Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Effect of plant essential oils on the growth of *Botrytis cinerea* Pers.: Fr., *Penicillium italicum* Wehmer, and *P. digitatum* (Pers.) Sacc., diseases

Rana Samara[1]*, Tawfiq Qubbaj[2], Ian Scott[3], Tim Mcdowell[3]

[1] Horticulture and Agricultural Extension, Palestine Technical University-Kadoorie, Tulkarm, Palestine

[2] Department of Plant Production and Protection, Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Nablus, Palestine

[3] London Research and Development Centre, Agriculture and Agri-Food Canada, Canada

DOI: 10.24425/jppr.2021.139240

Received: April 07, 2021
Accepted: July 02, 2021

*Corresponding address:
r.samara@ptuk.edu.ps

Responsible Editor:
Piotr Kaczyński

Abstract

The current study was conducted to evaluate the effect of eight Palestinian indigenous plant essential oils (EOs) under *in vitro* and *in vivo* conditions against *Botrytis cinerea* Pers.: Fr., *Penicillium italicum* Wehmer, and *Penicillium digitatum* (Pers.) Sacc., three common post-harvest pathogens of tomato and strawberry fruits. *In vivo* tests showed that thyme, sesame and sage EOs exhibited high antifungal activity against *B. cinerea* on strawberry and tomato fruits, compared with rosemary, mint and eucalyptus. *In vitro* agar, disk-diffusion tests showed that *B. cinerea*, *P. digitatum* and *P. italicum* mycelium growth was completely inhibited when treated with clove and sage EOs caused 50% inhibition of *B. cinerea* and *P. italicum* mycelium growth. Fruit decay and fruit quality index values measured in total soluble solids and fruit flesh firmness showed that EO coated strawberries had significantly less fruit decaying and ripping compared to control, while EO coated tomatoes showed no significant difference compared to control. EO constituents fall into different chemical classes, including sterols, caffeoylquinic acids, flavonoids, terpenoids, coumarins, and acetylenes. Chemical analysis of the EO preparations using gas chromatography-mass spectrometry determined that the main components in sesame oil were octadecenoic acid-(56%) and hexadecanoic acid (26%), while clove oil consisted of eugenol (53%). In the other EOs, the principal compounds were: menthol (44% in mint oil), eucalyptol (37% in sage oil), while bornanone (18% in rosemary oil) and γ-terpinene (21% in thyme oil) were present at lower concentrations. The EO of sage plants could potentially be a useful alternative to synthetic pesticides to control post-harvest diseases and prolong the shelf life of fruit products.

Keywords: chemical analysis, growth inhibition, indigenous essential oils, strawberry

Introduction

Strawberry (*Fragaria ananassa* Duchesne) var. *ananassa* Bailey is a highly perishable non-climacteric fruit which is highly susceptible to fungal decay during post-harvest handling, storage and marketing. In comparison, tomato (*Solanum lycopersicum* L.) var. Izmer is a climacteric fruit with a high respiratory peak associated with a high ethylene production rate after harvest, and is also susceptible to fungal decay during post-harvest handling, storage and marketing. Several fungi such as *Alternaria alternata* (Fr.: Fr.) Kessler., *Aspergillus niger* Tiegh., *Botrytis cinerea* Pers.: Fr., *Rhizoctonia solani* Kühn, *Phytophthora* spp., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Penicillium* spp. and *Rhizopus stolonifer* (Ehrenb: Fr) Vuill. are common post-harvest pathogens causing high yield losses of both strawberries and tomatoes in most regions of the world (Snowdon 1990). Storage at low temperatures and frequent SO_2 fumigation during storage are the main methods

used to control post-harvest diseases in many countries (Crisosto *et al.* 1994). However, there are restrictions on this application in several countries because of the harmful effects of the residual activity and the damage caused to fruits due to reductions in their polyphenol and total antioxidant content (Taylor 1993). Therefore, an alternative control method is required to reduce strawberry and tomato post-harvest decay without harming the environment or consumers.

The role of indigenous plant extracts and essential oils (EOs) as potential pesticides have been reported from studies that examined their efficacy against plant diseases and insects (Sighamony *et al.* 1986; Matusinsky *et al.* 2015; Mossa 2016). Many plant compounds with antioxidant, anti-inflammatory and repellent activities are the main components which have been identified as crop protectants for common insect pests and diseases (Sighamony *et al.* 1986; Matusinsky *et al.* 2015). Potential new components include crude plant extracts and their isolates that have been previously reported in folklore remedies and medicines due to their antimicrobial activities (Sabbobeh *et al.* 2016; Jaradat *et al.* 2017). The Palestinian flora is diverse and unique, with more than 3,000 plant species belonging to over 130 families, many of which have been recorded as folk medicines against several infectious diseases (Jaradat *et al.* 2017; Abu-Darwish and Efferth 2018). For example, tea made of fresh or dry leaves of thyme, sage, or mint leaves have been used to treat colds, flu, cough, stomachache, and as an antiseptic mouthwash. EOs of eucalyptus, sesame or rosemary have been used to rub on children's chests to relieve cough-related symptoms, sore throat, hoarseness, and other cough-related symptoms. Many studies have been carried out to screen EOs from these plants for antifungal activity and potential use against post-harvest pathogens on fruits and vegetables (Abdollahi *et al.* 2011; Shirzad *et al.* 2011; Boubaker *et al.* 2016). However, no studies report the effect of plant EOs on the post-harvest quality of strawberries and tomatoes. Therefore, there is an opportunity to screen these indigenous herbal and wild-type plant extracts as potential natural pesticides to control or inhibit pathogens safely. This study aimed to assess' the potential use of eight commercial EO preparations to control post-harvest fungal decay and enhance the fruit quality and marketability of strawberries and tomatoes.

Materials and Methods

The present investigation was conducted during 2018 and 2019 in Kadoorie Agricultural Research Center (KARC) laboratories, Palestine Technical University, Palestine, and Agriculture and Agri-Food Canada (AAFC), London Research and Development Centre, Canada.

Fungal isolation and maintenance

The post-harvest fungi *B. cinerea*, *P. italicum*, and *P. digitatum* were isolated from infected strawberry, lemon, and orange fruits, respectively. Infected fruits were surface sterilized with 1% sodium hypochlorite (NaOCl) for 1 min, washed three times with distilled water and left to air dry. Small pieces of the infected fruits were placed in Petri plates containing potato dextrose agar (PDA). Then the plates were incubated at 20 ± 2°C for 4 days and observed for fungal growth. Isolates were then purified by mono-spore isolation, and transferred to fresh PDA plates. They were maintained at the laboratory of the KARC and cultured on potato dextrose agar (PDA) at 22°C (*B. cinerea*) and 25°C (*P. italicum, P. digitatum*) as reported by Gilchrist-Saavedra (1997).

Pathogen morphological identification

Fungal species were then identified on the basis of pathogenic characteristics (color, mycelium growth rate, and type and shape of the colony), the morphology of the colony, and microscopic observations of conidiophores and conidia. After 3 or 4 days of incubation, mycelia were mounted in water, and conidial masses were observed by bright-field microscopy using an Inverted Microscope – Optika XDS-2 Trinocular (AIPTEK International GmbH, München, Germany). Images were recorded with an AIPTEK HD1080P digital camera (AIPTEK International GmbH, München, Germany).

In vitro antifungal activity of plant EO

Commercial preparations of eight EO processed from medicinal plant leaves or seeds were obtained from Palsame® essential oils (Jenin-Palestine). According to the manufacturer's protocol, plant seeds were used to obtain EOs by either hydrodistillation or cold pressing using a Clevenger apparatus. Plant leaf EOs were obtained by hydrodistillation using a Clevenger apparatus. The distilled essential oils were stored in a refrigerator at 4°C until the inhibition test was used (Table 1). The indigenous plant EO antimicrobial activities were evaluated against the isolated pathogens using the agar disk-diffusion method, described by Bhalodia and Shukla (2011).

To determine the *in vitro* inhibitory effects of each EO on fungal growth, 1% EO was incorporated into the PDA, then poured into five Petri dishes and left to solidify. Single agar disks (0.3 cm in diameter) covered with germinated fungal conidia were transferred separately to serve as a source of inoculums. Each agar disk was placed in the center of Petri dishes containing PDA with the corresponding EO tested. Petri dishes

Table 1. List of commercial essential oils from medicinal plants used in the study

Sr. No.	Scientific name	Common name	Family
1	*Rosmarinus officinalis*	rosemary	Lamiaceae
2	*Eugenia caryophyllus*	clove	Myrtaceae
3	*Mentha spicata*	mint	Lamiaceae
4	*Salvia fruticosa*	sage	Lamiaceae
5	*Citrullus colocynthis*	bitter cucumber	Cucurbitaceae
6	*Eucalyptus obliqua*	eucalyptus	Myrtaceae
7	*Sesamum indicum*	sesame	Pedaliaceae
8	*Thymus vulgaris*	thyme	Lamiaceae

were sealed with parafilm to reduce the loss of the test EO. Plates without the EOs were used as controls. The plates were incubated for 5–7 days at 35 ± 2°C (Al-Reza *et al.* 2010). The percent inhibition (*PI*) of the radial growth of the mycelium growth was measured after 24 h and 48 h using the following equation suggested by Zabka and Pavela (2013):

$$PI = (DC - DT)/(DC) \times 100,$$

where *DC* = the colony diameter of the control sets [mm] of control culture and *DC* = the colony diameter of the treated sets [mm].

EO effective concentrations

The EO effective concentration values (EC_{50} and EC_{90}) were determined for each EO. First, stock solutions (10×) of each EO were prepared, followed by serial dilutions in water to prepare four concentrations of 10, 100, 250, and 500 µl · ml^{-1}, which were incorporated into the PDA for each tested EO, as described above. One PDA disk covered with fungal growth (0.5 cm in diameter) was placed in the plate's center, and the dishes were covered with parafilm. Petri dishes without EO acted as a control. After 3 days, the percent inhibition of mycelium growth was measured as described above. Four EOs were evaluated for their antifungal activity against the radial growth and spore germination of *B. cinerea*, *P. italicum*, and *P. digitatum*.

In vivo antifungal activity and fruit quality effects of plant EOs

Conidia of *B. cinerea* were recovered from 2–3-week old cultures by adding 10 ml of sterile water to each Petri plate. The conidia suspension was filtered through three layers of sterile cheesecloth. The conidial suspension concentration was calculated using a hemocytometer (ART.No.1280, Ningbo Hinotek Technology Co., Ltd. Zhejiang, China) and then adjusted to 10^5 conidia · ml^{-1} fungal suspension (Gilchrist-Saavedra 1997). A drop of Tween 80 was added to the suspension and vortexed for 20 min before being used. Tomato and strawberry fruits were fresh, not chemically treated, and obtained from a commercial market. All the fruits were disinfected (Lopez-Reyes *et al.* 2010) in a sodium hypochlorite solution (2.5%) for 2 min, followed by washing with distilled water (×3). Tomato and strawberry fruits were randomly distributed into five replicates per EO treatment. A thin EO layer was applied to each treated fruit's surface by dipping fruits into the EO and then air-dried for 1 hr. Then 3–4 wounds were made using a micro-syringe, and a *B. cinerea* suspension was sprayed onto the fruit surface and air-dried. The fruits were placed in sterile commercial packages and stored in a 7°C refrigerator for 2 weeks.

Fruit quality assessments were based on fruit total soluble solids (TSS) and flesh firmness, as described by Samara *et al.* (2017). Fruit firmness was measured using a FT Fruit Tester (Wagner FDK Force Gage). Two or three drops of the extracted juice were placed on the prism of a digital refractometer (PR-32α, Atago®, City, Japan) using a range of 0–32°Brix for measurements of TSS.

Gas chromatography-mass spectrometry analysis

For all EO analyses, an Agilent 5975C MSD with 7890A GC System fitted with an Agilent 7683 Series Injector and Auto-sampler, and G1701 EA Chromatography Workstation software (Agilent Technologies Inc., Wilmington, DE, USA) was used. All analyses were carried out as described by Mothana *et al.* (2013). Briefly, aliquots of 1 µl of each EO were injected in pulsed-splitless mode (30 s) into the GC-MS and a Duraguard DB-5ms GC Column (30 m × 0.25 mm ID and film thickness of 0.25 µm, duraguard 10 m, JandW Scientific, Folsom, CA, USA) with helium as the carrier gas at a constant flow rate of 1 ml · min^{-1}. The separation was

Table 2. *In vitro* antifungal activity of essential oils (EOs) against the radial growth and spore germination of *Botrytis cinerea, Penicillium digitatum* and *P. italicum* based on percent inhibition (±Std) of mycelium growth, measured by the percent inhibition of radial growth

Essential oil	Mean percent inhibition	±Std
B. cinerea		
Clove, *E. caryophyllus* L.	100 a*	0
Sage, *S. officinalis* L.	52 b	13
Mint, *M. piperita* L.	36 bc	28
Rosemary, *R. officinalis* L.	29 c	16
P. italicum		
Clove, *E. caryophyllus* L.	100 a	0
Sage, *S. officinalis* L.	53 b	23
Mint, *M. piperita* L.	64 b	12
Rosemary, *R. officinalis* L.	24 c	10
P. digitatum		
Clove, *E. caryophyllus* L.	100 a	0
Sage, *S. officinalis* L.	25 b	13
Mint, *M. piperita* L.	18 c	1
Rosemary, *R. officinalis* L.	34 b	25

*within a column, means followed by the same letter are not significantly different (Duncan's multiple range test at $p > 0.05$)

achieved using the following temperature program: injection at 50°C hold for 1 min, increased at 5°C · min^{-1} to 300°C, followed by an isothermal hold at 300°C for 9 min. The solvent delay was set for 5.25 min, with a total run time of 60 min. The injection inlet and transfer line temperatures were 280°C, and the MS source and quadrupole were 230°C and 180°C, respectively. The MS was operated in electron impact mode with ionization energy of 70 eV, and the scan range was set from m/z 40 to 700. All samples were injected three times consecutively. The compounds were then identified by mass and fragmentation pattern against the NIST11 database and standards squalane, octanone (for quantification) and caryophyllene were analyzed.

Statistical analysis

Then EC_{50} and EC_{90} values and their 95% confidence limits (CL 95%) were calculated from Probit regressions using SAS software (SAS Institute, Inc. Cary, NC, USA). The percent inhibition and inoculation results were analyzed using ANOVA as a general linear model (PROC GLM) procedure. Duncan's multiple range test was used to compare the means (SAS Institute, 1998).

Results and Discussion

In vitro antifungal activity of plant EO

The effect of commercial EO preparations on isolated saprophytes and secondary colonizer pathogens was evaluated. Most tested EOs (clove, sage, mint, thyme, rosemary) showed notable antifungal activity with all concentrations tested against the three fungal species. The inhibition of radial growth and spore germination of *B. cinerea, P. digitatum, P. italicum* was most significant for the following four EOs in decreasing order of activity: clove (*E. caryophyllus* L.) > sage (*S. officinalis* L.) > mint (*M. piperita* L.) > rosemary (*R. officinalis* L.) (Table 2). Clove oil completely inhibited the mycelium growth of the three pathogens, while sage oil reduced the mycelium growth by 52 and 53% for *B. cinerea* and *P. italicum*, respectively. Clove has previously been reported to have many uses in medicine, including antiviral, antimicrobial, and antifungal properties (Hamini-Kadar *et al.* 2014) and clove EO was strongly inhibitory against fungal mycelial growth such as *Candida*, *Aspergillus* and dermatophyte clinical strains (Pinto *et al.* 2009).

EO effective concentrations

The EO effective concentration values (EC_{50} and EC_{90}) were calculated from log-dose probit regressions of the pathogen growth inhibition regression (Table 3) to compare the EOs. The slopes, intercepts, χ^2 statistics of all EC were computed using different significance levels and their 95% confidence limits (CLs) based on log(EC) variances. The lowest EC_{50} values (highest inhibition rate recorded) were for clove EO, while the highest EC_{50} values (lowest inhibition rate recorded) were for rosemary EO for the three pathogenic fungi tested.

The 48 h EC_{50} values for clove, sage, mint and rosemary EOs against *B. cinerea* ranged from 268.8 to

Table 3. EC_{50} and EC_{90} values (with corresponding 95% confidence limits) for *B. cinerea, P. italicum,* and *P. digitatum* fungal growth after 48 h exposure to four different EOs using agar disk-diffusion. The percent inhibition (*PI*) of mycelium growth was measured by the percent inhibition of radial growth. The results are presented as EC_{50} and EC_{90} with corresponding 95% confidence limits (CL), Pearson Chi-square results, degree of freedom (*df*) and regression equations

Essential oil	*B. cinerea*							
	no.*	regression equations[1]	χ^2 (*df*)	slope±SE	LC_{50}** [µl · ml^{-1}]	(95% CL)[2]	LC_{90}** [µl · ml^{-}]	(95% CL)
Clove	25	$y = 0.105x + 0.7212$	324.3 (1)	6.03±0.28	411.7	374.6–454.4	3,689	2,929–4,860
Sage	25	$y = 0.1475x + 0.245$	270.5 (1)	4.75±0.25	817.6	697.7–983.4	18,188	11,658–31,853
Mint	25	$y = 0.1973x - 1.0209$	303.0 (1)	3.11±0.17	268.8	230.7–314.6	14,034	9,005–24,329
Rosemary	25	$y = 0.0841x + 1.6589$	234.0 (1)	9.1577±0.53	1,280.0	1,122.0–1,504.0	7,126	5,244–10,559
Essential oil	*P. italicum*							
	no.	regression equations	χ^2 (*df*)	slope±SE	LC_{50} [µl · ml^{-1}]	(95% CL)	LC_{90} [µl · ml^{-}]	(95% CL)
Clove	25	$y = 0.0583x + 1.3446$	573.8 (1)	10.09±0.43	255.6	240.1–271.6	855.1	773.3–959.5
Sage	25	$y = 0.2379x + 0.3957$	52.7 (1)	4.77±0.36	$48E^3$	$16E^3$–$314E^3$	$703E^4$	$838E^3$–$280E^6$
Mint	25	$y = 0.2166x + 0.6561$	55.31 (1)	5.05±0.38	$37E^3$	$13E^3$–$202E^3$	$3.6E^6$	$521E^3$–$99E^6$
Rosemary	25	$y = 0.4804x - 1.0271$	13.8 (1)	3.80±0.33	$4.5E^6$	$665E^3$–$3.4E^{13}$	$1.1E^{12}$	$525E^6$–$7.3E^{22}$
Essential oil	*P. digitatum*							
	no.	regression equations	χ^2 (*df*)	slope±SE	LC_{50} [µl · ml^{-1}]	(95% CL)	LC_{90} [µl · ml^{-}]	(95% CL)
Clove	25	$y = 0.0583x + 1.3446$	557.8 (1)	10.03±0.43	340.68	320.4–362.4	1,063.0	953.7–1,205.0
Sage	25	$y = 0.2379x + 0.3957$	51.03 (1)	4.78±0.37	$53E^3$	$17E^3$–$374E^3$	$793E^4$	$896E^3$–$360E^6$
Mint	25	$y = 0.2166x + 0.6561$	57.5 (1)	5.26±0.41	$30E^3$	$11E^3$–$144E^3$	$223E^4$	$368E^3$–$47E^6$
Rosemary	25	$y = 0.4804x - 1.0271$	19.5 (1)	3.88±0.33	$5.3E^6$	$245E^3$–$1.5E^{10}$	$3.4E^{10}$	$109E^6$–$1.04E^{17}$

*number of samples used in the bioassay; **EC_{50} and EC_{90} values [µl · ml^{-1}]
[1]regression equations estimated by probit regression; [2](95%) confidence limits for EC_{50} and EC_{90}

1,280 µl · ml^{-1}, while 48 h EC_{50} values against *P. italicum* and *P. digitatum* were generally greater, 255.6 to 5.3E^{6} µl · ml^{-1}. The 48 h EC_{90} values for clove, sage, mint and rosemary exposure against *B. cinerea* were again lower than for the other two pathogens and ranged from 3.6E^{3} to 1.8E^{4} µl · ml^{-1}, compared to 855.1 to 1.1E^{12} µl · ml^{-1}.

In vivo antifungal activity of plant EOs

Eight Palestinian indigenous EOs had inhibitory effects on the fungal growth of *B. cinerea* in inoculated strawberries and tomatoes (Fig. 1A and B). Sage, sesame and thyme EOs were the most inhibitory of *B. cinerea* on both strawberries and tomatoes. Similar results were reported for thyme, sage and sesame against *B. cinerea* and another post-harvest pathogen, *Penicillium expansum* (Fraternale *et al.* 2005; Park 2011; Sabbobeh *et al.* 2016). Phillips *et al.* (2012) observed that citrus EO reduced spore germination of pathogens *P. chrysogeum*, *A. niger* and *A. alternata*. The plant EOs showed different activities in inhibiting disease development in strawberries and tomatoes. Although clove EO was the most active in the *in vitro* study, *B. cinerea* on tomato fruits was only partially inhibited by clove EO (Fig. 1A and B). In contrast, sage, thyme, and sesame EO caused a 10–18% inhibition of mycelium's radial growth on tomato (Fig. 1A) and 25–30% inhibition of radial growth of mycelium on strawberry (Fig. 1B). The least effective was thyme EO in reducing the infection caused by *B. cinerea.* According to Vitoratos *et al.* (2013), grey mold (*B. cinerea*) on strawberries was entirely inhibited by lemon EO at 0.05 µl · ml^{-1}. Also, lemon (*Citrus limon* L.) EO at the same concentration caused a 39% reduction of *B. cinerea* on cucumber. Fungal infection was only noticed 5 days after treatment with oregano EO. After 6 days with lemon EO the results were comparable to the controls where infections were evident 48 h after being placed in storage at 22°C. The antimicrobial activities of the indigenous plant EOs of *Thymus* species against the isolated fungal species *Penicillium digitatum, P. italicum* and *Geotrichum citri-aurantii* have also been recorded (Boubaker *et al.* 2016). According to Al-Zubairi *et al.* (2017), EO antioxidant activity is more significant with increased phenolic and flavonoid content or synergistic interactions between the chemical compounds (Moura Martins *et al.* 2020). Other research has documented *Artemisia arborescens* (L.) EO inhibitory effects against enterobacteria, *Listeria monocytogenes* strains (Militello *et al.* 2011).

Fruit quality

Comparative fruit decay and fruit quality index values were based on total soluble solids and flesh firmness (Figs 2 and 3). All tested EOs showed promising inhibition of disease severity, but fungal decay effectiveness was dependent on the type and concentration of EO. The total soluble solids content of tomato fruits (TSS) measured as Brix ranged from 6% for the control and 4–6% for coated fruits but was not significantly different (F = 1.64; p = 0.1378) (Fig. 2A). Measurements of tomato fruit firmness were also not significantly different (F = 1.45; p = 0.2012) from the control fruits (6.2 kgf) and EO coated fruits (4.4–5.9 kgf) (Fig. 2B). The TSS of strawberry fruits ranged from 1.6% for the control to 0.2–1% for the coated fruits, giving a significant difference (F = 3.57; p = 0.0054) between

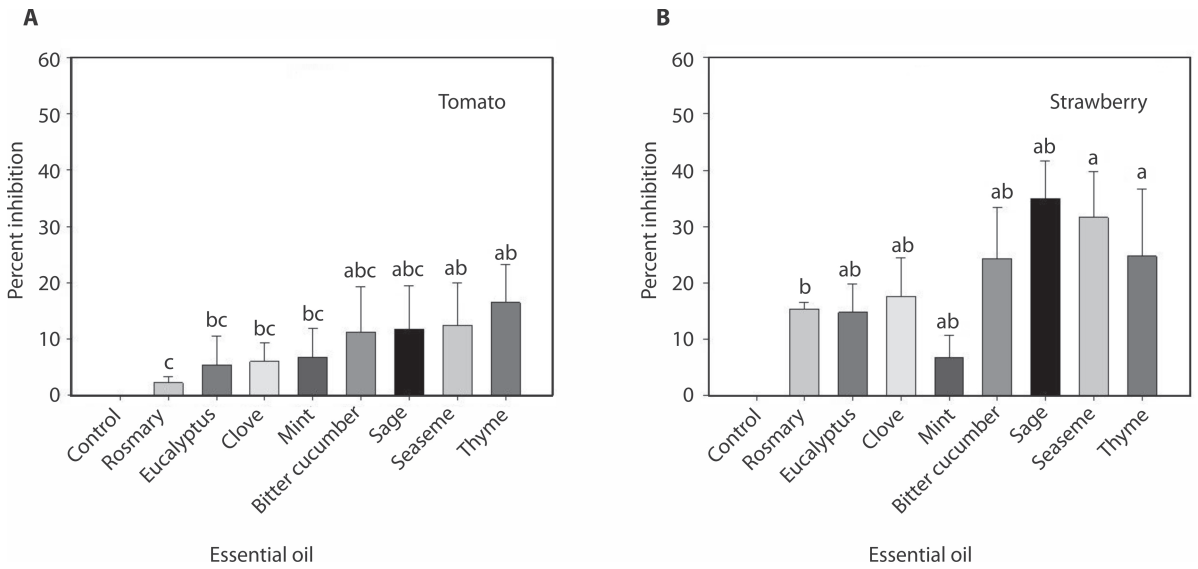

Fig. 1. *In vivo* percent inhibition ± mean standard deviation (±Std) activity produced by eight plant EOs against the growth and spore germination of *B. cinerea* on tomato (A) and strawberry fruit (B), measured by the percent inhibition of radial growth. Means followed by the same letter are not significantly different (Duncan's multiple range test, $p > 0.05$)

treated and non-treated fruits in fruit ripeness values (Fig. 3A). Measurements of strawberry fruit firmness were also significantly different ($F = 3.57$; $p = 0.0054$) between control fruits (4.8 kgf) and EO coated fruits (0.38–1.7 kgf) (Fig. 3B). Similar results were found when thyme EO was applied to strawberries (Martinez *et al.* 2018) and tomato fruits (Camele *et al.* 2012). Clove EO inhibited the growth of *Monilinia fructicola* (G. Winter) on nectarine fruits (Lazar-Baker *et al.* 2011), while cumin EO inhibited the growth of *B. cinerea* on strawberry fruits (Asghari Marjanlo *et al.* 2009).

There is potential for plant EOs to be used on fruit as a post-harvest treatment to reduce and inhibit fungal infection under storage conditions. Thyme, sage and sesame EOs provided a significant reduction in fruit decay indices and reduced pathogen growth. They can extend the shelf life of the treated fruits for a short period, which is required for handling and shipping to local markets, without significantly affecting the fruit quality.

The antifungal activity of thyme and clove was enhanced with higher EO concentrations (Hosseini *et al.* 2008). Thyme oil had the most significant antifungal activity while fennel (*Foeniculum vulgare* Mill), summer savoury (*Satureja hortensis* L.), and sweet basil (*Ocimum basilicum* L.) oils showed less antifungal activity (Abdollahi *et al.* 2011; Shirzad *et al.* 2011). Also,

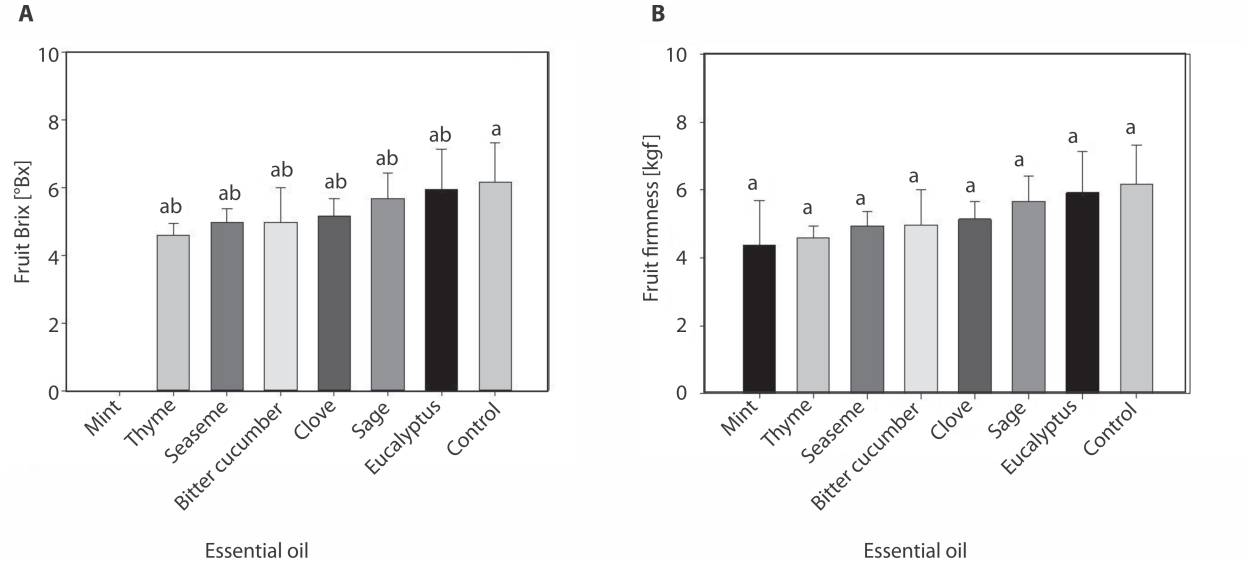

Fig. 2. Comparison of the mean fruit total soluble solids ± mean standard deviation (±Std) (A) and mean fruit firmness and ripeness (±Std) (B) of tomato fruits treated *in vivo* with six plant EOs against the growth and spore germination of *B. cinerea*. Means followed by the same letter are not significantly different (Duncan's multiple range test, $p > 0.05$)

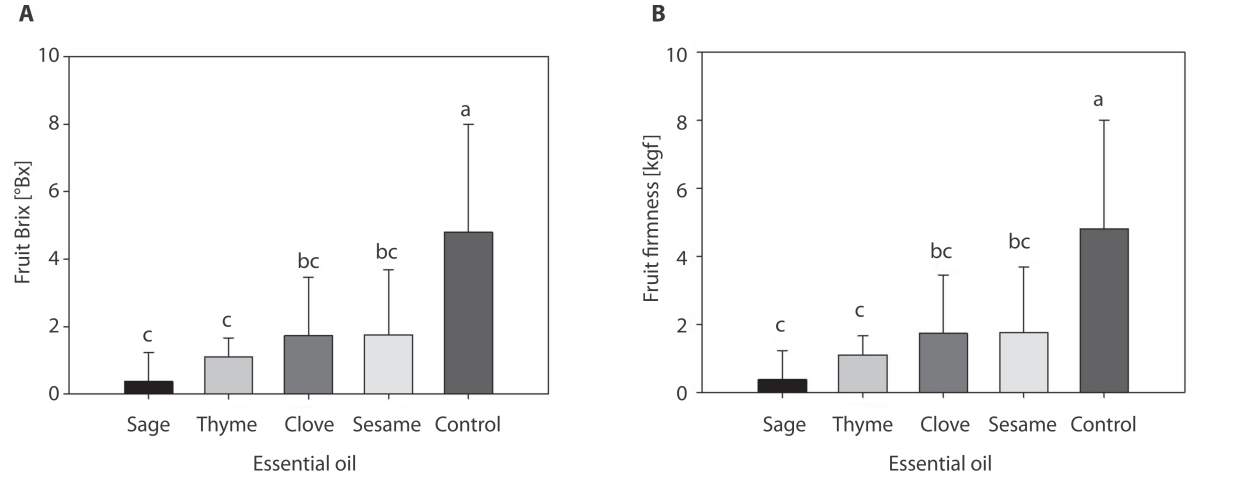

Fig. 3. Comparison of the mean fruit total soluble solids (A) and mean fruit firmness and ripeness ± mean standard deviation (±Std) (B) of strawberry fruits treated *in vivo* with four plant EOs against the growth and spore germination of *B. cinerea*. Means followed by the same letter are not significantly different (Duncan's multiple range test, $p > 0.05$)

thyme EO has a strong aromatic odor as well as antiseptic, antioxidant, antibacterial and antifungal properties (Park 2011). Javed *et al.* (2013) reported that thyme has been used for centuries in folk medicine, food preservation and phytopharmaceutical preparations. The current results indicated that it could extend the shelf life of treated fruits. Future work on these plants must be oriented to identify each essential oil's active components, action mechanism, and phytotoxicity based on the application rates.

Gas chromatography-mass spectrometry analysis

The GC-MS total ion chromatogram (TIC) (Fig. 4) and the chemical composition of the essential oils tested during this study are listed in Table 4. In general, the primary compounds found in sage, clove, mint and thyme were eucalyptol (37%), eugenol (53%), menthol (44%), and terpinene (21%), respectively. Chemical compounds found in rosemary oil were bornanone (18%), eucalyptol (16%), caryophyllene (5%), alpha-terpineol (5%) and endo-borneol (4%). These chemicals have been associated with insect repellant activity (Momen *et al.* 2001), and antifungal activity against *Aspergillus* spp. and other pathogens, including fungi (Baratta *et al.* 1998). The principal compounds in sage oil were eucalyptol (37%), β- and α- pinene (16 and 5%), caryophyllene (11%), myrcene (6%), and camphor (3%). These compounds were associated with antimicrobial activity against pathogens, such as yeasts, molds and gram-positive bacteria (Chaieb *et al.* 2007; Porte *et al.* 2013; Grzegorczyk-Karolak *et al.* 2019). Terpenoid compounds were found to be effective against insects

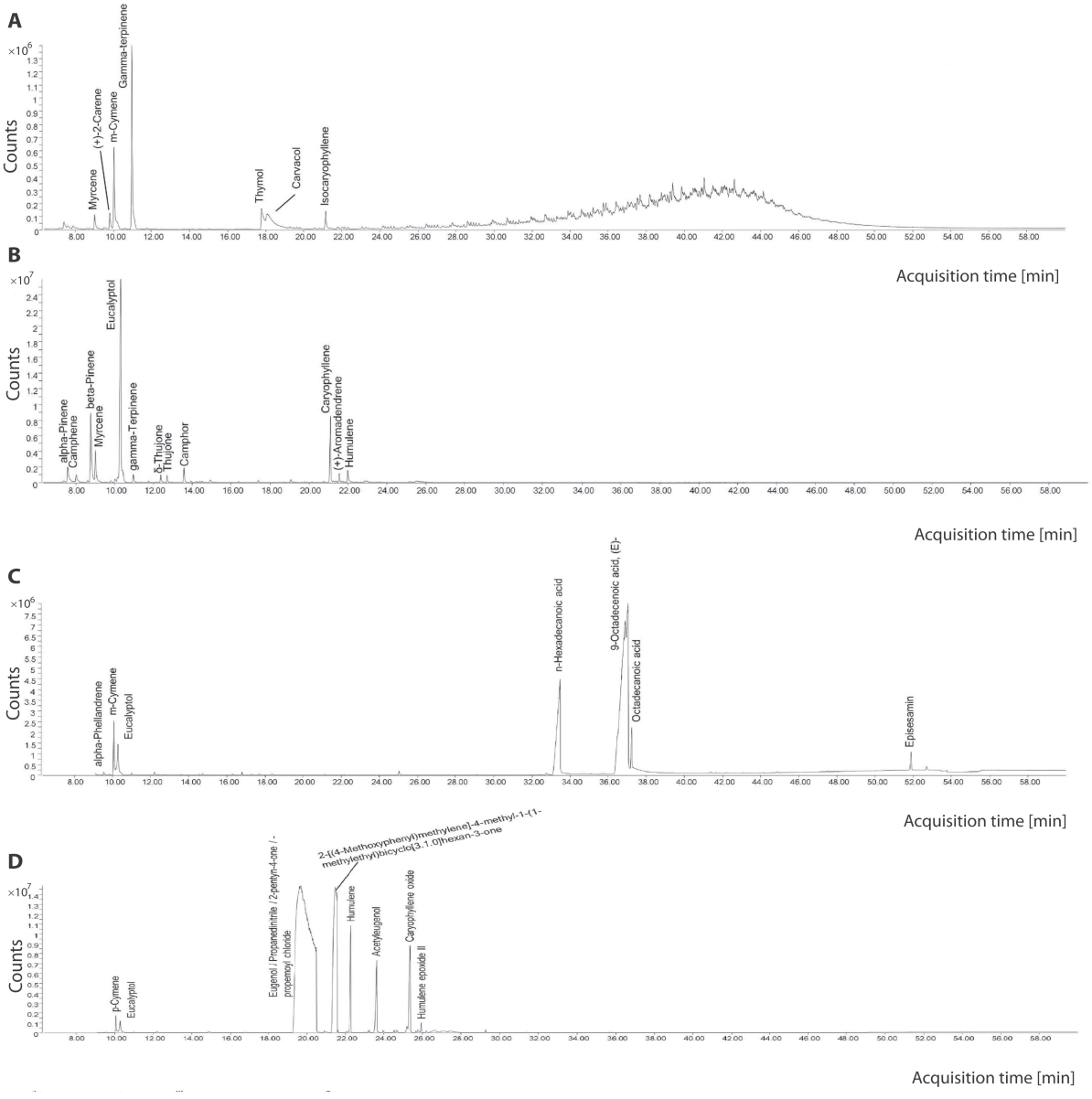

Fig. 4. The GC-MS total ion chromatogram (TIC) of EO of (A) Thyme, (B) Sage, (C) Sesame, (D) Clove, (E) Rosemary, (F) Bitter cucumber, (G) Eucalyptus, (H) Mint

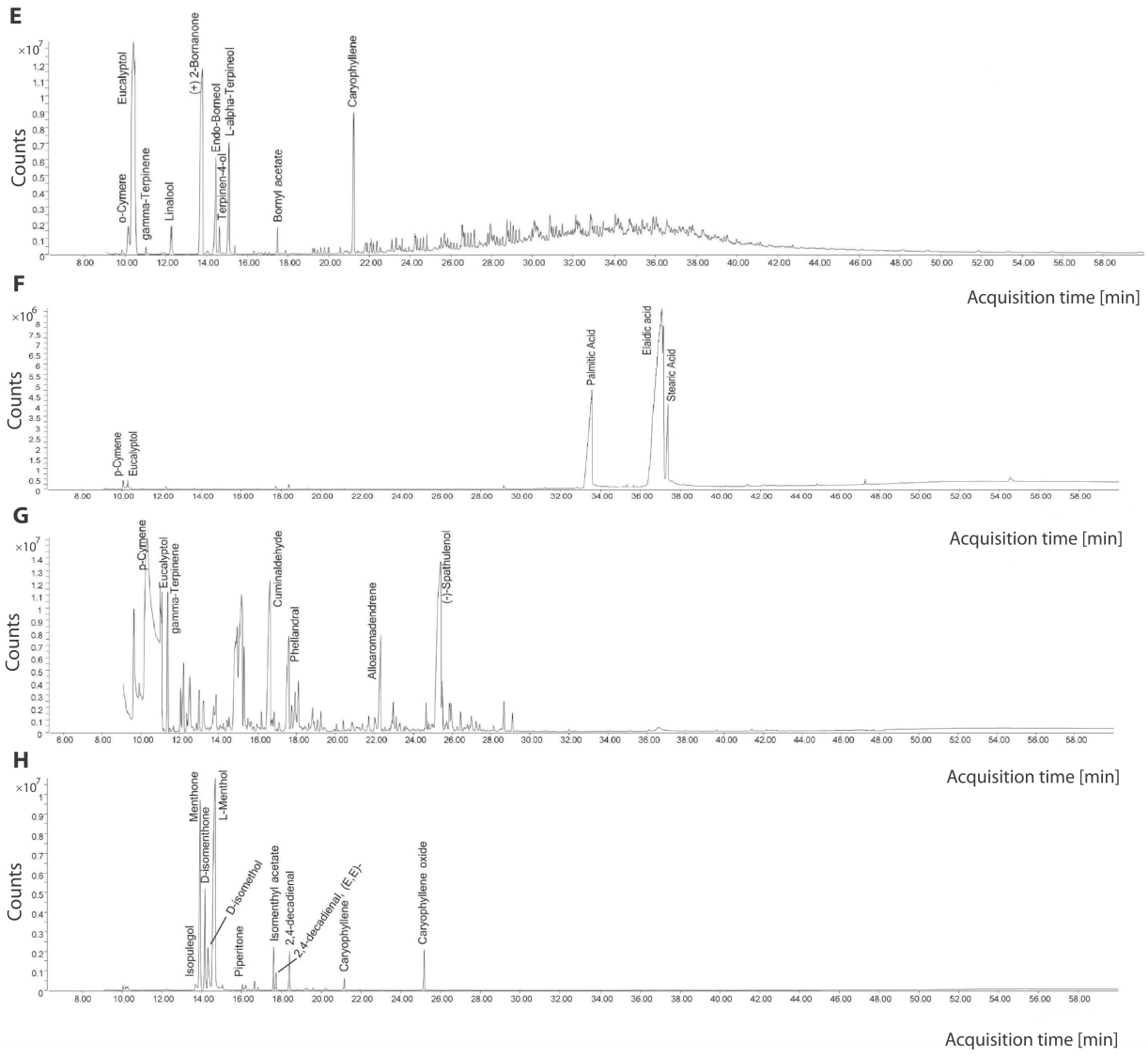

Fig. 4. The GC-MS total ion chromatogram (TIC) of EO – *continuation*

and other related microorganisms (Olsen 2000). The major compounds found in mint were menthol (44%), menthone (22%), and iso-menthone and iso-menthol compounds (9 and 6%, respectively). Menthol was reported to inhibit the mycelial growth of several plant diseases tested and showed higher antifungal impact than synthetic fungicides. Both chemicals inhibited seed germination and seedling growth of broadleaf weeds (Kordali *et al.* 2008). It was also observed to cause high mortality against stored insect pests (Rozman *et al.* 2006). The major compounds found in clove were eugenol and acetyl eugenol (53 and 5%, respectively), propenoyl chloride (10%), humulene (7%), and toluene (5%). Eugenol is highly toxic to insects and was reported to have repellant activity (Obeng-Ofori and Reichmuth 1997). Other studies showed that eugenol has antifungal, anti-carcinogenic, anti-allergic, anti-mutagenic, antioxidant and antimicrobial activities (Javed *et al.* 2013; Thosar *et al.* 2013; Shah *et al.* 2014; Rajkowska *et al.* 2016). In comparison, eugenol, acetyl eugenol, iso-eugenol and β-caryophyllene were the main chemicals in clove oil from another study (Politeo *et al.* 2010). These phenolic compounds are believed to be responsible for the inhibitory effects of clove oils on tested microbes (Shoaib *et al.* 2014). Many dental pharmaceutical medications contain eugenol due to its reported antimicrobial, antiseptic and antispasmodic activities (Nejad *et al.* 2017). In contrast, the significant chemicals of sage EO are linalool, linalyl acetate, geranyl acetate, (E)-β-ocimene, and caryophyllene oxide (Fraternale *et al.* 2005).

The major compounds found in thyme were carvacrol and thymol; both have anti-inflammatory, antimicrobial, anti-inflammatory, and antioxidant properties (Abu-Lafi *et al.* 2008). Thyme EO was used to enhance growth and productive performance via modification

Table 4. Major chemical composition, retention time and percentage of essential oil (thyme, sage, sesame, clove, rosemary, bitter cucumber, eucalyptus and mint) obtained with GS-MS analysis

Compound name	*RT**	Thyme	Compound name	*RT*	Sage
gamma-Terpinene	10.96	21%	Eucalyptol	10.47	37%
m-Cymene	10.15	11%	beta-Pinene	9.15	16%
n-Tetracosane	42.62	11%	Caryophyllene	21.17	11%
Carvacrol	18.09	8%	Myrcene	9.17	6%
Thymol	17.94	5%	alpha-Pinene	7.68	5%
Isocaryophyllene	21.16	2%	Camphor	13.59	3%
(+)-2-Carene	9.81	2%	Humulene	22.09	3%
Heptacosane	40.86	2%	Camphene	8.13	2%
Myrcene	9.12	2%	gamma-Terpinene	10.92	2%
Hexadecane	41.19	2%	Aromadendrene	21.67	2%
Compound name	*RT*	Sesame	Compound name	*RT*	Clove
9-Octadecenoic acid, (E)-	37.02	56%	Eugenol	19.37	53%
n-Hexadecanoic acid	33.47	26%	2-Propenoyl chloride	20.16	10%
m-Cymene	10.07	5%	Humulene	22.16	7%
Octadecanoic acid	37.21	3%	Toluene	21.46	5%
Eucalyptol	10.29	2%	Acetyleugenol	23.59	5%
p-Cymene	10.18	2%	Propanedinitrile	19.73	5%
Episesamin	51.90	2%	Caryophyllene oxide	25.07	3%
3-Amino-4-piperonyl-5-pyrazolone	52.71	0.4%	Dimethylethylborane	21.95	3%
D-Alanine, N-(2,5-ditrifluoro-methyl-benzoyl)-, heptyl ester	37.20	0.4%	2-Pentyn-4-one	20.14	1%
alpha-phellandrene	9.54	0.4%	p-Cymene	10.20	1%
Compound name	*RT*	Rosemary	Compound name	*RT*	Bitter cucumber
(+)-2-Bornanone	14.03	18%	Elaidic acid	37.23	46%
Eucalyptol	10.47	16%	Palmitic acid	33.42	31%
Caryophyllene	21.17	5%	Stearic acid	37.31	10%
L-.alpha.-Terpineol	15.14	5%	Lumiflavine	33.55	5%
Endo-Borneol	14.47	4%	2-Ethylimidazole	37.29	2%
Propane, 2-nitro-	10.63	3%	p-Cymene	10.20	1%
o-Cymene	10.25	1%	Eucalyptol	10.47	1%
Linalool	12.20	1%	Bi-1-cyclohexen-1-yl, 3,3,3',3',5,5,5',5'-octamethyl-	54.54	1%
4-Chlorobutyric acid, 4-isopropylphenyl ester	10.00	1%	trans,trans-2,4-Decadienal	18.08	0.3%
Terpinen-4-ol	14.64	1%	Squalene	47.27	0.3%
Compound name	*RT*	Eucalyptus	Compound name	*RT*	Mint
p-Cymene	10.30	26%	L-Menthol	14.68	44%
Spathulenol	25.40	12%	Menthone	13.93	22%
Cuminaldehyde	16.61	6%	D-isomenthone	14.17	9%
Phellandral	17.60	5%	D-isomenthol	14.32	6%
alpha-Phellandrene	9.64	3%	Caryophyllene oxide	25.17	4%
Alloaromadendrene	22.31	3%	2,4-Decadienal	18.41	3%
Cryptone	15.17	3%	Isomenthyl acetate	17.61	3%
gamma-Terpinene	11.36	3%	2,4-Decadienal, (E,E)-	17.74	1%
Linalol	12.49	2%	Caryophyllene	21.15	1%
1-Chloroethyl sulfone	10.37	2%	Isopulegol	13.69	1%

*retention time – the time taken for a solute to pass through a chromatography column

and activation of gastrointestinal tract structures and functions, as well as inhibiting and preventing cancer (Alagawany *et al.* 2015). It was also found to have insecticidal and acaricidal activities (Chaieb *et al.* 2007). Phenolic compounds identified in this study have been associated with potent antioxidant and anti-inflammatory activities (Park 2011). Among them, the primary chemical compounds in plant EOs include sterols, flavonoids, terpenoids, coumarins, caffeoylquinic acids, and acetylenes, which support their potential use in the control of pests and diseases as well as in food and pharmaceutical industries (Bora and Sharma 2011).

Conclusions

In vitro tests showed that clove inhibited 100% of the mycelium growth of *B. cinerea*, *P. digitatum* and *P. italicum*, while sage inhibited 50% of the growth of the first two tested pathogens. At the same time, some other EOs delayed fruit decay and fruit quality index values. Chemical analysis showed that EO constituents fall into different chemical classes, including sterols, caffeoylquinic acids, flavonoids, terpenoids, coumarins, and acetylenes. Results showed that EO treatments could be used as natural fungicides and could potentially extend the shelf life of tomato and strawberry fruits during storage. Palestinian flora could play an even more critical economic role and be used for medicinal and research purposes.

Acknowledgements

The scientific work was supported by the Zamala Fellowship Program 2017/2018 sponsored by the Bank of Palestine and Welfare Association. Part of this research was also supported by the Palestine Technical University-Kadoorie (PTUK). The authors thank many capable students for their assistance in the fieldwork, and laboratory technicians for their technical assistance, support and culture maintenance. Special thanks go to Palsame® essential oils Company for providing essential oil samples for this study.

References

Abdollahi A., Hassani A., Ghosta Y., Meshkatalsadat M.H., Shabani R. 2011. Screening of antifungal properties of essential oils extracted from sweet basil, fennel, summer savory and thyme against post-harvest phytopathogenic fungi. Journal of Food Safety 31: 350–356. DOI: https://doi.org/10.1111/j.1745-4565.2011.00306.x

Abu-Darwish M.S., Efferth T. 2018. Medicinal plants from near east for cancer therapy. Frontiers in Pharmacology 9: 56. DOI: https://doi.org/10.3389/fphar.2018.00056

Abu-Lafi S., Odeh I., Dewik H., Qabajah M., Hanuš L.O., Dembitsky V.M. 2008. Thymol and carvacrol production from leaves of wild Palestinian *Majorana syriaca*. Bioresource Technology 99: 3914–3918. DOI: https://doi.org/10.1016/j.biortech.2007.07.042

Alagawany M., El-Hack M.A., Farag M.R., Tiwari R., Dhama K. 2015. Biological effects and modes of action of carvacrol in animal and poultry production and health-a review. Advances in Animal and Veterinary Sciences 3: 73–84. DOI: https://doi.org/10.14737/journal.aavs/2015/3.2s.73.84

Al-Reza S.M., Yoon J.I., Kim H.J., Kim J.S., Kang S.C. 2010. Anti-inflammatory activity of seed essential oil from *Zizyphus jujuba*. Food Chemistry Toxicology 48: 639–643. DOI: https://doi.org/10.1016/j.fct.2009.11.045

Al-Zubairi A., Al-Mamary M., Al-Ghasani E. 2017. The antibacterial, antifungal, and antioxidant activities of essential oil from different aromatic plants. Global Advanced Research Journal of Medicine and Medical Sciences 6: 224–233.

Asghari Marjanlo A., Mostofi Y., Shoeibi S., Fattahi M. 2009. Effect of cumin essential oil on post-harvest decay and some quality factors of strawberry. Journal of Medicinal Plants 3: 25–43.

Baratta M.T., Dorman H.D., Deans S.G., Biondi D.M., Ruberto G. 1998. Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. Journal of Essential Oil Research 10: 618–627. DOI: https://doi.org/10.1080/10412905.1998.9700989

Bhalodia N.R., Shukla V.J. 2011. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* L., an ethnomedicinal plant. Journal of Advanced Pharmaceutical Technology & Research 2: 104. DOI: https://doi.org/10.4103/2231-4040.82956

Boubaker H., Karim H., El Hamdaoui A., Msanda F., Leach D., Bombarda I., Vanloot P., Abbad A., Boudyach E.H., Ait Ben Aoumar A. 2016. Chemical characterization and antifungal activities of four *Thymus* species essential oils against post-harvest fungal pathogens of citrus. Industrial Crops and Products 86: 95–101. DOI: https://doi.org/10.1016/j.indcrop.2016.03.036

Bora K.S., Sharma A. 2011. The genus *Artemisia*: a comprehensive review. Pharmaceutical Biology 49: 101–109. DOI: https://doi.org/10.3109/13880209.2010.497815

Camele I., Altieri L., De Martino L., De Feo V., Mancini E., Rana G.L. 2012. *In vitro* control of post-harvest fruit rot fungi by some plant essential oil components. International Journal of Molecular Sciences 13: 2290–2300. DOI: https://doi.org/10.3390/ijms13022290

Chaieb K., Hajlaoui H., Zmantar T., Kahla Nakbi A.B., Rouabhia M., Mahdouani K., Bakhrouf A. 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. Phytotherapy Research 21: 501–506. DOI: https://doi.org/10.1002/ptr.2124

Crisosto C.H., Smilanick J.L., Dokoozlian N.K., Luvisi D.A. 1994. Maintaining table grape post-harvest quality for long distant markets. p. 195–199. In*:* Proceedings of the International Symposium on Table Grape Production. American Society for Enology and Viticulture, ASEV, June 28–29. Anaheim, CA, USA.

Fraternale D., Giamperi L., Bucchini A., Ricci D., Epifano F., Genovese S., Curini M. 2005. Composition and antifungal activity of essential oil of *Salvia sclarea* from Italy. Chemistry of Natural Compounds 41: 604–606. DOI: https://doi.org/10.1007/s10600-005-0221-9

Gilchrist-Saavedra L. 1997. Practical guide to the identification of selected diseases of wheat and barley. CIMMYT.

Grzegorczyk-Karolak I., Kuźma Ł., Lisiecki P., Kiss A. 2019. Accumulation of phenolic compounds in different in vitro cultures of *Salvia viridis* L. and their antioxidant and an-

timicrobial potential. Phytochemistry Letters 30: 324–332. DOI: https://doi.org/10.1016/j.phytol.2019.02.016

Hamini-Kadar N., Hamdane F., Boutoutaou R., Kihal M., Henni J.E. 2014. Antifungal activity of clove (*Syzygium aromaticum* L.) essential oil against phytopathogenic fungi of tomato (*Solanum lycopersicum* L.) in Algeria. Journal of Experimental Biology and Agricultural Sciences 2: 447–454.

Hosseini M.H., Razavi S.H., Mousavi S.M.A., Yasaghi S.A.S., Hasansaraei A.G. 2008. Improving antibacterial activity of edible films based on chitosan by incorporating thyme and clove essential oils and EDTA. Journal of Applied Sciences 8: DOI: https://doi.org/2895–2900.10.3923/jas.2008.2895.2900

Javed H., Erum S., Tabassum S., Ameen F. 2013. An overview on medicinal importance of *Thymus vulgaris*. Journal of Asian Scientific Research 3: 974.

Jaradat N.A., Zaid A.N., Al-Ramahi R., Alqub M.A., Hussein F., Hamdan Z., Mustafa M., Qneibi M., Ali I. 2017. Ethnopharmacological survey of medicinal plants practiced by traditional healers and herbalists for treatment of some urological diseases in the West Bank/Palestine. BMC Complementary Medicine and Therapies 17: 255. DOI: https://doi.org/10.1186/s12906-017-1758-4

Kordali S., Cakir A., Ozer H., Cakmakci R., Kesdek M., Mete E. 2008. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresource Technology 99: 8788–8795. DOI: https://doi.org/10.1016/j.biortech.2008.04.048

Lazar-Baker E., Hetherington S., Ku V., Newman S. 2011. Evaluation of commercial essential oil samples on the growth of post-harvest pathogen *Monilinia fructicola* (G. Winter) Honey. Letters in Applied Microbiology 52: 227–232. DOI: https://doi.org/10.1111/j.1472-765X.2010.02996.x

Martinez K., Ortiz M., Albis A., Gilma Gutiérrez Castañeda C., Valencia M.E., Grande Tovar C.D. 2018. The effect of edible chitosan coatings incorporated with *Thymus capitatus* essential oil on the shelf-life of strawberry (*Fragaria* × *ananassa*) during cold storage. Biomolecules 8: 155. DOI: https://doi.org/10.3390/biom8040155

Matusinsky P., Zouhar M., Pavela R., Novy P. 2015. Antifungal effect of five essential oils against important pathogenic fungi of cereals. Industrial Crops and Products 67: 208–215. DOI: https://doi.org/10.1016/j.indcrop.2015.01.022

Militello M., Settanni L., Aleo A., Mammina C., Moschetti G., Giammanco G.M., Blàzquez M.A., Carrubba A. 2011. Chemical composition and antibacterial potential of *Artemisia arborescens* L. essential oil. Current Microbiology 62: 1274–1281. DOI: https://doi.org/10.1007/s00284-010-9855-3

Momen F.M., Amer S.A.A., Refaat A.M. 2001. Repellent and oviposition-deterring activity of rosemary and sweet marjoram on the spider mites *Tetranychus urticae* and *Eutetranychus orientalis* (Acari: Tetranychidae). Acta Phytopathologica et Entomologica Hungarica 36: 155–164. DOI: https://doi.org/10.1556/aphyt.36.2001.1-2.18

Mossa A.T.H. 2016. Green pesticides: Essential oils as biopesticides in insect-pest management. Journal of Environmental Science and Technology 9: 354. DOI: https://doi:10.3923/jest.2016.354.378

Mothana R.A., Al-Said M.S., Al-Yahya M.A., Al-Rehaily A.J., Khaled J.M. 2013. GC and GC/MS analysis of essential oil composition of the endemic soqotraen *Leucas virgata* Balf.f. and its antimicrobial and antioxidant activities. International Journal of Molecular Sciences 14: 23129–23139. DOI: https://doi.org/10.3390/ijms141123129

Moura Martins C., de Morais S.A., Martins M.M., Cunha L.C., da Silva C.V., Teixeira T.L., Santiago M.B., de Aquino F.J., Nascimento E.A., Chang R., Martins C.H. 2020. Antifungal and cytotoxicity activities and new proanthocyanidins isolated from the barks of *Inga laurina* (Sw.) Willd. Phytochemistry Letters 40: 109–120. DOI: https://doi.org/10.1016/j.phytol.2020.10.001

Nejad S.M., Ozgunes H., Basaran N. 2017. Pharmacological and toxicological properties of eugenol. Turkish Journal of Pharmaceutical Sciences 14: 201–206. DOI: 10.4274/tjps.62207

Obeng-Ofori D., Reichmuth C.H. 1997. Bioactivity of eugenol, a major component of essential oil of *Ocimum suave* (Wild.) against four species of stored-product Coleoptera. International Journal of Pest Management 43: 89–94. DOI: https://doi.org/10.1080/096708797229040

Olsen R.W. 2000. Absinthe and gamma-aminobutyric acid receptors. p. 4417–4418. In: Proceedings of the National Academy of Sciences of the United States of America, 97. DOI: https://doi.org/10.1073/pnas.97.9.4417

Park J.B. 2011. Identification and quantification of a major antioxidant and anti-inflammatory phenolic compound found in basil, lemon thyme, mint, oregano, rosemary, sage, and thyme. International Journal of Food Sciences and Nutrition 62: 577–584. DOI: https://doi.org/10.3109/09637486.2011.562882

Phillips C.A., Laird K., Allen S.C. 2012. The use of Citri-V™® An antimicrobial citrus essential oil vapour for the control of *Penicillium chrysogenum*, *Aspergillus niger* and *Alternaria alternata in vitro* and on food. Food Research International 47: 310–314. DOI: https://doi.org/10.1016/j.foodres.2011.07.035

Pinto E., Vale-Silva L., Cavaleiro C., Salgueiro L. 2009. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on Candida, *Aspergillus* and dermatophyte species. Journal of Medical Microbiology 58: 1454–1462. DOI: https://doi.org/10.1099/jmm.0.010538-0

Politeo O., Carev I., Burčul F., Jukić M., Ajduković P., Tadijana V., Miloš M. 2010. Screening of anti-acetylcholineesterase and antioxidant activity of extracts from selected Croatian plants. In: Proceedings of the 10th Congress of the Croatian Society of Biochemistry and Molecular Biology, 15–18 September 2010, Opatija, Hrvatska, Croatia.

Porte A., Godoy R.L.O., Maia-Porte L.H. 2013. Chemical composition of sage (*Salvia officinalis* L.) essential oil from the Rio de Janeiro State (Brazil). Revista Brasileira de Plantas Medicinais 15: 438–441. DOI: https://doi.org/10.1590/S1516-05722013000300018

Rajkowska K., Nowak A., Kunicka-Styczyńska A., Siadura A. 2016. Biological effects of various chemically characterized essential oils: Investigation of the mode of action against *Candida albicans* and HeLa cells. RSC Advances 6: 97199–97207. DOI: https://doi.org/10.1039/C6RA21108A

Rozman V., Kalinović I., Liška A. 2006. Bioactivity of 1,8-cineole, camphor and carvacrol against rusty grain beetle (*Chryptolestes ferrugineus* Steph.) on stored wheat. In: Proceeding of the 9th International Working Conference on Stored Product Protection, 15–18 October 2006, Abrapos, Passo Fundo, Brazil.

Sabbobeh R., Hejaz H., Jahajha A., Al-Akhras S., Al-Jaas H., Abu-Lafi S. 2016. Antioxidant an antimicrobial activities of the leaf extract of *Salvia palaestina*. Journal of Applied Pharmaceutical Science 6: 76. DOI: https://doi: 10.7324/JAPS.2016.600113

Samara R., Hunter D.M., Stobbs L.W., Greig N., Lowery D.T., Delury N.C. 2017. Impact of *Plum Pox Virus* (PPV-D) infection on peach tree growth, productivity and bud cold hardiness. Canadian Journal of Plant Pathology 39: 218–228. DOI: https://doi.org/10.1080/07060661.2017.1336489

SAS Institute, 1998. SAS Users Guide, Statistics. Version 2. SAS Institute, Cary, NC.

Shah A., Jani M., Shah H., Chaudhary N., Shah A. 2014. Antimicrobial effect of Clove oil (Laung) extract on *Enterococcus faecalis*. Journal of Advanced Oral Research 5: 36–38. DOI: https://doi.org/10.1177/2229411220140307

Shirzad H., Hassani A., Ghosta Y., Abdollahi A., Finidokht R., Meshkatalsadat M. 2011. Assessment of the antifungal activ-

ity of natural compounds to reduce postharvest gray mould (*Botrytis cinerea* Pers.: Fr.) of kiwifruits (*Actinidia deliciosa*) during storage. Journal of Plant Protection Research 51 (1): 1–6. DOI: https://doi.org/10.2478/v10045-011-0001-4

Shoaib A., Saeed G., Ahmad S. 2014. Antimicrobial activity and chemical analysis of some edible oils (Clove, Kalonji and Taramira). African Journal of Biotechnology 13: 4347–4354. DOI: https://doi.org/10.5897/AJB2014.13683

Sighamony S., Anees I., Chandrakala T.S., Osmani Z. 1986. Efficacy of certain indigenous plant products as grain protectants against *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.). Journal of Stored Products Research 22: 21–23. DOI: https://doi.org/10.1016/0022-474X(86)90042-1

Snowdon A.L. 1990. Color atlas of post-harvest diseases and disorders of fruits and vegetables. Vol. 1. In: "General Introduction and Fruits". CRC Press, Boca Raton FL.

Taylor S. 1993. Why sulfite alternatives. Food Technology 47: 14.

Thosar N., Basak S., Bahadure R.N., Rajurkar M. 2013. Antimicrobial efficacy of five essential oils against oral pathogens: An *in vitro* study. European Journal of Dentistry 7: 71–77. DOI: https://doi/org/10.4103/1305-7456.119078

Vitoratos A., Bilalis D., Karkanis A., Efthimiadou A. 2013. Antifungal activity of plant essential oils against *Botrytis cinerea, Penicillium italicum* and *Penicillium digitatum*. Notulae Botanicae Horti Agrobotanici 41: 86–92. DOI: https://doi.org/10.15835/nbha4118931

Zabka M., Pavela R. 2013. Antifungal efficacy of some natural phenolic compounds against significant pathogenic and toxinogenic filamentous fungi. Chemosphere 93: 1051–1056. DOI: https://doi.org/10.1016/j.chemosphere.2013.05.076