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Effect of Chitosan and Calcium Gluconate on Fruit Quality and Storability of Crimson Seedless Grape Cultivar

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ABSTRACT

This investigation was conducted for two consecutive seasons (2021 & 2022) in El-Baramon farm located in Mansoura City, Dakahlia Governorate, Egypt, to study influence of chitosan and calcium gluconate on growth, yield and fruit quality as well as storability of Crimson Seedless grape cultivar. Seven-year-old vines were cultivated 2*3 meters apart in clay soil with flood irrigation and trellised using the Spanish baron system. In the final week of February, vines were cane-pruned and bud loaded with 96 buds per vine. Nine treatments were conducted as follows: foliar spraying with chitosan at the two concentrations (0.5 or 1%) were applied either separately or in combination with calcium gluconate at the two concentrations (1% or 2%), beside to the control treatment. All different concentrations of chitosan and calcium gluconate were foliar sprayed twice: after fruit set stage when the berry diameter reaches 2-3 mm and at veraison stage (20% berry color). Clusters of all treatments were stored for 4 weeks at ± 0ºC and 90 - 95% RH. The combined application of 1% chitosan and 2% calcium gluconate exhibited the most favorable results in terms of acquiring the best aspects of vegetative growth, increasing yield and enhancing cluster quality attributes as well as improving the storability of Crimson Seedless grape via reducing physiological loss in weight, decay and shattering, maintaining firmness and delaying the changes in total soluble solids, acidity and total anthocyanin.

Keywords: Grapes- Crimson- Chitosan- Calcium- Storability.

INTRODUCTION

Chitosan is a natural polysaccharide extracted from the low-acetylated form of chitin, consisting principally of N-acetyl glucosamine and glucosamine. It is produced from crab squid, shrimp shells, shells, lobster and cell walls of filamentous fungi (Kumaresapillai et al., 2011 and Kaya et al., 2015). Due to its diverse properties, it has become one of the most popular biopolymers and the leading plant excitability molecule since the last decade (Malerba and Cerana, 2015).

The application potential of chitosan has been studied on several horticultural crops, towards stimulating defense mechanisms, increasing plant growth and physiological activities, improving agronomic traits, increasing yields and enhancing the storability due to its nature a good semi-permeable barrier to water loss and the rate of respiration as well as fruit shrinkage, thus delaying ripening and senescence (Petriccione et al., 2015 and Bautista Baños et al., 2017).

Calcium plays a crucial role in plant growth and development, as well as activating enzymes and sending signals to coordinate cellular activities, in addition to maintaining various cell functions and enhancing cellular membrane stability, which is reflected on increased cell firmness (Conway et al., 2002 and White and Broadley, 2003).

Numerous horticultural crops have been researched for the use of calcium, which has been associated with enhancing growth, productivity and fruit quality traits as well as delaying the ripening and the processes of senescence (Vicente et al., 2007, Ilyas et al., 2014 and Al-Ahmadi, 2015). Organic calcium salts like lactate, citrate, or gluconate boast antioxidant capacity, improves nutritional value without tasting bad, and are more bioavailable compared to an inorganic salt like calcium chloride (Fekry, 2018).

Crimson Seedless grapes are characterized by being a seedless cultivar, attractive, fragile, red and late maturing. However, they face a major problem in their production, represented by the fact that their clusters lack sufficient color intensity, with the color not being uniform between the berries (Cameron, 2005).

The goal of this investigation is to study the influence of chitosan and calcium gluconate on growth, yield and fruit quality as well as storability of Crimson Seedless grape cultivar.

MATERIALS AND METHODS

This investigation was conducted for two consecutive seasons (2021 & 2022) in El-Baramon farm located in Mansoura City, Dakahlia Governorate, Egypt. Seven- year-old Crimson Seedless grapevines were used for this investigation. Vines were grown at 2*3 meters apart in clay soil shown in **(Table 1)** with flood irrigation and trellised using the Spanish baron system. In the final week of February, vines were cane-pruned and bud loaded with 96 buds per vine.

Table (1). Physical and chemical analysis of the experimental soil

Characters	Values
Sand %	19.83
Silt %	22.71
Clay %	57.46
Texture	Clay
PH (1:2.5)	7.83
Organic carbon %	1.92
$CaCO_3$ %	1.74
E.C. (1:5 extract) (mmhos/1 cm)	0.63
N (%)	0.27
P (%)	0.14
K (%)	0.33

One hundred and thirty five uniform vines were selected. Every five vines were used as a replicate and each treatment constitutes of three replicates.

Nine treatments were conducted as follows:

1. Chitosan at 0.5%
2. Chitosan at 1%
3. Calcium gluconate at 1%
4. Calcium gluconate at 2%
5. Chitosan at 0.5%+ calcium gluconate at 1%
6. Chitosan at 0.5%+ calcium gluconate at 2%
7. Chitosan at 1%+ calcium gluconate at 1%
8. Chitosan at 1%+ calcium gluconate at 2%
9. Control

All treatments were foliar sprayed twice: after fruit set stage when the berry diameter reaches 2-3 mm and at veraison stage (20% berry color).

Preparation of conducted treatments

Both chitosan and calcium gluconate were purchased from sigma chemical company. Chitosan was extracted by dissolving in 1% glacial acetic acid (Du et al., 1997), while calcium gluconate was prepared by dissolving in water before incorporating 0.5% acetic acid. All conducted treatments as well as control had Tween-80 (0.5% v/v) to enhance the solutions' wetting qualities and their adhesion to the surface of the fruits (Zhao et al., 2009).

Parameters assessed

1- Vegetative growth parameters

During the second week of July, five non-fruiting shoots were selected to measure average shoots length (cm), number of leaves/shoot and leaf area (cm^2) which taken from the tip of the shoot at the sixth and seventh nodes according to Montero et al. (2000). In addition, the total chlorophyll content of leaves (mg/g F.W.) was measured according to Mackinny (1941).

2- Yield and its physical attributes

Representative random samples of twelve clusters/vine were harvested at maturity when TSS/acid ratio reached 24-28 (Tourky et al., 1995). The following attributed were assessed: yield/vine (kg), cluster weight (g), berry weight (g), berry size (cm^3) and berry firmness (g/cm^2).

3- Chemical attributed of berries

Total soluble solids (TSS %) and total acidity as (g tartaric acid/100 ml juice) were determined as ascribed to A.O.A.C. (1995). TSS/acid ratio was calculated. Also, total anthocyanin (mg/100 g fresh weight) was identified ascribed to Husia et al. (1965).

4-Storability

At maturity stage, when TSS/acid ratio reached 24-28 as ascribed to Tourky et al. (1995). Clusters were harvested and placed in three perforated cartoon boxes (the 1st for the loss of weight, the 2nd for decay and the last for fruit quality traits at different sampling dates: 0, 1, 2, 3, and 4 weeks of cold storage); each box was replicated three times, and each replicate consisting of three cluster. All treatments were cold stored at ± 0ºC and 90-95% relative humidity.

Fruit physical properties

- Weight loss percentage was calculated according to the following equation: [weight loss (g)/the initial cluster weight (g)] X 100.
- Decay percentage was identified according to the following equation: [weight of the decayed berries/the initial cluster weight (g)] X 100.
- Shattering percentage was calculated according to the following equation: [weight of the shattered berries / the initial cluster weight (g)] X 100.
- Berry firmness (g/cm^2) was measured by using a texture analysis tool.

Fruit chemical properties

Total soluble solids (TSS %) and total acidity as (g tartaric acid/100 ml juice) were determined as ascribed to A.O.A.C. (1995). Also, total anthocyanin (mg/100 g fresh weight) was identified ascribed to Husia et al. (1965).

Trial Design and Statistical Analysis

For this experiment, a completely randomized design was applied. The current data's statistical analysis was performed in accordance with Snedecor and Cochran (1980). At the 5% level, averages were compared using the new LSD values (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

1. Vegetative growth characteristics

As shown in **Table (2),** it is evident that shoot length, number of leaves per shoot, leaf area and leaf total chlorophyll content were significantly increased by foliar spraying of chitosan or calcium gluconate at different concentrations either individually or in combined form as compared to control. The combined application of chitosan at 1% and calcium gluconate at 2% significantly achieved the highest magnitudes of these parameters, while the least ones were obtained by control for both seasons.

The enhancement of vegetative growth traits as a result of the use of chitosan may be due to its physiological influence on cell division, photosynthesis and chlorophyll synthesis (Dzung et al., 2011 and Al-Ahmadi, 2015). Regarding the stimulating influence of calcium gluconate on vegetative growth traits, it might be because of its involvement in cell wall and carbohydrate synthesis (Ilyas et al., 2014).

The outcomes attained are consistent with El-Kenawy (2017) on Thompson Seedless grape, Khalil et al. (2020) and El-Senosy (2022) on Flame Seedless grape as well as Khalil and Badreldin (2021) and Bedrech and Farroh (2022) on Crimson Seedless grape; they reported that vegetative growth features were enhanced by chitosan application. On the other hand, Abdel Ghany et al. (2023) and Mohamed and Mohamed (2023) mentioned that calcium application improved vegetative growth aspects of Flame Seedless grape cultivar.

Table (2). Effect of chitosan (Chito) and calcium gluconate (CaGu) on vegetative growth characteristics of Crimson Seedless grapevines during 2021 and 2022 seasons

Characteristics	Shoot length (cm)		Number of leaves/shoot		Leaf area (cm^2)		Total chlorophyll (mg/g F.W)	
Treatments	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
0.5%Chito	152.1	158.5	25.4	26.9	165.7	170.5	18.43	20.15
1%Chito	158.1	164.5	26.1	28.1	174.1	176.2	18.89	20.58
1%CaGu	148.6	156.9	24.1	26.1	165.3	169.3	17.91	19.43
2%CaGu	156.3	159.8	25.7	27.6	170.6	173.7	18.86	20.47
0.5%Chito + 1%CaGu	162.2	167.1	26.8	28.5	177.8	182.1	19.56	21.14
0.5%Chito + 2%CaGu	165.7	172.9	27.1	28.9	180.9	182.9	19.92	21.41
1%Chito + 1%CaGu	171.5	178.1	27.9	30.1	182.2	184.3	20.19	21.64
1%Chito + 2%CaGu	176.7	181.6	28.7	30.6	184.3	185.7	20.43	21.85
Control	143.7	150.1	23.1	24.9	162.7	167.8	16.16	18.51
New L.S.D at 5%	4.3	3.1	0.6	0.4	1.5	1.2	0.19	0.16

2- Yield and its physical attributes

Data in **Table (3)** demonstrated that the foliar spraying of chitosan or calcium gluconate at different concentrations either solely or in combined form markedly enhanced yield/vine, cluster weight, berry weight, berry size and berry firmness in comparison with control. Highest significant yield and berry physical characteristics were obtained by the combined application of chitosan at 1% and calcium gluconate at 2%, while the least ones were obtained by control for both seasons.

The improvement of yield and physical properties of berries as a result of the use of chitosan may be due to its physiological effect on cell division, photosynthesis and chlorophyll synthesis (Dzung et al., 2011 and Al-Ahmadi, 2015). Concerning the promoting influence of calcium gluconate on yield and berry physical properties may be due to decreased sensitivity of the cell wall to enzymatic hydrolysis by preventing the pectin methylesterase and polygalacturonase enzymes activity and thus preventing cell disintegration and collapse (Abdel Ghany et al., 2023).

The acquired outcomes are consistent with El-Kenawy (2017) on Thompson Seedless grape, Khalil et al. (2020) and El-Senosy (2022) on Flame Seedless grape as well as Bedrech and Farroh (2022) on Crimson Seedless grape; they showed that yield and cluster weight were enhanced by chitosan application. On the other hand, Mohamed and Mohamed (2023) mentioned that calcium application improved the yield and fruit quality attributes of Flame Seedless grape.

Table (3). Effect of chitosan (Chito) and calcium gluconate (CaGu) on yield and its physical attributes of Crimson Seedless grapevines during 2021 and 2022 seasons

Characteristics	Yield/vine (Kg)		Average cluster weight (g)		Average berry weight (g)		Average berry size (cm^3)		Average berry firmness (g/cm^2)	
Treatments	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
0.5%Chito	14.46	14.88	439.3	453.7	4.07	4.14	3.95	4.04	480.4	483.6
1%Chito	14.84	15.08	444.3	458.5	4.15	4.23	4.04	4.12	494.3	509.3
1%CaGu	14.32	14.73	436.5	450.4	4.05	4.09	3.92	4.01	468.7	476.2
2%CaGu	14.67	14.96	440.9	455.9	4.11	4.19	3.98	4.07	482.6	500.8
0.5%Chito + 1%CaGu	15.03	15.27	448.6	462.8	4.19	4.28	4.09	4.17	507.2	516.8
0.5%Chito + 2%CaGu	15.17	15.39	452.7	466.4	4.22	4.32	4.12	4.21	514.7	523.2
1%Chito + 1%CaGu	15.30	15.54	456.6	469.6	4.24	4.35	4.15	4.26	523.2	528.6
1%Chito + 2%CaGu	15.42	15.65	458.9	471.2	4.29	4.38	4.17	4.29	527.5	533.9
Control	14.25	14.58	435.8	448.7	4.02	4.17	3.88	3.95	441.9	456.9
New L.S.D at 5%	0.11	0.08	1.9	1.6	0.03	0.02	0.02	0.03	4.1	3.8

3- Chemical attributed of berries

As shown in **Table (4)**, it is evident that TSS, total acidity, TSS/acid ratio and berry skin content of total anthocyanin were significantly influenced by foliar spraying of chitosan and calcium gluconate at different concentrations either individually or in combined form as compared to control. The combined application of chitosan at 1% and calcium gluconate at 2% significantly achieved the lowest values of TSS, TSS/acid ratio and total anthocyanin as well as the highest values of acidity, whereas, control had the lowest values of acidity as well as the highest values of TSS, TSS/acid ratio and total anthocyanin for both seasons.

The enhancement of berry chemical properties as a result of the use of chitosan may be due to its physiological effect on cell division, photosynthesis and chlorophyll synthesis (Dzung et al., 2011 and Al-Ahmadi, 2015). Regarding the stimulating effect of calcium gluconate on berry chemical properties, it may be due to its role in cell division and carbohydrate metabolism (Ilyas et al., 2014).

The outcomes achieved are consistent with El-Kenawy (2017) on Thompson Seedless grape, Khalil et al. (2020) and El-Senosy (2022) on Flame Seedless grape as well as Khalil and Badreldin (2021) and Bedrech and Farroh (2022) on Crimson Seedless grape; they reported that chemical properties of berries were enhanced by chitosan application. On the other hand, Abdel Ghany et al. (2023) and Mohamed and Mohamed (2023) mentioned that calcium application improved berry chemical properties of Flame Seedless grape cultivar.

Table (4). Effect of chitosan (Chito) and calcium gluconate (CaGu) on berry chemical attributes of Crimson Seedless grapevines during 2021 and 2022 seasons

Characteristics	TSS (%)		Total acidity (%)		TSS/acid ratio		Total anthocyanin (mg/100g F.W)	
Treatments	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
0.5%Chito	16.22	16.38	0.63	0.61	25.75	26.85	33.29	34.13
1%Chito	16.19	16.37	0.66	0.63	24.53	25.98	32.65	33.58
1%CaGu	16.24	16.41	0.62	0.61	26.19	26.90	33.74	34.67
2%CaGu	16.21	16.38	0.65	0.62	24.94	26.42	32.87	33.79
0.5%Chito + 1%CaGu	16.17	16.34	0.67	0.63	24.13	25.94	32.38	33.16
0.5%Chito + 2%CaGu	16.15	16.33	0.67	0.64	24.10	25.52	32.07	32.97
1%Chito + 1%CaGu	16.14	16.33	0.68	0.64	23.74	25.52	31.82	32.58
1%Chito + 2%CaGu	16.11	16.32	0.69	0.65	23.35	25.11	31.73	32.41
Control	16.27	16.43	0.61	0.59	26.67	27.85	34.17	35.13
New L.S.D at 5%	0.02	0.01	0.01	0.02	0.43	0.39	0.27	0.24

Storability

Fruit physical properties

- **Weight loss percentage**

Data presented in **Table (5)** show that fruit weight loss percentage gradually increased with prolonging the cold storage period for all treatments in both seasons. It can be seen that the pre-harvest application of chitosan or calcium gluconate at different concentrations either in solely or in combined form significantly reduced the rise in fruit weight loss percentage as compared to the control with storage period advanced. The lowest value of weight loss was obtained by chitosan at 1% and calcium gluconate at 2%, while the highest percentage of this one was significantly attained by control during cold storage for both seasons.

The increase in fruit weight loss during cold storage may be due to transpiration and respiration (Hafez et al., 2012). Such results are in accordance with those mentioned by Abdel Wahab (2015), who indicated that weight loss % increased as cold storage proceeds.

The chitosan coating smooth the pericarp's surface and builds a semi-transparent layer that acts as a protective barrier that blocks transpiration, which reduces fruit weight loss, as well as decreases O_2 consumption, which lowers respiration rate and ethylene production, leading to extend the fruit's shelf life (González-Aguilar et al., 2009 and Trung et al., 2011). Furthermore, the chitosan coating shields the fruit's skin from mechanical harm and seals minor wounds to prevent dehydration and preserve tissue rigidity, thereby the chitosan retain the fruit's moisture content and preserves it for an extended amount of time with a generally acceptable quality (Krishna and Rao, 2014 and Chauhan et al., 2014).

These results are consistent with those of El-Kenawy (2017) on Thompson Seedless grape, El-Mahdy et al. (2018) on Red Roomy grape, Sabir et al. (2019) on Alphonse Lavallee grape; they demonstrate that chitosan application significantly decreased the rise in the weight loss percentage during cold storage.

Calcium effectively contributes to the maintenance of membrane functionality and integrity. Therefore, the reduced phospholipid loss and ion leakage in calcium-treated fruits are the cause of the lower weight loss (Lester and Grusak, 1999).

The remarkable performance of calcium gluconate in reducing weight loss can be attributed to its key role in retarding enzymatic activities that cause moisture loss and deterioration (Han et al., 2004, Atress et al., 2010 and Jouki and Khazaei, 2012)

The outcomes obtained are similarly comparable to those attained by Abdel Wahab et al. (2014) on Crimson Seedless grape, Abdel Gayed et al. (2017) on Early Swelling peach and Fekry (2018) on guava, they found that calcium application significantly decreased the rise in the weight loss percentage during cold storage.

Table (5). Effect of chitosan (Chito) and calcium gluconate (CaGu) on weight loss (%) of Crimson Seedless grapes during cold storage in 2021 and 2022 seasons

Date (D)	**2021, season**					
	Storage period (week)					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	0.00	1.82	2.79	5.28	9.54	3.89
1%Chito	0.00	1.39	2.37	4.86	8.34	3.39
1%CaGu	0.00	1.99	3.01	5.60	10.27	4.18
2%CaGu	0.00	1.57	2.61	5.07	9.09	3.67
0.5%Chito + 1%CaGu	0.00	1.29	2.08	4.64	8.18	3.24
0.5%Chito + 2%CaGu	0.00	1.04	1.99	4.40	7.81	3.05
1%Chito + 1%CaGu	0.00	0.82	1.74	2.98	6.53	2.42
1%Chito + 2%CaGu	0.00	0.58	1.60	2.68	6.16	2.20
Control	0.00	2.23	3.31	5.80	10.81	4.43
Means (D)	0.00	1.41	2.39	4.59	8.53	
New LSD at 5% (T) =	1.36					
New LSD at 5% (D) =	1.02					
New LSD at 5% (TXD) =	3.05					
Date (D)	**2022, season**					
	Storage period (week)					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	0.00	1.61	2.43	4.51	8.02	3.31
1%Chito	0.00	1.23	2.06	4.15	7.01	2.89
1%CaGu	0.00	1.76	2.62	4.79	8.63	3.56
2%CaGu	0.00	1.39	2.27	4.33	7.64	3.13
0.5%Chito + 1%CaGu	0.00	1.14	1.81	3.97	6.87	2.76
0.5%Chito + 2%CaGu	0.00	0.92	1.73	3.76	6.56	2.59
1%Chito + 1%CaGu	0.00	0.73	1.51	2.55	5.49	2.06
1%Chito + 2%CaGu	0.00	0.51	1.39	2.29	5.18	1.87
Control	0.00	1.97	2.88	4.96	9.08	3.78
Means (D)	0.00	1.25	2.08	3.92	7.16	
New LSD at 5% (T) =	1.27					
New LSD at 5% (D) =	0.95					
New LSD at 5% (TXD) =	2.84					

- **Decay percentage**

As shown in **Table (6),** a noticeable upward trend in the percentage of fruit decay was pronounced with the extension of the cold storage duration for all treatments in both seasons. It can be observed that the pre-harvest application of chitosan or calcium gluconate at different concentrations either alone or in combined form significantly reduced the increase in fruit decay percentage compared to the control with storage period advanced. Control clusters recorded the highest significant percentage of fruit decay at the end of cold storage period. On the other hand, the combined application of chitosan at 1% and calcium gluconate at 2% significantly recorded the lowest percentage of fruit decay during cold storage for both seasons.

Decay is one of the main fundamental post-harvest items affecting the decline in quality of horticulture crops. Fruits are affected by post-harvest diseases and disturbances during storage as a consequence of physiological changes and the process of ageing (Prusky and Keen, 1993).

Chitosan can be ascribed to the building of a thin layer on the surface of the fruit (Badawy and Rabea, 2009) and controls gray rot postharvest decay (Romanazzi et al., 2009). Furthermore, treatment with chitosan exhibited beneficial outcome in protecting the integrity of membrane and increasing the phenolic compounds and antioxidant enzymes activity (Kumari et al., 2015).

These results are in line with those attained by El-Kenawy (2017) on Thompson Seedless grape, El-Mahdy et al. (2018) on Red Roomy grape, Sabir et al. (2019) on Alphonse Lavallee grape; they found that chitosan application significantly decreased the rise in the decay percentage during cold storage.

In fruit tissue, calcium ions are incorporated and this strengthens the cell wall, especially the central lamella that holds the cell together. Consequently, improving the resistance of fruits to enzymes produced by fungal diseases (Hernandez-Munoz et al., 2008).

The acquired results are consistent with Abdel Wahab et al. (2014) on Crimson Seedless grape, Abdel Gayed et al. (2017) on Early Swelling peach and Fekry (2018) on guava, they found that calcium application markedly reduced the increase in the decay percentage during cold storage.

Table (6). Effect of chitosan (Chito) and calcium gluconate (CaGu) on decay (%) of Crimson Seedless grapes during cold storage in 2021 and 2022 seasons

	2021, season					
Date (D)	Storage period (week)					
Treatment (T)	0	1	2	3	4	Means (T)
0.5%Chito	0.00	0.69	1.72	4.48	6.44	2.67
1%Chito	0.00	0.44	1.65	4.01	6.15	2.45
1%CaGu	0.00	0.84	1.81	4.76	6.76	2.83
2%CaGu	0.00	0.55	1.68	4.32	6.35	2.58
0.5%Chito + 1%CaGu	0.00	0.37	1.61	3.85	5.87	2.34
0.5%Chito + 2%CaGu	0.00	0.29	1.52	3.58	5.72	2.22
1%Chito + 1%CaGu	0.00	0.22	1.44	3.43	5.62	2.14
1%Chito + 2%CaGu	0.00	0.08	1.31	3.32	5.13	1.97
Control	0.00	0.99	2.61	5.37	7.46	3.29
Means (D)	0.00	0.50	1.71	4.12	6.17	
New LSD at 5% (T) =	0.11					
New LSD at 5% (D) =	0.08					
New LSD at 5% (TXD) =	0.25					
	2022, season					
Date (D)	Storage period (week)					
Treatment (T)	0	1	2	3	4	Means (T)
0.5%Chito	0.00	0.54	1.49	3.83	5.41	2.25
1%Chito	0.00	0.35	1.42	3.43	5.17	2.07
1%CaGu	0.00	0.66	1.56	4.07	5.68	2.39
2%CaGu	0.00	0.43	1.45	3.69	5.34	2.18
0.5%Chito + 1%CaGu	0.00	0.29	1.39	3.29	4.93	1.98
0.5%Chito + 2%CaGu	0.00	0.23	1.31	3.06	4.81	1.88
1%Chito + 1%CaGu	0.00	0.17	1.24	2.93	4.72	1.81
1%Chito + 2%CaGu	0.00	0.06	1.13	2.84	4.31	1.67
Control	0.00	0.78	2.25	4.59	6.27	2.78
Means (D)	0.00	0.39	1.47	3.53	5.18	
New LSD at 5% (T) =	0.07					
New LSD at 5% (D) =	0.05					
New LSD at 5% (TXD) =	0.16					

- **Shattering**

Data presented in **Table (7)** showed that shattering percentage gradually increased with the prolongation of the cold storage period for all treatments in both seasons. It can be seen that the pre-harvest application of chitosan or calcium gluconate at different concentrations either alone or in combined form significantly reduced the rise in the percentage of shattering as compared to the control with storage period advanced. The lowest percentage of shattering was obtained by chitosan at 1% and calcium gluconate at 2%, while the highest percentage of this one was significantly attained by control during cold storage.

Chitosan reduce carbohydrate metabolism change during storage, which is associated with decreasing shattering percentage (Shiri et al., 2013).

The acquired results are consistent with El-Kenawy (2017) on Thompson Seedless grape, who demonstrated that chitosan application significantly reduced the increase in the shattering percentage during cold storage.

The effect of calcium in reduction of shattering percentage during storage might be due as it is one of the main ingredients of pectin and helps to reinforce the construction of membranes and cell walls as well as increases adherence berries (Rohani et al., 1997).

The outcomes obtained are similarly comparable to those attained by Abdel Wahab et al. (2014) on Crimson Seedless grape, who mentioned that calcium application significantly reduced the shattering percentage during cold storage.

Table (7). Effect of chitosan (Chito) and calcium gluconate (CaGu) on shattering (%) of Crimson Seedless grapes during cold storage in 2021 and 2022 seasons

	2021, season					
Date (D)	**Storage period (week)**					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	0.00	1.69	2.46	2.67	3.17	2.00
1%Chito	0.00	1.43	2.18	2.47	2.94	1.80
1%CaGu	0.00	2.24	2.84	3.05	3.43	2.31
2%CaGu	0.00	1.63	2.32	2.58	3.03	1.91
0.5%Chito + 1%CaGu	0.00	1.34	2.12	2.38	2.64	1.70
0.5%Chito + 2%CaGu	0.00	1.21	1.69	1.98	2.31	1.44
1%Chito + 1%CaGu	0.00	1.17	1.34	1.68	1.91	1.22
1%Chito + 2%CaGu	0.00	0.86	1.18	1.46	1.77	1.06
Control	0.00	2.48	3.07	3.42	4.08	2.61
Means (D)	0.00	1.56	2.13	2.41	2.81	
New LSD at 5% (T) =	0.12					
New LSD at 5% (D) =	0.09					
New LSD at 5% (TXD) =	0.27					
	2022, season					
Date (D)	**Storage period (week)**					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	0.00	1.31	2.07	2.21	2.56	1.63
1%Chito	0.00	1.11	1.83	2.04	2.37	1.47
1%CaGu	0.00	1.71	2.39	2.52	2.77	1.88
2%CaGu	0.00	1.26	1.95	2.13	2.44	1.56
0.5%Chito + 1%CaGu	0.00	1.04	1.78	1.97	2.13	1.38
0.5%Chito + 2%CaGu	0.00	0.94	1.42	1.64	1.86	1.17
1%Chito + 1%CaGu	0.00	0.91	1.13	1.39	1.54	0.99
1%Chito + 2%CaGu	0.00	0.67	0.99	1.21	1.43	0.86
Control	0.00	1.92	2.58	2.83	3.21	2.11
Means (D)	0.00	1.21	1.79	1.99	2.26	
New LSD at 5% (T) =	0.09					
New LSD at 5% (D) =	0.07					
New LSD at 5% (TXD) =	0.20					

- **Firmness**

As shown in **Table (8),** a significant progressive decrease in berry firmness was evident with the prolongation of the cold storage duration for all treatments in both seasons. It can be observed that the pre-harvest application of chitosan or calcium gluconate at different concentrations either alone or in combined form significantly reduced the loss in fruit firmness compared to the control with storage period advancement. Control clusters recorded the lowest significant value of fruit firmness, whereas the combined application of chitosan at 1% and calcium gluconate at 2% significantly recorded the highest value of fruit firmness during cold storage for both seasons.

Fruit Firmness is correlated with the cell walls structure, as changes in the cell compartment structure, including the reduction in galactose and hemicellulose, pectins dissolution and hydrolyzing enzymes activity which breakdown fruit tissues during storage (Cheng et al., 2008).

The preservation of the firmness by chitosan-treated fruits may be due to the inhibition of cellular degradable enzyme activity and the reduction of cell wall degradation and thus lowering of respiration and ripening processes (Bal, 2013).

These results with chitosan treatment are in line with those of Ali et al. (2011) on papaya, Hong et al. (2012) and Chawla et al. (2018) on guava fruits; they found that chitosan application significantly decreased the reducing in the fruit firmness during cold storage.

The calcium ions in fruits are linked to firmness and are thought to reinforce the middle lamella in the cell walls by binding the cells with each other, which slows down the ripening and senescence processes (Vicente et al., 2007).

The acquired results are consistent with Abdel Wahab et al. (2014) on Crimson Seedless grape, Abdel Gayed et al. (2017) on Early Swelling peach and Fekry (2018) on guava, they found that calcium application significantly reduced the decrease in the fruit firmness during cold storage.

Table (8). Effect of chitosan (Chito) and calcium gluconate (CaGu) on berry firmness (g/cm^2) of Crimson Seedless grapes during cold storage in 2021 and 2022 seasons

Date (D)	2021, season					
	Storage period (week)					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	480.4	461.2	433.5	394.5	319.6	417.9
1%Chito	494.3	474.6	446.1	405.9	328.8	430.0
1%CaGu	468.7	449.9	422.9	384.9	311.7	407.6
2%CaGu	482.6	463.3	435.5	396.3	321.0	419.7
0.5%Chito + 1%CaGu	507.2	486.9	457.7	416.5	337.4	441.1
0.5%Chito + 2%CaGu	514.7	494.1	464.4	422.6	342.3	447.6
1%Chito + 1%CaGu	523.2	502.3	472.2	429.7	348.0	455.1
1%Chito + 2%CaGu	527.5	506.4	476.0	433.2	350.9	458.8
Control	441.9	424.2	398.8	362.9	293.9	384.4
Means (D)	493.4	473.7	445.2	405.2	328.2	
New LSD at 5% (T) =	3.2					
New LSD at 5% (D) =	2.4					
New LSD at 5% (TXD) =	7.2					
Date (D)	**2022, season**					
	Storage period (week)					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	483.6	464.3	436.4	397.2	321.7	420.6
1%Chito	509.3	488.9	459.6	418.2	338.8	443.0
1%CaGu	476.2	457.1	429.7	391.0	316.7	414.1
2%CaGu	500.8	480.7	451.9	411.2	333.1	435.5
0.5%Chito + 1%CaGu	516.8	496.1	466.4	424.4	343.8	449.5
0.5%Chito + 2%CaGu	523.2	502.3	472.2	429.7	348.0	455.1
1%Chito + 1%CaGu	528.6	507.4	477.0	434.1	351.6	459.7
1%Chito + 2%CaGu	533.9	512.6	481.8	438.5	355.1	464.4
Control	456.9	438.6	412.3	375.2	303.9	397.4
Means (D)	503.3	483.1	454.1	413.3	334.7	
New LSD at 5% (T) =	2.9					
New LSD at 5% (D) =	2.2					
New LSD at 5% (TXD) =	6.5					

Fruit chemical properties

- **Total soluble solids (TSS)**

Data presented in **Table (9)** show that TSS percentage gradually increased with the extension of the cold storage period for all treatments in both seasons. It can be noticed that the pre-harvest application of chitosan or calcium gluconate at different concentrations either alone or in combined form significantly decreased the increase in TSS percentage as compared to the control with storage period advancement. The lowest percentage of TSS was obtained by chitosan at 1% and calcium gluconate at 2%, while the highest percentage was attained by control during cold storage for both seasons.

The increase in TSS in the fruits as the storage period advances may be due to an increase in the dry matter percentage by metabolic activity, respiration and moisture lost during the transpiration (Nandaniya et al., 2017)

The delay in increasing the TSS content of fruits coated with chitosan may be due to changes in the fruit internal atmosphere, represented by increasing in the level of carbon dioxide and reducing in the level of oxygen, which reduces metabolic activity, moisture loss, and respiration rate (Hong et al., 2012, Das et al., 2013 and Zahran et al., 2015)

These results are in line with those attained by Liu et al. (2014) on plums, Chawla et al. (2018) on guava fruits and Sabir et al. (2019) on Alphonse Lavallee grape; they found that chitosan application significantly decreased the rise in TSS percentage during cold storage.

The decrease in TSS% with Ca treatment could be due to its film-forming attributes, which reduce moisture loss, thus retaining the soluble solids concentration or it could be due to its role in retarding the metabolic activity of the fruits throughout storage (Han et al., 2004 and Chulaki et al., 2017).

The outcomes obtained are similarly comparable to those attained by Abdel Wahab et al. (2014) on Crimson Seedless grape, Abdel Gayed et al. (2017) on Early Swelling peach and Fekry (2018) on guava, they found that calcium application significantly reduced the increase in TSS percentage during cold storage.

Table (9). Effect of chitosan (Chito) and calcium gluconate (CaGu) on TSS (%) of Crimson Seedless grapes during cold storage in 2021 and 2022 seasons

	2021, season					
Date (D)	**Storage period (week)**					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	16.22	16.71	17.54	18.51	20.04	17.80
1%Chito	16.19	16.68	17.51	18.47	20.01	17.77
1%CaGu	16.24	16.73	17.56	18.53	20.07	17.83
2%CaGu	16.21	16.70	17.53	18.50	20.03	17.79
0.5%Chito + 1%CaGu	16.17	16.66	17.49	18.45	19.98	17.75
0.5%Chito + 2%CaGu	16.15	16.63	17.47	18.43	19.96	17.73
1%Chito + 1%CaGu	16.14	16.62	17.46	18.42	19.94	17.72
1%Chito + 2%CaGu	16.11	16.59	17.42	18.38	19.91	17.68
Control	16.27	16.76	17.60	18.56	20.10	17.86
Means (D)	16.19	16.67	17.51	18.47	20.00	
New LSD at 5% (T) =	0.08					
New LSD at 5% (D) =	0.06					
New LSD at 5% (TXD) =	0.18					
	2022, season					
Date (D)	**Storage period (week)**					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	16.38	16.87	17.71	18.69	20.24	17.98
1%Chito	16.37	16.86	17.70	18.68	20.23	17.97
1%CaGu	16.41	16.90	17.75	18.72	20.28	18.01
2%CaGu	16.38	16.87	17.71	18.69	20.24	17.98
0.5%Chito + 1%CaGu	16.34	16.83	17.67	18.64	20.19	17.94
0.5%Chito + 2%CaGu	16.33	16.82	17.66	18.63	20.18	17.92
1%Chito + 1%CaGu	16.33	16.82	17.66	18.63	20.18	17.92
1%Chito + 2%CaGu	16.32	16.81	17.65	18.62	20.17	17.91
Control	16.43	16.92	17.77	18.75	20.30	18.03
Means (D)	16.37	16.86	17.70	18.67	20.22	
New LSD at 5% (T) =	0.05					
New LSD at 5% (D) =	0.04					
New LSD at 5% (TXD) =	0.11					

- **Total acidity**

As shown in **Table (10),** a significant progressive decrease in the total acidity was clear with the prolongation of the cold storage duration for all treatments in both seasons. It can be observed that the pre-harvest application of chitosan or calcium gluconate at different concentrations either alone or in combined form significantly reduced the low in total acidity compared to the control with storage period advanced. Control berries recorded the lowest significant percentage of total acidity, whereas, the combined application of chitosan at 1% and calcium gluconate at 2% significantly recorded the highest percentage of total acidity during cold storage for both seasons.

The gradual decrease in the acidity of fruits during their storage at low temperatures could be due to the transformation of acids into sugars and the consumption of organic acids in the process of respiration (Chulaki et al., 2017).

The higher acidity in fruits coated with chitosan can be assigned to lower metabolic activities and rates of respiration, thus banning loss of organic acids throughout cold storage (Hernandez-Munoz et al., 2008).

The outcomes obtained are similarly comparable to those attained by Liu et al. (2014) on plums, Chawla et al. (2018) on guava fruits and Sabir et al. (2019) on Alphonse Lavallee grape; they found that chitosan application gave the high levels of titratable acidity during cold storage.

The high acidity in Ca-treated fruits throughout cold storage may be due to reduced hydrolysis of organic acids, which oxidize at a slower rate as a result of reduced respiration (Gupta et al., 2011).

The acquired results are consistent with Abdel Wahab et al. (2014) on Crimson Seedless grape, Abdel Gayed et al. (2017) on Early Swelling peach and Fekry (2018) on guava, they found that calcium application maintain higher rates of acids during cold storage.

Table (10). Effect of chitosan (Chito) and calcium gluconate (CaGu) on acidity (%) of Crimson Seedless grapes during cold storage in 2021 and 2022 seasons

Date (D)	2021, season					
	Storage period (week)					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	0.63	0.60	0.57	0.53	0.49	0.57
1%Chito	0.66	0.63	0.60	0.56	0.51	0.59
1%CaGu	0.62	0.60	0.57	0.53	0.48	0.56
2%CaGu	0.65	0.62	0.59	0.55	0.50	0.58
0.5%Chito + 1%CaGu	0.67	0.64	0.61	0.57	0.52	0.60
0.5%Chito + 2%CaGu	0.67	0.64	0.61	0.57	0.52	0.60
1%Chito + 1%CaGu	0.68	0.65	0.62	0.58	0.52	0.61
1%Chito + 2%CaGu	0.69	0.66	0.63	0.59	0.53	0.62
Control	0.61	0.59	0.56	0.52	0.47	0.55
Means (D)	0.65	0.63	0.60	0.55	0.50	
New LSD at 5% (T) =	0.02					
New LSD at 5% (D) =	0.02					
New LSD at 5% (TXD) =	0.04					
Date (D)	**2022, season**					
	Storage period (week)					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	0.61	0.59	0.56	0.52	0.46	0.55
1%Chito	0.63	0.60	0.57	0.54	0.48	0.56
1%CaGu	0.61	0.59	0.56	0.52	0.46	0.55
2%CaGu	0.62	0.60	0.57	0.53	0.47	0.56
0.5%Chito + 1%CaGu	0.63	0.60	0.57	0.54	0.48	0.56
0.5%Chito + 2%CaGu	0.64	0.61	0.58	0.55	0.48	0.57
1%Chito + 1%CaGu	0.64	0.61	0.58	0.55	0.48	0.57
1%Chito + 2%CaGu	0.65	0.62	0.59	0.56	0.49	0.58
Control	0.59	0.57	0.54	0.51	0.45	0.53
Means (D)	0.62	0.60	0.57	0.54	0.47	
New LSD at 5% (T) =	0.01					
New LSD at 5% (D) =	0.01					
New LSD at 5% (TXD) =	0.02					

Total anthocyanin

Data presented in **Table (11)** show that the total anthocyanin gradually increased with the prolongation of the cold storage duration for all treatments in both seasons. It can be seen that the pre-harvest application of chitosan or calcium gluconate at different concentrations either alone or in combined form significantly decreased the increase in total anthocyanin as compared to the control with storage period advanced. The lowest value of the total anthocyanin was acquired by chitosan at 1% and calcium gluconate at 2%, while the highest value of this one was significantly attained by control during cold storage.

The retardation of color development in fruits coated with chitosan and Ca can be assigned to slow respiration rate and decreased ethylene production, which controlled the increase of anthocyanin content (Suntharalingam, 1996 and Varasteh et al., 2012).

The outcomes obtained are similarly comparable to those attained by Abdel Wahab et al. (2014) on Crimson Seedless grape, Abdel Gayed et al. (2017) on Early Swelling peach and Fekry (2018) on guava, they found that chitosan and calcium application retard colour development during cold storage.

Table (11). Effect of chitosan (Chito) and calcium gluconate (CaGu) on total anthocyanin (mg/100g F.W) of Crimson Seedless grapes during cold storage in 2021 and 2022 seasons

Date (D)	2021, season					
	Storage period (week)					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	33.29	34.29	36.00	37.98	41.14	36.54
1%Chito	32.65	33.63	35.31	37.25	40.35	35.84
1%CaGu	33.74	34.75	36.49	38.50	41.69	37.03
2%CaGu	32.87	33.86	35.55	37.50	40.62	36.08
0.5%Chito + 1%CaGu	32.38	33.35	35.02	36.95	40.01	35.54
0.5%Chito + 2%CaGu	32.07	33.03	34.68	36.59	39.63	35.20
1%Chito + 1%CaGu	31.82	32.77	34.41	36.31	39.32	34.93
1%Chito + 2%CaGu	31.73	32.68	34.32	36.20	39.21	34.83
Control	34.17	35.20	36.95	38.99	42.22	37.51
Means (D)	32.75	33.73	35.42	37.36	40.46	
New LSD at 5% (T) =	1.19					
New LSD at 5% (D) =	0.89					
New LSD at 5% (TXD) =	2.67					
Date (D)	**2022, season**					
	Storage period (week)					
Treatment (T)	**0**	**1**	**2**	**3**	**4**	**Means (T)**
0.5%Chito	34.13	35.15	36.91	38.94	42.17	37.46
1%Chito	33.58	34.59	36.32	38.31	41.49	36.86
1%CaGu	34.67	35.71	37.50	39.56	42.84	38.05
2%CaGu	33.79	34.80	36.54	38.55	41.75	37.09
0.5%Chito + 1%CaGu	33.16	34.15	35.86	37.83	40.98	36.40
0.5%Chito + 2%CaGu	32.97	33.96	35.66	37.62	40.74	36.19
1%Chito + 1%CaGu	32.58	33.56	35.24	37.17	40.26	35.76
1%Chito + 2%CaGu	32.41	33.38	35.05	36.98	40.05	35.57
Control	35.13	36.18	37.99	40.08	43.41	38.56
Means (D)	33.60	34.61	36.34	38.34	41.52	
New LSD at 5% (T) =	1.03					
New LSD at 5% (D) =	0.77					
New LSD at 5% (TXD) =	2.31					

Conclusion

From the above findings, it can be inferred that the growth, yield, fruit quality attributes and storability of Crimson Seedless grape cultivar can be easily enhanced by the combined pre-harvest application of 1% chitosan plus 2% calcium gluconate in acquiring the best aspects of vegetative growth, increasing yield and improving cluster quality attributes as well as enhancing the storability of Crimson Seedless grape via decreasing the physiological loss of weight, decay and shattering, maintaining firmness and delaying the changes in TSS, acidity and total anthocyanin as compared to control during extended cold storage.

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الملخص العربى

تأثير الشيتوزان وجلوكونات الكالسيوم على جودة الثمار والقدرة التخزينية لصنف العنب الكريمسون سيدلس

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أجريت هذا البحث خلال موسمين متتاليين (2021 و 2022) بمزرعة البرامون بالمنصورة التابعة لمحافظة الدقهلية، مصر وذلك لدراسة تأثير الشيتوزان وجلوكونات الكالسيوم على النمو والمحصول وجودة الثمار وكذلك القدرة التخزينية لصنف العنب الكريمسون سيدلس. تمَّت زراعة الكرمات البالغة من العمر سبع سنوات على مسافة 2 × 3 أمتار في تربة طينية مع نظام الري بالغمر ومرباة باستخدام التدعيم بالتكاعيب الأسبانية. في الأسبوع الأخير من شهر فبراير، تمَّ تقليم الكرمات تقليما قصبيًا مع حمولة 96 برعم/كرمة، واشتملت الدراسة على تسع معاملات على النحو التالي: الرش الورقى بالشيتوزان عند تركيزى (0.5 أو 1%) وجلوكونات الكالسيوم عند تركيزى (1 أو 2%) تمَّ إجرائها إما بشكل منفرد أو بالاشتراك مع بعضهما البعض، بالإضافة إلى معاملة الكونترول. تمَّ إجراء جميع المعاملات فى ميعادين مختلفين وهما: بعد مرحلة العقد عندما يصل قطر الحبات إلى 2-3 ملم وفي مرحلة بداية التلوين عندما تصل نسبة التلوين إلى 20%. تمَّ تخزين العناقيد لجميع المعاملات لمدة أربعة أسابيع عند درجة الصفر المئوى ورطوبة نسبية عن 90-95٪. أظهرت الدراسة أن الإضافة المشتركة لكل من الشيتوزان عند تركيز 1% وجلوكونات الكالسيوم عند تركيز 2% أعطت أفضل النتائج من حيث الحصول على أفضل صفات للنمو الخضري وزيادة المحصول وتحسين خصائص الجودة للثمار وكذلك القدرة التخزينية لصنف العنب الكريمسون سيدلس عن طريق تقليل الفقد الفسيولوجي في الوزن، ونسبة التالف والفرط وكذلك الحفاظ على درجة الصلابة للحبات وتأخير التغيرات في المواد الصلبة الذائبة الكلية، الحموضة وصبغة الأنثوسيانين.

الكلمات الدالة: العنب، الكريمسون ، الشيتوزان، الكالسيوم ، القدرة التخزينية