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Essential oils as an alternative postharvest treatment to control fusariosis, caused by *Fusarium verticillioides*, in fresh pineapples (*Ananas comosus*)

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ABSTRACT

Fusariosis of the pineapple is an aggressive disease which needs to be controlled during postharvest. Essential oils have been studied with the intention of incorporating them into integrated pest management, to avoid or reduce the use of synthetic fungicides. In-vitro assays showed that thyme oil was the best essential oil for controlling mycelial growth of *Fusarium verticillioides*. Because of its fungicidal effect, four concentrations of thyme oil (100, 250, 500 and 1000 μL L^{-1}) were tested in-vivo. The results showed that after 21 d at 8 °C plus 7 d of shelf-life at 20 °C, the reduction of the severity of *F. verticillioides* on pineapples treated with 1000 μL L^{-1} of thyme oil (50.1%) was higher ($p < 0.05$) than with other treatments. Moreover, application of 1000 μL L^{-1} thyme oil treatment reduced mass loss, and retained color and firmness of fruit. Treated fruit also showed a low translucency index and delayed changes in total soluble solids, titratable acidity, potential of hydrogen and maturity index. Sensory parameters also scored better in 1000 μL L^{-1} thyme- oil treated fruit than in untreated controls during cold storage. These results suggest that thyme oil may potentially be used for controlling fusariosis in pineapples during postharvest, without negative effects on its physicochemical and sensory qualities.

1. Introduction

Pineapple (*Ananas comosus* L.) is a tropical and subtropical non-climacteric fruit that is well known for its juiciness texture, high nutritional value, and pleasant flavor (Liu et al., 2017). Its production in Ecuador ranks third after bananas and mangoes (FAO, 2016). However, due to the prompt water loss and its active metabolism, pineapple deteriorates quickly after harvest (Wijesinghe and Sarananda, 2002; Hong et al., 2013).

The improvement of effective methods to preserve postharvest quality, including treatments using controlled atmosphere (Kader, 1994), heat (Wilson-Wijeratnam et al., 2005), 1-methylcyclopropene (Selvarajah et al., 2001), waxing (Hu et al., 2012) and postharvest salicylic acid (Lu et al., 2011) have been widely reported. However, cold storage is the predominant method used to slow fruit deterioration and to maintain nutritional values in terms of consumer conception (Bartolomé et al., 1995). Since low temperature induces chilling injury symptoms in pineapple, the range of optimal temperature of conservation is around 7.5–12 °C (Paull and Chen, 2003).

Moreover, this fruit is susceptible to a number of fungal diseases, of which fusariosis caused by *F. verticillioides* is the most severe (Stepien et al., 2013). In pineapple, this disease is one of the most destructive during the postharvest period, and during preharvest it affects virtually all parts of the plant (Ploetz, 2003). Affected areas on fruit arise off-color, become sunken, are light to dark brown, and are covered with mycelium of the pathogen and a brownish exudate (Ploetz, 2006).

Before storing the fruit at the packinghouse, pineapple is usually treated with a chemical fungicide prochloraz to control several pathogens during the postharvest period (Abdullah, 2011).

However, the application of synthetic fungicides to control postharvest degradation of fruit commodities would have to be regulated in several countries, due to their possible negative effects like carcinogenicity, teratogenicity, high and acute toxicity, long persistence, environmental pollution and side effects on human beings (Feng and Zheng, 2007). Consumers demand attractive fruit, free from diseases and toxic residues. Moreover, the minimum residue limits present in the comestible portion of the fruit are often limited by strict regulations of importing countries. Thus, the search for new decay control strategies is necessary, and natural antifungal compounds such as essential oils are gaining increasing attention as alternative means for controlling rot (Sellamuthu et al., 2013a; Farzaneh et al., 2015).

In a novel study, aimed at decreasing the negative consequences of agrochemicals, the potential of natural products with bioactive compounds and their use as tools in fungal pathogen control have been

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shown (Oliveira de Souza et al., 2015). For prolonging fruit shelf-life and in order to preserve fruit quality essential oils have been used as antifungal compounds, and can be used as food preservatives to reduce microbial spoilage (Guerreiro et al., 2015). Hence, essential oils, due to their natural antimicrobial activity, could be a promising advance to reduce the risk of chemical fungicide handling to control postharvest decay in fruit (Maqbool et al., 2011). The effectiveness of essential oils treatments will be determined by natural resistance of the fruit commodity. Essential oils inhibit pathogens mainly due to their direct effect on spore germination and mycelial growth by altering the cellular metabolism of the microorganisms (Regnier et al., 2010; Sivakumar and Bautista-Baños, 2014). Their application has no risk to human health, presents antioxidant properties (Sivakumar and Bautista-Baños, 2014), increases fruit quality and extends shelf-life (Plaza et al., 2004). However, one of the limitations of the use of natural products from plant extracts is the likelihood of inducing alterations in fruit sensory quality. Fruit acceptability could decrease due to off-flavor, and changes in aroma and flavor caused by the intense odor of essential oils (Perdones et al., 2012; Sangsuwan et al., 2016).

For all these reasons, this work consists of four objectives. First, to examine the in-vitro effect of several essential oils by means of the control of radial mycelial growth of *F. verticillioides* and to select one essential oil based on its inhibition effect; second, to test the effect of essential oil at different concentrations on decay reduction in artificially inoculated fruit (*in-vivo*); third, to determine the effect of essential oil, at different concentrations, on physicochemical quality, and finally to analyze the impact of the more effective essential oil concentration on sensory parameters during pineapple cold storage period.

2. Materials and methods

2.1. Plant material and essential oils

Pineapple (*Ananas comosus* var. MD-2) fruit was harvested at a commercial orchard located in Santo Domingo de los Tsáchilas in Ecuador, and rapidly transferred to the pathology laboratory. Upon arrival, fruit was selected based on uniformity in fruit size and color without physical damage (0.5 matured grade following the scale range explained below). The grade of ripeness was defined by visual appreciation of the peel. The scale ranges from 0 to 5 with 0: all eyes were totally green; 1: < 20% of the eyes were predominantly yellow; 2: 20–40 % of the eyes were tinged with yellow; 3: up to 65% of the eyes were predominantly yellow; 4: 65–90 % of the eyes were fully yellow; and 5: > 90% of the eyes were fully yellow and no more than 20% of the eyes were reddish orange (Hong et al., 2013).

Essential oils of thyme (*Thymus vulgaris* L.; main volatile compounds: thymol, carvacrol and *p*-Cymene); mint (*Mentha* x *piperita* L.; main volatile compounds: menthol, menthone and menthyl acetate); rosemary (*Rosmarinus officinalis* L.; main volatile compounds: 1,8-cineole, α-pinine and camphor) and lavender (*Lavandula angustifolia* Mill.; mail volatile compounds: linalool, ocimene and terpin-4-ol) were used in this study. Essential oils with a purity of 100% were obtained from Azolea oils (Quito, Ecuador). Information about volatile compounds was provided by Azolea oils.

2.2. Pathogen strain

F. verticillioides strain HPN-16 was isolated from infected pineapple fruit in the Food Science and Biochemistry Department laboratory at the Escuela Politécnica Nacional (Quito, Ecuador) and identified by DNA amplification of Internal Transcribed Spacer (ITS) region (results not shown). Potato dextrose agar medium (PDA) (Difco™, Le Pont de Claix, France) was used for the maintenance of strain and the culture was sustained at 25 °C for 10 d.

2.3. In-vitro antifungal activity of essential oils on F. verticillioides mycelial growth

Antimicrobial tests were performed according to Soylu et al. (2010), with modifications, in order to assess the impact of essential oils *F. verticillioides* HPN-16. For determining contact effect, each essential oil with 10 μL L^{-1} Tween 80 (0.001% (v/v)) was diluted and dispersed into PDA media, previously sterilized at 121 °C for 15 min, at 100, 250, 500 and 1000 μL L^{-1}. Essential oils were added when medium was still warm. Then, it was dosed into glass Petri dishes (90 × 20 mm in diameter) at 40–45 °C of temperature. The controls received the same amount of PDA without any essential oil. *F. verticillioides* HPN-16 was inoculated, by plating a mycelia streak of the fungus obtained from actively growing cultures on PDA dishes in the center of each plate.

Petri dishes, sealed with Parafilm M®, were incubated in the dark at 25 °C. The mean radial mycelial growth of the pathogen was determined by measuring the diameter of the colony in two perpendicular directions, 6 and 12 d after inoculation.

Four replicate plates were used for each concentration. Mean growth values were obtained and then converted to the inhibition percentage of mycelial growth in relation to the control treatment using the formula, MGI (%) = [(dc-dt)/dc] x 100; dc and dt represent mycelial growth diameter in control and treated Petri plates, respectively. No mycelial growth, it was reported as a fungicidal effect, while temporary inhibition of mycelial growth and growth of fungus resumed after 12 d of inoculation, it was reported as a fungistatic effect.

2.4. In-vivo effects of essential oil on decay development in artificially inoculated pineapples

Following the harvest, fruit were immersed completely in a sodium hypochlorite solution (5%) (Fast Concentrated Chlorite, Quito, Ecuador) for about 2 min, rinsed with tap water, and allowed to air-dry at room temperature (Karaca et al., 2014).

Spores were removed from the surface of the cultures, suspended in 5 mL of sterile distilled water containing 0.05% (v/v) Tween80, and filtered through four layers of sterile cheesecloth in order to remove any adhering mycelia. Spore concentration of *F. verticillioides* HPN-16 was determined using a hemocytometer and adjusted to 10^6 conidia mL^{-1} by adding sterile distilled water.

Surface-disinfected pineapples were punctured twice in the equatorial zone with a sterile steel rod with a probe tip 3 × 3 mm^2 and 3 mm deep. An aqueous suspension of *F. verticillioides* HPN-16 (100 μL) at 10^6 conidia mL^{-1} was applied to each puncture and allowed to dry for 2 h (Zhang et al., 2007). Puncture-inoculated fruit were divided into six groups: i) CK (Control fruit): pre-inoculated and non-treated; ii) CK + F: pre-inoculated and sprayed with the synthetic fungicide prochloraz (3 mL L^{-1}); the rest of the treatments were pre-inoculated and sprayed with essential oil at: iii) 100 μL L^{-1}; iv) 250 μL L^{-1}; v) 500 μL L^{-1} and vi) 1000 μL L^{-1}. Solutions of essential oil were prepared with 10% (v/v) of Tween80. Pineapples were sprayed until to cover their whole surface, crown included (approximately 3 mL per fruit).

For each treatment, five pineapples constituted a single replicate and every test was replicated four times. After treatment, all fruit were stored at 8 °C for 21 d (relative humidity RH = 80%), plus 7 d at 20 °C (RH = 85%) to simulate shelf-life period. Rot dynamics information was obtained by measuring the diameter of the lesion and expressed as a severity (mm) produced by *F. verticillioides* HPN-16, as presence of decay, after 7, 14 and 21 d of cold storage, plus a 7 d of shelf-life. The experiment was repeated two times.

2.5. In-vivo effects of essential oil on postharvest physicochemical quality

The effect of the essential oil on postharvest quality parameters of pineapple was tested. Firstly, freshly harvested fruit was artificially inoculated, treated and then stored as described above in the