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The Impact of Foliar Spray of Chitosan Nano-particles and Bulk Form on Crimson Seedless Grapevine Quality and Productivity

Salwa A. Bedrech [1] and Kh. Y. Farroh [2]
[1] Viticulture Department, Horticulture Research Institute, [2] Nano-technology and Advanced Materials Central Lab., Agriculture Research Centre, Giza, Egypt.

THE influence of Chitosan $(C_6H_{11}NO_4)_n$ with different molecular weights along with the Chitosan Nano-particles were studied, as supplements and alternative to mineral fertilizers due to their natural properties, on the growth and productivity of seven years-old Crimson Seedless cv. grapevine. The foliar spray was applied in order to determine the optimum rates that would promote yield quality by comparing the bulk Chitosan (CS) with the Chitosan (CS) Nano-particles. The experiment was conducted in 2019 and 2020 with a preliminary season 2018, comprised seven treatments (Control, Chitosan bulk form at 1, 5, 10 cm^3/ L and Chitosan Nano-particles at 100, 150, 200 ppm). Results indicated that Chitosan (CS) Nano-particles at 200 ppm was significantly the most effective in stimulating all growth parameters followed by bulk Chitosan at 5 cm^3/ L in relation to the control treatment and all other treatments. It has been shown an increase in average yield, cluster weight, berry size, shoot length, anthocyanin, TSS % and leaf pigments content besides its cost effective through reducing the amount of bulk fertilizers.

Keywords: Chitosan, Nano-particles, Chitin, Crimson seedless, Grapevines**.**

Introduction

Chitin is a white, hard, inelastic, nitrogenous polysaccharide found in the exoskeleton as well as in the internal structure of invertebrates. It is composed of β (1-4)-linked 2-acetamido-2-deoxy-β -D-glucose1 (*N*-acetylglucosamine) (Fig.1). It is often considered as cellulose derivative, even though it does not occur in organisms producing cellulose.

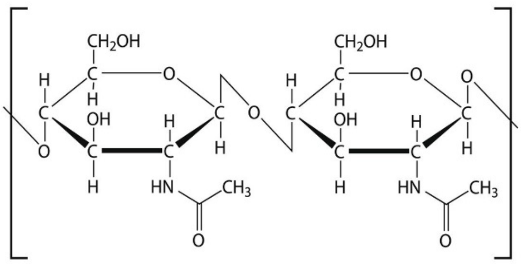

Fig.1. Chemical structure of chitin.

Recently, chitin and its derivatives have shown great effect in being an alternative to mineral fertilizers due to their natural properties. (Jeon et al., 2001). Chitosan is a natural compound prepared mainly from chitin, which is the main component of the skeleton of crustaceans, and it is used as a cheap polymer non-toxic and safe for health. (Kurita*,* 2006).

It was stated in a previous study on pepper plant that foliar application of Chitosan increased yield production in pepper plant. Therefore, Chitosan is considered as an important anti-transpiring to save the water used in irrigation (Bittelli et al., 2001). The quality of the berries has a great impact on the marketing process of table grapes all over the world. Producers often use mineral fertilizers to increase the berries size, which led to cause some health problems. However, some they are still used due to their important role in increasing the berry diameters and consequently a higher yield. Wherefore, there is a need to look for

Corresponding author: Salwa A. Bedrech, E-mail: greengalaxy@hotmail.com.com, Tel. 01155547971
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safer alternative agriculture practices to fulfill this aim, among which was nano-fertilizers applied as a supplement, to increase grape productivity and reducing the use of chemical fertilizers (Yaseen et al., 2020).

Previous research mentioned that applying Chitosan directly on the leaves of many horticultural plants, has stimulant effects on growth through enhancing and yield, shoot length and number of leaves in (*Hibiscus esculentus* L.) (Mondal et al., 2012). Similarly, in Greek oregano, plant growth was stimulated by applying CS (Yin et al., 2012). In addition, foliar spraying with CS improved the vegetative growth and yield of strawberries *(Fragaria chiloensis* L.*)* (El-Miniawy et al., 2013). Moreover, CS applied to freesia corms led to increasing the plant height, leaf and shoot numbers (Salachna and Zawadzi´nska, 2014). In another studies done by Sathiyabama et al. (2014) on tomato plants and Wang et al. (2015) on wheat (*Triticum aestivum* L.), it was mentioned that the yield components were improved due to foliar spraying of CS at different growth stages.

In general, all the nanostructures have small sized particles ranged from 1 to 100 nm which explains the ease of entering the stomata and the cell walls that ranging from 10 to 80 µm. At this scale, matters display new chemical with electric and magnetic properties. Since Materials at the nanoscale have large surface area to volume ratio, this unique characteristic gives them the potential to behave differently from the same material at the bulk form and make them useful and profitable as well. Chitosan Nano-particles is considered as one of this engineered materials that have excellent, natural and nontoxic physicochemical (Agnihotri et al., 2004). In addition, it easily entered the stomata when applied to the leaf surface with the gas uptake (Abd Elaziz et al., 2016).

Chitosan has been reported to have antimicrobial property to some pathogen species as well as an elicitor of resistance in plants, particularly Systemic Acquired Resistance (Modina et al., 2009).

Chitosan acts as an antibacterial even though chitin is not a component of bacterial cells (Jia et al., 2001). It has been discovered that Chitosan activates chitin-induced defense mechanisms found in the plant cell membranes by mimicking those compounds that influence the plant's response if it is attacked by any organisms that contain chitin in their exoskeletons. (Day et al., 2001). It has been suggested that Chitosan may be very effective in resisting plant viruses by modulating the plant's response to infection. Moreover, Rabea et al. (2005) found in an experiment done on the cotton leaf worm that the Chitosan killed all the larvae.

The beneficial goal of this investigation is to produce grapevines with a high quality and in sufficient quantity. Besides elucidating the influence of various concentrations of Chitosan whether in its bulk or Nano-particles form in order to increase yield.

Materials and Methods

This experiment was performed during the consecutive seasons 2019 and 2020 on seven years-old Crimson seedless cv. grapevines. The vineyard has a sandy loam soil and irrigated through drip irrigation system. Vines were trellised by Spanish parron system. Pruning is done during the last week of December leaving a total load of 60 buds per vine (20 fruiting spurs x 3 buds). One hundred and five uniform vines were chosen for this study. All the vines received the same farming practices during the years of investigation.

All treatments were applied thrice as a foliar application and sprayed first at the beginning of the growth stage starting from shoots having 15-20 cm length and the second just after berry setting and the third one month later as follow:

- Control
- Chitosan 1 cm^3/ L
- Chitosan 5 cm^3 / L
- Chitosan 10 cm^3/ L
- Chitosan Nano-particles 100 ppm
- Chitosan Nano-particles 150 ppm
- Chitosan Nano-particles 200 ppm

The Randomized complete block design is adopted for this experiment which included 7 treatments, 15 vines for each treatment divided into 3 replicates (5 vines/replicate).

Physiochemical characterization and preparation method of Chitosan Nano-particles

The Materials used

Chitosan (CS) (molecular weight 50,000-190,000 Da, degree of deacetylation 75-85% and viscosity: 20-300 cP), acetic acid and sodium tripolyphosphate (TPP). All the chemicals used in this study were used without further purification, which were purchased from Sigma-Aldrich, USA chemical company.

Preparation method

The method of ionic gelation is used to transfer the bulk Chitosan into CS NPs (Calvo et al., 1997). This method uses the electrostatic interaction between a negatively charged polyanion group such as sodium tripolyphosphate (TPP) and a Chitosan amine group. First, at room temperature, we prepared an aqueous solution of Chitosan (0.2% w/v) by dissolving it in acetic acid solution (1% v/v) and then, the TPP solution (0.06% w/v) is added as drops with severe and continuous stirring for half an hour. Then the obtained suspension of molecules was centrifuged at 12000 g for another half hour and in deionized water, the granules were resuspended. The suspension of CS NPs is then dried freeze before being used or analyzed again. A (HR-TEM) microscope was used to record the CS NPs morphology. The solution of Chitosan Nano-particles was sonicated for 5 min to reduce particle build-up. On a carbon-coated copper grid three drops of the sonicated solution were deposited using a micropipette and then left to dry.

A morphological evaluation was carried out using HR-TEM images of CS NPs deposited on the grid. Dynamic light scattering (DLS) and Zeta Potential volume measurement were performed using a zeta nanoscaler. The chemical composition of the as-prepared CS NPs was evaluated using X-ray diffraction (XRD) technique. The corresponding XRD pattern was recorded in scanning mode (X' PERT PRO, Analytical PAN, The Netherlands) operated by a Cu K radiation tube (= 1.54 A A) at 40 kV and 30 mA.

Measurements

Morphological measurements

- Leaf area (cm^2): At harvest in the last week of July, leaves samples of 30 leaves for each treatment (10 leaves per replicate) were chosen randomly and their areas were determined by using the leaf area meter
- Shoot length (cm): it was determined by measuring the fruiting shoots at harvest time.

Yield

- Average yield (Kg/vine)
- Average cluster weight (g.)
- Average berry size (cm^3)

Chemical characteristics of berries and leaves

At harvest, when berry TSS% reached about 18-20 %, 15 clusters were randomly chosen as samples from each treatment (Badr and Ramming, 1994) and they are subjected to chemical analysis as follow:

- Total soluble solids (TSS %) and titratable acidity according to (A.O.A.C., 1985) and TSS / acid ratio was calculated.
- Total anthocyanin in berry skin (mg/100g fresh weight) using the spectrophotometer (Yilidz and Dikmen (1990).
- Leaf content of chlorophyll: mature leaves were collected from the 7th positions from the apex and calculated by using the nondestructive Minolta chlorophyll meter model SPAD 502 (Wood et al., 1992).

The economic feasibility study

Statistical analysis

The New L.S.D method was used at 0.05 in order to compare the means according to Snedecor and Cochran (1980).

Results and Discussion

Analysis result

High Resolution Transmission Electron Microscope (HR-TEM) analysis result

The HR-TEM provide us with some features for the particle shape and particle size. We can notice from the Typical TEM micrograph of the Chitosan Nano-particles in Fig.1 that they a size of about 18.2 nm besides their spherical shape.

Dynamic Light Scattering (DLS) Analysis

DLS was used to measure hydrodynamic diameter in the nanometer range. The size of CSNPs was 18.1 nm and zeta potential 48.6 mV (Fig. 2 (A) and (B)).

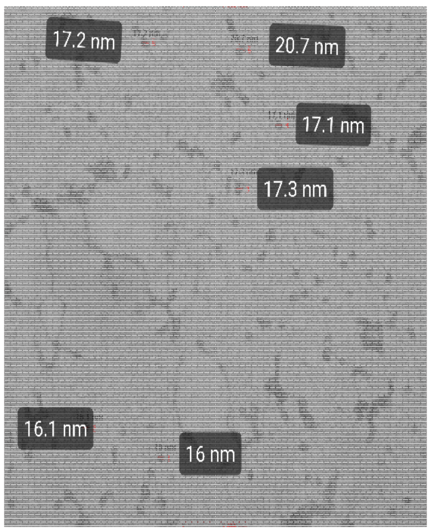
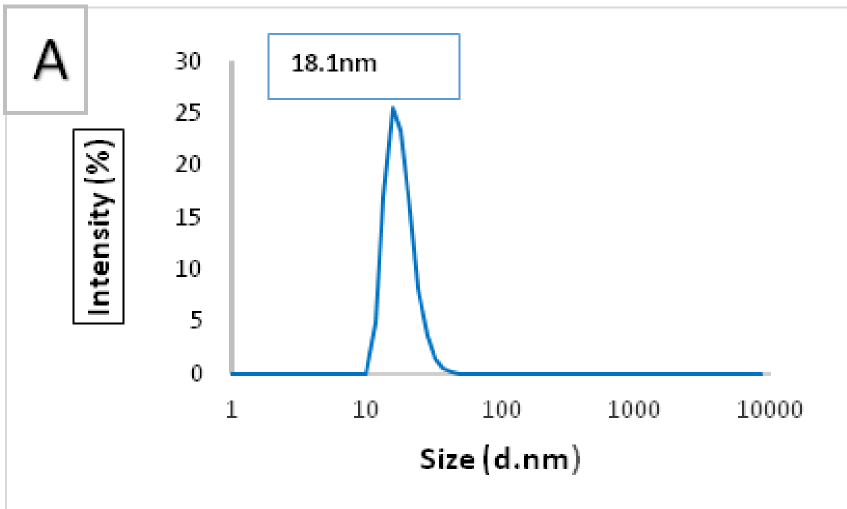
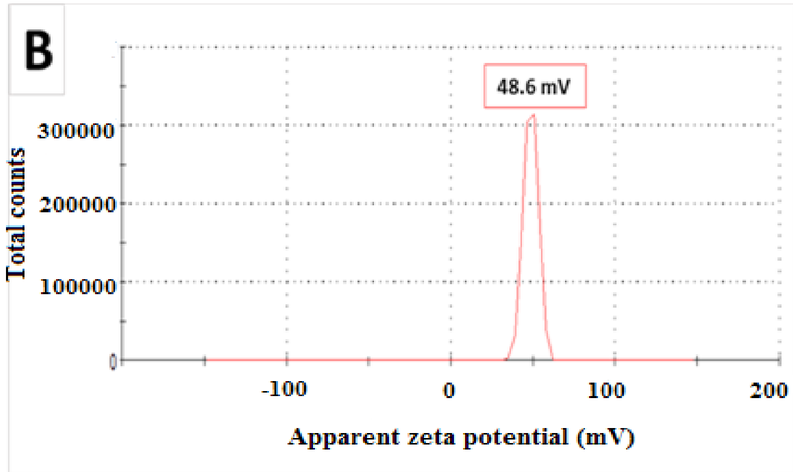


Fig. 1. TEM image of Chitosan Nano-particles.



(A) **Particle size**



(B) Zeta potential

Fig. 2. DLS analysis of CSNPs.

- X-Ray diffraction (XRD) pattern of Chitosan Nano-particles

X-Ray diffraction patterns of Chitosan Nano-particles is displayed in Fig.1. We can notice that the diffractograms has no peak. We note that Chitosan Nano-particles consist of a dense network structure of intertwined polymer chains, and the TPP counter ions lead to linking these chains to each other. The XRD caused greater disarray in the chain alignment in the Nano-particles after the cross-links.

Morphological measurements

Leaf area (cm^2)

Leaf area is considered as a crucial factor that affects yield and fruit quality (Table, 1). From the obtained data, we can clearly notice that treatments are significantly different giving the highest values from Chitosan Nano-particles at 200 ppm followed by the bulk Chitosan at 5 cm^3/L. Similar results showed that treating the Chinese cabbages with Chitosan products increased the plants growth more than standard mineral fertilizer (Spiegel et al., 1998). In addition, Chitosan has the ability to store dry product due to its high thermal and chemical stability as well as its high content of nitrogen for which is used as a source of energy and nitrogen (Sharp, 2013). Moreover, Khalil et al. (2020) declared that treating 'Flame seedless' grapevines thrice by chitosan at 200 ppm was very effective in increasing the leaf area giving the highest value.

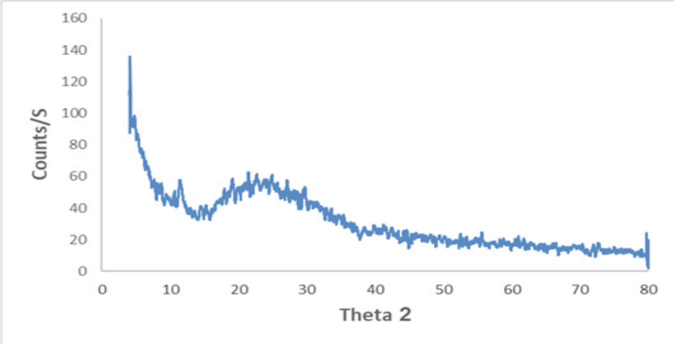


Fig. 3. X-ray diffraction patterns of Chitosan Nano-particles.

Shoot length (cm)

It is obvious in Table (1) that all treatments stimulated shoot length with superior significant values obtained with using Chitosan Nano-particles at 200 ppm followed by the bulk Chitosan at 5 cm^3/L and all the other results are linear to those obtained for the leaf area. In an agreement with the present results were those mentioned by Ait Barka et al. (2004) who found that grapevine sprayed by Chitosan has significantly improved growth. Besides, Harada et al. (1995) declared that application of Chitosan on soybean increased shoot length.

Yield

Yield, cluster weight and berry size

Foliar application of Chitosan Nano-particles at 200 ppm was significantly effective in increasing grapevine yield, cluster weight and berry size followed by the bulk Chitosan at 5 cm^3 / L, which has a slight increase than the higher concentration 10 cm^3 / L in yield and shoot length, comparing with the other treatments and control (Table,1). These results agree with Mona (2015) who proved that Chitosan stimulates plant growth by enhancing cell division similar to gibberellins. Similarly, Shabana and Farroh (2018) stated that the best results in enhancing fruit yield, fruit weight as well as fruit quality, were almost obtained with Chitosan Nano-particles at 200 ppm applied as a foliar spraying on tomato plant.

In addition, Leung and Giraudat (1998) found that Chitosan affects the pathways comprising Jasmonic acid that perform as some ABA-like activities, whereby an increase in ABA reduces transpiration by closing plant stomata maintaining biomass production and yield. Moreover, Lee et al. (1999) declared that Chitosan treatment increases the yield and marketability of soybean. However, increasing concentrations of Chitosan from 50 to 200 ppm and its dosage to thrice caused a progressive promotion on yield and cluster weight of Flame seedless grapevines (Khalil et al., 2020).

Chemical characteristics of berries and leaves

Total soluble solids (TSS %), Titratable acidity % and TSS / acid ratio

All treatments have a great impact on the chemical analysis of berries and showing a significant difference among them (Table 2). Spraying with Chitosan Nano-particles at 200 ppm followed by the bulk Chitosan at 5 cm^3/L was attached with berries high quality through increasing TSS %, and reducing total acidity % in relative to the concentrations. On the other hand, the lower concentration 100 ppm and 150 ppm gave the least values and did not differ significantly as well. In a trial done by Gad et al. (2016), it was found that Chitosan Nano-particles increased the TSS % and TSS / acid ratio of peach fruits cv. Desert Red. In addition, Khalil et al. (2020) stated that treating Flame seedless grapevines thrice with Chitosan at 200 ppm was very effective in improving berries quality in terms of increasing TSS and TSS / acid ratio. Besides, regarding grapevine reproductive performance and yield, obtained outcomes are coordinated, with those of Abd Elsattar et al. (2020) revealing that high values of total soluble solids (TSS%) and TSS/acid ratios and the lowest percentages of total acidity (TA), were achieved in both seasons when boric acid in combination with Chitosan, was applied.

Total anthocyanin content in berry skin

The positive action of Chitosan on total anthocyanin content in berry skin of Crimson seedless grapevines is displayed in Table (2). It is obvious that the highest values were associated with increasing the concentration of Chitosan Nano-particles, where best results were obtained from 200 ppm concentration. Oppositely, for bulk Chitosan there are no significant differences between the higher two concentrations 5 cm^3/L and 10 cm^3/L in both seasons. Similarly, Park et al. (2004) stated that treating Flame seedless grapevines with Chitosan significantly was very effective in improving berries quality in terms of increasing TSS and TSS/ acid ratio. The promotion was associated with increasing concentration of Chitosan. Also Ferri et al. (2009) stated that Chitosan increases the anthocyanins in berry skin. Silva et al. *(*2020) noticed an increment in anthocyanin recorded from the application of Chitosan Nano-particles.

Leaf pigments content (chlorophyll)

Concerning the total chlorophyll content, it was positively stimulated by applying both Chitosan Nano-particles and bulk form (Table, 2). Significant differences among treatments were seen, where best results were obtained from Chitosan Nano-particles at 200 ppm followed by the bulk Chitosan at a concentration of 5 cm^3/L. The experiment tested by Górnik et al. (2008) clarified that Chitosan significantly increased chlorophyll content in the leaves of 'Chrupka Zlota' grapevine. The beneficial influence of Chitosan in the rise of total chlorophyll levels may be ascribed to the increase of the photosynthesize production which entered in chlorophyll formation as stated by Sheikha and Malki, (2011). Besides, increasing the NPK uptake and promoting the transmission of nitrogen to the leaves, which consequently increase chlorophyll content (Abd EL-Gawad and Bondok, 2015). In a study that assess the effect of Chitosan Nano-particles on yield and quality, it was found that foliar spraying treatments enhanced, chlorophyll and the best treatments were almost obtained with CSNPs at 200ppm followed by bulk Chitosan (Shabana and Farroh, 2018).

The economic feasibility study

The cost per feddan of Chitosan in both forms bulk and Nano-particles, yield and the percentage of the increase in net profit over the control are displayed in Table (3). From the obtained data for each treatment, we can calculate the amount and cost saved by using the chitosan Nano-particle instead of bulk ones. It is obvious that the best treatment of Chitosan np at 200 ppm is more cost effective (784 L.E/Fed) whereas the percentage of the increase in net profit over the control for both seasons was (33.7% and 32.8 %) which compensates its higher price than the bulk Chitosan at its highest concentration 10 cm^3/L (10,500 L.E/Fed) with a percentage of increase in the net profit over the control (22.2% and 22.4%).

TABLE 1. Effect of spraying with chitosan bulk and Nano-particles on morphological measurements of Crimson seedless grapevine during 2019 and 2020 seasons.

Treatments	Average yield (Kg)		Cluster weight (g)		Berry size (cm^3)		Leaf area (cm^2)		Shoot length (cm)	
	2019	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
Control	9.8	10.0	432.9	441.2	1.8	1.8	110.5	111.6	155.2	155.8
Chitosan 1 cm^3/ L	10.6	10.7	468.4	481.0	2.1	2.2	112.6	113.4	160.7	161.0
Chitosan 5 cm^3/ L	13.5	13.7	629.6	660.8	2.8	2.9	127.7	127.9	175.6	175.4
Chitosan 10 cm^3/ L	12.6	12.9	576.0	605.4	2.7	2.8	123.3	123.5	173.3	172.8
Chitosan Nano-particles 100 ppm	11.1	11.8	501.3	543.6	2.3	2.5	116.5	117.0	165.9	166.2
Chitosan Nano-particles 150 ppm	11.8	12.4	535.2	578.1	2.5	2.6	119.3	120.2	170.2	171.3
Chitosan Nano-particles 200 ppm	14.8	14.9	689.9	722.3	3.0	3.0	131.5	130.5	182.6	186.4
New L.S.D at 5%	**0.9**	**0.9**	**0.61**	**0.62**	**0.1**	**0.1**	**2.0**	**1.6**	**3.0**	**3.0**

TABLE 2. The Effect of spraying with chitosan bulk and Nano-particles on berries and leaves chemical analysis of Crimson seedless grapevine during 2019 and 2020 seasons.

Treatments	TSS %		Acidity %		T.S.S/ acid ratio		Total anthocyanin (mg/100g)		Total chlorophyll SPAD	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	17.5	17.4	0.63	0.65	27.7	26.7	17.4	18.2	38.8	38.3
Chitosan 1 cm^3/ L	18.1	18.3	0.60	0.61	30.1	30.0	20.1	21.0	39.5	39.0
Chitosan 5 cm^3/ L	20.9	21.0	0.52	0.50	40.1	42.0	27.2	27.7	43.8	43.8
Chitosan 10 cm^3/ L	20.7	20.9	0.51	0.51	40.5	40.9	26.9	27.5	43.3	43.5
Chitosan Nano-particles 100 ppm	18.6	19.1	0.59	0.57	31.5	33.5	22.5	23.1	41.0	41.5
Chitosan Nano-particles150 ppm	19.4	19.7	0.56	0.53	34.6	37.1	24.9	25.0	42.9	42.8
Chitosan Nano-particles 200 ppm	21.3	21.5	0.48	0.48	44.1	44.7	30.8	32.4	44.9	44.6
New L.S.D at 5%	**0.2**	**0.3**	**0.02**	**0.01**	**0.3**	**0.3**	**0.5**	**0.5**	**0.4**	**0.6**

TABLE 3. The economic study of the cost per feddan for each treatment in both seasons (2019-2020).

Treatments	Amount used in cm^3/L per vine	Amount used in L/fed (700vines)	Price of litre (L.E)	Cost for feddan (L.E)	Cost for two seasons	Price of yield/fed (L.E)		The increase of net profit over the control (%)	
						2019	2020	2019	2020
Control	-	-	-	-	-	68,600	70,000	-	-
Chitosan 1 cm^3/ L	1.0	0.7	750	525	1,250	74,200	74,900	7.5	6.5
Chitosan 5 cm^3/ L	5.0	3.5	750	2,625	5,250	94,500	95,900	27.4	27.0
Chitosan 10 cm^3/ L	10.0	7.0	750	5,250	10,500	88,200	90,300	22.2	22.4
Chitosan Nano-particles 100 ppm	0.10	0.07	2,800	196	392	77,700	82,600	11.7	15.3
Chitosan Nano-particles 150 ppm	0.15	0.10	2,800	280	560	82,600	88,900	16.9	21.2
Chitosan Nano-particles 200 ppm	0.20	0.14	2,800	392	784	103,600	104,300	33.7	32.8

Conclusion

Various application levels along with forms of Chitosan whether bulk or Nano-particles had significant effects on yield, physical and chemical characteristics of berries and leaves. The highest yield was obtained from treated the vines with 200 ppm of Chitosan Nano-particles thrice. On the other hand, spraying Chitosan at $5cm^3/L$ was the most effective in its bulk form. Thus, it can be summarized from the present study results that foliar application of Chitosan Nano-particles is cost-effective as it used in very small quantities and is also safer than mineral fertilizers and suitable for reducing environmental pollution.

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Conflicts of interest

We declare that there are no conflicts of interest related to the publication of this study.

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تأثير الرش الورقى بالكيتوزان النانو على صفات الجودة والإنتاجية فى العنب.

سلوى عادل بدرش [١] و خالد يحيى فروح [٢]
[١] قسم بحوث العنب - معهد بحوث البساتين - [٢] المعمل المركزي للنانوتكنولوجي والمواد المتقدمة - مركز البحوث الزراعية - الجيزة - مصر.

تمت دراسة تأثير الكيتوزان $n\ (C_{٦}H_{١١}NO_{٤})$ مع الأوزان الجزيئية المختلفة وجزيئات الكيتوزان النانو كمكملات سمادية ، على جودة النمو وإنتاجية المحصول لكرمات العنب صنف الكريمسون سيدلس عمر سبع سنوات. تم تطبيق الرش الورقي من أجل تحديد أفضل جرعة من شأنها تحسين المحصول وخصائص الحبات من خلال مقارنة كتلة الكيتوزان العادى بجزيئات الكيتوزان النانو. أجريت التجربة في عامي ٢٠١٩ و ٢٠٢٠ بموسم تمهيدي ٢٠١٨ ، واشتملت على سبعة معاملات و هى، الكونترول، الكيتوزان عند تركيز ١ ، ٥ ، ١٠ سم ^{٣} / لتر و جزيئات الكيتوزان النانو بتركيز ١٠٠ ، ١٥٠ ، ٢٠٠ جزء في المليون. أشارت النتائج إلى أن جزيئات النانو كيتوزان عند ٢٠٠ جزء في المليون كانت الأكثر فاعلية في تحفيز جميع صفات النمو يليها الكيتوزان العادى بنسبة ٥٪ نسبة إلى الكونترول و جميع المعاملات الأخرى. وقد لوحظ زيادة في المحصول الكلى ووزن العنقود وحجم الحبات وطول الفرع، بالإضافة إلى الخصائص الكيميائية للحبات والأوراق مثل نسبة المواد الصلبة الذائبة والأنثوسيانين ومحتوى الأوراق من الكلوروفيل و هذا بجانب قلة التكلفة من خلال استخدام كميات اقل فى صورة النانوعن الأسمدة العادية.