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VITAMINS APPLICATION AND PERSIMMON (*Diospyros kaki* L. CV 'COSTATA'), FRUIT QUALITY

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ABSTRACT

The present investigation was conducted during two seasons (2007 and 2008) on Japanese persimmon trees located at commercial orchard, cv Costata, which considered the main and early ripening cultivar. Spraying trees with ascorbic acid (AA) and cyanocobalamine (B_{12}) in single or combined treatments were applied at different stages of development. Generally, fruit firmness values were gradually reduced from zero time of storage till the end of storage period in all treatments of AA and vitamin B_{12} alone or in combination. The influence of both AA and B_{12} in maintaining fruit firmness at harvest time and during the storage studied or shelf-life' resulted in increasing fruits firmness than the control. Total Soluble Sugars increased by applying AA and B_{12} sprays alone or in combination at different concentrations. Total tannins content of fruits decreased significantly during shelf-life period. Generally all treatments of AA and B_{12} alone or in combinations decreased total tannins content. Total phenolic contents significantly increased in the persimmon fruit as affected by different treatments during per-harvest, period, whereas AA alone at 0.5 and 1% have achieved the highest contents of total phenolic substances followed by AA combined with B_{12} sprays at two season of the study at harvest time. Ion leakage percentage increased with advancing the shelf-life period till the end of storage to 15 days, as affected by different treatments applied of AA and B_{12} alone or in combination. It is apparent that tree sprayed with AA and B_{12} has less ion leakage than control fruits during shelf-life period.

INTRODUCTION

Persimmon (*Diospyros kaki* L.) is one of the most important fruit was recently cultivated in Dakahlia province, where cultivated area has reached to about 750 feddans according to the last statistics of Ministry of Agriculture in Egypt (2006). Its fruit contains a higher content of dietary fibers, total and major phenolic substances, vitamins, and trace elements, by which are fruits preferable for healthy diet (Gorinstein, *et al*., 2001; Zisheng, 2006**).** Originally, it was cultivated in China and Japan. It is also known as a Chinese date palm, as referred to as the "apple of Orient" belongs to the genus *Diospyros* in the ebony family Ebenaceae. The name *Diospy-ros* is derived from the Greek name; grain of jove, referring to the edible fruits. The genus was contained approximately 200 species which were distrusted in wide ranges. The most of them are native to tropical and subtropical regions, and embraces evergreen and deciduous trees or shrubs (Austin, 2004).

Persimmon, 'Costata' cv is considered one of the important astringent and/early cultivar which is very easy to soften after harvesting and

handling at ambient temperature (Zisheng, 2006). Although, Egypt has a great potential to produce high fruit quality and export to other countries, Fruits marketability is still limited to local market. This may be due to the delicate nature of fruit, poor post-harvest technique for handling and transporting and storage facilities (Özdemir et al., 2009), as well as the common technique for controlling fruit ripening processes under ambient temperature. The rate of postharvest deterioration is affected by temperature as reviewed by Lo'ay, (2010). Even it used to prevent many post-harvest aspects such as, controlling insect pests, fungal rots, and increasing chilling injury tolerance. Therefore, heat application is being extensively studied as commercial method to control fruit ripening (Zisheng, 2006).

Several authors have been used the ascorbic acid (AA) on trees for its a stimulatory effect, since, it could be involved in a wide range of different important functions as an antioxidant quencher of active oxygen species (AOS) and protection of photosynthesis systems I and II with conjunction of carotenoids functions. Moreover, AA was associated with other antioxidant enzymes functions such as superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX) and catalase (CAT) (Lo'ay, 2005).

Higher plants neither synthesize nor require vitamin B_{12} because they contain cobalamin-independent methioninesynthase (*Met*) pathway by which cobalamin will metabolize in plant cell (Smith et al., 2007). In plants *Met* serves as a precursor for a variety of metabolic processes, including protein synthesis, as the prime methyl donor for a large number of biological methylations, polyamine synthesis and ethylene synthesis. Since methionine synthase is also required for both the regeneration and the de novo biosynthesis of *Met*. It is the convergence point for two major biochemical domains in cellular metabolism, the *Met* biosynthetic pathway and the one-carbon cycle (Zeh et al., 2002)

In Egypt, no study has been reported on spraying kaki fruit trees by B_{12} and AA solutions to increase fruit quality during shelf-life period. Therefore, the objective of this study is a trial to exploit the physiological roles of B_{12} and AA for prolonging the shelf-life of 'Costata' cv and improving fruit quality.

MATERIALS AND METHODS

Fruit materials and experimental setup

This investigation was conducted during two seasons (2007 and 2008) on Japanese persimmon trees of 20 years old Costata cv and located in "Fisha-banna" village, Dakhalia province. Costata cultivar was budded onto *D. virginiana* rootstock, it belongs to the major group of astringent cultivars and can be subdivided on the base of response of pollination on the color of flesh to the subdivision of constant cultivars which no changes in flesh color occurs after pollination and fruit set, (APC). The 'Costata' cv considered to be an early cultivar among the other kaki cultivars in Egypt. Trees were selected approximately vigorously uniform in their size and free from diseases. Regular horticultural practices were performed according to the standard

commercial system of production of this cultivar. Trees were planted at 4x4 meters apart in clay loamy soil. Trees were sprayed with AA and B_{12} solutions which were applied in single or in combination applications at different stage of development; the application of solutions was applied at four times as pre-harvest treatments as follows:

1) At the beginning of new leaves emergence.
2) At the onset of fruit set.
3) At reaching diameter of fruit to more than 2.5-3.5 cm.
4) At reaching diameter of fruit to more than 4.5-6 cm.

The following treatments were applied: AA at 2.8 mM and 5.6 mM and B_{12} at 0.36 and 0.74 µM. The combinations between them are AA 2.8 mM + B_{12} 0.36 µM; AA 2.8 mM + B_{12} 0.74 µM; B_{12} 0.36 µM + AA 5.6 mM; AA 5.6 mM + B_{12} 0.74 µM and spraying with tap water as a control. Fruits were harvested at 50% yellow and 50% orange (Oz, 2011), the 945 fruits were harvested in uniform. The fruit were distributed according to treatments; only each treatment was 105 fruit which divided into three replicates (35 fruits). All treatments were stored at room temperature at 26±2^{o}C and relative humidity 71%. Nine fruits were collected every 5 days intervals for chemical measurements.

Measurements

Fruit firmness: At maturity stage, Effegi hand-held, penetrometer Facchini, Alfonsine, Italy Effegi-penetrometers supplemented with a plunger 8.0 mm diameter and were used to measure firmness of kaki fruits. Values were recorded in triplicate then the average was estimated as Ib/Inch^2, according to (Watkins and Harman, 1981).

Total Soluble Sugars was determined by using phenol 18% and sulphuric acid 96% and the absorbance was spectrophotometrically recorded at 490 nm, according to the method described by Sadasivam and Manickam (1992). A standard curve was prepared by plotting the known concentrations of glucose solution (100 µg ml^{-1} of glucose) against respective optical density (OD) values of each. From the standard curve, the amount of total soluble sugars actually present in the sample was determined.

Total tannins were determined in fruit juice by titrating 5 ml of juice against 0.1 *N* of potassium permanganate ($KMnO_4$) using indigo-carmine as indicator. A O A C (1980).

Total phenolic content was determined in fresh fruits by using Folin–Ciocalteau's phenol reagent which consists of 2.5% sodium molybdate and 19% sodium tungstate according to (Gutfinger 1981). About 0.1g of air dried fruits were dissolved, separately in 1 ml distilled water. Aliquots of 0.1 ml from previous solution was taken and mixed with exactly 2.8 ml of distilled water, 2.0 ml of 2% (w/v) sodium carbonate and finally 0.1 ml of 50% (v/v) of Folin–Ciocalteau's reagent. Mixture was incubated for 30 minutes at room temperature and the absorbance of the resulting color was measured at 750 nm against distilled water as blank, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. For quantitatively determination a standard curve of gallic acid (0-200mg/l) was prepared in the same manner. Total phenolic contents were expressed as milligram gallic acid equivalent GAE g^{-1} based on fresh weight.

Ion leakage: Samples of 9 fruits were collected at 5-day intervals. Disks (7 mm diameter) of peel and pulp tissue were cut from five different parts of each fruit using a cork-borer. The disks were washed three times in demineralized water and placed in 10 ml 0.4 M mannitol in demineralized water at 24°C for 3h (Lo'ay 2005). Electrical conductivity of the aqueous phase was measured using conductivity meter, after which the tissue samples were killed by heating in water bath at 100°C for 20 minutes. This cooking process allows the release of all electrolytes from the tissue. Once cooled to room temperature the conductivity was re-measured and the relative electrolyte leakage from the uncooked pulp and peel samples was calculated as follows:

$$IL\ \% = \left(\frac{\text{Conductivity after 3 hour}}{\text{Conductivity after boiling}}\right) * 100$$

Statistical analysis:

Complete randomized block design was carried out with three replications according to Snedecor and Cochran (1972). Averages were compared using the LSD values at 5% level of probability.

RESULTS AND DISCUSSION

Fruit firmness

Data in Table 1 showed that the fruit firmness values were gradually reduced from Zero time of shelf-life till the end of storage period in all treatments of AA and vitamin B_{12} and control fruits. While, the firmness values of control fruits were lower than treated fruits in both seasons of study.

Table 1: Effect of pre-harvest treatments of spraying AA and B_{12} on persimmon fruits firmness (lb $Inch^{-2}$) during shelf-life period.

Treatments	2007				LSD at 5%	2008				LSD at 5%
	Storage period in days					Storage period in days				
	0	5	10	15		0	5	10	15	
AA 2.8 mM	17.30	15.50	13.00	12.03	0.54	17.30	14.66	13.16	12.03	0.09
AA 5.6 mM	17.90	15.63	12.56	11.40	0.11	17.50	15.00	14.06	12.30	0.94
B_{12} 0.36 µM	17.20	14.50	12.93	11.10	0.11	16.40	13.96	12.83	11.60	0.09
B_{12} 0.74 µM	16.60	14.53	12.16	10.55	0.11	16.70	13.93	11.63	10.46	0.09
AA 2.8 mM + B_{12} 0.36 µM	17.30	14.96	12.50	10.60	0.11	16.90	13.36	12.23	10.33	0.09
AA 2.8 mM + B_{12} 0.74 µM	16.40	15.23	12.36	11.46	0.11	16.90	13.66	11.53	10.03	0.07
AA 5.6 mM + B_{12} 0.36 µM	17.10	15.70	12.93	11.83	0.11	17.10	13.30	12.23	10.20	0.09
AA 5.6 mM + B_{12} 0.74 µM	16.80	13.66	12.80	10.20	0.11	16.50	13.00	11.66	10.26	0.54
Control	16.20	13.56	11.76	9.56	0.11	16.50	12.60	11.50	9.70	0.11
LSD at 5%	**0.09**	**0.09**	**0.34**	**0.09**	-----	**0.09**	**0.47**	**0.04**	**0.08**	----

These results reflect the influence of both AA and B_{12} in maintaining fruit firmness during shelf-life; this may be decreasing the softening of persimmon fruit under shelf-life conditions by using AA treatments at different concentration. Thereafter, AA keeps the pectinase enzyme at reductive

status by quenching AOS during shelf-life period (Lo'ay 2005). However, fruits firmness declined rapidly compared with control fruit when fruit immersed in B_{12} solution at different concentration. It may be related with B_{12} generates ethylene production by which generating ripping processes during shelf-life. It could be also illustrated that the hydrolysis process of cell wall polysaccharides enhanced more rapidly as affected by immersing in B_{12} solutions (Lo'ay 2010).

Total Soluble Sugars:

It is clear from the data presented in Table 2 that a pertinent effect of pre-harvest treatment by AA and B_{12} sprays alone or in combination at different concentrations applied on the total sugars of persimmon fruits was observed in both seasons of the study. A clear increasing trend was observed at the end of shelf-life period. The obtained data are in accordance with those reported by different authors that metabolic processes may enhance by AA and B_{12}. Increasing of total sugars during shelf-life period may be due to that B_{12} treatment activate both carbohydrate pathways of Calvin and pentose phosphate and glycolysis (Asensi-Fabado and Munne´-Bosch, 2010). Afterword, many metabolic processes are enhanced which are diverted toward glucose, fructose and sucrose increases during shelf-life period (Bubba et al., 2009).

Table 2: Effect of pre-harvest treatments of spraying AA and B_{12} on total sugar content (100 µg ml^{-1} of glucose) of persimmon fruits during shelf-life period.

Treatments	2007				LSD at 5%	2008				LSD at 5%
	Storage period in days					Storage period in days				
	0	5	10	15		0	5	10	15	
AA 2.8 mM	13.19	14.03	14.62	15.23	0.05	14.19	14.77	15.37	16.23	0.05
AA 5.6 mM	13.47	14.31	14.87	15.48	0.07	14.47	15.05	15.64	16.49	0.01
B_{12} 0.36 µM	13.35	14.19	14.77	15.38	0.07	14.35	14.92	15.51	16.36	0.01
B_{12} 0.74 µM	13.60	14.44	15.05	15.66	0.07	14.60	15.17	15.76	16.62	0.05
AA 2.8 mM + B_{12} 0.36 µM	13.75	14.59	15.19	15.80	0.09	14.75	15.32	15.91	16.74	0.01
AA 2.8 mM + B_{12} 0.74 µM	14.02	14.86	15.44	16.04	0.54	15.00	15.58	16.17	16.99	0.54
AA 5.6 mM + B_{12} 0.36 µM	13.91	14.74	15.34	15.95	0.07	14.90	15.48	16.06	16.90	0.07
AA 5.6 mM + B_{12} 0.74 µM	14.19	15.03	15.65	16.26	0.05	15.11	15.67	16.27	17.14	0.01
Control	13.08	13.91	14.46	15.07	0.01	14.13	14.70	15.31	16.23	0.15
LSD at 5%	**0.06**	**0.08**	**0.33**	**0.03**	**----**	**0.36**	**0.04**	**0.009**	**0.03**	**----**

Total tannin content

It is evident from data presented in Table 3 that holding Costata fruits at 20±5 ^{o}C, led to a sharp decrease in total tannins content with expanding the storage period. Generally all treatments of AA and B_{12} alone or in combinations were enhanced the decline of total tannins content. Removal of astringency correlated with the amount of soluble tannins, while avoiding softening is necessary for maintaining fruit quality and extending persimmon shelf-life control fruits clearly had the lowest tannins values after 15 days of storage in both seasons. It can be concluded that treatments of AA and B_{12}

may resulted in reducing tannins content with abnormal softening for persimmon fruits. It suggested that B_{12} provoked ethylene synthesis by recycling of sulphur atom of methionine, then it activates 1-aminocyclopropane-1-carboxylate synthase to produce ethylene (Ravanel et al., 1998). So, generating fruit ripening via B_{12} application during their shelf-life by increasing ethylene synthesis, since, the kaki fruit is classified as climacteric fruit (Nakano et al., 2003).

Table 3: Effect of pre-harvest treatments of spraying AA and B_{12} on tannin % of persimmon fruits during shelf-life period.

Treatment	2007				LSD at 5%	2008				LSD at 5%
	Storage period in days					Storage period in days				
	0	5	10	15		0	5	10	15	
AA 2.8 mM	1.81	1.67	1.38	1.17	0.01	1.88	1.70	1.45	1.09	0.05
AA 5.6 mM	1.80	1.64	1.30	1.09	0.07	1.87	1.65	1.40	1.11	0.05
B_{12} 0.36 µM	1.82	1.34	1.17	0.93	0.01	1.86	1.25	1.10	0.93	0.05
B_{12} 0.74 µM	1.81	1.55	1.23	0.78	0.01	1.88	1.40	1.05	0.85	0.05
AA 2.8 mM + B_{12} 0.36 µM	1.80	1.63	1.33	1.02	0.05	1.85	1.65	1.40	1.05	0.05
AA 2.8 mM + B_{12} 0.74 µM	1.81	1.60	1.27	1.11	0.05	1.85	1.59	1.23	1.09	0.01
AA 5.6 mM + B_{12} 0.36 µM	1.81	1.41	1.11	0.88	0.01	1.87	1.30	1.06	0.92	0.05
AA 5.6 mM + B_{12} 0.74 µM	1.82	1.43	1.17	0.86	0.01	1.86	1.22	1.00	0.87	0.54
Control	1.83	1.65	1.40	1.25	0.05	1.90	1.72	1.55	1.30	0.07
LSD at 5%	**0.04**	**0.03**	**0.04**	**0.009**	**-----**	**0.03**	**0.05**	**0.33**	**0.03**	**----**

Total phenolic contents

Table 4 shows that total phenolic contents increased in the persimmon fruit as affected by vitamin treatments compared with control treatment at per-harvest period, whereas AA alone at 2.8 mM and 5.6 mM resulted in the highest contents of total phenolics followed by AA combined with B_{12} sprays at two season of the study at harvest time. The highest amount of phenolic substances may reflect higher antioxidant activity, which related to proper quality of persimmon fruits under room storage till the end of storage period.

Table 4: Effect of pre-harvest treatments of spraying AA and B_{12} on total phenolics content (mg GAE g^{-1}FW) of persimmon fruits during shelf-life period.

Treatment	2007				LSD at 5%	2008				LSD at 5%
	Storage period in days					Storage period in days				
	0	5	10	15		0	5	10	15	
AA 2.8 mM	6.52	4.51	3.24	2.76	0.01	8.25	6.39	5.11	3.57	0.01
AA 5.6 mM	7.34	4.70	3.48	3.30	0.07	8.92	6.92	5.39	3.90	0.05
B_{12} 0.36 µM	5.82	3.66	2.50	1.69	0.05	6.90	5.42	3.36	1.99	0.05
B_{12} 0.74 µM	5.52	3.64	2.44	1.57	1.62	6.65	5.24	3.21	1.89	0.05
AA 2.8 mM + B_{12} 0.36 µM	5.77	3.85	2.87	1.89	0.01	7.25	5.77	3.60	1.96	0.05
AA 2.8 mM + B_{12} 0.74 µM	5.22	3.75	2.75	2.54	0.05	7.12	5.73	3.42	1.87	0.01
AA 5.6 mM + B_{12} 0.36 µM	6.25	3.86	3.17	2.84	0.02	7.99	5.85	4.41	2.50	0.05
AA 5.6 mM + B_{12} 0.74 µM	5.92	4.36	3.12	2.58	0.05	7.90	5.81	4.12	3.33	0.05
Control	5.17	3.49	2.23	1.38	0.01	6.31	4.57	2.99	1.81	0.01
LSD at 5%	**0.009**	**0.99**	**0.03**	**0.03**	**-----**	**0.04**	**0.009**	**0.03**	**0.04**	**------**

The content of total phenolics and radical scavenging activity of different persimmon cultivars showed positive and highly relationship. Applications of AA are mainly responsible for the expected antioxidant activities. The obtained results are in harmony with those reported by Kaur, (2002) and Jang *et al.* (2010). The applied treatments of both AA alone or in combinations with those of vitamin B_{12} resulted in decreasing pattern of total phenolic substances during the shelf-life periods, but the contents were higher than the control fruits. The results may reflect the proper quality of persimmon fruits, and revealed to the beneficial effect of the applied treatments.

Ion leakage%

Ion leakage percentage increased with advancing the storage period till the end of storage to 15 days, as affected by different treatments applied of AA and B_{12} alone or in combination (Table 5). It's apparent from data of the same table that fruits sprayed with AA and B_{12} has less ion leakage than control fruits. The differences were significantly varied among the applied treatments and control one. These responses of both AA and vitamin B_{12} during storage period and shelf-life could be related to that substance, keep enhancing indigenous ascorbic acid and ß-carotene and α-Tocopherol in fruit tissues (Loay 2010). Since the compounds may be considered as antioxidants which play a pertinent role to scavenge active oxygen species during shelf-life (A sensi-Fabado and Munne-Bosch, 2010).

Table 5: Effect of pre-harvest treatments of spraying AA and B_{12} on Ion leakage %) of persimmon fruits during shelf-life period.

Treatments	2007				LSD at 5%	2008				LSD at 5%
	Storage period in days					Storage period in days				
	0	5	10	15		0	5	10	15	
AA 2.8 mM	45.60	50.20	54.40	63.90	0.11	53.20	61.40	65.20	71.40	0.11
AA 5.6 mM	44.70	47.10	52.40	60.70	0.11	51.70	59.00	63.70	70.50	0.55
B_{12} 0.36 µM	46.60	52.70	55.60	64.70	0.11	53.70	63.20	66.20	74.70	0.11
B_{12} 0.74 µM	46.30	52.70	57.70	64.20	0.11	55.80	63.80	65.80	75.70	0.11
AA 2.8 mM + B_{12} 0.36 µM	48.30	52.70	60.10	65.10	0.11	54.20	62.30	66.30	75.60	0.11
AA 2.8 mM + B_{12} 0.74 µM	49.20	54.40	61.10	64.20	0.11	57.70	64.90	67.40	76.20	0.11
AA 5.6 mM + B_{12} 0.36 µM	50.00	52.40	57.10	62.40	0.55	56.70	62.10	66.30	74.70	0.11
AA 5.6 mM + B_{12} 0.74 µM	49.70	54.10	60.60	65.60	0.11	58.80	63.90	66.80	73.40	0.11
Control	54.90	59.50	65.20	71.90	0.11	66.10	71.40	73.30	83.90	0.11
LSD at 5%	**0.34**	**0.09**	**0.09**	**0.09**	**------**	**0.09**	**0.34**	**0.09**	**0.09**	**------**

In conclusion, treating kaki fruit with different B_{12} and AA solutions increases total sugars and decreases fruit firmness and total tannin after 15 days of shelf-life time. It is considered that B_{12} a good chemical to control kaki quality. Also, AA a good soluble antioxidant to prevent oxidative reactions during shelf-life.

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تطبيقات الفيتامينات على أشجار الكاكى صنف الكوستاتا وجودة الثمار

السيد البدوى طه البلز ، محمد عبد الرحيم عراقى ، لؤى عبد اللطيف و محمد رزق ابراهيم الديب

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اجريت هذه الدراسة خلال موسمين متتاليين (2007-2008) لمعاملات ما و بعد الحصاد على الكاكى اليابانى لصنف كوستاتا الموجود فى مزرعة خاصة بمحافظة الدقهلية – مركز ميت غمر والذى يعتبر من الأصناف مبكرة النضج. حيث تم رش الأشجار بمحلولى حمض الأسكوربيك (فيتامين ج) وفيتامين ب 12 فى صورة السينانوكوبلامين منفردين أو مخلوطين على الاشجار وتم الرش فى مراحل مختلفة من نمو و تطور الثمار.

وعموماً فإن صلاحية الثمار تناقصت تدريجياً من وقت الحصاد حتى إنهاء مرحلة تخزين فى جو الغرفة بخلاف الثمار الكنترول فقد لوحظ نقصان صلابة الثمار تدريجيا بالمقارنة بمعاملة الكنترول و بالاخص عند الرش بمحلول الاسكوربيك بتركيز 5.6 ملليمول الاكثر صلابة عند الانتهاء من التجربة ، كما اعطت هذا التركيز تأثير واضح على انخفاض نسبة النفاذية الخلايا و الخفاظ على كمية الفينول الكلى و لكن التأثير الواضح للمعاملة فيتامين ب12 و حمض الاسكوربيك بالتركيز العالى 5.6 ملى مول و 0.74 ميكروكول ادى الى زيادة السكريات الكلية للثمار وإنخفاض فى محتوى المادة التانينية خلال فترة التخزين على درجة حرارة الغرفة 26±2 درجة م و رطوبة نسبية 71% خلال فترة 15 يوماً من التخزين. ولوحظ الحفاظ على المواد الفينولية تحت تأثير المعاملات التى بها الاسكوربيك اكثر من المحتوية على فيتامين ب12 كما أدى المعاملة بالرش بالاسكوربيك إلى إنخفاض تدهور الأنسجة النباتية مما يدل على حيوية جدر الخلايا خلال مرحلة النضج على درجة حرارة الغرفة بخلاف معاملات الكنترول والمعاملات الاخرى بإستخدام كل من فيتامين ج وفيتامين ب12.

قام بتحكيم البحث

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