

Effect of stingless bee propolis (*Heterotrigona itama*) coating on reducing anthracnose and preserving postharvest quality of wax apple (*Syzygium samarangense*)

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Abstract

Water extract propolis (WEP) was evaluated as an edible coating to reduce anthracnose in wax apples (*Syzygium samarangense*) due to its antifungal properties. The antifungal effects of WEP at concentrations of 10%, 12%, and 14% were assessed *in vitro* and *in vivo*. Coated wax apples were stored at 10°C with 80–90% relative humidity for 15 days, with evaluations conducted at three-day intervals. In the *in vitro* test, growth inhibition of *Colletotrichum gloeosporioides* increased with WEP concentration, with inhibition rates of 41.67%, 46.67%, and 50.00% for 10%, 12%, and 14% WEP, respectively. Similarly, *in vivo* experiments demonstrated that all tested WEP concentrations completely inhibited anthracnose development in artificially inoculated fruits stored under the same conditions. Additionally, changes in fruit weight loss, total soluble solids (TSS), and disease severity index were analysed. Fruits coated with 14% WEP exhibited significantly lower weight loss (1.5%) on day 15 ($p < 0.05$), indicating that higher concentrations of WEP effectively create a moisture barrier, preserving fruit quality. The application of WEP as a fruit coating significantly reduced anthracnose development, maintained fruit quality, and has the potential to enhance the market value of wax apples.

1. Introduction

Wax apple (*Syzygium samarangense*), which is a non-climacteric tropical fruit, is also known by names such as rose apple, Java apple, and water apple. It is cultivated widely across tropical and subtropical regions, including Malaysia, Indonesia, Thailand, and China (Simirgiotis *et al.*, 2008). In Malaysia, wax apple is primarily grown by smallholders, with an estimated 3,300 hectares under cultivation, generating an annual production value of over USD 9 million (Al-Obaidi *et al.*, 2018). Although having a relatively low respiration rate, wax apple is highly perishable due to its edible and delicate skin, which makes it particularly vulnerable to fungal infections. One of the most common diseases affecting this fruit is anthracnose. Initial reports of anthracnose in wax apple have identified *Colletotrichum* species as the causal agents, including *C. gloeosporioides* in Malaysia (Al-Obaidi *et al.*, 2017) and *C. siamense* in China (Yao *et al.*, 2023). Infected fruits typically exhibit pale-brown

to dark, sunken circular lesions that lead to pre-harvest rot. *Aspergillus niger* has also been found in diseased wax apple samples collected in Johor, Malaysia (Al-Obaidi *et al.*, 2023). Currently, the disease is controlled using chemical fungicides such as mancozeb, carbendazim, and chlorothalonil (Lee, 2005). Although these treatments are effective, long-term use of these chemicals raises concerns about environmental contamination, human health risks, and the development of pathogen resistance. As a result, there is growing interest in safer and more sustainable alternatives, particularly biocontrol methods. Recent studies have shown promise in using natural compounds to manage fungal infections in wax apples. For instance, treatments incorporating aloe vera, cinnamon oil, and salicylic acid have demonstrated efficacy in controlling black rot (Supapvanich *et al.*, 2017; Rosli *et al.*, 2022). Additionally, microbial agents such as *Brevibacillus brevis* have shown potential as biological control agents (Jianme Che *et al.*, 2015). Among the emerging natural

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alternatives, propolis, a resinous substance produced by bees, has attracted increasing attention. In Malaysia, the rise of stingless beekeeping has resulted in a growing supply of propolis, which is often considered an agricultural byproduct. Notably, propolis exhibits strong antioxidant and antimicrobial properties, even higher than those of honey (Ahmad *et al.*, 2019). The two main stingless bee species reared locally are *Heterotrigona itama* and *Geniotrigona thoracica*, with *H. itama* showing superior antibacterial and antioxidant activity (Ibrahim *et al.*, 2016; Putra *et al.*, 2025). These bioactivities are largely attributed to its high phenolic content (Woźniak *et al.*, 2020). Previous studies have demonstrated the antifungal efficacy of propolis against pathogens affecting crops such as bananas (Mohamad Areff *et al.*, 2022). Although ethanol-based propolis extracts show better properties (Putra *et al.*, 2025), water-based extracts are more environmentally friendly and cost-effective, especially for large-scale agricultural applications. In fact, water extracts of propolis have been successfully used to manage disease in strawberries (Omar *et al.*, 2020). Given its bioactive potential and sustainable availability, propolis could serve as an alternative strategy for managing anthracnose in wax apples. Therefore, this study aims to investigate the antifungal effects of water-extracted *H. itama* propolis, both in vitro and in vivo, with the goal of reducing disease severity and preserving the postharvest quality of wax apples.

2. Materials and methods

2.1 Sample preparation

Raw propolis was collected from a beekeeper from Marang, Terengganu. Samples were cleaned of any defects, debris and dead bees. Cleaned propolis was stored at a blast freezer at -80°C for a few h before it was crushed down into powder (Sun *et al.*, 2015). Fresh wax apples were selected for the uniformity in size, shape and the presence of physical injuries. The wax apples were washed with tap water and rinsed with distilled water, and left air-dried at room temperature.

2.2 Extraction of propolis

Propolis powder was mixed with sterile distilled water at a ratio of 1:10 and agitated on a water bath shaker at 150 rpm for 3 days. The mixture was centrifuged and filtered with Whatman Grade No. 1 filter paper with the help of a vacuum filter. The solvent in the mixture was dried in a rotary evaporator (Buchi R-200) for about 3 to 4 h and left in a water bath at 50°C until the solvent had totally evaporated. All final extracts were stored at -20°C until used.

2.3 Source of pathogen and inoculum preparation

The *Colletotrichum* sp. obtained from the Culture Collection of Laboratory for Pest Disease and Microbial Biotechnology (LAPDiM), Universiti Malaysia Terengganu. Only *Colletotrichum* species that had been confirmed as a pathogen (anthracnose disease) were chosen and subcultured onto potato dextrose agar (PDA) to confirm fungus viability. The pure culture was grown for 10 days at 25°C. The *Colletotrichum* cultures were flooded with sterile distilled water and gently dislodged the spores using a glass rod. The spore suspensions obtained were filtered to remove mycelial fragments (Lane *et al.*, 2012). A haemocytometer was used to obtain 2×10^6 conidial/mL of spore suspension.

2.4 In vitro assay

The *in vitro* analysis of water extract propolis (WEP) was done using the poison plate technique (Fiori *et al.*, 2000). Different concentrations of WEP (10% WEP (v/v), 12% WEP (v/v) and 14% WEP (v/v)) were tested against *Colletotrichum* sp. The WEP was added to the PDA after it cooled down from the autoclave, mixed by shaking the bottle, then gently poured into petri dish plates. A 5 mm diameter disc from seven-day-old *Colletotrichum* cultures was used and placed at the centre of the poison plates agar in an aseptic condition. All poison plates agar were incubated for 7 days at 30°C. The mycelium of the colony was measured daily. Both data obtained for control and treatments were transformed into percent inhibition of mycelium by using the following formula (Skidmore and Dickenson, 1976):

$$\% \text{ Mycelium inhibition} = \frac{\text{Mycelium growth (Control - Treatment)}}{\text{Control}} \times 100$$

2.5 In vivo application

All samples used were washed with tap water, rinsed with sterile distilled water and air dried. Wax apples were dipped in water extract propolis (WEP) with different concentrations 10%, 12% and 14% for 3 min and dried. Samples were inoculated by spraying with *Colletotrichum gloeosporioides* spore suspension of 2×10^6 conidia/mL. All inoculated samples were placed in a plastic container as a damp chamber and incubated at 10°C with 90-95% relative humidity and analysed at a 3-day interval.

2.6 Parameter evaluation

All samples were analysed for post-harvest quality, which included weight loss, disease severity assessment (DSI) and total soluble solids (TSS). The weight loss of wax apples was measured from each treatment using an electronic balance for 15 days at a 3-day interval. The percentage of weight loss was calculated using the

equation:

$$\% \text{ Weight loss} = \left(\frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \right) \times 100$$

To determine the effect of treatments on the total soluble solids of wax apple, the fruits were squeezed to extract the juice. One to 2 drops of the juice were placed on a hand-held refractometer (Atago, MODEL REF103), and the reading ($^{\circ}$ Brix) were recorded. The diameter of the lesion of anthracnose on wax apple was observed every two days and measured using a scale in Table 1.

The overall disease severity index (DSI) was calculated as follows (Mak *et al.*, 2004):

$$\text{DSI} = \frac{\sum (\text{number of symptomatic fruits} \times \text{severity index})}{N \times Z} \times 100$$

Where N = Total of sample fruits and Z = Highest rating scale.

Table 1. Disease scale

Disease scale	Description	Inference
0	No symptoms on fruits	No infection
1	1-25% of the inoculated area covered with lesions	Mild infection
2	26-50% of the inoculated area covered with lesions	Moderate infection
3	51-75% of the sample are infected and softening	Severe infection
4	>76% and rotting	Very severe/ devastating

2.7 Statistical analysis

The experimental design for this study is a complete randomised design (CRD) with replicates of each treatment. Data were analysed using SPSS Programme version 20, subjected to analysis of variance (ANOVA).

3. Results and discussion

3.1 In vitro antifungal assay

The mycelial growth of *Colletotrichum gloeosporioides* was significantly inhibited ($p \leq 0.05$) by all water extracts of propolis (WEP) treatments (Figure 1). The strongest inhibition was recorded with 14% WEP treatment after seven days of incubation. A similar trend was reported by Omar *et al.* (2020), where propolis application effectively suppressed fungal development in strawberries. Similarly, Menses *et al.* (2009) demonstrated that propolis derived from Colombia exhibited antifungal activity against *Colletotrichum* and *Botryodiplodia* species. Flavonoids are widely recognised as the principal bioactive compounds in propolis, playing a key role in antifungal efficacy, particularly against *Colletotrichum* spp. (Costantine,

2022). Propolis is known to contain various phenolic acids, phenolic acid esters, and flavonoids, with major compounds including pinocembrin, pinobanksin and its 3-O-acetate, chrysin, galangin, and phenethyl esters of caffeic and ferulic acids (Bankova *et al.*, 2002). These compounds are often present in high concentrations and contribute substantially to their antimicrobial activity. Additionally, the presence of terpenes may contribute to its antimicrobial properties (Menses *et al.*, 2009). However, the chemical composition of propolis varies depending on its geographical origin, and its antimicrobial activity is likely the result of a synergistic interaction between flavonoids and other bioactive constituents, rather than the effect of a single compound (Katircioğlu and Mercan, 2006).

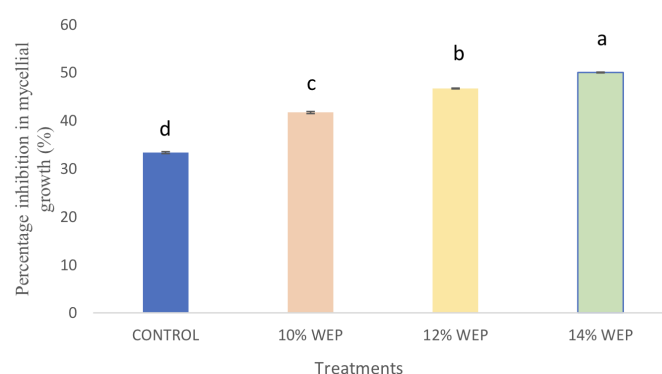


Figure 1. The effects of different concentrations of WEP solution (10%, 12% and 14% along with control on percentage inhibition of *Colletotrichum gloeosporioides* mycelial growth after 7 days). Bars with different letters indicate significant differences among treatments ($p < 0.05$).

3.2 In vivo antifungal assay

The maximum disease severity index (DSI) was observed for the control (60%). The application of water extract of propolis (WEP) significantly suppressed disease development in the inoculated fruits after 15 days of storage (Figure 2), resulting in 0% disease severity index (DSI) ($p < 0.05$). These *in vivo* antifungal findings are consistent with those reported by Pastor *et al.* (2011), who observed a marked reduction in microbial activity in table grapes coated with hydroxypropyl methylcellulose containing 1.5% ethanolic extract of propolis (EEP). Likewise, Yang *et al.* (2010) demonstrated the effectiveness of ethyl acetate extracts of Chinese propolis in controlling blue and green mould in citrus fruits. Omar *et al.* (2020) also reported minimal DSI values when 14% WEP was applied to strawberries inoculated with a similar fungal pathogen, indicating the cross-fruit consistency of propolis antifungal action. These results strongly support the potential of propolis as a natural alternative to synthetic fungicides in postharvest disease management. When used as an edible coating, propolis may form a semi-permeable barrier that regulates gas exchange (O_2 and CO_2), moisture, and

solute movement, effectively reducing respiration and oxidative processes, as well as limiting water loss. The severity of *C. gloeosporioides* infection is visibly lower in propolis-treated fruits, as shown in Figure 3, where the 14% WEP treatment clearly preserved the external appearance and commercial value of the wax apples, making them visually acceptable and marketable even after storage.

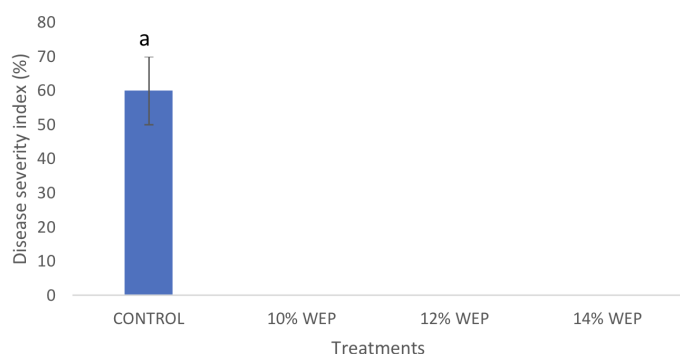


Figure 2. The effects of different concentrations of WEP solution (10%, 12% and 14% along with control) on anthracnose disease severity index on inoculated wax apples (10°C, 90% RH) after 15 days. Bars with different letters indicate significant differences among treatments ($p < 0.05$).

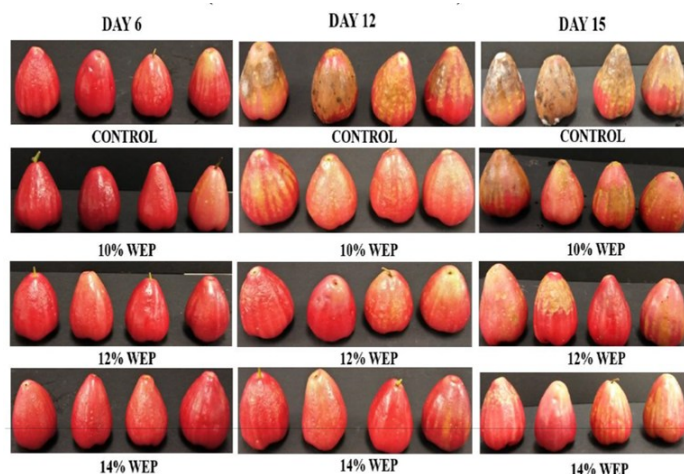


Figure 3. Visual appearance of inoculated wax apple with *Colletotrichum gloeosporioides* with and without WEP treatment for 15 days of storage

3.3 Weight loss

Coating with 14% WEP significantly reduced ($p < 0.05$) weight loss in wax apples to 1.52% after 15 days, compared to 5.45% in the uncoated control (Figure 4). All samples experienced weight reduction over time, but uncoated fruits lost the most. This moisture loss is likely minimised by the propolis content, which enhances the coating's ability to act as a dual-action barrier, physically through its resin-based composition and biologically through its natural antifungal properties (Pastor et al., 2011). By limiting moisture escape, WEP coating helps retain firmness and overall fruit quality during storage. Edible coatings are known to delay dehydration by creating a partial barrier to gases and water vapour, thus

slowing respiration and surface drying. Earlier studies also reported similar benefits in fruits like cherries and mandarins, where propolis-treated samples can retain weight and skin quality without visible physiological damage during storage (Özdemir et al., 2005; Çandır et al., 2009). Weight loss in fresh produce typically results from ongoing respiration and evaporation postharvest, and is affected by environmental factors such as temperature, humidity, and air movement. The obvious lower weight loss in 14% WEP-coated fruits suggests the coating provided sufficient protection, helping preserve visual appeal and marketability over the storage period.

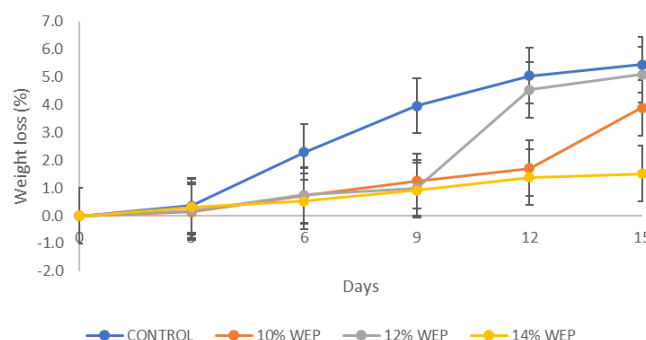


Figure 4. The effects of different concentrations of WEP solution (10%, 12% and 14% along with control) on the weight loss (%) of wax apples during 15 days of storage

3.4 Total soluble solids

No significant difference ($p > 0.05$) in TSS values was observed between the control and WEP-treated wax apples throughout the storage period (Figure 5). Although the 10% WEP treatment exhibited a higher TSS at day 0, this value declined over time, while the TSS of other treatments remained relatively stable. In fruit, TSS is commonly associated with ripening and sugar accumulation. However, wax apple is a non-climacteric fruit, which typically undergoes minimal metabolic changes after harvest (Vendrell et al., 2001). As such, the ripening-related rise in TSS is unlikely to occur postharvest. In addition to that, the lack of clear change across treatments suggests that the propolis treatments did not induce physiological stress or trigger any metabolic response that would affect sugar

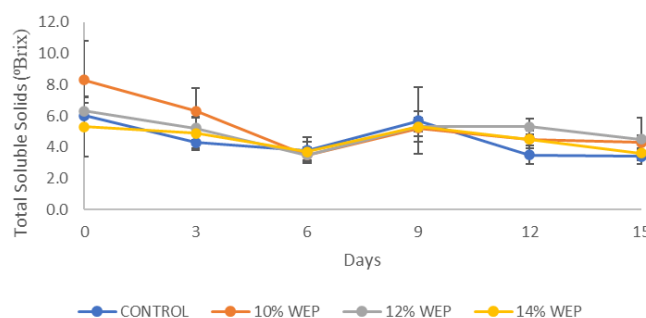


Figure 5. The effects of different concentrations of WEP solution (10%, 12% and 14% along with control) on total soluble solids contents of wax apples for 15 days.

metabolism. This stability in TSS further supports the conclusion that postharvest application of WEP has no adverse effect on internal fruit quality.

4. Conclusion

This study demonstrated the effectiveness of propolis, particularly at 14% concentration, in controlling common postharvest diseases in wax apples. In addition to disease suppression, the treatment significantly reduced weight loss—an issue often observed in infected fruits that compromises their overall quality. The reduced disease severity in propolis-coated fruits also helped maintain the cosmetic appearance of wax apples, making them more marketable even after storage. Further investigations using propolis derived from different stingless bee species may provide valuable insights into variations in antifungal efficacy.

Conflict of interest

The authors declare no conflict of interest.

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Declaration of generative AI in scientific writing process

During the preparation of this work, the author(s) used [ChatGPT/Grammar correction] in order to [improve language and clarity]. The author(s) have reviewed and edited the content as needed. The author (s) will take(s) full responsibility for the content of the published article.

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