Effect of Cumin Essential Oil on Postharvest Decay and Some Quality Factors of Strawberry

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Receive: 5 May 2009 **Acceptance:** 10 Aug. 2009

Abstract

Background: An essential oil is a liquid that is generally distilled from the leaves, stems, flowers or other elements of a plant.

Objective: Cumin (*Cuminum cyminum* L.) Essential Oil (CEO) was analyzed to determine oil component. Antifungal effect of CEO was determined by *in vitro* technique. In second stage, effect of different concentrations of CEO by PDM on postharvest life of strawberry (*Fragaria×ananassa* Duch.) fruit, cultivar Selva, was studied.

Methods: Antifungal effect of CEO was carried out with Paper Disk Method (PDM) and Solution Method (SM). In second stage Fruits were infected artificially by *Botrytis cinerea* spore, and then treated by different concentration of CEO. The rate of weight loss, fruit firmness, Total Soluble solids (TSS), titrable acidity, pH, ascorbic acid and color of fruits were determined at 0, 3, 6, 9, 12 and 15 days after the beginning of storage. The degree of infection on fruit was rated using a scale of 0 to 8, Fruit surface color was measured by Chromameter (CR 400, Minolta), TA by titration method, ascorbic acid content by iodometric method TSS content (Brix°) by refractometer and pH by pH meter.

Results: PDM was effective as antifungal. Storage life of the strawberry fruits was increased by the use of CEO significantly, by inhibition of fungal infection compared to controls. No significant fruit firmness, total soluble solids were observed in treated fruits compared to controls at all determination times. CEO treated fruit maintained higher L^* at 3 and 6 day in compare with control. Titrable acidity, pH and ascorbic acid were significant in some stages.

Conclusion: Results of present paper confirmed antifungal effect of CEO in both *in vitro* and on fruit postharvest. However, more studies are required to recommendation of CEO as a commercial and natural antifungal for increase postharvest on horticultural crops.

Keywords: Strawberry, Cumin, Antifungal, Postharvest, Essential oil

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Introduction

Strawberry (*fragaria×ananassa* Duch.) is a nonclimactric fruit characterized by short postharvest life, often estimated less than a week even under ideal conditions at 8˚C [49]. It must be harvested at full maturity to achieve maximum quality in terms of visual appearance (freshness, color and absence of decay or physiological disorders), texture (firmness, juiciness and crispness), flavor and nutritional value (vitamins, minerals, dietary fiber and phytonutrients). Loss of quality is frequently due to the onset of rots, often caused by *Botrytis cinerea* [13, 15, 18, 29, 39, 48, 53]. This mold is the most frequently postharvest diseases affecting strawberry in worldwide and most economically significant postharvest pathogen of strawberry fruits [10, 25]. Several pre and postharvest technologies have been used to control their decay [40] to extend the shelf life of strawberries. These include hot water treatments [17, 52], heat treatment [16] control atmospheres [20, 26, 31, 50], ultraviolet light [2, 32, 27], biological natural active products [8, 19], biological control [50] and chemical control [9, 11, 23, 54]. Most of these treatments could be effective, however, these have adversely affect on color, flavor, aroma or texture [17, 20, 22, 24, 37] and several studies have been shown that the compounds used in these chemical fungicides caused strain resistance representing a potential risk for the environment and human health [1, 14, 33]. So that, particularly during postharvest storage and handling, it is important to encourage the rapid development of alternative approaches to plant disease control.

Among the various alternatives, natural plant products, including essential oils that are biodegradable and eco-friendly, are catching the attention of scientists worldwide. Such products from higher plants are bio-efficacious, economical, and environmentally safe and can be ideal candidates for use as agrochemicals.

Essential oils are volatile, natural, complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites. In nature, essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivores by reducing their appetite for such plants [6].

Cumin (*Cuminum cyminum* L.) is an aromatic plant in the family Apiaceae. Its fruit, known as cumin seed, is a lateral fusiform with abundant content of essential oil. In indigenous medicine, cumin seeds have long been considered as a stimulant and carminative and are used for therapeutic purposes [21].

The aim of this work was study the effect of different cumin essential oil concentrations on the decay control as well as post-harvest life, firmness, color parameters, pH, soluble solids content, titrable acidity, ascorbic acid of strawberry fruits during storage.

Material and Methods

Essential oil analyses

Cumin essential oil was purchased from Zardband Pharmaceuticals - Medicinal Plants Production Company, Tehran, Iran and was analyzed in Research Institute of Forests and Rangelands of Iran.

Gas chromatography

GC analysis were performed using a Shimadzu GC-9 a gas chromatograph equipped with a DB-5 fused silica column (J & W Scientific Corporation) (30 m × 0.25 mm i.d., film thickness 0.25 μm). Detector (FID) temperature was 280 °C and injector

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temperature was 300 °C. Helium was used as carrier gas with a linear velocity of 32 cm/s. The percentages of compounds were calculated by the area normalization method, without considering response factors.

Gas chromatography–mass spectroscopy

GC–MS analyses were carried out in a Varian 3400 GC–MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm); oven temperature was 50–240 °C at a rate of 4 °C/min, transfer line temperature 260 °C, carrier gas, helium, with a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1 s, and mass range 40–300 amu.

Identification of components

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature. The retention indices were calculated for all volatile constituents using a homologous series of alkanes [12, 41].

***In vitro* antifungal activity**

First, Antifungal activity was studied by using a contact assay (*in vitro*), which produces hyphal growth inhibition. Briefly, potato dextrose agar (PDA) plates were prepared using 8 cm diameter glass Petri dishes. *In vitro* experiment was conducted in a Completely Randomized Factorial Design with two treatments; including five concentration treatments (0, 250, 250, 500 and 1000) $\mu L.L^{-1}$ CEO as first factor and two using method as second factor, and three replications. Two methods were used for essential oil treatment on PDA medium; "Paper Disk Method" and "Solution Method". In PDM, different concentration of CEO was treated on the Paper Disk and was located separately from PDA medium in the Petri dishes. In SM the CEO was dissolved in tween 80 -water solution (5% v/v) [35] and required amounts of the solutions /Petri dish (0, 250, 500, 750 and 1000 $\mu L.L^{-1}$ concentrations) were added to each of the PDA plates containing 20 ml of agar at 45 °C. *B. cinerea* was isolated from strawberry and had cloned. Mycelium inoculation was carried out by Singh *et al*, 2005, method [42]. A 0.5 mm Disk of mycelium was located on PDA medium. The treated medium was incubated in 24˚ C and mycelium growth was determined daily. Inhibitory percent was determined according to the following formula.

IP= dc-dt×100/dc

IP= Inhibitory percent, dc= mycelium growth diameter in control and dt= mycelium growth diameter in essential oil treated Petri dish.

Effect of CEO on postharvest decay and some qualities factors of strawberry

Pathogen inoculation on strawberry fruits

B. cinerea Link was isolated from infected strawberry fruit. The culture was maintained on potato-dextrose agar (PDA) at 4°C, and fresh cultures were grown on PDA plates before use. Spore suspensions were prepared by removing the spores from the sporulation edges of a 20-day-old culture with a bacteriological loop, and suspending them in sterile distilled water. Spore concentration was determined with a hemocytometer, and adjusted as required with sterile distilled water. Uniform strawberries were selected by shape, size and color. This fruit were treated by Sodium hypochlorite (100 $\mu L.L^{-1}$). Then fruits were dipped in prepared suspension and located in room temperature for 2 h in order to

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fixed fungal inoculation.

According to the *in vitro* experiment, PDM method was used. Watman Paper (number 1) was treated by required essential oil and located in the fruit continuing 1 L plastic bags separately. Experiment was conducted in a Completely Randomized Design, including five treatments (0, 60, 250, 500 and 1000 $\mu L.L^{-1}$) CEO, and three replications. 2 kilograms of fruits was used for each replicate. Essential oil-treated and untreated fruits together with controls were transferred into 1 L plastic containers and were steeked in order preventing loss of essential oil and then put into the cold storage (3˚C). Determinations were carried out 0, 3, 6, 9, 12 and 15 days after the beginning of storage.

Effect of CEO on gray mold decay development in artificially inoculated fruits

To evaluation of the effect of CEO on development of natural decay, 9 scaling unit was used. The severity of decay was visually evaluated during and following exposure to essential oil. The degree of infection on fruit was rated using a scale of 0 to 8, where 1= no infection; 2= trace infection low than 10%, 3= infection between 10- 20%, 4= infection between 20- 30%, 5= infection between 30- 40%, 6= infection between 40- 50%, 7= infection between 50- 65%, 8= infection between 65- 80% and 10=infection more than 80%.

Effect of CEO on postharvest quality of strawberry

Weight loss

In order to determine any weight loss during the storage of the fruits, both treated and untreated fruits were weighed 0, 3, 6, 9, 12 and 15 days after treatments.

Fruit firmness

Firmness values of each individual strawberry were measured at two points of the equatorial region by using with a 5 mm diameter probe. The probe descended towards the sample at 2.0 mm s^{-1} and the maximum force (N) was defined as firmness.

Surface color measurement

Fruit surface color was measured on 10 fruit from each replicate at two opposite sides using a chromameter (CR 400, Minolta), which provided CIE L^*, a^*, and b^* values. Negative a^* values indicate green and higher positive a^* values red color. Higher positive b^* values indicate a more yellow skin color. These values were then used to calculate hue degree (h°=arctangent $[b^*/a^*]$), where 0°= red–purple, 90°= yellow, 180°= bluish green, and 270°= blue, and Chroma ($C^*= [a^{*2}+b^{*2}]^{1/2}$), which indicates the intensity or color saturation. ΔE was recorded by $\Delta E =[\Delta L^2+ \Delta a^2+ \Delta b^2]^{1/2}$

Titratable acidity, Total Soluble Solids and pH

Titratable acidity was measured using titration method. To do that, 10 mL fruit juice was added to 60 mL distilled water plus a few drops of phenolphthalein and titrated with 0.1N NaOH up to pH 8.1. The results were expressed as g of citric acid per 100 g fresh weight. Total soluble solids content was determined using Atago (Japan) N1 refractometer at 20˚C and expressed as ˚Brix. The pH of fruit juice was measured using a Jenway 3320 pH meter calibrated by pH 4 and 7 buffer solutions.

Ascorbic acid

The iodometric method was used to

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determine the ascorbic acid content of pressed fruit juice. Results were expressed as milligrams of ascorbic acid per 100 g sample.

Statistical analysis

The data was analyzed using MSTATC statistical software and the means were compared by Duncan's multiple range test.

Result and Discussion

The identified components of cumin essential oil are listed in table 1 in order of their elution time from the HP-5 column. A total of 14 compounds were identified from all the samples, accounting for 88.46 % of the total compositions of individual samples. Components were mainly composed of monoterpenes ($C_{10}H_{16}$). The oil consisted of compounds with γ-Terpinene accounting for 25.5 % of the total constituents. Besides γ-Terpinene, the other major compounds were Cumin aldehyde (24.9 %) and β-Pinene (19.74 %). Significant amounts of ρ-Cymene (12.9%), P-menth-1-en-7- al (5.3%) were also detected (table 1).

Effect of ECO on, *in vitro* mycelium growth

Different method of using CEO in medium had significant effect on inhibition of mycelium growth ($p < 0.05$). PDM method was effective than SM. All concentrations of CEO in PDM method controlled the mycelium growth in contract only concentration of 1000 μ $L.L^{-1}$ controlled fugal growth completely (table 2 and Fig 1). It seems that in SM method essential oil loss antifungal effect with low concentration.

Table 1- Composition of the cumin essential oil from Iran

No	Compound	RI*	Percent
1	α-Thujene	935.3	0.43
2	α-Pinene	492	1.4
3	Camphene	955.5	0.3
4	Sabinene	976.7	0.45
5	β-Pinene	980.4	19.74
6	Myrcene	990.6	0.6
7	α-Phellandren	1002.6	1.04
8	α-Terpinene	1013	0.073
9	ρ-Cymene	1019.8	12.9
10	β-Phellandrene	1023.2	1.14
11	γ-Terpinene	1047.9	25.5
12	Cumin aldehyde	1228.3	24.9
13	P-menth-1-en-7- al	1260.5	5.3
14	Nonanal-dimethyl acetate	1264.7	1.8

* Retention Index

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Table 2- Effect of CEO on inhibition of mycelium growth in various techniques

Treatments (µ L.L-1)	Mycelium growth 6 day after infection (SD)	Mycelium inhibition growth 6 day after infection(SD)
PDM*		
Control (with Tween 80)	7.5 ^{a}	0
250	0 ^{h}	100
500	0 ^{h}	100
750	0 ^{h}	100
1000	0 ^{h}	100
SM**		
Control	7.5 ^{a}	0
250	4.66 ^{cd}	37.86
500	1.46 ^{fg}	82
750	1 ^{gh}	86.6
1000	0 ^{h}	100

Data represent means of three replicates compared by Duncan's multiple range test ($p < 0.01$).
* Paper disk method
** Solution Method

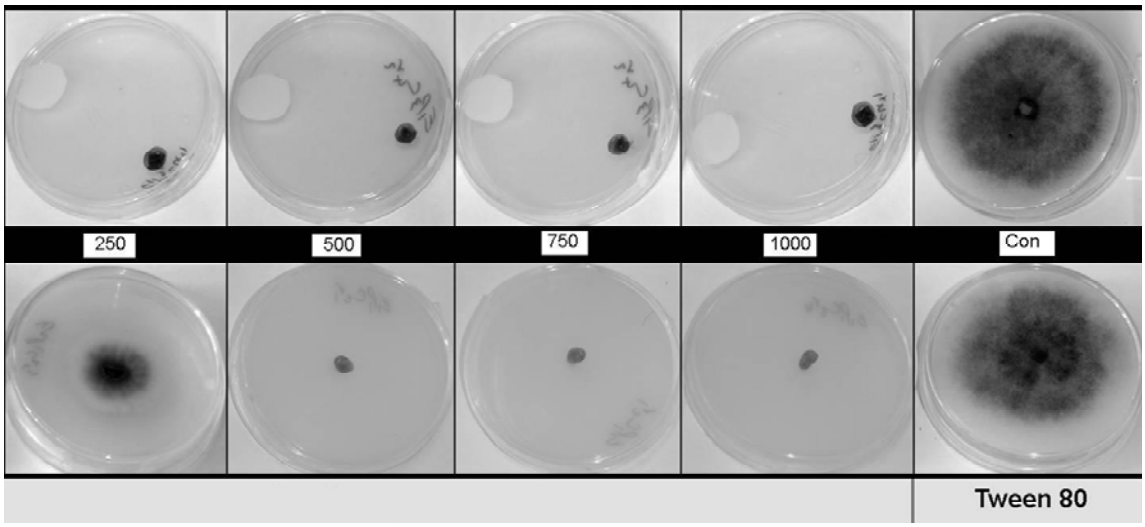


Fig. 1- Effect of different concentrations of CEO on inhibition of *B. cinerea* mycelium growth in various techniques; the row above is PDM and the row below is SM.

CEO could control fungal pathogen, as shown from other essential oils have been identified against various fungi [4, 7, 43]. Other author attributed this function to the phenolic compounds. The amphipathicity of these compounds can explain their interactions with biomembrane and thus the antimicrobial activity [44] probably γ-Terpinene could play antifungal role because of its similarity chemical proportion to phenolic compound. Also Cumin aldehyde is an aldehyde (CH=O) and carbon bund of this compound could produce partial negative free electron that play a vital role by bund with N of micro organism and could inhibit microorganism growth [36]. The resurgence of interest in natural control of plant pathogens and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required. Recently our group and various publications have documented the antifungal activity of essential oils and plant

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extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel [5, 30, 51] *in vitro* result show that CEO could be as a candidate to natural antifungal in food preservation technology.

Effects of CEO on Surface color measurement of strawberry

In control fruit, lightness and hue angle values were significantly ($p < 0.05$) decreased during 3 and 6 day, indicating less color intensity (Fig. 2 - 7). CEO treated fruit maintained higher L^* at 3 and 6 day in compare with control. However, No significant lightness and Hue angle were observed in treated fruits compared to the controls at other determination times. In contrast, hue, Chroma, b^* and ΔE stayed constant among treatments (Fig. 2-7). Application of CEO significantly slowed down these changes (lightness and hue) during early shelf-life days. Results of this study show that CEO treated fruits with Paper Disk Method not only have not adverse effect on color parameter but also maintained up fruit Lightness and hue angle in compare with control. Most of other reports with other treatments had shown adversely affect color, flavor, aroma or texture in strawberry [17, 20, 22, 24, and 37]. So, it is benefit as postharvest factor, however future studies are necessary to find the optimal concentration of CEO to support our results about color parameters.

Effect of CEO on storage life and fungal infection symptoms of the fruits

CEO-treated fruit better maintained ($p < 0.01$) and have low severity of decay scores, whereas non-treated fruit showed increased fruit deterioration (Fig. 8). Previous reports indicated that reduced fruit decay during postharvest treatments with volatile compounds for several produce including raspberry and kiwifruit [46, 47]. Essential oil has been known to be secondary metabolite in plants responding to biotic stress and to undergo profound changes in plants interacting with fungal (and other) pathogens [6]. Essential oil of cumin mainly conjugated to compounds that have known as phenolic compounds, responsible for plants order and test that accumulate in some plants cells and had been shown useful effect of these compounds for pathogen control [38] it is known that oxidation products of phloridzin (an ortho-dihydroxyphenolic compound) inhibit fungal are thought to inhibit the apple scab fungus Venturia inaequalis. pectinases. Fungal pectinases hydrolyze pectin, a cell wall compound that is abundant in the middle lamella and plays a role in cell adhesion. Thus, by inhibiting pectinases, the ability of the fungus to hydrolyze and invade the plant cell wall would be compromised [45]. It seems that similar role was done by phenolic compound of cumin essential oil such as γ-Terpinene, Cumin aldehyde and etc. These findings reveal that exogenous essential oil may have an anti-fungal function in strawberry fruits. Botrytis (gray mold) is the most important fungus that has been detected in strawberry fruit samples limiting its storage life.

Effects of CEO on other postharvest quality of strawberry

Fruit firmness

No significant weight losses were observed in treated fruits compared to the controls and at all determination times (Fig. 9). Similar results were shown with Eucalyptus vapor that enrichment resulted in no change in fruit firmness during or following vapor exposure of both strawberry and main tomato crop [43]. Other previous reports on fruit firmness indicated that cherries and grapes were

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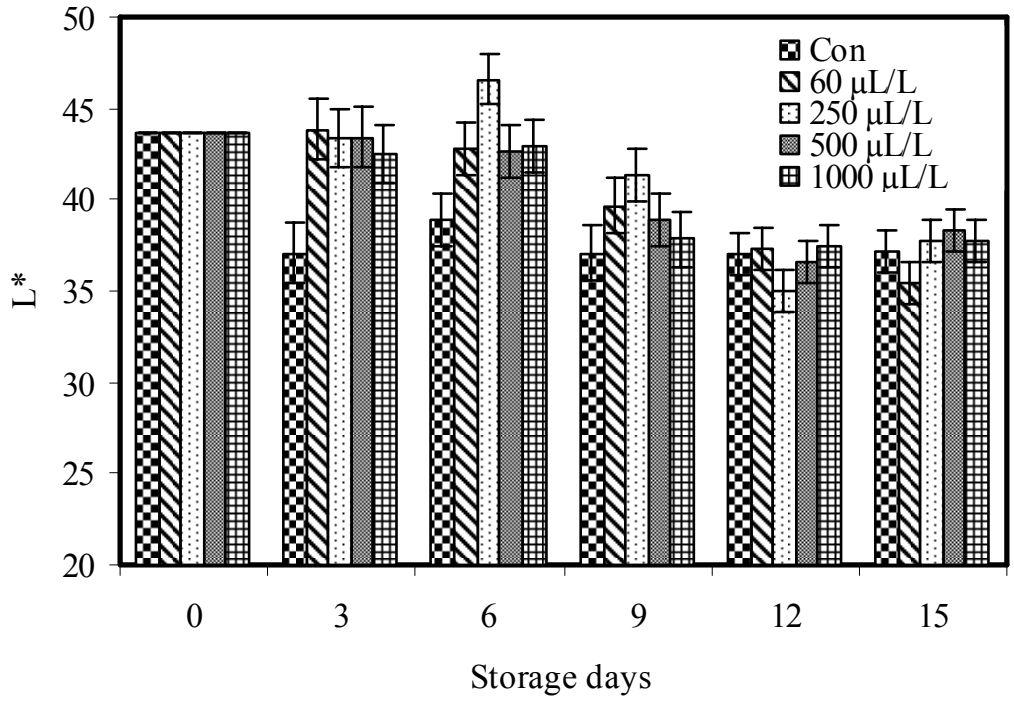


Fig. 2- Effect of cumin essential oil on L* of strawberry fruits, cv. Selva, during storage

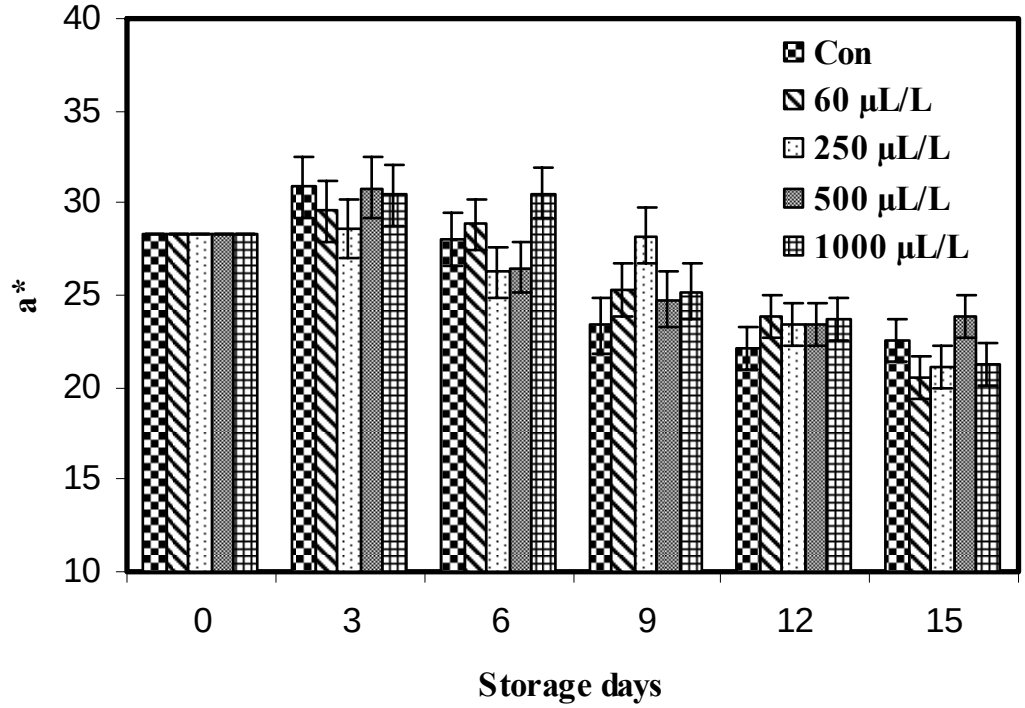


Fig. 3- Effect of cumin essential oil on a* of strawberry fruits, cv. Selva, during storage

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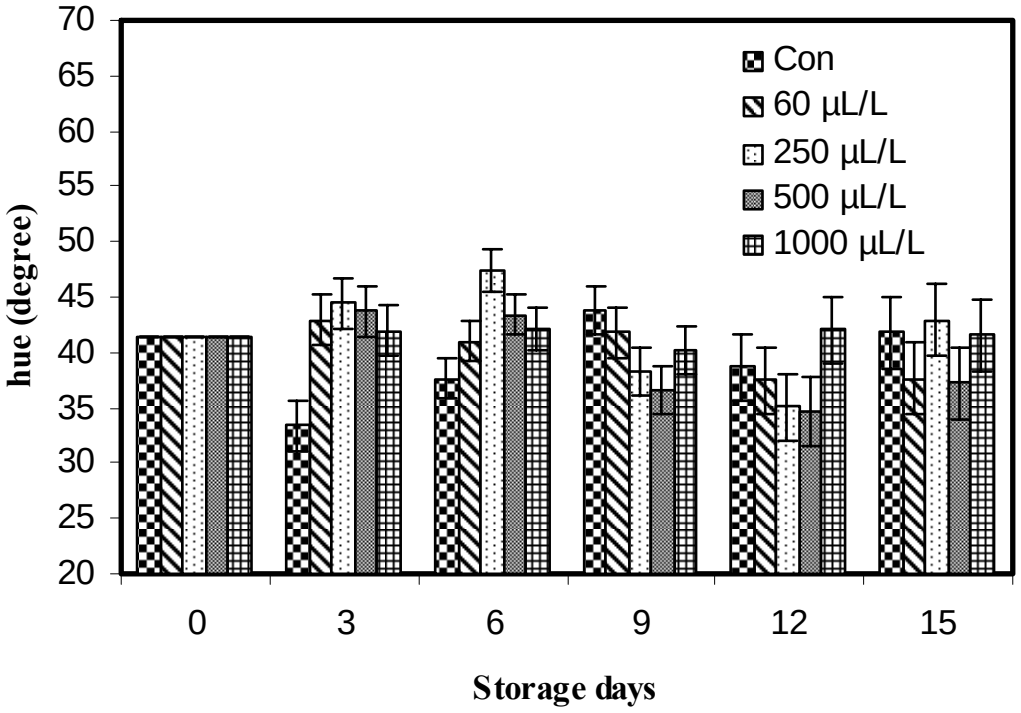


Fig. 4- Effect of cumin essential oil on hue angle of strawberry fruits, cv. Selva, during storage

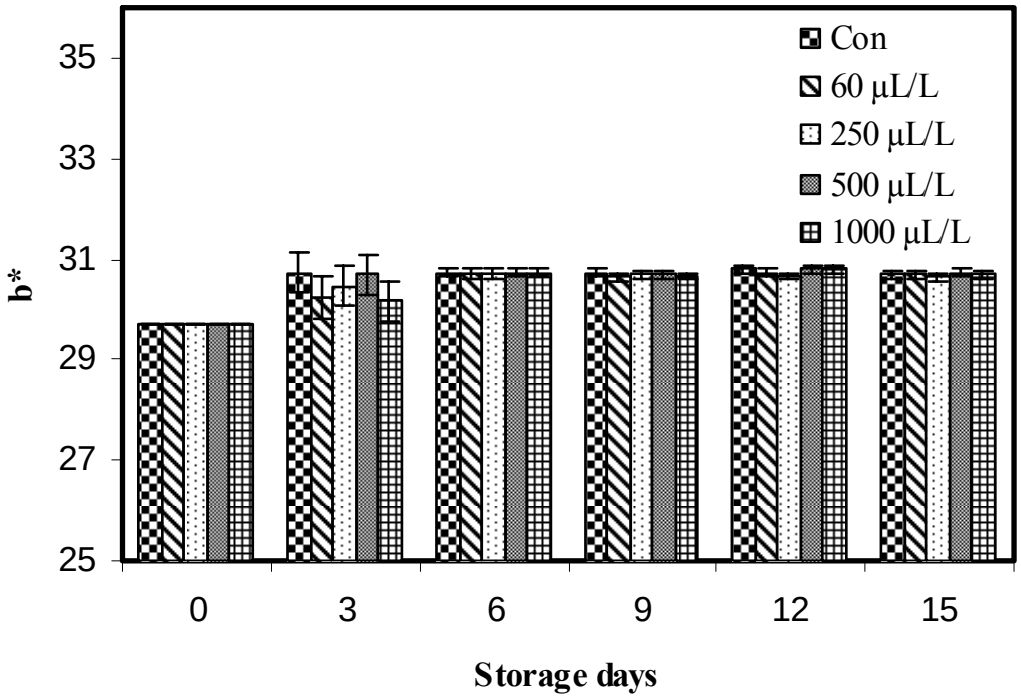


Fig. 5- Effect of cumin essential oil on b^* of strawberry fruits, cv. Selva, during storage

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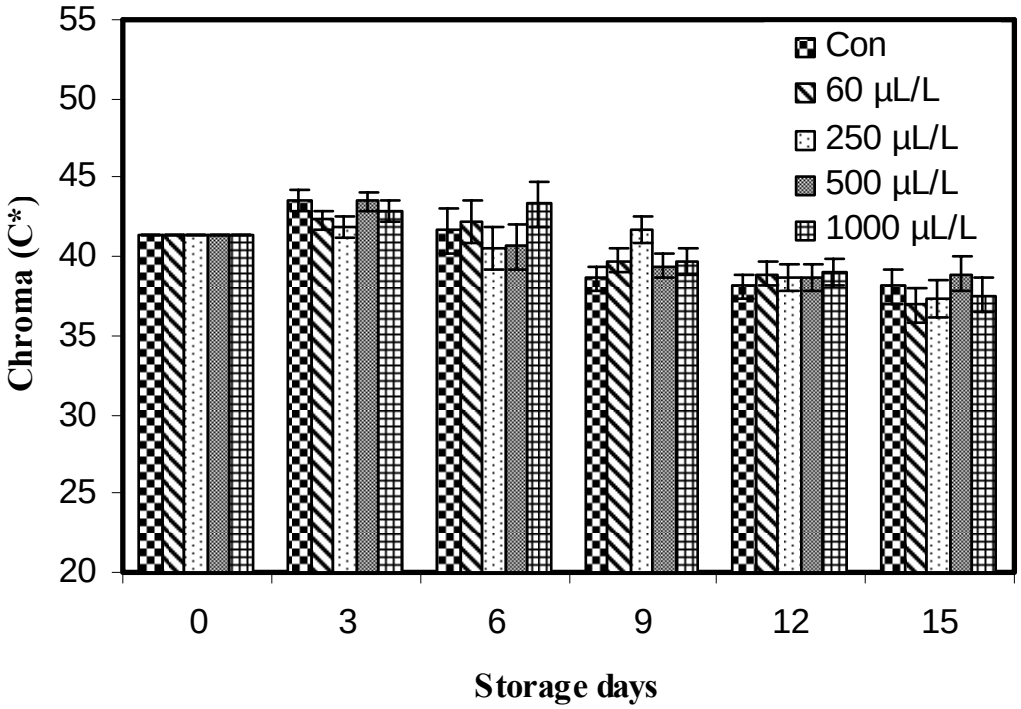


Fig. 6- Effect of cumin essential oil on Chroma of strawberry fruits, cv. Selva, during storage

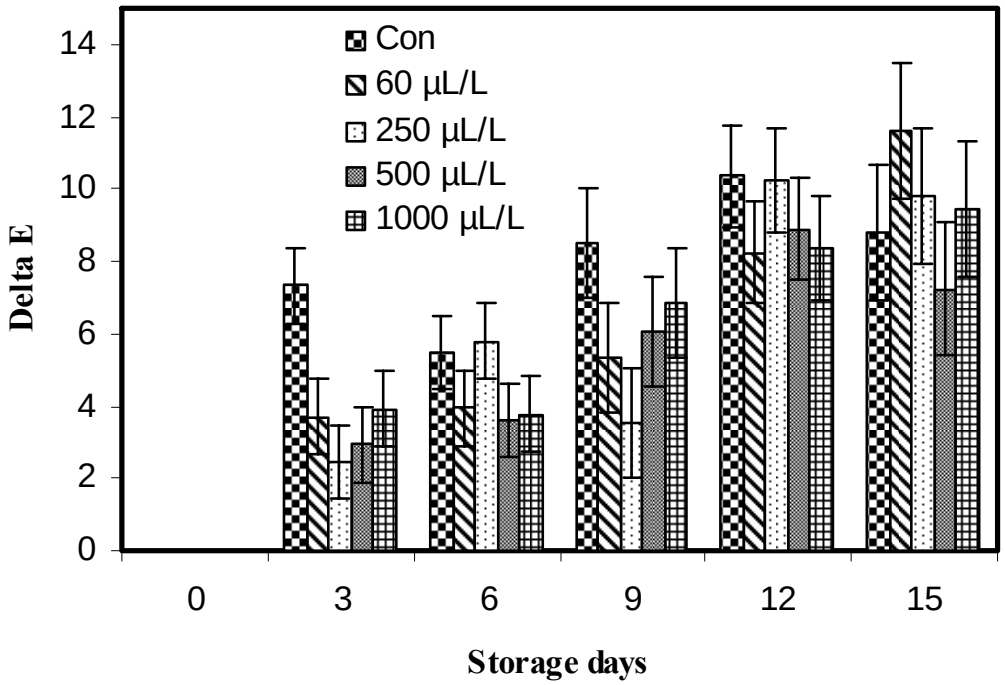


Fig. 7- Effect of cumin essential oil on ΔE of strawberry fruits, cv. Selva, during storage

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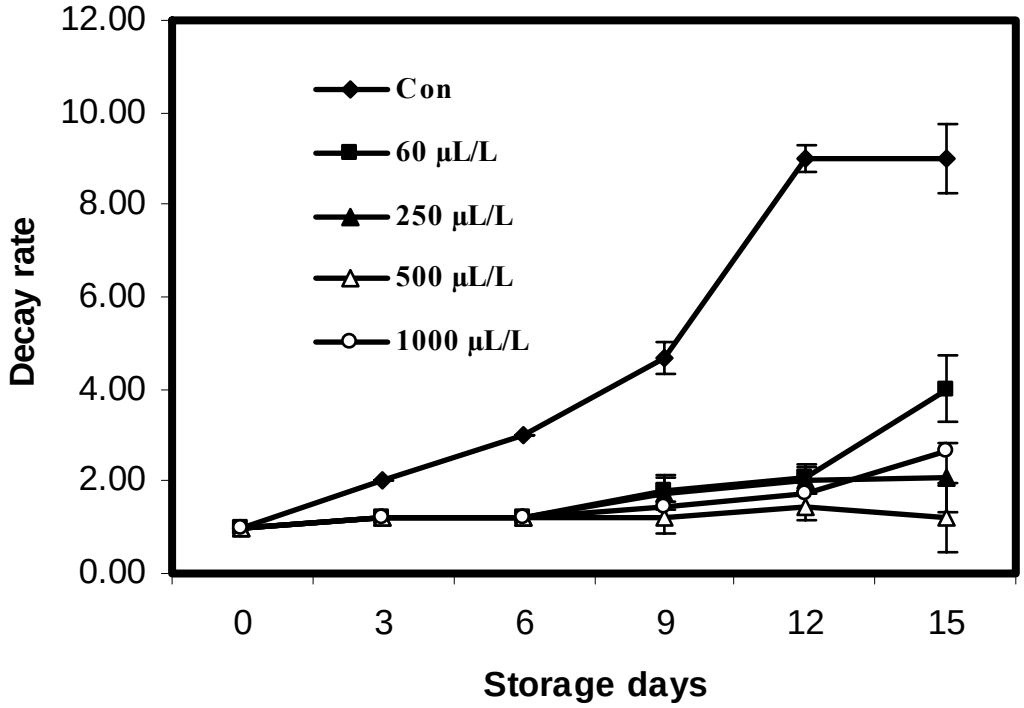


Fig. 8- Effect of cumin essential oil on Decay rate of strawberry fruits, cv. Selva, during storage

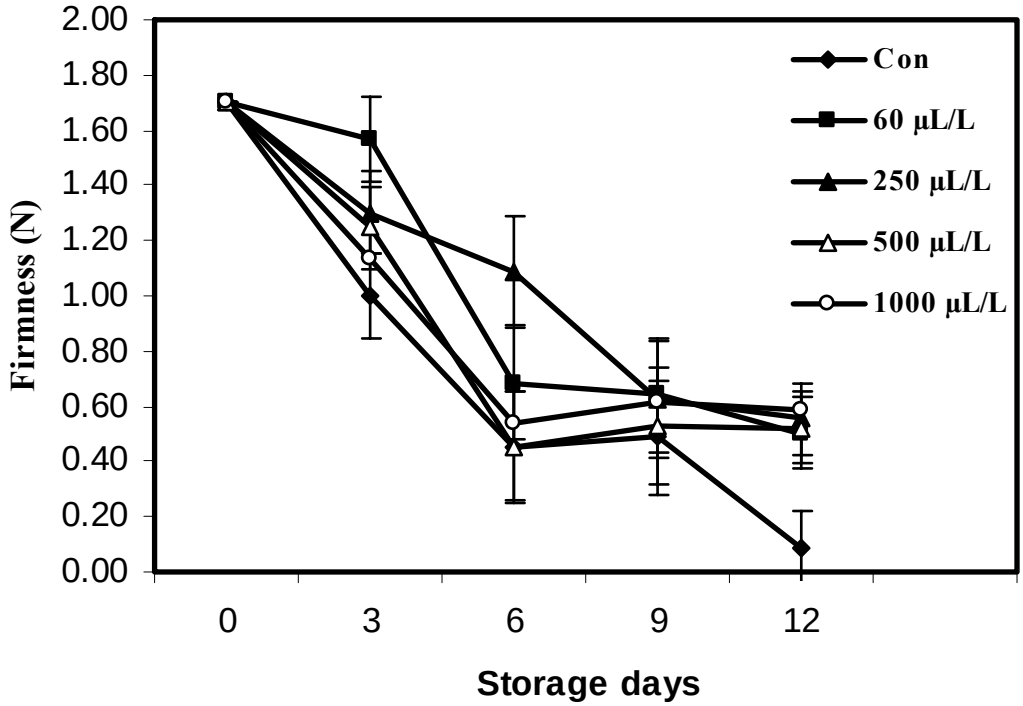


Fig. 9- Effect of cumin essential oil on firmness of strawberry fruits, cv. Selva, during storage

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Unaffected, after exposure to eugenol, thymol or menthol vapors [28, 40] However, Cinnamon vapor concentration had effective rule in fruit firmness [43].

Weight loss

The percentage of weight loss was very low for fruit treated by CEO and was significant at day 3 ($p < 0.01$), possibly due to increased respiration rates and fungi infection. However, no significant weight losses were observed in treated fruits compared to the controls at other determination times (Fig. 10). Previous experiments using eugenol, thymol or menthol vapors revealed benefits due to reduced weight loss in cherries and grapes [28, 40]. Similar results were finding with eucalyptus and cinnamon oil in strawberry and tomato [43].

Titratable acidity, Total Soluble Solids and pH

No significant TSS was observed in treated fruits compared to the controls at all determination times (Fig. 11). In similar results; Basil essential oil spray emulsion (0.16% v/v) treatment on banana to control crown rot disease did not have any significant effect on TSS after induced ripening [3]. Also, Cinnamon and eucalyptus vapor had any significant effect on TSS on tomato but increased TSS level in strawberry [43]. There was observed significant difference among treatment in titrable acidity at 3, 12 and 15 days ($p < 0.05$). Treated fruit have high TA in compared to control, but there was constant pattern in days 12 and 15 (Fig. 12). However other oil did not have any significant effect on TA on tomato and strawberry [43]. Only significant pH difference was finding among treatment at 12 and 15 days and no significant pH were observed in treated fruits compared to the controls and at early determination times. It seems that decomposition of cell wall in days of 12 and 15 causes to this irregular pattern (Fig. 13). Our results confirmed other study results by day 9 [43].

Ascorbic acid

There was significant deference among treatment at 3 and 12 days ($p < 0.05$). But no significant ascorbic acid difference was observed in treated fruits compared to the controls at all other determination times (Fig. 14). It seem that beginning of fungal infection Cause to such irregular pattern in days of 3 and 12. Probably ascorbic acid decreased by fungal infection duo to cell wall break down by progress of time.

Discussion

In conclusion, data presented in this study show that CEO treatments using by PDM could be safe and natural fungicide and could be used to prevent infection of strawberry during storage. Concentration o 60 $\mu L.L^{-1}$ of

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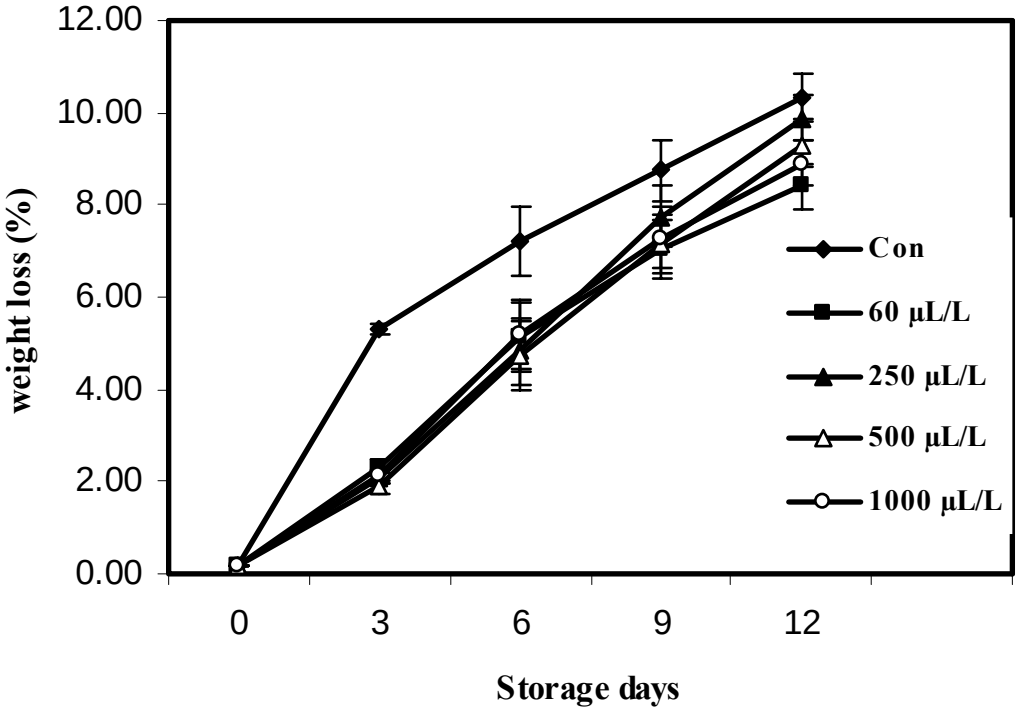


Fig. 10- Effect of cumin essential oil on weight loss of strawberry fruits, cv. Selva, during storage

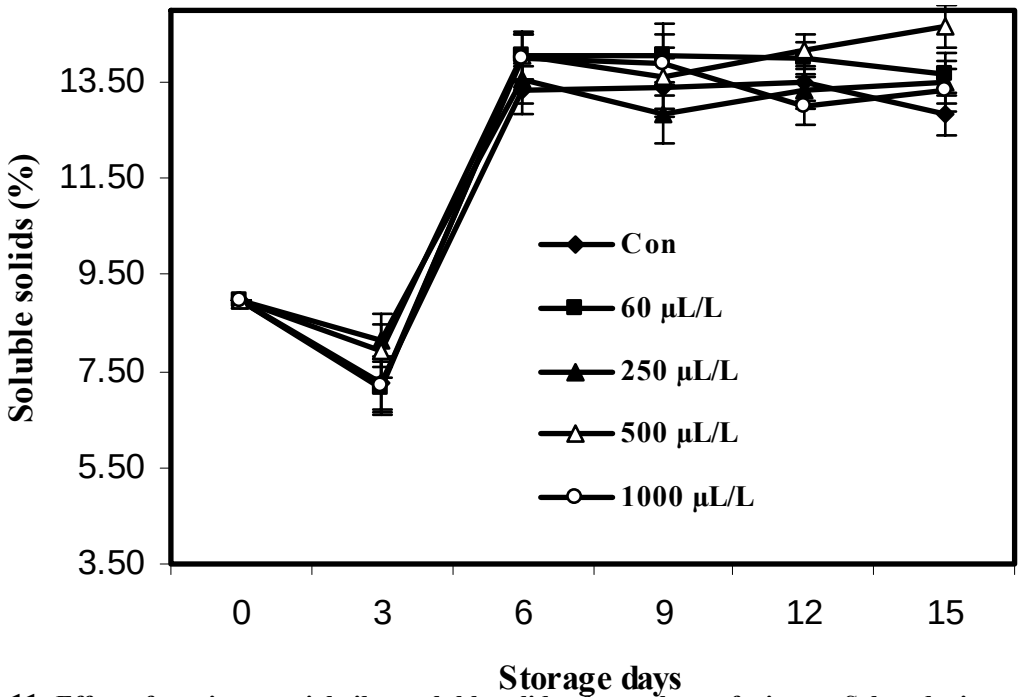


Fig. 11- Effect of cumin essential oil on soluble solids of strawberry fruits, cv. Selva, during storage

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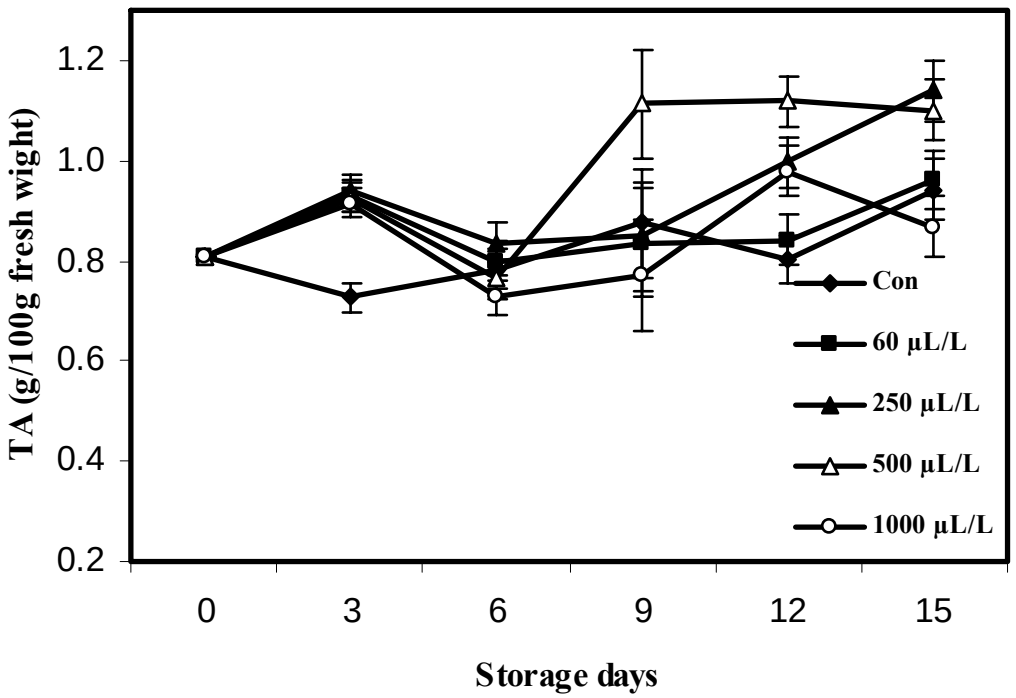


Fig. 12- Effect of cumin essential oil on titrable acidity of strawberry fruits, cv. Selva, during storage

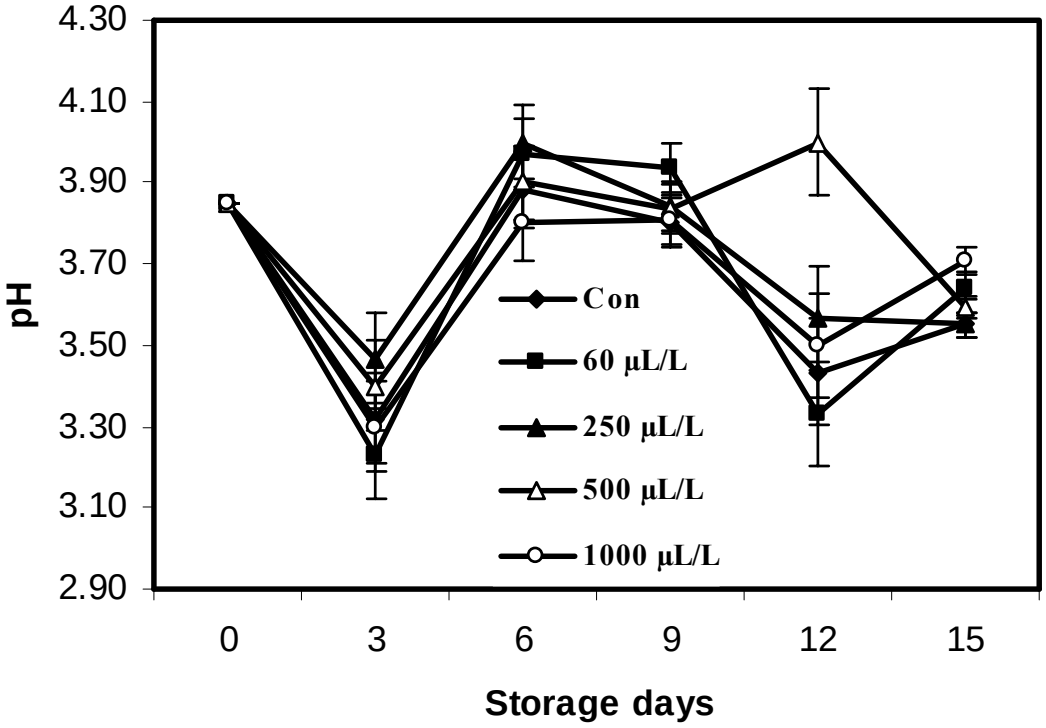


Fig. 13- Effect of cumin essential oil on pH of strawberry fruits, cv. Selva, during storage

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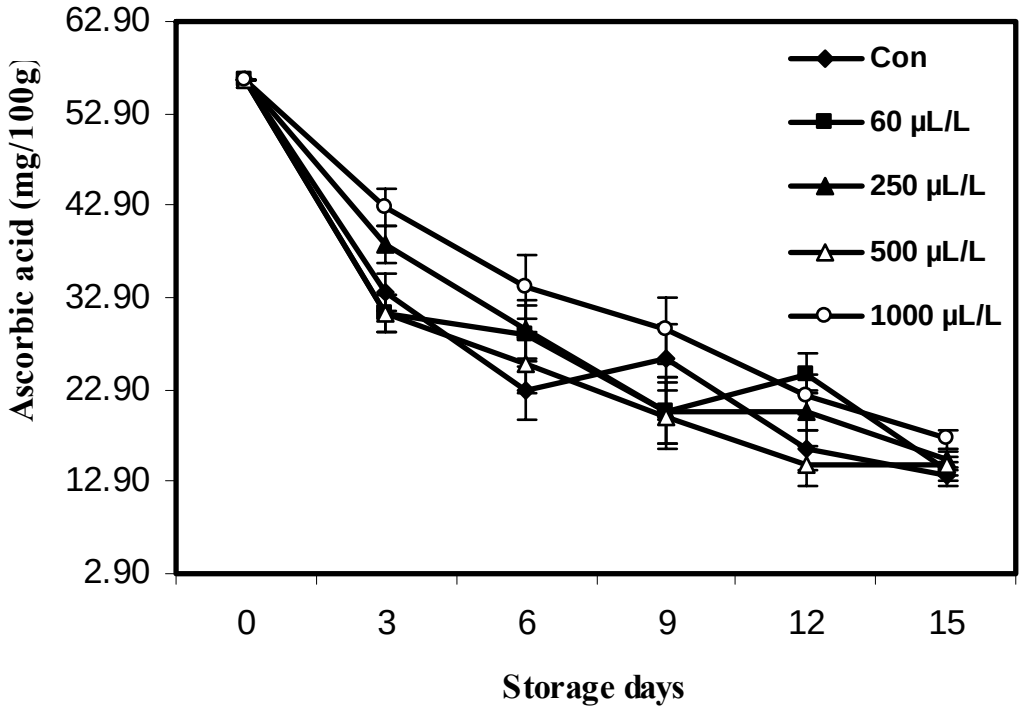


Fig. 14- Effect of cumin essential oil on titrable acidity of strawberry fruits, cv. Selva, during storage

CEO could be control fungal infection on fruit as well as high concentration that can be use with minimum effect on ardor and test. CEO can extend shelf life for over the minimum period required to transit strawberries to foreign markets, and without notable adversely affect quality. However, future studies are necessary to fully understand the mechanisms by which CEO may affect as a fungicide and increase their post-harvest life. Using CEO by PDM could be worldwide; although, further research is needed to establish a commercial recommendation.

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