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ORIGINAL ARTICLES

Response of Washington navel orange to thyme and clove oils as natural postharvest treatments under cold storage conditions

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ABSTRACT

This work was carried out during two successive seasons of 2011 and 2012 to evaluate the effect of some natural postharvest treatments i.e. thyme and clove oils at 2 and 3% on some fruit quality parameters of Washington navel orange fruits under cold storage (5±2˚C& 90% R.H.). Briefly, fruit weight loss %, decay %, T.S.S % and respiration rate (ml CO_2/kg fruits/hr) of Washington navel orange fruits were increased in most cases with prolonging the storage period. Whereas, total acidity % and ascorbic acid (mg/100 ml juice) were decreased with advancing the storage period. However, the lowest values of weight loss %, decay % and respiration rate as well as the best results of shelf life (days) ,besides the highest values of total acidity % and ascorbic acid were gained by 3% clove oil treatment.

Key words: Washington navel orange, post-harvest, thyme oil, clove oil and fruit quality.

Introduction

Orange industry is important for Egyptian national income. The cultivated orange area in Egypt reached 314115 feddans in 2010, while the productive area reached 241102 feddans producing 2401020 tons of orange fruits (Ministry of Agriculture and Land Reclamation Statistics, Egypt, 2010). Although the greatest quantities of orange productivity yearly in Egypt, the exported quantities of fresh orange fruits to the outer markets are still limited. Thus, any directed effort for maintaining fruit quality and reducing post harvest losses is necessary for increasing our national income.

Storage is essential for extending the marketing period of fruits, regulating their supply to the market and for transportation to long distance. The marketing period could be extended by pre-cooling, storage under low temperature and some pre-storage treatments. There is a dispute need to study, how the marketing period could be extended and how to reduce the loss of fruits and supply of orange fruit frequently and over long period of time.

In recent years, consumers have become more concerned about application of chemicals on food products because synthetic preservatives release residues on foods that have negative effects on human health and the environment (Namiki 1990). Besides, the use of synthetic compounds have significant drawbacks, such as increasing cost, handling hazards, concerns about residues on food and threat to human environment (Paster and Bullerman, 1988). Therefore, there has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds. Those are, in the first place, extracts and essential oils of spices and herbs (Smid and Gorris, 1999). As natural foodstuffs, spices and herbs appeal to all who question safety of synthetic food additives and demand high-quality products that at the same time are safe and stable (Brul and Coote, 1999).

The use of biocontrol agents with plant extracts i.e. lemon, citronella, clove, mint, thyme, and oregano oils in plant disease control has been employed by (Samson, 1984). Antifungal activities of essential oils from Thymus and Mentha species have been reported in other studies as well (Bourdy *et al.,* 2000; Sokovicet *et al.*, 2009). Also, Thyme and clove oils presented inhibitory effects on food spoilage (Viuda *et al.,* 2007a). The thyme oil had strong fungicidal effect (Mironescua and Georgescub, 2008; Hadizadeh *et al.,* 2009). Clove extracts showed inhibitory effects on the fungi and showed abnormal conidia, malformations as swollen, often septated and pale color hypha (Suwitchayanon and Kunasakdakul, 2009).

Zeng *et al.,* (2012) suggested that clove extract might be a viable alternative to synthetic fungicides to extend the postharvest storage period and maintain fruit quality of navel orange. On the other hand, Fatemi *et al.,* (2011) showed that thyme essential oil at 1000ppm decreased the decay and scored the highest level of ascorbic acid of Valencia orange.

Therefore, based on the above-mentioned points, the aim of this study was to evaluate the potential use of natural essential oils like thyme and clove in controlling the losses and enhancing quality and marketability of Washington navel orange fruits.

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Materials and Methods

This study was conducted during two successive seasons of 2011 and 2012 on mature Washington navel orange fruits, to evaluate the effect of emulsifying fruits with some natural products on physical and chemical properties under cold storage.

Harvested fruits (at full colour stage) were directly transferred to the laboratory at the Agricultural Development Systems (ADS) project, Faculty of Agriculture, Cairo University. Defective fruits including wounded and other disorders were excluded, the remained fruits were washed with tap water and air dried.

Experimental fruits were divided into five similar groups. Each group was subjected to one of the following treatments as a fruit coating by using a hand sprayer.

1- Control (tap water plus tween 80)
2- Thyme oil at 2%
3- Thyme oil at 3 %
4- Clove oil at 2%
5- Clove oil at 3%

Each treatment was replicated three times and each replicate was about 15 kg weight placed as one layer in three carton boxes (60×40×15 cm). Experimental boxes were stored at 5±2°C and 90% relative humidity for 63 days (9 weeks).

The emulsion of thyme or clove oils were prepared by mixing oil with tween 80 in water, Ju *et al.,* (2000).

Gas chromatography analysis of thyme and clove essential oils are presented in Tables (1&2) according to Viuda *et al.,* (2007b) and Bhuiyan *et al.,* (2010), respectively.

Table 1: Constituents of thyme oil and its relative percentages of total chromatogram area, Kovats Index and retention time estimated by GC-MS.

Compounds	KIa	(%)
α-thujene	928	2.10
α-pinene	936	5.16
camphene	951	1.30
sabinene	974	1.66
β-pinene	977	0.65
β-myrcene	992	2.69
α-terpinene	1019	4.05
p-cymene	1026	5.79
limonene	1031	5.09
1,8-cineole	1034	2.93
γ-terpinene	1060	9.21
cis-sabinene hydrate	1070	7.65
terpinolene	1089	1.56
linalool	1104	7.12
1-terpineol	1125	0.95
dihydrocarveol	1144	0.89
verbenol	1148	1.15
camphor	1151	--
isoborneol	1162	--
borneol	1172	4.07
terpinen-4-ol	1181	13.15
α-terpineol	1195	5.84
verbenone	1211	5.69
cuminal	1226	--
bornyl acetate	1288	0.38
2-caren-10-al	1289	--
carbicol	1293	--
thymol	1296	2.27
carvacrol	1304	0.13
α-terpinyl acetate	1353	0.84
eugenol	1370	--
β-caryophyllene	1426	0.71
α-humulene	1460	Tr.
cyclogermacrene	1501	0.13
δ-cadinene	1528	0.35
SEM		0.65

–Not detected. Tr: Trace (Area≤ 0.06%). (KIa) Kovats Index in DB-5 column in reference to n-alkanes (C8–C32). (SEM) Standard Error of the means.

Table 2: Chemical constituents of the essential oil from clove oil estimated by GC-MS.

S/No.	Name of constituents	%
1.	α-Pinene	0.33
2.	β-Phellandrene	0.12
3.	β-Pinene	0.45
4.	α-Phellandrene	0.09
5.	α-Terpinene	0.31
6.	m-Cymene	0.16
7.	Limonene	2.08
8.	Eucalyptol	5.78
9.	γ-Terpinene	0.17
10	Linalool	0.14
11	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	0.04
12	2-Heptanol acetate	---
13	4-Terpineol	0.45
14	Methyl Salicylate	0.20
15	Terpinyl acetate	0.59
16	Chavicol	0.08
17	Eugenol	74.28
18	Benzyl acetate	---
19	4-Terpineo	0.45
20	Caryophyllene	3.85
21	Copaene	0.17
22	•-Caryophyllene	1.52
23	Alloaromadendrene	0.05
24	α-Cubebene	0.02
25	Germacrene D	0.38
26	α-Guaiene	0.06
27	γ-Elemene	0.21
28	β-Bisabolene	0.06
29	Benzoic acid, 3-(1-methylethyl)	---
30	δ-Cadinene	0.21
31	Benzene, 1-ethyl-3-nitro	---
32	Guaiene	0.09
33	Caryophyllene oxide	0.78
34	Globulol	0.38
35	Ledol	0.16
36	Humulane-1,6-dien-3-ol	0.51
37	Cedr-9-ene	0.16
38	Cubenol	0.19
39	Elixene	---
40	α-Cadinol	2.43
41	Megastigma-4,6(E),8(Z)-triene	---
42	Juniper camphor	0.17
43	Kauran-18-al, 17-(acetyloxy)-	0.13
44	Alloaromadendrene oxide-(1)	0.11
45	α -Amorphene	---
46	Germacrene D	0.38
47	(+)- Cycloisosativen	---
48	Nerolidyl acetate	0.06

Changes in some physical and chemical fruit properties were determined at seven days intervals.

1. Fruit physical properties:

1.1. Fruit weight loss percentage:

The initial weight of Washington navel orange fruits was recorded in each treatment and at weekly interval, then fruit weight loss% was calculated by weighing the same fruits at each interval and at the end of cold storage duration using the following formula:

$$\text{Fruit weight loss \%} = \frac{\text{Initial weight} - \text{Weight at specific interval}}{\text{Initial weight}} \times 100$$

1.2. Fruit decay percentage:

The decayed fruits of each treatment were discarded and weighed. The weight of such discarded fruits related to the initial weight of fruits per each treatment was estimated and decay percentage was calculated.

1.3. Shelf life (days):

At the end of cold storage period, samples of the treated fruits were taken and left at room conditions (20±5˚C and 70-75% R.H.) and the number of days at which treated fruits still with good appearance were counted and shelf life was determined.

2. Fruit chemical properties:

Total soluble solids (T.S.S) of fruit juice were measured using a hand refractometer and expressed as a percent. Moreover, fruit total acidity (grams of citric acid per 100ml of juice and ascorbic acid (V.C) content (milligrams ascorbic acid per 100ml fruit juice) were estimated according to A.O.A.C. (1985). Besides, respiration rate was measured by carbon dioxide produced from the fruits after harvest (Initial reading was scored under room temperature), then every two weeks and at the end of cold storage and CO_2 levels produced by the fruit were calculated as ml CO_2/kg fruits/hr according to Pesis and Ben-Arie (1984) & Lurie and Pesis (1992).

Statistical Analysis:

Data obtained in the two studied seasons were subjected to the analysis of variance according to Snedecor and Cochran (1989), least significant differences (L.S.D.) was used to differentiate the obtained values.

Results and Discussion

Effect of thyme and clove oils treatments on:

1. Fruit physical properties:

1.1. Fruit weight loss percentage:

With respect to the effect of the tested post harvest treatments**,** data in Table (3) indicate that in both seasons, all tested treatments succeeded in reducing weight loss percentage of Washington navel orange fruits in comparison with untreated fruits "control" with superiority of 3% clove oil treatment, followed descendingly by 3% thyme oil treatment. The remained treatments induced less reductive effect on fruit weight loss % as compared with control.

Table 3: Effect of thyme and clove oils treatments on weight loss % of Washington navel orange fruits under cold storage at 5±2°C during 2011and 2012 seasons.

Treatment	2011 season									
	Storage periods (weeks)									
	1	2	3	4	5	6	7	8	9	Mean
Control	0.69	1.32	1.96	2.55	3.13	4.24	5.31	6.53	8.23	3.78 a
Thyme oil at 2%	0.58	1.27	1.95	2.35	2.96	3.85	4.80	5.53	6.44	3.30 b
Thyme oil at 3%	0.58	1.02	1.79	2.27	2.70	3.57	4.21	5.03	6.04	3.02 d
Clove oil at 2%	0.64	1.12	2.00	2.20	2.91	3.79	4.53	5.25	6.32	3.20 c
Clove oil at 3%	0.55	1.06	1.73	2.16	2.70	3.34	4.07	4.62	5.86	2.90 e
Mean	0.61 I	1.16 H	1.89 G	2.31 F	2.88 E	3.76 D	4.58 C	5.39 B	6.58 A	
L.S.D for the interaction effect between treatments and storage periods at 5%= 0.271										
Treatment	2012 season									Mean
Control	0.51	1.09	1.72	2.56	3.40	4.40	5.06	6.00	7.81	3.62 a
Thyme oil at 2%	0.52	1.09	1.70	2.43	3.24	4.20	4.80	5.67	7.11	3.42 b
Thyme oil at 3%	0.49	1.07	1.62	2.22	2.87	4.03	4.66	5.10	6.57	3.18 c
Clove oil at 2%	0.51	1.07	1.70	2.46	2.70	4.10	4.73	5.26	6.62	3.24 c
Clove oil at 3%	0.47	1.03	1.67	2.10	2.92	3.97	4.48	5.03	6.05	3.08 d
Mean	0.50 I	1.07 H	1.68 G	2.35 F	3.03 E	4.14 D	4.75 C	5.41 B	6.83 A	
L.S.D for the interaction effect between treatments and storage periods at 5%= 0.266										

Looking at the effect of storage periods, tabulated data in the same Table declare that weight loss percentage was steadily increased as the storage period prolonged, So, nine weeks cold storage duration registered significantly the highest weight loss percentages, followed descendingly by eight weeks cold storage period. On the contrary, the lowest weight loss percentages were recorded by one week cold storage duration followed ascendingly by two weeks cold storage period in both seasons. The rest storage periods came in between in this sphere in both seasons of this study.

Considering the interaction effect between the tested post-harvest treatments and storage periods, Table (3) demonstrates that the interactions of one week cold storage duration recorded statistically the lowest percentages of fruit weight loss, particularly 3% cloves oil- treated fruits in both seasons. On the opposite, the highest percentages of fruit weight loss were produced by nine weeks storage duration interactions, especially untreated fruits "control" in both seasons. The other interactions of the tested storage periods occupied intermediate

positions between the above mentioned two categories.

Weight loss from harvested horticultural crops is mainly due to water loss through transpiration process, while some weight loss is due to loss of carbon in respiration process, but this is only a minor part of the total (Hardenburg *et al.,* 1990).

The positive effect of eugenol, thymol, and menthol in reduction of weight loss in essential oil treated sweet cherries was reported by Serrano *et al.,* (2005). In addition, thymol and eugenol decreased weight loss in the table grape (Valverde *et al.,* 2005; Valero *et al.,* 2006 and Abdollahi *et al.,* 2012). In this respect Badawy *et al.,* (2011) on orange, Rabiei *et al.,* (2011) and Shirzadeh and Kazemi (2012) on apple and Hassani *et al.,* (2012) on apricot fruits emphasized the obtained results of thyme and clove essential oils.

1.2. Fruit decay percentage:

Referring to the effect of the post harvest treatments, Data in table (4) indicated that clove oil- treated fruits showed to be the most effective treatments in inducing the lowest fruit decay percentages, while un-treated fruits had higher fruit decay percentages in both seasons.

Table 4: Effect of thyme and clove oils treatments on decay % of Washington navel orange fruits under cold storage at 5±2°C during 2011and 2012 seasons.

Treatment	2011 season									
	Storage periods (weeks)									
	1	2	3	4	5	6	7	8	9	Mean
Control	0.00	0.00	0.00	3.63	8.96	19.45	28.43	39.21	52.51	16.91 a
Thyme oil at 2%	0.00	0.00	0.00	1.91	5.30	15.33	24.65	34.24	44.32	13.97 b
Thyme oil at 3%	0.00	0.00	0.00	0.00	5.21	13.64	22.80	33.66	41.50	12.89 bc
Clove oil at 2%	0.00	0.00	0.00	1.73	3.38	11.92	20.74	31.23	39.27	12.03 cd
Clove oil at 3%	0.00	0.00	0.00	0.00	3.23	10.03	21.72	29.16	35.85	11.11 d
Mean	0.00 F	0.00 F	0.00 F	1.45 F	5.22 E	14.07 D	23.67 C	33.5 B	42.69 A	
L.S.D for the interaction effect between treatments and storage periods at 5%= 5.29										
Treatment	2012 season									Mean
Control	0.00	0.00	1.73	1.73	7.11	19.21	30.18	42.13	50.63	16.97 a
Thyme oil at 2%	0.00	0.00	0.00	0.00	6.99	18.95	30.69	41.58	48.53	16.30 a
Thyme oil at 3%	0.00	0.00	0.00	0.00	5.17	16.81	25.71	35.56	43.52	14.09 b
Clove oil at 2%	0.00	0.00	0.00	0.00	5.14	13.48	22.06	35.49	43.43	13.29 bc
Clove oil at 3%	0.00	0.00	0.00	0.00	5.32	13.14	20.34	33.34	39.15	12.37 c
Mean	0.00 F	0.00 F	0.35 F	0.35 F	5.95 E	16.32 D	25.80 C	37.62 B	45.05 A	
L.S.D for the interaction effect between treatments and storage periods at 5%= 4.63										

Evaluating the effect of storage periods, data presented in Table (4) illustrate that fruit decay percentage showed a steadily increment with extending the storage duration in both seasons of this study.

With regard to the interaction effect between the tested post-harvest treatments and storage periods, it is obvious from Table (4) that the interactions of nine weeks storage period scored higher fruit decay percentages in comparison with the corresponding ones of storage durations. Generally, all interactions of one, two and three weeks storage durations produced healthy fruits free from decay and recorded zero decay percentage in both seasons (irrespective the control at three weeks in the second season). On the opposite, all interactions of nine weeks storage duration, especially untreated fruits recorded statistically the highest fruit decay percentage in both seasons. The remained interactions registered inbetween values in this concern.

Many spices and herbs exert antifungal activity due to their essential oil fractions. Application of essential oils for postharvest diseases control of fresh product, as a novel emerging alternative to hazardous anti-fungal treatments will allow a safer and environmentally more acceptable management of post harvest diseases (Hadizadeh *et al*., 2009). The inhibitory effects of plant oils might be regarded to which act as cidal agent against fungal growth and showed abnormal conidia and malformations as swollen, often septated and pale color of hypha (Suwitchayanon and Kunasakdakul, 2009). In addition, some essential oils showed inhibitory effect on pectinase and cellulose enzymes (Dubey *et al.,* 2007 & 2008). Pectinase and cellulase enzymes produced by fruit rotting fungi play a prominent role in disease development during host pathogen interaction (Yakoby *et al.,* 2000). Therefore, essential oils can inhibit the fungi growth by acting on enzymes related to an early stage pathogenesis in the fruits. Some authors related the antifungal property of essential oils to their major compounds especially phenolic compounds such as thymol and carvacrol (Nychas, 1995; Rasooli and Mirmostafa, 2003). On the other hand, thymol and eugenol, as the main components available in tested essential oils of thymus and cloves, respectively , showed high antimicrobial activity against fungal decay on sweet cherry (Serrano *et al.,* 2005) and table grape (Valverde *et al.,* 2005; Valero *et al.,* 2006). Also, Rasooli and Owlia (2005) showed that thyme oil, which is rich in thymol, caused severe damage to cell walls, cell membranes and cellular organelles such as mitochondria of tested fungi which seem to be destroyed.

The gained results of thyme and clove essential oils in reducing fruit decay go in line with findings of Badawy *et al.,* (2011) on orange, Shirzad *et al.,* (2011) on kiwifruit, Abdollahi *et al.,* (2012) on grape, and Hassani *et al.,* (2012) on apricot. Also, Fatemi *et al.,* (2011) showed that thyme essential oils at1000ppm decreased decay compared to the control in inoculated Valencia orange.

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1.3. Shelf life (days):

Data in Table (5) indicate that all tested postharvest treatments progressively increased shelf life of Washington navel orange fruits with significant differences in most cases when compared with control in both seasons. However, 3% clove oil- treated fruits showed to be the most effective treatment for inducing the highest value of shelf life, followed in a descending order by 2% clove oil- treated fruits in both seasons.

The obtained results of essential oil treatments on extending the shelf life of Washington navel orange are in harmony with those of Serrano *et al.,* (2005) on sweet cherry, Valverde *et al.,* (2005) and Valero *et al.,* (2006) on table grape and Bosquez-Molina *et al.,* (2010) on papaya. They mentioned that application of essential oil constituents such as thymol, carvacrol, eugenol and menthol enhanced the shelf life of fruits.

Table 5: Effect of thyme and clove oils treatments on shelf life (days) at 20±5°C of Washington navel orange fruits after nine weeks cold storage at 5±2°C during 2011and 2012 seasons.

Treatment	Shelf life (days)	
	2011 season	2012 season
Control	11.70 C	12.00 C
Thyme oil at 2%	12.70 C	12.00 C
Thyme oil at 3%	13.30 BC	13.00 BC
Clove oil at 2%	14.00 ABC	13.30 ABC
Clove oil at 3%	15.00 A	14.30A

2. Fruit chemical properties:

2.1. Total soluble solid percentage (T.S.S %):

It was obvious from data in Table (6) that all tested post-harvest treatments affected T.S.S % of Washington navel orange fruits in both seasons. However, the highest values of T.S.S % were scored by untreated fruits "control" in both seasons. Besides, the treatments of thyme oil at 2% or 3% in the first season and the treatments of thyme oil at 2% and clove oil at 2% in the second one induced high increments in this concern.

With regard to the effect of storage periods, data in Table (6) clear that the values of T.S.S % of Washington navel orange fruits were increased with advancing storage period till reach to the high increasing at seven weeks storage, then start to decrease gradually to the end of storage periods at nine weeks in both seasons. Briefly, the highest values of this parameter were scored by control and 2% thyme oil- treated fruits under storage period at seven weeks showed its superiority in this sphere in both seasons.

Table 6: Effect of thyme and clove oils treatments on total soluble solid % of Washington navel orange fruits under cold storage at 5±2°C during 2011and 2012 seasons.

Treatment	2011 season										
	Storage periods (weeks)										
	0	1	2	3	4	5	6	7	8	9	Mean
Control	12.73	13.00	13.27	13.50	13.57	13.77	13.80	13.80	13.73	13.77	13.49 a
Thyme oil at 2%	12.73	12.93	13.07	13.17	13.33	13.50	13.67	13.80	13.60	13.40	13.32 b
Thyme oil at 3%	12.73	12.90	12.97	13.13	13.27	13.47	13.53	13.67	13.73	13.70	13.31 b
Clove oil at 2%	12.73	12.80	12.83	12.90	13.20	13.17	13.50	13.73	13.67	13.63	13.22 c
Clove oil at 3%	12.73	12.73	12.73	12.87	13.00	13.20	13.20	13.32	13.37	13.43	13.06 d
Mean	12.73 H	12.87 G	12.97 F	13.11 E	13.27 D	13.42 C	13.54 B	13.66 A	13.62 A	13.59 A	
L.S.D for the interaction effect between treatments and storage periods at 5%= 0.2505											
Treatment	2012 season										Mean
Control	11.23	11.77	12.00	12.20	12.50	13.00	13.03	13.37	12.60	12.27	12.40 a
Thyme oil at 2%	11.23	11.60	11.60	11.93	12.10	12.73	12.83	13.30	13.23	12.73	12.33 a
Thyme oil at 3%	11.23	11.33	11.40	11.70	11.93	12.07	12.47	12.87	12.90	12.20	12.01 c
Clove oil at 2%	11.23	11.50	11.60	11.80	11.90	12.13	12.80	13.00	13.03	12.67	12.17 b
Clove oil at 3%	11.23	11.40	11.40	11.60	11.90	11.87	12.50	12.70	12.90	12.43	11.99 c
Mean	11.23 H	11.52 G	11.60 G	11.85 F	12.07 E	12.36 D	12.73 B	13.05 A	12.93 A	12.46 C	
L.S.D for the interaction effect between treatments and storage periods at 5%= 0.3651											

2.2. Total Acidity percentage:

Data in Table (7) reveal that total acidity of Washington navel orange fruits were statistically increased by all studied treatments as compared with control in both seasons. However, the highest values of fruit total acidity of Washington navel orange was scored by 3% clove oil treatment in both seasons. Also, the treatments of clove oil at 2% and 3% gave high increments in this parameter in both seasons.

Regarding the effect of storage periods, data in the same Table shows that there is gradual decrease in fruit acidity % of Washington navel orange with advancing the storage period under cold storage at 5 ˚C in both seasons. Hence, stored Washington navel orange fruits for nine weeks recorded the lowest values in this sphere, while stored Washington navel orange fruits for zero or one week registered the highest values in this respect. This trend was true in both seasons of this study.

As for the interaction effect between the tested post-harvest treatments and storage periods, data in Table (7) indicate that all combinations of the tested storage periods succeeded in decreasing fruit acidity of Washington navel orange as compared with the combinations of zero storage period. However, the lowest values of this parameter were gained by the combination of nine storage period, especially those of untreated fruits in both seasons.

Table 7: Effect of thyme and clove oils treatments on total acidity % of Washington navel orange fruits under cold storage at 5±2°C during 2011and 2012 seasons.

Treatment	2011 season										
	Storage periods (weeks)										
	0	1	2	3	4	5	6	7	8	9	Mean
Control	1.03	0.84	0.78	0.74	0.70	0.60	0.60	0.58	0.56	0.55	0.70 d
Thyme oil at 2%	1.03	0.94	0.87	0.87	0.79	0.67	0.65	0.60	0.60	0.57	0.76 c
Thyme oil at 3%	1.03	0.94	0.89	0.89	0.77	0.67	0.66	0.65	0.65	0.63	0.78 b
Clove oil at 2%	1.03	0.96	0.84	0.84	0.80	0.70	0.68	0.67	0.66	0.60	0.78 b
Clove oil at 3%	1.03	0.99	0.90	0.90	0.80	0.70	0.69	0.69	0.69	0.67	0.81 a
Mean	1.03 A	0.93 B	0.86 C	0.85 C	0.77 D	0.67 E	0.66 EF	0.64 F	0.63 F	0.60 G	
L.S.D for the interaction effect between treatments and storage periods at 5%= 0.0723											
Treatment	2012 season										Mean
Control	1.03	0.86	0.70	0.68	0.64	0.58	0.57	0.58	0.55	0.55	0.67 d
Thyme oil at 2%	1.03	0.93	0.77	0.74	0.63	0.64	0.62	0.60	0.60	0.60	0.72 c
Thyme oil at 3%	1.03	1.02	0.90	0.74	0.68	0.67	0.66	0.63	0.61	0.58	0.75 b
Clove oil at 2%	1.03	0.93	0.80	0.74	0.71	0.70	0.65	0.63	0.61	0.61	0.74 b
Clove oil at 3%	1.03	1.02	0.80	0.80	0.75	0.74	0.68	0.67	0.62	0.61	0.77 a
Mean	1.03 A	0.95 B	0.79 C	0.74 D	0.68 E	0.67 E	0.64 F	0.62 FG	0.60 G	0.59 G	
L.S.D for the interaction effect between treatments and storage periods at 5%= 0.056											

2.3. Ascorbic acid (mg/100 ml juice):

Data in Table (8) showed that all tested post-harvest treatments scored highly significant increment in V.C (ascorbic acid mg/100ml juice) of orange fruits as compared with control in both seasons. However, 2% clove oil treatment in the first season and 3% clove oil treatment in the second one statistically induced the highest value of V.C, followed in a descending order by 3% thyme oil treatment in the first season and 2% clove oil treatment in the second season.

As for the effect of storage periods, data in Table (8) reveal that there is gradual decrease in V.C of orange fruits with prolonging the cold storage period. So, stored Washington navel orange fruits for nine weeks scored the lowest values of this parameter as compared with other different storage periods in both seasons. While Washington navel orange fruits stored for zero or seven days scored significantly highest values of this parameter as compared to the long storage period of nine weeks in both seasons.

Referring to the interaction effect between the tested post-harvest treatments and storage periods, data in the same Table indicate that regardless of the initial reading (zero day storage period) the combinations of one week storage periods is being the most effective ones in inducing the highest values of this parameter, especially those interacted with 3% thyme oil treatment in the first season and 3% clove oil treatment in the second season. On the opposite, the lowest values of this parameter were gained by the combination of nine weeks storage periods, particularly those of untreated fruits “control" in both seasons. The rest treatments came in between the above mentioned treatments in both seasons.

The obtained results of thyme and clove essential oils in improving fruit chemical properties of Washington navel orange go in line with the findings of Rabiei *et al.,* (2011) and Shirzadeh and Kazemi (2012) on apple and Hassani *et al.,* (2012) on apricot fruits. Also, Fatemi *et al.,* (2011) on Valencia orange showed that the highest level of V.C was observed in thyme treatment at 1000ppm and the highest total acid value was observed in non-inoculated fruits of thyme treatment with 100ppm concentration. But, the highest soluble solids content was

observed in the control without inoculation. On the reveres, quality parameters of kiwifruit such as T.S.S, T.A and V.C reduced in kiwifruits treated with essential oil (Shirzad *et al.*, 2011).

Table 8: Effect of thyme and clove oils treatments on ascorbic acid (mg/100ml juice) of Washington navel orange fruits under cold storage at 5±2°C during 2011and 2012 seasons.

Treatment	2011 season										
	Storage periods (weeks)										
	0	1	2	3	4	5	6	7	8	9	Mean
Control	48.63	45.96	43.46	42.39	40.76	40.73	40.12	38.59	38.48	37.08	41.62 b
Thyme oil at 2%	48.63	46.42	44.05	42.47	39.44	40.88	40.79	39.23	38.74	37.84	41.85 ab
Thyme oil at 3%	48.63	47.00	44.80	43.55	43.24	40.88	40.68	40.08	39.66	38.09	42.66 a
Clove oil at 2%	48.63	46.36	44.80	43.12	42.92	42.25	41.38	40.23	39.22	38.58	42.75 a
Clove oil at 3%	48.63	46.80	44.82	43.33	36.53	42.88	41.56	40.27	39.38	38.80	42.30 ab
Mean	48.63 A	46.51 B	44.39 C	42.97 D	40.58 EF	41.52 E	40.91 EF	39.68 FG	39.10 GH	38.08 H	
L.S.D for the interaction effect between treatments and storage periods at 5%= 2.959											
Treatment	2012 season										Mean
Control	48.31	46.26	46.17	44.31	42.27	41.56	41.25	40.16	39.28	36.18	42.58 c
Thyme oil at 2%	48.31	46.15	45.52	44.58	44.28	43.34	42.79	42.60	40.72	40.42	43.87 b
Thyme oil at 3%	48.31	47.20	45.51	44.85	44.36	44.29	43.43	42.97	40.93	40.75	44.26 ab
Clove oil at 2%	48.31	47.18	46.96	46.29	44.53	43.36	43.28	43.06	41.08	40.69	44.47 ab
Clove oil at 3%	48.31	47.88	46.91	46.69	45.22	44.53	43.97	43.22	42.72	41.26	45.07 a
Mean	48.31 A	46.93 B	46.21 BC	45.34 C	44.13 D	43.42 DE	42.94 EF	42.40 F	40.95 G	39.86 G	
L.S.D for the interaction effect between treatments and storage periods at 5%= 2.743											

2.4. Respiration rate (ml CO_2/kg fruits/hr):

It was quite clear from data in Table (9) that the respiration rate of Washington navel orange fruits was greatly decreased by all the examined post-harvest treatments, with superior 3% clove oil treatment in both seasons.

With respect for the effect of storage periods, Table (9) indicate that respiration rate of Washington navel orange fruits were greatly increased with prolonging storage periods in both seasons. Therefore, irrespective of the initial values (zero day cold storage); the lowest respiration rate values were scored by two weeks storage period, whereas the highest values were registered at the end of cold storage for nine weeks in both seasons.

Regarding the interaction effect between the tested post-harvest treatments and storage periods, data in Table (9) reveal that the lowest respiration rate of Washington navel orange were gained by the combinations of two weeks storage period, especially those combined with clove oil at 3% in both seasons. On the contrary, the highest respiration rates of Washington navel orange were recorded by those stored under cold storage for nine weeks particularly, those of untreated ones in both seasons.

The gained results of essential oils in this respect are in harmony with the analogous ones mentioned by Rabiei *et al.*, (2011) and Shirzadeh and Kazemi (2012) who found that treated apple fruits with thyme and lavender essential oils decreased ethylene production during cold storage.

Table 9: Effect of thyme and clove oils treatments on respiration rate (ml CO_2/kg fruits/hr) of Washington navel orange fruits under cold storage at 5±2°C during 2011and 2012 seasons.

Treatment	2011 season						
	Storage periods (weeks)						
	0	2	4	6	8	9	Mean
Control	11.65	3.21	4.20	4.31	8.30	10.61	7.05 a
Thyme oil at 2%	11.65	2.60	3.30	3.61	6.10	9.79	6.18 b
Thyme oil at 3%	11.65	2.50	3.03	3.30	5.90	8.43	5.80 c
Clove oil at 2%	11.65	3.00	2.81	3.30	5.60	8.90	5.88 c
Clove oil at 3%	11.65	2.40	2.80	3.00	5.20	7.90	5.49 d
Mean	11.65 A	2.74 F	3.23 E	3.50 D	6.22 C	9.13 B	
L.S.D for the interaction effect between treatments and storage periods at 5%= 0.309							
Treatment	2012 season						Mean
Control	11.60	2.29	3.82	5.15	7.11	9.20	6.53 a
Thyme oil at 2%	11.60	1.97	3.81	4.54	6.71	8.80	6.24 b
Thyme oil at 3%	11.60	1.86	3.31	4.17	5.55	6.45	5.49 d
Clove oil at 2%	11.60	1.75	3.76	4.56	5.72	6.66	5.68 c
Clove oil at 3%	11.60	1.57	3.34	4.11	5.02	5.15	5.13 e
Mean	11.60 A	1.89 F	3.61 E	4.51 D	6.02 C	7.25 B	
L.S.D for the interaction effect between treatments and storage periods at 5%= 0.230							

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References

A.O.A.C., 1985. Association of Official Agriculture Chemist. Official Methods of Analysis 4th ed. pp: 495-510. Benjamin Franklin Station, Washington. D.C., U.S.A.

Abdollahi, A., A. Hassani, Y. Ghosta, I. Bernousi, M.H. Meshkatalsadat, R. Shabani and S.M. Ziaee, 2012. Evaluation of essential oils for maintaining postharvest quality of Thompson seedless table grape. Natural Product Research, 26(1): 77-83.

Badawy, F.M.I., M.A.N. Sallam, A.R. Ibrahim and M.R. Asran, 2011. Efficiency of some essential oils on controlling green mold of orange and their effects on postharvest quality parameters. Plant Pathology Journal (Faisalabad), 10(4): 168-174.

Bhuiyan, M.D.N.I., B. Jaripa, C.N. Nemai and A. Farhana, 2010. Constituents of the essential oils from leaves and buds of clove (*Syzigium caryophyllatum* (L.) Alston). African Journal of Plant Science, 4(11): 451-454.

Bosquez-Molina, E., E. Ronquillo-de Jesus, S. Bautista-Banos, J.R. Verde-Calvo and J. Morales-Lopez, 2010. Inhibitory effect of essential oils against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in stored papaya fruit and their possible application in coatings. Postharvest Biology and Technology, 57(2): 132-137.

Bourdy, G.S.D., L.R. Chvez and D.M. Deharo, 2000. Medicinal plants uses of the tacana, an Amazonian Bolivian ethnic group. J. Ethnopharmacol, 70: 87-109.

Brul, S. and P. Coote, 1999. Preservative agents in foods: Mode of action and microbial resistance mechanisms. Int. J. Food Microbiol., 50: 1-17.

Dubey, R.K., J.R. Kumar and N.K. Dubey, 2007. Evaluation of *Eupatorium cannabinum* Linn. oil in enhancement of shelf life of mango fruits from fungal rotting. World J. Microbiol. Biotechnol., 23: 467-473.

Dubey, R.K., J.R. Kumar, J.P.N. Chansouria and N.K. Dubey, 2008. Evaluation of *Amomum subulatum* Roxb. oil as a source of botanical fungi toxicant for the protection of mango fruits from fungal rotting. J. Food Saf., 28: 400-412.

Fatemi, S., M. Jafarpour, S. Eghbalsaied, A. Rezapour and H. Borji, 2011. Effect of essential oils of *Thymus vulgaris* and *Mentha piperita* on the control of green mould and postharvest quality of *Citrus Sinensis* cv. Valencia. African Journal of Biotechnology, 10(66): 14932-14936.

Hadizadeh, I., B. Peivastegan and H. Hamzehzarghani, 2009. Antifungal activity of essential oils from some medicinal plants of Iran against *Alternaria alternate*. Am. J. Applied Sci., 6(5): 857-861.

Hardenburg, R.E., A.E. Watada and C.Y. Wang, 1990. The commercial storage of fruits, vegetables and florist and nursery stocks. Agri. Handbodk Number 66, USDA, pp: 19.

Hassani, A., Z. Fathi, Y. Ghosta, A. Abdollahi, M.H. Meshkatalsadat and R.J. Marandi, 2012. Evaluation of plant essential oils for control of postharvest brown and gray mold rots on apricot. Journal of Food Safety, 32(1): 94-101.

Ju, A.T., J.H. Warcup, W.M. Wright and G.J.F. Pugh, 2000. The effect of fungicide on certain chemical and microbial of soil. Soil Biol. Biochem., 6(4): 263-267.

Lurie, S. and E. Pesis, 1992. Effect of acetaldehyde and anaerobiosis as postharvest treatment on the quality of peaches and nectarines. Postharvest Biol. And Technol., 1: 317-326.

Ministry of Agriculture and Land Reclamation Statistics, Egypt, 2010. Economic Affairs Sector. Bulletin of the Agricultural Statistics (In Arabic).

Mironescua, M. and C. Georgescub, 2008. Preliminary researches on the effect of essential oils on moulds isolated from surfaces. Journal of Agroalimentary Processes and Technologies, 14: 30-33.

Namiki, M., 1990. Antioxidant/antimutagens in food. Food Sci. Nutr., 29: 273-300.

Nychas, G.J.E., 1995. Natural antimicrobials from plants. In: Gould GW (ed). New Methods Food Preserv., Blackie Academic Professional, London, pp: 58-89.

Paster, N. and L.B. Bullerman, 1988. Mould spoilage and mycotoxin formation in grains as controlled by physical means. Int. J. Food Microbiol., 7: 257-265.

Pesis, E. and R. Ben-Arie, 1984. Involvement of acetaldehyde and ethanol accumulation during induced deastringency of persimmon fruits. J. Food Sci., 49: 896-899.

Rabiei, V., E. Shirzadeh, H.R. Angourani and Y. Sharafi, 2011. Effect of thyme and lavender essential oils on the qualitative and quantitative traits and storage life of apple 'Jonagold' cultivar. Journal of Medicinal Plants Research, 5(23); 5522-5527.

Rasooli, I. and P. Owlia, 2005. Chemoprevention by thyme oils of *Aspergillus parasiticus* growth and aflatoxin production. Phytochemistry, 66: 2851-2856.

Rasooli, I. and S.A. Mirmostafa, 2003. Bacterial susceptibility and chemical composition of essential oils from *Thymus kotschyanus* and *Thymus persicus*. J. Agric. Food Chem., 51: 2200-2205.

Samson, J.A., 1984. Tropical fruits.Tropical agricultural series. Longman Inc., New York, pp: 64-118.

Serrano, M., D. Martinez-Romero, S. Castillo, F. Guillen and D. Valero, 2005. The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. Innov. Food Sci. Emerg. Technol., 6: 115-123.

Shirzad, H., A. Hassani, Y. Ghosta, A. Abdollahi, R. Finidokht and M.H. Meshkatalsadat, 2011. Assessment of the antifungal activity of natural compounds to reduce postharvest gray mould (Botrytis cinerea Pers.: Fr.) of kiwifruits (*Actinidia deliciosa*) during storage. Journal of Plant Protection Research, 51(1): 1-6.

Shirzadeh, E. and M. Kazemi, 2012. Effect of essential oils treatments on quality characteristics of apple (*Malus domestica* var. Gala) during storage. Trends in Applied Sciences Research, 7(7): 584-589.

Smid, E.J. and L.G.M. Gorris, 1999. Natural antimicrobials for food preservation, in Handbook of Food Preservation. Ed. M.S. Rahman, Marcel Dekker, New York, pp: 285-308.

Snedecor, W. and W.G. Cochran, 1989. Statistical Methods, 8th ed. Iowa State Univ. Press Ames. Iowa. U.S.A.

Sokovicet, M.D., J.P.D. Vukojevic, D.D. Marin, V. Brkic, L.J. Vajs and L.D. Van, 2009. Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities. Molecules, 14: 238-249.

Suwitchayanon, P. and K. Kunasakdakul, 2009. *In vitro* effects of clove and turmeric extracts controlling crucifer pathogens. Journal Agricultural Technology, 5(1): 193-199.

Valero, D., J.M. Valverde, D. Martínez-Romero, F. Guillén, S. Castillo and M. Serrano, 2006. The combination of modified atmosphere packaging with eugenol or thymol to maintain quality, safety and functional properties of table grapes. Postharvest Biol. Technol., 41: 317-327.

Valverde, J.M., F. Guillén, D. Martínez-Romero, S. Castillo, M. Serrano and D. Valero, 2005. Improvement of table grapes quality and safety by the combination of modified atmosphere packaging (MAP) and eugenol, menthol, or thymol. J. Agric. Food Chem., 53: 7458-7464.

Viuda, M.M., Y.N. Ruiz, J.L. Fernandez and J.A. Perez, 2007a. Antifungal activities of thyme, clove and oregano essential oils. Journal of Food Safety, 27(1): 91-101.

Viuda, M.M., Y.N. Ruiz, J.L. Fernandez and J.A. Perez, 2007b. Chemical composition of the essential oils obtained from some spices widely used in Mediterranean region. Acta Chim. Slov., 54: 921-926.

Yakoby, N., S. Freeman, A. Dinoor, N.T. Keen and D. Prusky, 2000. Expression of pectate lyase from *Colletotrichum gloeosporioides* in *C. magna* promotes pathogenicity. Mol. Plant Microbe Interact., 13: 887-891.

Zeng, R., A. Zhang, J. Chen and F. Yong-Qi, 2012. Postharvest quality and physiological responses of clove bud extract dip on 'Newhall' navel orange. Scientia Horticulturae, 138: 253-258.