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Effect of Preharvest Application of Some Antioxidants on The Fruit Yield, Quality and Storability of "Manfalouty" Pomegranate Fruits (*Punica granatum* L.)

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ABSTRACT

Pomegranate (*Punica granatum* L.) is one the most important sub-tropical fruits due to its nutritional, pharmacological and therapeutic values. The aim of this work was to study the effects of some antioxidants, salicylic acid (SA) and ascorbic acid (ASA) on the yield, cracking and quality of fruits at harvest as well as fruit chilling injuries, weight loss % and quality at cold storage and shelf life of "Manfalouty" cultivar. Seven treatments, ASA at 250 or 500 ppm SA at 250 or 500 ppm, ASA+ SA at 250 + 250 or 500 + 500 ppm and control were applied three times (July, August, September). All treatments of SA and / or ASA improved total yield, fruit weight, fruit juice %, TSS and TSS/TA ratio at harvest. In fruits with ASA at 500ppm induced the highest total yield, fruit weight and TSS in both seasons followed by T2 (ASA at 250ppm) at harvest. However, T7 (SA+ASA at 500+500ppm) recorded the highest aril weight % and juice % at harvest for both seasons. All treatments significantly reduced the weight loss and chilling injuries at cold storage and shelf life during both seasons.

Keywords: Pomegranate, Ascorbic acid, Salicylic acid, Cold storage, Shelf life

Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest and most important fruit crops in tropical and sub-tropical regions. However, its spread and consumption were very low till the end of last century due to the difficulty of aril extractions for eating (Kahramanoğlu, 2019). By the beginning of the 21th century, many reports cleared the nutritional, pharmacological and therapeutic values of pomegranate fruits which have a potential effect as strong antioxidative and anti-inflammatory (Kahramanoğlu, 2019; Vučić *et al.*, 2019), which made it to be called a "Super fruit". Thus, the demands and production of pomegranates were highly increased in the last years which was estimated 3.8 million tons in 2017 compared to 3 million tons in 2014 (Kahramanoğlu, 2019). Recently, in Egypt, production and consumption of pomegranate fruits are highly increasing which made it the seventh producer over the world. As well as, for exportation, according to the Ministry of Agriculture and Land Reclamation, Egypt exported 90969 tons of pomegranate fruits in the period of January to November 2020, which recorded the pomegranates as the fifth crop in term of exported fruit crops (Elsawy, 2020). According to the Ministry of Agriculture and Land Reclamation Statistics (2016), the total area devoted for pomegranate was 85415 fed (35,888 ha), with production of 269070 tons. Pomegranate cv. "Manfalouty" is one of the most successfully cultivars that is growing in Upper Egypt, especially in Assiut Governorate (El-Orabi *et al.*, 2020).

Pomegranate fruits are subjected to many damages during the growing season, especially with "Manfalouty" cultivar which is classified as a sensitive to fruit cracking that decreases the fruit yield and marketing value (Abdel Aziz *et al.*, 2017; El-Orabi *et al.*, 2020), as well as during storage such chilling injury, weight loss, curliness, decay and the decrease in fruit quality during the shelf life or cold storage. In spite of the pomegranate fruit is classified as a non-climacteric, it still subjected to qualitative and quantitative losses during postharvest life due to weight loss, chilling injuries, husk scald and decay (Kader *et al.*, 1984). The most noticeable symptoms of fruit chilling injury are pitting in fruit surface and discoloration in fruit peel and arils that made it susceptible to decay (Kader *et al.*, 1984; Fawole and Opara, 2013). Moreover, cracking incidence in pomegranate fruit is one of the most important

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physiological disorder that cause great loss in fruit yield and value which account 3.64%, 6.30% and 19.40% for early, mid and late season, respectively, from the total fruit yield (Abdel Aziz *et al.*, 2017) Therefore, it was important to search for some natural compounds that able to improve the productivity of fruit trees and maintaining the quality of the fruits during storage and shelf life, as well as to reduce the effect of stresses (biotic and abiotic stresses) and the sensitivity of fruit crops to them.

Antioxidants such as salicylic acid and ascorbic acid are safe compounds capable of reducing the stresses effect (biotic and abiotic stresses) and or sensitivity of fruit crops to stresses that made them are of great importance from both of the theoretical and practical point of view (Khan *et al.*, 2011; Khan *et al.*, 2015; Paciolla *et al.*, 2019). Also, their application increased the fruit yield and quality of many fruit crops (Bondok *et al.,* 2011; Osman, 2014; Ayed, 2014; Akl *et al.*, 2014; Mohamed-Attiat, 2016; Abdel Aziz *et al.*, 2017 and Abd-El-Rhman *et al.*, 2017).

Salicylic acid (SA) belongs to a group of plant phenolic compounds and being involved in regulating plant growth, development and disease resistance (Raskin, 1992; Shah, 2003; Beckers and Spoel, 2006**.** They protect the plant cells by preventing the accumulation of reactive oxygen species (ROS) (Taiz and Zeiger, 2002; Joseph *et al.*, 2010). SA is a phenolic growth regulator which enhanced plant photosynthesis, uptake and transport of nutrients, cell division and the biosynthesis of plant pigments (Abdel Aziz *et al.*, 2017; Sharma *et al.*, 2020), which demonstrated to play a definite role on increasing the tolerance of many fruit crops to different stresses such as heat, salinity and heavy metal stresses. It is also considered as a natural plant hormone that inhibits ethylene biosynthesis and delays the fruit senescence (Erogul and Özsoydan, 2020). Beckers and Spoel (2006) reported that the SA is involved in systemic acquired resistance. There are several studies indicating the beneficial influences of SA on preserving fruit quality and reducing decay percentage (Ennab *et al.* 2020; Lokesh *et al.*, 2020; Haider *et al.* 2020).

Moreover, ascorbic acid [ASA or vitamin C (VC)] is physiologically active antioxidant compound that plays a key substrate for the detoxification of ROS which could protect the plants against various environmental stresses like heavy metal, heat and salinity (Shalata and Neumann, 2001; Vwioko *et al.*, 2008; Hagagg *et al.*, 2020). Ascorbic acid plays multiple roles in many developmental processes including cell division and cell wall expansion leading to improve plant growth, photosynthesis and increase yield of the crop (Pignocchi and Foyer, 2003). Vitamin C also, was reported to be in the defense response against many pathogens (Boubakri *et al.*, 2017). As well, exogenous application of ASC acts as an inducer of disease resistance in different plant-pathogen interactions (Egan *et al.*, 2007; Botanga *et al.*, 2012; Fujiwara *et al.*, 2013 and Li *et al.*, 2016).

Therefore, this study aimed to investigate the effects of preharvest applications of some antioxidants (ascorbic acid and salicylic acid) for reducing fruit cracking, improving the fruit yield and quality at harvest as well as storability during cold storage and shelf life of pomegranate fruits under Assiut Governorate conditions.

Materials and Methods

The present study was carried out on "Manfalouty" pomegranate trees (*Punica granatum* L.) grown in clay soil in a private orchard situated at El Badary, Assiut Governorate, Egypt in 2016 and 2017 seasons Healthy trees and uniformly in growth were selected for this study. The age of the trees was approximately 15 years old at the beginning of the experiment and they were planted at 5x5 m apart.

Application of antioxidant treatments:

Seven treatments were applied with three replicates for each (1 replicate = 1 tree) as follows:

Treatments

T_1	Spraying with water (control)**,**
T_2	Spraying with ascorbic acid (ASA) at 250 ppm,
T_3	Spraying with ascorbic acid (ASA) at 500 ppm,
T_4	Spraying with salicylic acid (SA) at 250 ppm,
T_5	Spraying with salicylic acid (SA) at 500 ppm,
T_6	Spraying with ASA (250 ppm) plus SA (250ppm),
T_7	Spraying with ASA (500 ppm) plus SA (500 ppm**)**

The trees were sprayed three times (at the first week of July, August and September, respectively). Trees were sprayed in early morning by using a back gun sprayer 20 liters even runoff.

At harvest date (early October), which was detected according to commercial criteria based on fruit size, weight, skin color and total soluble solids (TSS), the fruits were manually and carefully harvested and total yield was calculated, then the fruits were examined and separated into sound and cracked for each replicate (tree). The percentage of cracked fruits was calculated. For each treatment, about 200 to 250 fruits (about 90 kg) were directly brought to the laboratory of Agricultural Research Station (Arab Elawamer), Assiut Governorate. The fruits of each treatment were divided into 3 groups. The first one was to examine the physical and chemical characteristics of the fruits at the harvest date (about 12 to 15 fruits). The second group (about 40 to 45 fruits, 15 to 20 kg for each treatment) was stored at 5 ± 1 °C and 85-90 % relative humidity (RH), and used to measure the weight loss every three weeks during 12 weeks of cold storage. Finally, the third one was the rest of fruits (about 140 to 190 fruits, 65 to 70 kg each), packed in 12 carton boxes, 5-6 kg (12-15 fruits) each, divided into four batches, each batch has three replicates, one carton for each, stored also at 5 ± 1 °C and 85-90 % RH. Every three weeks, one batch (three cartons) from above mentioned (after three, six, nine and 12 weeks of cold storage), was removed then divided into two groups. The first group was used to examine fruit quality properties and decay index at the day of extraction of cold storage. The second one was placed on room temperature for five days of shelf life then the weight loss percentage, fruit quality properties and decay index were recorded.

Table 1: Average monthly meteorological data of Assiut weather station during two years 2016 and 2017.

Month	Max T (°C)	Min T (°C)	RH %	W.S / km/h	Sunshine
			2016		
January	19.0	5.10	60.3	15.2	8.90
February	24.5	8.30	50.7	14.5	9.70
March	28.0	13.1	41.0	17.0	9.90
April	35.1	17.1	31.5	17.0	10.3
May	36.1	20.0	27.7	20.3	11.4
June	40.7	24.6	28.0	19.5	12.3
July	37.4	24.1	37.9	19.5	12.2
August	37.5	24.1	36.8	19.5	11.9
September	35.0	21.6	43.5	21.7	10.8
October	32.8	17.7	49.5	19.2	10.0
November	27.0	12.7	54.7	15.1	9.40
December	19.9	6.30	59.7	16.8	9.00
			2017		
January	19.3	5.30	55.3	14.8	8.90
February	20.5	6.30	52.6	14.5	9.70
March	25.3	11.0	42.5	17.2	9.90
April	31.3	15.5	36.6	17.3	10.3
May	36.3	20.0	31.4	16.2	11.4
June	37.4	23.4	34.6	21.0	12.3
July	39.1	25.3	32.7	16.3	12.2
August	37.8	24.6	38.8	17.6	11.9
September	35.3	20.9	44.6	20.7	10.8
October	30.3	16.5	47.0	17.2	10.0
November	25.1	10.9	54.6	15.2	9.40
December	23.2	9.00	58.8	14.6	9.00

Max T = Maximum temperature (°C) Min T = Minimum temperature (°C), RH= Relative humidity (%) W.S = Wind speed (Km/h)

1- Yield

At harvest time the fruits per each tree was harvested and weighed as kg/tree.

2- Fruit cracking percentage

The percentage of cracked fruits was calculated as follows:

Cracking (%)/tree = (weight of cracked fruits per tree / Total yield per tree) x 100.

3-Physical characteristics

1- **Fruit weight** (g): was estimated as average weight of 10 fruits per replicate.
2- **Weight loss** %: calculated as a percentage from the initial weight.
3- **Chilling injured fruit %**: The weight of discarded fruits mainly due to chilling injury was recorded after every storage period (three weeks) and five days of shelf life at room temperature, and calculated as a percentage from the total weight of fruits.
4- **Peel weight %**: was calculated as a percentage of the fruit weight.
5- **Aril weight %**: was calculated as a percentage of the fruit weight.
6- **Juice weight %**: was expressed as percent of fruit weight (W/W).

4- Chemical juice constituents

1- The total soluble solids (TSS) were determined by using a hand refractometer.
2- Titratable acidity (TA %) as citric acid was determined by titration using 0.1 N of NaOH according to (A.O.A.C., 2000).
3- TSS / TA ratio was calculated by dividing TSS on TA.

Statistical analysis

All treatments of the field experiment were arranged in a randomized complete block design with three replicates. However, the storage treatments were arranged in a split-plot design, storage periods assigned in main plot and spraying with antioxidant treatments was considered as sup-plot with three replicates. The data was statistically analyzed by Statistix 8.1 software (Analytical Software, 2005), according to Snedecor and Cochran (1990) using L.S.D. at the level of 0.05.

Results

Effect of ascorbic acid and salicylic acid on yield, cracking and physico-chemical attributes of pomegranate fruit at harvest

Fruit yield/tree

The results in Table 2 indicate that all treatments were significantly increased yield compared to the non-treated control. The highest fruit yield was obtained by spraying trees with T3 (ascorbic acid (ASA) at 500ppm) followed by T2 (ascorbic acid at 250ppm), while the control treatment gave the lowest yield in both seasons.

Fruit cracking (%)

The results in Table 2 reveal that the highest fruit cracking % was recorded with control treatment by 10.70% and 9.85% for the 1st and 2nd seasons, respectively. On the other hand, the lowest fruit cracking% in the 1st season was 6.91% with T7 (ASA at 500 ppm + salicylic acid (SA) at 500 ppm), while in the second season it was 5.67% with T3. In general, all treatments recorded fruit cracking % lower than control treatment.

Physical properties of fruits

Fruit weight (g)

The results clearly show a significant effect on fruit weight (Table 2). T3 surpassed the other foliar spraying treatments, where it gave the highest fruit weight (394.6g and 409.8g) in both seasons, respectively. On the other hand, the lowest fruit weight (380.5g and 382.5g) in both seasons, respectively, was obtained by the control treatment.

Fruit peel%

In terms of their impact on the percentage of fruit peel, the highest percentage was observed by control treatment (46.8 % and 40.2 % during both of seasons, respectively). However, the lowest value of fruit peel percentage was recorded by T7, which recorded 39.4% and 33.9% in the first and second seasons, respectively. Moreover, no significant differences were found among T7 (ASA, 500 ppm +

SA, 500 ppm), T2 (ASA, 250ppm), T3 (ASA, 500ppm), T4 (SA, 250ppm) and T5 (SA, 500 ppm) treatments.

Fruit aril (%)

The treatment 7 induced the highest percentage of fruit aril in both seasons followed by T6 with no significant differences. The lowest percentage was recorded by the control treatment (Table 2).

Fruit juice%

The results in Table 2 show that the highest fruit juice % was found by T7, whereas the lowest was observed with control treatment (T1). In general, all levels of SA alone or in combination with ASA recorded significantly higher juice % compared to the control. Similar trend was observed in both seasons the study.

C- Chemical properties

Total soluble solids (TSS), Acidity (TA) % and TSS/TA ratio:

The highest TSS% was achieved by T3 which had no significant differences with T2 and T4 (Table 2). On the other hand, the lowest TSS% was noticed at control treatment (T1), which had no significant differences with T5 and T6 in the first season. As well, in the second season the highest TSS% was recorded by T2 and T3 but no significant differences with the rest treatments were found.

Regarding TA, the results in Table 2 show that the highest acidity value was obtained with control treatment compared to other treatments in both seasons. While T2 and T4 recorded lowest TA for 1st and 2nd seasons, respectively.

Table 2: Effect of ascorbic acid and salicylic acid on yield and physico-chemical fruit attributes of Manfalouty pomegranate fruits at harvest.

Treatments	Cross yield/ tree (kg)	Fruit creaking (%)	Fruit weight (g)	Fruit peel weight (%)	Aril weight (%)	Juice (%)	TSS (%)	TA (%)	TSS/ A ratio
				Season 2016					
T_1	79.50	10.70	380.5	46.8	53.2	37.19	14.13	1.39	10.17
T_2	96.55	7.49	390.7	44.9	55.1	38.97	14.80	0.96	15.42
T3	100.45	7.10	394.6	42.9	57.1	39.15	14.97	1.14	13.13
T_4	89.77	8.31	388.7	43.0	57	39.70	14.60	1.22	11.97
T_5	87.45	7.90	386.9	40.8	59.2	39.02	14.40	1.23	11.71
T_6	88.19	7.21	387.5	39.5	60.5	41.94	14.47	1.27	11.39
T_7	85.70	6.91	383.8	39.4	60.6	42.89	14.33	1.28	11.20
L.S.D. 0.05 :	5.53	1.19	3.12	1.13	3.53	1.77	0.44	0.11	0.47
				Season 2017					
T1	86.60	9.85	382.5	40.2	59.8	38.40	14.00	1.49	9.40
T2	102.50	6.22	404.1	38.9	61.1	39.88	14.97	1.36	11.01
T3	107.54	5.67	409.8	37.9	62.1	38.70	14.97	1.31	11.43
T4	100.39	6.86	400.9	38.2	61.8	40.95	14.23	1.16	12.27
T5	99.29	6.87	399.2	37.9	62.1	41.92	14.63	1.20	12.19
T6	91.59	8.62	395.9	35.1	64.9	40.84	14.50	1.19	12.18
T7	88.50	7.21	389.2	33.9	66.1	42.92	14.50	1.32	10.98
L.S.D. 0.05 :	3.76	0.32	1.47	2.74	2.38	1.35	0.87	0.29	0.88

T_1 Control
T_2 ASA at 250 ppm
T_3 ASA at 500 ppm
T_4 SA at 250 p.pm
T_5 SA at 500 ppm
T_6 ASA (250 ppm) plus SA (250ppm)
T_7 ASA (500 ppm) plus SA (500 ppm)

Results in Table 2 showed clearly that the T2 gave the highest TSS/TA value (15.42) in 1st season, whereas in 2nd season T4 recorded the highest one (12.27) compared to the control which recorded the lowest TSS/TA during 2016 and 2017 seasons.

Effect of ascorbic acid and salicylic acid on weight loss, fruit decay and physico-chemical attributes of fruits during cold storage and shelf life during:

Weight loss percentage

Results in Table (3) clear that fruit weight loss percentage was markedly increased with advancing storage period during the cold storage. As a general view, all treatments induced significant reduction in fruit weight loss % during cold storage periods as well as during shelf life through the two studied seasons. T6 gave the lowest level of fruit weight loss % (4.24%, 5.55% in cold storage in 2016 and 2017 seasons, respectively). However, no significant differences were found among T3, T5 and T6. Also, the interaction between treatments and cold storage periods showed a significant reduction in weight loss % during the two studied seasons as well as in the interaction between treatments and shelf life periods. Moreover, the lowest values of weight loss (%) during shelf life were recorded by the fruits under T5 and T4 in 2016 and 2017 seasons, respectively, compared with the other treatments.

Table 3: Effect of ascorbic acid and salicylic acid on weight loss (%) of Manfalouty pomegranate fruits during cold storage and shelf life through 2016 and 2017 seasons.

Treatments	Cold storage					Shelf life				
	Storage period									
	3W	6W	9W	12W	Mean	3W	6W	9W	12W	Mean
					Season 2016					
T_1	2.95	3.33	6.64	12.67	**6.40**	12.01	12.91	7.78	4.73	**9.36**
T_2	2.56	3.48	5.95	7.55	**4.89**	9.56	7.55	6.49	3.15	**6.69**
T3	2.24	3.55	4.61	7.52	**4.48**	9.30	8.45	6.35	3.16	**6.82**
T_4	3.27	4.61	6.24	8.89	**5.75**	9.00	8.65	5.32	3.60	**6.64**
T_5	2.26	3.57	5.04	7.22	**4.52**	8.76	9.06	4.17	3.51	**6.38**
T_6	2.15	3.18	4.66	6.98	**4.24**	10.75	9.74	5.89	4.97	**7.84**
T_7	2.57	3.38	5.06	8.44	**4.86**	8.57	10.19	5.59	4.55	**7.23**
Mean	**2.57**	**3.59**	**5.46**	**8.47**		**9.71**	**9.51**	**5.94**	**3.95**	
L.S.D. 0.05 :	**(P)**: 0.17 **(T)**: 0.40 **(PxT):** 0.80					**(P)**: 0.49 **(T)**: 0.37 **(PxT):** 0.74				
					Season 2017					
T_1	2.97	4.78	8.81	11.97	**7.13**	9.78	7.30	5.54	5.57	**7.05**
T_2	2.34	4.37	6.12	9.28	**5.53**	8.31	9.66	5.09	3.49	**6.64**
T3	2.41	4.40	5.51	9.66	**5.50**	8.21	7.33	4.13	4.28	**5.99**
T_4	2.83	5.51	6.19	9.67	**6.05**	9.07	5.22	4.97	3.58	**5.71**
T_5	3.03	4.46	5.61	9.52	**5.66**	9.93	9.24	3.77	3.84	**6.70**
T_6	3.75	4.07	6.11	8.26	**5.55**	8.22	7.10	4.86	4.33	**6.13**
T_7	2.77	4.27	5.69	10.52	**5.81**	9.58	9.22	4.27	3.88	**6.74**
Mean	**2.87**	**4.55**	**6.29**	**9.84**		**9.01**	**7.87**	**4.66**	**4.14**	**6.42**
L.S.D. 0.05 :	**(P)**: 0.22 **(T)**: 0.36 **(PxT):** 0.71					**(P)**: 0.23 **(T)**: 0.33 **(PxT):** 0.66				

W = week

T_1 Control
T_2 ASA at 250 ppm
T_3 ASA at 500 ppm
T_4 SA at 250 p.pm
T_5 SA at 500 ppm
T_6 ASA (250 ppm) plus SA (250ppm)
T_7 ASA (500 ppm) plus SA (500 ppm)

Fruit chilling percentage

At the end of cold storage or shelf life, in general, the results showed that all treatments markedly reduced chilled fruit percentage compared to the control treatment. However, the percentage of chilled fruits was increased with progress of storage periods or shelf life. The most significant treatments in reducing chilled fruits were T6 and T7 in the first season and T2 in the second season. Furthermore, during shelf life, salicylic acid 500ppm (T5) markedly reduced the percentage of chilled fruits comparing with all other treatments in the first season and both ascorbic acid at 250ppm (T2) and ascorbic acid at 500ppm plus salicylic acid 500ppm (T7) in the second season (Table 4).

Juice weight %

Results in Table (5) clear that fruit juice content was gradually and significantly decreased from the beginning until the end of cold storage. While, during the shelf life, this decrease was not significant during 2016 and 2017 seasons. As shown in Table (5), it could be obviously noticed that all treatments induced significant differences in juice weight % of fruits either during cold storage or shelf life in 2016 and 2017 seasons. However, T6 (ascorbic acid at 250ppm plus salicylic acid at 250ppm) gave the

Table 4: Effect of ascorbic acid and salicylic acid on chilling injury (%) of Manfalouty pomegranate fruits during cold storage and shelf life through 2016 and 2017 seasons.

Treatments	Cold storage					Shelf life				
	Storage period									
	3W	6W	9W	12W	Mean	3W	6W	9W	12W	Mean
					Season2016					
T1	16.67	27.78	33.33	50.00	**31.95**	0.00	9.45	24.00	33.44	**16.72**
T2	0.00	0.00	18.45	48.22	**16.67**	0.00	0.00	7.99	26.35	**8.59**
T3	0.00	15.56	35.44	49.03	**25.01**	0.00	0.00	16.42	33.58	**12.50**
T4	0.00	16.67	34.12	49.21	**25.00**	0.00	0.00	15.89	34.11	**12.50**
T5	0.00	0.00	10.01	23.32	**8.33**	0.00	0.00	0.00	16.67	**4.17**
T6	0.00	0.00	0.00	14.67	**3.67**	0.00	0.00	16.34	33.64	**12.50**
T7	0.00	0.00	0.00	16.67	**4.17**	0.00	0.00	0.00	32.98	**8.25**
Mean	**2.38**	**8.57**	**18.76**	**35.87**		**0.00**	**1.35**	**11.52**	**30.11**	
L.S.D. 0.05 :	**(P)**: 0.96	**(T)**: 1.25	**(PxT)**: 2.50			**(P)**: 1.28	**(T)**: 0.60	**(PxT):** 1.20		
					Season2017					
T1	0.00	7.77	18.65	55.00	**20.36**	0.00	0.00	24.89	36.67	**15.39**
T2	0.00	0.00	9.22	24.12	**8.34**	0.00	0.00	10.07	22.16	**8.06**
T3	0.00	10.34	19.11	45.55	**18.75**	0.00	6.34	21.80	30.33	**14.62**
T4	0.00	9.30	16.71	40.67	**16.67**	0.00	0.00	22.10	35.10	**14.30**
T5	0.00	7.64	17.37	25.00	**12.50**	0.00	0.00	18.87	31.12	**12.50**
T6	0.00	8.23	21.22	36.43	**16.47**	0.00	0.00	7.97	25.37	**8.34**
T7	0.00	6.14	18.87	38.12	**15.78**	0.00	0.00	9.14	25.00	**8.54**
Mean	**0.00**	**7.06**	**17.31**	**37.84**		**0.00**	**0.91**	**16.41**	**29.39**	
L.S.D. 0.05 :	**(P)**: 1.37	**(T)**: 1.45	**(PxT):** 2.90			**(P)**: 2.08	**(T)**: 0.82	**(PxT):** 1.64		

W = week

T1 Control
T2 ASA at 250 ppm
T3 ASA at 500 ppm
T4 SA at 250 p.pm
T5 SA at 500 ppm
T6 ASA (250 ppm) plus SA (250ppm)
T7 ASA (500 ppm) plus SA (500 ppm)

Table 5: Effect of ascorbic acid and salicylic acid on **juice** (%) of Manfalouty pomegranate fruits during cold storage and shelf life through 2016 and 2017 seasons.

Treatments	Cold storage					Shelf life				
	Storage period									
	3W	6W	9W	12W	Mean	3W	6W	9W	12W	Mean
					Season2016					
T1	37.16	36.77	36.27	35.93	**36.53**	41.86	41.38	41.28	41.25	**41.44**
T2	38.76	38.73	38.67	38.44	**38.65**	41.67	41.65	41.61	41.60	**41.63**
T3	38.93	38.52	38.27	38.67	**38.60**	41.53	41.38	41.24	41.17	**41.33**
T4	39.61	39.55	39.38	39.34	**39.47**	41.74	41.43	41.29	41.12	**41.40**
T5	38.95	38.89	38.69	38.26	**38.70**	40.81	40.77	40.36	40.15	**40.52**
T6	42.73	42.47	42.44	42.18	**42.46**	38.83	38.81	38.63	38.34	**38.65**
T7	41.63	41.42	41.34	41.11	**41.38**	40.96	40.83	40.79	40.01	**40.65**
Mean	**39.68**	**39.48**	**39.29**	**39.13**		**41.06**	**40.89**	**40.74**	**40.52**	**40.80**
L.S.D. 0.05 :	**(P)**: 0.45	**(T)**: 0.79	**(PxT):** 1.58			**(P)**: NS	**(T)**: 0.52	**(PxT):** 1.05		
					Season2017					
T1	38.48	38.21	38.20	37.67	**38.14**	42.63	42.23	41.44	41.29	**41.90**
T2	39.77	39.44	39.24	39.17	**39.41**	41.25	40.91	40.37	40.03	**40.64**
T3	38.38	38.15	38.09	38.08	**38.18**	41.51	41.07	40.57	40.23	**40.85**
T4	40.69	40.65	40.56	40.07	**40.49**	40.77	40.56	40.30	40.26	**40.47**
T5	41.85	41.32	41.26	41.10	**41.38**	41.58	41.69	40.80	40.61	**41.17**
T6	40.83	40.81	40.30	40.03	**40.49**	41.50	41.41	40.78	40.30	**41.00**
T7	42.90	42.72	42.22	42.09	**42.48**	41.81	41.66	40.94	40.70	**41.28**
Mean	**40.41**	**40.19**	**39.98**	**39.74**		**41.58**	**41.36**	**40.74**	**40.49**	**41.04**
L.S.D. 0.05 :	**(P)**: 0.61	**(T)**: 0.58	**(PxT):** 1.16			**(P)**: NS	**(T)**: 0.92	**(PxT):** 1.84		

W = week

T1 Control
T2 ASA at 250 ppm
T3 ASA at 500 ppm
T4 SA at 250 p.pm
T5 SA at 500 ppm
T6 ASA (250 ppm) plus SA (250ppm)
T7 ASA (500 ppm) plus SA (500 ppm)

highest value (42.46%) of juice weight % followed by T7 (fruits treated with ASA at 500ppm plus SA at 500ppm) (41.38%), while un-sprayed fruits (T1) gave the lowest value of juice weight % (36.53%) during 2016 season. In 2017, T7 (ascorbic acid at 500ppm plus salicylic acid at 500ppm) gave the highest value of juice weight % per fruits (42.48%), followed by T5 (salicylic acid at 500ppm) (41.38%), while the fruits sprayed with ascorbic acid at 500ppm (T3) or un-sprayed trees (T1) recorded the lowest value of juice weight % under cold storage.

On the other hand, regarding the shelf life, fruits treated by ASA at 250ppm (T2) recorded the highest value of juice weight %. While, ASA at 250ppm plus SA at 250 ppm (T6) gave the lowest value of juice weight % during shelf life of 2016 season. However, un-sprayed fruits (T1) gave the highest value of juice weight % compared with T4 (SA at 250 ppm) which recorded the lowest value during the shelf life of 2017 season.

Total soluble solids (TSS), titratable acidity % (TA, %) and (TSS/TA ratio) in juice:

Results in table (6) clear that total soluble solids percentage was markedly and gradually increased with advancing of storage period or shelf life in 2016 and 2017 seasons. The application of ascorbic acid at 250ppm (T2) gave the lowest total soluble solids percentage values during the cold storage in both study seasons without significant differences compared to the other treatments except with T3 and T5 in 1st season and T5 in 2nd one. Similar trend was noticed during shelf life, where T2 recorded the lowest TSS value whereas T5 gave the highest one. Hence, there is a positive relationship between the total soluble solids percentage and storage period.

Table 6: Effect of ascorbic acid and salicylic acid on **TSS** (%) of Manfalouty pomegranate fruits during cold storage and shelf life through 2016 and 2017 seasons.

	Cold storage					**Shelf life**				
.	**Storage period**									
Treatments	**3W**	**6W**	**9W**	**12W**	**Mean**	**3W**	**6W**	**9W**	**12W**	**Mean**
					Season2016					
T_1	14.50	14.80	15.20	16.13	**15.16**	14.80	15.27	15.33	16.40	**15.45**
T_2	14.70	14.90	14.96	15.07	**14.91**	14.87	14.93	14.97	15.13	**14.98**
T3	15.20	15.33	15.40	15.57	**15.38**	15.30	15.40	15.57	15.67	**15.49**
T_4	14.77	14.93	15.33	15.67	**15.18**	14.87	15.07	15.40	15.73	**15.27**
T_5	14.93	15.20	15.60	15.90	**15.41**	15.33	15.40	15.67	15.97	**15.59**
T_6	14.67	15.13	15.47	15.70	**15.24**	14.87	15.17	15.50	15.73	**15.32**
T_7	14.87	15.13	15.20	15.35	**15.14**	15.00	15.20	15.27	15.60	**15.27**
Mean	**14.81**	**15.06**	**15.31**	**15.63**		**15.01**	**15.21**	**15.39**	**15.75**	
L.S.D. 0.05 :	**(P)**: 0.25	**(T)**: 0.40	**(PxT): 0.80**			**(P)**: 0.36	**(T)**: 0.25	**(PxT):** 0.51		
					Season2017					
T_1	14.93	15.20	15.60	16.00	**15.43**	15.00	15.30	15.73	16.13	**15.54**
T_2	14.87	15.13	15.33	15.53	**15.22**	15.00	15.30	15.40	15.60	**15.33**
T3	15.00	15.30	15.50	15.73	**15.38**	15.07	15.33	15.53	15.73	**15.42**
T_4	14.93	15.00	15.34	15.87	**15.29**	15.33	15.40	15.50	16.10	**15.58**
T_5	14.93	15.47	15.93	16.07	**15.60**	15.30	15.60	16.00	16.10	**15.75**
T_6	14.67	15.53	15.80	16.13	**15.53**	14.73	15.60	15.83	16.13	**15.57**
T_7	14.53	15.33	15.35	15.60	**15.20**	14.80	15.40	15.60	15.67	**15.37**
Mean	**14.84**	**15.28**	**15.55**	**15.85**		**15.03**	**15.42**	**15.66**	**15.92**	
L.S.D. 0.05 :	**(P)**: 0.65	**(T)**: 0.35	**(PxT):** 0.71			**(P)**: 0.13	**(T)**: 0.12	**(PxT):** 0.24		

W = week

T_1	Control	**T_5**	SA at 500 ppm
T_2	ASA at 250 ppm	**T_6**	ASA (250 ppm) plus SA (250ppm)
T_3	ASA at 500 ppm	**T_7**	ASA (500 ppm) plus SA (500 ppm)
T_4	SA at 250 p.pm		

Results in table (7) showed that titratable acidity content was decreased with the advancing of storage period. Such trait was gradually and significantly decreased from the beginning of storage either during cold storage or shelf life. The application of ascorbic acid at 250ppm plus SA at 250ppm (T6) or control (T1) had higher values of total acidity percentage (1.04) and (1.03) in the first seasons, while, (1.03) and (1.05) in the second season. Thus, the highest values of total acidity percentage (0.91 and 0.98%) were obtained in both seasons, respectively, under shelf life.

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Table 7: Effect of ascorbic acid and salicylic acid on acidity (%) of Manfalouty pomegranate fruits during cold storage and shelf life through 2016 and 2017 seasons.

	Cold storage					Shelf life				
	Storage period									
Treatments	**3W**	**6W**	**9W**	**12W**	**Mean**	**3W**	**6W**	**9W**	**12W**	**Mean**
					Season2016					
T_1	1.19	1.08	1.02	0.84	**1.03**	1.09	0.93	0.84	0.76	**0.91**
T_2	0.90	0.85	0.77	0.76	**0.82**	0.93	0.92	0.81	0.75	**0.85**
T3	0.89	0.86	0.83	0.79	**0.84**	1.08	0.93	0.73	0.70	**0.86**
T_4	0.95	0.80	0.72	0.71	**0.80**	0.99	0.82	0.79	0.77	**0.84**
T_5	1.11	1.01	0.98	0.90	**1.00**	0.97	0.87	0.82	0.75	**0.85**
T_6	1.12	1.11	1.02	0.91	**1.04**	1.08	0.92	0.84	0.68	**0.88**
T_7	1.03	0.99	0.87	0.84	**0.93**	1.10	0.87	0.80	0.76	**0.88**
Mean	**1.03**	**0.96**	**0.89**	**0.82**		**1.03**	**0.89**	**0.80**	**0.74**	**0.87**
L.S.D. 0.05 :	**(P)**: 0.02	**(T)**: 0.02	**(PxT):** 0.05			**(P)**: 0.04	**(T)**: 0.03	**(PxT):** 0.06		
					Season2017					
T_1	1.17	1.10	1.01	0.93	**1.05**	1.11	1.09	0.94	0.79	**0.98**
T_2	1.00	0.81	0.74	0.73	**0.82**	0.84	0.80	0.76	0.75	**0.79**
T3	1.05	0.92	0.83	0.78	**0.90**	1.04	0.89	0.83	0.67	**0.86**
T_4	1.17	0.98	0.86	0.78	**0.95**	1.04	0.97	0.97	0.93	**0.98**
T_5	1.15	0.96	0.90	0.79	**0.95**	1.19	0.94	0.89	0.67	**0.92**
T_6	1.12	1.19	0.92	0.89	**1.03**	0.99	0.98	0.68	0.54	**0.80**
T_7	1.13	1.01	0.97	0.75	**0.97**	1.01	0.98	0.93	0.74	**0.92**
Mean	**1.11**	**1.00**	**0.89**	**0.81**		**1.03**	**0.95**	**0.86**	**0.73**	**0.89**
L.S.D. 0.05 :	**(P)**: 0.15	**(T)**: 0.06	**(PxT):** 0.11			**(P)**: 0.09	**(T)**: 0.06	**(PxT):** 0.11		

W = week

T_1 Control
T_2 ASA at 250 ppm
T_3 ASA at 500 ppm
T_4 SA at 250 p.pm
T_5 SA at 500 ppm
T_6 ASA (250 ppm) plus SA (250ppm)
T_7 ASA (500 ppm) plus SA (500 ppm)

Table 8: Effect of ascorbic acid and salicylic acid on **TSS** /acid ratio of Manfalouty pomegranate fruits during cold storage and shelf life through 2016 and 2017 seasons.

	Cold storage					Shelf life				
	Storage period									
Tretments	**3W**	**6W**	**9W**	**12W**	**Mean**	**3W**	**6W**	**9W**	**12W**	**Mean**
					Season2016					
T_1	12.18	13.70	14.90	19.20	**15.00**	13.58	16.42	18.25	21.58	**17.46**
T_2	16.33	17.53	19.43	19.83	**18.28**	15.99	16.23	18.48	20.17	**17.72**
T3	17.08	17.83	18.55	19.71	**18.29**	14.17	16.56	21.33	22.39	**18.61**
T_4	15.55	18.66	21.29	22.07	**19.39**	15.02	18.38	19.49	20.43	**18.33**
T_5	13.45	15.05	15.92	17.67	**15.52**	15.80	17.70	19.11	21.29	**18.48**
T_6	13.10	13.63	15.17	17.25	**14.79**	13.77	16.49	18.45	23.13	**17.96**
T_7	14.44	15.28	17.47	18.27	**16.37**	13.64	17.47	19.09	20.53	**17.68**
Mean	**14.59**	**15.95**	**17.53**	**19.14**		**14.57**	**17.04**	**19.17**	**21.36**	**18.03**
L.S.D. 0.05 :	**(P)**: 1.00	**(T)**: 1.16	**(PxT):** 2.33			**(P)**: 0.88	**(T)**: 0.80	**(PxT):** 1.60		
					Season2017					
T_1	12.76	13.82	15.45	17.20	**14.81**	13.51	14.04	16.73	20.42	**16.18**
T_2	14.87	18.68	20.72	21.27	**18.88**	17.86	19.13	20.26	20.80	**19.51**
T3	14.29	16.63	18.67	20.17	**17.44**	14.49	17.22	18.71	23.48	**18.48**
T_4	12.76	15.31	17.84	20.35	**16.56**	14.74	15.88	15.98	17.31	**15.98**
T_5	12.98	16.11	17.70	20.34	**16.78**	12.86	16.60	17.98	24.03	**17.87**
T_6	13.10	13.05	17.17	18.12	**15.36**	14.88	15.92	23.28	29.87	**20.99**
T_7	12.86	15.18	15.82	20.80	**16.17**	14.65	15.71	16.77	21.18	**17.08**
Mean	**13.37**	**15.54**	**17.62**	**19.75**	**16.57**	**14.71**	**16.36**	**18.53**	**22.44**	**18.01**
L.S.D. 0.05 :	**(P)**: 1.00	**(T)**: 0.55	**(PxT):** 1.12			**(P)**: 1.82	**(T)**: 1.69	**(PxT):** 3.39		

W = week

T_1 Control
T_2 ASA at 250 ppm
T_3 ASA at 500 ppm
T_4 SA at 250 p.pm
T_5 SA at 500 ppm
T_6 ASA (250 ppm) plus SA (250ppm)
T_7 ASA (500 ppm) plus SA (500 ppm)

Concerning the effect of all treatments on TSS/TA ratio in juice, it could be observed that TSS/TA ratio in fruit juice was increased with increasing cold storage periods and shelf life during the two seasons. Moreover, in 2016, fruits treated with SA at 250ppm (T4) induced the highest value of TSS/TA ratio; followed by ASA at 250ppm (T2) then ASA at 500ppm (T3), with no significant difference among them. While ASA at 250 ppm plus SA at 250 ppm (T6) recorded the lowest level of TSS/TA ratio during the same season. On the other hand, in 2017, the application of ASA 250ppm (T2) gave the highest level of TSS/TA ratio in juice. However, control fruits (T1) produced the lowest level of TSS/TA ratio in juice. In addition, control (T1) resulted in the least level of TSS/TA ratio in juice during shelf life in the first season, while SA at 250 ppm (T4) recorded the least value in second season.

Discussion

The cultivated area of Pomegranates greatly spread in Egypt as well as over the world due to high demands of their high nutritional value (Kahramanoglu, 2019). Many problems face pomegranate fruits export and prolong storage such as weight loss, shrinking, chilling injury and maintaining fruit quality during transport and storage (Orabi *et al.*, 2020). Assiut governorate are exposed to heat stress as cleared in metrological data in Table (1), environmental conditions, including high temperatures, especially in the summer months affect productivity, yield, and marketable fruits.

From the above mentioned results, all treatments of preharvest application of salicylic acid (SA) and ascorbic acid (ASA) significantly increased yield and fruit weight in both seasons. T3 (ASA at 500ppm), recorded the highest yield by 26.35 and 24.18 % increasing compared to control for both seasons respectively, followed by T4 (SA, 250ppm). It was previously proved that SA increased cell division and cell expansion (Hayat *et al.*, 2005). The results of present study are in line with the previous results obtained by Abdel Aziz *et al.* (2017) on pomegranates who found that the application of SA from 50 to 200 ppm significantly increased fruit yield. Also, the application of SA at 1mM and 2mM increased the average of peach fruit weight by 20 and 16 %, respectively, as well as the fruit dimensions (diameter and length) were also significantly increased (Erogul and Özsoydan, 2020). Similar results were obtained on olives (Hagagg *et al.*, 2020) and grapevine (Marzouk and Kassem, 2011) by application of SA and ASA. ASA and SA could play as auxinic action that might increase fruit size and number (Ragab, 2002). They also could decrease the abscission of fruit.

Fruit cracking (splitting), is the most important physiological disorder faces pomegranate growers and reduces pomegranate fruit yield, quality, marketability and exportability (Ahmed *et al.*, 2014; Abdel Aziz *et al.*, 2017; Singh *et al.*, 2020). The present results showed that all treatments significantly decreased fruit cracking % compared to the control. In general, our results showed lower cracking percentage compared to the previous studies, about 30 % (in control treatment) Abdel Aziz *et al.*, (2017), to 65% Singh *et al.*, (2020). The different observation reasons of fruit cracking were previously reported and might be due to inconstancy of day and night temperature, imbalance of water relation, sharp temperature, low humidity and high evapo-transpiration during fruit growth and development as well as unbalanced nutrition, irregular irrigation and cultivar (Taiz and Zeiger, 2002; Singh *et al.*, 2020). The beneficial effect of SA on decreasing fruit cracking was previously reported (Abdel Aziz et al 2017), who found that the application SA at 200 ppm three times reduced fruit cracking in pomegranate from 20 and 19.9 % to 6.9 and 5.7 % in both seasons. SA and ASA are enhancing plant photosynthesis (Pignocchi and Foyer, 2003; Sharma *et al.*, 2020), improving uptake and transport of nutrients that could contribute in decreasing fruit cracking and /or increase the tolerance of plant to inconstant conditions that cause fruit cracking.

The deterioration of pomegranate during cold storage is mainly due to the chilling injuries. Our present results revealed that all treatments greatly and significantly reduced chilled fruits % (discarded fruits) compared to the control. SA alone or combined with ASA was more effective in suppressing fruit chilling. The percentage of chilled fruits was reduced to less than 4% by T6 (SA, 205ppm and ASA, 250ppm) compared to 30% with control treatment in first season. The application of SA and / or ASA induced the systemic resistance that improve the tolerance of fruit to abiotic and / or biotic stresses (Yao and Tian, 2005; Chan and Tian 2006; Shah *et al.*, 2011). Commercially pomegranate fruits are stored at 5 ± 1 °C to prolong fruits storability. Under these conditions fruits are subjected to chilling injuries such as discoloration in fruit peel and arils. The application of antioxidants compounds such as SA and ASA could increase the activities of antioxidant enzymes that decrease the activity of cell wall degrading enzymes and increase the fruit postharvest life as well as delaying fruit ripening (Vlot *et al.*, 2009; Khademi and Ershadi, 2013). Moreover, they induce plant tolerance to different stresses such as high and low temperature (Khan *et al.*, 2013a Muzammil *et al.*, 2014). SA at 0.5 mM can moderate heat stress by increasing pro-production and restriction of the stress ethylene formation under heat stress (Khan *et al.*, 2013b).

The above mentioned results revealed that the lower rate of fruit weight loss (%) was recorded by SA and/ or ASA treated fruits during the cold storage and shelf life. The low rate of weight loss may be due to lower rates of respiration and transpiration (Manthe *et al.*, 1992; Sartaj *et al.*, 2013). TSS values were generally increased in the fruits during the cold storage or shelf life that could be due to the reduction in fruit weight and subsequently fruit juice concentration (Khademi and Ershadi, 2013). TSS% was higher by application of different treatments that could be as above mentioned due to the reduction of respiration rate (Sartaj *et al.*, 2013). Also, Chanikan *et al.*, (2015) reported that the application of SA could slow the rate of respiration that also may decrease the decline in TA. Our results showed significant effects on TA that in contrast with those found by Ranjbaran *et al.*, (2011) on grapevines, where they found no significant effects of SA on TSS or TA. The beneficial effect of SA and ASA on reducing weight loss, chilling injury, reducing decay and improving fruit quality of many fruit crops during the storage were previously reported (Yao and Tian, 2005; Asghari and Aghdam, 2010; Wei *et al.*, 2011; Bondok *et al.*, 2011; Khademi and Erachidi, 2013; Alejandra *et al.*, 2017; Ennab *et al.*, 2020; Lokesh *et al.*, 2020; Haggag *et al.*, 2020; Haider *et al.*, 2020).

Conclusion

In general, it could be concluded from the above mentioned results that the preharvest application of salcilcylic acid (SA) and/or ascorbic acid (ASA) improved total fruit yield per tree as well as most of fruit quality parameters such as fruit weight, fruit aril weight %, juiciness %, TSS and TSS/acid ratio at harvest date. In addition, they significantly reduced fruit cracking and peel weight % at harvest that increased the marketability % of the fruits. Moreover, the treatment of SA and or ASA improved the storability of Manfalouty pomegranate fruits at cold storage (12 weeks), as well as shelf life for five days. They significantly decreased the fruit chilling injury (discoloration) and fruit weight loss % and delayed the decline in fruit quality parameters at the cold storage and shelf life.

Conflict Of Interest

The authors declare no conflict of interest.

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