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Research Article
Postharvest Application of Some Essential Oils for Controlling Gray and Blue Moulds of Apple Fruits

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Abstract

Worldwide gray mould caused by *Botrytis cinerea* and blue mould caused by *Penicillium* spp. are the most important diseases attacking apple fruit during storage. Evaluating the effects of some essential oils i.e., eucalyptus, lemon grass and thyme on linear growth and spore germination of pathogenic fungi. Moreover, their efficacy on gray and blue moulds incidence of apple fruits was tested. All treatments significantly reduced the linear growth and spore germination of both tested fungi. Essential oils of eucalyptus, lemon grass and thyme at a concentration of 0.6 and 0.8% completely inhibit the linear growth and spore germination of *B. cinerea* and *Penicillium* sp. except that lemon grass at 0.6%. Sterilized water containing essential oils of eucalyptus, lemon grass and thyme at different concentrations, i.e., 0.0, 0.6 and 0.8% (v/v) were tested to study their effect against gray and blue mould incidence of apple fruits. Results indicated that all treatments significantly reduced the disease incidence and rotted part tissue (Disease severity). The most effective treatment was eucalyptus and thyme at 0.8%, which reduced the disease incidence more than 83.8 and 82.7% and rotted part tissue by 85.9 and 88.5% for gray and blue moulds, respectively. The highest reduction was obtained with eucalyptus and thyme at concentrations of 0.6% and lemon grass at 0.8% which reduced both diseases incidence and rotted part tissue more than 74.0 and 75.8%, respectively. Meanwhile, other treatments showed moderate effect. It could be suggested that essential oils could make them an excellent treatment for controlling postharvest diseases of apple fruits.

Key words: Eucalyptus, lemon grass, thyme essential oils, gray mould, blue moulds, apple fruits

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Worldwide gray mould caused by *Botrytis cinerea* Pers, Fr. and blue mould caused by *Penicillium* spp. are the most important diseases attacking apple fruit during storage (Sholberg *et al*., 2003; Pianzzola *et al*., 2004; Janisiewicz and Peterson, 2004; Janisiewicz *et al*., 2005).

Using of chemical fungicides gave satisfactory control against mould infection but have residual harmful effect to human and environment (Eckert and Ogawa, 1988). Moreover, successive use of fungicides could lead to developing to some significant fungal isolates resistant to use fungicides. Therefore, alternative fungicide treatments are needed for the management of postharvest diseases of fruits (Abd-El-Latif and Abd-El-Kareem, 2009; El-Shahawy *et al*., 2015).

The potential of using essential oils by spraying or dipping fruits for controlling postharvest diseases has been reported by several investigators (Kanherkar *et al*., 2007). Essential oils are naturally including several fractions which have insecticidal and fungicidal activities against some important plant pathogens (Kanherkar *et al*., 2007).

Allelopathic effect of eucalyptus (*Eucalyptus globulus*) against plant pathogens was reported by several investigators (Suryawanshi *et al*., 2007; Kanherkar *et al*., 2007).

The essential oil of thyme (*Thymus vulgaris* L.) and its major component, thymol had antifungal activity against plant pathogenic fungi (Plaza *et al*., 2004; Angelini *et al*., 2006) as well as plant diseases of several fruits and vegetables (El Sherbieny *et al*., 2002; Klaric *et al*., 2007).

Essential oil extracted from lemon grass (*Cymbopogon citratus* Stapf.) has antifungal activity against several plant pathogens (Nguefack *et al*., 2007). The purpose of the present study is evaluating the effects of Eucalyptus, limon grass and thyme essential oils on linear growth and spore germination of pathogenic fungi. Moreover, their efficacy on gray and blue moulds incidence of apple fruits.

MATERIALS AND METHODS

Source of pathogenic fungi and apple fruits: Pathogenic isolates of *Botrytis cinerea* and *Penicillium* sp. the causal agent of gray and blue mould diseases were kindly obtained from Department of Plant Pathology, National Research Centre, Giza, Egypt. Meanwhile, apple fruits cv. Anna were obtained from commercial markets Egypt.

***In vitro* inhibition of linear growth of pathogenic fungi by different concentrations of some essential oils:** Essential oils of eucalyptus, lemon grass and thyme obtained from Delta Aromatic Company, Egypt at different concentrations i.e., 0.0, 0.2, 0.4, 0.6 and 0.8% (v/v) were prepared. Oil solutions were added to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized petri plates (9 cm diameter). Plates were individually inoculated at the center with equal disks (5 mm diameter) of 10 day old culture of *Botrytis cinerea* and *Penicillium* sp. (obtained from Plant Pathology, Department NRC Egypt). Five plates were used as replicates for each particular treatment. Inoculated plates were incubated at 20±2°C. The average linear growth of fungi tested was calculated after 10 days.

***In vitro* inhibition of spore germination of pathogenic fungi by different concentrations of some essential oils: Preparation of spore suspension:** Spores suspension were prepared by inoculated sterilized PDA medium with disk (6 mm diameter) taken from 10 days old cultures of *B. cinerea* and *Penicillium* sp. Plates were incubated at 20±2°C for 10 days. Spores of *B. cinerea* and *Penicillium* sp. were harvested and transferred in sterilized water (containing 0.01% Tween 80) to obtain spore suspension which was adjusted to 10^6 spores/mL using hemocytometers slide (Palou *et al*., 2001). Essential oils of eucalyptus, lemon grass and thyme at different concentrations i.e., 0.0, 0.2, 0.4, 0.6 and 0.8% (v/v) were prepared as mention before. One milliliter of each prepared spore suspension was placed in test tubes containing different concentrations of tested oils then were incubated at 20°C for 24 h. Germinated spores were counted microscopically and percentage of spore germination was calculated.

***In vivo*, testing of different concentrations of some essential oils on gray and blue moulds incidence of apple fruits:** Sterilized water containing essential oils of Eucalyptus, lemon grass and Thyme at different concentrations i.e., 0.0, 0.6 and 0.8% (v/v) were tested to study their effect against gray and blue moulds incidence on apple fruits. Fresh apple fruits apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Fruits were dipped in sterilized water containing eucalyptus, lemon grass and thyme at different concentrations i.e., 0.0, 0.6 and 0.8% (v/v) containing 0.01% Tween 80 for 3 min, then air dried. Inoculation of treated fruits was carried out by spraying fruits with spore suspension (10^6 spores/mL) of *B. cinerea* and *Penicillium* sp. then air dried. All treated or un-treated fruits were placed into carton boxes at the rate of 10 fruits/box. Each particular concentration as well as control treatment was represented by three carton box. All boxes were stored at 20±2°C for 20 days. Percentage of infected fruits (disease incidence) and rotted parts of fruits (disease severity) were recorded after 20 days.

$$\text{Rotted part of fruit (\%)} = \frac{\text{Rotted part weight}}{\text{Fruit weight}} \times 100$$

Statistical analysis: Tukey test for multiple comparison among means was utilized (Neler *et al*., 1985).

RESULTS

***In vitro* inhibition of linear growth of pathogenic fungi by different concentrations of some essential oils:** Essential oils of eucalyptus, lemon grass and thyme at different concentrations i.e., 0.0, 0.2, 0.4, 0.6 and 0.8% (v/v) were tested to study their inhibitory effect on linear growth and spore germination of *B. cinerea* and *Penicillium* sp. results in Table 1 and 2 indicate that all treatments significantly reduced the linear growth and spore germination of both tested fungi. Essential oils of eucalyptus, lemon grass and thyme at concentration of 0.6 and 0.8% completely inhibit the linear growth and spore germination of *B. cinerea* and *Penicillium* sp. except that lemon grass at 0.6%. The highest reduction was obtained with essential oils of Thyme at concentration of 0.4% which reduced the linear growth and spore germination by 67.7 and 80.6 and 74.4 and 79.1% for *B. cinerea* and *Penicillium* sp., respectively. Meanwhile, concentration of 0.2% was less effective.

***In vivo* effect of different concentrations of some essential oils on gray and blue moulds incidence of apple fruits:** Sterilized water containing essential oils of eucalyptus, lemon grass and thyme at different concentrations i.e., 0.0, 0.6 and 0.8% (v/v) were tested to study their effect against gray and blue moulds incidence of apple fruits. Results in Table 3 and 4 indicate that all treatments significantly reduced the diseases incidence and rotted part tissue (Disease severity).

The most effective treatment was eucalyptus and thyme at 0.8% which reduced the disease incidence more than 83.8 and 82.7% and rotted part tissue by 85.9 and 88.5% for gray and blue moulds, respectively. The highest reduction was obtained with eucalyptus and thyme at concentrations of 0.6% and lemon grass at 0.8% which reduced both diseases incidence and rotted part tissue more than 74.0 and 75.8%, respectively. Meanwhile, other treatments showed moderate effect.

Table 1: Linear growth of *B. cinerea* and *Penicillium* sp. as affected with different concentrations of some essential oils

	Botrytis cinerea		*Penicillium* sp.	
Treatments and concentration (%)	Linear growth	Reduction (%)	Linear growth	Reduction (%)
Eucalyptus				
0.2	67.0^{c}	25.6	74.0^{b}	17.8
0.4	37.0^{de}	58.9	41.0^{d}	54.4
0.6	00.0^{g}	100.0	00.0^{g}	100.0
0.8	00.0^{g}	100.0	00.0^{g}	100.0
Lemon grass				
0.2	74.0^{b}	17.8	78.0^{b}	13.3
0.4	45.0^{d}	50.0	48.0^{c}	46.7
0.6	4.0^{g}	95.6	12.0^{e}	86.7
0.8	0.0^{g}	100.0	00.0^{f}	100.0
Thyme				
0.2	45.0^{d}	50.0	47.0^{c}	47.8
0.4	21.0^{f}	76.7	23.0^{f}	74.4
0.6	0.0^{g}	100.0	00.0^{g}	100.0
0.8	0.0^{g}	100.0	00.0^{g}	100.0
Control				
0.0	90.0^{a}	0.0	90.0^{a}	0.0

Values with the same letter are not significantly different (p = 0.05)

Table 2: Average percentage of spore germination of pathogenic fungi as affected with different concentrations of some essential oils

	Botrytis cinerea		*Penicillium* sp.	
Treatments and concentration (%)	Spore germination	Reduction (%)	Spore germination	Reduction (%)
Eucalyptus				
0.2	45.0^{c}	51.6	51.0^{c}	44.0
0.4	18.0^{d}	80.6	22.0^{e}	75.8
0.6	00.0^{e}	100.0	00.0^{f}	100.0
0.8	00.0^{e}	100.0	00.0^{f}	100.0
Lemon grass				
0.2	62.0^{b}	33.3	62.0^{b}	31.9
0.4	41.0^{c}	55.9	39.0^{d}	57.1
0.6	00.0^{e}	100.0	00.0^{f}	100.0
0.8	00.0^{e}	100.0	00.0^{f}	100.0
Thyme				
0.2	39.0^{c}	58.1	44.0^{d}	51.6
0.4	18.0^{d}	80.6	19.0^{e}	79.1
0.6	00.0^{e}	100.0	00.0^{f}	100.0
0.8	00.0^{e}	100.0	00.0^{f}	100.0
Control				
0.0	93.0^{a}	0.0	91.0^{a}	0.0

Values with the same letter are not significantly different (p = 0.05)

Table 3: Percentage of gray and blue moulds incidence on apple fruits affected with different concentrations of some essential oils

	Disease incidence (%)			
Treatment and concentration (%)	Gray mould	Reduction (%)	Blue mould	Reduction (%)
Eucalyptus				
0.6	22.0^{c}	78.0	25.2^{c}	74.5
0.8	16.2^{d}	83.8	17.3^{d}	82.7
Lemon grass				
0.6	37.0^{b}	63.0	40.0^{b}	60.0
0.8	22.0^{c}	78.0	26.0^{c}	74.0
Thyme				
0.6	20.0^{c}	80.0	22.5^{c}	77.5
0.8	14.0^{d}	86.0	15.5^{d}	84.5
Control				
0.0	100.0^{a}	0.0	100.0^{a}	0.0

Values with the same letter are not significantly different (p = 0.05)

Table 4: Percentage of rotted part tissue caused by gray and blue moulds on apple fruits as affected with different concentrations of some essential oils

	Rotted part tissues (%)			
Treatment and concentration (%)	Gray mould	Reduction (%)	Blue mould	Reduction (%)
Eucalyptus				
0.6	21.0[c]	79.0	21.5[c]	78.5
0.8	14.1[d]	85.9	14.5[d]	85.5
Lemon grass				
0.6	34.0[b]	66.0	37.0[b]	63.0
0.8	21.0[c]	79.0	24.2[c]	75.8
Thyme				
0.6	20.0[c]	80.0	21.5[c]	78.5
0.8	13.0[d]	87.0	13.8[d]	86.2
Control				
0.0	100.0[a]	0.0	100.0[a]	0.0

Values with the same letter are not significantly different (p = 0.05)

DISCUSSION

Worldwide gray mould caused by *Botrytis cinerea* Pers, Fr. and blue mould caused by *Penicillium* sp. are the most important diseases attacking apple fruit during storage (Sholberg *et al*., 2003; Pianzzola *et al*., 2004; Janisiewicz and Peterson, 2004; Janisiewicz *et al*., 2005).

Using of chemical fungicides gave satisfactory control against mould infection but have a residual harmful effect to human and environment (Eckert and Ogawa, 1988). Moreover, some fungal isolates could be resistant to used fungicides. Therefore, alternative fungicide treatments are needed for the management of postharvest diseases of fruits (Abd-El-Latif and Abd-El-Kareem, 2009; El-Shahawy *et al*., 2015).

Essential oils are naturally including several fractions which have insecticidal and fungicidal activities against some important plant pathogens (Kanherkar *et al*., 2007; Nguefack *et al*., 2007). In the present study results indicated that *in vitro* trails essential oils of eucalyptus, lemon grass and thyme at concentration of 0.6 and 0.8% completely inhibit the linear growth and spore germination of *B. cinerea* and *Penicillium* sp. except that lemon grass at 0.6%. Moreover, *in vivo*, trails, sterilized water containing essential oils of eucalyptus, lemon grass and thyme at different concentrations i.e., 0.0, 0.6 and 0.8% (v/v) were tested to study their effect against gray and blue moulds incidence of apple fruits. Results indicated that all treatments significantly reduced the diseases incidence and rotted part tissue (Disease severity). The most effective treatment was eucalyptus and thyme at 0.8% which reduced the disease incidence more than 83.8 and 82.7% and rotted part tissue by 85.9 and 88.5% for gray and blue moulds, respectively. The highest reduction was obtained with eucalyptus and thyme at concentrations of 0.6% and lemon grass at 0.8% which reduced both diseases incidence and rotted part tissue.

The inhibitory effect of eucalyptus against plant pathogens was reported by Harborne and Tomas-Barberan (1991) and Byron and Hall (2002). In the present study, eucalyptus essential oil completely inhibited the linear growth spore germination of *P. digitatum* and *P. italicum*. Moreover, it reduced blue and green moulds of navel orange fruits. In this respect, Singh *et al*. (1992) reported that essential oils from Eucalyptus has antifungal effect against plant pathogenic fungi that inhibited the growth of *Fusarium* sp., *Colletotrichum* sp., *Aspergillus* sp. and *Alternaria alternata*. The effect of volatile oil from the leaves of eucalyptus against *Macrophomina phaseolina*, *Celletotrichum lindemuthianum*, *Fusarium oxysporum* f. sp. *lycopersici*, *Helminthosporium oryzae*, *Alternaria triticina*, *Rhizoctonia solani* and *A. solani* were evaluated. They found that the volatile oil strongly inhabited the radial growth of all tested fungi and the volatile oil was more effective than the synthetic fungicide, mancozeb (Ramezani *et al*., 2002). Moreover, aqueous extracts from fresh and dry leaves of eucalyptus and basil significantly inhibited radial growth of *P. aphanidermatium* and the extract from eucalyptus were more effective as fungitoxic against *P. phanidermatum* (Oluma and Garba, 2002). Furthermore, Patni and Kolte (2006) reported that eucalyptus leaf extract significant reduce the radial growth, sporulation and spore gemination of *A. brassicae*. They also revealed that under glass house condition eucalyptus spray gave significantly lesser number of spot/leaf, minimum sporulation intensity and minimum disease index. On the other hand, the effect of leaf extracts from eucalyptus for controlling soil-borne fungi was reported by Kanherkar *et al*. (2007). Similar results was obtained when soil amendment with leaves, stem, bark and fruit of eucalyptus, at 5% w/w showed significant increase in germination, shoot length, shoot weight, root length and root weight of chick-pea and mung bean plants. They added that the infection by *Fusarium* sp., *M. phaseolina* and *R. solani* was also reduced (Dawar *et al*., 2007).

The essential oil of thyme and its major component, thymol had antifungal activity against plant pathogenic fungi (Plaza *et al*., 2004; Angelini *et al*., 2006). Thymol is an essential oil component from thyme (*Thymus capitates* L.) and has been used as medicinal drug, food preventative and beverage ingredient as well as plant diseases of several fruits and vegetables (El Sherbieny *et al*., 2002; Klaric *et al*., 2007).

In the present study, thyme essential oils *in vitro* trails at concentration of 0.6 and 0.8% completely inhibit the linear growth and spore germination of both fungi. In this respect, Soliman and Badeaa (2002) reported that thyme was tested for its inhibitory effect against *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *F. moniliform*. Results indicated that thyme at concentrations 500 ppm completely inhibited all tested fungi. Similar results was obtained by Bouchra *et al*. (2003), they reported that thyme, clove and cinnamon essential oils completely inhibited *P. digitatum* and *P. italicum* growth either when added into the medium or by their volatiles. Moreover, Omidbaigi *et al*. (2007) reported that the fungistatic and fungicidal activities of the essential oils of thyme, myrtle, clove and lime against *A. flavus* and showed that the most effective essential oils were found from those of thyme and clove. Thyme and clove oils completely inhibited growth of *A. flavus*. Furthermore, thyme essential oil possesses a wide range spectrum of fungicidal activity against plant pathogenic fungi *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium pythium* sp., *F. oxysporam* f. sp. *lycopersici*, *Fusarium oxysporum* f. sp. *pisi*, *Verticilium albo-atrum*, *Rhizoctonia* sp. and *Clavibacter michiganensis* sub sp. *michiganesis* (Tanovic *et al*., 2007).

Essential oil extracted from lemon grass (*Cymbopogon citratus* Stap f.) has antifungal activity against several plant pathogens (Nguefack *et al*., 2007).

In the present study, results indicate that lemon grass reduced gray and blue moulds of apple fruits. In this respect, Abd El-Khair and Haggag (2007) reported that extract of lemon grass leaves was the best one in controlling both late and early blights. Moreover, Tzortzakis and Economakis (2007) recorded that lemon grass oil has antifungal activity against *Colletotrichum coccodes*, *B. cinerea*, *Cladosporium herbarium* Link; Fr., *Rhizopus stolonifer* (Ehreb. Fr.) Vuill and *A. niger in vitro*. Fungal spore production inhibition up to 70% at 25 ppm of lemon grass oil concentration. In the highest oil concentration (500 ppm) employed, fungal sporulation was completely inhibited. Lemon grass oil reduce spore germination and germ tube length of *C. coccodes*, *B. cinerea*, *C. herbarium* and *R. stolonifer*. It could be suggested that essential oils could be make them an excellent treatment for controlling post harvest diseases of apple fruits.

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