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Dried *Garcinia atroviridis* Crude Extracts Incorporated with Gum Arabic Coating Controlled Postharvest Anthracnose Diseases in Dragon Fruits

Nur Aina Adlan Mustafa¹, Paa Kwesi Bordoh² and Azhane Ahmad^{3*}

¹ School of Biosciences, University of Nottingham Malaysia Campus, 43500 Semenyih, Selangor, Malaysia

² Crops For the Future (CFF), Food Plus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia

³ Department of Agrotechnology, School of Food Science and Technology,

Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

ABSTRACT

The aim of this study was to examine the antifungal effect of dried *Garcinia atroviridis* fruits extract (GAFE) in combination with gum arabic (GA) against red-fleshed dragon fruits (*Hylocereus polyrhizus*) anthracnose disease caused by *Colletotrichum gloeosporioides* as well as to determine the efficiency of dried GAFE in maintaining the postharvest quality of dragon fruits. *In vitro* results showed that the inhibition in mycelial and conidial growth was dose-dependent and greatest inhibition was recorded at 15 mg/mL dried GAFE+10% GA. Such concentration also conferred fungistatic effects to fungal growth and spore germination *in vitro*. Concentration at 10 mg/mL dried GAFE+10% GA exhibited similar effectiveness comparable to commercial fungicide (Mancozeb at 3.2 mg/mL) to control anthracnose disease in dragon fruits. In addition, fruits treated with 10 mg/mL dried GAFE+10% GAFE+10% GA markedly suppress disease incidence and severity as well as effectively maintained SSC and TA level throughout cold storage. On the other hand, lower dried GAFE concentrations (1 mg/mL and 4 mg/mL dried GAFE+10% GA) suppressed weight loss

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E-mail addresses: azhane_ahmad@yahoo.com; azhane.ahmad@umt.edu.my (Azhane Ahmad) nurainaadlan@gmail.com (Nur Aina Adlan Mustafa) paakwesib73@gmail.com (Paa Kwesi Bordoh)

* Corresponding author

and retained tissue firmness throughout shelf life of dragon fruits. Coating application at highest concentration of 15 mg/mL dried GAFE+10% GA impaired physicochemical quality by displaying phytotoxic effect on the dragon fruits.

Keywords: Colletotrichum gloeosporioides, dried *Garcinia atroviridis,* gum arabic, *Hylocereus polyrhizus,* postharvest quality

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INTRODUCTION

The red-fleshed dragon fruits (*Hylocereus polyrhizus*) is cultivated in Southeast Asian countries including Malaysia (Nguyen, 2006). This rare fruits attracted worldwide attention due to the their prominent purplered colour, economic importance as a food source and antioxidant properties associated with its rich betacyanin source (Le Bellec, Vaillant, & Inbert, 2006). Initially introduced at the end of the 1990's in Perak, the cultivation has expanded enormously around 927.4 ha in 2006 with a grand total of produce about 2534.2 tons (Masyahit, Sijam, Awang, & Mohd Satar, 2009).

Besides abiotic conditions such as chilling injury, mechanical injury and moisture loss, pests and diseases infestation can also affect dragon fruits cultivation and storage (Cheah & Zulkarnain, 2008). Recently, Colletotrichum gloeosporioides has caused a severe threat to the cultivation of the fruits due to suitable environmental condition for the growth of the fungus (Masyahit et al., 2009). The symptomatic characterizations of anthracnose on dragon fruits are reddish-brown spots and chlorotic haloes that appear on the fruit and stem, which coalesced to rot (Masyahit et al., 2009). This pervasive fungus contributed to more than 50% postharvest losses in commodities (Paull, Nishijima, Reyes, & Cavaletto, 1997).

Gum arabic (GA), a dried adhesive exudate from the stems and branches of *Acacia* species has been developed into edible coatings and used as carriers of antimicrobial constituents through their incorporation with antimicrobial natural products to delay ripening and extend the shelf life of fruits such as papaya and banana (Ali, Cheong, Zahid, 2014; Maqbool et al., 2011). Additionally, this natural biopolymer provides a physical and chemical barrier against the growth of microbes on the fruit surface (Ali, Maqbool, Ramachandran, & Alderson, 2010).

Traditionally, synthetic fungicides such as Propineb and Difenoconazole are used to control anthracnose caused by *Colletotrichum* species on several fruits and vegetables (Hoa, 2008). Nevertheless, consumers' trend of a healthier lifestyle demands the fresh produce to be free from synthetic fungicide. Applying single fungicide continuously on fresh fruits and vegetables could develop resistant fungal strains and decrease the effectiveness of fungicide against the target organisms (Edirisinghe, Ali, Maqbool, & Alderson, 2014; Maqbool et al., 2011).

Additionally, some microbial growth on fresh produce has been controlled by using various chemicals-based washing and sanitizing agents such as chlorine (Ali, Goh, & Yeoh, 2016). Despite their antimicrobial effect on fruits, its use has been restricted due to their carcinogenicity, long degradation time and high residual toxicity which cause serious effect to human health and ecosystem (Zahid, Maqbool, Siddiqui, Manickam, & Ali, 2015). Alternative approach using natural products such as plant parts (fruit, leaf, seed, root and pulp) which confer antimicrobial properties due to their bioactive secondary metabolites are used to control several phytopathogens (Tripathy & Dubey, 2004).

Garcinia atroviridis Griff. ex T. Anders, a medium-sized tree, which is common in Peninsular Malaysia especially in the northern states is one medicinal plants with significant antimicrobial activity. Apart from its culinary use, the sun-dried slices of the fruits are also used in folkloric medicine such as a post-partum medication and weight management on a short-term basis, and also rich in antioxidants and other secondary metabolites such as xanthone, benzophenones, flavonoids, biflavanoids (Minami, Takahashi, Kodama, & Fukuyama, 1996; Waterman & Hussain, 1983) and lactones and phenolic acid (Joseph, Jayaprakasha, Selvi, Jena, & Sakariah, 2005) which are beneficial for human health (Nursakinah et al., 2012).

Additionally, in vitro studies of dried G. atroviridis showed their wide antibacterial effects against Bacillus subtilis B28 & B29, MRSA, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa as well as antifungal properties against phytopathogen Clasdosporium herbarum (Mackeen, Ali, & Lajis, 2000) while being non-cytotoxic in mammalian cells (Mackeen et al., 2012). However, the antifungal effect of dried G. atroviridis to control postharvest anthracnose in dragon fruits caused by plant pathogen C. gloeosporioides has not been reported.

Considering the antifungal properties of *G. atroviridis* and the fact that it is generally regarded as safe (GRAS), biodegradable

and environmentally friendly, the study examined the antifungal effect of dried *G. atroviridis* fruits extract (GAFE) in combination with GA against dragon fruits anthracnose disease caused by *C. gloeosporioides*. Finally, the efficacy of dried GAFE in maintaining the postharvest qualities of dragon fruits was further evaluated.

MATERIALS AND METHODS

Materials

Red-fleshed dragon fruits were purchased from a local supplier in Semenyih, Selangor, Malaysia. Fruits were chosen and assorted to be unvarying in size, colour, and shape, free from diseases, blemishes and physical injury. Fruits were washed with distilled water and air-dried at ambient temperature $(25\pm1 \ ^{\circ}C)$ before coating treatment. Sundried *Garcinia atroviridis* was obtained from a local supplier in Ipoh, Perak, Malaysia while gum arabic powder was purchased from Sigma-Aldrich, USA.

Isolation and Identification of *C. gloeosporioides* from Dragon Fruits

Fungal Isolation and Preparation of Cultures on PDA. *C. gloeosporioides* was isolated from infected dragon fruits exhibiting definite anthracnose symptoms. Small slices of the infected fruit with disease lesions were excised using a scalpel. Surface sterilization was conducted by dipping excised tissue in 1% sodium hypochlorite solution and 70% ethanol for 2 min each, followed by 3x washing in distilled water. The washed tissue was dried on a tissue paper, plated on Potato Dextrose Agar (PDA) and incubated at 25 ± 1 °C for one week. The mycelial growth was observed and the colonies were re-isolated on fresh PDA plates to obtain pure cultures. The isolates were identified as *C. gloeosporioides* based on their morphological and cultural features (Barnett & Hunter, 1972) and preserved for further use.

Preparation of Crude *Garcinia* atroviridis Dried Fruits Extract

The crude extract was obtained by maceration using methanol as suggested by Mackeen et al. (2000) with slight modifications. Sundried sample was oven dried at 45 °C for 72 hours to remove moisture completely prior to grinding the materials into powdered form. The sample was pulverized using rotor beater mill. Approximately 500 g of the pulverized sample was macerated in 800 mL methanol for three days. Three times extraction was performed until the powerderd sample looked pale or white. The extract solution was pooled after 3x extraction before filtration. The filtered pooled solution was concentrated by evaporation under reduced pressure at 45 °C in water bath using a rotary evaporator (BUCHI Rotavapor R-200) to obtain crude extract. The extract was then kept at 4 °C until further use.

Preparation of Gum Arabic Solution

Gum arabic (10%) was prepared by dissolving 100 g of gum arabic powder in 1 L distilled water. The gum arabic solution was stirred for 60 min at 40 °C. The solution was then filtered through three layers of muslin cloth to remove undissolved impurities.

In vitro Antifungal Assay of Dried GAFE+10% GA Against *C. gloeosporioides* Antifungal Assay

The antifungal efficacy of crude extracts was determined by poisoned food technique based on inhibiton of radial mycelial growth and conidial germination of C. gloeosporioides (Balamurugan, 2014). PDA was amended with different concentrations of dried GAFE incorporated with 10% GA -1 mg/mL, 4 mg/mL, 10 mg/mL and 15 mg/ mL. The 10% GA and PDA was prepared separately in the ratio of 1:7. The amended PDA media was allowed to solidify prior to antifungal assay. Three control lines were used: PDA plates with commercial fungicide Mancozeb (3.2 mg/mL), PDA plates with 10% gum arabic as positive control and plates containing solely PDA as negative control. Mycelial plugs (7 mm) of C. gloeosporioides were placed at the centre of PDA plates and were incubated at 25±1 °C. In vitro assays were carried out in three replicates for each treatments including control.

Radial Mycelial Growth. Radial mycelial growth was measured daily until the control dishes reached the edge of the plate. The percentage inhibition in radial growth (PIRG) was recorded after 8 days of incubation according to the formula described by Sivakumar, Hewarathgamagae, Wijeratnam and Wijesundera (2002).

Inhibition of Conidial Germination. Conidial germination inhibition test was carried out using the cavity slide technique (Cronin, Yohalem, Harris, & Andrews, 1996). An aliquot of 100 µl of the spore suspension was pipetted onto each treatment plates including of controls (diluted to 10^{5} /mL conidia concentration using a hemocytometer). After 48 hours of incubation, the conidia were killed by adding 10 µl 2% sodium azide to each cavity. Approximately, 100 conidia per replicate were observed for germination in each treatment. The conidium was presumed to be germinated when the germ tube was partial or more than the length of the conidium. The percentage of inhibition in germination was calculated:

% germination inhibition = 1- (Gr/Gc) x 100 [1]

where,

Gr = Number of spore germination in the treatment;

Gc = Number of spore germination in the control

Scanning electron microscopy on spore and mycelial structure

Four spores and mycelial samples (1.5 mm³) were taken from all the treatments including control and mounted on aluminium stubs and viewed and photographed under the scanning electron microscope (Model Quanta 400 FESEM, FEI Company, USA).

In vivo Antifungal Assay of Dried GAFE+10% GA Against *C. gloeosporioides*

Preparation and Coating Application on Fruits. A stock solution of GAFE crude extracts was prepared at 33.9 mg/mL by dissolving the extracts with 10% GA. The working concentrations (1 mg/mL, 4 mg/mL, 10 mg/mL and 15 mg/mL) were prepared by adding 10% GA to the stock solution until a final volume of 1.5 L (volume enough to completely immerse the fruit). The working concentrations were homogenized for 30 min prior to use. Fruits were washed with 0.01% sodium hypochlorite, rinsed with distilled water and left to dry at 25±1 °C. Dragon fruits were immersed for 1 min in C. gloeosporioides spore suspension (10^5) spore/mL) and air-dried.

Subsequently, fruits were immersed in working concentrations (1 mg/mL, 4 mg/mL, 10 mg/mL, 15 mg/mL) for 1 min and air-dried fully. For negative and positive controls, fruits were immersed in spore suspension (10⁵ spores/mL) and commercialized fungicide solution Mancozeb (3.2 mg/mL concentration) for 1 min and air-dried fully. Prior to cold storage, dragon fruits were packed in cardboard carton boxes and stored at 10±1 °C, 80±5% RH.

Disease Incidence and Disease Severity. The disease incidence (DI) and disease severity (DS) was measured at 7 days interval for 21 days. Data for DI were determined based on the percentage of fruits exhibiting anthracnose out of the total amount of fruits per treatment (Cooke, 2006). DS was scored using a scale as per the method of Sivakumar et al. (2002) with some modification.

A scale of 0 was given when there was no sign of disease on the fruit surface. Scales 1, 2, 3 and 4 were given when the surface of the fruits were infected up to 25, 50, 75 and >75% with symptoms of anthracnose. Experiments were conducted in three replicates with 5 fruits per treatment (n=15).

Postharvest Physicochemical Fruit Quality Assessment

A digital balance (Model GF-6100, Japan) was used to weigh fruits from each treatment at day 0 and at 7 days intervals for 21 days. Total weight loss during storage intervals was measured by subtracting the initial and final fruit weight. Weight loss was expressed as percentage weight loss. The firmness of the fruit was measured using an Instron texture analyzer with an 8 mm plunger tip (Instron 5540, USA). The fruit was subjected to a puncture test at a constant speed of 20 mm/min on three points along the surface of the fruit.

Dragon fruits were cut into small pieces and the pulp was homogenized in a kitchen blender by grinding 20 g of the dragon fruit pieces in 80 mL distilled water. After blending, the filtrate (juice) was filtered using a muslin cloth. Soluble solid content (SSC) of dragon fruit pulp was analyzed using a Palettle Digital Refractometer (Model PR-32 α ; Atago Co, Ltd. Japan). An aliquot of 100 µl of the filtrate was pipetted onto the prism glass of the refractometer to obtain the reading. The refractometer was calibrated with purified water before analysis. The readings were multiplied by the dilution factor to obtain the SSC of the dragon fruits pulp.

Titratable acidity (TA) was measured using the remaining filtrate of SSC. A 5 ml aliquot of filtrate was titrated with 0.1 N NaOH until the pH reached an endpoint pink 8.1. The results were expressed as the percentage citric acid per 100 g of fresh weight. Physical and biochemical assessments were conducted in three replicates with 5 fruits per treatment (n=15).

Statistical Analysis

Experiments were conducted in a completely randomized design. Experimental data were analyzed using the analysis of variance (ANOVA) procedure in Genstat 16 version (VSN International Ltd, UK). The means separation was done using Fisher's unprotected LSD at p<0.05.

RESULTS

In vitro Antifungal Assay of Dried GAFE+10% GA Against *C. gloeosporioides*

The efficacy of dried GAFE to control mycelial and conidial growth was dose dependent. Plates with combined treatment of 15 mg/mL dried GAFE+10% GA exhibited the greatest fungistatic inhibition effect against *C. gloeosporioides* radial mycelial growth (Figure 1). Dried GAFE at 15 mg/mL, 10 mg/mL, 4 mg/mL and Mancozeb treated plates showed significant

fungistatic effect (p < 0.05) of up to 70.26, 61.29, 30.16 and 55.5% respectively compared to negative control (only PDA) after 10 days. Based on Figure 1, the inhibitory effect on mycelial growth was not significant in the positive control of PDA (only 10% GA) indicating that GA does not confer antifungal effect.

The effect of the dried GAFE in inhibiting conidia germination was also studied. Spores from negative control (PDA only) and positive control plates (PDA+10% GA) germinated and grew to form long hyphae (germ tube) (Figure 2a). In contrast, the crude extract markedly inhibited conidial germination after 48 hours of incubation in dark (Figures 2b and 2c). The efficacy of the extract to inhibit conidial germination was also dose dependent and was highest effects were at 15 and 10 mg/ mL dried GAFE+10% GA.

Based on the conidial germination assay, scanning electron microscopy (SEM) was performed to examine the mode of action of dried GAFE on morphology of *C. gloeosporioides* fungal hyphae. Figure 3a showed that fungal hyphae in the control plates appeared elongated and normal meanwhile fungal hyphae in GAFE-treated plates were shrunk, wrinkled and distorted as compared to the control (Figure 3b).



Figure 1. Inhibition percentage of dried *Garcinia atroviridis* fruit extract (GAFE) incorporated with 10% gum arabic (GA) on *C. gloeosporioides* mycelial growth with respect to the negative control after 8 days at ambient temperature $(25\pm1 \text{ }^\circ\text{C})$. The vertical bars represent standard error of means for three replicates



Figure 2. Inhibition of conidia germination (a) in the Potato Dextrose Agar (PDA) control and PDA+10% gum arabic (GA) plates (b) 1 mg/mL+10% GA and 4 mg/mL+10% GA (c) 10 mg/mL+10% GA and 15 mg/mL+10% GA after 48 hours incubation in the dark. Red arrow indicates elongated form of germ tub



Figure 3. Scanning electron micrographs (SEM) of *C. gloeosporioides* spore and mycelial structure. Fungal hyphae in (a) control plates and (b) plates treated with dried GAFE+10% GA. Blue arrow indicates normal, elongated and healthy hyphae. Red arrows indicate shrunken, wrinkled and swollen hyphae while red box shows distortion of hyphae

Disease Incidence and Disease Severity

There were no significant effects (p>0.05) of dried GAFE treatments on disease incidence (DI) and disease severity (DS) after 21 days of cold storage. Irrespective of treatments, DI and DS increased significantly throughout storage, however DI and DS were the lowest at 10 mg/mL dried GAFE+10% GA as compared to the control (Figure 4a and 4b). The efficacy of 10 mg/mL dried GAFE+10% GA was also comparable to commercialized fungicide (Mancozeb) in controlling anthracnose disease in dragon fruits.

Integrated Management of Anthracnose Disease from Natural Extracts



Figure 4(a). Effect of dried GAFE in combination with 10% GA on disease incidence during 21 days of cold storage at 10 ± 1 °C. Vertical bars indicate standard error of means for three replicates



Figure 4(b). Effect of dried GAFE in combination with 10% GA on disease severity of dragon fruits caused by *C. gloeosporioides* during 21 days of cold storage at 10 ± 1 °C. Vertical bars indicate standard error of means for three replicates

Postharvest Physicochemical Fruit Quality Assessment

Weight loss of dragon fruit was significantly affected (p < 0.05) by GAFE and GA treatments throughout cold storage (Figure 5a). The lowest weight losses were observed in control fruits (4.5%) on final day of

storage. Meanwhile, highest doses of GAFE at 15 and 10 mg/mL+10% GA experienced highest weight loss of 5.9% and 5.7% respectively (Figure 5a). There were no significance differences (p>0.05) between

all dried GAFE the treatments on weight loss and regardless the treatment, the weight loss increased significantly (p<0.05) until the end of storage periods.

The firmness of dragon fruit declined gradually throughout cold storage (Figure 5b). Fruits treated with 15 and 10 mg/ mL dried GAFE+10% GA experienced lowest firmness loss with 25% and 24% respectively at the end of storage. In contrast, higher firmness loss of 29% and 27% were recorded in control fruits and 1 mg/mL dried GAFE+10% GA treated fruits respectively (Figure 5b). Nevertheless, there was no significance difference (p>0.05) amongst dried GAFE treatments on tissue firmness throughout cold storage.

SSC of dragon fruits stored at cold storage were slightly reduced but there were no significance difference (p>0.05) between control and GAFE treated-fruits (Figure 5c). Fruits treated with 10 mg/ mL dried GAFE+10% GA maintained higher SSC compared to other treatments throughout storage. As ripening takes place, an increment in SSC was coincident with TA decline. Fruits treated with extract at 10 mg/mL incorporated with 10% GA recorded highest acidity value compared to the control (Figure 5d). However there was no significant difference (p>0.05) amongst GAFE treatments throughout storage.



Figure 5. Effect of the dried GAFE incorporated with 10% GA on weight loss of dragon fruits during 21 days of cold storage at 10 ± 1 °C. Vertical bars indicate standard error of means for three replicates

Integrated Management of Anthracnose Disease from Natural Extracts



Figure 5. Effect of the dried GAFE incorporated with 10% GA on tissue firmness of dragon fruits during 21 days of cold storage at 10 ± 1 °C. Vertical bars indicate standard error of means for three replicates



Storage time (days)

Figure 5. Effect of the dried GAFE incorporated with 10% GA on soluble solid content of dragon fruits during 21 days of cold storage at 10±1 °C. Vertical bars indicate standard error of means for three replicates

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Figure 5. Effect of the dried GAFE incorporated with 10% GA on titrable acidicity (TA) of dragon fruits during 21 days of cold storage at 10 ± 1 °C. Vertical bars indicate standard error of means for three replicates

DISCUSSIONS

Mycelial growth and conidial germination were clearly affected at higher concentrations of dried GAFE (15>10>4>1 mg/mL)+10% GA. Among all the treatments tested, the 15 mg/mL of GAFE+10% GA was found to be most effective in controlling fungal growth and spore germination in vitro. Dried GAFE significantly inhibited C. gloeosporioides growth and germination and that the inhibition was due to the presence of bioactive compounds which strongly exerted antifungal properties. The higher crude extract concentrations may contain higher accumulation of secondary metabolites thus resulting in stronger effects of fungal inhibition in vitro. Dose dependent antifungal effect of natural products against C. gloeosporioides has been reported in earlier literatures (Ali, Chow, Zahid, & Ong, 2013; Ali et al., 2014; Rahman, Mahmud,

Kadir, Abdul Rahman, & Begum, 2008).

Based on the SEM results, it can be observed that the GAFE extract caused distortion, swelling and shrivelling of fungal hyphae. Crude extract of natural products may result in structural alteration of the hyphal morphology and interference of plasma membrane of fungi (Ait Barka, Eullaffroy, Clément, & Vernet, 2004). The actual mode of action of GAFE extract is still unclear however the extract might have caused disruptions of the mycelial growth and an inactivation of the spore germination due to its high antifungal properties.

On the other hand, findings with dried GAFE extract were not apparent in the *in vivo* study. DI and DS in dragon fruits were prevalent at highest concentration (15 mg/mL) in combination with 10% GA

probably due to phytotoxicity arising from the concentrated extract. The phytotoxic effect might render some host cells more vulnerable to fungal pathogen attack resulting in tissue damage, thus may reduce disease resistance to anthracnose (Yulia, Shipton, & Coventry, 2006).

However, lower concentrations such as 4 and 1 mg/mL dried GAFE+10% GA may have insufficient amount of bioactive compound to inhibit the fungal growth, therefore compounded the DI in dragon fruits. Meanwhile, at concentration of 10 mg/mL extract markedly inhibited progression of DI and DS. This could be due to fact that at such concentration when combining with 10% GA might be the optimum concentration to reduce the development of anthracnose caused by C. gloeosporioides. Treatment with dried GAFE not only provides antifungal effects but the synergistic interaction between GA coatings by limiting respiration rate in fruit which might reduce the incidence of anthracnose.

Fruit weight loss is closely related to respiration rate and moisture evaporation through the skin (Hernández-Muñoz, Almenar, Valle, Velez, & Gavara, 2008). Higher weight loss was observed in fruits coated with higher concentrations of dried GAFE (15 and 10 mg/mL)+10% GA. This study revealed that low concentrations of GAFE extract (1 and 4mg/mL)+10% GA was more effective compared to higher concentrations, explaining higher composite coating may resulted higher weight loss in the fruit due to anaerobic respiration and subsequently leading to respiratory heat. Studies by Ghasemnezhad, Shiri and Sanavi (2010) and Zahid, Ali, Siddiqui and Maqbool (2013) also reported higher concentration of natural products showed higher weight loss in apricot and dragon fruits respectively.

Softening is commonly associated with progressive activity of hydrolase enzymes acting on plant cell wall during ripening onset (Ali, Chin, Marimuthu, & Lazan, 2004). Reduced tissue firmness was observed in all fruits regardless of treatments. However, firmness loss was more apparent in fruit treated with 15 and 10 mg/mL GAFE extract+10%GA compared to lower concentrations. A plausible explanation might be due to the decreased cohesion between fruit and the extract as the concentration of dried GAFE increases, which in turn prevent the change in internal atmosphere of the fruit resulting in the loss of textural firmness (Zahid et al., 2013).

It was well documented that respiration process requires breakdown of carbohydrate into sugar and use of organic acids as an energy source respectively (El-Anany, Hassan, & Rehab Ali, 2009). In all cases, SSC increment and TA reduction in dragon fruits were observed during storage. Coating with 10 mg/mL GAFE+10% GA was found to be the most effective by decelerating metabolic activities compared to other treatments. This is probably because of modification of the internal atmosphere that suppressed respiration rate of the fruit which also might slowed down SSC increment and TA reduction throughout storage period.

CONCLUSIONS

In conclusion, 15 mg/mL dried GAFE incorporated with 10% GA exhibited maximum inhibitory effect on mycelial and conidial growth. At such concentration, the extract has significant antifungal effect on dragon fruits' anthracnose in vitro. In addition, 10 mg/mL dried GAFE plus 10% GA was as efficient as the commercial fungicide during in vitro, markedly controlled DI and DS progression and maintained higher SSC and acidity value throughout storage period. Albeit its outstanding antifungal effectiveness in vitro, the dried GAFE at higher concentrations (15 and 10 mg/mL+10% GA) resulted undesirable outcomes in postharvest quality such as higher weight loss and hastened tissue firmness. Nevertheless, coating application of natural extract from dried GAFE incorporated with 10% GA can be considered as environmentally friendly alternatives that provide protection from postharvest anthracnose disease and also prolonging the shelf life of dragon fruits.

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REFERENCES

Ait Barka, E., Eullaffroy, P., Clément, C., & Vernet, G. (2004). Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. *Plant Cell Reporter*, 22(8), 608-614.

- Ali, A., Cheong, C., & Zahid, N. (2014). Composite effect of propolis and gum arabic to control postharvest anthracnose and maintain quality of papaya during storage. *International Journal of Agriculture Biology*, 16(6), 1117-1122.
- Ali, A., Chow, W., Zahid, N., & Ong, M. (2013). Efficacy of propolis and cinnamon oil coating in controlling postharvest anthracnose and quality of chilli (*Capsicum annuum* L.) during cold storage. *Food Bioprocess Technology*, 7(9), 2742-274.
- Ali, A., Goh, K. H., & Yeoh, W. K. (2016). Efficacy of ginger oil and extract combined with gum arabic on anthracnose and quality of papaya fruit during cold storage. *Journal of Food Science Technology*, 53(3), 1435-1444.
- Ali, A., Maqbool, M., Ramachandran, S., & Alderson, P. G. (2010). Gum arabic as a novel edible coating for enhancing shelf-life and improving postharvest quality of tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biology and Technology*, 58(1), 42-47.
- Ali, Z. M., Chin, L. H., Marimuthu, M., & Lazan, H. (2004). Low temperature storage and modified atmosphere packaging of carambola fruit and their effects on ripening related texture changes, wall modification and chilling injury symptoms. *Postharvest Biology Technology*, 33(2), 181-192.
- Balamurugan, S. (2014). In vitro antifungal activity of Citrus aurantifolia Linn plant extracts against phytopathogenic fungi Macrophomina phaseolina. International Letters of Natural Science, 13, 70-74.
- Barnett, H. L., & Hunter, B. B. (1972). *Illustrated genera of imperfect fungi*. (p. 24). Minneapolis, USA: Burgess Publishing Co.
- Cheah, L. S., & Zulkarnain, W. M. (2008). Status of pitaya cultivation in Malaysia. Seminar on Pitaya Production, Market and Export Challenges and Prospects, Putrajaya, Malaysia:

International Tropical Fruits Network. Retrieved March 12, 2016, from http://www.itfnet.org/ source/mainpage/newsAndEvent/contents/PDF_ Pitaya_Seminar/Status%20of%20pitaya%20 cultivation%20in%20Malaysia.pdf

- Cooke, B. M. (2006). Disease assessment and yield loss. In B. M. Cooke, D. G. Jones, & B. Kaye (Eds.), *The epidemiology of plant diseases* (2nd ed.) (pp. 43-80). Dordrecht, Netherlands: Springer.
- Cronin, M. J., Yohalem, D. S., Harris, R. F., & Andrews, J. H. (1996). Putative mechanism and dynamics of inhibition of apple scab pathogen *Venturia inequalis* by compost extracts. *Soil Biology and Biochemistry*, 28(9), 1241-1249.
- Edirisinghe, M., Ali, A., Maqbool, M., & Alderson, P. G. (2014). Chitosan controls postharvest anthracnose in bell pepper by activating defenserelated enzymes. *Journal of Food Science Technology*, 51(12), 4078–4083.
- El-Anany, A. M., Hassan, G. F. A., & Rehab Ali, F. M. (2009). Effects of edible coatings on the shelflife and quality of Anna apple (*Malus domestica* Borkh) during cold storage. *Journal of Food Technology*, 7(1), 5-11.
- Ghasemnezhad, M., Shiri, M., & Sanavi, M. (2010). Effect of chitosan coatings on some quality indices of apricot (*Prunus armeniaca* L.) during cold storage. *Caspian Journal of Environment Science*, 8(1), 25-33.
- Hernández-Muñoz, P., Almenar, E., Valle, V. D., Velez, D., & Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria×ananassa*) quality during refrigerated storage. *Food Chemistry*, 110(2), 428-435.
- Hoa, N. V. (2008). Current research activities and the development of good agricultural practice (GAP) for pitaya in Vietnam. Seminar on Pitaya Production, Market and Export-Challenges and

Prospects, Putrajaya, Malaysia: International Tropical Fruits Network. Retrieved February 24, 2016, from http://www.itfnet.org/source/ mainpage/newsAndEvent/contents/PDF_Pitaya_ Seminar/Current%20research%20activities%20 and%20development%20of%20good%20 agricultural%20practices%20(GAP)%20for%20 pitaya%20in%20Vietnam.pdf

- Joseph, G. S., Jayaprakasha, G. K., Selvi, A. T., Jena, B. S., & Sakariah, K. K. (2005). Antiaflatoxigenic and antioxidant activities of *Garcinia* extracts. *International Journal of Food Microbiology*, 101(2), 153-160.
- Le Bellec, F., Vaillant, F., & Imbert, E. (2006). Pitahaya (*Hylocereus* spp.): A new fruit crop, a market with a future. *Fruits*, 61(4), 237-250.
- Mackeen, M., Ali, A., & Lajis, N. (2000). Antimicrobial, antioxidant, antitumor-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* Griff. ex T. Anders. *Journal of Ethnopharmacology*, 72(3), 395-402.
- Mackeen, M., Lim, Y. M., Amran, M., Mat, N., Lajis, N. H., & Ali, A. M. (2012). Noncytotoxic and antitumour-promoting activities of *Garcinia* acid esters from *Garcinia atroviridis* Griff. ex T. Anders (Guttiferae). *Evidence-Based Complementary and Alternative Medicine*, 2012, 1-5.
- Maqbool, M., Ali, A., Alderson, P. G., Mohamed, M. T. M., Siddiqui, Y., & Zahid, N. (2011). Postharvest application of gum arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biology and Technology*, 62(1), 71-76.
- Masyahit, M., Sijam, K., Awang, Y., & Mohd Satar, M. (2009). The first report of the occurrence of anthracnose disease caused by *Colletotrichum* gloeosporioides on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia. *American Journal* of *Applied Sciences*, 6, 902-912.

- Minami, H., Takahashi, E., Kodama, M., & Fukuyama, Y. (1996). Three xanthones from *Garcinia* subelliptica. Phytochemistry, 41(2), 629-633.
- Nguyen, D. L. (2006). Current difficulties and solutions for enhancing production and export of Vietnamese fruit. Proceedings of International Seminar on Enhancing Export Competitiveness of Asian Fruits (pp. 34-46). Bangkok, Thailand: United Nations Asian and Pacific Centre for Agricultural Engineering and Machinery.
- Nursakinah, I., Zulkhairi, H., Norhafizah, M., Hasnah, B., Zamree, M. S., Farrah, S. I., ... Hamzah, F. H. (2012). Nutritional content and *in vitro* antioxidant potential of *Garcinia atroviridis* (asam gelugor) leaves and fruits. *Malaysian Journal of Nutrition*, 18(3), 363-371.
- Paull, R. E., Nishijima, W., Reyes, M., & Cavaletto, C. (1997). Postharvest handling and losses during marketing of papaya (*Carica papaya* L.). *Postharvest Biology Technology*, 11(3), 165-179.
- Rahman, M., Mahmud, T., Kadir, J., Abdul Rahman, R., & Begum, M. (2008). Antimicrobial activities of chitosan and calcium chloride on *in vitro* growth of *Colletotrichum gloeosporioides* from papaya. *Pertanika Journal of Tropical Agricultural Science*, 31(2), 223-232.
- Sivakumar, D., Hewarathgamagae, N. K., Wijeratnam, R. S. W., & Wijesundera, R. L. C. (2002). Effect of ammonium carbonate and sodium bicarbonate on anthracnose of papaya. *Phytoparasitica*, 30(5), 1-7.
- Tripathi, P., & Dubey, N. K. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biology Technology*, 32(3), 235-245.
- Waterman, P. G., & Hussain, R. A. (1983). Systematic significance of xanthones, benzophenones and biflavonoids in *Garcinia*. *Biochemical Systematics and Ecology*, 11(1), 21-28.

- Yulia, E., Shipton, W., & Coventry, R. (2006). Activity of some plant oils and extracts against *Colletotrichum gloeosporioides*. *Plant Pathology Journal*, 5(2), 253-257.
- Zahid, N., Ali, A., Siddiqui, Y., & Maqbool, M. (2013). Efficacy of ethanolic extract of propolis in maintaining postharvest quality of dragon fruit during storage. *Postharvest Biology Technology*, 79, 69-72.
- Zahid, N., Maqbool, M., Siddiqui, Y., Manickam, S., & Ali, A. (2015). Regulation of inducible enzymes and suppression of anthracnose using submicron chitosan dispersions. *Scientia Horticulture*, 193, 381–388.