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Enhancement Quality and Storability of "Anna" Apple Fruits by some Pre-Harvest Foliar Applications

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ABSTRACT

This experiment was carried out during two seasons of 2016 and 2017 in order to study the effect of pre-harvest foliar application with Putrescine, ascorbic acid, salicylic acid and citric acid at (200, 400, 600) ppm and control (water only) on fruit quality and storability of 10-year-old "Anna" apple trees budded on MM 106 rootstock, grown in clay soil under drip irrigation system in a private orchard at Kafr El-Dawar region, El-Behira governorate, Egypt. The fruits were stored for 60 days at 2° C and 85–95% R.H. and assessed at harvest time and every 20 days to determine the changes in fruit quality characteristics during cold storage. Results showed that, TSS, total sugars, vitamin C, firmness and anthocyanin contents were significantly increased while, weight loss, physiological and pathological disorders, acidity and chlorophylls a, b significantly decreased by pre-harvest foliar application with Putrescine, ascorbic acid, salicylic acid and citric acid. Moreover, Putrescine at 600 ppm and salicylic acid, ascorbic acid and citric acid at (400 and 600) ppm were more effective on improving fruit quality at harvest time and during cold storage of "Anna" apple in both seasons.

During storage periods, results indicated that, fruit weight loss, physiological disorders, pathological disorders, TSS, total sugars and anthocyanin contents significantly increased while, firmness, acidity, vitamin C and chlorophylls a, b significantly decreased during cold storage. It could be concluded that, preharvest spraying with Putrescine, ascorbic acid, salicylic acid and citric acid led to markedly increase quality and extended the storage periods at (2˚ C with 85-95% R.H.) of "Anna" apple fruits.

Keywords: "Anna" Apple, Putrescine, Salicylic Acid, Citric Acid, Ascorbic Acid, Fruit Quality, Storability.

Introduction

Apples (*Malus domestica* Borkh.) are one of the most popular fruits in many regions and cultures around the world. The demand for a high fruit quality of apple with attract color and longer storability is increasing. The awareness of consumers about the nutritive value of apples, especially the antioxidant content, is driving towards many attempts to enhance anthocyanin formation in apple fruit skins. Even in northern countries, there have been many attempts to use safe applications to accelerate color formation in order to avoid either the rainy season or the frost period. It is important to keep in mind that fruits of apple is a regular part of the daily meal offered to students around the world.

Growers of apple have been facing many production problems that hinder them and represent a main obstacle towards obtaining high quality fruits with the desired coloration and texture. They are dealing with a false fruits as the core tissues differ from the edible part in firmness, respiration rate and ethylene production rate. However, the whole apple fruit is classified as climacteric which makes them very sensitive to applied chemicals, to various abiotic stresses and even to slight motion on the branch they were in touch with other branches. Since apple fruits are autocatalytic, they are very sensitive to ethylene whether the internal or the atmospheric ethylene which reflect on the rapid loss of tissue firmness and reduce their keeping quality. Moreover, apple fruit is very sensitive to bruising, heating or ever some mild fluctuations during transportation from the field to the storage facility or to the retail market.

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Several compounds have been used to retard ripening and extend the storage period. For instance polyamines are group of natural compounds that are believed to have anti-senescence function by inhibition of the formation of enzymes essential to the synthesis of ethylene (Ke and Romani, 1988). Where, polyamines play an important role in the senescence process. Apelbaum *et al*., 1981 and Lee *et al.*, 1997, also, reported that, polyamines play an inhibitory role on ethylene production through inhibition of ACC synthesase and ACC oxidase, thus delaying ethylene emission. Polyamines have been reported to reduce softening, delay senescence and reduce decay in several fruits (Safiner and Baldi 1990, Kramer *et al*., 1991).

Ascorbic, citric and salicylic acids are considered with auxinic action, since they have synergistic effect on catching or chelating the free radicals which could result in extending the shelf life of plant cells and stimulating growth aspects is reported by Rao *et al.,* 2000. In the meantime, ascorbic acid is considered a regulator of plant growth. Citric acid also, plays an essential role in signal transduction system, membrane stability and functions, activating transporter enzymes, metabolism and translocation of carbohydrates (Smirnoff, 1996). Moreover, salicylic acid significantly reduced the quality loss in peaches (Wang *et al*., 2006) found that, salicylic acid has been quoted that its exogenous treatment carried away from the application surface to the other tissues to bring forth its response. Salicylic acid and its derivatives are widely in use to enhance fruits postharvest life by controlling their firmness. In addition, Khademi and Ershadi, 2013 reported that salicylic acid increased storability and lowered weight loss and fungal decay and has positive effect on firmness, total phenol contents delayed the fruit senescence of peach fruits and had significantly increased firmness and acidity of peach fruits (El-Shazly *et al*., 2013) and maintained greater firmness reduced TSS and fruit losses during cold storage and extend postharvest life of peach (Awad, 2013).

Therefore, this investigation was carried out during the two successive seasons of 2016 and 2017 in order to study the effectiveness of pre-harvest spraying of Putrescine, ascorbic acid, salicylic acid and citric acid treatments on fruit quality and extend the storage period at (2°C with 85-95% R.H.) of “Anna” apple trees.

Materials and Methods

The present study was conducted during the two successive seasons 2016 and 2017 on 10-year-old “Anna” apple trees (*Malus domestica* Borkh.) budded on MM 106 rootstock, grown in clay soil, spaced at 4×4m, under drip irrigation system in a private orchard at Kafr El-Dawar region, El-Behira governorate, Egypt. Thirty nine uniform trees, free from various physiological and pathological disorders received common horticultural practices were selected for investigation. Treatments included water as the control, Putrescine at (200, 400, 600) ppm, salicylic acid at (200, 400, 600) ppm, ascorbic acid at (200, 400, 600) ppm and citric acid at (200, 400, 600) ppm were sprayed on 20 April, 15 May during 2016 and 2017, respectively, The non-ionic surfactant Tween 20 at 0.05% (v/v) was added to all treatments to reduce the surface tension and increase the contact angle of sprayed droplets. Trees were sprayed to the run off using a hand sprayer. The treatments were sprayed at the time corresponding to specific fruit growth stages established by monitoring the progress of the sigmoid curve, and before the onset of climacteric ethylene. The study treatments were arranged in a factorial experiment in randomized complete block design. Five replications were used per each treatment. Thus thirty nine trees were used in this study. At harvest, a random sample of fourteen fruits from each replication was taken to the laboratory, washed with tap water, surface sterilized for 3 min in 0.05% sodium hypochlorite, rinsed quickly in distilled water to remove the residues of such sterilizer, then left for air drying and the initial fruit weight was determined. All fruits were then placed into fiberboard boxes with vented plastic liners and stored for 60 days at 2^{o} C in air (85-95% relative humidity), during cold storage period (every 20 days in both seasons), the following measurements were taken:

Fruit firmness (Newton):

Apple fruits firmness was determined on red and green cheek as ($Ib/inch^2$) using Effigi Pressure Tester (mod. Ft327). The results of these measurements were converted to (Newton) using the following equation:

$$Newton = Ib/inch^2 * 4.448$$

Fruit weight loss percentage:

It equals the amount of water loss through evaporation and transpiration plus the amount of dry matter lost by respiration. It was determined by weighting the fruits. The fruits were weighted periodically during storage every 20 days and their weight loss was calculated as follows:

$$\text{Weight Loss \%} = \frac{\text{Initial Weight} - \text{Weight at Sampling Date}}{\text{Initial Weight}} \times 100$$

Fruit disorders percentage:

Fruits affected with either pathological or physiological disorders were counted by visual and calculated as a percentage to the initial number of fruits per each sample (replicate) and treatment too.

Total soluble solids percentage:

TSS% was determined in apple fruit juice using a hand refractometer according to Chen and Mellenthin (1981).

Acidity percentage:

Fruit juice acidity was determined according to (A.O.A.C., 1985) by titration with 0.1 N sodium hydroxide using phenolphthalein as an indicator and expressed as malic acid percentage.

Vitamin C (mg/100ml Juice):

Vitamin C content was determined in fruit juice using 2,6- dichlorophenol-indo-phenol blue dye as mg ascorbic acid per 100 ml Juice. (A.O.A.C., 1985).

Total sugars percentage:

Total sugars were determined by using the phenol sulfuric acid method (Smith, 1956), and the concentration was calculated from a standard curve of glucose (mg. per gm. fresh weight of fruit tissue).

Anthocyanin (mg/100g):

Anthocyanin was determined on red and green cheeks of the fruit according to the method of Fuleki and Francis (1968**)** as follow: 10 grams of fresh peel, was extracted by using 20 ml of the extraction solution (85% (V/V) ethyl alcohol 95% + 15%(V/V) HCl 1.5N), the mixture was left for the extraction of anthocyanin for 2 weeks, 2 ml of the filtrate was used to determine the optical density at 535 nm, after adding 5 ml of the extraction solution. The blank was just the used extraction solution, using spectrophotometer.

Statistical analysis:

Data of the present study were subjected to the analysis of variance test (ANOVA) in a factorial experiment as complete randomized block design (RCBD). Where, the first factor was for thirteen treatments mentioned before, the second factor was for storage period. The least significant differences (LSD) at the 5% level of probability were calculated using a computer program Costat according to Snedecor and Cochran (1980).

Results and Discussion

Fruit firmness at the red cheek:

Results presented in Table (1) showed that, in both seasons, all treatments caused a significant increase in fruit firmness at red cheek compared with control. Moreover, in the two seasons of study, the statistical analysis showed that the highest two concentrations (400 and 600) ppm of Putrescine, salicylic acid, ascorbic acid and citric acid are more effective in increasing fruit firmness at red cheek as compared with control and the concentration 200 ppm of all used treatments. In harmony with these results are those obtained by Farag *et al.* (2007) working on "Desert Red" peach cultivar and the data indicated that, Putrescine either at 5 or 10 mM significantly retarded the loss of fruit firmness in a consistent manner in the two seasons as compared with the control. However, Putrescine at 5 mM was as effective as Putrescine at 10 mM on fruit firmness in both seasons. In addition, El-Badawy (2013) working on Canino apricot trees. The results showed that, the highest fruit firmness value was scored by the high level of antioxidants.

Table 1: Effect of preharvest spraying with Putrescine and some antioxidants treatments on fruit firmness (Newton) at red cheek of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	45.75	43.85	42.38	41.84	**43.45**	45.04	43.22	41.72	40.84	**42.71**
Putrescine at 200 ppm	50.54	49.67	48.10	44.70	**48.25**	51.37	50.05	48.79	46.08	**49.07**
Putrescine at 400 ppm	51.49	50.71	48.51	45.79	**49.12**	52.44	51.31	49.19	46.62	**49.89**
Putrescine at 600 ppm	52.57	51.67	49.11	46.33	**49.92**	53.37	52.12	49.35	47.85	**50.67**
Ascorbic Acid at 200 ppm	47.88	47.48	44.70	43.07	**45.78**	48.85	51.42	45.48	43.74	**47.37**
Ascorbic Acid at 400 ppm	48.92	48.35	45.45	43.96	**46.67**	50.02	48.95	46.48	44.44	**47.47**
Ascorbic Acid at 600 ppm	49.92	49.15	47.45	45.65	**48.04**	50.65	49.68	48.05	46.35	**48.68**
Salicylic Acid at 200 ppm	47.74	47.24	44.72	42.62	**45.58**	48.75	47.94	45.75	43.47	**46.48**
Salicylic Acid at 400 ppm	48.85	47.84	45.65	43.77	**46.53**	49.82	48.67	46.68	44.42	**47.40**
Salicylic Acid at 600 ppm	49.77	48.92	47.38	45.58	**47.91**	50.52	49.52	48.05	46.24	**48.58**
Citric Acid at 200 ppm	48.30	47.45	44.83	43.18	**45.94**	49.00	51.35	45.60	43.82	**47.44**
Citric Acid at 400 ppm	48.70	48.43	44.92	44.14	**46.55**	49.58	48.95	46.65	45.11	**47.57**
Citric Acid at 600 ppm	49.99	49.27	47.58	45.27	**47.78**	50.29	49.68	48.11	46.35	**48.61**
Mean	**49.98**	**49.64**	**46.74**	**44.72**		**49.98**	**49.45**	**46.92**	**45.02**	
LSD at $_{0.05}$	**T: 0.48 D: 0.27 T×D: 0.97**					**T: 1.06 D: 0.59 T×D: 2.14**				
T: Treatments D: Storage periods (Days) T×D: Interaction										

Regarding the effect of storage periods on the changes in fruit firmness at red cheek was decreased with increasing storage period, and the differences among all tested storage period were statistically significant compared with initial date in the two experimental seasons, except for the second date in 2017 season. This reduction in decreasing fruit firmness by Putrescine, may be due to their cross-linkage to the pectin substances carboxyl groups in the cell wall and lead to strengthening of cell wall and consequently decreasing cell wall degrading enzymes activities of pectin methyl esterase (PME), pectin esterase (PE) and polygalactouronase (PG) (Valero *et al.*, 2002). The role of Putrescine in maintaining fruit firmness has been reported for peach (Zokaee Khosroshahi and Esna-Ashari, 2008) and pear (Franco-Morq *et al.*, 2005).

In agreement with these results are those obtained by Alizade-Dashqapu *et al.* (2011) on peach fruit and they reported that fruit firmness continuously decreased during storage and various treatments significantly reduced fruit softening. However, 'Put' treatments could maintain fruit firmness during storage. Lower quality fruit was observed when fruit was immersed in distilled water only. Also, Davarynejad *et al.*, (2015) investigated and compare the effect of Putrescine and salicylic acid on quality properties of plum fruits during storage and they reported that the fruit flesh firmness decreased significantly during storage at 4° C in both materials (Putrescine and salicylic acid). The flesh firmness was affected by Putrescine and salicylic acid treatments, since control fruits had significantly lower firmness during storage, while the highest firmness values were obtained with 4 mmol/L Putrescine and 4 mmol/L salicylic acid treatments.

Fruit firmness at the green cheek:

Results in Table (2) showed that, in both seasons, all treatments caused a significant increase in fruit firmness at green cheek compared with control. Moreover, in the two seasons of study, the statistical analysis showed that the highest two concentrations (400 and 600) ppm of Putrescine, salicylic acid, ascorbic acid and citric acid were more effective in increasing fruit firmness at green cheek as compared with control and the concentration 200 ppm of all used treatments.

Table 2: Effect of preharvest spraying with Putrescine and some antioxidants treatments on fruit firmness (Newton) at green cheek of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	46.71	45.03	43.72	42.36	**44.45**	46.00	44.41	43.15	41.81	**43.84**
Putrescine at 200 ppm	50.98	50.01	48.50	45.37	**48.71**	51.75	50.55	49.54	46.12	**49.49**
Putrescine at 400 ppm	51.97	50.93	48.96	46.18	**49.51**	53.07	52.17	49.37	46.88	**50.37**
Putrescine at 600 ppm	52.97	52.11	49.58	46.85	**50.38**	53.67	53.01	50.12	47.95	**51.19**
Ascorbic Acid at 200 ppm	48.56	47.52	45.18	43.65	**46.22**	49.18	47.74	45.95	44.52	**46.85**
Ascorbic Acid at 400 ppm	49.35	48.75	45.95	44.25	**47.07**	49.81	49.15	46.62	44.85	**47.61**
Ascorbic Acid at 600 ppm	50.39	49.59	47.80	45.98	**48.44**	50.92	49.65	48.22	46.52	**48.83**
Salicylic Acid at 200 ppm	48.11	47.62	45.15	42.92	**45.95**	49.21	47.88	46.45	43.66	**46.80**
Salicylic Acid at 400 ppm	49.25	48.21	46.22	44.54	**47.05**	49.95	48.87	46.92	45.30	**47.76**
Salicylic Acid at 600 ppm	50.22	49.17	47.68	46.11	**48.29**	50.75	49.58	48.18	46.88	**48.85**
Citric Acid at 200 ppm	49.12	47.08	45.55	43.78	**46.38**	50.38	47.77	46.35	43.82	**47.08**
Citric Acid at 400 ppm	49.98	49.02	45.58	44.92	**47.37**	51.32	49.52	46.18	45.52	**48.14**
Citric Acid at 600 ppm	50.42	49.68	48.12	45.85	**48.52**	51.57	50.27	49.04	46.58	**49.37**
Mean	**50.53**	**50.09**	**47.23**	**45.24**		**50.58**	**49.27**	**47.39**	**45.42**	
LSD at $_{0.05}$	**T: 0.51 D: 0.29 T×D: 1.04**					**T: 0.73 D: 0.41 T×D: 1.47**				
T: Treatments D: Storage periods (Days) T×D: Interaction										

In agreement with these results are those obtained by El-Shazly *et al.* (2013) working on "Swelling" peach trees and they found that salicylic acid had significantly higher fruit firmness than ascorbic and citric acids.

As for the effect of storage periods on the changes in fruit firmness at green cheek was decreased with increasing storage period, and the differences among all tested storage periods were statistically significant compared with initial date in the two experimental seasons. These results are in harmony with those obtained by Hosseini *et al.* (2017) studying the effect of pre-harvest foliar spraying with Putrescine (at 0.5, 1 and 2 mM) on quality and postharvest life of *Pyrus communis* cv. Spadona during cold storage. Fruit quality was detected at harvest and after 3, 6, 9, 12, 15, 18 and 21 weeks of storage at 0±1°C, 80-85% relative humidity. Their results showed that, fruit softening increased during storage but the rate of changes was significantly lower in fruit treated with Putrescine at 1 and 2 mM. Furthermore, higher doses of Putrescine were effective in terms of prolonging the storage and marketability of fruits more than 127-142 days. In conclusion, pre-harvest application of Putrescine could be an effective means for extending the postharvest life of pear cv. Spadona. In addition Hadian-Deljou *et al.* (2017) studied the effects of pre- and post-harvest application of salicylic acid on fruit quality of ‘Red Delicious’ apples during 193 days cold (0±0.5°C) storage. Their results showed that, fruits showed signs of softening during storage. Firmness of apples was initially decreased in both control and SA-treated fruits.

Fruit weight loss:

Data of studying the effect of preharvest spraying with Putrescine and some antioxidants treatments on fruit weight loss of "Anna" apple fruits in 2016 and 2017 seasons at harvest and during cold storage are listed in Table (3). Results of both seasons, generally, indicated that all enhanced treatments decreased fruit weight loss as compared with the control. In addition, the statically analysis showed that Putrescine at (400 and 600) ppm, salicylic acid at (400 and 600) ppm, ascorbic acid at (400 and 600) ppm and citric acid at (400 and 600) ppm were more effective on decreasing fruit weight loss than the control and the concentration of 200 ppm of all used treatments.

Table 3: Effect of preharvest spraying with Putrescine and some antioxidants treatments on weight loss percentage of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	0.00	1.88	2.43	3.59	**1.97**	0.00	2.00	2.57	3.70	**2.07**
Putrescine at 200 ppm	0.00	1.53	1.64	2.52	**1.42**	0.00	1.44	1.58	2.51	**1.38**
Putrescine at 400 ppm	0.00	1.43	1.54	1.82	**1.20**	0.00	1.35	1.49	1.75	**1.15**
Putrescine at 600 ppm	0.00	1.35	1.52	1.66	**1.13**	0.00	1.28	1.47	1.61	**1.09**
Ascorbic Acid at 200 ppm	0.00	1.41	1.58	2.32	**1.33**	0.00	1.34	1.52	2.22	**1.27**
Ascorbic Acid at 400 ppm	0.00	1.37	1.55	1.73	**1.16**	0.00	1.35	1.49	1.67	**1.13**
Ascorbic Acid at 600 ppm	0.00	1.24	1.43	1.55	**1.05**	0.00	1.20	1.37	1.49	**1.02**
Salicylic Acid at 200 ppm	0.00	1.39	1.57	2.29	**1.31**	0.00	1.30	1.48	2.22	**1.25**
Salicylic Acid at 400 ppm	0.00	1.38	1.55	1.71	**1.16**	0.00	1.30	1.50	1.63	**1.11**
Salicylic Acid at 600 ppm	0.00	1.29	1.46	1.58	**1.08**	0.00	1.21	1.40	1.52	**1.03**
Citric Acid at 200 ppm	0.00	1.47	1.57	2.26	**1.32**	0.00	1.40	1.50	2.15	**1.26**
Citric Acid at 400 ppm	0.00	1.39	1.54	1.71	**1.16**	0.00	1.32	1.50	1.65	**1.12**
Citric Acid at 600 ppm	0.00	1.26	1.41	1.55	**1.05**	0.00	1.16	1.37	1.48	**1.00**
Mean	**0.00**	**1.39**	**1.57**	**1.99**		**0.00**	**1.36**	**1.56**	**1.97**	
LSD at 0.05	**T: 0.08 D: 0.04 T×D: 0.15**					**T: 0.07 D: 0.04 T×D: 0.15**				
T: Treatments D: Storage periods (Days) T×D: Interaction										

Regarding the effect of storage periods on the changes in fruit weight loss was significantly increased with increasing storage period, and the differences among all tested storage periods were statistically significant compared with initial date in the two experimental seasons. These reduction in weight loss in fruit during storage could be due to the water exchange between the internal and external atmosphere, the transpiration rate being accelerated by cellular breakdown (Woods, 1990). In this sense, the Putrescine treatment modified or consolidated the cell disposition and delayed the removal of epicuticular waxes which play an important role in water exchange through the skin, and then lower weight loss would occur. With regard to the results, the Putrescine treatments showed significantly less of weight loss during storage being a negative correlation between Putrescine concentrations and weight loss. Weight loss, also, is caused by both dehydration and consumption of soluble solids during respiration. salicylic acid could decrease water loss and respiration rate through controlling degradation of cell wall and reducing ethylene biosynthesis (Srivastavam and Dwivedi, 2000), respectively. These results are in harmony with those obtained by Hafez *et al.* (2010), they sprayed Le Conte pear trees twice with citric acid at concentration of (0.0, 50 and 100 ppm) and ascorbic acid at concentration of (0.0, 50 and 100 ppm). They showed that, all treatments decreased the weight loss (%). Khademi and Ershadi (2013) working on peach fruit cv. Elberta to study the effect of postharvest application of salicylic acid on fruit quality and storage life. Results showed that, all SA treatments prevented weight loss in comparison with the control. Moreover, Hosseini *et al.* (2017) studied the effect of pre-harvest foliar spraying with Putrescine (at 0.5, 1 and 2 mM) on quality and postharvest life of *Pyrus communis* cv. Spadona during cold storage. The results showed that, weight loss increased during storage but the rate of changes was significantly lower in fruit treated with Putrescine at 1 and 2 mM. In conclusion, pre-harvest application of Putrescine could be an effective mean for extending the postharvest life of pear cv. Spadona.

Fruit physiological disorders:

Data in Table (4) showed that, in both seasons, all mentioned pre-harvest treatments significantly reduced physiological disorders percentage of "Anna" apple fruits compared with control. Moreover, in the two seasons of study, the statistically analysis showed that Putrescine at 600 ppm, salicylic acid at 600 ppm, ascorbic acid at 600 ppm and citric acid at 600 ppm were more effective on decreasing fruit disorders than that other treatments.

Table 4: Effect of preharvest spraying with Putrescine and some antioxidants treatments on physiological percentage disorders of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	0.00	1.33	1.66	3.00	**1.50**	0.00	1.50	2.00	3.27	**1.69**
Putrescine at 200 ppm	0.00	0.66	1.33	1.67	**0.92**	0.00	0.83	1.67	2.03	**1.13**
Putrescine at 400 ppm	0.00	0.33	0.66	1.33	**0.58**	0.00	0.50	0.93	1.63	**0.76**
Putrescine at 600 ppm	0.00	0.00	0.32	0.66	**0.25**	0.00	0.32	0.60	1.00	**0.48**
Ascorbic Acid at 200 ppm	0.00	0.33	1.17	0.32	**0.71**	0.00	0.50	1.00	1.33	**0.71**
Ascorbic Acid at 400 ppm	0.00	0.00	0.67	0.83	**0.38**	0.00	0.30	0.50	0.83	**0.41**
Ascorbic Acid at 600 ppm	0.00	0.00	0.00	0.66	**0.17**	0.00	0.23	0.37	0.67	**0.32**
Salicylic Acid at 200 ppm	0.00	0.33	0.66	1.17	**0.54**	0.00	0.50	0.93	1.43	**0.72**
Salicylic Acid at 400 ppm	0.00	0.00	0.34	0.66	**0.25**	0.00	0.33	0.60	1.03	**0.49**
Salicylic Acid at 600 ppm	0.00	0.00	0.00	0.33	**0.08**	0.00	0.17	0.53	0.57	**0.32**
Citric Acid at 200 ppm	0.00	0.32	0.67	1.16	**0.54**	0.00	0.50	0.83	1.33	**0.67**
Citric Acid at 400 ppm	0.00	0.00	0.33	1.00	**0.33**	0.00	0.30	0.63	1.16	**0.53**
Citric Acid at 600 ppm	0.00	0.00	0.32	0.67	**0.25**	0.00	0.00	0.50	0.83	**0.33**
Mean	**0.00**	**0.26**	**0.63**	**1.12**		**0.00**	**0.46**	**0.85**	**1.32**	
LSD at $_{0.05}$	**T: 0.42**		**D: 0.23**		**T×D: 0.85**	**T: 0.43**		**D: 0.24**		**T×D: 0.96**
T: Treatments	**D: Storage periods (Days)**					**T×D: Interaction**				

Regarding the effect of storage periods on the changes in fruit physiological percentage data in Tables (5 and 6), showed that fruit physiological were found in both sampling date after 20, 40 and 60 days of storage in 2016 and 2017 seasons. Differences were significant compared with zero time in both seasons of study. To further explain the role of polyamines in enhancing fruit storability, (Kramer *et al*., 1991) worked on McIntosh and Golden Delicious apples and showed that polyamines inhibited the development of brown core (chilling injury) in McIntosh fruits. Also, these results agreement with those obtained by Martinez-Romero *et al*. (2000) who found that exogenous application of Putrescine (1Mm) at harvest on Babygold 6 peach cultivar followed by cold storage and the data showed that the treatment was effective in reducing the susceptibility of fruit damage by mechanical compression with fruits having lower volume and surface of the damage zone.

Moreover, these results are in harmony with those obtained by Hafez *et al.,* (2010) sprayed Le Conte pear trees twice with citric acid at concentration of (0.0, 50 and 100 ppm) and ascorbic acid at concentration of (0.0, 50 and 100 ppm). All treated and untreated pear fruit were stored at 0 ± 1° C and 85 – 90% relative humidity (RH) for 75 days and additional one week at room temperature (20-25° C) as stimulated marketing period. They showed that, treated and control fruits withstand free from chilling injury and pathogenic rot up to 45 days of cold storage. While, almost treatments prevented chilling injury symptoms and fruit deterioration up to 60 days of cold storage.

Fruit pathological disorders:

Results of the present investigation, presented in Table (5) showed that, in both seasons, all treatments caused a significant decrease in fruit pathological disorders compared with control. Moreover, in the two seasons of study, the statistically analysis showed that Putrescine at 600 ppm, salicylic acid at 600 ppm, ascorbic acid at 600 ppm and citric acid at 600 ppm were more effective on decreasing fruit decay than other treatments.

Table 5: Effect of preharvest spraying with Putrescine and some antioxidants treatments on pathological disorders percentage of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	0.00	0.87	1.50	2.50	**1.22**	0.00	1.03	1.67	2.67	**1.34**
Putrescine at 200 ppm	0.00	0.17	1.00	1.17	**0.58**	0.00	0.27	1.13	1.30	**0.67**
Putrescine at 400 ppm	0.00	0.00	0.83	1.33	**0.54**	0.00	0.13	0.93	1.07	**0.53**
Putrescine at 600 ppm	0.00	0.00	0.47	0.67	**0.28**	0.00	0.00	0.63	0.87	**0.37**
Ascorbic Acid at 200 ppm	0.00	0.10	0.83	1.00	**0.48**	0.00	0.17	1.00	1.13	**0.57**
Ascorbic Acid at 400 ppm	0.00	0.00	0.60	0.97	**0.39**	0.00	0.00	0.70	1.06	**0.44**
Ascorbic Acid at 600 ppm	0.00	0.00	0.43	0.80	**0.31**	0.00	0.00	0.48	0.88	**0.34**
Salicylic Acid at 200 ppm	0.00	0.13	0.77	0.93	**0.46**	0.00	0.20	0.90	1.10	**0.55**
Salicylic Acid at 400 ppm	0.00	0.00	0.36	0.80	**0.29**	0.00	0.00	0.47	0.93	**0.35**
Salicylic Acid at 600 ppm	0.00	0.00	0.28	0.67	**0.24**	0.00	0.00	0.40	0.82	**0.30**
Citric Acid at 200 ppm	0.00	0.17	0.90	1.13	**0.55**	0.00	0.20	1.03	1.30	**0.63**
Citric Acid at 400 ppm	0.00	0.00	0.63	1.00	**0.41**	0.00	0.13	0.68	1.20	**0.50**
Citric Acid at 600 ppm	0.00	0.00	0.60	0.50	**0.28**	0.00	0.00	0.57	0.67	**0.31**
Mean	**0.00**	**0.11**	**0.71**	**1.04**		**0.0c**	**0.16**	**0.82**	**1.15**	
LSD at $_{0.05}$	**T: 0.36**		**D: 0.20**		**T×D: 0.73**	**T: 0.35**		**D: 0.19**		**T×D: 0.70**
T: Treatments		**D: Storage periods (Days)**				**T×D: Interaction**				

As for the effect of storage periods on the changes in fruit decay percentage data in Table (5), showed that fruit decay were found in both sampling dates after 20, 40 and 60 days of storage in 2016 and 2017 seasons. Differences were significant compared with zero time in both seasons of study. These results are in agreement with those obtained by Khademi and Ershadi (2013) working on peach and they reported that, no fungal decay was visually observed on fruits during the first two weeks of storage. Fruits treated with different concentrations of salicylic acid had significantly lowered decay incidence than control one's during storage period. However, salicylic acid at 2 mM concentration was the most effective to reduce fungal decay at the end of storage time. Surprisingly, fruits treated by SA at 4 mM showed a high decay incidence after 28th day of storage. Although SA does not have direct fungicidal effects. Moreover, Abd El Wahab (2015) studying the effect of pre-harvest application of Putrescine, salicylic acid or ascorbic acid + citric acid on the decay of "Canino" apricot fruits. The sprayed fruits were stored up to 4weeks at 0 °C and 90–95% R.H. and fruit quality was evaluated at harvest, during cold storage and after 5 days as market-life. Results show that, the all pre harvest treatments with added calcium nitrate reduced decay in all storage periods as Putrescine or salicylic acid were the best treatments, followed by amino acids then antioxidants (ascorbic acid and citric acid) in descending order as compared with control treatment under study. Moreover, Ghasemnezhad *et al.* (2010) reported that, the percentage of decayed fruits increased gradually with prolonging storage period.

Total soluble solids:

Data in Table (6) showed that, in both seasons, all mentioned pre-harvest treatments significantly increased total soluble solids content of "Anna" apple fruits, except for Putrescine at 200 ppm in 2016 season where the difference was not big enough to be significant compared with control. In addition, in both seasons, the statistical analysis showed that Putrescine at 600 ppm, salicylic acid at (400 and 600) ppm, ascorbic acid at (400 and 600) ppm and citric acid at (400 and 600) ppm were more effective on increasing fruit total soluble solids content than that other treatments.

Table 6: Effect of preharvest spraying with Putrescine and some antioxidants treatments on total soluble solids percentage of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	10.67	10.83	11.42	11.75	**11.17**	10.29	10.60	11.17	11.50	**10.89**
Putrescine at 200 ppm	10.83	10.90	11.58	11.90	**11.30**	11.03	11.23	11.70	11.97	**11.48**
Putrescine at 400 ppm	11.20	11.45	11.75	12.00	**11.60**	11.40	11.63	11.90	12.20	**11.78**
Putrescine at 600 ppm	11.38	11.57	11.82	12.42	**11.80**	11.60	11.90	12.12	12.60	**12.06**
Ascorbic Acid at 200 ppm	11.17	11.57	11.78	12.20	**11.68**	11.38	11.73	11.97	12.40	**11.87**
Ascorbic Acid at 400 ppm	11.40	11.63	11.90	12.38	**11.83**	11.57	11.83	12.20	12.57	**12.04**
Ascorbic Acid at 600 ppm	11.63	11.80	12.30	12.75	**12.12**	11.80	12.05	12.57	12.92	**12.34**
Salicylic Acid at 200 ppm	10.97	11.33	11.69	12.12	**11.53**	11.23	11.58	11.90	12.33	**11.76**
Salicylic Acid at 400 ppm	11.42	11.58	11.90	12.25	**11.79**	11.57	11.87	12.10	12.48	**12.01**
Salicylic Acid at 600 ppm	11.55	11.71	12.18	12.75	**12.05**	11.77	11.85	12.47	12.97	**12.27**
Citric Acid at 200 ppm	11.23	11.62	11.80	12.23	**11.72**	11.38	11.80	12.00	12.35	**11.88**
Citric Acid at 400 ppm	11.44	11.70	12.01	12.47	**11.90**	11.63	11.90	12.27	12.67	**12.12**
Citric Acid at 600 ppm	11.67	11.97	12.07	12.83	**12.13**	11.77	12.08	12.23	13.00	**12.27**
Mean	**11.27**	**11.51**	**11.86**	**12.31**		**11.42**	**11.70**	**12.05**	**12.46**	
LSD at $_{0.05}$	**T: 0.15 D: 0.08 T×D: 0.30**					**T: 0.25 D: 0.14 T×D: 0.50**				
T: Treatments D: Storage periods (Days) T×D: Interaction										

Regarding the effect of storage periods on the changes in fruit total soluble solids were significantly increased with increasing storage periods, and the differences among all tested storage periods were statistically significant compared with the initial date in the two seasons of study. During storage, the increase in content of total soluble solids was probably due to concentrated juice content as a result of dehydration and hydrolysis of polysaccharides. During storage, all treatments showed increases in content of total soluble solids, although the increases were significantly lower in treatment of Putrescine than in control treatment. This effect of Putrescine can be attributed to low levels of the respiration rate, ethylene production and delay in ripening process. According to data, one can say that there is an inversely relation between Putrescine concentrations and level of total soluble solids during storage.

The obtained results in this study agreed with those found in previous research such as Davarynejad *et al*. (2013) working on two apricot cultivars (‘Lasgerdi’ and ‘Shahrodi’). Their data revealed that the quality of apricot fruits was improved by the use of Putrescine treatment due to its effect on delaying the ripening processes. Moreover, Abd El Wahab (2015) studying the effect of pre-harvest application of Putrescine, salicylic acid or ascorbic acid + citric acid all with addition to calcium nitrate on the decay of "Canino" apricot fruits. The sprayed fruits were stored up to 4 weeks at 0 °C and 90–95% R.H. and fruit quality was evaluated at harvest, during cold storage and after 5 days as market-life. Results showed that all treatments delayed the changes in total soluble solids compared with control.

Acidity:

Data concerning the effect of various treatments on fruit acidity of "Anna" apple fruits in 2016 and 2017 seasons, are shown in Table (7). In both seasons results revealed that, generally, all treatments significantly decreased fruit juice acidity compared with control. Moreover, in both seasons, the statically analysis showed that Putrescine at 600 ppm, salicylic acid at (400 and 600) ppm, ascorbic acid at (400 and 600) ppm and citric acid at (400 and 600) ppm were more effective on decreasing fruit acidity content than that other treatments.

Table 7: Effect of preharvest spraying with Putrescine and some antioxidants treatments on acidity percentage of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	0.74	0.69	0.64	0.59	**0.66**	0.81	0.72	0.69	0.64	**0.71**
Putrescine at 200 ppm	0.61	0.52	0.48	0.45	**0.52**	0.64	0.52	0.47	0.44	**0.51**
Putrescine at 400 ppm	0.57	0.50	0.45	0.43	**0.49**	0.60	0.49	0.45	0.42	**0.49**
Putrescine at 600 ppm	0.51	0.46	0.42	0.40	**0.45**	0.54	0.45	0.42	0.39	**0.45**
Ascorbic Acid at 200 ppm	0.54	0.49	0.46	0.40	**0.47**	0.57	0.48	0.45	0.39	**0.47**
Ascorbic Acid at 400 ppm	0.48	0.41	0.39	0.37	**0.41**	0.50	0.40	0.38	0.37	**0.41**
Ascorbic Acid at 600 ppm	0.46	0.37	0.35	0.33	**0.38**	0.48	0.37	0.35	0.32	**0.38**
Salicylic Acid at 200 ppm	0.54	0.49	0.46	0.40	**0.47**	0.58	0.48	0.45	0.40	**0.48**
Salicylic Acid at 400 ppm	0.50	0.42	0.39	0.38	**0.43**	0.52	0.42	0.38	0.37	**0.42**
Salicylic Acid at 600 ppm	0.46	0.38	0.36	0.34	**0.38**	0.50	0.38	0.36	0.32	**0.39**
Citric Acid at 200 ppm	0.54	0.49	0.46	0.39	**0.47**	0.57	0.48	0.45	0.38	**0.46**
Citric Acid at 400 ppm	0.48	0.42	0.39	0.37	**0.42**	0.50	0.41	0.38	0.37	**0.41**
Citric Acid at 600 ppm	0.44	0.37	0.35	0.34	**0.37**	0.48	0.36	0.34	0.33	**0.38**
Mean	**0.53**	**0.46**	**0.43**	**0.39**		**0.56a**	**0.46**	**0.43**	**0.39**	
LSD at $_{0.05}$	**T: 0.02**		**D: 0.01**		**T×D: 0.04**	**T: 0.02**		**D: 0.01**		**T×D: 0.03**
T: Treatments	**D: Storage periods (Days)**					**T×D: Interaction**				

As for the effect of storage periods, data indicated that fruit acidity significantly decreased in both seasons, with increasing the storage periods. This obtained trend of results agreed with those reported El-Badawy (2013) working on Canino apricot trees to study the effect of some antioxidants i.e ascorbic acid and citric acid each at 1000 or 2000ppm three times a year started from the first week of March with two weeks intervals. The results showed that, the lowest value of total acidity value was scored by the high level of antioxidants. Also, Abd El Wahab (2015) studying the effect of pre-harvest application of Putrescine, salicylic acid or ascorbic acid + citric acid all with addition to calcium nitrate on the decay of "Canino" apricot fruits. The sprayed fruits were stored up to 4weeks at 0 °C and 90–95% R.H. and fruit quality was evaluated at harvest, during cold storage and after 5 days as market-life. it showed that all treatments delayed the changes in total soluble solids compared with control. Moreover, Davarynejad *et al.* (2015) working on plum cv. 'Santa Rosa' fruits to investigate and compare the effect of Putrescine and salicylic acid on quality properties of plum fruits during storage and they found that, The levels of titratable acidity decreased significantly during storage at 4° C for both compounds (Putrescine and salicylic acid).

Vitamin C:

The data demonstrated in Table (8) declared that as an average for all used treatments, the initial of Ascorbic acid significantly increased in both seasons compared with control. Moreover, the statically analysis showed that Putrescine at 600 ppm, salicylic acid at 600 ppm, ascorbic acid at (400 and 600) ppm and citric acid at (400 and 600) ppm were more effective on increasing ascorbic acid (mg/100 juice) than that other treatments.

Table 8: Effect of preharvest spraying with Putrescine and some antioxidants treatments on vitamin C content (mg/100ml Juice) of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	4.70	4.60	4.43	4.27	**4.58**	4.60	4.53	4.37	4.23	**4.43**
Putrescine at 200 ppm	5.13	4.97	4.80	4.70	**4.90**	5.23	5.10	4.87	4.83	**5.01**
Putrescine at 400 ppm	5.33	5.06	4.90	4.83	**5.03**	5.43	5.20	5.00	4.90	**5.13**
Putrescine at 600 ppm	5.57	5.33	5.17	4.93	**5.25**	5.70	5.47	5.33	5.03	**5.38**
Ascorbic Acid at 200 ppm	5.30	5.12	5.01	4.85	**5.07f**	5.47	5.23	5.03	4.90	**5.16**
Ascorbic Acid at 400 ppm	5.50	5.20	5.08	4.98	**5.19**	5.60	5.33	5.17	5.07	**5.29**
Ascorbic Acid at 600 ppm	5.69	5.49	5.35	5.05	**5.39**	5.87	5.65	5.43	5.26	**5.55**
Salicylic Acid at 200 ppm	5.25	5.04	4.90	4.81	**5.00**	5.37	5.15	5.00	4.90	**5.11**
Salicylic Acid at 400 ppm	5.45	5.11	4.93	4.87	**5.09**	5.62	5.35	5.07	4.94	**5.25**
Salicylic Acid at 600 ppm	5.61	5.44	5.31	5.04	**5.35**	5.75	5.57	5.40	5.13	**5.46**
Citric Acid at 200 ppm	5.37	5.19	5.11	4.90	**5.14**	5.52	5.37	5.20	4.93	**5.26**
Citric Acid at 400 ppm	5.54	5.27	5.25	5.12	**5.29**	5.67	5.42	5.42	5.27	**5.45**
Citric Acid at 600 ppm	5.74	5.36	5.21	5.03	**5.44**	5.83	5.48	5.29	5.12	**5.43**
Mean	**5.40**	**5.17**	**5.06**	**4.91**		**5.51**	**5.30**	**5.12**	**4.96**	
LSD at $_{0.05}$	**T: 0.09 D: 0.05 T×D: 0.37**					**T: 0.11 D: 0.06 T×D: 0.21**				
T: Treatments D: Storage periods (Days) T×D: Interaction										

Data indicated that ascorbic acid values were decreased with prolonging the storage periods. This reduction in ascorbic acid was significant in the storage periods compared with the initial date in both experimental seasons. Ishaq *et al*. (2009) reported that the content of ascorbic acid decreased during storage could be due to the conversion of dehydroascobic to diketogulonic acid by oxidation. The effect of Putrescine may be ascribed to decreased or delayed ascorbate oxidase activity.

These results are in harmony with those obtained by Davarynejad *et al*. (2013) working on two apricot cultivars (‘Lasgerdi’ and ‘Shahrodi’). The data revealed that the quality of apricot fruits was improved by the use of Putrescine treatment due to its effect on delaying the ripening processes. Also, El-Badawy (2013) working on Canino apricot trees to study the effect of some antioxidants i.e ascorbic acid and citric acid. The results showed that, the highest vitamin C (mg/100 ml juice) value was scored by the high level of antioxidants. In addition, Davarynejad *et al.* (2015) working on plum cv. ‘Santa Rosa’ fruits to investigate and compare the effect of Putrescine and salicylic acid on quality properties of plum fruits during storage and they found that, The levels of ascorbic acid decreased significantly during storage at 4 °C for both compounds (Putrescine and salicylic acid).

Total sugars:

Results Table (9) showed that, in both seasons, all treatments caused a significant increase in total sugars content compared with control. Moreover, in two seasons of study, the statistical analysis showed that Putrescine at 600 ppm, salicylic acid at (400 and 600) ppm, ascorbic acid at (400 and 600) ppm and citric acid at (400 and 600) ppm were more effective on increasing total sugars content than other treatments.

Table 9: Effect of preharvest spraying with Putrescine and some antioxidants treatments on total sugars percentage of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	7.97	8.50	8.80	9.13	**8.60**	7.87	8.30	8.63	8.87	**8.42**
Putrescine at 200 ppm	8.43	8.73	9.30	9.97	**9.11**	8.63	8.90	9.50	10.20	**9.31**
Putrescine at 400 ppm	8.67	8.93	10.03	10.60	**9.56**	8.80	9.17	10.23	10.70	**9.73**
Putrescine at 600 ppm	9.17	9.53	10.30	10.80	**9.95**	9.50	9.73	10.53	10.97	**10.18**
Ascorbic Acid at 200 ppm	8.73	9.07	9.50	10.27	**9.39**	9.03	9.30	9.70	10.50	**9.63**
Ascorbic Acid at 400 ppm	8.97	9.23	10.33	10.70	**9.81**	9.23	9.37	10.57	10.97	**10.04**
Ascorbic Acid at 600 ppm	9.57	9.77	10.57	10.93	**10.21**	9.73	9.97	10.73	11.13	**10.39**
Salicylic Acid at 200 ppm	8.67	8.93	9.43	10.10	**9.28**	8.90	9.20	9.63	10.37	**9.53**
Salicylic Acid at 400 ppm	8.83	9.07	10.13	10.63	**9.67**	9.00	9.23	10.30	10.77	**9.83**
Salicylic Acid at 600 ppm	9.30	9.67	10.47	10.87	**10.08**	9.50	9.87	10.67	11.10	**10.29**
Citric Acid at 200 ppm	8.87	9.23	9.63	10.50	**9.56**	9.07	9.60	9.87	10.67	**9.80**
Citric Acid at 400 ppm	9.17	9.40	10.57	10.80	**9.98**	9.50	9.77	10.70	10.97	**10.24**
Citric Acid at 600 ppm	9.67	9.90	10.67	11.00	**10.31**	9.77	10.07	10.77	11.23	**10.46**
Mean	**8.92**	**9.23**	**9.98**	**10.48**		**9.12**	**9.42**	**10.14**	**10.65**	
LSD at $_{0.05}$	**T: 0.17 D: 0.09 T×D: 0.34**					**T: 0.22 D: 0.12 T×D: 0.45**				
T: Treatments D: Storage periods (Days) T×D: Interaction										

Regarding the effect of storage periods on the changes in fruit total sugars were increased with increasing storage periods, and the differences among all tested storage periods were statistically significant compared with the initial date in the two experimental seasons. These results are in harmony with those obtained by Abd El Wahab (2015) studying the effect of pre-harvest application of Putrescine, salicylic acid or ascorbic acid + citric acid all with addition to calcium nitrate on the decay of "Canino" apricot fruits. The sprayed fruits were stored up to 4weeks at 0 °C and 90–95% R.H. and fruit quality was evaluated at harvest, during cold storage and after 5 days as market-life. Results show that all treatments delayed the changes in total sugars compared with control. In addition, Ullah and Jawandha (2015) working on peach cv. SHAN-I-PUNJAB fruits to reduce the losses in which physiologically mature, uniform and healthy fruits where harvested and treated for 5-minutes in aqueous solutions of Putrescine at three different concentrations viz., 1.0, 2.0 and 3.0 mmol L^{-1} and they revealed that, post-harvest treatments of Putrescine were effective in delaying ripening and extending the post-harvest life of peach fruits under cold storage conditions. Putrescine at 3 mmol L^{-1} treatment was found most effective in maintaining the high palatability total sugars at the end storage period.

Anthocyanin content at red cheek:

Data in Table (10) showed that, in both seasons, all treatments caused a significant increase in fruit anthocyanin content at the red cheek, except for Putrescine at (400 and 600) ppm in 2017 season where the differences were not big enough to be significant compared with the control. Moreover, in the two seasons of this study, the statically analysis showed that salicylic acid at 600 ppm, ascorbic acid at 600 ppm and citric acid at 600 ppm were more effective on increasing fruit anthocyanin content at the red cheek than other treatments.

As for the effect of storage periods on the changes in fruit anthocyanin content at the red cheek, data showed that, it was increased with increasing storage period, and the differences among all tested storage period were statistically significant compared with initial date in the two experimental seasons. Anthocyanin's degrade by polyphenol oxidase during post-harvest, and this might be the main reason behind the reduction of anthocyanin compounds.

Table 10: Effect of preharvest spraying with Putrescine and some antioxidants treatments on anthocyanin (mg/100g) at red cheek of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	20	40	60	Mean	0	20	40	60	Mean
Control (Water Only)	15.86	16.72	17.45	17.86	**16.97**	16.22	17.07	17.72	18.29	**17.33**
Putrescine at 200 ppm	16.77	17.79	18.13	18.29	**17.74**	16.37	17.29	17.74	17.83	**17.46**
Putrescine at 400 ppm	17.02	18.04	18.37	18.99	**18.10**	16.51	17.60	17.92	18.57	**17.65**
Putrescine at 600 ppm	17.54	18.52	18.99	19.38	**18.61**	17.07	17.95	18.60	19.04	**18.17**
Ascorbic Acid at 200 ppm	17.61	18.28	18.86	19.57	**18.58**	17.22	17.78	18.57	19.16	**18.18**
Ascorbic Acid at 400 ppm	17.48	18.12	18.77	19.41	**18.44**	17.24	17.64	18.47	19.10	**18.11**
Ascorbic Acid at 600 ppm	18.04	18.60	19.64	21.15	**19.36**	17.63	18.17	19.22	20.68	**18.92**
Salicylic Acid at 200 ppm	17.70	18.38	19.11	19.85	**18.76**	17.33	17.87	18.71	19.45	**18.34**
Salicylic Acid at 400 ppm	18.37	18.98	19.42	19.72	**19.12**	18.04	18.45	18.94	19.34	**18.69**
Salicylic Acid at 600 ppm	20.02	20.16	20.47	21.31	**20.49**	19.51	19.69	20.11	20.81	**20.03**
Citric Acid at 200 ppm	17.53	18.18	18.75	19.47	**18.48**	17.15	17.61	18.37	19.12	**18.06**
Citric Acid at 400 ppm	17.36	18.00	18.65	19.27	**18.32**	16.94	17.60	18.22	18.82	**17.90**
Citric Acid at 600 ppm	17.92	18.48	19.44	21.05	**19.22**	17.54	18.21	19.25	20.48	**18.87**
Mean	**17.63**	**18.33**	**18.93**	**19.64**		**17.29**	**17.92**	**18.60**	**19.28**	
LSD at 0.05	**T: 0.41 D: 0.22 T×D: 0.82**					**T: 0.49 D: 0.27 T×D: 0.99**				
T: Treatments D: Storage periods (Days) T×D: Interaction										

The role of salicylic acid on anthocyanin production is unknown, one may hypothesize that salicylic acid could activate the key enzyme (Chalcone synthase) in the anthocyanin biosynthetic pathway (Godoy-Hernandez and Loyola-Vargas, 1997). Total anthocyanin has also been increased with storage period in both control and treated pomegranates (Sayyari *et al*., 2011). Our results agree with findings of Obinata *et al*. (2003) and Sayyari *et al*. (2011). This obtained trend of results agreed with those reported Hadian-Deljou *et al.,* (2017) studying the effects of pre- and post-harvest application of salicylic acid on fruit quality of 'Red Delicious' apples during 193 days cold (0±0.5°C) storage. Results showed that, anthocyanin content of apples revealed an increasing trend until the 60th day of storage while decreased right afterwards. Initially, effects of SA on anthocyanin content were significant. The highest contents of anthocyanin were obtained in the concentrations of 1 and 2 mM at pre-harvest treatments with no significant differences between one and with 1mM of post-harvest treatment.

Anthocyanin content at green cheek:

Results in Table (11) showed the effect of preharvest spraying with Putrescine and some antioxidants treatments on anthocyanin content at the green cheek of "Anna" apple fruits at harvest and during storage, in 2016 and 2017 seasons. Data showed that, in both seasons, all treatments caused a significant increase in fruit anthocyanin content at the green cheek compared with the control. Moreover, in the two seasons of this study, the statistical analysis showed that salicylic acid at 600 ppm, ascorbic acid at 600 ppm and citric acid at 600 ppm were more effective on increasing fruit anthocyanin content at the green cheek than other treatments.

Regarding the effect of storage periods on the changes in fruit anthocyanin content at the green cheek, data showed that, it was significantly increased with increasing storage period, and the differences among all tested storage period were statistically significant compared with initial date in the two experimental seasons. In agreement with these results are those obtained by El-Shazly *et al.* (2013) on "Swelling" peach trees (*Prunus persica* L.) and they found that, Results showed that, both ascorbic and citric acids caused the highest increase in anthocyanin content as compared with all other different agrochemicals.

Table 11: Effect of preharvest spraying with Putrescine and some antioxidants treatments on anthocyanin at green cheek of "Anna" apple fruits at harvest and during cold storage in 2016 and 2017 seasons.

Treatments	1st Season					2nd Season				
	Storage Periods (Days)					Storage Periods (Days)				
	0	**20**	**40**	**60**	**Mean**	**0**	**20**	**40**	**60**	**Mean**
Control (Water Only)	2.03	2.25	2.45	2.56	**2.32**	2.00	2.18	2.38	2.48	**2.26**
Putrescine at 200 ppm	2.39	2.42	2.52	2.60	**2.48**	2.40	2.43	2.53	2.61	**2.49**
Putrescine at 400 ppm	2.42	2.45	2.55	2.61	**2.51**	2.43	2.46	2.56	2.63	**2.52**
Putrescine at 600 ppm	2.45	2.48	2.58	2.68	**2.55**	2.46	2.49	2.60	2.70	**2.56**
Ascorbic Acid at 200 ppm	2.41	2.45	2.55	2.61	**2.50**	2.42	2.46	2.56	2.62	**2.52**
Ascorbic Acid at 400 ppm	2.44	2.49	2.56	2.66	**2.54**	2.45	2.51	2.57	2.67	**2.55**
Ascorbic Acid at 600 ppm	2.48	2.51	2.65	2.70	**2.58**	2.50	2.52	2.67	2.72	**2.60**
Salicylic Acid at 200 ppm	2.41	2.45	2.56	2.62	**2.51**	2.42	2.47	2.57	2.63	**2.52**
Salicylic Acid at 400 ppm	2.45	2.50	2.57	2.66	**2.54**	2.47	2.51	2.59	2.67	**2.56**
Salicylic Acid at 600 ppm	2.49	2.51	2.63	2.70	**2.58**	2.50	2.53	2.64	2.72	**2.60**
Citric Acid at 200 ppm	2.42	2.45	2.54	2.61	**2.50**	2.43	2.47	2.56	2.63	**2.52**
Citric Acid at 400 ppm	2.45	2.51	2.56	2.65	**2.54**	2.46	2.52	2.57	2.66	**2.55**
Citric Acid at 600 ppm	2.50	2.50	2.66	2.71	**2.59**	2.52	2.52	2.68	2.73	**2.61**
Mean	**2.41**	**2.46**	**2.57**	**2.64**		**2.42**	**2.47**	**2.58**	**2.65**	
LSD at $_{0.05}$	**T: 0.05 D: 0.02 T×D: 0.11**					**T: 0.05 D: 0.03 T×D: 0.11**				
T: Treatments D: Storage periods (Days) T×D: Interaction										

Conclusion

Generally, it could be concluded that, the treatments of Putrescine, ascorbic acid, salicylic acid and citric acid as a pre-harvest spraying on "Anna" apple trees had more pronounce effect on fruit quality at harvest time and during cold storage as a compared with control. Furthermore, the high concentration 600 ppm for all used treatments led to marked increase in fruit firmness, TSS, total sugars, vitamin C and anthocyanin also, lowest value of fruit weight loss, fruit disorders and acidity contents compared with others.

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