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Influence of chitosan coating combined with spermidine on anthracnose disease and qualities of 'Nam Dok Mai' mango after harvest

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ABSTRACT

Chitosan (CTS) combined with spermidine (SPD) was applied as fruit coating for 'Nam Dok Mai' mango (*Mangifera indica* L.) compared with CTS and SPD treatments after harvest and stored at 25 ± 2 °C for 9 days. Influence of all treatments on anthracnose disease and qualities of mango fruit was investigated after inoculation fruit with *Colletotrichum gloeosporioides*. Inoculated fruit coated with 1% CTS combined with 0.1 ppm SPD exhibited the smallest area of lesion development (0–1 cm); while non-coating inoculated fruit presented the most severe fungal decay (4–5 cm). Furthermore, inoculated fruit coated with 1% CTS combined with 0.1 ppm SPD showed higher plant defense mechanisms than control and other treated fruits. These phenomena were represented by the production of high levels of H_2O_2 and phenolic compounds during storage and the induction of defense enzyme activities including chitinase, β-1,3-glucanase and peroxidase whereas fruit treated with only CMS or SPD expressed lower effect on induction of plant defense mechanisms. Therefore, synergistic effect of chitosan and spermidine combination can increase the ability to inhibit anthracnose disease development on 'Nam Dok Mai' mango fruit. Delayed mango fruit softening by 1% CTS combined with 0.1 ppm SPD was in correlation with reduced soluble pectin content during ripening stage. The results obtained suggested that 1% CTS combined with 0.1 ppm SPD had potential to improve firmness and delay deterioration processes of 'Nam Dok Mai' mango fruit after harvest.

1. Introduction

Around the world, mango (*Mangifera indica* L.) is a popular tropical fruit with its high qualities and nutritional properties. Mango is an important export fruit of Thailand to which the national total production reached 33,000 tons in 2015 (Office of Agricultural Economics (OAE), 2016: online). However, mango is a climacteric fruit, making it perishable after harvest during the ripening process. Furthermore, a major disease of mango fruit that causes reduction of fruit qualities in both preharvest and postharvest is anthracnose stemming from *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Akem, 2006). This disease reduces shelf life of mango tremendously. Therefore, treatments used to control the pathogen development are essential for maintaining fruit qualities.

Chitosan, a deacetylated form of chitin, is a natural carbohydrate polymer (Rinaudo, 2006). Chitosan has been eagerly used in agriculture because of its advantageous effects on plant growth and its biodegradable property (Pichyangkura and Chadchawan, 2015). Postharvest coating is well recognized as an effective application on fruits to extend shelf life and maintain fruit quality during transportation and storage (Olivas et al., 2008; Mattiuz et al., 2015; Munhuweyi et al., 2017). Chitosan could create modified atmosphere when used as fruit coating which could lead to an enhanced shelf life and maintain qualities of fruit during storage. Generally, pathogenesis-related (PR) proteins are defense enzymes that are induced in plants by pathogen infection (Ohashi and Oshima, 1992). PR proteins can be separated into 17 families by protein property (Sels et al., 2008). Chitinase, β-1,3-glucanase and peroxidase (POD) are major PR proteins that gene expressions are also increased when plant is attacked by pathogens. Phenylalanine ammonia-lyase (PAL) is one of the major enzymes in plant defense mechanism that activates after pathogen infection (Passardi et al., 2004). Previous studies indicated that chitosan coating had the potential to prolong shelf life by inducing antioxidant properties, reducing respiration rate, ethylene production, and transpiration and controlling decay of many fruits and vegetables (Hong et al., 2012; Dhall, 2013; Zhang et al., 2013; Jongsri et al., 2016a,b). Chitosan was reported as an exogenous elicitor that could induce plant defense mechanism by increasing activity of defense enzymes such as chitinase, β-1,3-glucanase

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and POD in chitosan coated grapevine leaves, mango and pear fruits (Trotel-Aziz et al., 2006; Jitareerat et al., 2007; Meng et al., 2010). Preharvest chitosan spray and postharvest chitosan coating significantly induced the activities of PAL and POD of table grape fruit while activities decreased in control fruit. Thus, chitosan promoted protection in grape fruit against latent infection of pathogens (Meng et al., 2008). In addition, levels of H_2O_2, lignin, phytoalexin and phenolic compounds were increased in many plants treated with chitosan (Bhaskara-Reddy et al., 1999; Agrawal et al., 2002). However, the physiological differences of fruit ripening phenomenon in response to chitosan treatment could be due to the molecular weight of the applied chitosan. Jongsri et al. (2016b) reported that among the chitosan coatings tested on 'Nam Dok Mai' mango fruit, 1% high molecular weight chitosan (Mw. 360,000 Da) could prolong shelf life of 'Nam Dok Mai' mango fruit by delaying respiration rate and ethylene production and inhibiting disease occurrence.

Polyamines (PAs), mainly diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm), are organic compounds that are present in all living organisms (Handa and Mattoo, 2010). PAs also play a role in the developmental processes in plant, such as morphogenesis, fruit ripening and responses to biotic and abiotic stresses (Galston and Sawhney, 1990; Ziosi et al., 2006). Changes in PAs content have been correlated with fruit growth during the cell division stage of several annual and woody crops, suggesting that biosynthesis is associated with post-fertilization growth and development of ovary tissues (Slocum and Galston, 1985). During climacteric fruit ripening, pectin is the major component of the primary cell wall and middle lamella which are degraded by pectin degrading enzymes leading to fruit softening (Posé et al., 2015). Polygacturonase (PG) and pectin methyl esterase (PME) are mainly cell wall degrading enzymes that cause the reduction of cell wall structure (Sozzi, 2004). In 1992, Charney et al. studied the effect of three PAs (Put, Spd and Spm) on PME activity in soybean and orange. They presented that PAs could inhibit PME activity by interacting with negative charges of pectic substrate that condition the binding of PME. This report indicated that PAs could control the esterification of pectin within cell wall. Therefore, PAs might have a role in all regulatory mechanisms in which cell-wall enzymes were involved. Furthermore, exogenous PAs could induce levels of endogenous PAs in litchi fruits so it could delay browning, peroxide level and ethylene production (Jiang and Chen, 1995). Previous study by Santivipanond et al. (2012) presented that 0.1 ppm spermidine was the best treatment for maintaining firmness and delaying chemical changes of 'Hom Thong' banana fruit. Jongsri et al. (2016a) used a combination of 1% chitosan and 0.1 ppm spermidine to coat 'Nam Dok Mai' mango and resulted in higher firmness than other treatments. However, there are still only few reports about the effect of polyamine on postharvest response of mango fruit.

Although chitosan and polyamines have been known to control decay and prolong storage life in fruit and vegetable, no reports have been published regarding the use of the combination of chitosan and spermidine to control postharvest disease in mango. The coating application as antimicrobial compounds, elicitor or protective of decay needs to be further explored. We hypothesized that combining spermidine at 0.1 ppm and 1% chitosan coating could present a greater result in the defense mechanisms and delay deterioration processes on 'Nam Dok Mai' mango.

2. Materials and methods

2.1. Plant material

Mature green mango fruit (*Mangifera indica* L. cv. Nam Dok Mai) was harvested from a commercial orchard in Nakornratchasrima province (90–100 days after fruit set). Fruit was selected for uniformity in size, color, shape and without any blemishes and disease symptoms and transferred to the Laboratory within 4 h.

2.2. Fungal isolation and culture

The phytopathogenic fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. was isolated from diseased tissues of symptomatic mango fruit and identified by morphological technique (Barnett and Hunter, 1998) and molecular technique (White et al., 1990; Bunyard et al., 1994) by DNA sequencing performed by National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. Koch's postulate was used to confirm the causal agent of the disease (Juangbhanich, 1988). Pure culture was grown on potato dextrose agar (PDA) at room temperature for 7 days before usage.

2.3. Experimental treatments

Fruit was washed under running water and left to air dry in the laboratory. Mango fruit was surface sterilized by immersion in 70% ethanol for 1 min and prepared for inoculation by inflicting one 1-mm-deep wound in the middle of each fruit with a sterile needle. Each wound was then inoculated with 10 µL conidial suspension (10^6 conidia/mL) of *C. gloeosporioides*. The inoculated fruit was incubated overnight at room temperature before dipping treatments (Yenjit et al., 2010). The experiment comprised of 6 treatments: (1) control treatment (inoculated with distilled water) (2) inoculated with *C. gloeosporioides* then dipped in distilled water (3) inoculated with *C. gloeosporioides* then dipped in 0.5% acetic acid solution (4) inoculated with *C. gloeosporioides* then dipped in 1% chitosan (CTS) solution (A.N. Lab Thailand: Mw. 360,000 Da, %DD = 84.90 ± 0.72) combined with 0.1 ppm spermidine (SPD) (Sigma) (5) inoculated with *C. gloeosporioides* then dipped in 1% CTS solution (6) inoculated with *C. gloeosporioides* then dipped in 0.1 ppm SPD solution. After treatments, fruit was stored at 25 ± 2 °C for 9 days. There were 3 replications in each treatment and each replication consisting of 2 samples. The result was measured every 3 days during storage.

2.4. Disease severity

The diameter of lesion (cm) was measured and calculated after inoculation every 3 days (Jitateerat et al., 2007).

2.5. Physico-chemical analysis

2.5.1. H_2O_2 content

One gram of mango pulp was grinded in liquid nitrogen then cold phosphate buffer (pH 6.5) containing hydroxylamine was added. Tube content was mixed and centrifuged at 8000 rpm for 25 min at 4 °C. Supernatant was added with 0.3% titanium sulphate in 20% H_2SO_4 (v/v) and centrifuged at 8000 rpm for 15 min at 4 °C. H_2O_2 content was measured at 410 nm by spectrophotometrically assay following Jana and Choudhuri (1982). According to standard curve, H_2O_2 content was shown as µmol/g FW.

2.5.2. Total phenolic content

The Folin-Ciocalteu assay, adapted from Ramful et al. (2010), was used for determining total phenolic present in the fruit extracts. One gram of mango pulp was added to 80% methanol. Tube content was centrifuged at 9000 rpm for 20 min at 4 °C. Plant extract and distilled water were mixed with Folin-Ciocalteu reagent (Merck) and incubated at room temperature for 3 min. After incubation, 20% sodium carbonate was added to tube and incubated for 40 min in a water bath at 40 °C. The absorbance of the blue coloration formed was read at 685 nm. Results were expressed in mg of gallic acid/g FW.

2.5.3. Phenylalanine ammonia-lyase activity

One gram of mango pulp was used for analyzing following the method of D'Cunha et al. (1996). Mango pulp was homogenized with extraction buffer (Tris-HCl pH 7.0) then centrifuged at 13,000 rpm at