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Antifungal Activity of Five Plant Essential Oils as Fumigant Against Postharvest and Soilborne Plant Pathogenic Fungi

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A total of 39 essential oils were tested for antifungal activities as volatile compounds against five phytopathogenic fungi at a dose of 1 µl per plate. Five essential oils showed inhibitory activities against mycelial growth of at least one phytopathogenic fungus. *Origanum vulgare* essential oil inhibited mycelial growth of all of the five fungi tested. Both *Cuminum cyminum* and *Eucalyptus citriodora* oils displayed *in vitro* antifungal activities against four phytopathogenic fungi except for *Colletotrichum gloeosporioides*. The essential oil of *Thymus vulgaris* suppressed the mycelial growth of *C. gloeosporioides*, *Fusarium oxysporum* and *Rhizoctonia solani* and that of *Cymbopogon citratus* was active to only *F. oxysporum*. The chemical compositions of the five active essential oils were determined by gas chromatography-mass spectrometry. This study suggests that both *E. citriodora* and *C. cyminum* oils have a potential as antifungal preservatives for the control of storage diseases of various crops.

Keywords : antifungal activity, apple gray mold, *Cuminum cyminum*, *Eucalyptus citriodora*, essential oil

Synthetic fungicides are currently used as the primary means for the control of plant diseases. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicides among fungal pathogens, and high development cost of new chemicals. The uses of plant-derived products as diseases control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance.

Essential oils are concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from plants. They may provide potential alternatives to the control agents currently used because the compositions of essential oils are rich of bioactive chemicals and commonly used as fragrances and flavoring agents for foods and beverage (Isman, 2000). They were previously reported to have biological activities such as antifungal (Soliman and Badeaa, 2002), antibacterial (Dorman et al., 2000), insecticidal (Isman, 2000) and nematicidal effects (Pandey et al., 2000).

In this study, the inhibitory effects of 39 essential oils as volatile compounds were determined against two postharvest pathogenic fungi, *Botrytis cinerea* and *Colletotrichum gloeosporioides* and three soilborne pathogenic fungi, *Fusarium oxysporum*, *Pythium ultimum* and *Rhizoctonia solani*. In addition, major constituents were determined in the five active essential oils. Fumigation activities of two essential oils, *Eucalyptus citriodora* and *Cuminum cyminum* were evaluated against the development of apple gray mold.

Materials and Methods

Fungal cultures. The five phytopathogenic fungi such as *B. cinerea*, *C. gloeosporioides*, *F. oxysporum*, *P. ultimum*, and *R. solani* were maintained and grown on potato dextrose agar medium.

***In vitro* antifungal activity test.** All essential oils used in this study were purchased from JinAh Food & Cosmetic additives Co., Anyang, Korea and listed in Table 1. For the *in vitro* antifungal activity test, PDA was poured into commercially available half-plate separated Petri plates (90 mm × 15 mm, SPL Life Science, Korea). The agar plugs of actively growing cultures on the PDA were placed on half of the PDA and sterilized paper disc was placed on the other side. An aliquot (1 µl) of the each essential oil was added onto a paper disc in a plate (equal to 22.7 $\mu l\ l^{-1}$ in air) and allowed only volatile compounds to be the causative agents for mycelial growth inhibition. The plate was sealed with Paraflim immediately after adding each essential oil and incubated for 3 days at 25°C except for *B. cinerea* at 20°C. Plates in three replicates were used for each treatment. The radius of fungal mycelia was measured and compared with that of untreated control. Minimum inhibitory concentration (MIC) and median effective concentration (EC_{50}) values were determined for essential oils against fungal growth of fungi. Essential oils were diluted in

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acetone and 10 μl of each dilution was applied to assay plate at the final concentration of 0, 0.1, 0.5, 1, 5 or 10 μl per plate. For control plate, 10 μl of acetone was added. Each experiment was performed in three replicates.

Gas chromatography-mass spectrometry analyses of essential oils. Five essential oils displayed *in vitro* antifungal activities were analyzed by gas chromatography with mass spectrometry (GC-MS) (GC-MS QP5050, Shimadzu, Kyoto, Japan). A capillary column DB5 (30 m ×0.32 mm i.d., 0.25 μm film thickness; J&W Scientific Inc., Folsom, CA, USA) with cross linked 5% phenylmethylsilicone was used. The initial temperature of the column was held at 40°C for 5 min, programmed at 5°C min^{-1} to 200°C. The injection port and interface were set at 220°C and 200°C, respectively. Helium was the carrier gas at a flow rate of 2.2 ml min^{-1}. Components were identified on the basis of comparison of their relative retention time and mass spectra with those of standards.

***In vivo* antifungal activity of essential oils against apple gray mold.** The essential oils of *C. cyminum* and *E. citriodora* diaplyed potent *in vitro* antifungal activity against *B. cinerea* were evaluated for their effects to the development of *B. cinerea* on artificially inoculated apples. Each fruit was wound with 8 mm diameter cork borer, and then 8 mm diameter mycelial plug of *B. cinerea* was inoculated. The inoculated fruits were arranged in a moistened 7 litre container as two replicates of 6 fruits for each treatment. The paper discs were placed in the center of containers and then essential oils of *C. cyminum* and *E. citriodora* were added on discs. The essential oils was applied at two dosages of 5 and 10 μl l^{-1} in air for *C. cyminum* oil and 4 and 8 μl l^{-1} in air for *E. citriodora* oil. The boxes were sealed and stored at 20°C. After 5 days, the radii of rotted symptoms were measured from edge of the agar inoculum plug on the fruits surface. The experiment was conducted twice.

Results

Antifungal activities of plant essential oils were tested against five phytopathogenic fungi such as *B. cinerea*, *C. gloeosporioides*, *F. oxysporum*, *P. ultimum* and *R. solani*. The oils were treated to each bioassay plate which allows only volatiles to be the causative agents for any microbial inhibition. Among 39 essential oils, *Origanum vulgare* oil alone inhibited all of the phytopathogenic fungi tested by the inhibition rates of 55% for *B. cinerea*, 78% for *C. gloeosporioides*, 70% for *F. oxysporum*, 93% for *P. ultimum* and 68% for *R. solani* (Table 1). Both *C. cyminum* and *E. citriodora* oils showed fungal growth inhibitory activities ranged from 41% to 83% and from 50% to 91%, for the tested fungi except for *C. gloeosporioides*, respectively (Table 1). The essential oil of *Thymus vulgaris* showed *in*

Table 1. Inhibitory activity of the vaporous phases of various plant essential oils against the mycelial growth of the five plant pathogenic fungi

Plant species (common name)	Inhibition rate (%)				
	BC[a]	CG	FO	PU	RS
Artemisia absinthium (wormwood)	–[b]	–	–	–	–
Artemisia dracunculus (tarragon)	–	–	–	–	–
Artemisia vulgaris (mugwort)	–	–	–	–	–
Cinnamomum camphora (camphor)	–	–	–	–	–
Citrus limon (lemon)	–	–	–	–	–
Citrus paradise seed (grapefruit)	–	–	–	–	–
Citrus paradise fruit (grapefruit)	–	–	–	–	–
Citrus sinesis (orange sweet)	–	–	–	–	–
Cuminum cyminum (cumin)	67	–	62	41	83
Cupressus sempervirens (cypress)	–	–	–	–	–
Cymbopogon citratus (lemongrass)	–	–	66	–	–
Eucalyptus citriodora (lemon eucalyptus)	91	–	57	50	87
Eucalyptus globulus (blue gum eucalyptus)	–	–	–	–	–
Eucalyptus radiate (narrow-leaved peppermint)	–	–	–	–	–
Gaultheria procumbens (wintergreen)	–	–	–	–	–
Ginkgo biloba leaf (ginko)	–	–	–	–	–
Juniperus communis (junifer)	–	–	–	–	–
Lavandula spica (lavenda)	–	–	–	–	–
Majorana hortensis (marjoram)	–	–	–	–	–
Matricaria recutita (german chamomile)	–	–	–	–	–
Melaleuca alternifolia (tea tree)	–	–	–	–	–
Melaleuca quinquenervia (niaouli)	–	–	–	–	–
Melissa officinalis (lemon balm)	–	–	–	–	–
Mentha piperita (peppermint)	–	–	–	–	–
Mentha pulegium (pennyroyal)	–	–	–	–	–
Ocimum basilicum (basil)	–	–	–	–	–
Origanum vulgare (oregano)	55	78	70	93	68
Petroselinum sativum (parsely)	–	–	–	–	–
Pinus sylvestris (pine)	–	–	–	–	–
Prunus dulcis (bitter almond)	–	–	–	–	–
Rosemarinus officinalis (rosemary)	–	–	–	–	–
Ruta graveolens (rue)	–	–	–	–	–
Salvia lavenduuilfolia (sage spanish)	–	–	–	–	–
Salvia sclarea (clary sage)	–	–	–	–	–
Sassafras albidum (sassafras)	–	–	–	–	–
Thuja plicata (cedarleaf)	–	–	–	–	–
Thymus vulgaris (thyme red)	–	60	76	–	50
Tnancetum annuum (tansy blue)	–	–	–	–	–
Vetiveria zizanioides (vertiver)	–	–	–	–	–

[a]BC, *Botrytis cinerea*; CG, *Colletotrichum gloeosporioides*; FO, *Fusarium oxysporum*; PU, *Pythium ultimum*; RS, *Rhizoctonia solani*
[b]–, no inhibition observed.

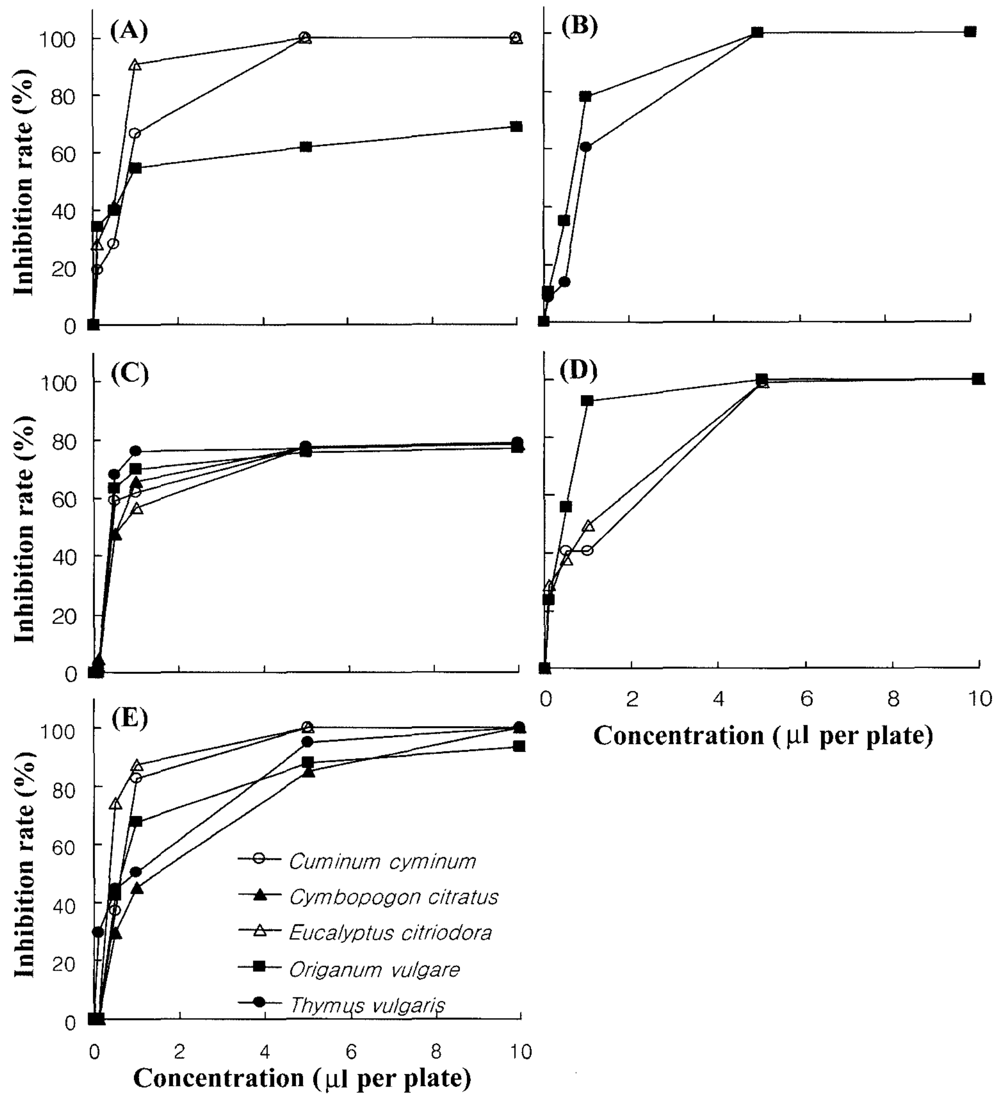

Fig. 1. Concentration-dependent effects of essential oils on the mycelial growth of phytopathogenic fungi. (A), *Botrytis cinerea*; (B), *Colletotrichum gloeosporioides*; (C), *Fusarium oxysporum*; (D), *Pythium ultimum*; (E), *Rhizoctonia solani*.

vitro antifungal activity against *C. gloeosporioides*, *F. oxysporum* and *R. solani* with inhibition rate of 50% to 76% (Table 1). Both *B. cinerea* and *P. ultimum* were most affected by *T. vulgaris* oil. On the other hand, *Cymbopogon citratus* oil suppressed the mycelial growth of only *F. oxysporum* and the other fungi were not susceptible to the oil (Table 1).

The concentration dependent effects of the five active essential oils on the mycelial growth of five fungi were evaluated (Fig. 1). EC_{50} values of the essential oils against five fungi were ranged from 0.4 to 1.2 μl per plate which were equal to 9.1 and 27.3 μl l^{-1} in air, respectively. For *B. cinerea*, essential oils of *C. cyminum* and *E. citriodora* were significant inhibitors with MIC values of 5 μl per plate. *C. gloeosporioides* was effectively inhibited by *O. vulgare* and *T. vulgaris* oils with the MIC (EC_{50}) values of 5 (0.5) μl per plate for *O. vulgare* oil and 5 (0.7) μl per plate for *T. vulgaris* oil. Antifungal activities of the 5 essential oils against *F. oxysporum* were moderate with EC_{50} values of from 0.3 to 0.8 μl per plate but their MIC values were not determined within the performed concentrations. Essential oil of *O. vulgare* strongly inhibited fungal growth of *P. ultimum* and the MIC and EC_{50} values of *O. vulgare* oil were 0.5 and 5 μl per plate, respectively. The fungal growth of *R. solani* also inhibited by all five essential oils and among them *C. cyminum* and *E. citriodora* oils showed potent inhibitory effects with the MIC values of 5 μl per plate.

The chemical compositions of five antifungal essential oils were listed in Table 2. The major components of each

Table 2. Chemical compositions of essential oils

Essential oil	Component	Percentage (%)
Cuminum cyminum	β-Pinene	23
	γ-Terpinene	19
	Cuminaldehyde	18
Cymbopogon citratus	Geranial	43
	Neral	30
	Limonene	10
Eucalyptus citriodora	Citronellal	73
	Isopulegol	6.7
Origanum vulgare	Carvacrol	59
	ρ-Cymene	22
	Thymol	6.5
Thymus vulgaris	Thymol	38
	ρ-Cymene	30
	γ-Terpinene	6.7

[a]Quantification of each constituent was estimated by area normalization.

Table 3. Antifungal effects of *Cuminum cyminum* and *Eucalyptus citriodora* essential oils on apple gray mold

Essential oil	Concentration ($\mu l\ l^{-1}$ in air)	Control value[a] (%)
Cuminum cyminum	5	19 ± 9.8
	10	33 ± 11
Eucalyptus citriodora	5	43 ± 2.3
	10	70 ± 3.6

[a]Control value is average of 6 fruits from two separate experiments.

essential oil were β-pinene (23%), γ-terpinene (19%) and cuminaldehyde (18%) for *C. cyminum* oil, geranial (43%), neral (30%) and limonene (10%) for *C. citratus* oil, citronellal (73%) and isopulegol (6.7%) for *E. citriodora* oil, carvacrol (59%), ρ-cymene (22%) and thymol (6.5%) for *O. vulgare* oil and thymol (38%), ρ-cymene (30%) and γ-terpinene (6.7%) for *T. vulgaris* oil.

The essential oils of *C. cyminum* and *E. citriodora* showing strong *in vitro* antifungal activity against *B. cinerea* were evaluated for their control efficacies against the decay of apple fruits caused by *B. cinerea*. The essential oils were applied in two dosages, 4 and 8 $\mu l\ l^{-1}$ in air (28 and 56 μl per container) for *E. citriodora* and 5 and 10 μl l^{-1} in air (35 and 70 μl per container) for *C. cyminum*. The essential oil of *E. citriodora* suppressed the development of Botrytis lesions with control values of 38% at a concentration of 4 $\mu l\ l^{-1}$ and 59% at a concentration of 8 $\mu l\ l^{-1}$ (Table 3) (Fig. 2). *C. cyminum* oil reduced gray mold lesions by 19% and 37% at dosage of 5 $\mu l\ l^{-1}$ and 10 $\mu l\ l^{-1}$, respectively (Table 3). The incidences of disease were reduced by increasing dosages of the applied essential oils.

Discussion

To develop environment-friendly alternatives to synthetic fungicides for the control of fungal plant diseases, the interest on essential oils has been increased. In this study, we investigated the antifungal activities of 39 essential oils as volatile compounds against five soilborne or postharvest disease pathogens by exposure to vaporous phases of the oils. As the results, the essential oils of *C. cyminum*, *C. citratus*, *E. citriodora*, *O. vulgare* and *T. vulgaris* were active to at least one plant pathogenic fungus.

The essential oil of *O. vulgare* showed the broadest

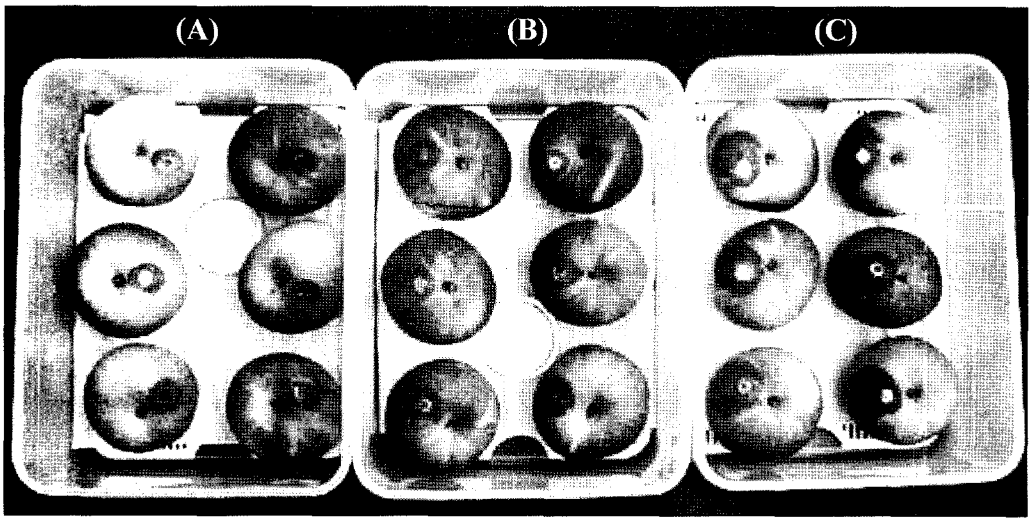

Fig. 2. *In vivo* antifungal activity of *Eucalyptus citriodora* oil aginast apple gray mold. Each fruit was wound and then was inoculated with mycelial plug of *Botrytis cinerea*. The essential oil was added on paper discs in the center of containers at the concentration of 8 (A), 4 (B) and 0 (C) $\mu l\ l^{-1}$ in air. The boxes were sealed and incubated for 5 days at 20°C. The radius of rotted symptom was measured from edge of the agar inoculum plug on the fruit surface.

antifungal spectrum in this study. The inhibitory effect against human pathogenic fungi and antibacterial activities of *O. vulgare* oil were reported previously (Adam et al., 1998; Sivropoulou et al., 1996). The main constituents of *O. vulgare* oil were identified as carvacrol, ρ-cymene and thymol and this result is accordance with the previously published report (Bozin et al., 2006). The volatile terpenes such as carvacrol, ρ-cymene and thymol were thought to be responsible for the antifungal activity of *O. vulgare* oil (Holley and Patel, 2005). In real, Bouchra et al. (2003) reported carvacrol and thymol as strong inhibitors of *B. cinerea in vitro*. ρ-Cymene, a constituent of *O. vulgare* oil showed synergistic activity with thymol against fungi (Pina-Vaz et al., 2004).

The essential oil of *C. cyminum* inhibited the mycelial growth of the phytopathogenic fungi except for *C. gloeosporioides* and also suppressed the development of apple gray mold. The antifungal and antibacterial activities of the essential oil have been reported by many scientists (Iacobellis et al., 2005; Pawar et al., 2006). Rahman et al. (1999) reported that the essential oil applied into medium at a concentration of 200 μg ml^{-1} inhibited the mycelial growth of *Pseudoallescheria boydii* by 88% and *F. oxysporum* f. sp. *lycopersici* by 19%. In our study, *B. cinerea* and *R. solani* were strongly inhibited by exposure to vaporous phase of *C. cyminum* oil with EC_{50} values of 0.5 ml l^{-1}. The main constituents of essential oil of *C. cyminum* were β-pinene, γ-terpinene and cuminaldehyde, identical to previous report by Iacobelli et al. (2005). Both β-pinene and γ-terpinene, the two main components of *C. cyminum* oil, showed antifungal activity against various fungi when treated as a sole component (Hammer et al., 2003).

E. citriodora oil showed the same antifungal spectra as *C. cyminum* oil in this study. In addition, it effectively suppressed the development of apple gray mold. Ramezani et al. (2002) reported that the oil of *E. citriodora* possessed a wide spectrum of fungicidal activity. It was also reported to have insecticidal and nematicidal activities (Isman, 2000; Pandey et al., 2000). The antifungal activity of *E. citriodora* oil was attributed to citronellal as a volatile compound which is the major constituent of oil. The antifungal activity of citronellal against several species of *Aspergillus*, *Penicillium* and *Eurotium* were previously reported using a vapor-agar contact method, similar to the method used in this study (Nakahara et al., 2003). In this study, *E. citriodora* oil showed the most potent inhibitory effect on *B. cinerea*. It also displayed dose-dependent inhibition rates on gray mold in an *in vivo* test.

T. vulgaris oil as volatile compounds inhibited mycelial growth of three phytopathogenic fungi such as *C. gloeosporioides*, *F. oxysporum* and *R. solani*, but was not active to *B. cinerea* and *P. ultimum* at a dosage of 1 μl per plate. The essential oil of *T. vulgaris* inhibited various fungi including food spoilage, mycotoxin producing fungi and postharvest pathogenic fungi (Nguefack et al., 2004; Reddy et al., 1997). Reddy et al. (1997) reported that two clonal types of *T. vulgaris* oils controlled decay of strawberry fruits caused by *B. cinerea* up to 74% and 76%, respectively. In this study, *T. vulgais* oil did not inhibit mycelial growth of *B. cinerea*. This result may come from low dosage of the treated oil. The major compounds of essential oil of *T. vulgaris* used in this study were thymol and ρ-cymene, identical to those of thymol chemotype of *T. vulgaris* (Giordani et al., 2004).

C. citratus oil in this study, at a dosage of 1 μl per plate, showed *in vitro* antifungal activity against only *F. oxysporum* among the five fungi tested. The bioactive properties of essential oil of *C. citratus* were reported for the food spoilage and mycotoxin producing fungi, *Fusarium moniliforme*, *Aspergillus flavus* and *A. fumigatus* (Nguefack et al., 2004; Paranagama et al., 2003). The chemical composition of *C. citratus* oil used in this study was identical to that of the previous report (Chisowa et al., 1998). Adegoke et al. (2000) reported the inhibitory effect of limonene, the constituent of *C. citratus* oil, against *A. flavus* and *A. parasiticus*, and they confirmed that limonene caused membrane injury on membrane of susceptible organism.

This study demonstrated the *in vitro* antifungal activities of essential oils against phytopathogenic fungi and potential use of essential oils as antifungal preservatives for the control of gray mold caused by *B. cinerea* on apple fruits. However, for the development of essential oils as alternatives of synthetic fungicides, further studies are required to evaluate phytotoxicity of essential oils for application on plants and sensory quality of treated fruits and vegetables.

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