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Carboxymethylcellulose coating associated with essential oil can increase papaya shelf life

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ABSTRACT

The use of essential oils (EO) associated with coatings in the post-harvest treatment of papaya is a little studied alternative to avoid post-harvest losses. Therefore, the antifungal activity of *Eucalyptus staigeriana, Lippia sidoides* and *Pimenta pseudocaryophyllus* essential oils (EOs) was tested *in vitro* against *Colletotrichum gloesporiodes*, the causal agent of anthracnose in papaya. The EO with the highest activity was evaluated regarding its chemical composition, *in vivo* activity and its effects on papayas post-harvest quality, when associated with a carboxymethylcellulose coating. *L. sidoides* EO presented the highest *in vitro* antifungal activity, with thymol as the predominant compound in its composition. *In vivo*, the fruit treated with CMC associated with *L. sidoides* EO presented a reduction in disease severity and maintained post-harvest parameters, besides slowing the appearance of rot and shriveling in the fruit on the ninth day of storage, whereas in the control and treatment with only CMC, this behavior occurred on the fifth and seventh days, respectively. Thus, the association of *L. sidoides* EO with CMC was effective in the rise of papayas shelf life, preserving their post-harvest characteristics for nine days, indicating that this treatment can be considered a viable alternative for the extension of the fruit commercialization period.

1. Introduction

Papaya (*Carica papaya* L.) is considered one of the fruits with the highest importance worldwide, for being nutritious, since it contains high contents of ascorbic acid, pro-vitamin A, calcium and carotenoids, and for preventing the occurrence of degenerative diseases such as cancer, heart diseases and arteriosclerosis (Ali et al., 2011; Ong et al., 2013). In 2016, the world production of papaya reached 12.85 million tons, being Brazil the second biggest producer, with 11% of the world production (FAOSTAT, 2018).

This fruit is climacteric, has a short post-harvest life and during ripening becomes much more susceptible to infections by pathogens, because of the reduction in pericarp resistance and the increase in pulp softening, water availability and sugars (González- Aguilar et al., 2009). The main post-harvest deteriorating fungus that attacks the fruit is *Colletotrichum gloeosporioides*, which causes the disease named anthracnose, which severely depreciates the fruit, reducing its shelf life and market value (Ayón-reyna et al., 2017). The most employed method for reducing rot incidence in fruits, including papaya, is the application of synthetic fungicides; nevertheless, this practice may pose serious threats to consumers and the environment (Maqbool et al., 2011). Additionally, there has been a rise in claims for products and foods which, throughout their production chain, have not required the use of products that attack health and the environment. In this context, the use of essential oils (EO) as antimicrobial agents is a natural and safe alternative and can contribute to shelf life extension of these products (Trajano et al., 2009). EOs are natural, chemically complex, with variable composition and can be extracted from various parts of the vegetables (Reyes-Jurado et al., 2015; Rehman et al., 2016).

Many studies have demonstrated the potential of EOs from different plant species in phytopathogen growth inhibition (Combrinck et al., 2011; Reyes-Jurado et al., 2015; Liu et al., 2016). However, the use of EO in food preservation can be limited because of its application costs, its intense aroma and the potential toxicity from some compounds that are present. An interesting alternative to decrease EO concentration, reducing aroma and maintaining its efficiency, can be the association of the oils with the formulation of edible coatings (Perdones et al., 2012). The edible coatings are thin layers of a material made from biodegradable ingredients, which might be consumed as a part of the food. They are especially used to improve appearance and expand fruit

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preservation, since they provide selective barriers against respiration and the loss of moisture (Ali and Mahmud, 2007; Ali et al., 2011). Carboxymethylcellulose (CMC), derived from cellulose, has excellent properties for this purpose, acting as an efficient barrier to the permeation of ambient oxygen, besides being non-toxic and with a low cost (Tongdeesoontorn et al., 2011).

Some studies have evaluated the antifungal power of EOs on *Colletotrichum* sp. (Elshafie et al., 2016; Espana et al., 2017). Nevertheless, studies on the antifungal activity of EOs extracted from leaves of the species *Eucalyptus staigeriana*, *Lippia sidoides* and *Pimenta pseudocaryophyllus* on fruit-deteriorating fungi in the post-harvest are scarce, despite the proven antimicrobial action of these oils (Yokomizo and Nakaoka-Sakita, 2014; Herculano et al., 2015). Therefore, this study evaluated the *in vitro* antifungal activity of these EOs on *C. gloesporiodes*, and the one with the highest activity was determined. The chemical composition and the effect of the EO association with CMC was evaluated *in vivo* and its effects on the post-harvest quality parameters of papaya were also verified.

2. Material and methods

2.1. Essential oils and acquisition of the C. gloesporiodes isolate

The EOs were extracted from the leaves of *E. staigeriana* (Itatinga - SP, Brazil, 24°59′34.8″S 48°41′14.4″W), *L. sidoides* (Campinas - SP, Brazil, 22°47′42.5″S 47°06′40.4″W) and *P. pseudocaryophyllus* (Cananéia - SP, Brazil, 25°00′257″S 47°56′56″W) by hydrodistillation in a Clevenger equipment for 4 h at a maximum temperature of 100 °C until they reached boiling. Subsequently, the EOs were dried in anhydrous sodium sulfate and stored at −5 °C in an amber glass flask.

The pathogen was obtained from the direct isolation of fungal structures from infected papaya, derived from a conventional cultivation in Sooretama (ES, Brazil, 19°11′49″S 40°05′52″W). The fungus was cultivated in Potato-Dextrose-Agar (PDA) and maintained for 15 days in a growth chamber at 25 °C and photoperiod of 12 h. After growth, the fungus was morphologically identified by the method established by (Sutton, 1992).

2.2. In vitro antifungal activity of the EOs

The EOs were individually evaluated, in two binary combinations and one ternary combination, at the concentrations 0; 31; 62.5; 125; 250 and 500 μl L^{-1}, by the method of dilution in agar (Plaza et al., 2004). Thus, the following treatments were employed: *E. staigeriana*, *L. sidoides*, *P. pseudocaryophyllus*, M1 (*L. sidoides* + *E. staigeriana)*; M2 (*L. sidoides* + *P. pseudocaryophyllus);* M3 (*E. staigeriana* + *P. pseudocaryophyllus)*, M4 (*L. sidoides* + *P. pseudocaryophyllus* + *E. staigeriana)* and the control (without any EO or mixtures). Five repetitions were used for each concentration evaluated and the experiment was performed 4 times to confirm the results.

The pathogen was inoculated as a suspension of *C. gloesporiodes* containing 10^5 spores mL^{-1}. The treatments were maintained at 25 °C, with a 12-hour photoperiod, for 18 days, in an incubator of the BOD type. Mycelial growth was measured every two days (cm), from the diameter of each colony, taken in two perpendicular directions from each other, and the arithmetic mean of these two measures was calculated. To obtain the Minimum Inhibitory Concentration (MIC), the diameter of the fungal mycelial growth (mm), on the last day of incubation, was modeled using the sigmoidal function of the model of Gompertz (Gompertz, 1825). To select the best model, the following criteria were assumed: the lowest value of the mean square of the residue, independence and homogeneity of residues and the highest value of the coefficient of determination (R^2).

2.3. Chemical composition of the EO

The chemical composition was determined for the EO or mixture that presented the lowest MIC, and was performed by gas chromatography coupled to mass spectrometry, using a CGMS 2010 equipment (SHIMADZU). The volatile compounds were identified by the comparison of their Kovats Indexes (KI) and the calculated and observed mass spectra, with data published in the literature (Adams, 2007) and with the libraries of existing mass spectra (NIST, WebBook, NIST 07 and WILEY 8). Only the peaks with presence higher than 1% of the total chromatogram area were identified.

2.4. In vivo antifungal activity

The antifungal activity of the EO or mixture that obtained the lowest MIC was verified preventively in an *in vivo* test, in association with CMC coating. A CMC emulsion at 0.25% (Trigo et al., 2012) with purity (dry basis) of 99.74%, degree of substitution of 0.86, moisture of 6.2%, pH (solution at 1%, 25 °C) of 7.1 and viscosity (solution at 1%, 25 °C) of 2760 cP, was prepared with distilled water at 60 °C and constant stirring of 2000 rpm in a mechanical homogenizer (Fisatom - 713D) for 10 min.

The papayas were obtained from an organic cultivation in Poço Fundo (MG, Brazil, 21°46′59″ S, 45°57′13″ W), selected and sanitized by immersion in a sodium hypochlorite solution (200 μl L^{-1}) for ten minutes. Subsequently, some of the fruit were submerged, for 1 min, in a CMC solution containing EO; another portion of fruit was submerged in a CMC solution and fruit without any coating were used as control treatment. The fruit were then maintained at room temperature until the coating was dried.

The inoculum was performed with the deposition of 50 μl of a suspension of spores (10^6 spores mL^{-1}), on a 3-mm deep wound on the peel of those fruit, with a sterilized phytopathological needle. After this step, the fruit were stored in a wet chamber and incubated for 48 h at 25 °C with a 12-hour photoperiod. After this period, the fruit were removed from the wet chamber and stored at the same conditions of temperature and photoperiod.

The experimental design in this experiment was in a 3 × 6 factorial scheme, involving three treatments: Control (C); fruit with only CMC coating (F) and fruit with coating associated with EO (FEO); in six periods of evaluation (1, 2, 3, 4, 5 and 6 days after removal from the wet chamber). Each treatment consisted in four repetitions, each one composed of eight papayas.

The antifungal activity of the EO associated with CMC was measured by the disease incidence and severity in the inoculated fruit. Incidence (%) was evaluated at naked eye, by the presence or not of symptom in the point of inoculum, calculated based on the number of symptomatic fruit in relation to the total number of fruit in each treatment (Canaver and di Piero, 2011). Severity was evaluated by the measurement of lesion diameter (mm), in orthogonal directions, with a graduated digital caliper. From the mean value of lesion diameter over time, in each repetition, the Area Under the Disease Progress Curve (AUDPC) was calculated, according to (Simko and Piepho, 2012): $AUDPC = \Sigma\ [(yi + yi + 1)/2 \times (ti + 1 - ti)]$, in which yi refers to the mean diameter of the lesion at time ti; $yi + 1$ to lesion diameter at time $ti + 1$.

The results relative to AUDPC were evaluated by the program Statistical Analysis System model 9.3 (S. A. S Institute, 2010) and subjected to the analysis of variance (ANOVA) for the F test. The standard deviation of the means was calculated and the statistical difference of the means, at the significance level of 5% ($p < 0.05$), was determined by the Tukey test.

2.5. Effect of CMC associated with EO on the post-harvest quality of papaya

In this experiment, organic papayas (363.57 ± 69.78 g) were used