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EFFECT OF DIFFERENT CONCENTRATIONS OF PROPOLIS EXTRACT COATING ON POSTHARVEST QUALITY OF BANANA ARTIFICIALLY INOCULATED WITH *Colletotrichum gloesporioides*

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ABSTRACT

The coating is one of the methods to prevent postharvest loss in the food industry. Propolis seems to be promising as a coating due to its waxy properties, high antifungal activity and less toxicity. In this study, propolis ethanol extract coating was tested for the antifungal activity against *Colletotrichum gloeosporioides*, the causative fungus that caused anthracnose disease on bananas (*Musa acuminata*). All samples were artificially inoculated with *C. gloeosporioides* and the disease severity index (DSI) was measured. Other postharvest qualities of banana tested were weight loss, total soluble solids (TSS), colour and titratable acidity (TA). Results showed that the control banana was more susceptible to the fungal infection (60% necrosis) compared to the treated banana. The propolis coating successfully inhibited the fungus activity of *C. gloeosporioides* activity on a banana during storage. The higher concentration of extract coating seems to be better effective against the fungus. Moreover, the control banana showed higher weight loss (6.92%) and total soluble solid ($p<0.05$) compared to coated banana during storage. In conclusion, bananas treated with an 11% concentration of propolis coating are promising for improving the colour, total soluble solid content and titratable acidity and can inhibit artificially anthracnose disease caused by *C. gloeosporioides* on a banana.

Key words: Anthracnose disease, banana, coating, *Colletotrichum gloeosporioides*, propolis

INTRODUCTION

Banana is an important fruit in many tropical countries such as Malaysia, Thailand and Indonesia (Aurore *et al*., 2009). It is widely cultivated and ranks third place after grapes and citrus (FAO, 2000). It is highly consumed for its flavour, texture, nutritional value, and convenience (Robinson, 1996). Although 87% of bananas are produced for local food consumption (Bioversity International, 2008), banana is the most wasted fruit compared to other types of fruit which can reach up to 80 million pounds per year (The Guardian, 2017). It contains high water content and is highly respiration which leads to a faster rate of deterioration after being harvested (Chitarra & Chitarra, 2005; FAO, 2018). The high starch level in bananas makes it most liked by pathogens including *Colletotrichum* spp. (Jeffries *et al*., 1990). *Colletotrichum gloeosporioides* was found to be the causative agent for anthracnose disease in bananas (Intan Sakinah *et al*., 2013). Once it is infected, it will accelerate respiration and subsequently lead to faster deterioration. It can form latent lesions on fruits and slowly develop larger lesions that appear more rapidly when fruit are damaged (Meredith, 1960). Current postharvest practices do not often guarantee a good quality of bananas (Botrel *et al*., 2002).

Various kinds of post-harvest treatment had been conducted to reduce the postharvest losses such as controlled atmosphere (CA), modified atmosphere (MA) (Hailu *et al*., 2013) but due to the susceptibility of bananas towards improper storage, it does not sufficient in maintaining the losses (Daiuto *et al*., 2012). Many chemical pesticides and fungicides have been used to reduce the problem, but the increasing of the tolerance of pathogen towards the chemical treatment leads to higher doses of application and endangering human health. It may also cause deterioration of farmers' health, economic status and toxic contamination of the environment (Voorrips *et al*., 2004; Fakri *et al*., 2018).

Therefore, the application from natural sources such as propolis seems to be a promising treatment to reduce the anthracnose disease as well as prolong the usefulness of the banana (Vit *et al*., 2013). Propolis, a stingless bee by-product has a high amount of antioxidants and it is reported to have antifungal characteristics (Ahmad *et al*., 2019; Omar *et al*., 2020; Shehata *et al*., 2020) which is very beneficial to reduce the occurrence of plant disease. Similar to honey (Mahmood *et al*., 2021), the properties of propolis are also affected by seasons and plant sources (Anjum *et al*., 2019). However, its potential to reduce anthracnose disease is scarce. Based on a previous study by Omar *et al*. (2020), it was proven that the inhibitory properties of propolis produced from

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Heterotrigona itama towards *C. gloeosporioides* in strawberries. The immersion of fruits inside propolis coating can build up a film on the fruit pulp and it is being able to act as a barrier from penetrator and even from the gaseous exchange (Carvalho *et al*., 2013; Ali *et al*., 2015). Compared to water extract propolis, ethanolic extract propolis was reported with higher properties of antioxidant and antifungal properties (Kubilene *et al*., 2018) due to the higher polarity of ethanol towards the antioxidant compounds in propolis. By having this performance, it diverges on either to use as postharvest treatment as well as prolong the fruit's shelf life. Therefore, the objective of this study was to determine the effects of different concentrations of propolis extract coating on the postharvest quality of bananas artificially inoculated with *C. gloeosporioides.*

MATERIALS AND METHODS

Sample preparation

Raw propolis was collected from beehives of *H. itama* located at the Department of Forestry, Terengganu. Samples were cleaned by removing dead bees or any debris and stored at -20 ± 2 ºC (Omar *et al*., 2020) until further analysis. Freshly harvested bananas (stage 3) were purchased from local farmers in Terengganu, Malaysia. Samples taken were free from diseases and any illness that may cause differences of symptoms or any changes on the banana in the research period. Samples were then washed using acetic acid (3%) and rinsed with distilled water and air-dried at ambient temperature (25 ± 2 ºC).

Ethanolic extraction of propolis (EEP)

The frozen propolis samples were grounded by using mortar and pastel to produce a fine powder (Omar *et al*., 2020). The powder then was mixed with 95% of ethanol food grade with the ratio of 1:1. The mixture was shaken using an incubator shaker for 5 days at 50 ºC. The mixture then was centrifuged at 15,000 rpm for 15 min and filtered using Whatman filter paper no.1 (Sigma Aldrich, Germany). The filtrate was evaporated using a rotary evaporator (Ibrahim *et al*., 2016) and diluted with distilled water to produce the selected concentration of 8% and 11% of ethanol extract propolis (Ali *et al*., 2015). These concentrations were chosen based on our preliminary data which was done on the in vitro analysis of the different concentrations of propolis ranging from 5 to 12% of ethanol extract.

Inoculum preparation

Pure culture of *C. gloeosporioides* was obtained from Laboratory for Pest, Disease and Microbial Biotechnology (LAPDiM), Universiti Malaysia Terengganu, Malaysia. Spore suspensions were prepared by using seven days old cultures of the pathogen. *C. gloeosporioides* was grown onto potato dextrose agar (PDA) (Oxoid, UK). The culture was flooded with sterile distilled water and dislodged the spore using a glass rod. The spore suspensions obtained were passed through filtration of sterile cheesecloth to remove mycelial fragments (Lane *et al*., 2012; Omar *et al*., 2020). Spore suspension (2×10^6 conidia/mL) was counted using a haemocytometer (SigmaAldrich, Germany) counting (Araujo *et al*., 2016).

Application of propolis coating on banana

Banana was dipped into ethanol extract propolis (EEP) containing 8% and 11%, respectively. All samples were inoculated with spore suspension by spraying with 2×10^6 conidia/mL of *C. gloeosporioides.* Samples without any coating were considered as the control sample. All samples were then kept at room temperature (25 ± 2 ºC) in a container called a damp chamber for 10 days and analysed at a two days interval.

Postharvest analysis

All samples were analysed for the postharvest parameter to determine the effect of ethanolic extracted propolis on the infected banana quality during storage. Parameters tested were weight loss, colour, total soluble solid, disease severity index and titratable acidity. Weight loss of bananas was conducted using an electronic balance (Maqbool, 2010). The weight loss of the samples was determined according to this formula:

Equation 1:

$$weight\ loss(\%) = \frac{Initial\ weight\text{-}final\ weight}{initial\ weight}$$

The colour changes of fruit during storage was determined using a colourimeter (Konica Minolta, Japan). The result was expressed in L*, a*, b* values (Tshwenyane *et al*., 2014). The total soluble solids (Brix^o) were determined using a handled refractometer (Model REF 103, Atago, Japan) to determine the sweetness of the fruit. The total acidity of the fruit was determined using the titratable acidity method. The aliquot of samples was titrated with 0.1 N NaOH using 2-3 drops of phenolphthalein as an indicator (Regina *et al*., 2016) Disease severity assessment of the fruit was determined by observing the diametre lesion of anthracnose. The assessment was done based on the given scale (Table 1). The overall disease severity index (DSI) was calculated as follows (Mak *et al*., 2004):

Equation 2:

$$DSI\ (\%) = \frac{\Sigma(Number\ of\ infected\ fruit\ vegetables \times severity\ index)}{Total\ number\ of\ sample \times highest\ rating\ scale} \times 100$$

Table 1. Disease severity index (Pereira *et al*., 2011)

Scale	Symptoms (%)
1	0
2	1-10
3	11-25
4	26-50
5	51-75
6	>75

Statistical analysis

The experimental design for this study is a complete randomised design (CRD). The data were subjected to analysis of variance (ANOVA) using IBM SPSS 23.

RESULTS AND DISCUSSION

Disease severity index

Disease severity index (DSI) recorded the lesion of the control banana up to 60% necrosis/lesion with a diameter of 8 cm on the surface of the banana (Figure 1). According to the scale that was used as shown in Table 1 by Pereira *et al*. (2011), the lesion for the control banana is on the scale of 5 while the coated banana is only on the scale of 1. Amani *et al*., (2009) also found the high lesion of disease severity on a non-treated banana which was up to 70% of necrosis. Thus, this study clearly showed that the propolis coating was able to inhibit the artificial anthracnose disease caused by *C. gloeosporioides.* The inhibitory disease of propolis against the *C. gloeosporioides* was potentially contributed by the high content of phenolic compounds in propolis (Ahmad *et al*., 2019). There were many studies reported on the relation of phenolic compounds and antifungal properties (Ansari *et al*., 2013; Carvalho *et al*., 2018; Joaquín-Ramos *et al*., 2020).

Total soluble solids

As shown in Figure 2, there is an increasing trend of total soluble solids (TSS) in all of the samples during storage (p<0.05). From day 2 until day 6, both of the treatments of the sample showed less soluble solid content compared to control samples (p<0.05) where the TSS of samples coated with 8% propolis was lower than 11%. The increasing TSS in all fruits can be explained by an increased fruit respiration rate due to the hydrolysis of starch into sugar (Mohapatra *et al*., 2011). Lower total soluble solid (TSS) in treated bananas indicates the delay in respiration and conversion of starch to sugar due to the lower severity of anthracnose disease. Besides, the waxy characteristics of propolis coating slowly prevent the gaseous exchange and the release of the ethylene production from the banana (Ali *et al*., 2014) which slow down the fruit respiration. However, soluble solids increased at the end of fruit ripening indicating the beginning of senescence. That increase might be related to fresh weight losses because of dehydration resulting in the concentration of sugars (Chitarra & Chitarra, 2005).

Weight loss

Propolis coating affected significantly (p≤0.05) on the lower weight loss of bananas during storage (Figure 3). All the bananas maintained turgidity and had a low weight loss which range from 4-6% which is still in the acceptable range for the weight loss of fruit (Kader, 2002). The lower weight loss in coated bananas might result in the prevention or the coating act as a vapour barrier affected those propolis extract and delaying the respiration rates. Besides, the disease occurrence which was severe in the control sample (Figure 1) was the main factor that contributed to the greater weight loss of bananas in this study.

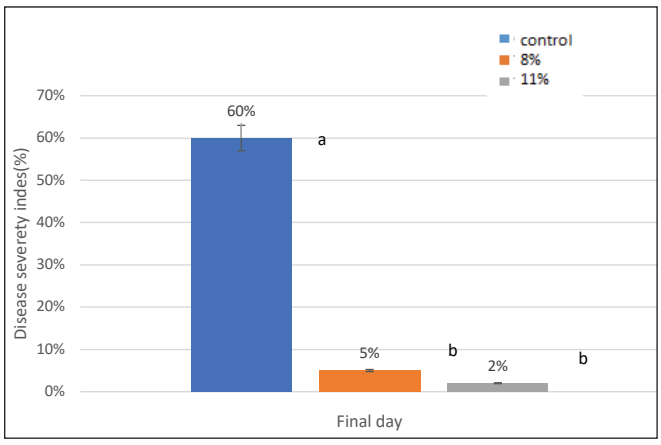

Fig. 1. Disease severity index of banana on the final day of assessment.

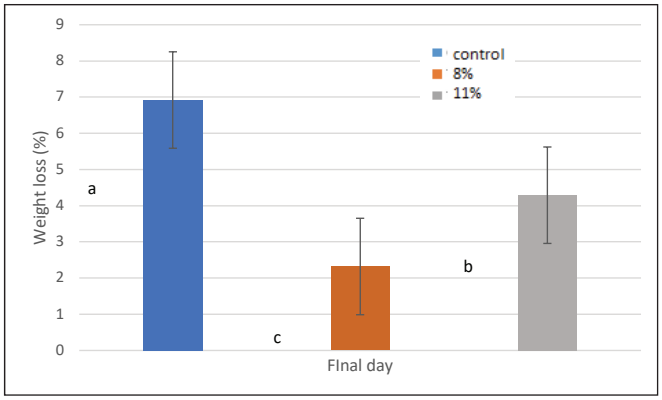

Fig. 2. Effect of different concentrations of EEP on the weight loss of banana during storage.

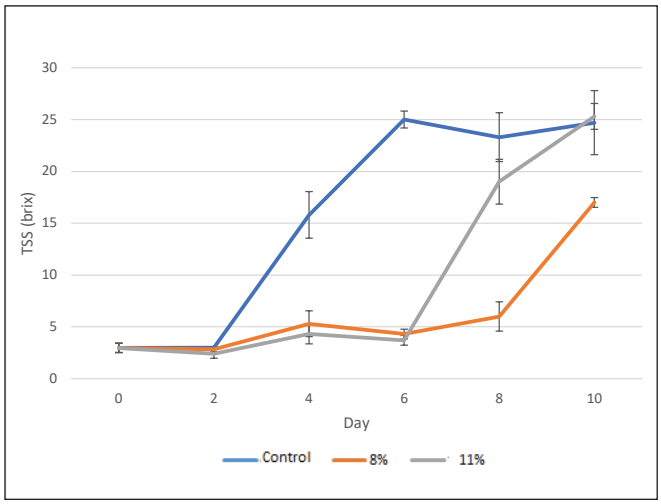

Fig. 3. Total soluble solids in bananas with different concentrations of EEP during storage.

Titratable acidity (TA)

The result in Figure 4 showed no significant difference (P>0.05) on the titratable acidity value for all samples during storage. But, the result tends to increase at the early storage time and decrease as the time gets longer. The significant decline of TA (p≤0.05) in the control banana can be seen on day 6 and day 10. The reading of TA was aligned and agreed by Botrel *et al*. (2002) who reported on the increasing value of TA related to the predominance of malic acid contained in the banana were at maximum when the peel of the banana was turned yellow and the level will reduce later until senescence. The lower value of TA was also recorded by Maqbool *et al*. (2010) on 'Berangan' which were coated with chitosan in their research. The malic acid contained in the banana were decreasing as the banana ripen it was used as a respiratory substrate and also converted into sugar (Chitarra & Chitarra, 2005) consequently.

Colour

Colour is one of the critical factors that determine the acceptance of the consumer on the quality of fruits (Costa *et al*., 2011). In this study, colour can also be used as an indicator of the ripening stage of bananas. There was no significant difference (p>0.05) for all colour changes (Figure 5a & 5b) during storage except b* value (yellowness) on day 4 and day 10 (Figure 5c). The lower value of b* for bananas coated with propolis results in delayed ripening compared to control bananas. As discussed earlier, the lower incidence of anthracnose and waxy properties of treated banana able to delay respiration and ripening (Passos *et al*., 2016) which indirectly reduce the yellowness of banana peel.

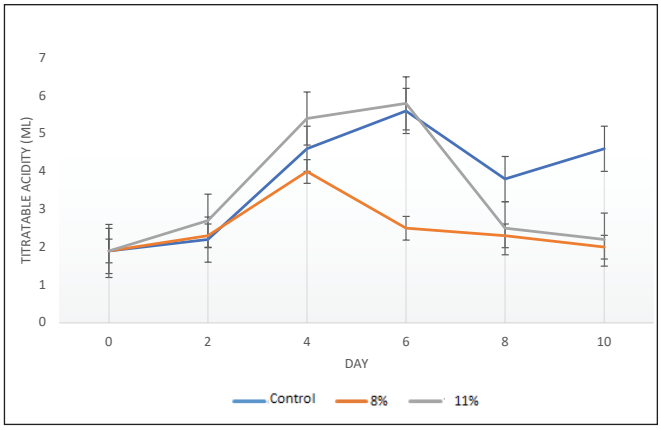

Fig. 4. Effect of different concentrations of EEP on the titratable acidity of banana during storage.

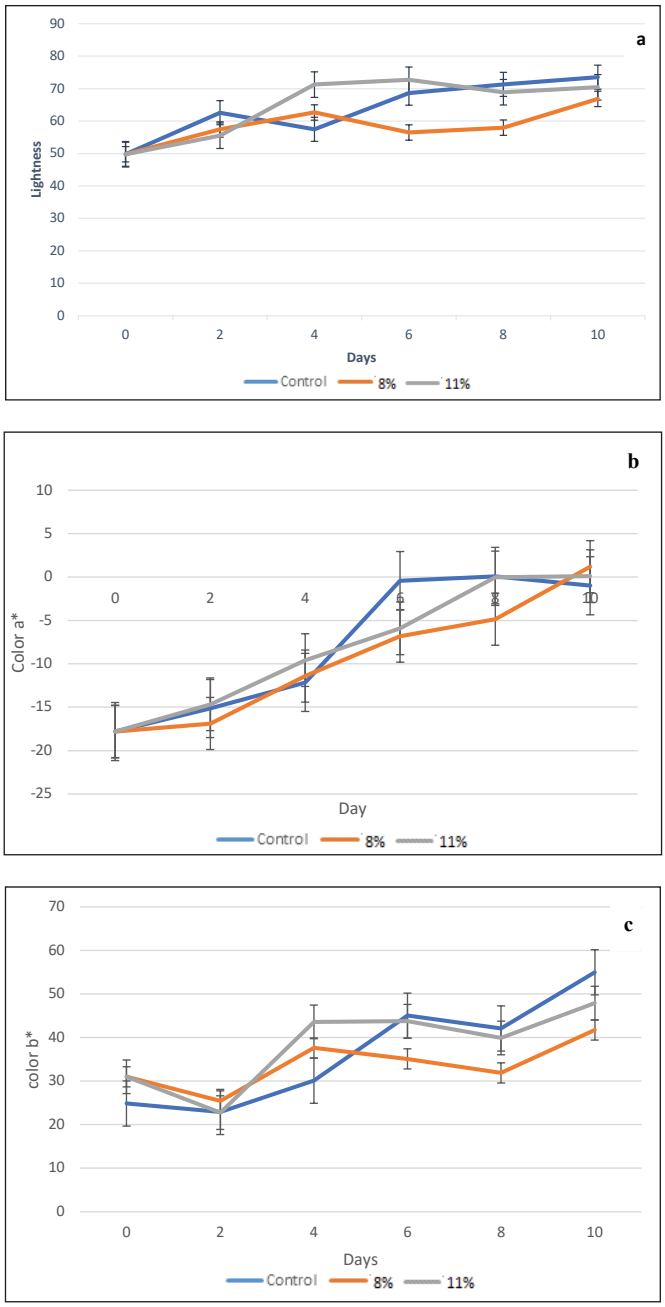

Fig. 5. (a-c). Effect of different concentration EEP on the colour (L*, a*, b*) of banana during storage.

CONCLUSION

This study clearly showed that treatment of ethanol extract propolis coating manage to retain most of the postharvest quality of the banana by reducing the anthracnose disease that was caused by *C. gloeosporioides*. The great inhibitory properties against the disease were potentially contributed by the antifungal and antimicrobial activity in the propolis. Banana treated with 11% concentration of propolis coating are promising for improving the colour, total soluble solid content and titratable acidity and able to inhibit artificially anthracnose disease caused by *C. gloeosporioides* on a banana. Further study needs to be done on the quantification of active compounds in propolis that contributed to the antimicrobial properties. The relation of ethylene or any parameter that can determine respiration and ripening also need to be analysed for a better explanation of the acceleration of ripening and disease severity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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