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Study the Effect of Spraying Pre-Harvest Treatments by Chitosan and Some Compounds as Normal and Nano Forms on Wonderful Pomegranate Fruits Storability

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Abstract

This research was carried out on a wonderful pomegranate cultivar at The National Research Centre, Researches and Production Station, at El-Nobaria district, El-Behaira Governorate, Egypt. Also, the post-harvest treatments were carried out in the laboratory at the Agricultural Development Systems (ADS) project, Faculty of Agriculture, Cairo University at (7°C – RH 90%) during two successive seasons (2020-2021 and 2021-2022). Wonderful cultivar pomegranate fruits in pre-harvest received seven applications of Chitosan 3g/L, Nano Chitosan 1.5 g/L, $CaCl_2$ 2g/L, Nano $CaCl_2$ 1g/L, K_2CO_3 1g/L, Nano K_2CO_3 0.5g/L and control. The results proved the applications of pre-harvest study on Wonderful cultivar pomegranate fruits recorded that using 1g/L Nano $CaCl_2$ showed the highest average of the chemical and physical characteristics of the fruits in two seasons under cold storage conditions (7°c and RH 90%). On the other hand, the data on the interaction between the treatments and the storage periods was recorded as the minimum average results of POD enzyme when using Nano $CaCl_2$ 1g/L after four weeks under cold storage in both seasons under study.

Keywords: wonderful, pomegranate, pre-harvest, enzyme

Introduction

The pomegranate (*Punica granatum*) is a fruit-bearing deciduous and rich in symbolic and mythological associations in many cultures and also a non-climacteric fruit, it should be harvested fully ripe to ensure optimal qualitative attributes. In addition, the abundant content of water in the fruit and numerous natural pores on the peel are subject to weight loss **(Kader *et al.*, 1984)**. The cultivated area reached (80515) feddan, with a productivity of (672064) ton **(Economic Affairs Sector, 2020)**. Chitosan, a high molecular-weight cationic polysaccharide produced by the deacetylation of chitin, is applied widely in postharvest because of its film-forming and biochemical properties **(Lin *et al.*, 2008; Jianglian and Shaoying, 2013; Shiekh *et al.*, 2013).** Nano chitosan has broad antimicrobial activity against fungal pathogens , but the bulk size limits its solubility which affects its antimicrobial property, have also raised concerns about adverse effects on environmental health, investigated undertaken to study the effect of nano chitosan and nano micronutrients on fruit drop, yield and quality **(Mishra *et al.*, 2023)** Calcium plays an important role in plant life cycle as it influence intake of nitrogen and boron, promotes early roots formation and growth, increase calcium content of food and feed crops. Also, promoted the early solidification and hardening of concrete **(Bai *et al.*, 2022 and Pandya *et al.*, 2023)**. K is involved in numerous biochemical and physiological processes vital to plant growth, yield, quality and stress **(Lester *et al.*, 2010)**.

The present investigation was undertaken to study the effect of spraying (Chitosan, Nano Chitosan, $CaCl_2$, Nano $CaCl_2$, K_2CO_3, Nano K_2CO_3) as pre-harvest treatments on fruit quality of Wonderful pomegranate under cold storage conditions.

Materials and Methods

The experimental study was conducted at The National Research Centre, Researches and Production Station, at El-Nobaria district, El-Behaira Governorate, Egypt. during both 2020-2021 and 2021- 2022 seasons on pomegranate fruits CV. wonderful. Fruits of pomegranate were selected carefully and harvested at the same maturity stage. The pomegranate fruits were used in this investigation to study and evaluate some

chemical properties therefore, the main objective of this experiment was to study the effect of some pre-harvest treatments with some chemical substances (compounds) on fruit quality under cold storage conditions (7°c and RH 90%). In this regard, the different seven pre-harvest treatments were concluded as follows: -

1- Chitosan application at 3.0 g/L.
2- Nano chitosan application at 1.5 g/L.
3- $CaCl_2$ application at 2.0 g/L.
4- Nano $CaCl_2$ application at 1.0 g/L.
5- K_2Co_3 application at 1.0 g/L.
6- Nano K_2Co_3 application at 0.5 g/L.
7- Tap water as (control).

Moreover, every treatment was replicated three times while each replicate was represented by (15) fruits whereas the experiment was arranged in a completely randomized design.

-Fruit quality assessments

a. **Total soluble solids (TSS° Brix)**: of the wonderful pomegranate were determined using a digital refractometer **(Model PR-32, Atago, Japan)** by squeezing the juice.

b. **Total Sugars %**: In ethanol extract, total sugars were determined by using the phenol-sulphuric acids methods **(Dubois *et al.*, 1956)** as follows: One ml of ethanol sugars extracted was mixed with phenol (0.5ml 5%) in a test tube and immediately followed by the addition of 5 ml of concentrated sulfuric acid then the mixture was shaken gently and left to cool. The blank contained all the reagents without fruit extract which was replaced with 1 ml 80% ethanol. The absorbance of the developed yellow-orange color was measured at 490 nm using a spectrophotometer. A standard curve was carried out using pure glucose with a suitable Figs concentration. The number of total sugars was calculated and expressed as a percentage.

c. **Total tetra table acidity (TA) %** was determined by titration with a standard solution of sodium hydroxide (0.1N), using phenolphthalein as an indicator **(A.O.A.C., 2010).** The results were expressed as percentages of anhydrous tartaric acid according to the following equation.

$$Total\ acidity = \frac{\text{M1 of NaOH x 0.0075}}{\text{M1 juice used}} \text{ X } 100$$

d. **Juice ratio:** 1-Weigh your 5 fruit and record the combined weight in grams. 2-Weigh the empty 1 or 2-liter jug and record the weight in grams. 3-Juice all 5 fruits using the juicer. 4-Apply even force and try to remove all the juice. 5-Strain the juice into the jug. 6-Weigh the juice and record the weight in grams, then subtract the weight of the jug. 7- Calculate the percentage juice content by dividing the juice weight by the total fruit weight. 8-Multiply this by 100 to get the percentage.

(Juice weight ÷ Fruit weight) x 100 = percentage (%) juice

e. **Vitamin C (L Ascorbic Acid): mg/100 mL juice**: Vitamin C content was measured by the colorimetric method described in **A.O.A.C (2010)** based on the reduction of 2, 6- di chlorophenol indophenol-sodium (DCIP), standardized with ascorbic acid. The fruit ascorbic acid extracts were titrated with DCIP solution until a light rose pink hue persisted for 30 seconds. The amount of DCIP solution used in the titration stage was determined and used to calculate vitamin C (100 mg mL-1 juice) content.

f. **Anthocyanin and Peroxidase enzyme (POD):** The method of Liquid Chromatography–Mass Spectrometry (LC-MS) has been widely used for qualitative analysis of anthocyanins and molecular weight and structure of anthocyanins can be identified which is effective for the identification of anthocyanins. Samples were frozen in a cold aqueous Methanol 80% (v/v). Then it was adjusted to 20 ml/g and stored at 2°C for 48 hours. Hormones were extracted according to (**Wasfy and Orrin, 1975).** The determination of plant hormones and total free amino acids (g/100g FW) as a Lysine was carried out by using High-Performance Liquid Chromatography (HPLC) finally, it was determined according to A.O.A.C. (2010).

Statistical analysis

The analysis of variance (ANOVA) was done for each season separately as a completely randomized design, according to procedures reported by Gomez and Gomez, (1984). The differences between mean values of treatments were compared by the least significant difference (LSD) test at 0.05 level of probability. Data were analyzed using (ANOVA) in the MSTAT-C software package **(Freed *et al.*, 1989)**.

Results and Discussion

a. **Effect of spraying pre-harvest treatments on TSS % of Wonderful pomegranate fruits under cold storage conditions**

Data in **Table (1)** showed that TSS content gave the maximum data with 1.0 g/l Nano $CaCl_2$ and the minimum data with control treatment in both seasons. Storage periods (Weeks) in **Table (1)** showed that The maximum TSS% was recorded after four weeks in this study and followed by a decrease after five and six weeks in both seasons. Data of interactions between treatments and storage periods (Weeks) has recorded that TSS content was not significantly affected by all treatments with the same Nano treatments but, Nano K_2CO_3 0.5g/L, Nano $CaCl_2$ 1g/L and Nano chitosan 1.5 g/l gave the maximum TSS content in the first season. However, data in the second season showed that the same maximum TSS content in fruits was recorded in Nano K_2CO_3 0.5g/L, K_2CO_3 1.0/L, $CaCl_2$ 2g/L, and Nano chitosan 1.5 g/l compared to the control and other treatments under the study. **Assar and Taghipour (2022)** showed that significant differences were detected only with chitosan application. Increasing TSS% with spraying with chitosan only or in combination with other treatments (**Meng *et al.*, 2008 and Ghasemnezhad *et al.*, 2013).** On the other hand**, Khalil and Aly (2013)** spraying treatment on pomegranate in TSS% decreased with 3% Ca, 0.3% B and 0.3% Zn. Carbohydrates, organic acids, and their rapid translocation to the fruits have led to the accumulation of more sugars and other soluble solids content in the fruits. Also, reported that the increase in the level of total soluble solids and sugar content in the fruit will help to lower the fruit acidity **(Aziz *et al.*, 2017; Meena *et al.*, 2018; Yadav *et al.*, 2018; Harhash *et al.*, 2019; and Ibtesam *et al.*, 2019).**

Table 1. The effect of spraying pre-harvest treatments on TSS % of Wonderful pomegranate fruits under cold storage conditions during (2020-2021 / 2021-2022) seasons.

Treatments	Storage periods (Weeks)							Means
	TSS % (2020-2021)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	14.65	14.75	15.40	16.10	16.15	15.95	15.00	**15.43**
Nano Chitosan 1.5 g/L	15.60	15.90	16.48	16.52	17.45	14.75	14.67	**15.91**
$CaCl_2$ 2g/L	15.95	16.10	16.15	16.70	16.70	16.45	13.90	**15.99**
Nano $CaCl_2$ 1g/L	15.30	15.75	16.15	16.85	17.05	16.85	14.70	**16.09**
K_2CO_3 1g/L	15.55	15.80	16.35	16.62	16.90	16.15	14.15	**15.93**
Nano K_2CO_3 0.5g/L	15.50	15.85	16.85	16.90	17.25	16.45	12.75	**15.94**
Control	14.45	14.70	15.90	16.42	16.25	16.20	13.73	**15.38**
Means	**15.29**	**15.55**	**16.18**	**16.58**	**16.82**	**16.11**	**14.13**	**------**
LSD 0.05	**Treatments (A)**=0.228			**Storage periods (Weeks) B** =0.220			**A×B**= 0.539	
Treatments	**Storage periods (Weeks)**							**Means**
	TSS % (2021-2022)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	14.60	14.97	15.00	15.77	15.97	15.40	15.10	**15.26**
Nano Chitosan 1.5 g/L	15.20	15.25	15.60	15.75	16.88	15.45	14.20	**15.48**
$CaCl_2$ 2g/L	15.97	16.37	16.47	16.50	16.90	15.38	14.53	**16.02**
Nano $CaCl_2$ 1g/L	15.83	15.67	16.00	16.37	16.63	16.60	15.20	**16.04**
K_2CO_3 1g/L	15.37	15.89	16.27	16.30	16.90	15.95	14.30	**15.85**
Nano K_2CO_3 0.5g/L	15.67	15.90	16.23	16.28	16.90	16.15	14.00	**15.88**
Control	14.17	14.57	14.75	15.07	16.30	16.10	13.55	**14.93**
Means	**15.26**	**15.52**	**15.76**	**16.00**	**16.64**	**15.86**	**14.41**	**------**
LSD 0.05	**Treatments (A)**= 0.381			**Storage periods (Weeks) (B)**=0.419			**A×B**= 1.028	

b. Effect of spraying pre-harvest treatments on total sugar of Wonderful pomegranate fruits under cold storage conditions

Regarding the total sugar data in **Table (2)** indicate that both treatments of Nano K_2CO_3 0.5g/L and K_2CO_3 1.0g/L were exhibited the highest average of total sugar in wonderful pomegranate fruits under pre-harvest stage under study in both seasons as compared with the control and other treatments. As for, the storage periods (Weeks) were significantly affected by increased time, the great average of total sugar recorded after four weeks under study as compared to the start and the end stages during both seasons of study. Data of total sugar interactions with treatments and the storage periods (Weeks) showed that the highest values of total sugar recorded with Nano K_2CO_3 0.5g/L after four weeks in both seasons respectively followed in descending order by K_2CO_3 1g/L, Nano $CaCl_2$ 1g/L, Nano Chitosan 1.5 g/L, $CaCl_2$ 2g/L, Chitosan 3 g/L and control, respactivly. **Al-Saif *et al.* (2023)** found that sugars percentages were

increased by the spraying of potassium nitrate at 3 and 2%. Also, **Mohamed *et al.* (2023)** found that the best-influencing treatment on the reducing sugars % was KNO_3 at 500 ppm which gave an average of 14.51% with an increment of 49.1% over the control. In another site, **Khalil and Aly (2013)** recorded that increase in total sugar content with 3% Ca, 0.3% B and 0.3% Zn.

c. Effect of spraying pre-harvest treatments on acidity %of Wonderful pomegranate fruits under cold storage conditions

Acidity percentage in **Table (3)** recorded that, acidity percentage was significantly affected by all treatments, storage periods (Weeks), and the interaction between them in both seasons. Also, the treatments in Table 3 showed that acidity % decreased with 0.5 g/l of Nano K_2Co_3 treatment than other treatments and control in the first season. However, in the second one, the treatment of Nano $CaCl_2$ 1.0 g/l recorded the lowest percentage of acidity compared to the other treatments used. Data of storage periods (Weeks) in Table 3 showed that acidity gave the lowest percentage after four weeks under study in both seasons. On the other hand, a gradual increased in acidity% start and end of this study in both seasons. Data in interactions between treatments and storage periods (Weeks) relative that acidity percentage gave the lowest results with 0.5 g/l Nano K_2Co_3 after four weeks in the first season but, in the second season, the lowest acidity % recorded in Nano $CaCl_2$ 1.0 g/l treatment in the same storage periods (Weeks).

Data harmony in the study with (**Al-Saif *et al.*, 2023** and **Mohamed *et al.*, 2023)** found that fruit acidity was lowered by the spraying of potassium nitrate at 2 and 3% (0.35 and 0.3; 0.37 and 0.34%), which gave the most positive influence as compared to the other treatments in both seasons and KNO_3 at 500 ppm treatment gave the lowest acidity percentage (0.93%). On the other hand, **Khalil and Aly, 2013; Davarpanah *et al.*, 2018 and Abd El-wahed *et al.*, 2021)** recorded that spraying calcium chloride in the pre-harvest stage gave the lowest percentage of acidity in pomegranate fruits.

Table (2): The effect of spraying pre-harvest treatments on total sugar content of Wonderful pomegranate fruits under cold storage conditions during (2020-2021 / 2021-2022) seasons.

Treatments	Storage periods (Weeks)							Means
	Total sugar content (2020-2021)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	10.72	10.87	10.92	11.53	12.02	11.22	9.580	**10.98**
Nano Chitosan 1.5 g/L	10.52	10.69	11.33	12.78	13.88	13.08	11.65	**11.99**
$CaCl_2$ 2g/L	11.32	11.47	12.13	12.25	13.35	12.55	10.87	**11.99**
Nano $CaCl_2$ 1g/L	11.19	11.34	13.48	14.74	13.94	11.87	**8.333**	**12.13**
K_2CO_3 1g/L	11.74	11.89	12.31	13.22	14.32	13.52	11.92	**12.70**
Nano K_2CO_3 0.5g/L	12.02	12.17	12.83	13.88	14.98	14.18	10.89	**12.99**
Control	10.08	10.23	10.89	11.30	10.50	10.20	9.030	**10.32**
Means	**10.214**	**9.18**	**8.468**	**7.675**	**7.297**	**7.693**	**8.977**	------
LSD 0.05	**Treatments (A)**=0.651 **Storage periods (Weeks)(B)**= 0.657 **A×B**= 1.611							
Treatments	**Storage periods (Weeks)**							**Means**
	Total sugar content (2021-2022)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	10.68	10.83	11.49	11.98	11.18	10.88	9.657	**10.96**
Nano Chitosan 1.5 g/L	10.48	10.63	11.29	12.74	13.51	13.04	11.73	**11.92**
$CaCl_2$ 2g/L	11.28	11.43	12.09	12.21	13.31	12.51	10.95	**11.97**
Nano $CaCl_2$ 1g/L	11.15	11.30	11.96	13.60	14.70	13.90	11.95	**12.65**
K_2CO_3 1g/L	11.70	11.85	12.51	13.18	14.28	13.48	12.00	**12.72**
Nano K_2CO_3 0.5g/L	11.98	12.13	12.79	13.84	14.94	14.14	10.97	**12.97**
Control	10.24	10.39	11.05	11.26	10.48	10.16	9.107	**10.39**
Means	**10.67**	**9.946**	**9.125**	**7.814**	**6.984**	**7.392**	**8.038**	------
LSD 0.05	**Treatments (A)**= 0.061 **Storage periods (Weeks) (B)**= 0.066 **A×B**= 0.162							

Table (3): The effect of spraying pre-harvest treatments on acidity % of Wonderful pomegranate fruits under cold storage conditions during (2020-2021 / 2021-2022) seasons.

Treatments	Storage periods (Weeks)							Means
	Acidity % (2020-2021)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	1.250	1.567	1.200	0.950	0.900	1.050	1.250	**1.167**
Nano Chitosan 1.5 g/L	1.200	1.700	1.150	1.050	1.050	1.000	0.950	**1.157**
$CaCl_2$ 2g/L	1.000	1.400	1.250	1.100	1.000	0.950	1.200	**1.129**
Nano $CaCl_2$ 1g/L	1.050	1.050	1.100	1.000	0.900	1.000	1.050	**1.021**
K_2CO_3 1g/L	0.850	0.900	0.950	0.800	0.800	0.850	1.050	**0.885**
Nano K_2CO_3 0.5g/L	1.300	1.000	0.950	0.650	0.450	0.500	0.750	**0.800**
Control	1.067	1.400	1.587	1.150	1.300	1.450	1.550	**1.358**
Means	**1.102**	**1.288**	**1.169**	**0.957**	**0.914**	**0.971**	**1.114**	------
LSD 0.05	**Treatments (A)**=0.175		**Storage periods (Weeks) (B)**= 0.102		**A×B**= 0.252			
Treatments	**Storage periods (Weeks)**							**Means**
	Acidity % (2021-2022)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	1.150	1.583	1.200	0.983	0.900	0.916	1.073	**1.115**
Nano Chitosan 1.5 g/L	1.500	1.287	1.100	0.716	1.000	1.067	1.183	**1.108**
$CaCl_2$ 2g/L	0.966	1.000	0.990	0.783	0.550	0.636	0.840	**0.823**
Nano $CaCl_2$ 1g/L	1.117	1.000	0.950	0.650	0.450	0.500	0.750	**0.774**
K_2CO_3 1g/L	1.000	1.083	1.100	1.000	0.866	1.200	1.283	**1.090**
Nano K_2CO_3 0.5g/L	0.750	0.883	1.183	1.083	0.816	0.816	1.000	**0.933**
Control	1.150	1.400	1.583	1.300	1.183	1.250	1.283	**1.307**
Means	**1.090**	**1.177**	**1.158**	**0.931**	**0.824**	**0.912**	**1.058**	------
LSD 0.05	**Treatments (A)**= 0.057		**Storage periods (Weeks)(B)**= 0.063		**A×B**= 0.154			

d. Effect of spraying pre-harvest treatments on juice ratio of Wonderful pomegranate fruits under cold storage conditions

Data of treatments in **Table (4)** recorded the highest juice ratio with Nano $CaCl_2$ 1g/L (52.78 and 54.06, during the 1st and 2nd seasons, respectively) as follows decrease $CaCl_2$ 2g/L, Nano K_2CO_3 0.5g/L, K_2CO_3 1g/L, Nano Chitosan 1.5 g/L, Chitosan 3g/L, while control was recorded as the lowest data of juice ratio in both seasons. In addition, storage periods (weeks) in **Table (4)** showed that the highest results of juice ratio recorded after four weeks (51.27 and 55.15, respectively) under study than other storage periods (weeks) were used in both seasons. Data of interactions between treatments with storage periods in Table 4 showed that the maximum average of juice ratio recorded with $CaCl_2$ 2.0g/L after four weeks in the study compared to other interactions of treatments and storage periods (weeks) in both seasons.

Khalil and Aly (2013) used some sprays of growth regulators and mineral nutrients. The results showed that the lowest fruit juice percentage from trees sprayed with $CaCl_2$ at 3% in both seasons. **Kamel *et al.* (2016)** They reported that the storage duration effect on the arils juice (%) of the juice content of Wonderful arils decreased at cold storage 5°C in comparison with fruits that at harvest time. **Al-Saif et al. (2023)** reported that fruit juice content was statistically increased by the spraying of potassium nitrate at 2 and 3% and calcium nitrate at 4%. On the other hand, **Assar and Taghipour (2022)** showed that juice had a significantly slower rate for treated arils than control.

Table (4): The effect of spraying pre-harvest treatments on juice ratio of Wonderful pomegranate fruits under cold storage conditions during (2020-2021 / 2021-2022) seasons.

The effect of spraying application treatments of Wonderful pomegranate fruits.

Treatments	Storage periods (Weeks)							Means
	juice ratio (2020-2021)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	41.72	45.33	50.38	51.04	52.28	37.61	31.87	**44.32**
Nano Chitosan 1.5 g/L	40.09	46.41	47.02	52.39	52.61	39.69	33.67	**44.55**
$CaCl_2$ 2g/L	43.52	44.62	47.95	49.61	60.59	46.79	40.72	**47.68**
Nano $CaCl_2$ 1g/L	45.23	51.61	54.44	57.42	50.98	60.10	49.65	**52.78**
K_2CO_3 1g/L	44.01	42.59	42.86	47.62	56.47	44.01	36.74	**44.90**
Nano K_2CO_3 0.5g/L	42.79	45.79	51.61	51.72	55.29	40.51	36.10	**46.26**
Control	44.94	63.80	33.86	33.58	30.70	30.59	25.08	**37.51**
Means	**43.19**	**48.59**	**46.87**	**49.05**	**51.27**	**42.76**	**36.26**	------
LSD 0.05	**Treatments (A)**=3.228 **Storage periods (Weeks) (B)**=3.508 **A×B=** 8.593							
Treatments	**Storage periods (Weeks)**							**Means**
	juice ratio (2021-2022)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	47.27	48.71	49.41	54.47	55.76	37.55	35.36	**46.93**
Nano Chitosan 1.5 g/L	41.53	51.01	54.28	56.00	57.30	45.26	32.74	**48.30**
$CaCl_2$ 2g/L	50.37	50.99	54.70	55.39	65.69	49.79	49.24	**53.74**
Nano $CaCl_2$ 1g/L	51.23	53.85	54.23	54.82	57.29	60.46	46.57	**54.06**
K_2CO_3 1g/L	43.99	49.74	56.14	56.79	60.54	47.11	37.78	**50.30**
Nano K_2CO_3 0.5g/L	50.65	51.86	55.86	56.33	58.05	42.86	38.57	**50.60**
Control	56.11	62.45	45.80	45.33	34.70	33.01	31.41	**44.11**
Means	**48.73**	**52.66**	**52.92**	**54.16**	**55.62**	**45.15**	**38.81**	------
LSD 0.05	**Treatments (A)**= 3.246 **Storage periods (Weeks) (B)**=4.051 **A×B=** 8.323							

e. Effect of spraying pre-harvest treatments on vitamin C content of Wonderful pomegranate fruits under cold storage conditions

Treatments in **Table (5)** showed that Vitamin C content gave the maximum data of Vitamin C content with 1.0 g/l Nano $CaCl_2$ and the minimum Vitamin C content with control treatment in both seasons. Storage periods (Weeks) in **Table (5)** showed that the maximum Vitamin C content was recorded after four weeks in the study and followed by a decrease at other weeks in both seasons under study. Data of interactions between treatments and storage periods (Weeks) has recorded that the maximum average of vitamin C content recorded with Nano $CaCl_2$ 1g/L after four weeks in the study compared to other interactions of treatments and storage periods (Weeks) in both seasons.

Table 5. The effect of spraying pre-harvest treatments on vitamin C content of Wonderful pomegranate fruits under cold storage conditions during (2020-2021 / 2021-2022) seasons.

Treatments	Storage periods (Weeks)							Means
	Vitamin C content (2020-2021)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	11.66	12.36	12.66	13.26	13.66	12.79	12.49	**12.70**
Nano Chitosan1.5 g/L	12.48	13.18	13.48	14.08	14.48	13.26	12.96	**13.42**
$CaCl_2$ 2g/L	14.51	15.18	15.48	16.08	14.72	14.42	11.48	**14.55**
Nano $CaCl_2$ 1g/L	14.02	14.72	15.02	15.62	16.02	15.46	15.16	**15.14**
K_2CO_3 1g/L	12.87	13.63	13.87	14.47	14.87	13.96	13.66	**13.91**
Nano K_2CO_3 0.5g/L	13.34	14.03	14.34	14.94	15.34	13.77	13.47	**14.17**
Control	12.60	12.97	13.14	12.68	12.43	12.16	10.96	**12.42**
Means	**13.06**	**13.72**	**14.00**	**14.45**	**14.50**	**13.69**	**12.88**	------
LSD 0.05	**Treatments (A)**=0.921 **Storage periods (Weeks) (B)**=0.887 **A×B=** 2.174							
Treatments	**Storage periods (Weeks)**							**Means**
	Vitamin C content (2021-2022)							
	zero	1st	2nd	3rd	4th	5th	6th	

Chitosan 3g/L	11.96	12.96	12.99	13.56	13.96	13.09	12.79	**13.05**
Nano Chitosan 1.5 g/L	12.78	13.48	13.78	14.41	14.78	13.56	13.23	**13.72**
$CaCl_2$ 2g/L	14.32	15.01	15.32	15.92	15.99	15.75	15.46	**15.39**
Nano $CaCl_2$ 1g/L	14.78	15.48	15.78	16.38	16.78	15.02	14.73	**15.57**
K_2CO_3 1g/L	13.17	13.87	14.17	14.77	14.84	14.26	13.96	**14.15**
Nano K_2CO_3 0.5g/L	13.97	14.44	14.84	15.37	15.11	13.97	13.70	**14.48**
Control	13.70	13.82	13.99	11.95	11.78	11.57	11.33	**12.59**
Means	**13.53**	**14.15**	**14.41**	**14.62**	**14.75**	**13.89**	**13.60**	------
LSD 0.05	**Treatments (A)**= 0.190			**Storage periods (Weeks) (B)**= 0.158		**A×B**= 0.388		

e. Effect of spraying pre-harvest treatments on anthocyanin content of Wonderful pomegranate fruits under cold storage conditions

Table (6) showed that the anthocyanin content was significantly affected by all treatments, storage periods (Weeks) and the interaction between them in both seasons. Also, Data of treatments recorded the highest anthocyanin content with Nano $CaCl_2$ 1g/L as follows decrease $CaCl_2$ 2g/L, Nano K_2CO_3 0.5g/L, K_2CO_3 1g/L, Nano Chitosan 1.5 g/L, Chitosan 3g/L, and control were recorded as the lowest data of anthocyanin content in both seasons. In addition, storage periods (Weeks) in **Table (6)** showed that the highest results of anthocyanin content recorded after four weeks under study than other storage periods were used in both seasons. Data of interactions between treatments with storage periods (Weeks) in **Table (6)** showed that the maximum average of anthocyanin content recorded with Nano $CaCl_2$ 1g/L after four weeks in the study compared to other interactions of treatments and storage periods (Weeks) in both seasons.

Table 6. The effect of spraying pre-harvest treatments on anthocyanin content of Wonderful pomegranate fruits under cold storage conditions during (2020-2021 / 2021-2022) seasons.

Treatments	**Storage periods (Weeks)**							**Means**
	Anthocyanin content (2020-2021)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	13.70	13.84	14.01	11.91	11.74	11.49	11.38	**12.58**
Nano Chitosan 1.5 g/L	13.66	13.78	13.95	13.77	13.53	13.41	12.63	**13.53**
$CaCl_2$ 2g/L	13.52	13.54	13.71	15.23	15.59	15.33	14.68	**14.51**
Nano $CaCl_2$ 1g/L	13.60	13.72	13.89	15.98	16.44	15.80	14.65	**14.87**
K_2CO_3 1g/L	13.77	13.93	14.10	14.63	14.66	14.01	9.96	**13.58**
Nano K_2CO_3 0.5g/L	13.75	13.87	14.04	14.33	14.78	14.57	14.24	**14.23**
Control	12.60	12.97	13.14	12.68	12.43	12.16	10.96	**12.42**
Means	**13.51**	**13.66**	**13.83**	**14.08**	**14.17**	**13.82**	**12.64**	------
LSD 0.05	**Treatments (A)**=0.831			**Storage periods (Weeks) (B)**=0.779			**A×B**= 1.909	
Treatments	**Storage periods (Weeks)**							**Means**
	Anthocyanin content (2021-2022)							
	zero	1st	2nd	3rd	4th	5th	6th	
Chitosan 3g/L	13.37	13.49	13.66	12.56	12.36	12.11	11.31	**12.70**
Nano Chitosan 1.5 g/L	13.67	13.79	13.98	13.83	13.67	13.37	12.50	**13.54**
$CaCl_2$ 2g/L	13.56	13.68	13.85	15.18	15.63	15.43	14.60	**14.56**
Nano $CaCl_2$ 1g/L	13.57	13.63	13.80	16.02	16.39	16.14	14.88	**14.92**
K_2CO_3 1g/L	13.71	13.86	14.03	14.25	14.69	14.50	13.95	**14.14**
Nano K_2CO_3 0.5g/L	13.77	13.95	14.12	14.38	14.74	14.49	14.25	**14.24**
Control	13.70	13.82	13.99	11.95	11.78	11.57	11.33	**12.59**
Means	**13.62**	**13.75**	**13.92**	**14.02**	**14.18**	**13.94**	**13.26**	------
LSD 0.05	**Treatments (A)**= 0.086			**Storage periods (Weeks) (B)**=0.093			**A×B**= 0.230	

f. Effect of spraying pre-harvest treatments on peroxidase enzyme (POD) of Wonderful pomegranate fruits under cold storage conditions

Data in **Table (7)** confirmed that the Peroxidase enzyme was significantly affected by all treatments, storage periods (Weeks) and the interaction between them in both seasons. The highest POD of fruits was recorded with control treatment, followed in descending order by Chitosan 3 g/L, Nano Chitosan 1.5 g/L, K_2CO_3 1g/L, Nano K_2CO_3 0.5g/L, $CaCl_2$ 2g/L and 1g/L Nano $CaCl_2$ in both seasons under the study of the pre-harvest stage. The storage period (Weeks) treatments showed the highest peroxidase enzyme after six weeks compared to the results at start the study in both seasons. Data on the interaction between the treatments and the storage periods (Weeks) was recorded as the minimum average results of POD when using 1g/L Nano $CaCl_2$ after four weeks in both seasons under study.

Table (7) The effect of spraying pre-harvest treatments on Peroxidase enzyme (POD) of Wonderful pomegranate fruits under cold storage conditions during (2020-2021 / 2021-2022) seasons.

treatments	Storage periods (Weeks)				Means
	Peroxidase enzyme (POD) (2020-2021)				
	Zero	2nd	4th	6th	
Chitosan 3g/L	11.81	11.51	11.00	15.16	**12.37**
Nano Chitosan 1.5 g/L	11.28	11.15	10.85	12.50	**11.45**
$CaCl_2$ 2g/L	10.76	10.52	10.22	10.67	**10.54**
Nano $CaCl_2$ 1g/L	11.19	9.56	9.26	11.38	**10.35**
K_2CO_3 1g/L	11.26	10.59	10.29	12.26	**11.10**
Nano K_2CO_3 0.5g/L	10.82	10.33	10.03	11.16	**10.59**
Control	12.03	11.90	11.60	15.80	**12.83**
Means	**11.31**	**10.79**	**10.46**	**12.70**	----------
LSD 0.05	**Treatments (A)**= 0.099 **Storage periods (Weeks) (B)**= 0.452 **A×B** = 1.108				

treatments	Storage periods (Weeks)				Means
	Peroxidase enzyme (POD) (2021-2022)				
	Zero	2nd	4th	6th	
Chitosan 3g/L	12.13	11.49	11.34	15.26	**12.56**
Nano Chitosan 1.5 g/L	12.07	10.64	10.49	12.95	**11.54**
$CaCl_2$ 2g/L	11.64	9.70	9.55	12.25	**10.79**
Nano $CaCl_2$ 1g/L	12.01	9.58	9.43	11.44	**10.62**
K_2CO_3 1g/L	11.31	11.26	10.84	12.26	**11.42**
Nano K_2CO_3 0.5g/L	11.68	9.90	9.75	12.06	**10.85**
Control	12.27	11.83	11.68	15.88	**12.91**
Means	**11.87**	**10.63**	**10.44**	**13.16**	----------
LSD 0.05	**Treatments (A)**= 0.294 **Storage periods (Weeks) (B)**= 0.254 **A×B** = 0.624				

The results of **Tables (5), (6) and (7)** confirmed with **(Khalil and Aly, 2013; Davarpanah *et al.*, 2018; Abd El-wahed *et al.*, 2021)** they recorded that the maximum anthocyanin content and Vitamin C content when using calcium chloride only or in combination with another mineral in the pre-harvest stage of pomegranate but the Peroxidase enzyme decreased in the same treatments.

Also, the same results were recorded with potassium or chitosan application treatments compared to control in other studies or other cultivars of fruit trees. The probable increase in the anthocyanin content of the fruit might be due to the combined application of nutrients especially potassium will enhance the fruit anthocyanin content which has a positive correlation with the anthocyanin accumulation in the fruit and also plays a crucial role in anthocyanin synthesis by increasing the translocation of sugars to the developing fruits, as well as act as a co-factor and stimulator of enzymes which are involved in the synthesis of anthocyanin and phenol compounds. The foliar application of chitosan will increase the expression of genes involved in the biosynthesis of flavonoid compounds such as flavonol synthase and anthocyanidin synthase which improves the anthocyanin contents in the fruits. According to **Abdel**

Fattah *et al.*, 2016; Khedr, 2021; Assar and Taghipour, 2022; Al-Saif *et al.*, 2023; El-Shereif *et al.*, 2023 and Mohamed *et al.*,2023.

Conclusion

From the aforementioned results and discussions, it could be concluded that the applications of some chemical substances as pre-harvest study on Wonderful cultivar pomegranate fruits recorded that using 1g/L Nano CaCl2 showed the highest average of the chemical and physical characteristics of the fruits in two seasons under cold storage condition (7°c and RH 90%).

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دراسة تأثير الرش بمعاملات ما قبل الحصاد بالشيتوزان وبعض المركبات بصورة عادية ونانو على قابلية تخزين ثمار الرمان وندرفل

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أجريت هذه الدراسة على ثمار أشجار الرمان صنف (وندرفل) بمزرعة المركز القومي للبحوث –محطة البحوث والانتاج – منطقة النوبارية – محافظة البحيرة – مصر. خلال موسمين متتاليين (2020–2021) و (2021–2022). حيث تم الرش كمعاملات قبل الحصاد بعدد سبع معاملات ببعض المركبات الكيماويه وكانت المعاملات كالأتي:–

1- الرش بالشيتوزان 3 جم / لتر.
2- الرش بالنانو شيتوزان 1.5 جم / لتر.
3- الرش بكلوريد كالسيوم 2جم/ لتر.
4- الرش بالنانو كلوريد كالسيوم 1جم/ لتر.
5- الرش بكربونات البوتاسيوم 1جم/ لتر.
6- الرش بنانو كربونات البوتاسيوم 0.5 جم/لتر.
7- معاملة المقارنة (أشجار رشت بالماء فقط).

وتم تخزين الثمار بعد أجراء معاملات ما قبل الحصاد عليها في الثلاجه علي درجة حرارة 7 درجة مئوية ونسبة رطوبة 90 % لمدة ستة اسابيع وقد اشارت النتائج المتحصل عليها خلال الدراسة إلي:

1- بالنسبه لتاثير معاملات الرش ببعض المركبات الكيماوية فقد اوضحت النتائج أن ثمار الرمان صنف (وندرفل) المعامله (نانو كلوريد الكالسيوم) بمعدل 1 جم/ لتر اعطت اعلي متوسط لكل الصفات الكيماويه المختبره تحت الدراسه (النسبه المئويه لكل من المواد الصلبه الذائبه الكليه– الحموضه– محتوي الثمار من السكريات الكليه– محتوي الثمار من حمض الاسكوربيك (V.C.) وكذلك صبغه الانثوسيانين) وايضا بعض الصفات الطبيعيه مثل معدل العصير بالثمار في حين انتجت هذه المعاملات اقل القيم المعنويه من محتوي الثمار من انزيم البيروكسيديز وعلي العكس من ذلك فان معامله المقارنه اعطت اعلي القيم من محتوي الثمار من انزيم البيروكسيديز واقل القيم المعنويه لكل الصفات سواء الطبيعيه او الكيماويه المختبره أثناء التخزين المبرد وكذلك اتفقا في كلا موسمى الدراسه.
2- أثبتت الدراسه فيما يخص تاثير فترات التخزين ان اعلي القيم معنويا لصفات (محتوي الثمار من TSS – السكريات الكليه– معدل العصير بالثمار – محتوي الثمار من الانثوسيانين وحمض الاسكوربيك) كانت مرتبطه بالاسبوع الرابع من االتخزين ثم بدات في الانخفاض التدريجي حتي الاسبوع السادس بينما كان العكس صحيحا مع كل من محتوي الثمار من الحموضه وانزيم البيروكسيديز في كلا الموسمين مع التخزين
3- وفيما يتعلق بتاثير التفاعل للعاملين (تاثير كل من االرش بالمواد الكيماويه وفترات التخزين) فقد اظهرت النتائج ان كل من عاملي التخزين انعكس تاثيرهما علي التفاعل بينهما حيث اعطت معامله التفاعل (نانو كالسيوم1% × الاسبوع الرابع من التخزين اعلي القيم المعنويه لصفات (TSS – السكريات الكليه– فيتامين سي – الانثوسيانين وكذلك معدل العصير بالثمار) بينما اعطت معامله المقارنه اعلي القيم المعنويه لكل من النسبه المئويه للحموضه الكليه ومحتوي الثمار من انزيك البيروكسيديز مقارنه بالمعملات الاخري خلال موسمين الدراسه.