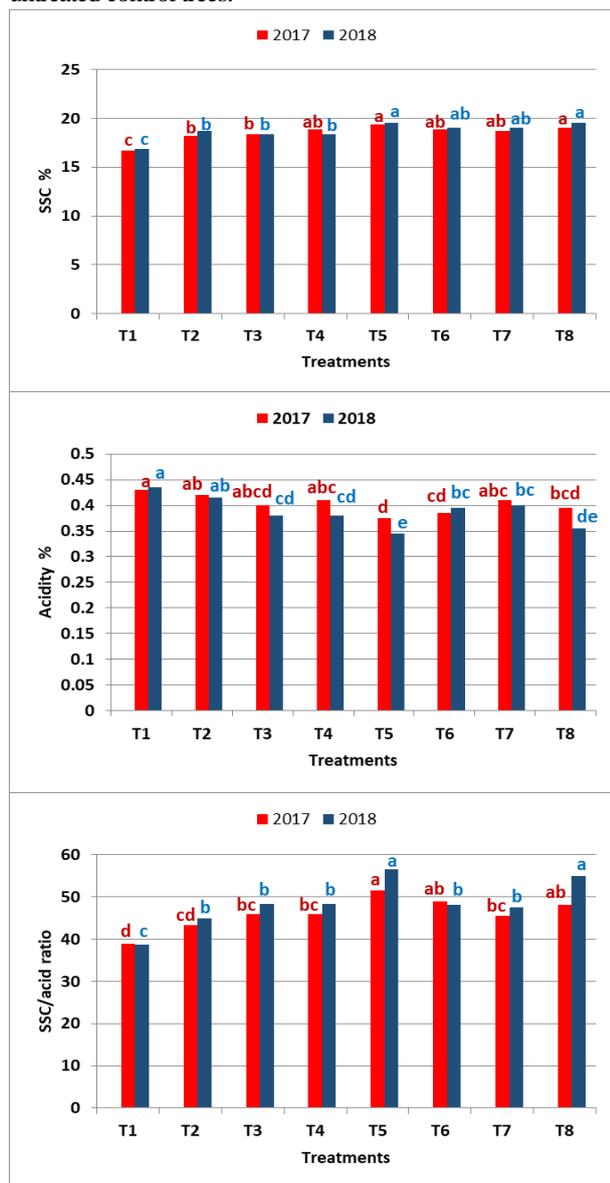
	Yield/vine (Kg)		Cluster weight (g)		Cluster length (cm)	
	2017	2018	2017	2018	2017	2018
T1 (100% of recommended dose, Control)	9.32	9.42	364.84	368.68	21.33	23.50
T2 (75% mineral + 1000 ppm Nano N)	12.15	11.71	509.23	470.07	23.50	25.00
T3 (75% mineral + 500 ppm Nano N)	12.04	11.69	456.91	508.61	25.67	27.67
T4 (75% mineral + 250 ppm Nano N)	11.43	12.50	457.77	473.06	24.17	27.00
T5 (50% mineral + 1000 ppm Nano N)	13.55	14.76	589.29	624.07	27.83	30.50
T6 (50% mineral + 500 ppm Nano N)	12.80	13.32	576.01	555.29	25.83	29.17
T7 (50% mineral + 250 ppm Nano N)	12.19	12.47	534.50	513.16	26.17	28.50
T8 (1000 ppm Nano N)	13.37	13.68	591.02	590.86	26.83	27.50
LSD 5%	1.17	1.34	66.37	78.18	3.02	4.61

**Berries chemical properties**

Concerning the effect of Nano chitosan – N fertilizer treatments on chemical properties of ‘King Ruby seedless’ berries, results in Fig. 2 reveal that all Nano-N treatments led to an increment in berries SSC% and SSC/acid ratio and a decrease in total acidity compared to control treatment. The highest SSC% and SSC/acid ratio were obtained in trees treated with 50% mineral plus 1000 ppm Nano-N in both seasons, without significant differences with those treated by T6, T7, and T8 in both seasons in case of SSC%. By comparison, the control treatment gave the lowest values of SSC percent and SSC/acid ratio and the highest values of

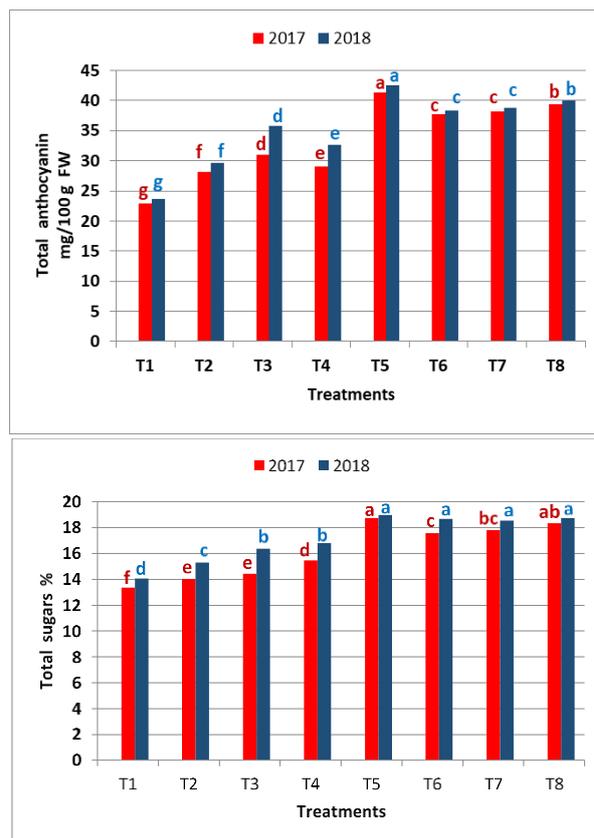
acidity in both seasons. Concerning total anthocyanin in skin berries, data illustrated in Fig. 3 clearly show that, among of all treatments, the highest significant values of total anthocyanin in skin berries (41.30 and 42.54 mg/100g FW in 2017 and 2018 seasons, respectively) were obtained with the treatment (T5) 50 % mineral + 1000 ppm Nano N.

In case of total sugars, a significant increase in total sugars could be observed with the increase of Nano-N concentration under 50% of the recommended dose of ammonium sulphate soil fertilizer compared to other treatments in the second season and insignificant differences were noticed between T5 and T8 in both tested seasons, whereas the lowest significant values of total anthocyanin 22.95 and 23.65 mg/100g FW and total sugars 13.36 and 14.05 % in both seasons, respectively were recorded in the untreated control trees.



**Fig. 2. Impact of Nano chitosan – N fertilizer on SSC percent, acidity percent and SSC/acid ratio of ‘King Ruby seedless’ berry juice during the seasons of 2017 and 2018.**

T1: 100% of recommended dose, Control, T2: 75% mineral + 1000 ppm Nano N, T3: 75% mineral + 500 ppm Nano N, T4: 75% mineral + 250 ppm Nano N, T5: 50% mineral + 1000 ppm Nano N, T6: 50% mineral + 500 ppm Nano N, T7: 50% mineral + 250 ppm Nano N, and T8: 1000 ppm Nano N.



**Fig. 3. Impact of Nano chitosan – N fertilizer on total anthocyanin in berries skin and total sugars of ‘King Ruby seedless’ berries during the seasons of 2017 and 2018.**

T1: 100% of recommended dose, Control, T2: 75% mineral + 1000 ppm Nano N, T3: 75% mineral + 500 ppm Nano N, T4: 75% mineral + 250 ppm Nano N, T5: 50% mineral + 1000 ppm Nano N, T6: 50% mineral + 500 ppm Nano N, T7: 50% mineral + 250 ppm Nano N, and T8: 1000 ppm Nano N.

**Discussion**

Several investigators evaluated the efficiency of nitrogen on vegetative growth of different grapevine cultivars, Nijjar (1985) reported that raising the rate of nitrogen increased the cane thickness of ‘Red Roomy’ grapevines. Similarly, Abd El-Razek *et al.* (2011) mentioned that high N-fertilization enhanced vegetative growth as it improved cane diameter and leaf area of ‘Crimson seedless’ grapevines. In addition, an increase in the leaf area of ‘Thompson seedless’ grapes resulted when N increased from 150 to 200 kg/ha (Delgado *et al.*, 2004).

The stimulative effect of nitrogen on growth characters may be due to the major role of nitrogen on protein and nucleic acids synthesis and protoplasm formation. That’s in role induced cell division and initiates meristematic activity for producing more tissues and organs. Thus, plant growth could be affected by the nitrogen amount (Marschner, 2012; Najm *et al.*, 2012). Also, it could be attributed to increasing the uptake of nitrogen and its associated role in chlorophyll synthesis, which in role hence the process of photosynthesis and carbon dioxide assimilation (Jasso-Chaverria *et al.*, 2005). The major impact of chitosan on plant growth may be due to the rise in the key enzyme activities of nitrogen metabolism (protease, nitrate reductase, and glutamine synthetase) and photosynthesis increase, which improved

the growth of plants (Gornik *et al.*, 2008; Mondal *et al.*, 2012). Besides, Sabir *et al.*, (2014) mentioned that the nano-calcite fertilizer increased significantly the vegetative growth of own-rooted 'Narince' grapevines.

All Nano N fertilizer treatments had significant positive impacts on leaf N, P, and K contents of 'King Ruby seedless' grapevines compared with control vines, such results are in accordance with those found by Davarpanah *et al.* (2017) on pomegranate, they reported that Nano-chelated fertilizer N used as a foliar application at the rate of 0.25 and 0.5 g N/L increased the concentration of N in leaves when compared with the control trees, but N foliar sprays did not affect significantly the leaf P and K concentrations. In this respect, Delgado *et al.* (2006) on 'Tempranillo' grapevines, Amiri *et al.* (2008) on apple, and Hasani *et al.* (2016) on pomegranate trees found an increase in the concentration of N in leaves after nitrogen fertilization. Similarly, Havlin *et al.* (2016) suggest that foliar N applied pre- and post-veraison can significantly improve grape N content. Grapes foliar fertilization is an effective measure to replenish the soil fertilizer deficiency, with characteristics of a small amount of fertilizer, high utilization, quick and clear effect (Liang *et al.*, 2003; Li *et al.*, 2009). Nano fertilizers are aimed to make nutrients more available to leaves, consequently increasing nutrient use efficiency (Suppan, 2013).

Nano-N treatments proved to be of great importance in enhancing yield and fruit physical and chemical properties compared with control treatment. The enhancement of yield and berry quality by foliar application of nitrogen are in accordance with those found by El-Otmani *et al.* (2002) on citrus, Amiri *et al.* (2008) on apple, and (Sarker and Rahim, 2013) on mango. Moreover, the study conducted by Sabir *et al.* (2014) on 'Narince' grapevines, Davarpanah *et al.* (2017) on pomegranate, and Ibrahim *et al.* (2019) on 'Superior seedless' grapevines mentioned that the application of foliar nano-size fertilizers positively influenced the yield and fruit quality. The significant effect of Nano-N fertilizer on yield could be due to the stimulative effect of chitosan on physiological processes and enhanced the transportation of nitrogen in the functional leaves which improved vegetative growth and development (Chibu and Shibayama, 2003; Gornik *et al.*, 2008). Chitosan-based materials have recently been used to manufacture nanoparticles able to supply chemicals and nutrients to plants efficiently (Kah *et al.*, 2013). Chitosan quickly absorbs easily into leaves and stems of the epidermis prolonging the contact time and promoting the absorption of bioactive molecules. The increases found in yield with foliar nitrogen fertilization can be attributed to the physiological and metabolic roles of nitrogen in flowering and fruit set, including supplying carbohydrates, which are needed for flower bud growth, flower initiation and development, ovule lifespan, effective pollination, and fertility (Lovatt, 1994; Stiles, 1999; Etehadnejad and Aboutalebi, 2014).

## CONCLUSION

Due to the significant role of nitrogen in vineyards, this investigation aimed to examine the use of a new and effective delivery method of N by studying the impact of Nano-fertilizer on 'King Ruby seedless' grapevines

compared to the traditional fertilizer application method. The present study clearly illustrates that the application of 1000 ppm Nano-nitrogen foliar fertilizer with 50% of the recommended dose of ammonium sulphate soil fertilizer positively influences vine growth, yield, berry quality, and leaf nutrient content of grapevines. Therefore, the strategy to supply N by foliar application, taking the advantage of the high mobility of this element, could be used as an aid in vineyard fertilizing and as a standard practice to support balanced yield and high quality performance.

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### تأثير سماد النتروجين النانو على المحصول وصفات ثمار العنب الكينج روبي

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أجريت هذه الدراسة خلال موسمي 2017 و2018 في مزرعة خاصة تابعة لمركز أجا محافظة الدقهلية على كرمات عنب كينج روبي عمرها عشرة أعوام ومنزوعة في تربة طينية طمييه وتروى بنظام الري بالغمر ومنزوعة على مسافة 2,5 × 2 م وذلك بهدف دراسة تأثير الرش الورقي بسماد النتروجين النانو المحمل على الشيتوسان بثلاث تركيزات (250 و500 و1000 جزء في المليون) مع التسميد الأرضي بسلفات النشادر بتركيز (50 و75%) من الجرعة الموصى بها على النمو الخضري والمحصول وجودة الحبات. أظهرت نتائج الدراسة أن جميع معاملات التسميد بالنتروجين النانو كانت ذات تأثير ايجابي في زيادة قيم النمو الخضري مثل (سمك الأفرخ والمساحة الورقية) والنسبة المئوية لكل من النتروجين والفوسفور والبولتاسيوم في أعناق الأوراق وكذلك زيادة معنوية في كمية المحصول ووزن العنقود ، كما أدت الى تحسين صفات الجودة في الحبات وذلك مقارنة بمعاملة الكنترول خلال موسمي الدراسة ، وكان واضحا من النتائج أن المعاملة (50% معدنى + 1000 جزء في المليون نانو نتروجين) تفوقت على باقى المعاملات بالنسبة لتحسين النمو الخضري والمحصول وصفات الجودة في الحبات خلال موسمي الدراسة.