Zagazig J. Agric. Res., Vol. 47 No. (1) 2020 39-50

Plant Production Science

http:/www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master

ALLEVIATING CHILLING INJURY OF OLIVE DURING COLD STORAGE BY SAFE COMPOUNDS AFTER HARVEST

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Received: 09/11/2019; Accepted: 26/12/2019

ABSTRACT: Recently, there has been a great concern about increasing the yield and storage of many olive cultivars in Egypt and countries around the world. However, there is a lack of storage facilities to reduce the losses of olives. This study provide some safe treatments after harvest to extend the cold storage of olives, while reducing or mitigating the chilling injury of stored fruits. Treatments were done by dipping fruits of each used cultivar, namely Manzanillo and Toffahi in one of the treatment solutions which were, the control (water), Naphthalene Acetic Acid (NAA) at 100 or 200 ppm, Lisophos (LPE) at 200 or 400 ppm, putrescine at 1 or at 2 mM, in addition to benzyladenine (BA) at either 100 or 200 ppm. After dipping for 20 min, olives were left for air drying at room temperature (22 ± 2°C) in foam plates, then stored in the cold store at (5 ± 1°C) and relative humidity of 95 %. Each solution contained the surfactant called Tween 20 at 0.05% (*V/V*). After each cold storage duration olives were left for 24 hrs on the bench at room temperature to warm up before assessing the physiological and chemical characteristics of fruits of each treatment. The results revealed that putrescine - treated fruits had higher chlorophyll content especially at 2 mM. Similar trend of results was obtained with LPE at 200 ppm or BA at 200 ppm. Meanwhile, there was a significant reduction of electrolyte leakage following the cold storage periods, especially with LOE at 400 ppm or putrescine at 1 mM when compared with the control. This study recommended dipping olive fruits after harvest and prior cold storage in Lisophos at 200 or 400 ppm or in putrescine solution at 1 mM to prolong the cold storage life and to reduce the injury of stored olives, while reducing their loss of quality.

Key words: Postharvest, growth regulators, olives chilling injury, lisophos.

INTRODUCTION

The olive (*Olea europea*) is one of the most important food crops in many countries around the Mediterranean sea and places with a Mediterranean climate especially in the arid areas. The trees have a distinguished stress tolerance such as drought and salt stresses.

Due to the high demand in Egypt, there has been a need to raise the production of olives. Since olives could be one of the best crops to increase the national income of Egypt. In addition to, the need to fulfill the demand to more yield for oil extraction. Thus, there is a need to store a great amount of olive fruits. Moreover, olive fruits are also needed for pickling as part of the traditional food in many countries.

There have been good efforts to decrease chilling injury by avoiding exposure of olive fruits to cold temperatures below 5°C, since they prefer 5 to 7.5°C and 90-95% relative humidity.

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There are relatively few attempts to alleviate chilling injury but they are not enough. Thus, there has been a lack of more research in order to alleviate chilling injury of olives. It is expected to find variations in chilling stress tolerance when the local cultivars are compared with adapted-imported cultivars such as Manzanillo. However, the utilization of safe-plant growth regulators to mitigate chilling stress of olives is still very scant. As shown in the literature, growth regulators such as salicylic acid, putrescine and Jasmonates were applied to reduce chilling injury of stored olives in the cold (**Cai *et al*., 2006; Asghari and Aghdam, 2010; Lu *et al*., 2010; Wang, 2010; Lu *et al.,* 2011**).

The main objective of this research was to alleviate the chilling injury symptoms on olive fruits by applying some growth regulators after harvest and before cold storage, then assess the characteristics of local and universal cultivars following cold storage.

MATERIALS AND METHODS

This study was conducted during two successive seasons 2014 and 2015 using olive fruits at full maturity (green stage), collected from a private orchard located in El Hammam District, Matrouh Governorate, uniform and free of visible defects as possible. Two commercial olive cultivars were used, one of them is local, namely Toffahi, and the other was adapted to the Egyptian agriculture, namely Manzanillo.

Fruits were surface sterilized by the next steps: Washed with tap water, dipped in sodium hypochlorite (NaOCl) for 3 minutes at concentration of 0.5 ml/l (0.05%, *V/V*), rinsed again quickly in distilled water, then fruits were left for air drying at room temperature.

Then after, the olive fruits of each cultivar were divided into 9 groups with 400 fruits in each group. Each group was randomly divided into 4 replicates with 100 fruits for each. Then fruits of each were dipped for 20 minutes in one of the treatments solutions as follows: Distilled water (the control), Naphthalene Acetic Acid (NAA) at 100 ppm or 200 ppm, Lisophos at 200 ppm or 400 ppm, putrescine at 1 mM or 2 mM and finally Kenzo as benzyladenine at 100 ppm or 200 ppm. The non-ionic surfactant Tween 20 at 0.05% (*V/V*) was added to all treatments to reduce the surface tension and to increase the contact angle.

The fruits were left for air drying, then packed in foam plates and wrapped with polyethylene stretch, after that they were stored in a commercial cold room at 5 ± 1°C and humidity of 95%. All assessments were conducted with four replicates per treatment.

Fruit quality assessment was made after 45 and 90 days in cold storage, then the data was expressed as the treatment effect regardless the storage time duration factors.

The fruit quality parameters were done after keeping fruits for 24 hr., from cold storage at room temperature.

Physical Characteristics

Water loss (%)

In order to determine the water loss (%), olive fruits were weighted at the beginning of the experiment after packing and thereafter 45 days and 90 days during the storage times.

Pit and flesh weights (g) were determined by using the total weight of ten olive fruits of each replication at the beginning, then after 45 and 90 days of cold storage.

Furthermore, the percentage of electrolyte leakage of fruit peel was calculated according to the method of **Ahrenes and Ingram (1988)**.

Moreover physiological disorders incidence after each duration of cold storage were assessed by rating units: 1= no disorders, 2= acceptable, 3 = commercially accepted, 4 = visibly unmarketable. After 24 hours of exposure to room temperature such as pitting, tissue browning and visible shriveling.

Chemical Characteristics

1. Chlorophylls a, b and beta-carotene contents were extracted, measured, calculated and expressed as mg/l according to the procedure of **Wintermans and Mats (1965)**.
2. The percentage of total soluble solids (TSS) was determined in olive fruit juice using a hand refractometer.
3. Total acidity was estimated as malic acid (g) per 100 ml according to the method described by **AOAC (1985)**.

4. Vitamin C content was measured and expressed as mg ascorbic acid/100 ml juice (**Egan *et al*., 1987**).

Statistical Analysis

Data were analyzed as a completely randomized design (CRD) with four replicates. Comparisons among means were made via the Least Significant Differences multiple ranges according to **Snedecor and Cochran (1980)**. The data were analyzed by using **SAS (2000)** program.

RESULTS

The Effect of Postharvest Treatments on" Manzanillo" Olives during Cold Storage for the Two Seasons 2014 and 2015

Some physical characteristics and physiological disorders

Moreover, the effect of various used treatments on some physical characteristics of "Manzanillo" olives, regardless the time factor, was reported in Table 1. The results revealed that all treated olives had similar weight to that found in the control. That was also the trend of results with regard to the influence of the treatments on pit weight and flesh weight of "Manzanillo" fruits in both seasons when compared with the control.

The influence of used treatment on weight loss, regardless the time was shown in Table 1. The results indicated that there was a consistent increase in such property caused by using BA at 100 ppm relative to the control in both seasons. Meanwhile, postharvest treatments of "Manzanillo" olives resulted in a significant increase in water loss, only in the second season related to the control. These treatments included LPE at 400 ppm, putrescine at 2 mM and BA at 200 ppm (Table 1).

With regard to the effect of applied treatments after harvest on some physiological disorders of "Manzanillo" olive fruits during the two seasons 2014 and 2015, it was evident that LPE at 400 ppm caused a significant reduction in fruit pitting in a consistent manner in both seasons as compared with the control. Similar trend of results was obtained with the application of some other treatments but was significant in one season only relative to the control. These treatments included putrescine at 1 mM as well as BA at 100 or 200 ppm.

Fruit tissue browning, as one of the disorders related to exposure to chilling was also assessed and reported in Table 2. The results revealed that there were variations in the level of tissue browning in relation to the used treatments of "Manzanillo" olives. The significant reduction was obtained with putrescine-treated fruits especially at 1mM. Some other treatments also resulted in reducing the magnitude of tissue browning in the second season such as BA at 100 or 200ppm. In a similar manner, LPE at 400 ppm and putrescine at 2 mM reduced tissue browning in the first season.

Moreover, visible shriveling of fruits was only increased significantly with the treatment of BA especially in the first season with both used concentrations but was reduced when olives were treated with either LPE at 400 ppm or putrescine at 1 or 2 mM during the second season.

The percentage of electrolyte leakage of "Manzanillo" olives during cold storage

With regard to the influence of various used treatments on the percentage of electrolyte leakage of "Manzanillo" olives, regardless the time factor, the results in Table 3 prove that all treatments were significantly effective on reducing such leakage when compared with the control. However, the magnitude of such reduction was similar in the cases of LPE at 200 and 400 ppm, in addition to putrescine at 1 and 2 mM. The least magnitude of electrolyte leakage reduction was found with the applications of LPE at 400 and 200 ppm.

Some chemical characteristics of "Manzanillo" olives

The results in Table 4 show the influence of postharvest treatments of "Manzanillo" olives on some chemical characteristics regardless the time factor. The results indicated that the content of chlorophyll a was significantly decreased by all treatments relative to the control especially in the second season only. However, chlorophyll b in the fruit was increased relative to the control by most treatments in the first season such as

Table 1. The effect of various postharvest applied treatments on some physical characteristics of "Manzanillo" olives during the two seasons 2014 and 2015

Treatment	Whole weight (g) (10 fruits)		Pit weight (g) (10 fruits)		Flesh weight (g) (10 fruits)		Water loss (%)	
	2014	2015	2014	2015	2014	2015	2014	2015
Control	48.21a*	48.22a	7.74abc	7.35ab	40.05a	40.87a	2.94b	2.76de
NAA 100 ppm	46.98a	48.52a	7.77abc	7.45a	38.78a	41.06a	3.01ab	2.65e
NAA 200 ppm	48.34a	47.28a	7.78abc	7.25ab	40.13a	40.02a	2.90b	2.76de
LPE 200 ppm	46.94a	47.46a	7.51c	7.25ab	39.00a	40.20a	3.70ab	2.87cd
LPE 400 ppm	48.05a	46.76a	8.17a	7.16ab	39.49a	39.6a	3.50ab	2.90c
Putrescine 1mM	47.42a	48.37a	7.65bc	7.51a	39.34a	40.85a	3.39ab	2.82cd
Putrescine 2mM	48.13 a	47.94a	7.77abc	7.31ab	39.93a	40.62a	2.88b	3.08b
BA 100 ppm	48.24a	46.25a	8.09ab	6.92b	39.72a	39.33a	3.88a	3.27a
BA 200 ppm	47.67 a	47.95a	7.85abc	7.44a	38.56a	40.51a	3.51ab	3.12b

* Means within the column having the same letters are not significantly different, in comparing treatments according to Least significant difference (LSD) at 0.05 level.

Table 2. The effect of various postharvest applied treatments on some physiologicalcal disorders of "Manzanillo" olives during the two seasons 2014 and 2015

Treatment	Pitting		Browning		Visible shriveling	
	2014	2015	2014	2015	2014	2015
Control	1.83bc	1.91a	1.58ab	1.66a	1.66a	1.5a
NAA 100 ppm	1.5de	1.66abc	1.41bc	1.66a	1.29c	1.33abc
NAA 200 ppm	1.66cd	1.75ab	1.83a	1.66a	1.5ab	1.33abc
LPE 200 ppm	1.75c	1.91a	1.66ab	1.66a	1.5ab	1.41ab
LPE 400 ppm	1.41e	1.5bc	1.25cd	1.5ab	1.33bc	1.16c
Putrescine 1mM	1.66cd	1.41c	1.08d	1.41b	1.33bc	1.16c
Putrescine 2mM	1.33e	1.83a	1.08d	1.5ab	1.33bc	1.25bc
BA 100 ppm	2ab	1.5bc	1.83a	1.41b	1.66a	1.33abc
BA 200 ppm	2.08a	1.66abc	1.83a	1.33b	1.41bc	1.25bc

Means within the column having the same letters are not significantly different, in comparing Least significant difference (LSD) at 0.05 level. These visible characteristics were assessed by using rating units: 1= no disorders, 2= acceptable, 3= commercially accepted, 4= visibly unmarketable.

Table 3. The effect of postharvest treatments on "Manzanillo" and "Toffahi" olives according to the percentage of electrolyte leakage regardless the time, after cold storage during season 2015

Treatment	Electrolyte leakage (%)	
	Manzanillo	Toffahi
Control	71.65a*	78.36a
NAA 100 ppm	63.00bc	72.15b
NAA 200 ppm	60.82c	69.72bc
LPE 200 ppm	51.06de	61.47c
LPE 400 ppm	45.38e	54.29d
Putrescine 1mM	59.38cd	59.43cd
Putrescine 2mM	54.70d	57.26cd
BA 100 ppm	63.22bc	69.28bc
BA 200 ppm	65.17b	68.19bc

* Means within the column having the same letters are not significantly different, in comparing Least significant difference (LSD) at 0.05 level.

Table 4. The effect of various postharvest applied treatments on some chemical characteristics of "Manzanillo" olives during the two seasons 2014 and 2015

Treatment	Chlorophyll a (mg/100 g)		Chlorophyll b (mg/100 g)		Carotenes (mg/100 g)	
	2014	2015	2014	2015	2014	2015
Control	0.207a*	0.256a	0.149d	0.188a	4.57e	4.34i
NAA 100 ppm	0.208a	0.223f	0.152d	0.158a	4.79d	4.93d
NAA 200 ppm	0.209a	0.229e	0.163abc	0.161a	4.69d	4.81e
LPE 200 ppm	0.209a	0.234d	0.162bc	0.168a	5.14ab	4.69f
LPE 400 ppm	0.208a	0.242c	0.163abc	0.173a	5.02bc	4.58g
Putrescine 1mM	0.210a	0.215g	0.170a	0.149a	5.22a	5.03c
Putrescine 2mM	0.206a	0.250b	0.165ab	0.183a	4.92c	4.44h
BA 100 ppm	0.210a	0.209h	0.156cd	0.145a	4.94c	5.15b
BA 200 ppm	0.207a	0.202i	0.162bc	0.222a	4.96c	5.26a

* Means within the column having the same letters are not significantly different, in comparing Least significant difference (LSD) at 0.05 level.

NAA at 200 ppm, LPE at 200 or 400 ppm, putrescine at 1 or 2 mM in addition to BA at 200 ppm.

Carotenes, on the other hand, were consistently increased by all postharvest treatments of "Manzanillo" olives in the two seasons. The greater magnitude of carotene increase was found with putrescine at 1 mM in the first season and BA at 200ppm in the second season relative to other treatments and to the control.

With regard to the changes in total soluble solids (TSS) in "Manzanillo" fruits in response to postharvest applied treatments, the results in Table 4 reveal that the significant alterations were obtained only by the second season applications but the changes of TSS during the first season were not significant by all treatments relative to the control. Moreover, fruit acidity was affected by using the treatments in the second season especially with putrescine at 1mM and LPE at 200 ppm.

Changes in vitamin C in "Manzanillo" fruits in response to the treatments, regardless the time of cold storage, were recorded in Table 5. The results showed significant changes in vitamin C by all treatments in the first season relative to the control. However, the consistent increase in vitamin C was found with some treatments in the second season such as NAA at 100 ppm, putrescine at 1 mM, in addition to BA at 100 or 200 ppm.

The Effect of Postharvest Treatments on "Toffahi" Olives During Cold Storage for the Two Seasons 2014 and 2015

Some physical characteristics and physiological disorders

Physical characteristics of used "Toffahi" olives in response to postharvest treatments, regardless the time factor, were reported in Table 6. The results showed that there were no significant differences in fruit weight when comparing the treatments with the control in both seasons with exception of case with BA treated olives in the first season and putersene 1mM in the second one. In addition, some variations were also reported in pit weight of "Toffahi" olives, especially with the application of NAA at 200 ppm, LPE at 400 ppm, and BA at 100 ppm that had lower pit weight than that of the control in the first season and as the putrescine in the second season. However, almost all treated "Toffahi" olives had similar flesh weight to that of the control in both seasons.

Furthermore, the percentage of water loss from "Toffahi" olives was not, in general, influenced by used treatments after harvest except with LPE treated fruits at 400 ppm or BA - treated fruits at 100 ppm in 2014 season (Table 6).

Regarding the effect of various treatments used on some physiological disorders, regardless the time factor, the results in Table 7 showed that pitting of "Toffahi" olives varied among treatments. Pitting was slightly higher with the applications of either LPE at 200 or 400 ppm or BA at 100 or 200 ppm relative to the control in the first season. However, such pitting was decreased with the application of NAA at 200 ppm and putrescine at 2 mM in 2014 and 2015 seasons, respectively. Meanwhile, fruit tissue browning was reduced by many treatments especially by putrescine at 2 mM or by BA at 100 ppm during both seasons and by NAA, LPE at 200 ppm putrescine at 2 mM and BA at 100 ppm in the first season, while with LPE at 400 ppm or putrescine at 1 mM or BA at 200 ppm, it was reduced in a significant manner only in the second season.

Furthermore, visible shriveling did not increase by used treatments in both seasons but was even reduced by some treatments such as NAA at 100 ppm and pturescine at 1 or 2 mM in the first season.

The percentage of electrolyte leakage of "Toffahi" olives during cold storage

The influence of various-postharvest treatments on the percentage of electrolyte leakage during cold storage of "Toffahi" fruits was reported in Table 3. The results showed that all treated "Toffahi" olives had lower electrolyte leakage than the control. The lowest magnitude of electrolyte leakage was obtained with LPE at 400 ppm followed by putrescine at 1 or 2 mM. These three treatments were equally effective on the leakage of electrolytes regardless the duration of cold storage. Moreover, the leakage of electrolytes was similar when comparing its magnitude by both used concentrations of NAA (at 100 and 200 ppm) or BA.

Table 5. The effect of various postharvest applied treatments on some chemical characteristics of "Manzanillo" olives during the two seasons 2014 and 2015

Treatment	TSS (%)		Acidity in flesh (mg/100ml)		Vitamin C in flesh (mg/100ml)	
	2014	2015	2014	2015	2014	2015
Control	14.02Ns*	10.43i**	0.20c	0.42e	4.14f	3.26d
NAA 100 ppm	14.01	10.56h	0.20c	0.48b	4.56e	3.74abc
NAA 200 ppm	14.07	10.74g	0.21bc	0.47bc	4.64de	3.65abcd
LPE 200 ppm	13.81	11.51b	0.21abc	0.45cd	4.59e	3.53bcd
LPE 400 ppm	13.66	11.64a	0.22a	0.44de	4.48e	3.46bcd
Putrescine 1mM	14.33	11.22d	0.22ab	0.49b	5.06bc	3.85ab
Putrescine 2mM	13.72	11.38c	0.20c	0.43de	4.89cd	3.35cd
BA 100 ppm	14.15	10.88f	0.20c	0.43de	5.20b	3.94a
BA 200 ppm	13.86	11.08e	0.20c	0.51a	5.51a	4.04a

Ns*: Not-significant.
**: Means within the column having the same letters are not significantly different, in comparing Least significant difference (LSD) at 0.05 level.

Table 6. The effect of various-applied treatments on some physiological characteristics of "Toffahi" olives during two seasons 2014 and 2015

Treatment	Whole weight (g) (10 fruits)		Pit weight (g) (10 fruits)		Flesh weight (g) (10 fruits)		Weight loss (%)	
	2014	2015	2014	2015	2014	2015	2014	2015
Control	119.56a*	101.45b	15.9a	12.17b	104.02abc	89.27b	2.46c	2.92a
NAA 100 ppm	119.25a	102.52b	14.6b	11.76b	105.00ab	90.75b	2.54c	2.57a
NAA 200 ppm	121.00a	105.97ab	14.69b	12.70ab	106.67a	93.26ab	2.50c	2.34a
LPE 200 ppm	117.16abc	104.76b	14.85b	12.42ab	102.67abc	92.34b	2.56c	2.24a
LPE 400 ppm	117.99ab	101.87b	14.57b	12.46ab	103.77abc	89.40b	3.90a	2.42a
Putrescine 1mM	116.15abc	114.58a	15.93a	12.50ab	100.57abc	102.07a	3.19abc	3.07a
Putrescine 2mM	114.64abc	107.38ab	15.38ab	13.2a	99.61abc	94.18ab	3.06bc	2.53a
BA 100 ppm	110.24c	104.60b	14.76b	12.32ab	95.83c	92.28b	3.42ab	2.76a
BA 200 ppm	111.78bc	102.53b	15.1ab	12.28ab	97.04bc	90.25b	2.88bc	2.36a

* Means within the column having the same letters are not significantly different, in comparing Least significant difference (LSD) at 0.05 level.

Table 7. The effect of various-applied treatments on some physiological disorders of "Toffahi" olives during two seasons 2014 and 2015

Treatment	Pitting		Browning		Visible shriveling	
	2014	2015	2014	2015	2014	2015
Control	1.41b	1.75abc	1.5a	1.58a	1.75a	1.66ab
NAA 100 ppm	1.5ab	1.91a	1.25b	1.66a	1.58b	1.91a
NAA 200 ppm	1.08c	1.83ab	1.00c	1.58a	1.66ab	1.5b
LPE 200 ppm	1.66a	1.66bc	1.16bc	1.66a	1.66ab	1.66ab
LPE 400 ppm	1.66a	1.58c	1.33ab	1.16cd	1.66ab	1.58b
Putrescine 1mM	1.33b	1.66bc	1.33ab	1.33bc	1.33c	1.66ab
Putrescine 2mM	1.41b	1.25d	1.00c	1.08d	1.41c	1.66ab
BA 100 ppm	1.66a	1.58c	1.16bc	1.5ab	1.66ab	1.58b
BA 200 ppm	1.66a	1.58c	1.33ab	1.33bc	1.66ab	1.58b

Means within the column having the same letters are not significantly different, in comparing Least significant difference (LSD) at 0.05 level. These visible characteristics were assessed by using rating units: 1= no disorders, 2= acceptable, 3= commercially accepted, 4= visibly unmarketable.

Some chemical characteristics of "Toffahi" olives

The results in Table 8 reveal that there were significant increases in chlorophyll a as influenced by used treatments after harvest. In both seasons, higher values than in the control fruits, were found by treatments of putrescine at 1 and 2 mM, BA at 100 and 200 ppm, as well as LPE at 200ppm in addition to NAA at 200 ppm in the second season.

Moreover, chlorophyll b was also influenced by many treatments, regardless the time factor, especially when compared with the control in both seasons. These significant increases in chlorophyll b in both seasons were obtained with the application of some treatments such as NAA at 200 ppm, LPE at 200 ppm, putrescine at 1 mM, in addition to the two used concentrations of BA. Such treatments were all able to lead to greater chlorophyll b values when compared with control (Table 8).

Meanwhile, carotene content was not significantly affected by used treatments after harvest as compared with the control (Table 8) in the first season eventhough in the second season, carotene content in the fruit was significantly increased by treatments such as LPE at 400 ppm, putrescine at 1 and 2 mM, in addition to NAA at both used concentrations (100 and 200 ppm).

The response of the TSS to various used treatments after harvest (Table 9) indicated that they were all effective on increasing TSS over the control in the second season in addition to putrescine (2 mM) and BA at either 100 ppm or 200 ppm in the first season.

Moreover, flesh acidity in "Toffahi" olives was significantly increased in the second season by both applied concentrations of NAA (100 and 200 ppm) and by putrescine (1 and 2 mM) but decreased with the use of BA (at 100 and 200 ppm), in addition to LPE at 200 ppm in the second season.

On the other hand, there were no significant changes in vitamin C in both seasons, since the control and all used treatments had similar values (Table 9).

Table 8. The effect of various postharvest applied treatments on some chemical characteristics of "Toffahi" olives during the two seasons 2014 and 2015

Treatment	Chlorophyll a (mg/100 g)		Chlorophyll b (mg/100 g)		Carotenes (mg/100 g)	
	2014	2015	2014	2015	2014	2015
Control	0.208d	0.178f	0.123d	0.119fg	3.40a	3.76f
NAA 100 ppm	0.210cd	0.178f	0.214abc	0.201b	3.41a	3.94d
NAA 200 ppm	0.212bcd	0.184e	0.133bc	0.128d	3.41a	3.84e
LPE 200 ppm			0.139ab	0.146b	3.43a	3.60h
LPE 400 ppm	0.212bcd	0.173g	0.128cd	0.118g	3.42a	4.04c
Putrescine 1mM	0.215abc	0.191d	0.135bc	0.123e	3.48a	4.20a
Putrescine 2mM	0.215abc	0.198bc	0.122d	0.124de	3.42a	4.11b
BA 100 ppm	0.216ab	0.196c	0.135bc	0.140c	3.48a	3.67g
BA 200 ppm	0.219a	0.208a	0.145a	0.150a	3.39a	3.52i

Means within the column having the same letters are not significantly different, in comparing Least significant difference (LSD) at 0.05 level.

Table 9. The effect of various postharvest applied treatments on some chemical characteristics of "Toffahi" olives during the two seasons 2014 and 2015

Treatment	TSS (%)		Acidity in flesh (mg/100 ml)		Vitamin C in flesh (mg/100 ml)	
	2014	2015	2014	2015	2014	2015
Control	13.96b*	10.29i	0.37a	0.42f	4.61a	3.81a
NAA 100 ppm	14.44ab	10.43h	0.37a	0.43d	4.59a	4.00a
NAA 200 ppm	14.19ab	10.60g	0.37a	0.42e	4.66a	3.91a
LPE 200 ppm	14.38ab	11.33b	0.37a	0.39h	4.71a	3.59a
LPE 400 ppm	14.45ab	11.48a	0.37a	0.45c	4.71a	4.11a
Putrescine 1mM	14.26ab	11.02d	0.38a	0.47a	4.72a	4.31a
Putrescine 2mM	14.57a	11.18c	0.38a	0.45b	4.80a	4.21a
BA 100 ppm	14.48a	10.75f	0.36a	0.40g	4.86a	3.69a
BA 200 ppm	14.48a	10.91e	0.36a	0.39i	4.79a	3.49a

*: Means within the column having the same letters are not significantly different, in comparing Least significant difference (LSD) at 0.05 level.

DISCUSSION

It has been reported that pitting of fruit tissues, browning and shriveling are the main symptoms of chilling injury (**Farag *et al*., 1992; Farag *et al*., 2005; Golding, 2019**). Thus the breakdown of sub-surface fruit tissues and their collapse results such pitting. Moreover, the tissue browning following chilling exposure was attributed to the activating of the polyphenoloxidase, especially on those phenols that are transported from the vacuoles (**Farag *et al*., 2005**). Such symptoms are usually visible after the exposure of chilled fruits to room temperature to show its toxic influence.

The increasing demand in Egypt for more olive production dictates the need for more yield and capability of storing a huge amount of the fruit at the convenient temperature. Meanwhile, olive fruits are sensitive to chilling stress. The symptoms of chilling injury have been developing in and out the fruit such as internal browning next to the pit or at the stem end in addition to the surface pitting. All the symptoms that are visible on the olive fruits are reflected on the increase in the percentage of electrolyte leakage after periods of cold storage. No wonder, it was obvious from the results to find more leakage of cell electrolytes as the cold storage duration of "Manzanillo" or "Toffahi" olives increased especially in the control fruit. Such increase in the leakage was supported by the findings of many researchers who worked on the assessment of cold stored olive fruits (**Kader *et al*., 1989 and 1990; Garcia and Streif, 1991; Kiritsakis *et al*., 1998; Wang, 2010**).

However, such leakage of electrolytes was significantly reduced when the fruits were pre-treated by the growth regulators, used in this study, especially LPE at 200 or 400 ppm in addition to the treatments of putrescine at 1 or 2 mM. The significant reduction in the leakage of cold stored olives that were treated by LPE could be attributed to its ability to delay tissue senescence, repair the plasma membrane damage and inhibit the activity of phospholipase D (the destructive enzyme) as reported by (**Farag *et al*., 1992; Ozgen *et al*., 1999**).

Furthermore, the positive influence of putrescine on the alleviation of chilling injury of the treated olives could be explained on the bases of its ability to work as anti-ethylene action compound and biosynthesis that is effective on reducing the damage even in a non-climacteric fruit such as olive (**Wang, 2000; Wang, 2010; Barman *et al.,* 2011; Yang *et al.* 2016**).

Thus, the trend of results obtained by some treatments on electrolyte leakage was further supported by the findings of the reduction in chlorophyll breakdown after cold storage of fruits especially again by LPE or putrescine. It was well documented that the treatments which delayed tissue senescence were also able to hinder the destruction of chlorophylls whether a or b (**Ozgen and Palta, 2003; Farag, *et al*., 2005**).

In line with the above findings 6-BA treatment was also able to protect against chilling injury of olive fruits. Such protection could be explained on the basis that BA could slow down the aging and senescence processes (**Mirdehghan and Rahemi, 2005; Mirdehghan *et al.*, 2007; Wang, 2010; Zheng *et al.,* 2012**).

As one of the major cytokinins (from the adenine group) various parameters related to delaying fruit senescence were retarded by 6-BA treatments, while vitamin C was increased as the cold storage duration increased but the flesh acidity was reduced while carotenes were increased.

In spite of the above changes, the magnitude of electrolyte leakage of BA-treated olives was still less than that of the control.

Moreover, NAA was also effective on reducing chilling injury of treated olive fruits of "Manzanillo" and "Toffahi" such postharvest efficacy was supported by many researchers who reported that NAA was able to reduce chilling injury (**Amoros *et al.,* 2004; Mashau *et al.,* 2012; Phani *et al.,* 2015**).

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تخفيف ضرر البرودة على ثمار الزيتون أثناء التخزين البارد باستخدام مواد طبيعية بعد القطف

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تزايد الاهتمام في الفترة الحالية بزيادة إنتاج ثمار الزيتون و تحسين القدرة التخزينية للعديد من أصنافه خاصة في مصر والكثير من الدول المنتجة حول العالم، ومع ذلك فان هناك احتياجا كبيرا لثلاجات التخزين لمواجهة زيادة الكميات المنتجة وتقليل الفاقد من الثمار، وتوفر هذه الدراسة بعض المعاملات الآمنة التي يمكن إجراؤها بعد الحصاد لإطالة القدرة التخزينية لثمار الزيتون مع التخزين المبرد وفي نفس الوقت تقليل أو منع أضرار البرودة على الثمار المخزنة، وقد تم معاملة الثمار بالغمس لمدة ٢٠ دقيقة سواء لثمار الصنف مانزانيللو والتفاحي في كل من المعاملات المستخدمة واستخدمت المادة الناشرة توين ٢٠ (بتركيز ٠,٠٥% حجم/حجم) لتقليل التوتر السطحي، واشتملت المعاملات على الكنترول (الماء)، NAA بتركيز ١٠٠ و ٢٠٠ جزء في المليون، الليزوفوس بتركيزي ٢٠٠ و ٤٠٠ جزء في المليون، البيوترسين بتركيزي ١ و ٢ مللي مولار، بالإضافة إلى البنزيل أدينين بتركيزين هما ١٠٠ و ٢٠٠ جزء في المليون، ثم تركت الثمار بعد المعاملة على درجة حرارة الغرفة (٢٢ ± ٢°م) لتجف بواسطة الهواء قبل تخزينها في الثلاجة (كل معاملة في طبق من الفوم، وكل مكررة بمفردها في طبق) وخزنت على درجة (٥ ± ١°م) لفترة ٤٥ يوما و ٩٠ يوما ورطوبة نسبية ٩٥%، وعقب كل فترة تخزين مبرد، تم تعريض كل المعاملات بمكرراتها لمدة يوم على درجة حرارة الغرفة لتدفئتها قبل إجراء التقييم والتحاليل للصفات الطبيعية والكيميائية، وقد أظهرت النتائج عدة تأثيرات ايجابية خلال فترة التخزين المبرد يمكن اختصارها في بعض الأمثلة مثل زيادة محتوى الكلوروفيل في الثمار المعاملة بالبيوترسين خاصة بتركيز (٢ مللي مولار) وكذلك تم الحصول على نتائج مشابهة باستخدام الليزوفوس (٢٠٠ جزء في المليون) أو البنزيل أدينين (٢٠٠ جزء في المليون)، كذلك وجد انخفاضا معنويا في نسبة التسرب الالكتروليتي بعد التخزين المبرد خاصة مع معاملات الليزوفوس بتركيز (٤٠٠ جزء في المليون) أو البيوترسين بتركيز (١ مللي مولار) بالنسبة للكنترول، وتوصي تلك الدراسة باستخدام عملية غمس ثمار الزيتون بعد الجمع وقبل التخزين المبرد في محلول الليزوفوس سواء ٢٠٠ أو ٤٠٠ جزء في المليون أو البيوترسين بتركيز ١ مللي مولار لإطالة القدرة التخزينية لثمار الزيتون من الصنفين بالتبريد مع تقليل أضرار البرودة و تقليل الفاقد.

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