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Assessment of peppermint, clove, cumin essential oils and silver nano particles on biochemical and shelf life of *Citrus limon* (L.)

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Natural and chemical compounds are used to increase the biochemical and shelf life of citrus fruits. The effect of nano silver particles and three essential oils: peppermint, carnation, cumin, were investigated on morphological, biochemical and shelf life of the lemon fruit. The first factor consisted of 13 different substances and the second factor was three the level of storage periods (zero, one, and two months after harvest). The results showed that the treatments significantly affected the fruit weight, diameter, length, decay percentage, total soluble solids, vitamin C, electrolyte leakage and titratable acidity. Peppermint essential oil showed a 70% reduction of decay percentage compared to with the control. Five percent of nano silver and 500 µL/L of clove essential oil maintained better fruit quality than control. Although the effects of silver nano-particle were significant, considering the environmental concerns we recommend using plant essential oils to prolong the postharvest shelf life of lemon fruits.

Keywords: *Citrus limon* (L.), Clove, Cumin, Decay percentage, Electrolyte leakage, Essential oils, Nano silver particles, Peppermint

Consumption of the fruits and vegetables in daily diets leads to decrease of dangerous ailments such as cancer and heart diseases. The presence of health promoting substances, for instance, vitamin C, polyphenols, flavonoids, carotenoids, and antioxidant are evident in fruits especially citrus. The main compounds of flavonoid in citrus include:hydroxyl cinnamic acid, ferulic acid, coumaric acid, cinnamic acid and caffeic acid[1].

Lisbon lemon (*Citrus Limon* L.) belongs to Rutaceae family. It is medium size, fleshy fruit with thick skin, few seeds (2-4), and very acidic. One of the advantages of this fruit is its density at the center of the tree which protects it from low and high temperature. This fruit has attracted special attention because of its high yield[2]. Postharvest techniques to reduce fruit waste are fundamental for the agricultural industry. Citrus fruits cannot be kept for long-time under ambient condition. Storage of citrus in high quality requires industrial and technical facilities and installations, which is very expensive. Under these conditions, the application of advanced methods for postharvest storage can be highly effective[3]. With applying the advanced approaches to extend the life of citrus fruits in industrial storage, the amount of wastes has been decreased by 5-10%. Losses of fruits, citrus fruits, in particular, lead to loss of different resources such as water, soil, human force and time resources and finally social-economical facilities of a country. Undesirable effects of fruit wastes are more than economic losses, but they are not clear and they are hidden from public sight[4].

The most important factor in the development of modern agriculture is changing in agricultural-related technologies. Among them, nanotechnology has provided suitable conditions to produce agricultural and nutritious products. Nanotechnology uses have been increased sharply in many agriculture areas such as recycle science, transforming food and agricultural leftovers to energy and other products, different nano-pesticides, and disease prevention. Silver has been

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Abbreviations: ANOVA, Analysis of variance; EC, Electrical conductivity; TA, Titratable acidity; TSS, Total soluble solids used as one of the disinfectants against harmful microorganisms[5,6]. Silver nano-particle has strong anti-bacterialproperties[7] and it is used as an anti-microbial cover on wooden and plastic surfaces[8]. But, there is little information about its effects on controlling plant pathogenic factors and the extension shelf-life of fruits and vegetables. On the other hand, considering the side effects and hazards of a chemical preservative, herbal plants, and natural compositions can be used alternatively to preserve fruits. Many reports have shown that essential oil (EO) has anti-microbial and anti-fungal properties. They are secondary metabolites of herbal plants that are natural, almost safe and completely biodegradable. Essential oils have antibacterial properties due to the phenolic compounds at high concentration[9] such as thymol, carvacrol, and eugenol[10-12]. Recently, EOs have been used successfully to control pathogenic diseases in fruits and vegetables[13].

Alikhani *et al.*[14] used mucilage of cactus (Prickly pear) and thyme EOs and their combination to cover strawberry and study their effects on storage period duration. The results showed that the rate of fruit microbial decay decreased and it led to keep freshness and percentage of soluble solids in the fruits. Karimi & Rahemi[15] studied the effects of essential oils of thyme, clove and Imazalil fungicide on *Penicillium italicum* decay of citrus fruits in cold storage. Using pure oil essence of thyme and clove on Valencia orange showed that they reduce decay from 90 to 12.5%. In a study by Aboutalebi & Mohammadi[16] effects of herbal plants essences on increasing quality and decreasing decay of Kino tangerine was investigated. The lowest percentage of decay was observed in mint essence treatment with 750 mg/L and the highest percentage of decay was observed in the control. Jamali *et al.*[17] studied the effect of soaking treatments with methyl salicylate on maintaining the quality of Moro orange, (*C. sinensis* cv Moro), during the storage period. The results showed that during storage the ratio of total soluble solids/titratable acidity (TSS/TA) decreased, and the rate of TA and bitterness increased. The thickness of the fruit skin was the highest when kept individually in plastic bags and by using methyl salicylate.

To the best of our knowledge, there is no research about the effect of silver nano-particles and herbal EOs to extend the storage period of *Citrus limon* L. Thus, this research was conducted to study the effect of different concentrations of silver nano-particles and many herbal essences on increasing the long-life of *Citrus limon* L.

Materials and methods

Essential oils and silver nano particle treatments

Uniform size, healthy and free of any diseases lemons fruits were harvested from a commercial orchard in Iran. The experiment was performed as a factorial arrangement based on a completely randomized design with three replications. The first experimental factors were silver nanoparticles, clove, cumin, and peppermint EOs at three levels and the second factor includes the duration of storage period at three levels (initial day, on day 30th and 60th).

Considering that in other studies on different fruits and vegetables[18-20], various and different concentrations of nano silver was used for shelf life. The authors have chosen concentrations that are in the range of other researchers' studies. Selected concentrations included 5 ppm, 15 ppm and 25 ppm of silver nano particles. The treatments were prepared by mixing different concentrations of stock solution (4000 ppm) of silver nano particles with distilled water. In addition, three different concentrations (100, 250 and 500 ppm) of former herbal EOs (Barij Essence Co. Iran) were prepared according to the previous studies[21]. In order to disinfect the fruits with silver nanoparticles and EOs, they were soaked indifferent concentrations of nano and EOs (peppermint, clove, and cumin) solutions for 2 min. Then, fruits were transferred to cold storage after drying at room temperature.

The percentage of electrolyte was measured using an electrical conductivity (EC) system. For this, six disks (10 mM in diameter) consisting of flavedo and albedo tissue were removed from each fruit. After rinsing two times with sterile distilled water, they were immersed in 40 mL deionized water with constant shaking for four hours. Then, conductivity was measured by a conductivity meter (value E1). The tubes containing disks were autoclaved and allowed to cool at room temperature. Solution conductivity was measured at this time and recorded as the final value (value E2). Electrolyte leakage was calculated as the percentage of the initial (E1) to final (E2) conductivity[22].

Total soluble solids (TSS) in the extract of each fruit were measured by a refractometer Atago at 20°C. Acidity was determined by potentiometric titration with 0.1 sodium hydroxide (NaOH) and phenolphthalein indicator using the below formula.

Acidity = the amount of used NaOH*0.064

Vitamin C content was measured by a titration[23] (an iodometric method) of iodine at potassium iodide and using starch as an indicator. Decay analysis[24] was carried out through observation and counting the number of decayed fruits at each repetition. Diameter and length of fruits were measured by a digital caliper. Furthermore, the weight of fruit was measured by a precise digital scale.

Statistical analysis of the data obtained during the experiments were analyzed by analysis of variance (ANOVA), using SAS software and mean comparisons were calculated with Duncan test to examine differences among means.

Results

The results showed that the treatments significantly affected all qualitative traits at 1% level (Table 1). Although, storage period and combinational effects of storage, EOs and silver-NP were not significant for characteristics such as diameter and length, acidity, weight of fruit flesh and refuse, While, TSS, vitamin C, decay percentage and percentage of electrolyte leakage were affected by storage period and its combinational effects with EOs and silver-NP (Table 2).

The results showed that fruits at the second and third level of peppermint treatments (250 and 500 mL) exhibited the lowest fruit length while the controls showed the highest. Also, the lower rate of decreasing of the fruit length occurred at the third level of silver-NP. The third level of peppermint (500 ppm) and third level of clove (500 ppm) EOs led to the lowest fruit acidity. However, the highest weight of

Table 1 — Variance analysis results of silver nano-particles, peppermint, carnation and caraway essence and storage period on studied traits

Source of variance	Freedom degree	Mean square of studied traits								
		Pulp weight (g)	Meat weight (g)	Electrolyte leakage	Decay (%)	Vitamin C	Acidity	Soluble solids	Fruit length (mm)	Fruit diameter (mm)
Material type	12	9.84**	6.48**	623.93**	89.56**	52.40**	552.54**	8.88**	7.58**	5.64**
Storage period	2	0.12 ns	0.31 ns	19013.11**	1846.79**	91.26**	3.17 ns	19.58**	0.36 ns	4.54 ns
Material type *storage period	24	1.43 ns	1.01 ns	270.31**	39.61**	6.97**	6.22 ns	1.86**	2.67 ns	2.15 ns
Error	78	1.78	1.09	22.74	7.05	1.83	12	0.66	2.65	2.02
Coefficient of variation		3.72	1.39	7.41	33.95	10.39	3.94	9.70	2.24	2.32

** indicate statistically significant differences at 1% probability level and 'ns' means none statistically significant differences.

Table 2 — Mean comparison of material type on studied characters using LSD test.

Treatments	Studied traits								
	Pulp weight	Meat weight	Electrolyte leakage (%)	Decay	Vitamin C	Acidity	Soluble solids	Fruit length (mm)	Fruit diameter (mm)
C	33.1	72.7	81.5	15.0	10.1	70.1	7.1	71.3	59.6
SN1	35.8	75.1	69.1	7.8	13.6	73.2	8.4	73.1	60.6
SN2	36.4	75.5	72.5	7.2	17.2	81.2	9.7	72.7	60.8
SN3	36.3	75.6	73.6	5.0	17.9	87.4	9.7	74.1	61.1
PE1	35.3	74.3	49.9	9.4	10.6	91.0	7.8	71.4	60.8
PE2	36.6	75.5	57.2	5.6	11.8	92.0	8.6	73.3	62.2
PE3	36.4	75.5	61.3	2.2	12.9	93.1	9.2	73.8	62.7
CnE1	34.9	74.4	66.7	11.1	10.1	91.9	6.7	71.1	60.9
CnE2	36.2	75.1	62.2	9.4	11.9	91.8	8.7	73.1	61.2
CnE3	36.9	76.0	64.3	6.1	13.2	93.0	9.1	73.6	62.0
CE1	35.5	74.8	59.1	8.9	12.4	91.6	7.2	71.8	61.2
CE2	35.5	75.0	60.1	7.8	13.3	92.0	7.8	72.3	60.9
CE3	37.1	75.6	58.2	6.1	14.1	92.3	9.2	72.7	61.3
LSD 5%	1.25	0.98	4.48	2.49	1.27	3.25	0.76	1.53	1.34
LSD 1%	1.66	1.30	5.93	3.31	1.68	4.31	1.01	2.03	1.77

C: control, SN: silver nano-particles, PE: peppermint essence, CnE: carnation essence, CE: cumin essence.

fruit flesh and fruit refuse observed at the third level of clove and cumin EOs.

According to the results, it is clear that the best treatments to preserve lemon fruits compared with the control were the third level of peppermint, clove and cumin EOs. Also, studying the slicing interactions for traits showed that the effect of their interactions was significant, and also, there was a significant difference between three time periods and type of materials (Table 3). Also, the slicing studying of material type based on time showed some of the materials didn't exhibit significant different during three time periods. These suggest that these materials could save these properties of the fruits. For instance, the first level of silver-NP (5 ppm) and the second level of cumin EO (250 ppm) could preserve the rate of existing soluble solids and vitamin C. In the present study, the treatments successfully extended the storage period up to three months (Table 3).

Discussion

Fungicidal activity of the essential oils have been confirmed in many experiments[25,26] but there islimited knowledge about the effect of these natural compounds on the quality of the fruits. Recently, the demands for natural and organic food products have been increased due to the public awareness and concern of chemical preservatives. Thus, these kinds of researches have attracted more attention to find the suitable alternative for artificial chemicals. The results of this research showed that different levels of peppermint, clove and cumin EOs and silver-NP significantly affected the morphological and biochemical traits of lemon fruits during three months storage period. In control, reduction of lemon fruit diameter was higher than all other different levels of EOs and silver-NP treatments. This trait has a major impact on consumer acceptance and marketing of the lemon fruit.

Most of the horticultural products have high amounts of organic acid which play an important role in the metabolism and the quality of the crops. One of the most important organic acids is citric acid. The rate of fruit acidity changes in different stages of its growth so that they are degraded and transformed into sugar and causes reduction of fruit acidity. Level of acidity and the type of presented acids in the fruit are very important factors for quality of taste, postharvest storage extension and also this parameter can be used asa harvest index in conjunction with total soluble solids[27].

Table 3 — Slicing of studied traits interaction

Source of variance	Freedom degree	Mean square of studied traits			
		Electrolyte leakage	Decay %	Vitamin c	Soluble solids
Slicing time on material type					
Harvest time	12	42.76	0.001 ns	4.48**	5.2**
One month storage	12	441.15**	53.13**	29.13**	5.43**
Three months storage	12	680.64**	115.49**	31.95**	1.99**
Slicing of material type on storage time					
C	2	3499.96**	608.33**	7.44**	0.009 ns
SN1	2	824.66**	136.11**	3.44 ns	0.29 ns
SN2	2	1050.57**	119.44**	49.25**	017 ns
SN3	2	969.68**	58.33**	58.78**	0.14 ns
PE1	2	208.57**	219.44**	5.86*	0.69 ns
PE2	2	1267.74**	77.78**	5086*	3.6**
PE3	2	20144.5**	44.44**	13.36**	3.94**
CnE1	2	3328.76**	311.11**	2.03 ns	10.87**
CnE2	2	1350.24**	219.44**	4.69 ns	11.94**
CnE3	2	2620**	102.78**	11.44**	3.65**
CE1	2	1595.9**	177.78**	1.19 ns	3.36**
CE2	2	2496.62**	144.44**	2.58**	1.23 ns
CE3	2	1029.72**	102.78**	9.03**	2.14*

*, **represent significantly different at 1% and 5% probability level, respectively and 'ns' represents none significantly different.

C: control, SN: silver nano-particles, PE: peppermint essence, CnE: carnation essence, CE: cumin essence

Vitamin C was increased by application of high concentrations of silver-NP and EOs. Structurally, vitamin C is one of the simplest vitamins and it is synthesized from glucose or other simple sugars in plants. This vitamin is degraded and hydrolyzed by the activity of oxidase ascorbic acid enzyme. The level of ascorbic acid remains almost unchanged on a plant, but it decreases after harvesting the plant fruit or other parts[27]. Studies showed that the reduction of vitamin C or other antioxidant enzymes increases the oxidative damage in the plants. Antioxidants increase fruit resistance against stresses and diseases[28].

The reason for the decreasing rate of ascorbic acid can be related to neutralizing free radicals. Due to increased oxidative metabolism, active aux in species increase and this led to destroying biological membranes.

Plants activate their antioxidant systems such as enzymes including ascorbate peroxidase or non-enzymatic systems, ascorbic acid (vitamin C) or α-tocopherol (vitamin E) to prevent cells from damages by reactive oxygen species[29,30]. Antioxidants are changed into oxide by giving the electron to reactive oxygen species and eliminate their oxidation power and their damages[31]. Decreasing ascorbic acid in plants can increase the production of free radicals that they react with polysaccharides in the cell wall and destroy the cell. Brasil & Siddiqui[32] demonstrated that the oxygen and duration of storage are the main reasons for decreasing the amount of ascorbic acid in orange juice. Also, Emamifar *et al.*[18] reported that packages containing silver nanocomposite keep more ascorbic acid compared with normal polyethylene in fresh orange juice.

The amount of total soluble solids in *citrus limon* L. were affected by different concentrations of peppermint, cumin, clove EOs, and silver-NP. Most changes which occur at the ripening time are related to the degradation of polymeric hydrocarbons especially existing sugars in the cell wall that eventually they lead to changes in fruit taste and tissue[2].

Acidity taste of the fruits relates to organic acids and often one of them dominates the other, which is accountable for the acidity of the fruits. Organic acids decrease quickly after harvest and the rate of reduction gradually become slower. This rate is slower in fruits like strawberry. Decreasing of acidity during ripening of the fruit is due to the role of organic acid in respiration or conversion into sugar[3].

Application of EO decreases the losses of fruits as well as guarantees the safety of products. The sensitivity of fungi to EOs varies significantly and depends on the type of EO, its dose and compositions[33]. A higher level of EOs and a higher concentration of silver-NP remarkably reduced the decay in lemon fruits. Anti-microbial effects of silver-NP a reduce to the disruption of bacterial normal activity such as respiration or catabolism. Furthermore, silver ions penetrate in the bacterial cell, interact with their DNA, attack respiration chain, eliminate its reproduction system and finally kill them[34]. Studies showed that silver nanocomposite has more anti-microbial effects than other nanocomposites[35].

Fruit flesh weight decreased significantly during the storage time and the highest rate of weight decrease belong to the control. EOs and silver-NP reduced flesh weight loss. The effects of the clove EO on weight loss of grapes[36] and cherry[37] have been reported which is in line with our study. Higher respiration is one of the reasons for weight loss in fruits[37]. Many studies showed that EOs can reduce the agricultural crops respiration after harvest[38,39].

A number of studies have shown that the EOs or nano silver treatment can improve the biochemical traits and extend the shelf life of fruits[40-43] which is in agreement with the results of this study. Since plant EOs has been natural and organic, they can be recommended to be used because they do not have toxicity for the environment or human.

Conclusion

Using silver-NP and EOs significantly increased the quality of citrus lemon after harvest. Nanoparticles and EOs maintain the quality of the fruits and extend the shelf-life of the fruits by decreasing the decay percentage, reduction of vitamin C, and electrolyte leakage. EOs can be used as a novel and cost-effective approach to extend the citrus lemon storage period. The results of this experiment showed that the effect of nano-particles on decay percentage reduction is similar to the effect of EOs. However, the application of EOs is recommended instead of silver-NP to reduce biological and environmental impacts of nanoparticles.

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