UNIVERSITI PUTRA MALAYSIA

# *EFFECTS OF TORCH GINGER ESSENTIAL OIL ON PATHOGENS, QUALITY AND STORAGE OF DRAGON FRUIT [Hylocereus polyrhizus (Weber) Britton & Rose]*

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# EFFECTS OF TORCH GINGER ESSENTIAL OIL ON PATHOGENS, QUALITY AND STORAGE OF DRAGON FRUIT [*Hylocereus polyrhizus* (Weber) Britton & Rose]

**By**

**KHAWARIZMI MOHD AZIZ**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science**

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**EFFECTS OF TORCH GINGER ESSENTIAL OIL ON PATHOGENS, QUALITY AND STORAGE OF DRAGON FRUIT [*Hylocereus polyrhizus* (Weber) Britton & Rose]**

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**October 2018**

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Dragon fruit is an exotic fruit which exhibits a non-climacteric pattern of respiration. This indicates it needs to be harvested from the tree when it is fully ripe since it cannot ripen after harvest. This had become a limiting factor for this fruit to have longer storage life. Dragon fruit also very susceptible to postharvest disease during storage. In addition, the bract of this fruit is easily dried and turn to brown few days after harvest which cause losses to its appearance. Thus, to overcome this problem, torch ginger leaf's essential oil (EO) was used as a natural antimicrobial agent for dragon fruit to extend the storage life as well as to maintain the fruit quality. The aim of this research was to evaluate the antimicrobial activity of torch ginger leaf's EO towards the isolated pathogen from dragon fruit and to investigate the effect of this EO incorporated into tapioca starch as edible coating on dragon fruit. In the first study, pathogen was isolated and identified from diseased dragon fruit. Second study was the continuation of the first study, where EO antifungal effect was tested against isolated pathogens *in vitro*. The experiment was conducted using completely randomized design. *Bipolaris cactivora* and *Fusarium incarnatum* were found to be the causal pathogen for dragon fruit during storage. Results of poison agar study portray that 0.5% EO was able to inhibit 60.22% of *B. cactivora* mycelium growth compared to 38.11% of *F. incarnatum*. However, the conidia germination study reflect that *B. cactivora* conidia were more resistant to EO where it showed higher germination as compared to *F. incarnatum*. Spore count showed that 0.5% of EO was able to completely stop the spore production of *F. incarnatum* while the same concentration only reduces the spore of *B. cactivora*.

The third experiment was done using completely randomized design in factorial arrangement (8 treatments x 3 weeks of storage). Different concentration of EO was incorporated into tapioca starch as coating for dragon fruit and stored at 5 ± 2 ºC for three weeks at 80 ± 5% relative humidity. The analysis was done at weekly interval where various physico-chemical analysis were analysed. Bract visual quality was found to be better with low concentration of EO which is the same for peel disease severity (DS) where 1% EO treatment showed lower DS. Water loss was found to be lower in coated fruit. Peel firmness and respiration rate were found to react differently towards different coating treatments. Glossiness was found to be higher in higher concentration of EO used. Ascorbic acids did not respond to treatments applied. However, other antioxidant assays were found to show higher concentration of antioxidant in fruit treated with 1% EO compared to control. Overall, findings in both *in vitro* and *in vivo* study it can be concluded that 2% EO was effective in controlling most parameters studied in *in vitro* study while fruit coated with 1% EO was found to be potentially beneficial to improve the postharvest storage of this fruit as portrayed by the result in *in vivo* study.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

**KESAN PATI MINYAK KANTAN TERHADAP PATOGEN, KUALITI DAN PENYIMPANAN BUAH NAGA [*Hylocereus polyrhizus* (Weber) Britton & Rose]**

Oleh

**KHAWARIZMI MOHD AZIZ**

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Buah naga adalah buah eksotik yang mempamerkan pola pernafasan bukan klimatik. Ini bermakna ia perlu dituai dari pokok apabila ia masak sepenuhnya kerana buah ini tidak boleh masak selepas dituai. Ini telah menjadi faktor yang mengehadkan jangka hayat penyimpanan buah yang lebih lama. Buah naga juga sangat mudah terdedah kepada penyakit pasca tuai semasa penyimpanan. Di samping itu, kelopak buah ini mudah kering dan berubah menjadi coklat beberapa hari selepas dituai dan menyebabkan penampilanya menjadi kurang menarik. Oleh itu, untuk mengatasi masalah ini, pati minyak daun kantan (EO) telah digunakan sebagai agen antimikrob semulajadi untuk memanjangkan jangka hayat penyimpanan serta mengekalkan kualiti buah semasa penyimpanan pasca tuai. Tujuan penyelidikan ini adalah untuk menilai aktiviti antimikrob EO daun kantan terhadap patogen dari buah naga dan untuk mengkaji kesan EO ini yang diadun ke dalam kanji ubi sebagai lapisan yang boleh dimakan untuk buah naga. Dalam kajian pertama, patogen telah diasingkan dan dikenal pasti dari buah naga yang berpenyakit. Kajian kedua adalah kesinambungan kajian pertama, di mana kesan antimikrob EO diuji terhadap patogen secara *in vitro*. Eksperimen ini dijalankan dengan menggunakan reka bentuk sepenuhnya rawak. *Bipolaris cactivora* dan *Fusarium incarnatum* didapati menjadi patogen untuk buah naga semasa penyimpanan pasca tuai. Hasil kajian racun agar menunjukkan bahawa 0.5% EO mampu merencat 60.22% pertumbuhan mycelia *B. cactivora* berbanding 38.11% daripada *F. incarnatum*. Walau bagaimanapun, kajian percambahan spora menunjukkan bahawa spora *B. cactivora* lebih tahan terhadap EO di mana ia menunjukkan percambahan yang lebih tinggi berbanding dengan spora *F. incarnatum*. Kiraan spora menunjukkan bahawa 0.5% EO mampu menghentikan sepenuhnya pengeluaran spora *F. incarnatum* sementara kepekatan yang sama hanya mengurangkan spora *B. cactivora*.

Kajian ketiga dilakukan dengan menggunakan reka bentuk sepenuhnya rawak dalam susunan faktorial (8 rawatan x 3 minggu penyimpanan). Kepekatan EO

yang berbeza diadun ke dalam kanji ubi sebagai lapisan boleh dimakan untuk buah naga dan disimpan pada 5 ± 2 ºC selama tiga minggu pada 80 ± 5% kelembapan relatif. Analisis dilakukan pada setiap minggu dimana pelbagai analisis fiziko-kimia dianalisis. Kualiti visual kelopak buah didapati lebih baik dengan kepekatan EO yang rendah dimana hal ini menunjukan pola yang sama untuk penyakit pada kulit buah (DS) di mana 1% rawatan EO menunjukkan DS yang lebih rendah. Kehilangan air didapati lebih rendah dalam buah yang disalut. Kekerasan kulit buah dan kadar pernafasan didapati menunjukan tindak balas berbeza terhadap rawatan salutan yang berlainan. Kilauan buah didapati lebih tinggi apabila kepekatan EO yang lebih tinggi digunakan. Asid askorbik tidak bertindak balas terhadap rawatan yang digunakan. Walau bagaimanapun, ujian antioksida lain didapati menunjukkan kepekatan antioksidaa yang lebih tinggi dalam buah yang dirawat dengan 1% EO berbanding rawatan kawalan. Secara keseluruhan, penemuan dalam kedua-dua kajian *in vitro* dan *in vivo* dapat disimpulkan bahawa 2% EO adalah berkesan dalam mengawal kebanyakan parameter yang dikaji dalam kajian *in vitro* manakala buah yang dilapisi dengan salutan 1% EO didapati berpotensi memberi manfaat untuk meningkatkan penyimpanan pasca tuai buah seperti yang digambarkan oleh hasil dalam kajian *in vivo*.

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I certify that a Thesis Examination Committee has met on 19 October 2018 to conduct the final examination of Khawarizmi Mohd Aziz on his thesis entitled “Effects of Torch Ginger Essential Oil on Pathogens, Quality and Storage of Dragon Fruit [*Hylocereus polyrhizus* (Weber) Britton & Rose]” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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| | |
|---|---|
| ºC | Degree Celsius |
| % | Percentage |
| & | And |
| µL | Microliter |
| µm | Micrometre |
| AA | Ascorbic acid |
| ATP | Adenosine triphosphate |
| ANOVA | Analysis of variance |
| BLAST | Basic Local Alignment Search Tool |
| bp | Base pair |
| CE | Crude extract |
| $C_2H_4$ | Ethylene |
| $CO_2$ | Carbon dioxide |
| CAM | Crassulacean acid metabolism |
| CO2 | Carbon dioxide |
| CRD | Completely randomized design |
| $C_2H_4$ | Ethylene |
| cm | Centimetre |
| $CaCO_3$ | Calcium carbonate |
| $C_6H_{12}O_6$ | Glucose |
| DNA | Deoxyribonucleic acid |
| DS | Disease severity |
| DPPH | 2,2-Diphenyl-1-picrylhydrazyl |
| EDTA | Ethylenediaminetetraacetic acid |
| etc | Etcetera |
| EO | Essential oil |
| FDA | Food and Drug Administration |
| g | Gram |
| GRAS | Generally recognized as safe |
| GU | Gloss unit |
| $H_2O$ | Water |

| | |
|---|---|
| HCl | Hydrochloric acid |
| HSD | Honestly significant difference |
| ITS | Internal transcribed spacer |
| kb | Kilobase |
| kcal | Kilocalorie |
| L | Litre |
| min | Minute |
| M | Molar |
| mA | Milliampere |
| MARDI | Malaysian Agriculture Research and Development Institute |
| ml | Millilitre |
| mM | Millimolar |
| mg | Milligram |
| mm | Millimetre |
| MIC | Minimum inhibitory concentration |
| MFC | Minimum fungicidal concentration |
| N | Newton |
| NaCl | Sodium chloride |
| nm | Nanometre |
| NaClO | Sodium hypochlorite |
| ROS | Reactive oxygen species |
| $O_2$ | Oxygen |
| PCR | Polymerase chain reaction |
| PDA | Potato dextrose agar |
| PDB | Potato dextrose broth |
| PVP | Polyvinylpyrrolidone |
| TA | Titratable acidity |
| TCA | Tricarboxylic acid cycle |
| SAS | Statistical Analysis System software |
| SSC | Soluble solids concentration |
| spp | Species |
| SDS | Sodium dodecyl sulfate |
| TF | Total flavonoids |

| | |
|---|---|
| TBE | Tris/Borate/EDTA buffer |
| TEF | Translation elongation factor |
| V | Volt |

# CHAPTER 1

# INTRODUCTION

The catchy name dragon fruit was believed due to its skin, having scalelike bracts which resemble the legendary creature (Small, 2009). Dragon fruit is a native to Mexico, Costa Rica, Nicaragua, Panama, Central America and South America (Lim, 2012; Small, 2009). It was originally domesticated by pre-Columbian Americans and it remains unknown across the world until mid 90's (Bellec & Vaillant, 2011). As a remark, this fruit cultivation had greatly increased in Southeast Asia (Small, 2009). Dragon fruit particularly red-fleshed dragon fruit (*Hylocereus polyrhizus*) are also known as strawberry pear, pearl fruit, *buah naga*, *mata naga* and pitaya (which also spelt as pitahaya or pitajaya) (Lim, 2012; Small, 2009; Zee, Yen & Nishina, 2004). In Malaysia, it is generally known as *buah naga*. Dragon fruit usually refers to three common commercialized species which are yellow dragon fruit with white pulp (*Selenicereus megalanthus*), red dragon fruit with red pulp (*Hylocereus polyrhizus*) and red dragon fruit with white pulp (*Hylocereus undatus*) (Barbeau, 1990; Bellec & Vaillant, 2011; Small, 2009). In Malaysia, *H. polyrhizus* has the highest market demand among all others dragon fruit due to its eye-catching red coloured pulp (Cheah & Zulkarnain, 2008). In Malaysia, the growing of *H. polyrhizus* had increased from 47.3 – 695.3 ha from 2002 to 2016, where Negeri Sembilan was the biggest producer in 2016 (Cheah & Zulkarnain, 2008; DOA, 2016).

The increase in *H. polyrhizus* production follows by higher challenges in maintaining the quality of this fruit, especially during postharvest storage. *H. polyrhizus* suffers a lot from postharvest disease during trading such as fruit rot caused by *Bipolaris cactivora* (Ben-ze, Assouline & Levy, 2011; He, Ho, Wu, Hou & He, 2012; Oeurn, Jitjak & Sanoamuang, 2015; Taba, Miyahira, Nasu, Takushi & Moromizato, 2007) and anthracnose by *Colletotrichum gloeosporioides* (Palmateer, Ploetz, Santen & Correll, 2007). Besides postharvest diseases, proper handling of this fruit is required right after harvest to retain its quality. Improper handling will cause the bract of this fruit to become dried or shrivelled and turn colour to brown few days after harvest (Hoa, Clark, Waddell & Woolf, 2006) which indirectly reduce the market value. This had been summarized on a report done by Nerd & Mizrahi (1999) and Wall & Khan (2008) which showed that mechanical injury, decay, and water loss are the most susceptible factors to dragon fruit. Furthermore, due to postharvest losses caused by disease, dragon fruit losses up to 44% of the marketable fruit during shipping (Valencia-botín & Livera-muñoz, 2005). Both postharvest diseases and improper handling will increase the transportation cost and thus reduces profit earns by traders as the cost of transportation must be included for fruit that is discarded at the end of the marketing chain.

This exotic fruit exhibits a non-climacteric pattern of respiration (Jamaludin, Ding & Hamid, 2011). This indicates it needs to be harvested from the plant when it fully ripens in order to get the best eating quality since it cannot ripen after harvest. This had become one of the limiting factors for dragon fruit to have a longer postharvest life. Dragon fruit is also very susceptible to water loss as well as postharvest diseases during storage as mentioned earlier (Freitas & Mitcham, 2013; Masyahit, Sijam, Awang & Ghazali, 2009; Oeurn et al., 2015). Currently, growers use fungicides to prevent the lesion due to pathogens. Although the use of fungicides is currently the primary means to deal with this problem, its usage is actually contributing to negative effect towards consumer health and the environment itself (soil, air etc.) (Bataller, González, Veliz & Fernández, 2012; Hong et al., 2014). On top of this, the use of fungicides often left some chemical residue on fruits skin (Gabler, Smilanick, Mansour & Karaca, 2010). This issue recently had promoted awareness among consumer about food safety of those chemically treated fruits. Hence, a safer way of treating and reducing the disease caused by these pathogens are demanded.

Plant secondary metabolites had attracted researchers attention lately. Most plants have a unique fraction of essential oils (EOs) which have decent inhibitory and fungicidal effect (Jayasena & Jo, 2013). EOs are considered as non-phytotoxic compounds and are potentially effective as an antimicrobial agent (Antunes & Cavaco, 2010). These natural compounds are generally recognized as safe (GRAS) for the environment and human health according to the United State Food and Drug Administration (FDA). As EOs have great potential to be used as an antimicrobial agent, it can provide great impact towards postharvest storage of fruit if it can be incorporated with an edible coating to suppress the microbial pathogen growth as well as providing a protective barrier for the fruit against water loss.

In Malaysia, torch ginger is a well-known plant especially when it comes to cooking. This plant EO was reported to contain various potential compounds that are effective in inhibiting microbes growth of wide range of plant pathogen (Susanti, Awang, Qaralleh, Mohamed & Attoumani, 2013). To the best of our knowledge, there is still no research had been done on the response of dragon fruit pathogen by using this particular EO as well as its potential to be used as antimicrobial in *H. polyrhizus* edible coating.

Hence to overcome all of the problem mentioned above, this research was conducted to use EO from torch ginger's leaves (*Etlingera elatior*) as natural antimicrobial agent incorporated with an edible coating to extend the postharvest life and maintain the quality of dragon fruit. Thus, in order to conduct this study, three specific objectives were set. These specific objective were:

i) To identify fungi pathogens associated with *H. polyrhizus* diseases.

ii) To determine the antimicrobial activity of *E. elatior*'s EO towards fungi pathogens that cause diseases to *H. polyrhizus*.

iii) To determine the effect of *E. elatior*'s EO incorporated into edible coating for *H. polyrhizus* towards its quality and storage life.

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