INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–1382/2014/16–6–1117–1122
http://www.fspublishers.org

Full Length Article

Composite Effect of Propolis and Gum Arabic to Control Postharvest Anthracnose and Maintain Quality of Papaya during Storage

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Abstract

Anthracnose caused by *Colletotrichum gloeosporioides* is the most prevalent postharvest disease of papaya that results in major economic losses. To investigate the effect of ethanolic extract of propolis (EEP) alone or in combination with gum arabic (GA) on control of postharvest anthracnoase and maintenance of fruit quality during storage, papaya fruit treated with 0.5, 1.0 and 1.5% EEP alone or in combination with 10% GA were stored at 13 ± 1°C and 80-90% RH for 28 days. Data regarding antifungal assays and fruit quality were taken at 7 days intervals. Fruit treated with 1.5% EEP exhibited highest (87%) reduction in mycelial growth. Application of EEP delayed the development of postharvest anthracnose and maintained quality of papaya fruit. Combine application of 1.5% EEP and 10% GA composite coating synergistically reduced the occurrence of antracnose and delayed the reduction of weight loss, fruit firmness, soluble solids concentration and titratable acidity in papaya fruit as compared to all other treatments and control. The results suggest that combine application of 1.5% EEP and 10% GA can be used effectively as a biofungicide for controlling postharvest anthracnose as well as maintaining quality of papaya fruit.

Keywords: *Colletotrichum gloeosporioides*; Disease control; Fruit quality; Gum arabic; Papaya; Propolis

Introduction

Papaya (*Carica papaya* L.) is one of the highly demanded tropical fruits by the international market as it is rich in *β*-carotene, ascorbic acid and antioxidant (Gayosso-García Sancho *et al.*, 2010). Being a climacteric fruit, papaya has a short shelf-life because drastic changes occur during postharvest phase which result in faster deterioration and poor marketability (Ali *et al*., 2011). Moreover, papaya is highly susceptible to anthracnose caused by *Colletotrichum gloeosporioides*, which further deteriorates its quality (Hewajulige *et al*., 2009).

Generally, control of papaya anthracnose could be achieved by prochloraz and propiconazole (Sepiah, 1993), yet this has resulted in the development of fungicide resistant pathogen strains (Cia *et al*., 2007). Furthermore, control of the disease via the application of fungicides has caused serious environmental and consumers health issues (Hewajulige *et al*., 2009). Due to increased awareness about health problems and environmental issues, eco-friendly and cost effective fruit preservation techniques to maintain quality as well as extend the shelf-life of fruits are being investigated (Maqbool *et al*., 2010a).

The use of edible coatings carrying natural antimicrobial compounds appears to be a novel approach in extending the shelf-life of strawberries and asparagus (Tzoumaki *et al*., 2009) and mangoes (Huang *et al.,* 2012). Gum arabic (GA) is a dried gummy exudate from the stems and branches of *Acacia senegal* and related species of *Acacia* (Ali *et al*., 2010). It is a hydrocolloid that possesses excellent water solubility property and low-viscosity at high concentration as compared to other gums (Ali *et al*., 2010).

Propolis is another natural resinous substance collected by honeybees (*Apis mellifera* L.) from various plant sources (Burdock, 1998). The biological action and chemical compositions of propolis could be varied with the geographic zones, collection times as well as plant source (Kujumgiev *et al*., 1999). Among the constituents of the propolis, studies have shown that flavonoids could have the most significant biological action in inhibiting the microbial activity (Burdock, 1998; Pastor *et al*., 2011), although benzoic acid and other derivatives found in propolis have been reported to possess antimicrobial activity (Özcan, 1999). Due to its chemical composition and antimicrobial properties, it has been widely used in the pharmaceutical industry and considered safe for human health (Zahid *et al*., 2013).

Recently, hydroxypropyl methylcellulose edible coatings containing propolis extract were developed to minimise the postharvest decay of table grapes (Pastor *et al*., 2011). However, no research work has been reported on the use of edible coating based on the combination of GA and ethanolic extract of propolis (EEP). Therefore, the objectives of this study were to investigate the efficacy of

To cite this paper: Ali, A., C.K. Cheong and N. Zahid, 2014. Composite effect of propolis and gum arabic to control postharvest anthracnose and maintain quality of papaya during storage. *Int. J. Agric. Biol*., 16: 1117–1122

EEP and GA in combination with EEP *in vitro* as well as *in vivo* to control anthracnose of papaya and also study their effects on postharvest quality of papaya during cold storage.

Materials and Methods

Experimental Material

Mature green Eksotika II papayas with colour index 2 (about 10% of yellow stage) were obtained from Exotic Star (M) Sdn Bhd, Kajang, Selangor. Papayas were sorted out to be uniform in size, free from any physical injury and fungal infection. GA powder was supplied by Jumbo Trading Co., Ltd. Bangkok, Thailand. Crude propolis, collected by honeybees (*Apis mellifera* L.) from China was supplied by Yi Wang Honey Garden (M) Sdn Bhd, Semenyih, Selangor. Crude propolis covered with aluminium foil was stored at 10°C in dark conditions before it was used in any extraction.

Isolation of *C. gloeosporioides*

Healthy papayas were placed in a humid chamber at room temperature (25°C) for few days. Isolation of *C. gloeosporioides* was done from the diseased papaya fruits once the anthracnose symptoms emerged. This was done by taking small pieces of the symptomatic tissues and placed on several dishes containing potato dextrose agar (PDA) (Difco Brand, USA). Each fungus grown on the dishes was morphologically characterised, and only the *C. gloeosporioides* was picked up. *C. gloeosporioides* was then sub-cultured in new dishes at room temperature (25°C) till pure culture was obtained.

Preparation of EEP and GA

Fifty grams ground propolis was mixed with 500 mL of 95% ethanol (1:10 w/v) by shaking them with orbital shaker (Model: Yih Der TS-520, Taiwan) at 1157 x *g* at room temperature (25°C) for 72 h in dark condition. The mixture was then filtered through a Whatman No. 1 filter paper to discard any wax that was insoluble in ethanol. The extracts dried by using a rotary evaporator (Model: Büchi Rotavapor R-200, Switzerland) at 40°C were weighed and adjusted to different concentrations of EEP by dissolving in appropriate amount of 70% ethanol.

GA powder was dissolved in purified water and was stirred at 40°C for 1 h using a hotplate magnetic stirrer (Model: LMS-HTS-1003, Bunkyo-Ku, Tokyo, Japan). The solution was filtered using four layers of cheesecloth to remove any impurities. It was then adjusted to pH 5.6 by adding 1N NaOH, using a pH meter (Model: Cyberscan pH510, Eutech Instruments Pte Ltd, Singapore).

Antifungal Assay of EEP

The fungal plugs (7 mm in diameter) of *C. gloeosporioides* were placed in the centre of petri dishes containing PDA amended with 0.5, 1.0, 1.5 and 2.0% (v/v) of EEP. Two controls were prepared. Petri dishes containing solely PDA served as first control, while petri dishes containing PDA and 70% of ethanol served as second control. Mycelial growth on each petri dish was measured and recorded for one week.

The *in vivo* antifungal assays of EEP alone and combination of GA plus EEP were performed. Fruit were washed with 0.01% (v/v) of sodium hypochlorite for 3 min and dried at room temperature (25°C). Washed fruit were immersed in *C. gloeosporioides* spore suspension (1 x 10^4 spore mL^{-1}) for 2-3 min and were dried at room temperature (25°C). Fruit were then dipped in ethanol (without propolis) and EEP at 0.5, 1.0, 1.5% and in composite coating solution made up of GA plus ethanol (without propolis) and GA plus EEP at 0.5, 1.0 and 1.5% for 2-3 min. The control fruit were soaked in purified water only. Concentration of GA was adjusted to 10% as at this concentration, it showed a significant effect in retaining the fruit quality (Ali *et al*., 2010). After application of treatments, all fruit were air dried and packed in cardboard boxes and stored at 13 ± 1°C and 80-90% RH. The antifungal assay was based on disease incidence (DI) and disease severity (DS). DI data were expressed as the percentage of fruit showing anthracnose out of the total number of fruit in each treatment, while the DS was scored in accordance to the following scale (1 = 0% of fruit surface rotten; 2 = 1-25%; 3 = 26-50%; 4 = 51-75% and 5 = 76-100%).

In vivo Quality Assay of EEP and GA

The quality analysis of papayas was carried out weekly for 4 weeks based on weight loss percentage, firmness, soluble solids concentration (SSC) and titratable acidity (TA).

Fruit were weighted using a digital balance (Model: GF-6100, A&D Company Limited, Japan). The differences between the initial weight at the start of experiment and the final weight at the end of storage were calculated and the results were expressed as weight loss percentage. Fruit firmness was determined based on the compression force needed to penetrate a hole in the fruit, by using an Instron Universal Testing Machine with a 6.0 mm diameter plunger tip, Single Column Model (Norwood, MA, USA) connected with a computer. The penetration rate was set at 20 mm min^{-1} and was expressed as Newton (N).

SSC was determined by using a Palettle Digital Refractometer (Model PR-32α, Atago Co, Ltd. Japan). The results were expressed in percentage. TA was determined by titrating the diluted juice with 0.1N NaOH to an endpoint pink (pH 8.1) using phenolphthalein (0.1%) as indicator. Results were expressed as percentage citric acid per 100 g fresh weight.

Experimental Design and Statistical Analysis

Experiments were carried out using completely randomised design (CRD). The data were subjected to analysis of variance (ANOVA) using computer software SPSS.

Statistical significance was assessed at $P \leq 0.05$ and means were separated using Least Significant Difference (LSD) test. For *in vitro* experiment, five petri dishes were used as treatment unit with four replicates. For *in vivo* experiments, 10 fruit were taken as treatment unit replicated four times.

Results

Mycelial growth of *C. gloeosporioides* was significantly ($P \leq 0.05$) reduced by all EEP treatments. The highest inhibition of mycelial growth was observed in 1.5% EEP treatment after 7 days of incubation (Fig. 1). Ethanol plates, which served as second control, contributed only toward 7.7% inhibition of mycelial growth, suggesting that the mycelial growth inhibitions were due to propolis action, instead of ethanol.

Disease incidence (DI) and disease severity (DS) increased as the storage period progressed (Fig. 2A and 3A). Control fruit and fruit treated with different concentrations of EEP showed significant ($P \leq 0.05$) differences in DI and DS during 4 weeks of cold storage. The most effective anthracnose control was achieved with 1.5% EEP coated fruit, followed by 1.0% EEP treatment. Fruit treated with ethanol did not show any fungicidal effects. On the other hand, significant ($P \leq 0.05$) reduction in both DI and DS was observed in papayas treated with GA incorporated with EEP (Fig. 2B and 3B). While DI and DS reached to the maximum at the end of storage period in control fruit. Similarly, ethanol did not prevent the papayas from anthracnose infection as the spoilage of the papayas treated with ethanol was as high as the control fruit.

At the end of storage period significant ($P \leq 0.05$) reduction in fruit weight loss was observed in all treated fruit as compared to the control (Fig. 4). Weight loss increased gradually in all fruit but the control achieved the highest weight loss percentage, while 1.5% EEP showed the minimum weight loss after 28 days of storage.

Firmness decreased in all the treatments but the highest decrease in firmness was observed in control fruit (Fig. 5). No significant ($P \leq 0.05$) difference was observed between the control fruit and the fruit treated with ethanol and different concentrations of EEP at the end of storage (Fig. 5A). While in case of composite coating maximum fruit firmness was maintained by 10% GA plus 1.5% EEP, followed by 10% GA plus 0.5% EEP (Fig. 5B).

Significant ($P \leq 0.05$) increase in SSC was found in the control fruit comparing to the coated fruit (Fig. 6). The SSC increased gradually and becomes two times higher in control fruit after 28 days of cold storage. TA decreased gradually in all the treatments but significant ($P \leq 0.05$) differences were between the uncoated fruit and the fruit coated with EEP and GA + EEP at the end of storage. The maximum retention of TA was observed in the fruit treated with 0.5% EEP. Fruit coated with composite coatings made up of GA + EEP showed better retention of TA as compared to EEP coated fruit (Fig. 7A).

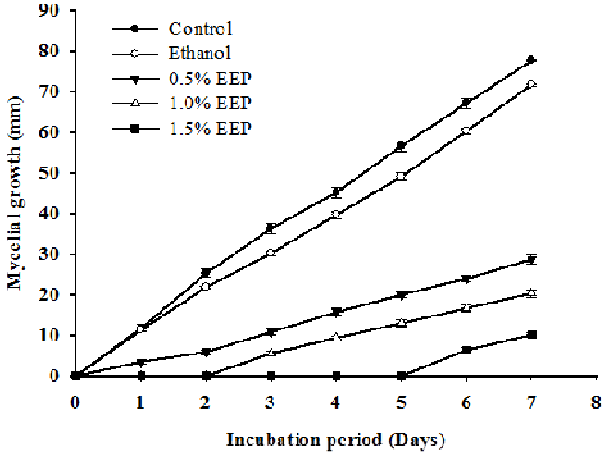

Fig. 1: Effect of different concentrations of ethanolic extract of propolis (EEP) on mycelial growth of *C. gloeosporioides* during of incubation period. Vertical bars indicate ± SE

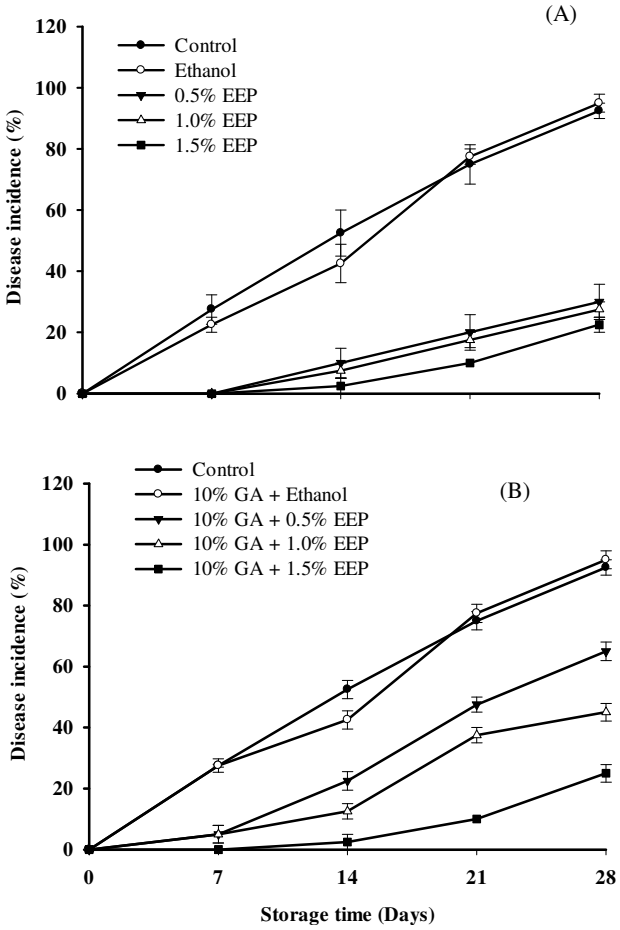

Fig. 2: Effect of different concentrations of ethanolic extract of propolis (EEP) (A) and gum arabic (GA) plus EEP (B) on disease incidence in inoculated papaya fruit during cold storage. Vertical bars indicate ± SE

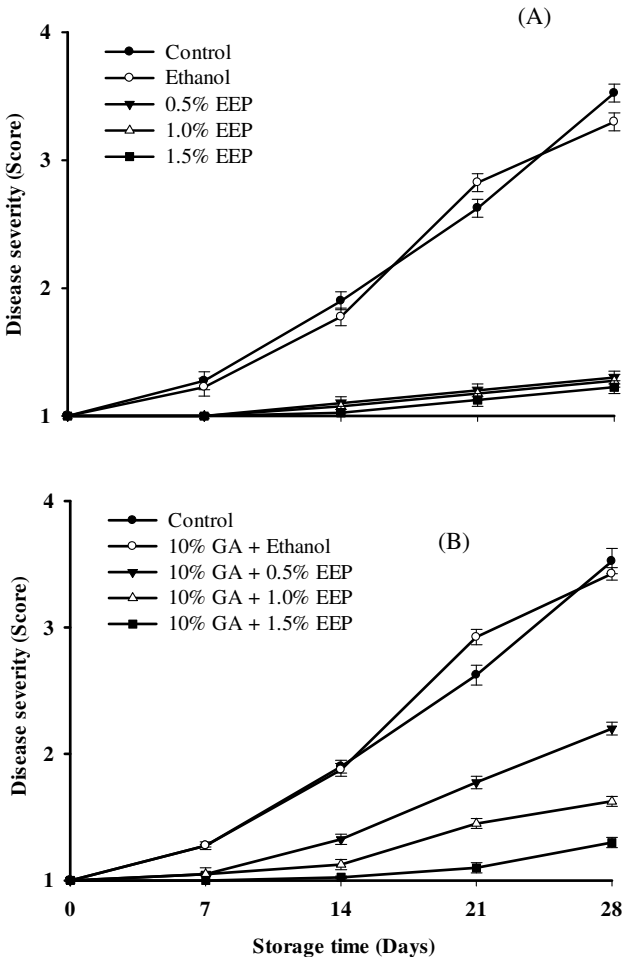

Fig. 3: Effect of different concentrations of ethanolic extract of propolis (EEP) (A) and gum arabic (GA) plus EEP (B) on disease severity in inoculated papaya fruit during cold storage. Vertical bars indicate ± SE

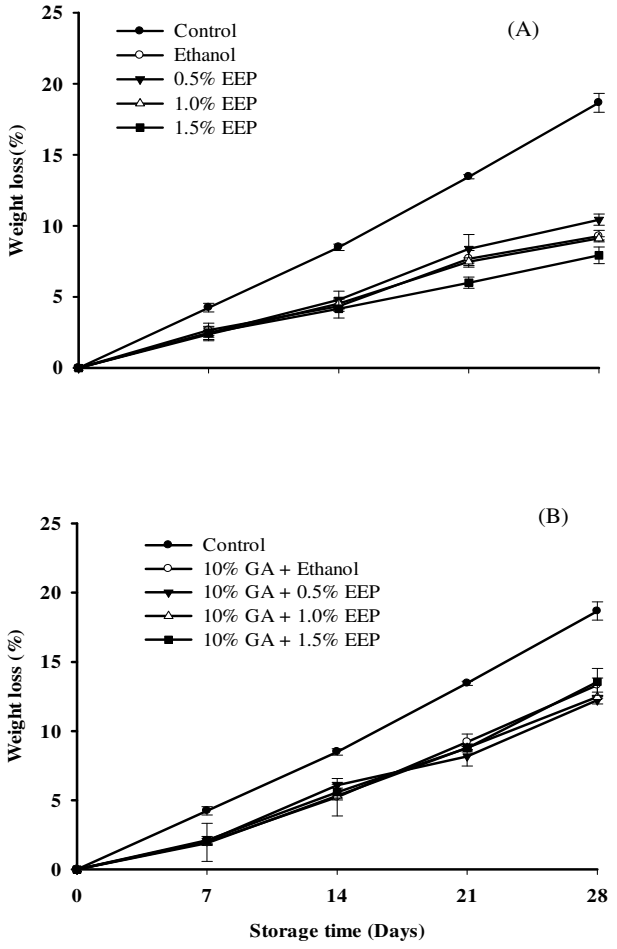

Fig. 4: Effect of different concentrations of ethanolic extract of propolis (EEP) (A) and gum arabic (GA) plus EEP (B) on weight loss in papaya fruit during cold storage. Vertical bars indicate ± SE

Discussion

The inhibitory effects of various concentrations of EEP against *C. gloeosporioides* were confirmed in the *in vitro* experiment of this study. Similarly, Meneses *et al.* (2009), also reported that propolis obtained from Colombia could also act as natural antifungal agent *Colletotrichum* sp. and *Botryodiplodia* sp. The presence of ethanol in the second control of this study showed very less inhibitory effect (7.7%) on fungal growth as compared to coated fruit. In earlier study, Katircioğlu and Mercan (2006) also observed no inhibitory effects of ethanol treatment against any of the microorganisms tested. Flavonoid is generally regarded as the main chemical in propolis, which has been found to contribute toward antimicrobial activities in propolis (Burdock, 1998). There are also evidences showing that antimicrobial activities of propolis could be affected by the presence of terpene (Meneses *et al*., 2009). Nevertheless, the chemical compositions of propolis differ with geographic locations and the biological action against the microbial should not be attributed solely to flavonoids as its antimicrobial activities can be a synergism between flavonoids and other chemical components available in the propolis (Katircioğlu and Mercan, 2006).

The results of this study had demonstrated the potential use of propolis treatment in controlling anthracnose caused by *C. gloeosporioides* in papayas. The inhibitory effects of propolis in the *in vivo* results were in agreement with *in vitro* results, as the disease incidence on fruit treated with EEP decreased with the increase of EEP concentration. In addition, the development of disease symptoms on fruit treated with EEP was delayed and the disease severity scores were also minimised. The exact mode of action of propolis in controlling the anthracnose was not clear; however its antifungal activity could be due to synergism effects brought by several major chemical compositions in it (Lu *et al*., 2005).

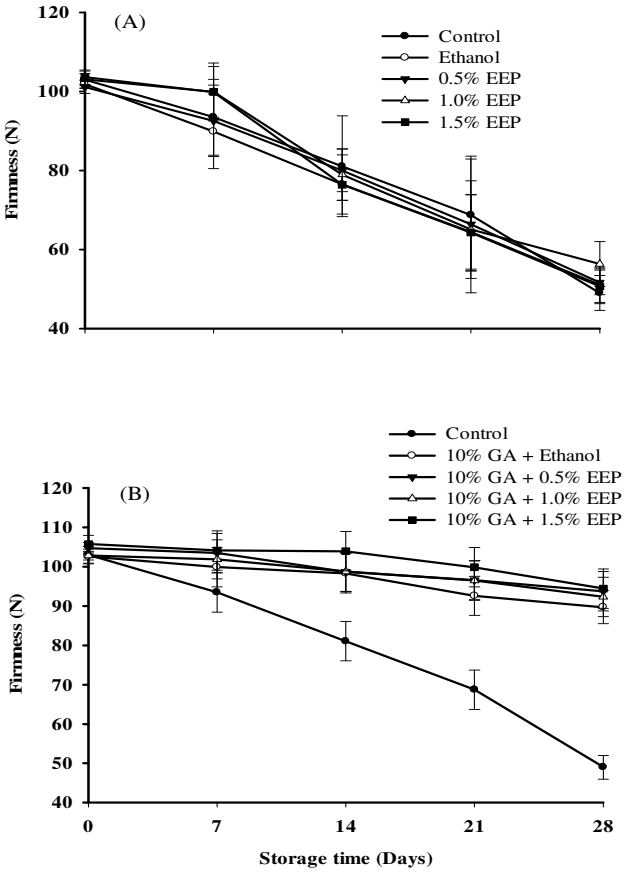

Fig. 5: Effect of different concentrations of ethanolic extract of propolis (EEP) (A) and gum arabic (GA) plus EEP (B) on firmness in papaya fruit during cold storage. Vertical bars indicate ± SE

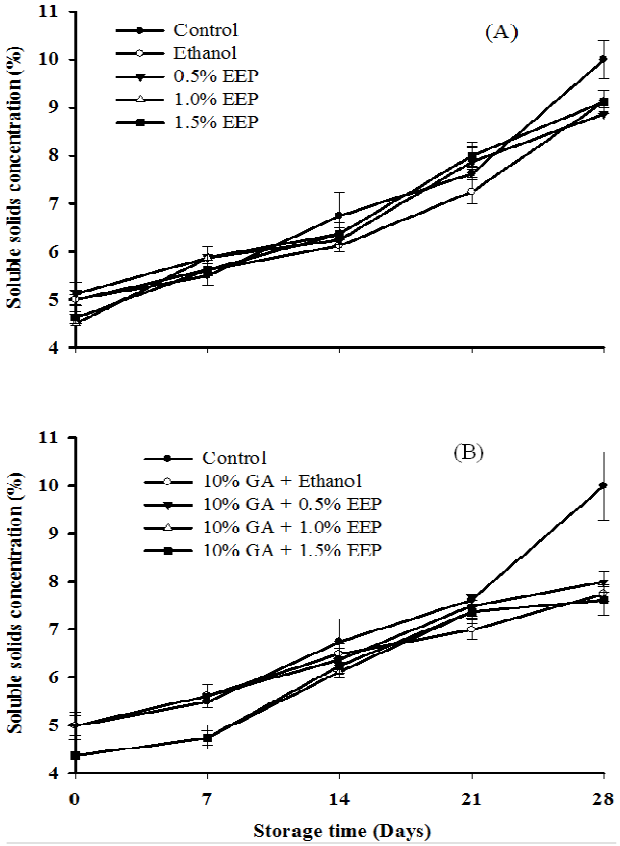

Fig. 6: Effect of different concentrations of ethanolic extract of propolis (EEP) (A) and gum arabic (GA) plus EEP (B) on soluble solids concentration in papaya fruit during cold storage. Vertical bars indicate±SE

The *in vivo* antifungal results are s in agreement with the findings by Pastor *et al.* (2011), who demonstrated greater reduction in microbial activities in table grapes coated with hydroxypropyl methylcellulose containing 1.5% EEP, as compared to the control fruit. Similarly, Yang *et al*. (2010) reported the potential use of Chinese propolis ethyl acetate extract in controlling blue and green moulds in citrus fruits. Results clearly revealed that propolis could be a promising source of natural antifungal in replacement to the fungicide applications in controlling postharvest disease in many fruits.

GA applied as edible coating on papayas might be created a semi-permeable barrier against the oxygen, carbon dioxide, moisture and solute movement, therefore limiting respiration and oxidation rates, as well as water loss of the fruit (Ali *et al.,* 2010). All these chances are due to the modified atmosphere generated by edible coatings, which partially sealed the pores on fruit skin and thus altering gaseous exchange and transfer rates (Lima *et al*., 2010). The present study had demonstrated the excellent film forming ability of GA in preserving the fruit quality, in term of weight loss, firmness, SSC and TA as compared to EEP.

The lower weight loss in ethanol treated fruit might be due to the fact that ethanol might have blocked stomata and guard cells, which slowed down active metabolic processes and maintained fruit weight. Weight loss increases gradually throughout the storage period, and it is due to the water loss driven by active metabolic processes, such as transpiration and respiration in the postharvest fruit (Abbasi *et al.,* 2011). Similar reduction in weight loss has been reported in banana (Maqbool *et al.* 2010b), and apple (El-Anany *et al.* 2009) with postharvest application of GA.

The application of composite coating resulted in modified atmosphere in fruit surfaces, which consequently retained the firmness of papaya fruit. The increase in softening in EEP treated fruit might be due to the fact that ethanol evaporated due to its volatile nature and left the hydrophobic propolis and water, which separated out later and left an open matrix for higher respiration, thus it could not]change the internal atmosphere (Zahid *et al*., 2013).

The decrease in TA with EEP concentration might be due to the open matrix of propolis after evaporation of ethanol, while the composite coating helped in retention of TA (Zahid *et al*., 2013).

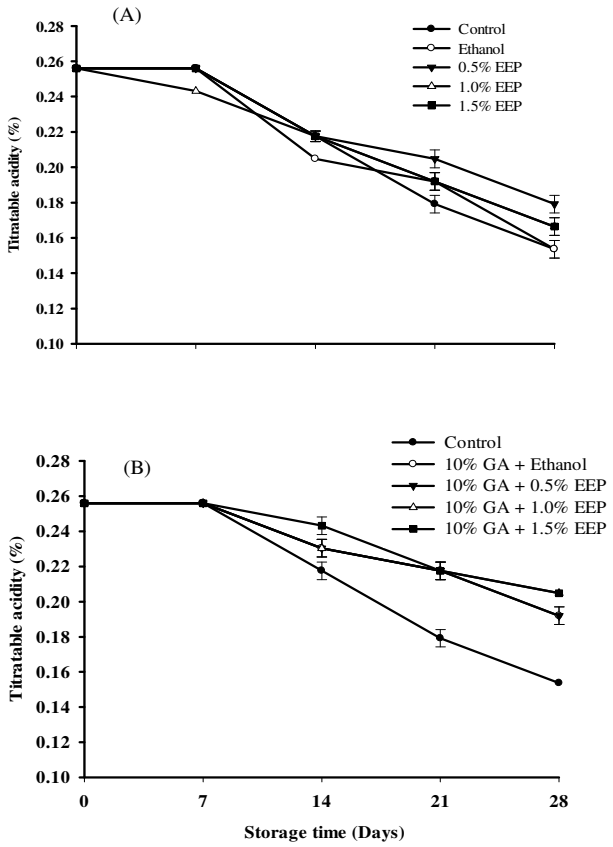

Fig. 7: Effect of different concentrations of ethanolic extract of propolis (EEP) (A) and gum arabic (GA) plus EEP (B) on titratable acidity in papaya fruit during cold storage. Vertical bars indicate ± SE

Nevertheless, with the lack of antifungal properties of GA, incorporating EEP into GA could give synergistic effect in reducing *C. gloeosporioides* on papaya as well as enhancing the postharvest shelf-life. In short, it can be concluded from this study that the composite coatings made up of 10% GA plus 1.5% EEP concentrations achieved more than 80% control of papaya and maintained quality during storage for up to 28 days. Therefore, the composite treatment of 10% GA plus 1.5% EEP can be used effectively for papaya growers and exporters as a postharvest biopesticides.

Acknowledgements

The authors thank the Ministry of Agriculture (MOA) Malaysia for providing financial support under the project grant (05-02-12-SF0031).

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(Received 21 December 2012; Accepted 22 April 2013)