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Effect of propolis on postharvest control of anthracnose and quality parameters of 'Kent' mango

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We dedicate this article to Dr. Marie-Noëlle Ducamp-Colin—*in memoriam*.

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ABSTRACT

The objective of this study was to evaluate the efficacy of propolis treatment for reducing anthracnose development on mango fruit of variety Kent; and compare its efficacy to chitosan treatment, which is already a well-studied natural produce. For *in vitro* experiments, Petri plates were amended with (0, 0.5, 1.0, 1.5, 2.0 and 2.5% v/v) propolis or (0, 0.25, 0.5, 1.0, 1.5 and 2.0% w/v) chitosan solutions. For the *in vivo* experiments, mangoes were infected with a spore suspension of *Colletotrichum gloeosporioides* and solution of either propolis (1.5%) or chitosan (1.5%) were used for controlling the pathogen development. The fruits were stored for 14 days at 12 ± 1 °C and 83 ± 2% RH, and then, for another 7 days at 23 °C, 82%. Lesion area, firmness, chemical composition, as well as color of the skin were used for evaluating and compare the performance of the two products. Even if the *in vitro* results demonstrated the net superiority of propolis for controlling the development of the pathogen, the *in vitro* results showed the opposite order when classifying the performance of the products with alive fresh produce. The results obtained suggested that propolis, as well as chitosan, may be used as treatment for controlling anthracnose, maintaining quality, and increasing the shelf life in 'Kent' mango. However, propolis was far from producing competitive results to chitosan treatment performance.

1. Introduction

Mango (*Mangifera indica*) is among the most widely produced fruit in the world (FAOSTAT, 2013). Among its cultivars, 'Kent' is dominating the production as it is widely accepted by consumers due to its excellent sensory attributes such as flavor, aroma, and attractive color (Robles-Sánchez et al., 2009). 'Kent' is a late ripening cultivar that produces big and oval fruit (650–850 g) with a reddish yellow–green peel; very sweet, aromatic, juicy and fibreless pulp; and small seed (Ángel et al., 2006).

Mango is a climacteric fruit. While ripening, it becomes much more susceptible to pathogen infections, due to the decrease in peel resistance and the increase of pulp softening, availability of water and sugars (Pfaffenbach et al., 2003).

Among postharvest diseases, anthracnose, caused by the fungus *Colletotrichum gloeosporioides*, is the most important biological constraint which restricts mango production in any tropical and subtropical regions around the world (Kamle et al., 2013; Tucho et al., 2014), and is the most serious disease of mango worldwide (Diedhiou et al., 2014). In unripe fruit, this disease acts as a quiescent infection and may manifest its symptoms only during or after the ripening process, when conditions for pathogen development are more favorable (Nelson, 2008; Iram and Ahmad, 2013) resulting in heavy postharvest losses (Kamle et al., 2013), up to 60% from some production regions (Devamma et al., 2014).

Disease control is normally performed using chemical products. However, due to the harmful effects of fungicides on the environment and human health, agrochemical application has been reduced. Furthermore, chemical application could induce the development of pathogen resistance to the active ingredients

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(Soares et al., 2008); therefore, alternative control strategies have to be developed.

Studies on the use of natural products for postharvest disease control of fruit and vegetables have shown promising results, indicating the possible development of natural fungicides, which are as effective as the synthetic ones, with the advantage of being safer to human and environment (Tripathi and Dubey, 2004). Natural products may present an antimicrobial effect, or act directly against phytopathogens *via* the inhibition of mycelia growth, spore production and/or germination. As they contain bioactive molecules, they may also act as resistance inducers that are able to either induce or activate plant defense mechanisms (Stadnik and Talamini, 2004).

Propolis, a resinous material collected by bees from plant exudates, has recently aroused the interest of scientists for the study of its constituents and biological properties (Sforcin and Bankova, 2011). Propolis application, either alone or in association with other treatments, has shown positive effects on the shelf life of fresh horticultural produce. Dragon berries (*Hylocereus polyrhizus*), when immersed in a propolis ethanolic extract at 0.5% concentration, presented an increase in shelf life without affecting their quality attributes (Zahid et al., 2013). Edible films of hydroxypropyl methylcellulose supplemented with propolis, produced an antimicrobial effect against the development of *Aspergillus niger* (Pastor et al., 2010). *In vitro* tests of Potato Dextrose Agar (PDA) combined with Chinese propolis alcoholic extract controlled the development of the fungus *Penicillium italicum* (Yang et al., 2011).

In recent years, chitosan, a high molecular mass polysaccharide which is soluble in organic acids, has been widely tested and used as edible film for fruit postharvest quality conservation. The natural coating formed by chitosan acts as a selective barrier for gas exchange, which results in reduced fruit metabolism and extends shelf life. For example, treatments with chitosan delayed ripening and extended shelf life in guava fruit during cold storage (Hong et al., 2012). In addition, antimicrobial properties have been identified for chitosan (Bautista-Banõs et al., 2006). Studies demonstrate that this polymer limited rots caused by *Botrytis cinerea* in 'Itália' grapes (Camili et al., 2007), strawberries (Romanazzi et al., 2013) and tomatoes (Badawy and Rabea, 2009).

The use of GRAS products (Generally Recognized As Safe), such as chitosan, is an ecologically sound alternative for controlling important pathogenic microorganisms. Applications of natural, safe, and environmentally friendly coatings could result in reduction of impact of postharvest diseases such as decrease of quality, reduction shelf life, and loses of fresh produce.

A particular attention should be given for comparing the results of different treatments in term of efficacy. As the concentration of an antifungal product is increased, the growth rate (GR) of the pathogen is expected to decrease. Based on this desirable result, the definition of the efficacy for controlling a pathogen is generally presented by a capability for reducing the GR of the pathogen (Iram and Ahmad, 2013; Diedhiou et al., 2014); the maximum efficacy being a GR of zero. By obtaining a higher efficacy as GR get smaller results on a negative slope (mm day^{-1}) calculated from the graphical representation of the size (mm) of the pathogen colony against the time (days or hours) required for reaching each colony dimension. The efficacy GR-based makes comparison more difficult to visualize generating confusion when comparing the GR-based efficacy of different treatments of products and/or concentrations. However, on a mathematics point of view, the efficacy could be redefined in a more positive term such as the efficacy value increases as the based-efficacy number increases. This could be obtained by inversing the GR value (mm day^{-1}) resulting on a GR^{-1}, which would be the growth pace (GP, day mm^{-1}), or the ration of the time (days) a mycelial colony was growing over the size (mm) it reached.

The objectives of this study were to evaluate the effect of propolis and compare its efficacy to chitosan treatments and control samples on anthracnose (*C. gloeosporioides*) development during *in vitro* and *in vivo* test using quality parameters of 'Kent' cultivar of mango.

2. Materials and methods

2.1. Raw material

Fresh harvested mango fruits of the cultivar 'Kent' at the commercially mature-green state were transported by plane from the Ivory Coast to Montpellier, France within 36 h after harvested. Mangoes of uniform mass (400–500 g), shape, and maturity, and free from any indication of mechanical injury, insect or pathogenic infection, were selected for the experiment.

The propolis product was from ethanolic extract at a concentration of 30% dried matter with 50% total soluble solids; flavonoids 20 mg L^{-1}; artepillin-C: 10.5 mg L^{-1}; ρ-coumaric acid 2.5 mg L^{-1}, obtained from Bioessens^{TM}, São Paulo, Brazil. The chitosan was a deacetylation degree 90%, viscosity 20–50 cps, density 0.6 g mL^{-1}, and obtained from France Chitine (Orange, France).

2.2. Microorganism and inoculum preparation

C. gloeosporioides [P85] was obtained from Culture Collection (Laboratory of Plant Pathology and Microbiology, Food Technology Institute, Campinas, Brazil) as the test microorganism.

A *C. gloeosporioides* spore suspension was prepared by growing fungus on PDA plates maintained at 29 °C for at least 14 days, and harvesting spores by suspension in 9 mL of 0.85% NaCl solution supplemented with Tween 80 at a concentration of 100 μL L^{-1}. The spore concentration was measured using a Malassez hemacytometer and adjusted by dilution using sterile saline solution until reaching a count of 10^5 *C. gloeosporioides* spores mL^{-1}. They were stored at 4 °C in tubes until they were used later in the same day.

2.3. In vitro experiments

Potato Dextrose Agar plates were amended with propolis diluted at concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5% v/v using Tween 80 (0.01%) as a surfactant. These concentrations were selected based on preliminary experiments performed in the laboratory. The control plates contained only PDA.

Chitosan solutions of 0.25, 0.5, 1.0, 1.5 and 2.0% w/v were prepared by dissolving 0.5, 1.0, 1.5 and 2.0 g of chitosan in 100 mL distilled water containing 0.5 mL (v/v) of lactic acid. To ensure the dissolution of chitosan was completed, the chitosan solution was heated at 70 °C and agitated constantly for 2 h. The pH of the solution was adjusted to 5.6 with NaOH 1 M. An acidic solution (pH 5.6), without chitosan, was used as a control. These solutions were infused into PDA plates.

The antifungal assays of propolis and chitosan were carried out based on the inhibition in radial mycelial growth and conidial germination of *C. gloeosporioides* on PDA using a "poison food technique". An agar disk (5 mm diameter) from a pure culture of *C. gloeosporioides* was placed at the center of 67.5 mm-diameter PDA plates and used as the control essay; while the 10 treatments consisted of adding to each PDA plate containing an infected disk one solution out of the 10 propolis or chitosan concentrations. Petri plates were incubated at 23 °C for at least 7 days. The radial mycelial growth was calculated as the average of two perpendicular diameter measurements with a transparent plastic ruler (±0.5 mm) on each sample showing any measurable colony development on a daily basis until at least one Petri dish out of the control samples was fully covered with mycelia. Six replicates for each treatment or control were performed for each antifungal product and concentration.