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Inhibitory effect of essential oils against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in stored papaya fruit and their possible application in coatings

E. Bosquez-Molina[a,*], E. Ronquillo-de Jesús[a], S. Bautista-Baños[b], J.R. Verde-Calvo[a], J. Morales-López[c]

[a] *Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco 186 Michoacán y la Purísima, Col. Vicentina D.F. 09340, Mexico*
[b] *Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, Carretera Yautepec-Jojutla km, 8.5 San Isidro Yautepec, Morelos 62731, Mexico*
[c] *INFOODS Valle de Aragón, Segunda Sección Nezahualcoyotl, Edo. De México 57100, Mexico*

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ABSTRACT

The aim of this study was to evaluate the fungicidal effect of the thyme and Mexican lime essential oils studies against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, and to determine the possibility of incorporating them in edible coatings to control postharvest diseases of papaya fruits.

For *in vitro* studies, both essential oils were tested to evaluate their effect on mycelial growth of *C. gloeosporioides* and *R. stolonifer* during given incubation times. For *in vivo* tests, fruit were dipped in the thyme and Mexican lime essential oils before and after inoculation. Non-inoculated fruits were similarly treated. A further experiment was carried out by dipping papayas in a coating formulated with both essential oils. Results indicated that the fungicidal effect was more evident with essential thyme than with Mexican lime oil. For the essential thyme oil, concentrations up to 0.060% stopped mycelial growth for both *C. gloeosporioides* and *R. stolonifer*. Papaya fruit dipped in both the essential oils experienced reduced decay caused by *C. gloeosporioides* and *R. stolonifer* by up to 50% and 40%, respectively, compared with the 100% infection observed in non-treated papayas. It was also observed that concentration was not a key factor in reducing the development of these two fungi as it occurred in *in vitro* studies. In papayas immersed in mesquite gum emulsion and formulated with both the essential oils, it was possible to reduce the disease incidence caused by *C. gloeosporioides* by 100% with the thyme and Mexican lime essential oils at 0.1% and 0.5%, respectively.

Conclusion: Thyme and Mexican lime essential oils demonstrated inhibitory effects against *C. gloeosporioides* and *R. stolonifer* growth in *in vitro* evaluations and over development of storage rots in papaya fruit. Incorporation of these two oils in mesquite gum-based coating gave excellent control of these two postharvest diseases.

Significance and Impact of the Study: Control of postharvest diseases of papayas by using synthetic fungicides is becoming increasingly difficult. Thyme and Mexican lime essential oils are considered as GRAS compounds. This investigation highlights the potential of using these two essential oils to control anthracnose disease and *Rhizopus* rot of papaya. We also confirmed that the mesquite-based gum coating formulated with thyme and Mexican lime oils accomplished what is expected in a film material: microorganism inhibition and shelf-life extension of papayas.

1. Introduction

Antimicrobial properties of essential oils from various plant species have been proved to affect and arrest fungal development *in vitro* and *in vivo* in various horticultural commodities. Thyme (*Thymus vulgaris*) essential oil has been found to be antibacterial and antifungal on various pathogenic microorganisms (Hammer et al., 1999; Vanneste and Boyd, 2002; Rassooli and Mirmostafa, 2003; Yang and Clausen, 2007). In postharvest studies, *in vitro* tests showed that fungi such as *Botrytis cinerea*, *Rhizopus stolonifer*, and *Alternaria alternata* were controlled 100% with thyme essential oil incorporated into nutrient agar at 500 mg L^{-1} (Plotto et al., 2003). In *in vivo* studies, control of *B. cinerea* of tomatoes was achieved after dipping them in a polysorbate and thyme oil (5 and 10 g L^{-1}) emulsion (Plotto et al., 2003). Mycelial growth of the fungus *Aspergillus niger* on onion bulbs was controlled with at concentrations up to 0.8 mL L^{-1}, whilst spore germination was reduced by more than 50% at concentrations from 0.4 to 0.8 mL L^{-1}. Further applications of thyme vapors to inoculated onion bulbs resulted in good control of black mould disease (Abd-Alla et al., 2006). *Alternaria citri*, an important pathogen of various citrus species was efficiently controlled in *in vitro* experiments when thyme

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* Corresponding author. Tel.: +52 55 5804 4711; fax: +52 55 5804 4712.
E-mail address: elbm@xanum.uam.mx (E. Bosquez-Molina).

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oil was added to potato dextrose agar at 250 and 1000 mg kg^{-1} concentrations (Arras and Usai, 2001; Soto-Mendívil et al., 2006). Additional experiments also concluded the fungicidal effect of thyme essential oil against *A. alternata* of tomatoes at 500 mg kg^{-1} concentration (Feng and Zheng, 2007).

Citrus fruit essential oils from various species have also shown a good fungicidal effect against *Penicillium digitatum* (Caccioni et al., 1998). Mexican lime (*Citrus aurantifolia*) essential oil is naturally antibacterial, antiviral, and fungicidal. Growth of the storage fungus *Aspergillus flavus* can be controlled with *C. aurantifolia* essential oil at 3000 mg kg^{-1} (Mishra and Dubey, 1994). Although scarce scientific evidence has been published about the fungicidal activity of this essential oil, popular knowledge among outdoor growers recommends the use of this natural repellent for its insecticidal value and fungicidal potential.

The importance of papaya in Mexico's economy is demonstrated by its wide distribution and substantial production in the tropical regions of this country. However, postharvest diseases caused by fungi greatly reduce the storage life of papayas, which frequently become unmarketable. The fungus *Colletotrichum gloeosporioides* has posed serious problems to Mexican producers because of conducive environmental conditions for the growth of this pathogen, and the development of resistance to synthetic fungicides. *C. gloeosporioides* infection is initiated in the field at early stages of fruit development and remains quiescent until the onset of fruit ripening when conditions favour growth of the fungus and lesions develop (Alvarez and Nishijima, 1987). Similarly, *R. stolonifer* is also a major factor limiting the postharvest life of papaya fruit. Infection takes place at harvest and during handling and once this happens, development and spread of the fungi to adjacent fruit is rapid, causing extensive 'nesting' (Snowdon, 1991). The low storage temperature that is widely used to control postharvest microorganisms in many fruits and vegetables is not viable for papayas, since they are classified as very sensitive to low temperatures (below 10 °C) (Kader et al., 2002). Other postharvest technologies such as hot water dips and irradiation do not eliminate anthracnose completely and these technologies are not economically accessible for all producers.

To date, the incorporation of antimicrobial agents for potential use in films, coatings, and packaging is under experimentation (Supakkul et al., 2003). It has been demonstrated in different food types such as meat, and bakery products that films containing essential oils of angelica, anise, cardamom, cinnamon, thymol, etc., inhibit various pathogenic moulds, bacteria, and yeasts (Cagri et al., 2004). Therefore, the incorporation of essential oils may be a convenient means by which postharvest disease may be controlled (Chung et al., 1998).

The objectives of our research were: (1) to identify the main components of the thyme and Mexican lime essential oils, (2) to evaluate the inhibitory effect of both the essential oils over *C. gloeosporioides* and *R. stolonifer* in *in vitro* growth, and (3) to evaluate their fungicidal effect, either alone or incorporated into an edible coating in artificially or naturally infected papayas during storage.

2. Materials and methods

2.1. Isolation of microorganisms

The isolates of *C. gloeosporioides* and *R. stolonifer* were obtained from infected papaya fruit from the region of Cuautla State of Morelos. Five-centimeter plugs of papaya tissue were disinfected with 1% sodium hypochlorite and placed at the center of a Petri plate containing potato dextrose agar (PDA, Bioxon). After a 7 d incubation period, the fungus was sub-cultured and identified according to established procedures (Burnett and Hunter, 1972; Streets, 1984). Monoconidial cultures were maintained in PDA plates at 25 ± 2 °C.

2.2. Characterization of the thyme and Mexican essential oils

2.2.1. Essential oil extraction by standard vacuum hydrodistillation

Five kilograms of clean rinds of Mexican limes were blended in water in a proportion of 60% of rinds to 40% of water for 10 min. The mixture was heated to a temperature of 60 °C for 2 h, while pressure was slowly decreased to 26.7 kPa. In the case of thyme, fresh leaves of thyme were purchased in the Mexico City warehouse market. Thyme leaves were ground in water (40% leaves by mass) and distilled under the same conditions as mentioned for Mexican lime.

2.2.2. Essential oil characterization

Analyses were carried out an HP 5890 Series II gas chromatograph directly coupled to an HP 5890 mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA). The capillary column used was an HP-FFAP (30 m × 0.25 mm i.d., film thickness 0.25 μm; Hewlett-Packard). A split injector was used at a ratio of 1:2; the injector and detector temperatures were 200 °C and 25 °C, respectively. Conditions were as follows: column temperature was held at 60 °C for 3 min and then increased at 0.083 °C s^{-1} to 250 °C; helium was used as carrier gas at a linear flow of 33 μL s^{-1}. Mass spectra were obtained at 70 eV and the ion source was at 250 °C. All the essays were performed three times. The identification of the analyzed compounds was accomplished by comparing their mass spectra with those of authentic compounds available from computerized spectral database (NIST/EPA/NIH 75K), or from published literature.

2.3. Effect of the essential oils of thyme and Mexican lime on the in vitro growth of C. gloeosporioides and R. stolonifer

2.3.1. In vitro evaluation

A PDA agar disk (5 mm diameter) of a pure culture of *C. gloeosporioides* and *R. stolonifer* isolates taken from the margins of 10- and 4-d-old cultures, respectively, was placed onto the center of a PDA plate of 5 cm of diameter mixed with the essential oils of thyme and Mexican lime at concentrations of 0.0, 0.015, 0.030, 0.045, 0.060, 0.075, 0.09 and 0.104% (v/w) and 0.014, 0.028, 0.0425,0.057, 0.071, 0.085 and 0.1% (v/w), respectively. Control Petri plates contained only PDA taken from the margins of cultures of the same age as above. Petri plates were incubated at ambient temperature (18–20 °C). Daily radial growth measurements were taken with a caliper until control Petri dishes were fully covered with mycelia. Four Petri plates for each treatment were considered for this evaluation. The experiment was repeated twice and data were pooled.

2.3.2. In situ evaluations

Fungicidal and preventive effects of the essential oil of thyme and Mexican lime against *C. gloeosporioides* and *R. stolonifer* of papaya fruits and over-naturally infected fruits.

Papaya cv. Maradol at the green to yellow color stage (Index 2) were obtained from a wholesale store in Mexico City. The mean initial total soluble solids and firmness of fruit were 8.8% and 12.6 N, coinciding with the typical initial ripening stage of papaya fruit. They were washed with sodium hypochlorite (1%), rinsed with distilled water, and dried at ambient conditions. The fruit were artificially inoculated with *C. gloeosporioides* and *R. stolonifer* before or after being dipped in the essential oils. Fruit were inoculated with *C. gloeosporioides* and after 12 h they were oil-treated whilst for *R. stolonifer*, treatments were done 4 h after inoculation. When oil treatment application was carried out before inoculation, fruit were left at ambient temperature (20–25 °C) until the surfaces were almost dry (approximately 2–3 h), and then inoculation was carried out. An additional experiment included fruit that were naturally infected. For all experiments, fruit were dipped for 20 min in the essential oils at 0.0, 0.1, 0.12 and 0.15% (w/w) of distilled water. The control treatments were dipped only in water before or after inoculation. After drying, they were evenly sprayed with a spore suspension of *C. gloeosporioides* (10^6 spores mL^{-1}) and *R. stolonifer* (10^5 spores mL^{-1}) and held at controlled temperature (20 °C) for 9 d.

2.4. Emulsion formulation with essential oils of thyme and Mexican lime and their fungicidal effect against C. gloeosporioides and R. stolonifer of papaya fruits

2.4.1. Preparation of emulsion

Mesquite gum-based edible emulsion films were prepared according to the method used by Bosquez-Molina et al. (2003). Aqueous dispersed phases of mesquite gum 10% (w/w) dispersion was prepared and used as the structural material in the emulsion formulations. Mesquite gum was dispersed in water by adding 5% (w/w) of glycerol as plasticizer and the mixture heated to 70 °C. At the same time, a blend of candelilla wax-mineral oil in a 2:1 ratio (17.5%, w/w) was heated to 70 °C and added drop-wise into the polysaccharide dispersion while stirring at maximum speed (133.3 s^{-1}) with a L4R Silverson homogenizer (Silverson Machines, Ltd., Waterside, Chesham, Bucks, U.K.) for 5 min. All emulsions were left to cool at room temperature. After that, thyme and Mexican lime essential oils were incorporated into the emulsion to obtain three film forming formulations of each essential oil at a final concentration of 0.05, 0.075 and 0.1% (w/w).

2.4.2. Treatments

As described earlier, fruit were conditioned and inoculated with *C. gloeosporioides* and *R. stolonifer*. Fruit were dipped in the three emulsion formulations. Storage time was similar as described earlier in the text. The control fruit were also treated with water.

2.4.3. Parameters evaluated for in vivo studies and statistical analyses of the data

At the end of the storage period, disease was evaluated as incidence (%), and severity index ranked from 0 to 4, where: 0 = 0% of fruit surface rotten, 1 = 1–5%, (initially damaged) 2 = 6–15% (lightly damaged), 3 = 16–30% (moderately damaged), 4 = >31% (severely damaged). Mean and standard deviations were carried out for *in vitro* experiments. For *in vivo* studies, each treatment consisted of five 25-fruit replicates. Treatments were arranged in a completely randomized design. Mean separation by Tukey's multiple range test ($P \leq 0.05$) was carried out. Experiments were repeated twice and data were pooled.

3. Results

3.1. Essential oils characterization

The heating process for 2 h of thyme leaves yielded 1-2 mL kg^{-1} of pale yellow liquid whilst the rinds of Mexican limes yielded 10 mL kg^{-1} of crystal-clear liquid. The thyme essential oil consisted of 23 principal compounds with γ-terpinene accounting for the majority of the total constituents (17.11%), followed by p-cymene (14.61%) and α-thujene (9.00%). Other components identified were α-pinene, camphene, β-pinene, α-terpinene, linalool, borneol, 4-carvomentehol, thymol and carvacrol which comprised 26% of the constituents (Table 1). The more prominent chemicals found in the Mexican lime essential oil consisted of D-limonene (45.10%), β-pinene (20.50%) and γ-terpinene (10.59%).

3.2. Effect of the essential oils of thyme and Mexican lime on in vitro growth of C. gloeosporioides and R. stolonifer

The inhibitory effects of thyme and Mexican lime oils against *C. gloeosporioides* and *R. stolonifer* are shown in Figs. 1 and 2. Essential thyme oil had a good fungicidal effect against these two fungi and growth of both was reduced as concentration increased. Evidently, concentrations up to 0.06% (v/w) completely reduced the development of *R. stolonifer*, while a slight rate of growth was observed in *C. gloeosporioides*. For the essential Mexican oil, the fungicidal

Table 1
GC–MS analyses of Mexican lime and thyme essential oils.

Thyme	% Area ± SD	Mexican lime	% Area ± SD
γ-Terpinene	17.11 ± 0.07	D-Limonene	45.10 ± 1.90
p-Cymene	14.61 ± 0.67	β-Pinene	20.50 ± 1.12
α-Thujene	9.00 ± 0.64	γ-Terpinene	10.59 ± 0.12
Thymol	7.28 ± 0.18	Citral	3.18 ± 0.02
β-Myrcene	7.23 ± 0.38	Neral	2.82 ± 0.16
Eucalyptol	6.66 ± 2.31	1S-α-Pinene	2.06 ± 0.17
Linalool	6.37 ± 0.10	α-Farnesene	1.84 ± 0.06
α-Terpinene	5.41 ± 0.43	β-Caryophyllene	1.46 ± 0.16
1-Octene-3-ol	3.36 ± 0.82	(−)β-Bisabolene	1.92 ± 0.66
3-Hexen-1-ol	2.60 ± 0.60	α-Trans-bergamotene	1.37 ± 0.18
Camphene	1.95 ± 0.03	β-Thujene	1.16 ± 0.03
Borneol	1.87 ± 0.93	α-Terpineol	0.84 ± 0.08
4-Carvomenthenol	1.72 ± 0.65	γ-Elemene	0.86 ± 0.12
d-Camphor	1.29 ± 0.77	Cyclohexene, 1-methyl-4-(1-methylethylidene)	0.66 ± 0.04
Carvacrol	0.98 ± 0.31	Geraniol	0.68 ± 0.06
3-Octanol	0.96 ± 0.00	Nerol	0.57 ± 0.07
β-Pinene	0.83 ± 0.00	3-Carene	0.45 ± 0.005
Sabinene	0.71 ± 0.00	δ-Terpinene	0.47 ± 0.04
m-Cymene	0.56 ± 0.38	ζ-Elemene	0.49 ± 0.08
α-Terpineol	0.40 ± 0.31	Germacrene D	0.49 ± 0.10
Terpinolene	0.38 ± 0.04	β-Elemene	0.33 ± 0.05
2-Hexenal	0.21 ± 0.00	4-Carene	0.29 ± 0.09
Methyl 2-methylbutyrate 2-methylbutyr	0.13 ± 0.00	α-Caryophyllene	0.18 ± 0.03
Others	8.38	Cis-α-bisabolene	0.17 ± 0.04
		Others	1.52

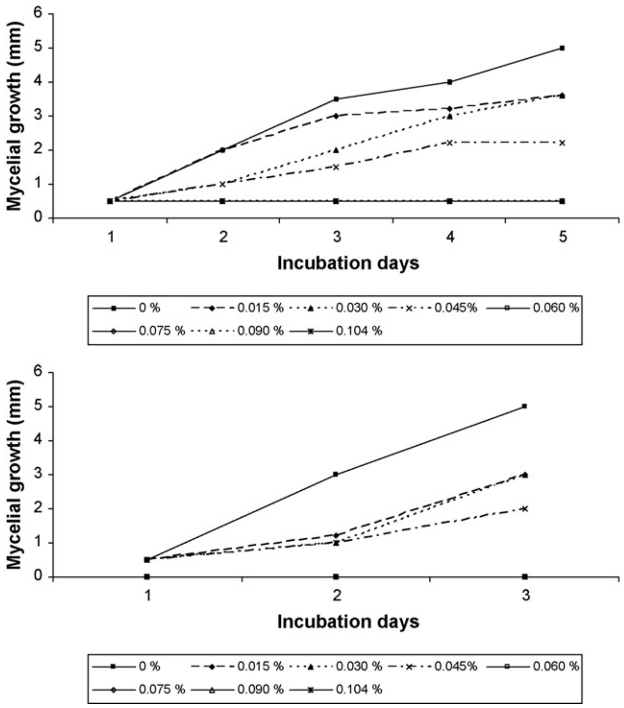

Fig. 1. Growth of the fungi a) *C. gloeosporioides* and b) *R. stolonifer* incubated at various concentrations of the thyme essential oil for 5 and 3 d, respectively.

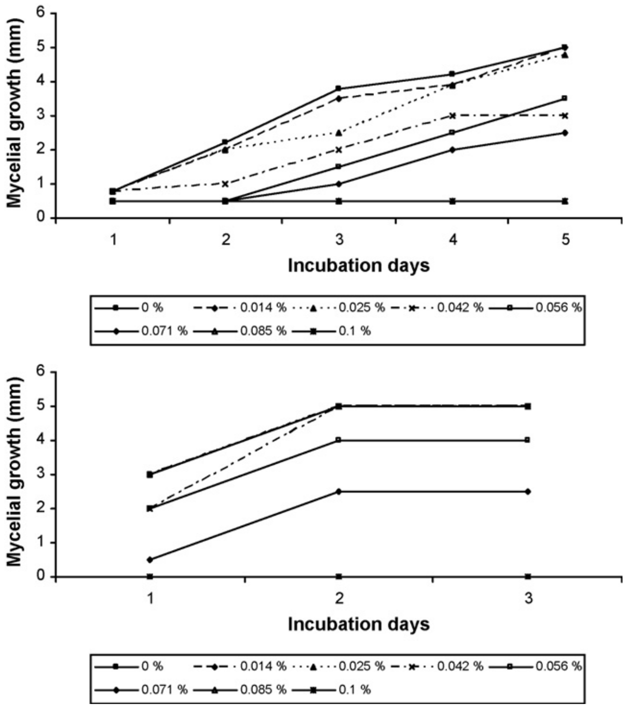

Fig. 2. Growth of the fungi a) *C. gloeosporioides* and b) *R. stolonifer* incubated at various concentrations of the Mexican lime essential oil for 5 and 3 d, respectively.

effect was not as effective as was observed with thyme oil, since the growth of *C. gloeosporioides* incubated with Mexican oil was stopped at the concentration of 0.085% (v/w) while the development of *R. stolonifer* was inhibited at the concentration of 0.10% (v/w).

3.3. Pre- and post-infection activity of C. gloeosporioides and R. stolonifer on artificially inoculated and naturally infected fruit

For *C. gloeosporioides* and *R. stolonifer*, there was a significant effect on disease incidence ($P \leq 0.05$) among treatments with thyme and Mexican oils treated before and after fruit inoculation (Table 2). Essential thyme oil controlled *C. gloeosporioides* most efficiently at

Table 2

Effect of thyme and Mexican lime essential oils on disease incidence and severity index of papaya fruit artificially inoculated by *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* before and after treatment application.

Treatments	*C. gloeosporioides*		*R. stolonifer*	
	Disease incidence (%)	Severity index	Disease incidence (%)	Severity index
Inoculated before treatment				
Thyme essential oil (% (v/w))				
Control	100a*	2	80ab	2.4
0.10	100a	1	40d	0.6
0.12	50b	2	60c	1.8
0.15	0c	0	80ab	1.
Mexican lime essential oil (% (v/w))				
Control	100a	3	100a	2.4
0.10	50b	1	100a	2.4
0.12	100a	4	100a	1.4
0.14	100a	4	80ab	2.2
Inoculated after treatment				
Essential thyme oil (% (v/w))				
Control	100a	2	100a	2
0.10	100a	2	60c	1
0.12	100a	2	80ab	1.4
0.15	50b	2	60c	1
Mexican lime essential oil (% (v/w))				
Control	100a	3	100a	2
0.10	100a	3	80ab	1.6
0.12	100a	3	80ab	2
0.14	50b	3	100a	1.6

Severity index: 0% = non, 1 = initially, 2 = lightly moderate, 3 = moderately and 4 = severely damaged.

[*] Means followed by the same letter are not significant different ($P \leq 0.05$) determined by Tukey's multiple test.

Table 3
Effect of thyme and Mexican lime essential oils on disease incidence and severity index of papaya fruit naturally infected by *C. gloeosporioides* and *R. stolonifer*.

Treatments	*C. gloeosporioides*		*R. stolonifer*	
	Disease incidence (%)	Severity index	Disease incidence (%)	Severity index
Thyme essential oil concentrations (%, w/w)				
Control	100a*	3	80ab	2.4
0.10	100a	1.5	100a	0.4
0.12	100a	2	20c	0.6
0.15	0b	0	20c	0.2
Essential Mexican lime oil concentrations (%, w/w)				
Control	100a	3	100a	2.4
0.10	100a	0.5	100a	1.4
0.12	50b	2	60c	0.6
0.14	0c	0	80ab	1.2

Severity index: 0% = non, 1 = initially, 2 = lightly moderate and 3 = moderately damaged.

* Means followed by the same letter are not significant different ($P \leq 0.05$) determined by Tukey's multiple test.

a concentration of 0.15% (w/w) when inoculation took place before treatments application. However, infection by this fungus could be reduced by 50% when papayas were dipped in thyme and Mexican essential oils at 0.12% (w/w) and 0.1% (w/w), respectively. Thyme essential oil at 0.15% and Mexican essential oil at 0.14% resulted in a 50% reduction of disease infection by *C. gloeosporioides* compared with the untreated papayas that presented 100% infection when fruit were inoculated after treatment application. Overall, the papayas treated with essential thyme oil had a severity index that varied from 0 to 2 (none to moderate damage), while in those treated with essential Mexican lime oil, the severity varied from 0 to 4 (none to severe damage). For *R. stolonifer*, the disease incidence was lower (40%) than untreated fruit and the remaining treatments when fruit were dipped in thyme oil at 0.10% (v/w). However, higher concentrations increased infection. Contrary to the results observed with *C. gloeosporioides*, the severity of the infection in fruit by *R. stolonifer* was lower. It ranged from 1 to 2.4 (initial to moderate damage).

On naturally infected fruit there was a significant reduction in decay incidence ($P \leq 0.05$) with the application of thyme and Mexican lime essential oils (Table 3). For *C. gloeosporioides* both essential oils at the highest concentration of 0.15% and 0.14% (w/w) totally controlled this fungus whilst for *R. stolonifer* the fruit infection was reduced by 80% with the thyme essential oil at 0.12 and 0.15% (w/w). The severity index associated with *R. stolonifer* was more sensitive to the essential oils than *C. gloeosporioides*.

3.4. Edible coating formulated with thyme and Mexican lime essential oils and their fungicidal effect against C. gloeosporioides and R. stolonifer of papaya fruit

In general, the mesquite gum-based emulsions prepared with both the essential oils had a significant ($P \leq 0.05$) fungicidal effect over *C. gloeosporioides* and *R. stolonifer* in papaya fruit (Table 4). The development of *C. gloeosporioides* was controlled 100% in those fruit dipped in the thyme essential oil at 0.1% (w/w) and in the Mexican essential lime oil at 0.05% concentration. However, application of thyme essential oil at the same concentration and 0.075% also resulted in a good control of this fungus (80 and 90%, respectively). For papayas inoculated with *R. stolonifer* and treated with both the essential oils, infection was reduced by 60%. The best concentration was that of thyme essential oil at 0.1%. The severity index in the inoculated papayas was also low compared with the untreated papayas. The range of disease intensity was from 0 to 0.6.

4. Discussion

The components identified in the thyme essential oil were in accord with those reported by other authors (Jukié and Milos, 2005; Porte and Godoy, 2008; Soto-Mendívil et al., 2006). In the case of the Mexican lime essential oil, Benvenuti et al. (2001), reported the same type of compounds in the essential oil of peels from the 'Sicilian' lemon variety but different concentrations were found for D-limonene (65%), β-pinene (10%) and γ-terpinene (10.3%). This reinforces the point that the composition of essential oils depends on the chemotype and biotype of the plant, the growing environmental conditions and the extraction process used (Vekiari et al., 2002).

Consistent with previous literature, we found that thyme oil had better fungicidal effects than Mexican lime oil. In accord with previous results, the concentration of thyme and Mexican oils was the major parameter affecting the *in vitro* growth of *C. gloeosporioides* and *R. stolonifer* rather than the incubation time (Abd-Alla et al., 2006; Feng and Zheng, 2007).

Except for the treatment with thyme essential oil at 0.1%, we could not observe (as we did in the *in vitro* studies) that increasing concentration of the tested oils reduced disease incidence. On the contrary, in some cases, increasing oil concentrations led to greater infection in the papayas. Plotto et al. (2003), mentioned that 'this could be the result of phytotoxic effects of the essential oils in the wound, changing the capacity of the fruit epidermis tissue to detain spore germination'.

Some antimicrobial edible films based on essential oils have been developed to control postharvest pathogenic microorganisms. For example, films impregnated with extracts of Chinese rhubarb (*Rheum palmatum*) and goldthread (*Coptis chinensis*) reduced the growth of bacteria and yeast in fresh strawberries (Chung et al., 1998). Other alternatives such as the inclusion of sachets impregnated with essential oils such as thymol oil during 35 d packaging reduced the development of moulds of table grapes (Martínez-Romero et al., 2005). To our knowledge, this is the first time that essential thyme and Mexican lime oils incorporated in an edible coating in papayas had beneficial effects in controlling *C. gloeosporioides* and *R. stolonifer*.

It is well documented that nonsystemic, synthetic fungicides and biological control agents are ineffective in controlling latent infections. Essential oils provide a wide variety of compounds as alternatives to synthetic fungicides; however, they have not been developed into products for postharvest treatments. Their potential as alternatives for disease control resides precisely in that their antimicrobial activity is not attributable to one specific mechanism so it is difficult to create resistance in the microorganisms (Burg, 2004).

This study has shown the potential of using essential oils in reducing these two important storage diseases of papaya fruit. In addition, another positive effect of thyme and Mexican lime is that they do not have the strong flavor that characterizes other essential oils considered as excellent antimicrobials such as garlic and cin-

Table 4
Effect of edible coatings formulated with thyme and Mexican lime essential oils over *C. gloeosporioides* and *R. stolonifer* infection of papaya fruit.

Pathogen	Treatment	Disease incidence (%)	Severity index
Control		100a*	3
C. gloeosporioides	Coating with 0.05% of thyme essential oil	20c	0.2
	Coating with 0.075% of thyme essential oil	10cd	0.2
	Coating with 0.1% of thyme essential oil	0e	0
	Coating with 0.05% of Mexican lime essential oil	0e	0
	Coating with 0.075% of Mexican lime essential oil	40b	0.2
	Coating with 0.1% of Mexican lime essential oil	40b	0.2
Control		100a	3
R. stolonifer	Coating with 0.05% of thyme essential oil	40b	0.4
	Coating with 0.075% of thyme essential oil	40b	0.4
	Coating with 0.1% of thyme essential oil	35b	0.4
	Coating with 0.05% of Mexican lime essential oil	40b	0.6
	Coating with 0.075% of Mexican lime essential oil	40b	0.4
	Coating with 0.1% of Mexican lime essential oil	40	0.4

Severity index: 0% = non, 1 = initially and 3 = moderately damaged.

* Means followed by the same letter are not significant different ($P \leq 0.05$) determined by Tukey's multiple test.

namon, which eventually affect the sensory characteristics of the commodity. Coating application extends the storage life of horticultural commodities since they cover the fresh produce by providing physical barriers to reduce loss of water vapor and aroma volatiles and delay the side effects of respiration. Keeping this in mind, our work will continue on validating this technology by studying the effect of the emulsion formulation type on essential oil release intensity and its impact on ripening and sensory quality of papayas.

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