Impacts of Paraffin Wax and Propolis on Controlling Crown Rot Disease and Maintaining Postharvest Quality of Banana

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Abstract. Crown rot disease is a major cause of losses during transportation, storage, and marketing of banana. In this study, the effects of paraffin wax, propolis extract and their combination on controlling crown rot disease and maintaining the quality of banana cv. Hom Thong were investigated. Crown of banana fruit were coated with 50% propolis, 100% paraffin wax and 100% paraffin wax combined with 50% propolis. Banana crown treated with 250 ppm prochloraz and non-coated fruit were used as the controls. All treatments were packed in corrugated box, stored at 13°C for 21 days (simulation for the transportation and storage) and then moved to ambient temperature for 6 days (simulation for the retail market). Results found that coating with paraffin wax combined with propolis showed the efficacy on controlling crown rot disease as same as using 250 ppm prochloraz. While coating with paraffin wax or propolis alone showed the incidence and severity of disease similar to non-coated fruit. Beside, coating with paraffin wax alone or combined treatment of paraffin wax and propolis could maintain some plant resistant mechanisms such as the content of total phenolic and the activity of phenylalanine ammonia lyase (PAL) in the crown of banana. For the quality of banana showed that coating with paraffin wax alone or the combination of paraffin wax and propolis were able to reduce weight loss, maintain firmness and color change of banana crown, and it did not affect color change of peel and firmness of banana fruit.

Keywords: Banana, Coating, Crown Rot Disease, Paraffin Wax, Propolis

1. Introduction
Banana is one of the most important fruit due to its high nutritive value, beneficial for health and multiple uses. In Thailand, there are several commercial cultivars, with ‘Hom Thong’ being the most popular for both domestic and export markets. However, this cultivar is susceptible to several diseases, especially crown rot disease. Crown rot is the most important postharvest disease of banana that can result in 10-86% losses [1]. This disease caused by several fungi including Colletotrichum musae, C. gloeosporioides, Lasiodiplodia theobromae, Alternaria triticina, Cladosporium oxysporum and Fusarium spp. [2; 3]. The rot symptoms begins with the tissues become black and soft, followed by mycelial development on the surface of the crown and penetrated to the finger stalks and progress to fingers. The application of chemical fungicide such as prochloraz and thiabendazole are the most commonly used method for controlling crown rot disease [1]. However, frequency uses of some fungicides have been problems due to development of disease resistance and potentially harmful
effects on the environment and human health. Therefore, the non-chemical treatments for disease suppression are necessary to the study.

Wax coatings are often used to extend the postharvest shelf life of fresh produce. The wax coating can block the opening of stomata and lenticels of fruits and vegetables, resulting to reduce the transpiration and respiration rate and also provide the barrier to microbial penetration [4]. In our previous studied the application of some coating substance including paraffin, shellac and sucrose fatty acid ester have been used for controlling crown rot disease. The result indicated that paraffin showed the most effective to reduce crown rot disease. Paraffin wax is obtained in the process of petroleum distillation, which it have been used to extend the shelf life of several fresh produce such as mango [5], lime [6] and banana [7]. In recent years, wax coatings have been developed with the addition of antimicrobial compounds against postharvest disease of fresh fruits and vegetables.

Propolis is a natural product from honey bees, with it known for having strong antibacterial, antifungal and antiviral properties [8]. Pobiega et al. (2019) reported that the components of propolis including several phenolic compounds such as cinnamic acid, caffeic acid, ferulic acid and flavonoids. Our previous studied found that 50% propolis showed completely inhibited mycelial growth of crown rot pathogens including C. musea and Fusarium sp.. Nowadays, the application of propolis have been used for prolonging the shelf life and improving the quality of various agricultural products. Moreover, the propolis extract can be added to the wax coating to improve the effectiveness of the wax for controlling disease. Ali et al. (2014) showed that a mixture of gum arabic and 1.5% propolis successfully reduced postharvest disease of papaya fruit. Similar to report of Barrera et al. (2015) who found that the combination of 1% chitosan and 5% propolis could reduce postharvest disease and maintain the quality of papaya during storage.

Therefore, the objective of this research was to investigate the effects of paraffin wax combined with propolis to control crown rot disease and maintain the postharvest quality of banana.

2. Materials and Methods

2.1. Preparation of Propolis
The propolis sample were collected from Ratchaburi province, Thailand. The frozen propolis were ground to fine powder in liquid nitrogen with a mortar and pestle. The powder was mixed with 70% ethanol and continuous shaking at 150 rpm for 72 h. The mixture was filtered with Whatman paper No. 1 and kept at 4°C in the dark condition.

2.2. Plant Materials and Experimental Procedure
Banana cv. Hom Thong at 75-80% maturity were harvested from the commercial orchard located in Pathumthani province, Thailand. The hand of banana were selected according to the uniformity of shape, weight (1.5-2.0 kg) and free from any diseases and damages. Banana samples were washed with tap water and dipped in 200 ppm sodium hypochlorite solution for 5 min. The crown surface of each hand was re-cut using a sterilized knife and air dried at ambient temperature. The sample were divided into 5 groups; the first group was non-coated fruit (control 1) and the second group was the banana crown were coated with 250 ppm prochloraz (control 2). Other three groups including the banana crown were coated with 50% propolis, 100% paraffin wax and 100% paraffin wax combined with 50% propolis, respectively. Afterward, all sample were dried about 1 h before packed in corrugated box, stored at 13°C and 85-90% RH for 21 days and then stored at 25°C for 6 days at simulated for the market conditions. Sampling was carried out every 7 days during stored at 13°C and every 2 days after moved to 25°C to evaluate incidence and severity of crown rot disease and quality of fruit including; weight loss, firmness of crown and fruit, color change of crown and peel and determined total phenolic content and PAL activity. Each treatment has three replications and one replication has two banana hand.

2.3. Incidence and Severity of Crown Rot Disease
Incidence of crown rot disease was expressed as a percentage of crown showing symptoms of discoloration and mycelial growth on the crown area. The severity of disease was assessed by the extent of total rotten area on each banana crown using 0-5 point scale as the follow: 0 = on discoloration and no mycelial growth on the crown area, 1 = 1-10%, 2 = 11-40%, 3 = 41-70%, 4 = 71-100% of discoloration or mycelial growth on the crown area and 5 = discoloration or mycelial growth extended to finger stalks [11].

2.4. Fruit Quality
Weight loss was determined as a percentage using the formula as (initial weight of banana – final weight of banana) / initial weight of banana x 100. Firmness of fruit and crown were measured using a texture analyzer (Model; TA-XT2, Stable micro-system, England). Fruit firmness was measured on the middle of fruit, with 5 mm diameter cylinder and penetrated to a depth of 5 mm at a rate of 10 mm per min. For the banana crown was measured on the cut surface using 5 mm diameter cylinder and penetrated to a depth of 5 mm at a rate of 20 mm per min. The average value of firmness of each fruit and crown were calculated and expressed in Newton (N). Color changes of peel and crown of banana were measured with a colorimeter (Model CR-400, Konica Minolta, Japan). The results were calculated and reported as ΔE value.

2.5. Total Phenolic and PAL Activity
Tissue of the banana crown were taken for analysis total phenolic and activity of PAL. The crown of sample were cut into small pieces, added liquid nitrogen to frozen and kept at -20°C until analysis. The extraction of total phenolic was 5 g of the crown tissue homogenized in 20 mL of 80% ethanol (v/v). The homogenate was centrifuged at 15,000 x g for 30 min at 4°C, then supernatant was collected for analysis. The content of total phenolic was determined according to the method of Singleton and Rossi (1965). The reaction mixture including; 1.58 mL of water, 100 µL of Foline Ciocalteu reagent and 300 µL of sodium carbonate. The mixture was mixed well before incubated at 37°C for 30 min and measured the absorption at 765 nm using spectrophotometer. The content of total phenolic was expressed as mg100 g$^{-1}$ fresh weight. For PAL activity, 5 g of sample was mixed with 50 mM sodium borate buffer (pH 8.8) and 0.2% polyvinylpolypyrrolidone (PPVP), then homogenized. The homogenate was centrifuged at 17,000 x g at 4°C for 30 min. The activity of enzyme was analyzed by mixing 1 mL of the extract with 2 mL of sodium borate buffer and 1 mL of 20 mM L-phenylalanine and mixed well. Then incubated at 37°C for 60 min and stopped the reaction by adding 1 mL of 1M hydrochloric acid. The absorption was measured using spectrophotometer at 290 nm and the result was expressed as the unit/ mg protein. Total protein in each tissue sample was measured following as the method of Bradford (1976).

3. Results and Discussion

3.1 Incidence and Severity of Crown Rot Disease
The combination treatment of paraffin wax and propolis significantly reduced both incidence and severity of crown rot disease as same as using 250 ppm prochloraz. Disease incidence developed faster in the non-coated (control 1), propolis and paraffin wax alone than the combined treatment and prochloraz (control 2). At day 14 of storage, the non-coated and the individual of propolis and paraffin wax treatments had 50% disease incidence, while the sample treated with the combined treatment and prochloraz did not show any visible decay until day 21 of storage. However, all treatments showed 100% of disease incidence when the samples were stored at 25°C (Figure 1A). In addition, the combined treatment of paraffin wax and propolis showed the efficacy on reduced severity of crown rot disease similar using prochloraz. By the end of storage, the combination and prochloraz treatments had 2.25 and 2.00 score of severity, while the single treatment of paraffin wax and propolis had 3.25 and 3.50 score, which no significant differences when compared with the control (3.50 score) (Figure 1B). These results indicate that a combination of paraffin wax and propolis treatment enhanced the benefits
of applying each treatment separately to reduce crown rot diseases in banana. This effect of the combined treatment on the reduction of disease might be attributed to coating cut surface of banana crown with paraffin wax could barrier against pathogenic infection, concurrently a coating material containing propolis, which propolis are very important source of antimicrobial activity [8].

![Figure 1](image1.png)

**Figure 1.** Effect of banana crown coated with 50% propolis, 100% paraffin wax and 100% paraffin wax combined with 50% propolis on the incidence (A) and severity (B) of crown rot disease during stored at 13°C for 21 days and 25°C for 6 days. Non-coated (control 1) and coated with 250 ppm prochloraz (control 2) treatments were used as the control. Vertical bars represent ± SE for triplicate samples.

3.2 Total Phenolic Content and PAL Activity

Phenolic compounds have been known as one of the indicator of plant defence. This result showed that total phenolic of the banana crown tended to decrease during storage. The highest level of total phenolic were found in the sample coated with paraffin alone and paraffin combined with propolis. Whereas, coating with propolis alone or prochloraz showed the low total phenolis content similar to non-coated treatment (Figure 2A). This result was correlated with the activity of PAL. PAL is the key enzyme involved in the biosynthesis of phenolic compound [14]. In this study found that PAL activity showed a continued deceased during storage. The single of paraffin wax and paraffin wax combined with propolis treatments provided a significance higher in the activity of PAL enzyme than non-coated treatment. After day 21 of storage, PAL activity in the control and propolis treatments showed a sharp decrease while the activity of PAL in the sample coated with paraffin wax and paraffin wax combine with propolis tended to remain until the end of storage (Figure 2B).

![Figure 2](image2.png)

**Figure 2.** Effect of banana crown coated with 50% propolis, 100% paraffin wax and 100% paraffin wax combined with 50% propolis on total phenolic content (A) and PAL activity (B) of banana crown during stored at 13°C for 21 days and 25°C for 6 days. Non-coated (control 1) and coated with 250 ppm prochloraz (control 2) treatments were used as the control. Vertical bars represent ± SE for triplicate samples.
3.3 Firmness and Color Change of Banana Crown

Crown tissue softening and discoloration are the important symptoms that corresponded to the severity of crown rot disease on banana fruit. In this study found that the application of paraffin wax combined with propolis and paraffin wax alone could delay crown softening and discoloration similar to using prochloraz. However, during storage at 13°C for 21 days a significant differences in the softening and discoloration of crown were not observed when compared among treatments. Thereafter, the sample were stored at 25°C the highest of firmness and lowest of color change of banana crown (ΔE value) were found in paraffin wax alone, paraffin wax combined with propolis and prochloraz. In contrast, the fruit coated with propolis alone showed the lowest of firmness and highest of color change of banana crown as same as non-coated fruit (Figure 3A-3B).

![Graph showing firmness and color change of banana crown](image)

**Figure 3.** Effect of banana crown coated with 50% propolis, 100% paraffin wax and 100% paraffin wax combined with 50% propolis on firmness (A) and color change (ΔE value) (B) of banana crown during stored at 13°C for 21 days and 25°C for 6 days. Non-coated (control 1) and coated with 250 ppm prochloraz (control 2) treatments were used as the control. Vertical bars represent ± SE for triplicate samples.

3.4 Quality of Banana

Quality of banana fruit after treated were investigated by observing weight loss, TSS content, fruit firmness and change in the color of banana peel. Weight loss of banana in all treatments increased progressively with extended storage time. Paraffin wax combined with propolis significantly reducing weight loss as same as fruit coated paraffin wax alone. Whereas, the sample coated with propolis alone and prochloraz showed on a significant difference compared with the control (Figure 4A). The effect of coating to reduce weight loss may be resulted by paraffin wax acts as the barrier to prevents the moisture loss and it have been reported the application of coating films on the reduction of weight loss were related to slowing down the respiration rate of fruit [16]. Moreover, coating films created a modified atmosphere around the fruit by providing a barrier to O₂ and CO₂, thereby reducing respiration and transpiration rates [17]. TSS content increased during banana ripening. Coating with paraffin wax, propolis and their combination did not effect on TSS content when compared with the control (Figure 4B). This result was correlated with decreased of firmness during period of storage. However, significant difference was not found among them (Figure 5A). Yellowing of the banana peel is one of physical manifestation of ripening that can be quantitatively indicated based on increases in ΔE value (Figure 5B). Color of the banana peel was changed very fast during storage, especially when the sample were stored at 25°C. However, a significant differences were not observed when compared among treatments. Thus, these results indicated that banana crown coated did not effect to the ripening of banana fruit during storage.
Figure 4. Effect of banana crown coated with 50% propolis, 100% paraffin wax and 100% paraffin wax combined with 50% propolis on weight loss (A) and TSS content (B) of banana during stored at 13°C for 21 days and 25°C for 6 days. Non-coated (control 1) and coated with 250 ppm prochloraz (control 2) treatments were used as the control. Vertical bars represent ± SE for triplicate samples.

Figure 5. Effect of banana crown coated with 50% propolis, 100% paraffin wax and 100% paraffin wax combined with 50% propolis on fruit firmness (A) and color change (ΔE value) of banana peel (B) during stored at 13°C for 21 days and 25°C for 6 days. Non-coated (control 1) and coated with 250 ppm prochloraz (control 2) treatments were used as the control. Vertical bars represent ± SE for triplicate samples.

4. Conclusion
The combination treatment of paraffin wax and propolis could reduce crown rot disease similar to use 250 ppm prochloraz and maintain some plant defense mechanism such as total phenolics and PAL activity. Coating with paraffin wax alone and paraffin wax combined with propolis were able to reduce weight loss, maintain firmness and color change of banana crown, and it did not affect to color change of peel and firmness of banana fruit.

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