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Cientific Paper

Strategic control of postharvest decay in peach caused by *Monilinia fructicola* and *Colletotrichum gloeosporioides*

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Abstract

In this work we report the postharvest control of *Monilinia fructicola* - causative agents of brown rot and *Colletotrichum gloesporioides* - causative agents of anthracnose. These fungi contribute significantly to the reduction of the marketing period of peach. The conventional control of these diseases can leave chemical residues on fruits and selecting resistant pathogenic strains. The use of essential oils can be a more appropriate alternative. Essential oil of *Eucalyptus globulus*, *Cinnamomum camphora* and *Cymbopogum citratus* were used in control. The results have demonstrated good control of these diseases manly using *C. camphora* and *C. citratus* in vitro and in vivo analysis beyond in vivo simulating cold storage. Our results in these essays indicate the possibility of using environmental-friendly essential oil in postharvest control of peaches and others fruits, without utilization of chemical fungicides.

Key words: postharvest disease; pathogenic fungi; environmental

El control de la deterioración post-cosecha en melocotón causada por *Monilinia fructicola* y *Colletotrichum gloeosporioides*

Resumen

En este trabajo fue relatado el control post-cosecha de *Monilinia fructicola* - agente causante de la podredumbre parda y *Colletotrichum gloeosporioides* - agente causante de la antracnosis. Estos hongos contribuyen significativamente a la reducción de la comercialización de melocotón. El control convencional de estas enfermedades puede dejar residuos químicos en las frutas y seleccionando estirpes patógenos resistentes. El uso de aceites esenciales puede ser una alternativa más adecuada. Los aceites esenciales de *Eucalyptus globulus*, *Cinnamomum camphora* y *Cymbopogum citratus* fueron utilizados como control. Los resultados demostraron un buen control de la enfermedad con *C. camphora* y *C. citratus* in vitro e in vivo y en el análisis de simulación de almacenamiento en frío. Los resultados de los ensayos indican la posibilidad de utilizar el aceite esencial en el control post cosecha de melocotón y otras frutas, sin el uso de fungicidas químicos.

Palabras clave: enfermedades post cosecha, hongos patógenos, medio ambiente

Controle da deterioração pós-colheita em pêssego causado por *Monilinia fructicola* e *Colletotrichum gloeosporioides*

Resumo

Neste trabalho foi relatado o controle pós colheita de *Monilinia fructicola* -agente causador da podridão marrom e *Colletotrichum gloesporioide*s – agente causador da antracnose. Estes fungos contribuem significativamente na redução da comercialização do pêssego. O controle convencional destas doenças

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podem deixar resíduos químicos nas frutas e selecionar estiperpes patogênicas resistentes. O uso de óleos essenciais pode ser uma alternativa mais adequada. Óleos essenciais de *Eucalyptus globulus, Cinnamomum camphora* e *Cymbopogum citratus* foram usados como controle. Os resultados demonstraram bom controle das doenças com *C. camphora* e *C. citratus* in vitro e in vivo e análise in vivo simulando armazenamento a frio. Os resultados dos ensaios indicam a possibilidade da utilização do óleo essencial no controle pós colheita de pêssego e outras frutas, sem a utilização de fungicidas químicos.

Palavras chave: doenças pós-colheita, fungos fitopatogênicos, meio ambiente.

Introduction

Postharvest diseases caused by fungal pathogens are a major problem affecting the quality and marketing value of fruit, with significant economic impact around the world. *Monilinia fructicola* cause brown rot, an extremely destructive disease occurring on stone fruit trees (*Prunus* spp.) and other rosaceous fruit trees (e.g. *Malus* spp. *and Pyrus* spp.) (BYRDE e WILLETTS, 1977). *C. gloeosporioides* is the causal agent of anthracnose, the main postharvest disease of some fruits, such as Papaya fruit (*Carica papaya* L.) (PALHANO et al., 2004). Both diseases can seriously reduce or even destroy a crop by affecting blossoms and fruits, either on the tree or after harvest.

Benzimidazole fungicides such as benomyl, thiophanate-methyl and sterol demethylation inhibitor fungicides (DMIs) were widely used in this control. In addition, many of these pathogens have become a serious threat to crops due to their resistance to known chemical control agents (MA e MICHAILIDES, 2005; ISHII, 2006). Elucidating non-chemical control methods to reduce postharvest decay is becoming increasingly important. Consumers are demanding less chemical residue on production, and the use of fungicides is becoming more restricted due to health concerns (RAGDALE e SISLER, 1994).

In this way, substantial efforts have been made on finding effective biocontrol agents. A number of different methods have reported promising alternatives to the use of synthetic fungicides, acting effectively on inhibiting postharvest decay of fruit. Ultraviolet light (CRISOSTO et al. 1998), some yeasts (KARABULUT e BAYKAL 2003), peptides and even acetic acid (SHOLBERG e GAUNCE, 1996) have been applied. The use of essential oil to biological control has been reported with success (ANTUNES e CAVACO, 2009). The aim of this work is show new alternatives to control the post-harvest decay caused by *M. fructicola* and *C. gloeosporioides* in peach fruits. With these intentions, the tests were done *in vitro* and *in vivo.*

Materials and Methods.

Collection of micro-organisms

Strain 887 of *M. fructicola* and A11/09 C. gloeosporioides were reactivated mycology collection of the Laboratory of Plant Pathology, University of Caxias do Sul. Strain 887 of *M. fructicola* was isolated from peach in the region of Caxias do Sul and isolate A11/09 *C. gloeosporioides* was isolated from guava in the region of Batley, MG.

Collection and drying plant

The botanical materials consisted of the leaves: *Cinnamomum* camphora NESS e EBERM *var. linaloolifera* Fujita (ho-sho), Cymbopogon citratus (lemongrass) and Eucalyptus globulus (eucalyptus), which were collected in the experimental field of the Institute of Biotechnology, University of Caxias do Sul; they were then dried in a dryer with forced airflow, at a temperature of 35° C for 48 hours or until there were no more changes on the weight of materials.Extractions of essential oils.

Extractions of essential oils

The extraction of essential oils of *C. camphora, C. citratus* and *E. globulus* was conducted at the Laboratory of Essential Oils, University of Caxias do Sul. For the extraction of essential oils we used Clevenger apparatus (Figure 1), using the method of hydro-distillation, for drag with water vapor. The extraction time was of two hours, uninterrupted. After this time, the readings were made of the essential oil yield (mL%, volume / weight) and then stored in amber bottle in the freezer.

After the extraction of the essential oils, we carried out the chemical analysis: qualitative in gas chromatography coupled to mass spectrometry (GC / MS) and quantitative gas chromatography (GC).

Inhibition potential of essential oils on growth and mycelial M. fructicola and C. gloeosporioides in vitro

In order to evaluate the effect of essential oils of *C. camphora, C. citratus* and *E. globulus* on the mycelial growth of pathogens, aliquots of 10, 50, 100, 150, and 200µL of each oil, previously autoclaved, were diluted in Twen 20 (1:1), and added to 100 ml of culture medium PDA (potato, dextrose agar) flux (≈ 50° C), avoiding the volatilization of essential oil. The culture medium was then distributed in Petri dishes, 20 mL per plate, and after solidification, was transferred to the center of each plate, a disc (0.5 cm diameter) of PDA colonized taken from a colony in growth *M. fructicola* or *C. gloeosporioides*. Control treatments were performed in two ways: i) the fungi were inoculated in the center of the plates only containing PDA culture medium, and ii) the fungi were inoculated in the center of plates containing PDA culture medium enriched with the chemical fungicide Orthocide (active ingredient captan) at a dose of 2.4 g L^{-1}. Tests were conducted in five replicates. Then, all treatments were incubated in a growth chamber with 12 h photoperiod at 25° C. Later, we quantified the percentage of mycelial growth in comparison with control, by measuring the diameter of the colonies, three, seven and 14 days after incubation by using a digital caliper.

Control M. fructicola and C. gloeosporioides in peach fruit

We collected randomly in the orchard, fruits in harvest time, the Chimarrita variety, grown on the estate of Francisco Conte, located in Flores da Cunha. The peaches were washed with detergent and water, being then immersed in a solution of sodium hypochlorite 0.5% and soon dipped twice in distilled water and left to dry on paper towel. After drying, three treatments were performed using the pathogen *M. fructicola*, which consisted of three replicates with five fruits for each repetition. The same was done with the pathogen *C. gloeosporioides*. In each of the fruits were four superficial incisions (only skin) scalpel in the area opposite to the stalk with a length of 2 mm. Immediately, the fruits were immersed for 10 minutes at:

Treatment 1- An aqueous syrup with optimal concentration of essential oil which showed satisfactory results in vitro tests.

Treatment 2 - Distilled water.

Treatment 3 - An aqueous syrup with Orthocide chemical fungicide (active ingredient captan) ata dosage of 2.4 g L^{-1}.

After each treatment, 1, 2 and 3, for each of the incisions was transferred an aliquot of a conidial suspension of *M. fructicola* and *C. gloeosporioides* at a concentration of 1107 cells mL^{-1}. The procedure was performed in a laminar flow hood previously sterilized to prevent contamination from the environment. After each treatment applied to the fruit, we made two different tests, listed below:

Test 1 - After inoculation, fruits were left in sealed plastic bags and kept in cold condition for 20 days and observed daily for the possible appearance of fruit decay. Test 2 - After inoculation, fruits were kept at room temperature for 10 days. Evaluations were performed at the third, fifth, seventh and tenth days, by determining the average diameter of lesions on fruits infected by the pathogens in each treatment. A statistical analysis was applied to the analysis of variance in order to verify the reliability of the results. Thereafter, the mean total scores were compared by the Tukey test at 5% probability.

Results and Discution

In the extraction process, we observed the yield of essential oils to establish a parameter on the economic feasibility. The yield of essential oil of *C. citratus* in mL% (volume/mass) was of 2.4%, while the essential oil *C. camphora* yielded 2.3% and for *E. globulus* the yield was 1.5%, of plant dry. The composition is shown in Table 1.

In vitro tests with pathogens *C. gloeosporioides* and *M. fructicola*, demonstrated that, after 3 days, mycelial growth of pathogens were completely inhibited by the application of essential oil of *C. camphora* at concentrations of 0.15 and 0.20%. The essential oil of *C. citratus* showed 100% of growth inhibition of both pathogens from 3rd day at concentrations from 0.05%. The essential oil of E. *globulus* showed inhibition on day 3rd of mycelial growth at concentrations of 0.15 and 0.20% of pathogen *C. gloeosporioides*, but at 7 and 14 days, there was growth of the pathogen at all concentrations tested, while for the pathogen *M. fructicola* did not inhibit mycelial growth.

The Orthocide fungicide (active ingredient captan) produced 100% inhibition of mycelial growth

Table 1. Essential oil composition of *C. citratus, C. camphora and E. globulus*

C. citratus
geranial (45.39%); neral (31.81%); mircene (9.82%); geraniol (2.11%); 6-methyl-hepten-2-one (2.03%); linalool (1.65%); geranic acid (1.10%); undecanone (0.86%).
C. camphora
linalool (93.40%); trans-cariofilene (1.90%); α-humulene (0.80%); D-germacrene (0.69%); canfor (0.66%)
E. globulus
1,8-cineol (61.30%); canfene (12.64%); α-pinene (5.80%); limonene (4.10%); vidiflorol (3.07%); aromadrendene (2.76%); p-cimene (1.05%); epiglobulool (0.73%); aloaromadendrene (0.65%)

on 3rd day, a reduction of the percentage of inhibition when evaluated on the 7th day was 92.5 and 93.5% for pathogens *C. gloeosporioides* and *M. fructicola*, respectively, but higher than the percentage of 88 and 90% on the 14th day of treatment, as shown in Figure 1 and 2.

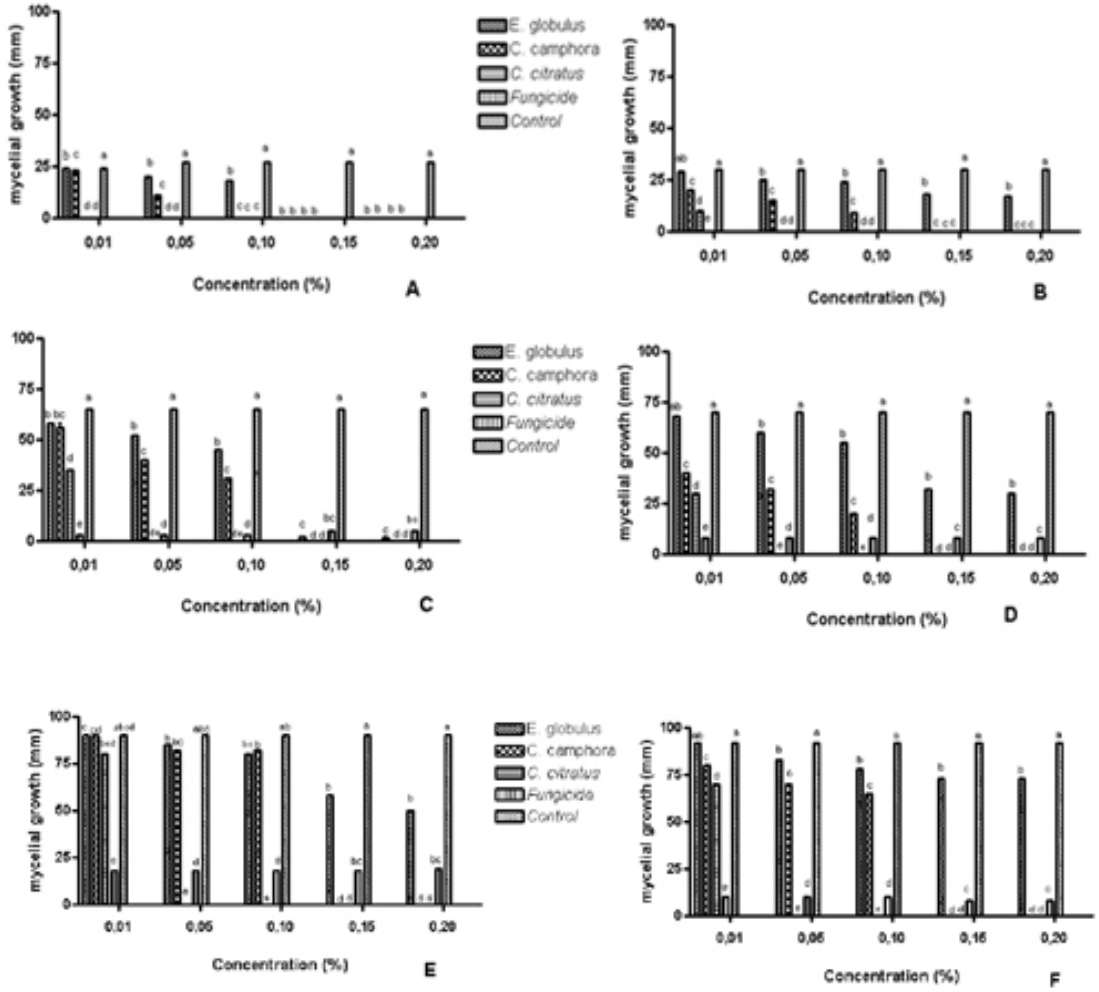

Figure 1. I*n vitro* effect under: **(A)** *C. gloeosporioides* e **(B)** *M. fructicola* in 3rd day, **(C)** *C. gloeosporioides* e **(D)** *M. fructicola* in 7th day and **(E)** *C. gloeosporioides* and **(F)** *M. fructicola* in the 14th day

Control in peach (In vivo)

Regarding the effect of the oils in the control of fruit rot, we found that the treatments reduced the incidence and severity of the disease, compared to control. The best performances, at room temperature, for control *C. gloeosporioides* were obtained with the essential oils of *C. camphora* and *C. citratus* both at concentrations of 0.20%, while for the control of *M. fructicola*, the best results were obtained with the essential oil of *C. citratus* 0.15 and 0.20%, both had similar control fungicide tested, as shown in Figure 2.

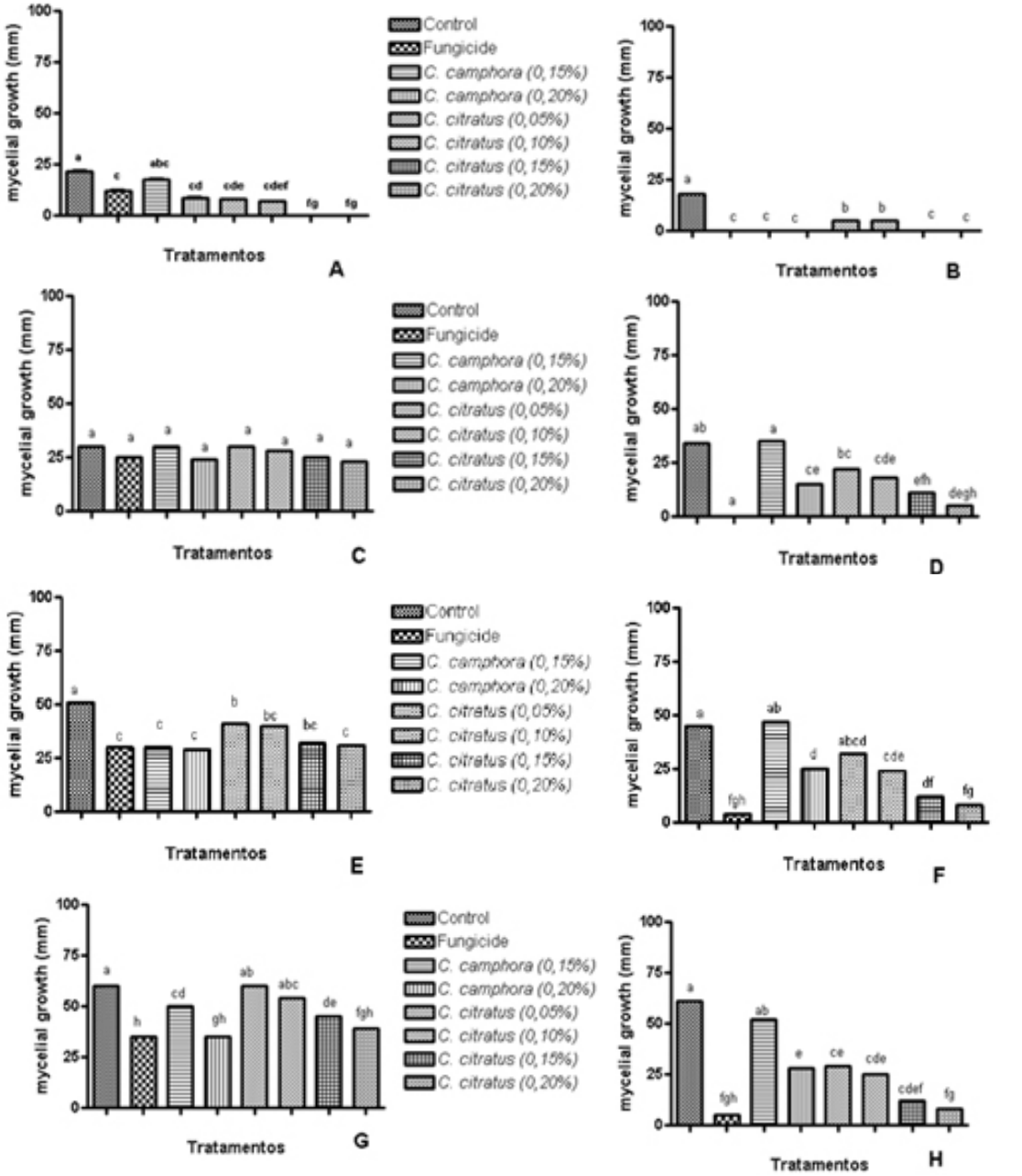

Figure 2. Test *in vivo*, at room temperature **(A)** *C. gloeosporioides* and **(B)** *M. fructicola* in 3rd day, **(C)** *C. gloeosporioides* and **(D)** *M. fructicola* in 5th day, **(E)** *C. gloeosporioides* and **(F)** *M. fructicola* in 7th day, and **(G)** *C. gloeosporioides* and (H) *M. fructicola* in the 14th day.

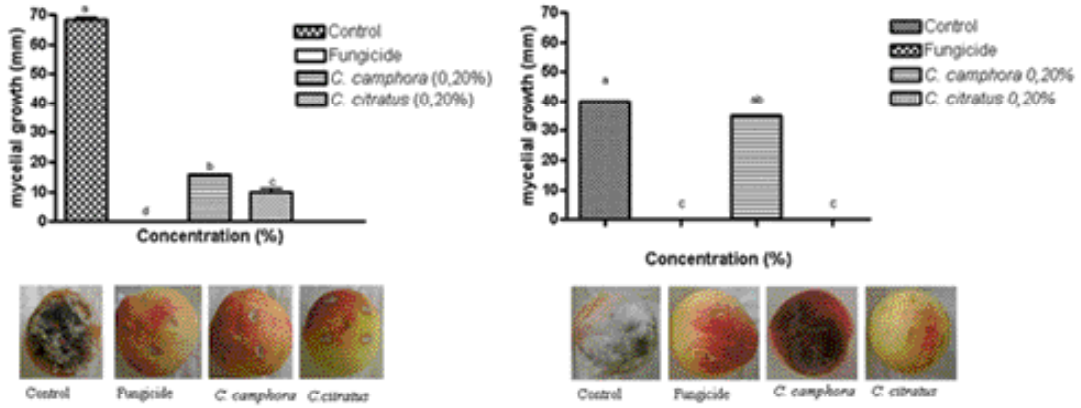

Figure 3. Test *in vivo,* in cold storage, with M. fructicola **(A)** and *C. gloeosporioides* **(B)** using essential oils using fungicide, and the essential oils of *C. camphora* and *C. citratus* to 0.20% on the 20th day of evaluation.

For the tests in cold, with *C. gloeosporioides,* the best performers were the essential oil of C. citratus, which showed inhibition of 85.7%, but with less control compared to the fungicide Orthocide (100%). While for the decay caused by *M. fructicola*, the results show that the essential oil of *C. citratus* control features similar to the fungicide tested (100%), contrary to what was reported by CARVALHO et al. (2009). This author showed that only the treatments with iprodione dichloran and disease severity remained significantly low, suggesting that it was because the fungicides have higher residual effect in relation to essential oil of clove and chlorine dioxide, tested for the control of *M. fructicola* and Rhizopus in post harvest of peaches.

The post-harvest is an indeterminate amount of time between harvest and consumption, in which there is a fundamental concern about losses caused by pathogens. *Monillinia fructicola*, and *Colletotrichum gloeosporioides*, are causal agents of brown-rot and anthracnose, respectively, which are latent in the fruit waiting appropriate conditions of temperature and humidity to attack. These diseases are the main cause of lost in marketing peach in world. This culture that first grown-up in China almost 4.000 years ago, has now spread worldwide. In Brazil the annual production is around 100.000 T (MADAIL e RASEIRA, 2008). The only control of these fungal problems in this moment is through chemical pesticides (fungicides), often indiscriminately.

One strategic way for substituting these chemicals, which normally cause health problems, is the utilization of natural products, such as essential oils. This technology is not new (ANTUNES e CAVACO, 2009). Essential oils are obtained from plant parts by distillation with water vapor or by expression of the pericarp of citrus fruits. They are called oils because of their oily-looking liquid state at room temperature, volatile and lipophylic. They are called essential because of their intense aroma in the majority of representatives (VITTI e BRITO, 2003). For instance, the investigation of inhibition of mycelial growth in vitro of *Glomerella cingulata* with essential oils of chamomile (*Matricaria chamomilla*), ginger (*Zingiber officinale*) and leaves of guava (*Psidium guajava*) and *Colletotrichum gloeosporioides* with chamomile (*Matricaria chamomilla*), guava (*Psidium guajava*) and marigold (*Tagetes patula*) were done with good results (ROZWALKA et al., 2008).

In similar work, ROSWALKA (2003) observed a 100% inhibition of mycelial growth of *C. gloeosporioides* until the 5th day, reducing their potential for inhibition to 62.77% on the 8th day of treatment with the essential oil of lemongrass (*C. citratus*). Marques et al. (2003) obtained, in the experiment *in vitro* with citral diluted to 60% (v/v) and the essential oil of *C. citratus* 50% (v/v), a total growth inhibition of the pathogen *C. gloeosporioides*, thereby showing that the essential oil of *C. citratus* was more efficient than the monoterpene. While ASTOLFI et al. (2007), working with the essential oil of *Cinnamomum camphora*, observed that the variation of the minimum inhibitory concentration for Gram-positive bacteria were 1.75 mg mL^{-1} (*Staphylococcus aureus*) to 2.50 mg mL^{-1} (*Staphylococcus epidermidis*) and gram-negative bacteria were 0.625 mg mL^{-1} (*Citrobacter freundii*) to 2.50 mg mL^{-1} (*Shigella flexneri*), proving to be a good antimicrobial agent.

Some strategies have been used for control *M. fructila in vitro* conditions. For instance, XU et al. (2008) had demonstrated that the utilization of *P. membranaefaciens* yeast with acid acetylsalicylic at 0-10ºC showed moderate effect under mycelial growth.

The methyl jasmoneate (MeJa) had a little inhibitory effect on the mycelia growth of *M. fructicola*. The MeJA alone did not reduce brown rot on sweet cherries (YAO e TIAN, 2005) 25º C and 0º C. Still, the extract from *Coptis chinensis* (a Chinese herb named "Huang Lian") demonstrates a strong inhibition to *M. fructicola*. The 50% effective concentration (EC50) of *C. chinensis* extract against *M. fructicola* was only 0.91 mg mL^{-1}. The EC50 and minimum inhibitory concentration (MIC) against *M. fructicola* were as low as 4.5 mg mL^{-1} and 46.9 mg mL^{-1}, respectively. In addition, the strong *in vivo* inhibition of berberine against *M. fructicola* was observed with no visual cytotoxicity, noted to peach fruits, even at berberine concentration of 400 mg mL^{-1}. Note that this was much higher than its MIC value (46.90 mg mL^{-1}).

In comparison, MARQUES et al. (2003) showed that the monoterpene citral 38.3% inhibited the mycelial growth of *C. gloeosporioides*, causal agent of anthracnose in papaya. A significant reduction was found in the concentration of 1.5% (v/v), where the inhibition was 61.1%, and the essential oil of *C. citratus* inhibited by 18.6 and 19. 9%, respectively, showing an inhibition less efficient than the monoterpene. While benomyl showed an inhibition of 71.6%.

The effect of curing treatments at different temperatures, exposure times and relative humidity (RH) to control brown rot was studied *in vivo*. Curing at 50° C for 2 h successfully increased brown rot control (95%) after fruit were incubated at 20° C and 85% RH for 5 d after treatment.

Another biocontrol agent was evaluated by LIU et al. (2006), for controlling post-harvest peach brown rot caused by *Monilinia fructicola.* Their best result was the significant activity, after five days of incubation at 22° C, of *Bacillus sp*. C06, which suppressed brown rot incidence by 92% and reduced lesion diameter by 88%, compared to the pathogen-only check.

In a study performed by SESTARI et al. (2008), in which were tested: UV-C radiation, ozonation, the application of calcium chloride, sodium hypochlorite, lime and potassium phosphite for control of postharvest decay of peaches (cv. Precocinho) during refrigerated storage (storage cold conditions), any treatment were effective to control decay, demonstrating the necessity of developing new alternatives to control these diseases. From the results obtained, it was proven the existence of biologically active secondary compounds with antifungal effect, but it is important to note some factors such as origin, botanical identification, collection, treatment plant and quality (MING, 1994). Also, how to use the plant material (dried or fresh), the extraction methods, as well as the concentrations used, will result in greater efficiency and credibility of results (ROSWALKA et al., 2008).

Conclusion

The essential oils of *C. camphora* and especially *C. citratus*, besides being a natural biological product, may have potential use for controlling fruit decay caused by *C. gloeosporioides* and *M. fructicola* in post-harvest peaches, with the advantage of minimizing the use of conventional fungicides to preserve the environment and consumer health protection.

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