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Post-Harvest Enhancing and *Botrytis cinerea* Control of Strawberry Fruits Using Low Cost and Eco-Friendly Natural Oils

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Abstract: This work investigates an experimental study for using low-cost and eco-friendly oils to increase the shelf life of strawberry fruit. Three natural oils were used: (i) *Eucalyptus camaldulensis var obtuse*, (ii) *Mentha piperita* green aerial parts essential oils (EOs), and (iii) *Moringa oleifera* seeds *n*-hexane fixed oil (FO). Furthermore, a mixture of EOs from *E. camaldulensis var obtusa* and *M. piperita* (1/1 *v*/*v*) was used. The treated fruits were stored at 5 °C and 90% relative humidity (RH) for 18 days. HPLC was used to analyse the changes in phenolic compounds during the storage periods. The effects of biofumigation through a slow-release diffuser of EOs (*E. camaldulensis var obtusa* and *M. piperita*), or by coating with *M. oleifera* FO, were evaluated in terms of control of post-harvest visual and chemical quality of strawberry fruits. The post-harvest resistance of strawberry fruits to *Botrytis cinerea* fungal infection was also evaluated. As a result, the EO treatments significantly reduced the change in visual and chemical quality of strawberry fruit. Additionally, changes in the titratable acidity of moringa FO-coated strawberry fruits were delayed. EO treatments improved total soluble solids, total phenols, ascorbic acid, antioxidants and peroxidase. *E. camaldulensis var obtusa* and *M. piperita* (1/1 *v*/*v*) EO-vapour fruit exhibited a slower rate of deterioration, compared to other treatments in all tested, in two experiments. The lowest colour change (ΔE) was observed inthe fruit treated with *E. camaldulensis var obtusa* EO and *M. oleifera* FO. HPLC showed changes in phenolic compounds' concentration, where *p*-coumaric acid, caffeic acid, gallic acid, ferulic acid and ellagic acid were mostly identified in the fruits treated with the oils. SEM examination confirmed the potential decrease in fungal growth as the fruits were treated with EOs. In conclusion, the treatment of EOs during different storage periods showed promising characterisations for strawberry fruit quality.

Keywords: strawberry; *Botrytis cinerea*; natural oil; shelf life; cold storage; postharvest; SEM; GC-MS; HPLC

1. Introduction

The strawberry (*Fragaria* × *ananassa*) is a highly perishable small fruit in the temperate zone and is an important and commercial product [1,2]. The strawberry has a high content of bioactive compounds such as anthocyanin, phenolic acid, flavonoids, tannins and

vitamin C [3,4]. The fruits' perishability is attributed to the high rates of respiration, softening, and water loss [5,6]. Due to the high metabolism of the fruit during storage, the fruits start to spoil very rapidly, sometimes even before reaching consumers [7,8].

Maintenance of the strawberry's quality during marketing is a challenge. Pathogen attack is an important problem in the spoilage of strawberries in storage. Grey mould fungi caused by *Botrytis cinerea* Pers.: Fr. is one of the most economically important pathogens of the strawberry [9,10]. Infection may occur in the flower, remain quiescent until the fruits mature, and then develop abundantly, causing fruit decay accompanied by profuse sporulation of the pathogen [11]. *B. cinerea* also causes enormous losses of quantity and quality of the fruits during shipping and marketing [12].

Therefore, it is required to manage strawberries appropriately in order to obtain a regulated market supply through post-harvest treatments to improve storage life. Due to consumer concerns over the safety of fruits containing synthetic chemicals, a lot of attention has been paid to naturally derived compounds or natural products [13–15]. Recently, the use of natural components such as natural extract or herbal oils has been one of the healthiest and safest methods to control post-harvest diseases; essential oils (EOs) include extensive secondary metabolites, which in most cases have antimicrobial, fungicidal antioxidant and bio-regulating properties [7,16].

Recently, some EOs have been reported to be effective in reducing decay, in quality maintenance and in the essential improvement of post-harvest life of many fruits (grape, strawberry, blueberry, raspberry, blackberry and fresh-cut fruit) [17]. In particular, aldehydes, phenols and ketones considerably inhibit pathogen growth [18–22]. Thymol, carvacrol and *p*-anisaldehyde have proven fungicidal activity and the EO rich in these components showed the highest inhibitory activity against many post-harvest pathogens, such as *Botrytis* spp. on peach and grey mould in eggplant, which are highly effective in controlling fungal pathogens [23,24]. EOs from a number of plants, including Eucalyptus (*Eucalyptus camaldulensis*), cinnamon (*Cinnamomum* ssp.) and cumin (*Cuminum cyminum*) could inhibit grey mould growth and increase the storage life and quality of strawberries [16,25–27] and *Botrytis* spp. on peach and *B. cinerea* in eggplant [28].

Moringa oleifera Lam. (Family Moringaceae) has several uses due to its composition of nutritional and bioactive compounds, including proteins, essential amino acids, carbohydrates, lipids, fibre, vitamins, minerals, fixed oil (FO), phenolic compounds, phytosterols and others [29–32]. The seeds are resistant to rancidity because they contain powerful antioxidants that act as natural preservatives [32]. The oil from the seeds can be used as a fertiliser in plantations to encourage the growth of other species [33], and it is also used as an edible coating to increase the shelf life of certain vegetables and fruit [34].

Therefore, the present investigation was designed with objectives to extend the marketable shelf life by evaluating the effectiveness of essential oils from the green aerial parts of *Eucalyptus camaldulensis var obtusa* and *Mentha piperita* as fumigation and *Moringa oleifera* seed fixed oil as a coating material, during a prolonged cold storage period and to assess the physico-chemical changes in strawberry fruits. Furthermore, chromatographic analyses, GC-MS, GC and HPLC were used to identify the chemical analysis of essential or fixed oils as well as the changes in polyphenolic compounds of treated strawberry fruits. Additionally, SEM examination was used to evaluate the antifungal activity of the oils and to show the extent of fungal growth on the treated fruits.

2. Materials and Methods

2.1. Experimental Location

In two successive experiments conducted during 2018 and 2019, fruits of strawberry (*Fragaria* × *ananassa* Duch.) cv. 'Florida Fortuna' were harvested at commercial maturity (red colour on 90% of fruit surface) at (30°35′34.5″ N, 30°42′58.4″ E), Behira Governorate, Egypt. Fortuna is an early season with high productivity, short day and semi-everbearing variety, and the fruit are mostly conical, firm yet juicy, glossy and bright to dark red in colour with a warm red interior and smooth appearance with stress tolerance, good setting,

long self-life (LSL) and bright red fruits of 16–20 g [35]. Fruits selected for their homogeneity in colour, size and absence of defects were delivered on the same day to the laboratory of Alex Postharvest Center (APHC), Faculty of Agriculture, Alexandria University, then washed with fresh water, air dried, and used in the post-harvest treatments [26].

2.2. Preparation and Chemical Analyses of the Natural Oils

Green aerial parts from *E. camaldulensis var obtusa* and *M. piperita* were separately hydrodistillated for 3 h via a Clevenger unit to extract the essential oils (EOs) [33]. The obtained EOs were kept dry in Eppendorf tube and stored at 4 °C in a refrigerator. Seeds of *M. oleifera* were ground to powder and about 100 g was soaked in 150 mL of *n*-hexane for 24 h. After the extraction process, the materials were filtered through a cotton plug, then with Whatman No. 1 filter paper, to remove any residues [32] and the dissolved fixed oil (FO) was obtained in *n*-hexane solvent. The solvent was evaporated, and the FO was obtained and stored in a sealed brown bottle at 4 °C in a refrigerator until needed.

Chemical constituents of the EOs from the green aerial parts of *E. camaldulensis var obtusa* and *M. piperita* were performed using GC–MS by means of GC-TSQ Quantum mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness) apparatus. The column oven temperatures and the chemical separation and identification conditions can be found in previous studies [19,36]. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database. Match factor with Xcalibur 3.0 data system of GC/MS, where the value $\geq$ 650 is acceptable to confirm the compounds [21,37–39], was measured. The chemical composition of *M. oleifera* seed FO was performed as shown in previous work [32].

2.3. Natural Oils' Fruit Treatments

Fruits were placed into 1 L polystyrene containers with Snap-on lids loaded separately with *E. camaldulensis var obtusa* (1%), *M. piperita* (1%) and a mixt of *E. camaldulensis var obtusa* and *M. piperita* (0.5/0.5 *v*/*v*) EOs. These were applied using filter papers (Whatman, No. 1). The EOs were allowed to vaporise inside the containers spontaneously at room temperature for 8 h [25], and other group was coated by spraying with moringa FO for 30 sec [34]. The containers were then transferred to storage at 5 °C in a cold room. Control samples were handled similarly without treatments by EOs or FO. Four fruits were used for every treatments.

2.4. Weight Loss and Firmness of Fruit

Fruit weight loss (%) from four fruits for every treatment was measured during the storage, where all fruits from all treatments were weighed at 0, 4, 14 and 18 days of storage. The fruit weight loss percentage was calculated using the following formula: Weight loss % = (A − B)/(A × 100); where A indicates the fruit weight at the time of harvest, and B indicates the fruit weight after storage intervals [40].

The firmness of the strawberries after each treatment during the storage periods was measured using a TA-1000 firmness analyser instrument with a penetrating cylinder of 1 mm diameter, to a constant distance (3 and 5 mm) inside the pulp of fruits, and at a constant speed of 2 mm per sec. the peak resistance was recorded per N [41]. The firmness was determined after 0, 4, 9, 14 and 18 days of storage as the average from 4 fruits for each treatment.

2.5. Colour Change Measurements

The change in colour was measured on two sides of each fruit using a Hunter lab colorimeter (HunterLab Labscan 600 spectrocolorimeter, version 3.0; Hunter Associates Laboratory Inc., Reston, VA, USA), according to the following formula [42]:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

where ΔE: The overall change in colour indices:

L index refers to black-to-white colour;
a index refers to green-to-red colour;
b index refers blue-to-yellow colour.

2.6. Fruit Chemical Parameters and Peroxidase Enzyme Activity

Total soluble solid (TSS %) as an average from 4 fruits from every each treatment was measured in the juice of the fruits using a portable digital refractometer (Atago Co. Ltd., model PR-1, Tokyo, Japan). Titratable acidity of fruits (TA) was analysed [40]. Ascorbic acid content (mg/100 g F.W.) was assessed using the 2,6-dichlorophenol indophenol titration method [40]. Fruit tissue (10 g) was homogenised with 90 mL of oxalic acid (3%) (Nice Chemicals Ltd., Kerala, India), the sample was then filtered using a filter paper (Whatman paper) and 25 mL of the filtrate was titrated by 2,6-dichlorophenol indophenol. Total phenolics content (TPC) was determined and expressed as mg GAE/Kg F.W. [43], while antioxidant activity was measured by using the free-radical scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [44] and calculated as a percentage of inhibition according to the following formula: Inhibition (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$, where $A_{control}$ and A_{sample} are the absorbance of the control fruits (without oil treatments) and fruits treated with oils, respectively. All previous characters were determined after 0, 4, 9, 14 and 18 days of storage. Peroxidase enzymes activity was determined by spectrophotometer according to the methods described by Hameda and Klein [45].

2.7. Fruit Extraction and HPLC Analysis of Phenolic Compounds of Treated Fruits

The extraction process was carried out on strawberry fruits treated or untreated with the natural oils (*E. camaldulensis var obtusa* EO, *M. piperita* EO, mixture of *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*), and *M. oleifera* FO). Four fruits were taken from each treatment, and about 30 g from each treatment's fruits was extracted by 60 mL methanol by soaking method for one week [46,47]. The extracts were then filtered through filter paper (Whatman No. 1) and then with a cotton plug. The extracts were concentrated and stored in brown vials for further analysis.

The phenolic compounds from the methanol extracts of each of the fruits treated with oils were identified by the Agilent ChemStation (HPLC- (Agilent, Santa Clara, CA, USA), which is composed of a quaternary pump and UV/Vis detector and C18 column (125 mm × 4.60 mm, 5 µm particle size). Chromatograms were obtained and analysed using HPLC. Phenolic compounds were separated by employing a mobile gradient phase of water/acetonitrile/glacial acetic acid (980/20/5, *v*/*v*/*v*, pH 2.68) and acetonitrile/glacial acetic acid (1000/5, *v*/*v*), operated at 30 °C with a flow rate of 1 mL/min and detected at 325 nm. All chemical standards (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA) [48].

2.8. Pathogen Inoculum Preparation

Botrytis cinerea was isolated from decayed strawberry fruits and maintained on potato dextrose agar (PDA) plates at 4 °C. Spore suspensions were prepared by removing the spores from the sporulation edges of a 10-day-old culture with a bacteriological loop and suspending them in sterile distilled water. Spore concentration was determined with a hemocytometer and adjusted to 1×10^5 spores/mL with sterile distilled water.

2.9. In Vitro Antifungal Activity

Antifungal assays of mint and camphor and their combination (1:1) and moringa were performed with the pour plate method. An amount of each oil was mixed with a sterilised, cooled PDA medium and poured into sterile Petri dishes (9 cm diameter) to obtain final concentrations of 0.05%. Then, a 5 mm plug of 7-day-old *B. cinerea* (from the margin of the plate) was placed in the centre of each Petri dish, mycelium side down. The dishes were sealed with parafilm and incubated at 25 °C for 7 days until the growth in the control

plates (without the EOs) reached the edge of the plates. Control plates contained PDA without tested oil. Radial colony growth (cm) was registered three times: 2, 5 and 7 days after inoculation (DAI) and incubated at 25 °C for 7 days until the growth in the control plates (without any treatment) reached the edge of the plates. Then, the antifungal index (AI) of the treatments was calculated as follows [49]:

Antifungal index (%) = [(C − T)/C] × 100, where C and T are the radial growth (mm) of fungus in the control and treated plates, respectively.

2.10. Susceptibility of Strawberry Fruits to B. cinerea and SEM Examination

Artificially inoculated fruits were conserved in a perforated plastic box. Strawberries of the same maturity and without disease symptoms, as assessed visually, were collected for further assays to determine the antifungal activity of the EOs and FO on strawberry fruits [26,34]. After the fruits had been treated with essential oils, wounds (ø 3 mm, 3 mm depth) were made vertically in the centre of each strawberry fruit using a sterile needle. Later, the fruits were individually inoculated with 20 µL of conidial suspension (1×10^5 spores/mL) into each wound. The boxes were closed with perforated lids and subsequently transferred from ambient temperature to 5 °C for storage. Lesion diameter (mm) was measured at 7 and 9 DAI. Antifungal activity was calculated as mycelial growth inhibition using the following formula: mycelial growth inhibition (%) = [(dc − dt)/dc] × 100, where dc is the lesion diameter in the control (mm), while dt is the lesion diameter in the treated samples (mm). Each treatment had 3 replicates, and each replicate had 6 fruits.

The control and treated pathogen mycelium were picked up respectively, and the morphology of mycelium was observed under a scanning electron microscope at 10 DAI. The scanning electron microscope was equipped with energy-dispersive spectroscopy (SEM-EDS) (JSM-IT200 Series, JEOL, Tokyo, Japan), where the fruit blocks (2 mm diameters), taken from those 10-day old fruits infected with *B. cinerea* and treated with tested oil were observed. Samples were fixed in 25 mL/L glutaraldehyde for 24 h at room temperature, and each sample was washed with 0.1 mol/L phosphate-buffered saline (pH 7.2) three times, for 15 min each, then fixed for another 1 h in 1% Osmium tetroxide (OsO_4) solution. Each sample was dehydrated in graded ethanol dehydration processes (30%, 50%, 70%) for 10 min and finally dehydrated with pure ethanol for 30 min and covered with a gold platinum layer using an ion coater. Finally, samples were observed and photographed by SEM at 20 KV.

2.11. Statistical Analysis

All data were tested for differences between treatments using the two-factor analysis of variance (ANOVA, general linear model) followed by Duncan multiple range test for $p < 0.05$ [50]. Data were subjected to statistical analysis for calculation of means, variance and standard error using CoStat Software Program Version 6.303 [51].

3. Results and Discussion

3.1. Chemical Characterisation of Oils

Table 1 presents the chemical composition of the essential oil (EO) from *E. camaldulensis var obtusa* and *Mentha piperita* green aerial parts, where the main compounds in *E. camaldulensis var obtusa* EO were eucalyptol (33.04%), spathulenol (21.15%), *p*-cymene (10.49%), γ-terpinene (6.55%), crypton (5.35%), phellandral (3.01%), thymol (2.69%) and terpinen-4-ol (2.45%), while the main compounds in *M. piperita* were pulegone (29.38%), isomenthone (17.23%), levomenthol (16.36%), eucalyptol or 1,8-cineole (7.46%), menthone (6.90%), aromadendrene (3.64%), endo-borneol (3.15%) and piperitone (3.02%). Previously, the EO from *M. piperita* leaves showed the presence of menthone, 1,8-cineole, menthyl acetate, caryophyllene, β-pinene and D-limonene as main compounds with values of 20.18, 15.48, 13.13, 4.82, 4.37 and 2.81%, respectively [19]. In addition, several studies reported that the EO from *Mentha* species were composed of different chemical compositions, with the abundant compounds menthol, eucalyptol, menthone, limonene, *trans*-carveol, pulegone,

β-caryophyllene and pipertitinone oxide [52–54]. The Algerian *Mentha* plant EO showed the presence of menthol, menthone, and menthyl acetate as major constituents [55].

Table 1. Percentages of phytochemical constituents of the essential oils from green aerial parts of *E. camaldulensis var obtusa* and *M. piperita* by GC-MS.

Chemical Compound	*E. camaldulensis var obtusa*	*M. piperita*
β-Pinene	0.39 (915) *	-
α-Phellandrene	0.8 (928)	-
Eucalyptol or 1,8-Cineole	33.04 (715)	7.46 (839)
Linalool	-	0.86 (879)
Sabinene	0.3 (918)	-
p-Cymene	10.49 (792)	-
endo-Borneol	-	3.15 (944)
γ-Terpinene	6.55 (856)	-
Isoterpinolene	0.28 (910)	-
Terpinen-4-ol	2.45 (875)	-
trans-3(10)-Caren-2-ol	0.39 (769)	
α-Terpineol	0.6 (908)	1.71 (907)
2-Undecanone	-	0.78 (864)
Menthone	-	6.90 (901)
Levomenthol	-	16.36 (873)
Isomenthone	-	17.23 (896)
Pulegone	-	29.38 (937)
β-Guaiene	-	0.98 (796)
Cubenol	-	0.37 (905)
Piperitone	0.2 (894)	3.02 (935)
p-Cymen-8-ol	0.16 (903)	-
Crypton	5.35 (879)	-
3-Isopropyl phenol	0.2 (950)	-
Nerolidol	-	1.14 (943)
α-Copaene	-	1.76 (959)
Cuminaldehyde	1.87 (893)	-
Phellandral	3.01 (894)	-
2-Caren-10-al	0.1 (841)	-
Carvacrol	0.17 (846)	-
Thymol	2.69 (885)	-
Cuminyl alcohol	0.88 (892)	-
β-Elemene	-	1.17 (928)
trans-α-Bergamotene	-	0.60 (897)
Aromadendrene	1.11 (909)	3.64 (918)
Cubenol	-	1.33 (928)
3-Methylenehexahydro-1-benzofuran-2(3H)-one	0.09 (718)	-
Dehydroaromadendrene	0.1 (746)	-
1,5-Dimethyltetralin	0.98 (741)	-
Ascaridol	0.18 (832)	-
Spathulenol	21.15 (794)	-
Isoaromadendrene epoxide	0.5 (775)	-
β-Eudesmol	-	1.10 (910)
7-Epi-cis-sesquisabinene hydrate	-	0.55 (853)
4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol	0.65 (731)	-
β-Caryophyllene oxide	-	0.82 (833)
β-Longipinene	0.16 (848)	-
α-Vetivol	0.12 (803)	-
Ledene oxide-(II)	0.69 (914)	-
α-Sinensal	0.1 (823)	-
Ylangenal	1.69 (802)	-

* Vlues are precentge of the compound (match factor, MF).

For the chemical composition of *M. oleifera* seeds fixed oil (FO), the full chemical analysis can be found in our previous work [32] as shown in Table 2, where the main compounds were oleic acid, *β*-sitosterol and *α*-tocopherol (vitamin E) with percentages of 59.7, 21.4 and 4.9%, respectively, as measured by GC-MS. After the methylation of fatty acids (FAs), the main fatty acid methyl esters (FAMEs) were oleic, palmitic, stearic, linolenic and arachidic, with values of 78.72%, 6.27, 5.63, 3.72 and 3.29%, respectively. The main FAMEs from *M. oleifera* oil were oleic and palmitic at percentages of 74.99 and 12.51%, respectively [56]. FAs of oleic, palmitic, stearic and linoleic with values 77.40, 12.97, 2.95 and 1.40%, respectively, were found in moringa seed FO [57].

Table 2. Chemical composition of the fixed oil from *M. oleifera* seeds *.

Chemical Group	Chemical Compounds	Percentage in the Oil (%)
Fatty acids	Oleic acid	59.7
	cis-Vaccenic acid	1.95
	6-Octadecenoic acid	1.03
	9-Octadecenoic acid	2.77
	Palmitoleic acid	0.68
	Erucic acid	0.97
	α-Ketostearic acid	0.06
Terpenoids	3-Carene	0.24
	Humulene	0.15
	α-Copaene	0.12
	Caryophyllene	1.06
	Estragole	0.29
Steroids	Stigmasterol	0.35
	β-Sitosterol	21.4
Esters	2-Chloropropionic acid, octadecyl ester	0.47
	Fumaric acid, 3-heptyl tridecyl ester	0.32
Vitamin E	*α*-Tocopherol	4.9
	β-Tocopherol	0.62
	γ-Tocopherol	1.5

* Data from Abbassy et al. [32].

3.2. Fruit Visual and Chemical Parameters

Figure 1 shows all the studied visual and chemical parameters of strawberry fruits treated with EOs and FO and different periods of storage. Significantly, there were different effects among natural oils and the storage days in all the studied parameters. The use of EOs observed the greatest effects in slowing down the weight loss, while moringa FO and the control sample showed the lowest effects. However, at the end of the storage period, a highly significant difference was found between treated and untreated strawberry samples after 18 days from the start of the storage period. The loss of weight in fresh fruit primarily reflects the respiration rate and moisture evaporation between the fruit tissue and surrounding air storage [58], which are influenced by post-harvest treatment and storage temperature [15]. Strawberry fruits are highly susceptible to a rapid loss of water due to the extremely thin skins of these fruits.

The effect of EO vapours could be associated with the formation of the fruit surface coating that modifies gas permeation and decreases respiration rate and water loss [59]. The EO fumigation was shown to decrease the dehydration process in fruit [60]. Our result was in agreement with a previous study on strawberries [26,41], cherries [13], grapes [61] and peaches [59] treated with eugenol, thymol, menthol and cinnamon vapours. Strawberry fruits' firmness is a very important visual quality of fresh market fruit in determining their post-harvest quality and shelf life [41].

Generally, firmness decreased during storage conditions in both seasons. The EOs' vapours also influenced the preservation of firmness of these fruits compared with untreated fruit (control) and fruit treated with moringa FO at 1%, which showed a significant decrease in firmness at 9, 14 and 18 days of storage, while vapour with mixed EOs from

E. camaldulensis var obtusa and *M. piperita* (1/1 *v*/*v*) resulted in higher firmness than other treatments at the end of the storage period for both seasons.

Similar to our findings, EO vapours decreased the firmness losses during cold storage conditions in the strawberry [26]. Furthermore, strawberry firmness was amended when lemon or orange EOs were used at shelf life storage of the fruit [2]. The marked effect observed in previous studies might be related to storage. This was probably due to the selective permeability of the coating material to gas and water transmission, thus reducing respiration rates, enzyme activities and most of the metabolic changes, thereby delaying ripening and over softening of the strawberry [62–65].

Total soluble solids (TSS%) were increased by time passed from harvest day in all treatments. At the end of the storage period, the lowest TSS was observed in fruits treated with mix of *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*). It seems that the increase in TSS was due to respiration during the storage period. EO fumigation reduced TSS consumption in strawberry fruits compared to control because of the decreased respiration as a result of reducing gas exchange [2,66,67]. Other reasons for the increase in TSS are degradation of carbohydrate, other material changes such as acids, increasing soluble pectin and fruit corruption [68,69]. Furthermore, it can be observed that the TSS increase is also correlated to the weight loss.

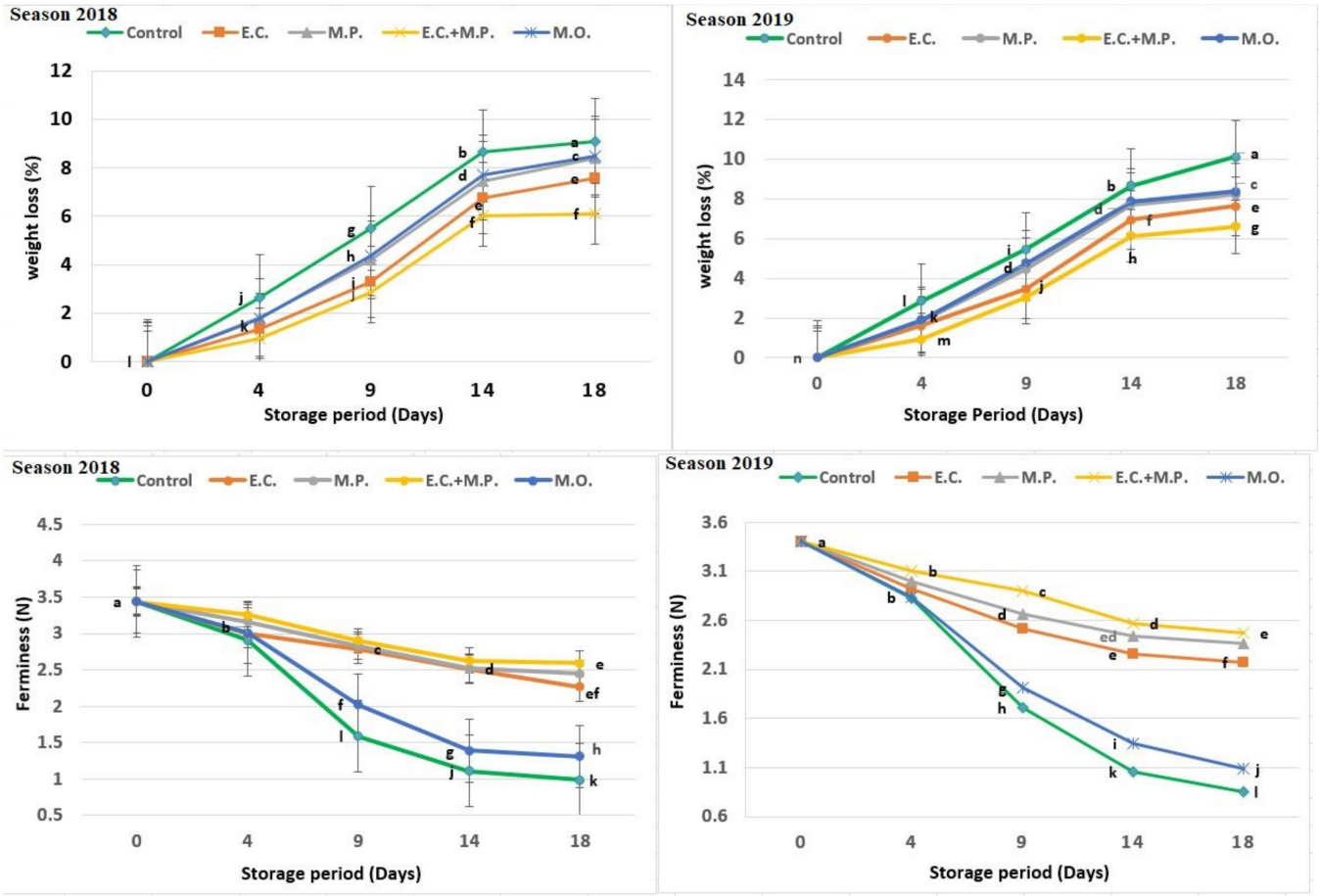

Figure 1. *Cont.*

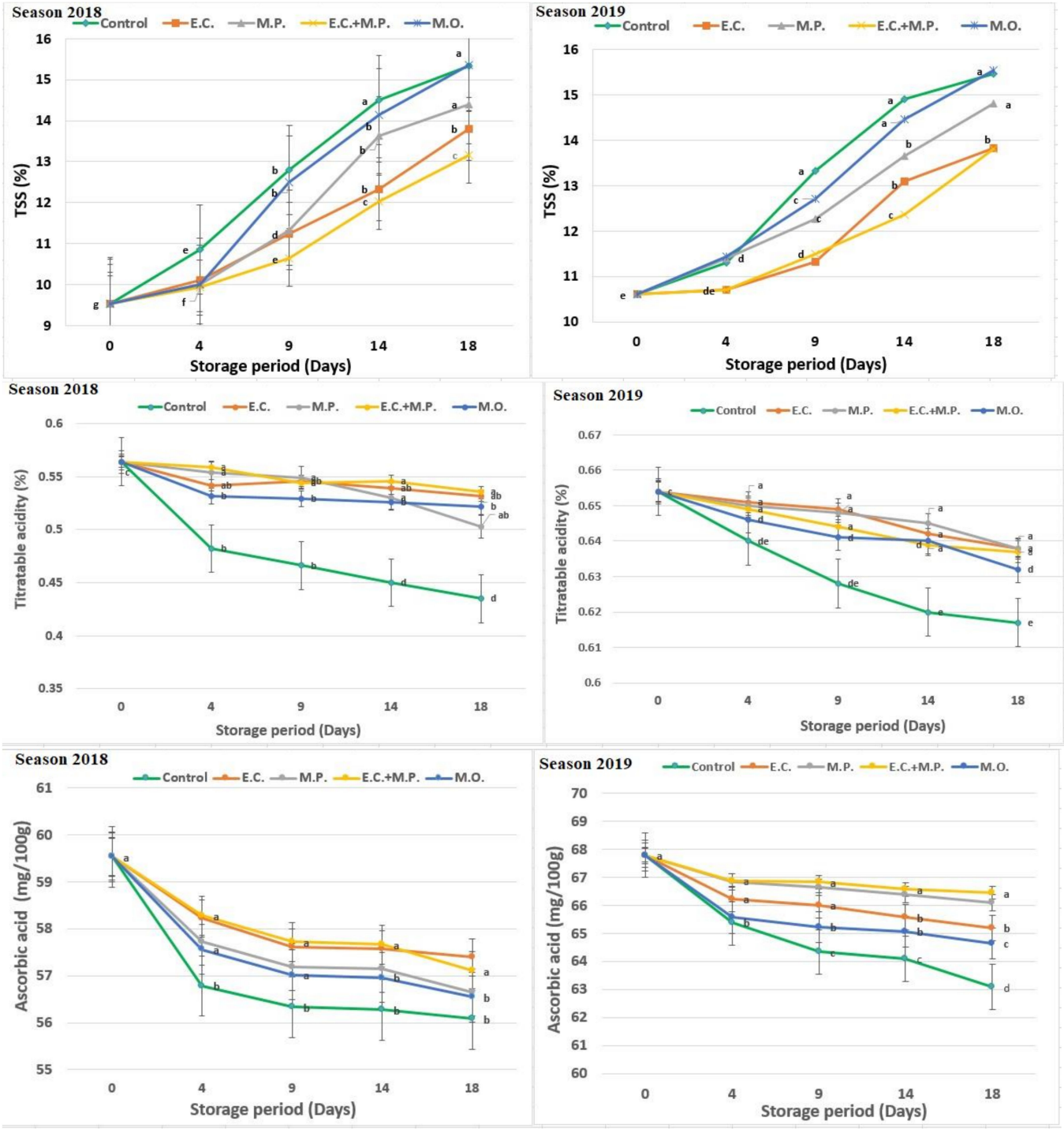

Figure 1. *Cont.*

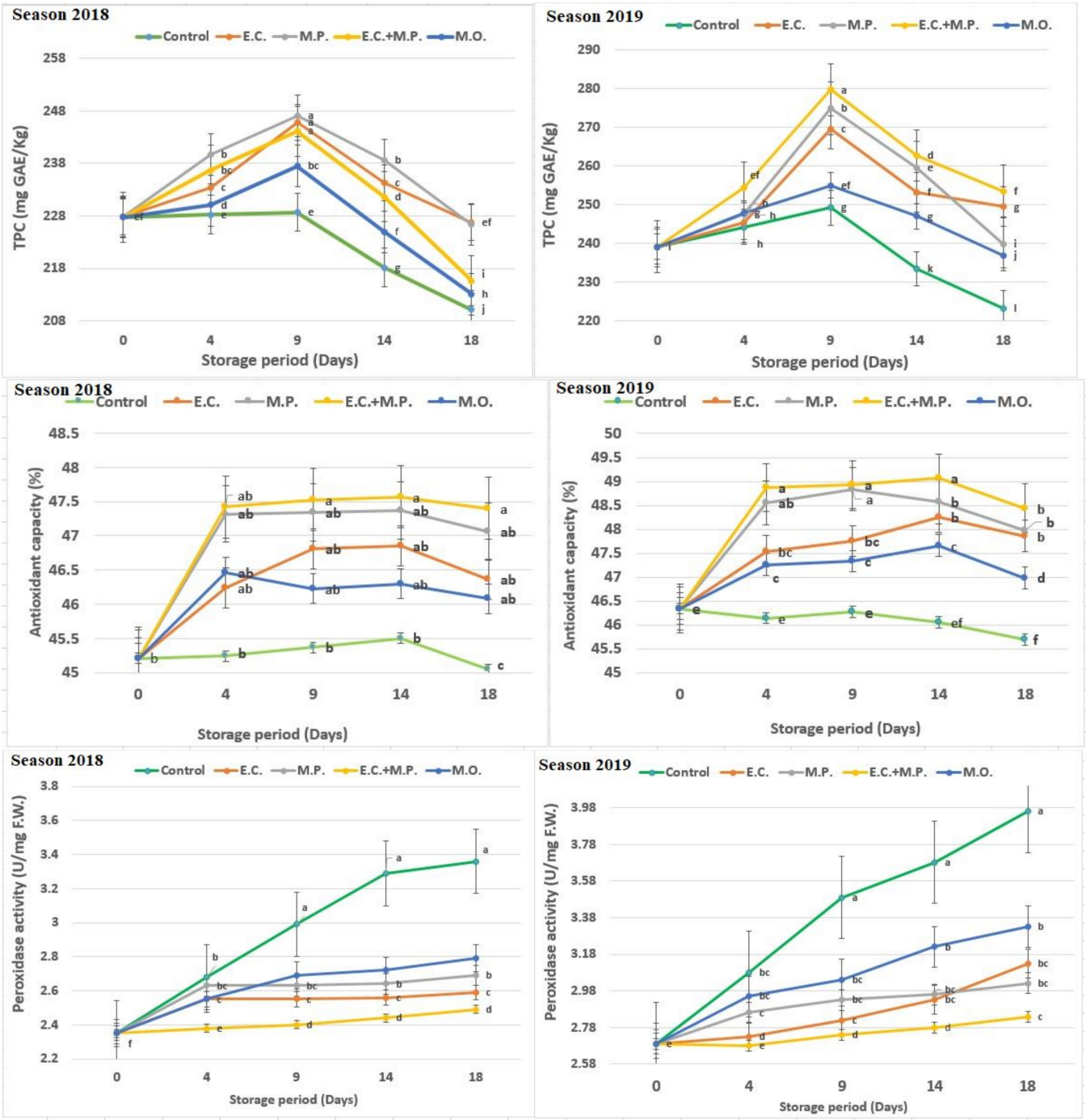

Figure 1. Visual and chemical parameters (means ± S.E) of strawberry fruits stored at 5 °C as affected by the application of natural oils at different storage durations in both seasons 2018 and 2019. Fruits treated with Control: without oils; E.C.: *E. camaldulensis var obtusa* EO; M.P.: *M. piperita* EO; E.C.+ M.P.: mix of *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*); Oil 4: *M. oleifera* FO. Letters (a-l) in figures explain that, means ± S.E of treatments with the same letter/s are not significant difference according to Duncan multiple range test for $p < 0.05$.

The percentage of titratable acidity (TA) value was decreased through the storage period and the lowest value was recorded in the last four days of the storage period, where all treatments had a reduced acidity value compared to the control treatment. The lowest value of acidity was obtained due to *E. camaldulensis var obtusa* and *M. piperita* mix EOs (1/1 *v*/*v*), while the highest value was obtained with the control treatment in both seasons.

TA is an important factor in maintaining the quality of fruits, which is directly related to the organic acid content present in the fruit [70,71], and the decrease in TA content could be due to the consumption of organic acids in fruits during respiration. In a similar study, treatments have a significant effect on the respiration process, which could result in reduction or delay of respiration and results in maintenance of TA content [58].

Ascorbic acid (AA) contents of all treated and untreated strawberry fruits were significantly decreased with the increase of storage periods from 0 to 18 days. This reduction could be related to its oxidation through superoxide and hydroxyl radicals in the strawberry fruits [72]. However, at the end of the storage period, the concentrations of AA were higher in fruits treated with oils compared to the control (untreated fruits). The maximum values of AA were observed in the strawberry fruits treated with the mix of *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*) followed by *E. camaldulensis var obtusa* EO and *M. piperita* EO compared with untreated fruits (control). This result might be attributed to the antioxidant properties of the EOs, which reduce the diffusion of oxygen, decrease the rate of respiration and consequently reduce the AA oxidation [62].

Total phenolic content (TPC, mg GAE/kg) continuously increased in the fruits treated with oils until 9 days from the start of the storage period, at which point the trend slowed down gradually and peaked on day 18. Mahmoud et al. [73] found that the TPC of the Hollywood plum was increased during cold storage. Piljac-Žegarac and Šamec [74] reported that small fruits like strawberries, raspberries, cherries and sour cherries stored at 4 °C exhibited slightly higher antioxidant activity values. Moreover, significant correlations between antioxidant capacity (AOC) and phenolic components in different fruits were established [75,76]. AOC was reduced during the storage time but not between treatments, and the changing trend in the control was more than with other treatments of the fruit. The fruits treated with *E. camaldulensis var obtusa* and *M. piperita* mix EOs (1/1 *v*/*v*) and *E. camaldulensis var obtusa* EO retained a higher AOC, reinforcing this attribute in the fruit at the end of the storage period in both seasons. During the storage period, the reduction in this property at the end of the storage time could be due to senescence and decomposition [77]. In addition, AOC decreases due to cell protection against the damage caused by free radicals, where EOs decrease the respiration rate and free radical production by means of moisture maintenance and CO_2 and O_2 exchange control [2,25]. On the other hand, the AOC was maintained with a high percentage in the oil-treated fruits compared to the untreated fruits at 5 °C, with an increase in the storage period, whereas a previous report showed that the fruits stored at 10 °C had higher antioxidant enzyme activities and AOC than those stored at 0 or 5 °C [78].

The peroxidase enzyme activity in all fruits increased with the extension of storage periods, and peroxidase in treated fruits with different EOs showed a lower level compared to control fruits. At the end of the storage period, the lowest peroxidase enzyme was in strawberries treated with the mix of *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*) followed by *E. camaldulensis var obtusa* and *M. piperita* EOs. Similar findings were reported by Badawy et al. [79], who observed that the use of EOs containing thymol (0.02%) or geraniol (0.04%) increased catalase (CAT) activity and reduced polyphenol oxidase (PPO) activity and peroxidase. These results suggested that the use of EOs improves the levels of oxyradical detoxification enzymes including CAT and decreases the PPO activity that prevents the oxidation damage and peroxidase, thus promoting prolongation of the shelf-life and preserving the quality of strawberries during storage.

3.3. Colour Measurements

The colour change measurements (Table 3) showed that the fruit samples treated with *E. camaldulensis var obtusa* EO and *M. oleifera* FO had the lowest ΔE. These lowest values of ΔE suggested that the treatments with those oils kept the fruits closest to their initial sample colour. Most other reports with other treatments showed that the treatments with EO significantly reduced the colour change in strawberry fruits [26,41].

Table 3. Chromatic parameters measured in the L*, a* and b* color system of strawberry fruits as affected by the application of natural oils after 18 days of the storage at 5 °C.

Oils		Season 2018				Season 2019			
		L*	***a****	***b****	**Δ*E***	***L****	***a****	***b****	**Δ*E***
Initial sample color		40.00	40.49	24.25	-	40.68	40.02	25.10	-
Control	Untreated fruits	34.19	34.12	15.40	12.63	36.69	34.69	12.89	13.90
E.C.	*E. camaldulensis var obtusa* EO	37.88	36.89	22.64	4.62	37.28	36.36	26.51	5.19
M.P.	*M. piperita* EO	35.53	31.55	20.26	10.90	30.56	35.83	21.87	11.41
E.C. + M.P.	*E. camaldulensis var obtusa* + *M. piperita* EOs (1/1 *v*/*v*)	33.27	36.51	20.08	9.04	33.94	31.58	22.16	11.19
M.O.	*M. oleifera* FO	36.79	36.32	23.54	5.37	39.94	36.48	21.68	4.97

3.4. Phenolic Compounds of Oil-Treated Fruits by HPLC

Figure 2 and Table 4 show the changes in phenolic compounds in the methanol extract (ME) from the oil-treated or untreated strawberry fruits stored at 5 °C for 18 days compared to the original fruit sample (not stored). Myricetin was found only in the ME from the original fruit sample. Syringic acid ranged from 8.12 to 13.30 μg/mL, *p*-coumaric acid from 8.09 to 25.51 μg/mL and eugenol was decreased from 35.16 μg/mL (original fruit) to 18.05 μg/mL (untreated fruit), but it was not detected in the ME from all the fruits treated with oils. Vanillin acid was detected only in the ME from fruits treated with the EOs from *E. camaldulensis var obtusa* and *M. piperita*. Caffeic acid was found in the ME of all fruit samples and ranged from 5.36 μg/mL (treated from with Moringa FO) to 19.63 μg/mL (treated fruits with the mixture of EOs from *E. camaldulensis var obtusa* and *M. piperita* (1/1 *v*/*v*)). *p*-Hydroxybenzoic acid was detected only in the ME from the fruits treated with *E. camaldulensis var obtusa* EO (7.12 μg/mL). Pyrogallol was detected in the ME from fruits treated with *E. camaldulensis var obtusa* EO (28.5 μg/mL) and a mix of *E. camaldulensis var obtusa* and *M. piperita* mix EOs (1/1 *v*/*v*) (14.51 μg/mL).

Gallic acid ranged from 5.12 to 12.66 μg/mL but was not detected in fruits treated with a mix of *E. camaldulensis var obtusa* and *M. piperita* (1/1 *v*/*v*)) EOs or moringa FO. Ferulic acid ranged from 6.12 μg/mL to 21.12 μg/mL in the ME from fruits treated with *M. oleifera* FO and the mix of *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*), respectively. A high amount of α-tocopherol was detected in the ME of fruits treated with *M. oleifera* FO (22.01 μg/mL) and in the fruits treated with a mix of EOs from *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*) (7.45 μg/mL) and was not detected in the ME from other fruits. Salicylic acid was not detected in the ME from the fruits treated with oils, but it was detected in the original fruit sample (9.12 μg/mL) and the untreated fruit (9.56 μg/mL). Catechol was identified in the ME from fruits treated with mix of *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*) (5.18 μg/mL) and *M. oleifera* FO (6.23 μg/mL).

According to the above results concerning the HPLC analysis of the changes of phenolic compounds, there are differences in the concentrations of the identified compounds among the fruits treated with EOs or FO. Phenolic compounds such as quercetin and kaempferol glycosides, *p*-coumaric acid and ellagic acid were identified in strawberry fruits [80–83]. It was reported that ellagic acid was the most abundant phenolic compound in the strawberry [80,84], but it was found strictly as a compound or combined with glycosides and ellagitannins [85]. *p*-OH benzoic acid, *p*-coumaric acid glucoside, ferulic acid derivative and caffeic acid were identified in strawberry cultivars [86]. Phenolic compounds identified in strawberry fruits containing ellagic acid were reported to have a high antioxidant capacity [87].

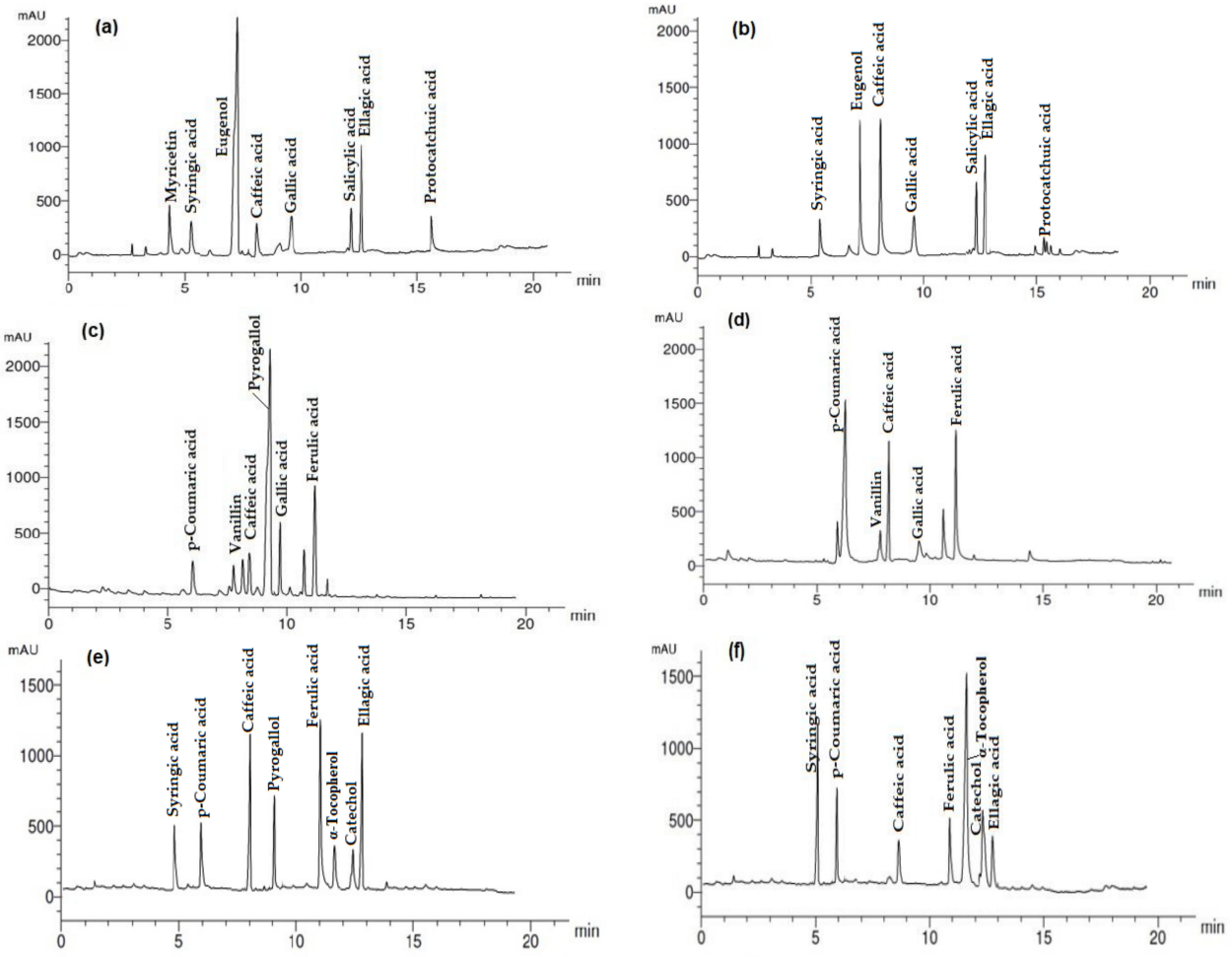

Figure 2. HPLC chromatograms of polyphenolic compounds detected in the methanol extracts of strawberry fruits from (**a**) the original fruit sample; (**b**) untreated fruits; and treated fruits with (**c**) *E. camaldulensis var obtusa* EO; (**d**) *M. piperita* EO; (**e**) *E. camaldulensis var obtusa* and *M. piperita* mix EOs (1/1 *v*/*v*) and (**f**) *M. oleifera* FO.

Table 4. HPLC analysis of polyphenolic compounds in strawberry fruits treated with natural oils after 18 from the storage period.

RT (min)	Compound	Phenolic Compounds (μg/mL) in Methanol Extract of Strawberry Fruits Treated with *					
		0 Day *	Control	E.C.	M.P.	E.C. + M.P.	M.O.
4.5	Myricetin	10.33	ND	ND	ND	ND	ND
5.1	Syringic acid	9.14	8.12	ND	ND	9.22	13.30
6.0	*p*-Coumaric acid	ND	ND	8.23	25.51	9.68	8.09
7.0	Eugenol	35.16	18.05	ND	ND	ND	ND
7.8	Vanillin	ND	ND	7.55	5.42	ND	ND
8.0	Caffeic acid	6.47	16.26	6.98	18.87	19.63	5.36
8.5	*p*-Hydroxybenzoic acid	ND	ND	7.12	ND	ND	ND
9.02	Pyrogallol	ND	ND	28.5	ND	14.51	ND
9.8	Gallic acid	8.16	7.14	12.66	5.12	ND	ND
11.0	Ferulic acid	ND	ND	18.09	20.11	21.12	6.12
11.5	α-Tocopherol	ND	ND	ND	ND	7.45	22.01
12.0	Salicylic acid	9.12	9.56	ND	ND	ND	ND
12.5	Catechol	ND	ND	ND	ND	5.18	6.23
13.0	Ellagic acid	17.36	8.49	ND	ND	18.33	5.14
15.6	Protocatchuic acid	10.68	2.21	ND	ND	ND	ND

ND: not detected; * 0 day: the orginal fruit sample; Control: Untreated fruits; E.C.: *E. camaldulensis var obtusa* EO; M.P.: *M. piperita* EO; E.C. + M.P.: mixt of *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*), and M.O.: *M. oleifera* FO.

3.5. Inoculation and Infection Process

Firstly, after 18 days from the storage period of the treated fruits with the natural oils, it was observed a fungal growth over untreated fruits, and treated fruits with *E. camaldulensis var obtusa* EO and *M. oleifera* FO (Figure 3).

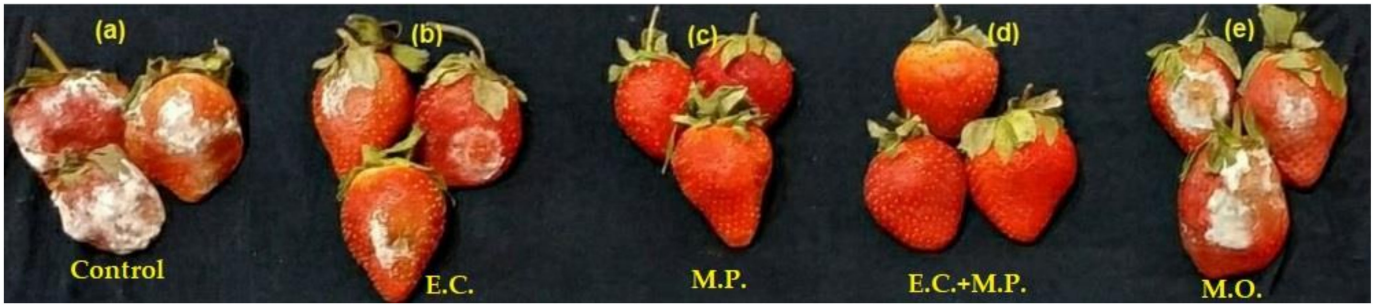

Figure 3. Visual observation of the treated fruits with oils after 18 days of storage at 5 °C. (**a**) untreated fruits; and treated fruits with (**b**) *E. camaldulensis var obtusa* EO; (**c**) *M. piperita* EO; (**d**) *E. camaldulensis var obtusa* and *M. piperita* mix EOs (1/1 *v*/*v*) and (**e**) *M. oleifera* FO.

Therefore, we conducted the primary in vitro experiment of the antifungal activity of the tested oils examined at 0.05% concentration as shown in Table 5 and Figure 4. All tested oils had different degrees of antifungal activity against *B. cinerea* in terms of the radial colony growth (cm) as measured at 2, 4 and 7 days from incubation. No radial growth from the *M. piperita* and the *E. camaldulensis var obtusa* and *M. piperita* (1/1 *v*/*v*) EOs in Petri dishes were noted throughout the experimental period.

Table 5. Radial colony growth (cm) on potato dextrose agar medium with oils for *Botrytis cinerea* isolated from strawberry fruits.

Treatment		Radial Colony Growth (cm)		
		2 DAI	**4 DAI**	**7 DAI**
Control	Untreated	5.23 * a	7.08 a	9.00 a
E.C.	*E. camaldulensis var obtusa* EO	1.22 b	2.57 c	2.57 c
M.P.	*M. piperita* EO	0.00 c	0.00 d	0.00 c
E.C. + M.P.	*E. camaldulensis var obtusa* + *M. piperita* EOs (1/1 *v*/*v*)	0.00 c	0.00 d	0.00 c
M.O.	Moringa FO	4.97 a	6.13 b	9.00 a

DAI: days after inoculation; * Means with the same letter within the same column indicate non-significant differences among treatments ($p < 0.05$) at 2, 4 and 7 DAI.

The highest antifungal activity was recorded for *M. piperita* EO and *E. camaldulensis var obtusa* and *M. piperita* mix EOs (1/1 *v*/*v*) with an antifungal index of 100%, while *E. camaldulensis var obtusa* EO showed a low to moderate effect on the radial growth of *B. cinerea* with antifungal indices of 76.67, 63.7 and 31.1% at 2, 4 and 7 DAI, respectively (Table 6). On the other hand, moringa FO demonstrated that reduction in mycelial growth of *B. cinerea* was very weak and reached 4.97 and 13.42% at 2 and 4 DAI, with no difference being observed in radial growth from the non-amended oil control at day 7 (Table 6). The application of EOs is a very attractive and eco-friendly method to control post-harvest diseases.

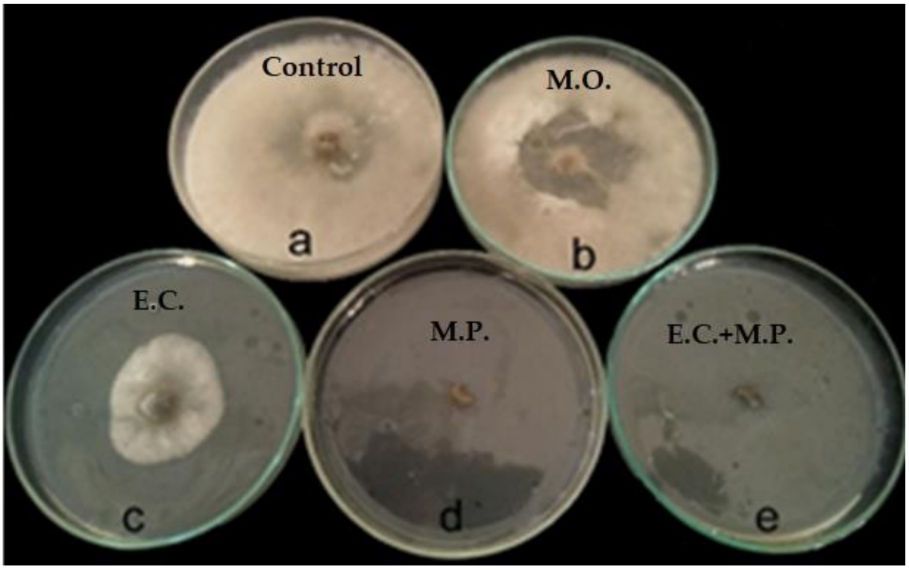

Figure 4. In vitro antifungal activity of the tested oils against *B.* cinerea. (**a**) control; (**b**) moringa FO; (**c**) *E. camaldulensis var obtusa* EO; (**d**) *M. piperita* EO; (**e**) combination of *E. camaldulensis var obtuse–M. piperita* (1/1 *v*/*v*) EOs.

Table 6. Antifungal activity of *M. piperita*, *E. camaldulensis var obtuse* and their combination and moringa oils against *B. cinerea*.

Treatment		Antifungal Index (%)		
		2 DAI	4 DAI	7 DAI
E.C.	*E. camaldulensis var obtusa* EO	76.67	63.70	31.11
M.P.	*M. piperita* EO	100.00	100.00	100.00
E.C. + M.P.	*E. camaldulensis var obtuse* + *M. piperita* EOs (1/1 *v*/*v*)	100.00	100.00	100.00
M.O.	Moringa FO	4.97	13.42	0.00

The infection rate (%) of strawberry fruits caused by *B. cinerea* is shown in Table 7, where it increased from 7 to 10 days after the infection, but the lowest value was observed in fruits treated with *M. piperita* EO and the mix EOs after 10 days with percentages of 18% and 20%, respectively, compared to the control (87%). The lowest lesion diameters (cm) were also found in the fruits treated with *M. piperita* EO and the mix EOs (Table 8). The mycelial growth inhibition of *B. cinerea* (%) in the fruits treated with *M. piperita* EO and the mix EOs was 38.78 and 46.94 %, respectively (Table 9).

Table 7. Infection rate (%) of strawberry fruits caused by *Botrytis cinerea* after treatment with different oils.

Treatments		Infection Rate (%)	
		7 DAI	10 DAI
Control	Untreated	53 a	87 a
E.C.	*E. camaldulensis var obtusa* EO	24 c	52 b
M.P.	*M. piperita* EO	12 d	18 c
E.C. + M.P.	*E. camaldulensis var obtusa* + *M. piperita* EOs (1/1 *v*/*v*)	15 d	20 c
M.O.	Moringa FO	40 b	80 a

DAI—days after inoculation; the same letter within the same column indicates non-significant differences among treatments ($p < 0.05$) at 7 and 10 DAI.

Table 8. Rot caused by *Botrytis cinerea*. Lesion diameter (cm) on strawberry fruits after treatment with different oils.

Treatments		Lession Diameter (cm)	
		7 DAI	10 DAI
Control	Untreated	1.4 a	2.45 a
E.C.	*E. camaldulensis var obtusa* EO	0.66 c	2.00 b
M.P.	*M. piperita* EO	0.65 c	1.5 c
E.C. + M.P.	*E. camaldulensis var obtusa* + *M. piperita* EOs (1/1 *v*/*v*)	0.55 c	1.3 c
M.O.	Moringa FO	1.25 b	2.35 a

DAI—days after inoculation; the same letters within the same column indicate non-significant differences among treatments ($p < 0.05$) at 7 and 10 DAI.

Table 9. Mycelial growth inhibition determined according to the diameters of the lesions for different treatments at 7 and 10 DAI.

Treatments		Inhibition of *B. cinerea* (%)	
		7 DAI	10 DAI
Control	Untreated	-	-
E.C.	*E. camaldulensis var obtusa* EO	52.86	18.37
M.P.	*M. piperita* EO	53.57	38.78
E.C. + M.P.	*E. camaldulensis var obtusa* + *M. piperita* EOs (1/1 *v*/*v*)	60.71	46.94
M.O.	Moringa FO	10.71	4.08

The visual observation of the fruits inoculated with *B. cinerea* 10 days after inoculation is shown in Figure 5. The mycelial morphology of *B. cinerea* grown on strawberry fruit treated with the studied oils is observed by SEM at 10 days (Figure 5). There were regular, uniform and complete mycelia with smooth surfaces, relatively strong and with high spore production in the control (Figure 5a). The fungal mycelial growth of *B. cinerea* was decreased after 10 days in the fruits treated with *E. camaldulensis var obtusa* EO (Figure 5b), but fungus hyphae are shown in dense growth. Fruit surfaces treated with *M. piperita* EO (Figure 5c) and the combined *E. camaldulensis var obtusa* + *M. piperita* EOs (Figure 5d) showed great morphological changes, including irregular growth of the mycelium, formation of verrucous surface, shrinkage, collapse and hollowing of hyphae. The morphology of mycelium *B. cinerea* grown on strawberry fruit surfaces treated with moringa FO (Figure 5e) was abnormal growth, lysis, shrinkage, reduced hyphal length and diameters with lower production of conidia compared to the control treatment. Recently, our group and various publications have documented the antifungal activity of EOs and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed fennel and *Cinnamomum camphora* [2,16,41,88]. The in vitro result showed that the EOs could be candidates for a natural antifungal in food preservation technology.

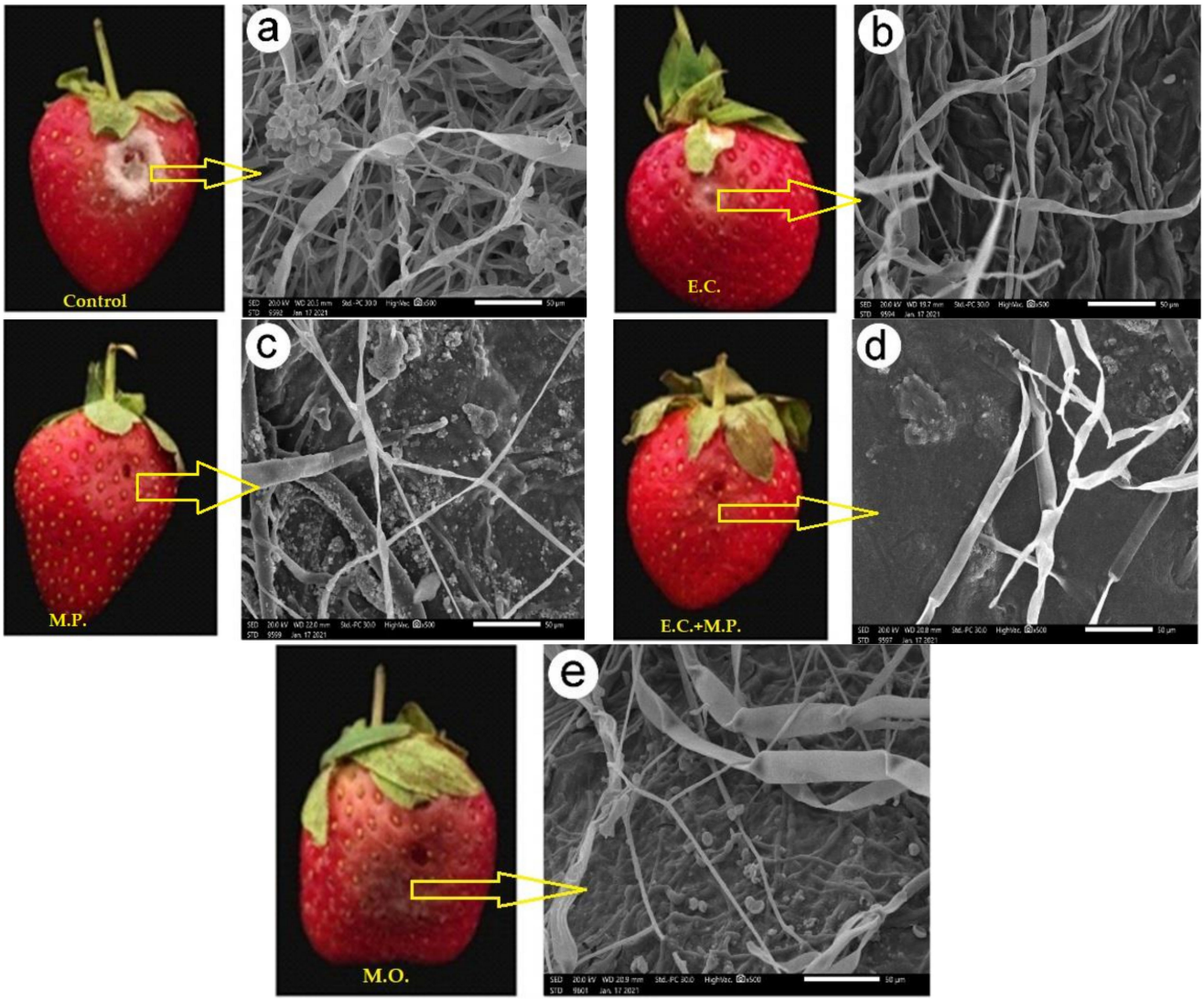

Figure 5. Visual observation and SEM photos of the treated and untreated fruits with oils after 10 days from the fungal inoculation and storage at 5 °C. (**a**) Untreated fruits and treated fruits with (**b**) *E. camaldulensis var obtusa* EO, (**c**) *M. piperita* EO, (**d**) mixture of *E. camaldulensis var obtusa* and *M. piperita* EOs (1/1 *v*/*v*) and (**e**) *M. oleifera* FO.

The beneficial effect of EO from *M. peperita* against *B. cinerea* was achieved after 15 days of storage by immersing cherry tomato fruits into this oil [89]. Medicinal and aromatic plants (MAPs) are very important in various fields, such as the pharmaceutical, perfumery and cosmetic industries [90]. In the last few years, the food industry has primarily used EOs as flavorings, and they represent an interesting source of natural antimicrobials for food preservation [91]. Carvacrol, cymene and γ-terpinene, the main chemical compounds from several EOs, showed potential antifungal activity against selected fungi including *B. cinerea* [92,93]. Their findings are in agreement with the results of this study, where GC–MS analysis showed that an EO mixture of *Eucalyptus* and *M. peperita* (1/1 *v*/*v*) exhibited the strongest antifungal activity.

The antimicrobial activity of EOs has been investigated against a large number of fungi [94,95]. *E. citriodora*, EO was found to exhibit *B. cinerea* fungitoxicity on grapes, with 100% growth inhibition [96]. Thyme and lemongrass have revealed great potential in post-harvest disease control [60].

4. Conclusions

This study aimed to determine the post-harvest changes occurring in CV Florida Fortuna strawberries exposed to three natural oils and to determine the proper natural oil for post-harvest quality studies. The present findings show both the mix of *E. camaldulensis var obtusa* and *M. piperita* (1/1 *v*/*v*) and *M. piperita* reduced decay symptoms and inhibited the mycelium growth of post-harvest pathogen *B cinerea*. In general, the essential oil treatments proved to be the most effective in improving post-harvest life, as evidenced by the values of the parameters assessed in this research. *E. camaldulensis var obtusa* and *M. piperita* (1/1 *v*/*v*) treatment reduced weight loss % and firmness loss and preserved the chemical quality of the strawberry during the storage period, while the lowest amount of colour change was achieved when *E. camaldulensis var obtusa* EO and *M. oleifera* FO were applied. Results from the present study show that natural oil treatments on strawberry fruit could constitute a safe and natural fungicide and could be used to prevent infection of strawberries during cold storage. This would extend shelf life over the minimum period required to transit strawberries abroad without notable adverse effects on the quality of the fruit. In the future, a new study will be performed to increase the post-harvest life using a combination of some essential oils which may affect as a fungicide. The application of natural oils represents a promising tool for reducing post-harvest losses and preserving quality in strawberries.

Author Contributions: Conceptualization, methodology and formal analysis, D.Y.A.-E., H.S.H., M.Z.M.S. and D.A.K.; validation, D.Y.A.-E., M.Z.M.S., D.A.K., A.A.A.-H., H.M.A. and H.S.H., designed the experiment, D.Y.A.-E., M.Z.M.S., D.A.K., A.A.A.-H., H.M.A. and H.S.H., conducted laboratory analyses, D.Y.A.-E., M.Z.M.S., D.A.K., A.A.A.-H., H.M.A. and H.S.H.; funding, A.A.A.-H. and H.M.A.; wrote parts of the manuscript and interpreted the results, D.Y.A.-E., M.Z.M.S., D.A.K., A.H.S., M.A. and H.S.H.; contributed reagents and materials, D.Y.A.-E., M.Z.M.S., D.A.K., A.A.A.-H., H.M.A. and H.S.H. All authors have read and agreed to the published version of the manuscript.

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