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Short communication

Antimicrobial activity of chitosan enriched with lemongrass oil against anthracnose of bell pepper

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ABSTRACT

Essential oils have been investigated as potential additives for polysaccharide based coatings. In this study the effectiveness of combining lemongrass essential oil with chitosan as an edible coating for bell pepper was examined. Lemongrass essential oil at concentrations of 0.5% and 1.0% was incorporated into 0.5% and 1.0% chitosan solution and evaluated as a means of controlling anthracnose of bell pepper in vitro and in vivo. Fungal growth was effectively controlled by 0.5% and 1.0% EO in vitro. The application of 1.0% chitosan was determined as an effective concentration, and was used in subsequent in vivo analysis as a base coating for maintaining the safety and quality of fresh bell peppers stored at room temperature for 21 days. EO was found to be less effective in vivo, however the combination of EO with CH did enhance the antimicrobial activity of the coating. Quality of the fruits, as determined by weight loss, firmness, colour, SSC and TA, was maintained throughout the storage by the chitosan coating solely and in combination with EO. The in vivo results demonstrated that, as an edible coating, the application of 1.0CH + 0.5 EO was significantly better at maintaining the fruit quality, however, with the anthracnose disease incidence results in consideration, chitosan individually was more effective in the extension of fruit shelf life.

1. Introduction

The shelf-life of fresh fruits and vegetables in tropical and subtropical areas is widely affected by anthracnose disease. A variety of Colletotrichum species are responsible for causing anthracnose in various fruits, with the major causal agent of anthracnose in bell pepper being *Colletotrichum capsici* (Pakdeevaraporn, Wasee, Taylor, & Mongkolporn, 2005). Anthracnose has been primarily controlled by the application of fungicides. Nonetheless, numerous negative effects on the environment and human health have been attributed to the persistent application of chemical fungicides (Ranasinghe, Jayawardena, & Abeywickrama, 2003). This has increased consumer awareness of food safety and prompted demands for healthier and environmentally friendly food products.

Various edible formulations are increasingly applied as environmentally conscience and healthy alternatives for fungicides to maintain the quality of fresh produce. Edible coatings create a modified atmosphere around the perishable

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product, allowing the extension of shelf life through the control of gas exchange and prevention of excessive moisture loss. Chitosan has been applied as a biodegradable edible coating for numerous tropical fruits for the control of anthracnose (Ali, Tengku, Kamaruzaman, & Siddiqui, 2010; Perdones, Sánchez-González, Chiralt, & Vargas, 2012; Zahid, Ali, Manickam, Siddiqui, & Maqbool, 2012). The water vapour permeability of chitosan based coatings can be improved by incorporation of lipid substances such as essential oils to lower the incidence of fruit dehydration during storage (Perdones et al., 2012).

Incorporation of essential oils in polysaccharide based edible coatings has gained interest in the agricultural sciences owing to the bactericidal and fungicidal properties associated with these volatile compounds (Maqbool, Ali and Alderson, 2010). An essential oil of particular interest is lemongrass essential oil which contains flavonoids and phenolic compounds (Abu-seif, Abdel Fattah, Abo Sreia, Shaaban, & Ramadan, 2009). The antimicrobial activity of lemongrass essential oil has been demonstrated against a range of microorganisms, and a few studies incorporated this essential oil into edible coatings applied on fruits such as banana and papaya (Maqbool et al., 2011); and pineapple (Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan, 2014). Moreover, lemongrass essential oil has been successfully used in various formulations for the control of anthracnose of a range of fruits such as avocado (Mpho, Sivakumar, Sellamuthu, & Bautista-Banos, 2013); banana and papaya (Maqbool et al., 2011); and mango (Duamkhanmanee, 2008).

Biopesticides play an important role in the protection of anthracnose susceptible fruits such as bell pepper and there is a constant urge to enhance the activity of existing treatments. The incorporation of essential oils in chitosan coatings has been regarded as an alternative option for the control of anthracnose and it is a postharvest tool that is worthy of further exploration. Therefore, this study aims to enrich chitosan with lemongrass essential oil, for the additive antimicrobial benefits associated with lemongrass oil, and to test it as a potential postharvest treatment against anthracnose of bell pepper.

2. Materials and methods

2.1. Plant material

Physiologically mature green bell peppers (*Capsicum annuum* L.) were purchased from a commercial market in Semenyih, Malaysia and were selected for uniformity in size and shape, and absence of external injury. Fruits were washed with tap water, followed by 0.05% sodium hypochlorite, rinsed with distilled water and air-dried at ambient temperature with the assistance of a standing fan (Ali, Maqbool, Ramachandran, & Alderson, 2010; Ali, Tengku, et al., 2010). The healthy fruits were then divided into six groups of fruits selected for uniformity in colour and size.

2.2. Isolation of Colletotrichum capsici

C. capsici was isolated from bell pepper fruits with anthracnose symptoms. The pathogen was isolated from diseased tissues and incubated on Potato Dextrose Agar (PDA) medium at 25 °C for 7 days. The colonies were then sub-cultured and the identity of the fungus was confirmed based on growth morphology patterns and cultural characteristics; cultures with sickle shaped spores of size 17–18 by 3–4 μm were identified as *C. capsici* (Barnett & Hunter, 1972; Chanchaichaovivat, Ruenwongsa, & Panijpan, 2007). The selected colonies were selected and continuously sub-cultured on fresh PDA dishes and Koch's postulates were used to verify their virulence and pathogenicity.

2.3. Preparation of chitosan and lemongrass oil coating

Chitosan solutions of concentrations 0.5% and 1.0% at pH 5.6–5.9, using 1 N NaOH, were prepared using low molecular weight chitosan (CH) (Ali, Maqbool, et al., 2010; Ali, Tengku, et al., 2010). Following that, 0.5% (v/v) and 1.0% (v/v) lemongrass essential oil (EO) were added to both chitosan solutions to prepare a total of four different chitosan solutions (0.5% CH with 0.5% EO, 0.5% CH with 1.0% EO, 1.0% CH with 1.0% EO and 1.0% CH with 0.5% EO). Tween 80 (R&M Chemicals, Malaysia) was incorporated to enhance both the water vapour permeability (WVP) and mechanical properties. To ensure incorporation of the lemongrass essential oil in chitosan, the solutions were mixed using a homogeniser (IKA RW 14 basic, Germany) for 15 min set at 1500 rpm. Fruits were dipped into the respective solution for 2 min and allowed to air dry with the assistance of a standing fan.

2.4. In vitro antifungal assay

The antifungal activity of the four solutions of chitosan and essential oil was assayed in vitro by mixing the solutions with PDA in liquid form. An additional five treatments were prepared as controls: 0.5% CH, 1.0% CH, 0.5% EO, 1.0% EO and PDA only. From the pure *C. capsici* cultures, fungal plugs (2 mm) were taken and positioned on the centre of the PDA plates. The plates were incubated at 25 °C for 21 days, during which mycelial growth was monitored and daily measurements were taken. The results were presented as percentage of mycelial growth inhibition as compared to control.

2.5. In vivo antifungal assay

Pure *C. capsici* cultures were incubated at ambient temperature until sporulating cultures were observed and the concentration was determined using a haemocytometer (Hirschmann EM Techcolor, Germany) and diluted to obtain a spore suspension of 1×10^5 spores/ml (Perdones et al., 2012). Ten microlitres of the prepared spore suspension were dropped onto holes punctured on the fruits using a sterile cork borer of a diameter of 5 mm, before the coatings were applied on the fruits. Based on the in vitro experiment, the treatments for in vivo experiments were selected as 1.0% CH; 0.5% EO; 1.0% EO; 1.0% CH + 0.5% EO; 1.0% CH + 1.0% EO; untreated control. The fruits were incubated in open air for 24 h before storage in corrugated boxes at room temperature for 21 days. Disease incidence (DI) was assessed on a weekly basis and was expressed as the percentage of fruits bearing the anthracnose symptoms out of the total number of fruits per treatment.