EFFICACY OF PLANT EXTRACTS TO CONTROL FUNGAL POST-HARVEST ROT

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EFFICACY OF PLANT EXTRACTS TO CONTROL FUNGAL POST-HARVEST ROT

by

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In the name of Allah, the Beneficent, the Merciful

بسم الله الرحمن الرحيم

يَا مَعْشَرَ الْجِنِّ وَالْإِنْسِ إِنِ اسْتَطَعْتُمْ أَنْ تَنْفُذُوا مِنْ أَقْطَارِ

السَّمَوَاتِ وَالْأَرْضِ فَانْفُذُوا لَا تَنْفُذُونَ إِلَّا بِسُلْطَانٍ

صدق الله العظيم

To my beloved mother, father and my brothers

Who gave me love, guidance and support

To the one who stood by me and made every effort to help me

My wife Rana

To my beloved kids

Ayat and Khilad

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Ghassan Faris Atiyah Al- Samarrai

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization
WHO	World Health Organization
BIA	Biopesticides Industry Alliance
GPP	Growing Plants for Pharmaceutical Production
LC50	Lethally Cytotoxicity
CRD	Completely Randomized Design
BST	Brine Shrimp Test
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MIC	Minimum Inhibitory Concentration
MAP	Modified atmosphere packaging
TLC	Thin-layer chromatography
GC-MS	Gas chromatography–mass spectrometry
LC-MS	Liquid chromatography mass spectrometry
MRLs	Maximum residue levels
PDA	Potato Dextrose Agar
PLA	Polylactic acid

Keberkesanan Ekstrak Tumbuhan untuk Mengawal Kulat Pereputan Selepas Tuaian

ABSTRAK

Racun kulat (fungi) digunakan secara meluas dalam pertanian konvensional untuk mengawal penyakit tumbuh-tumbuhan. Kesan penggunaannya untuk tempoh yang lama boleh mengancam kesihatan. Tambahan pula, masyarakat moden semakin mengutamakan kesihatan lantaran menyedari kesan daripada penggunaan racun kulat yang tersisa. Kini, penyakit-penyakit tumbuhan dikawal dengan menggunakan racun kulat sintetik. Bagaimanapun, alternatif lain iaitu racun perosak biologi yang mesra alam seperti bahan-bahan botanikal atau kawalan biologi semakin mendapat perhatian masyarakat global. *Penicillium digitatum*, *Aspergilius niger* dan *Fusarium Sp*, agen-agen penyebab kulat hijau, Black rot dan Fusarium rot, patogen lepas tuai yang penting yang mengakibatkan kerugian yang serius dalam sitrus setiap tahun, selain daripada buah-buahan komersial yang lain. Kajian semasa lebih cenderung kepada pengaplikasian botani, sebagai alternatif untuk racun kulat sintetik menjalankan pemeriksaan antimikrobial mereka, kajian tempoh hidup, toksikologi, analisis kromatografi, pembalut lepas-tuai dan pemilihan ekstrak tumbuhan anti-kulat yang terbaik pada pengasingan anti-kompaun kulat yang aktif menggunakan analisis statistik yang berbeza dan ANOVA. Kajian ini juga fokus kepada kesan tetap daripada ekstrak tumbuhan terpilih dalam kawalan lepas-tuai buah reput selain daripada keberkesanan kos bagi tujuan komersial. Sepuluh ekstrak etanol (kepekatan 500-5000 ppm) *Cerbera odollam* L. (Pong-pong), *Capsicum frutescence* L. (Cili), *Azadirachta indica* L. p(Semambu), *Cymbopogon nardus* L. (Serai), *Zingiber officinale* L. (Halia), *Andrographis paniculata* L. (Chirayta hijau), *Curcuma longa* L. (Kunyit), *Syzygyum aromaticum* L. (Cengkih), *Murraya koenigii* L. (Daun kari), *Swietenia macrophyllai* L. (Mahogani) telah menjalani ujian aktiviti anti-kulat bagi kulat hijau sitrus, '*Black rot'* dan '*Fusarium rot*' serta dibandingkan dengan racun kulat kawalan (Guazatine, 1000 ppm). Jangka hayat ekstrak tumbuhan mentah telah dikaji bergantung kepada aktiviti anti-kulat berdasarkan kaedah penyimpanan yang berbeza (peti sejuk, keadaan bilik, dan luar). Ekstrak terbaik dipilih berdasarkan ekstrak tumbuhan anti-mikrob yang menunjukkan perencatan kulat lebih daripada 70-90%, kestabilan yang lebih panjang dan keberkesanan di bawah keadaan simpanan yang berbeza. Ketoksikan (LC50) ekstrak anti-kulat terbaik ditentukan melalui Ujian *'brine shrimp'* (BST). Analisis kromatografi GC-MS digunakan untuk menentukan baki ekstrak tumbuhan dalam buah-buahan yang disembur dengan ekstrak tumbuhan. Keberkesanan ekstrak tumbuhan (semburan) digandingkan dengan bahan-bahan pembalut mesra alam (asid Polylactic, pectin dan suratkhabar) telah ditentukan semasa penyimpanan/transit untuk mengawal pereputan patogen dengan menggunakan pembungkusan atmosfera yang diubahsuai (MAP) untuk meningkatkan jangka hayat dan kualiti buah-buahan semasa penyimpanan. Serta eksperimen lain dengan menambahkan lapisan (chitosan, kanji dan parafin minyak) bersama pembalut buah-buahan bagi meningkatkan lagi kualiti buah selain mengkaji. Ekstrak tumbuhan yang menunjukkan kesan paling baik dalam meningkatkan jangka hayat dan kualiti buah-buahan telah dijalankan analisis '*biocompound'* dengan menggunakan kromatografi LC-MS . Ekstrak mentah dari Pong-pong dan Cili menunjukkan zon perencatan kulat dalam media PDA pada 3000ppm (kepekatan 100%), manakala Semambu, Serai, Halia mencatatkan lebih daripada (70%) perencatan kulat; tumbuh-tumbuhan lain menunjukkan kesan yang rendah, kurang daripada (50%) bagi kepekatan yang sama. Kajian jangka hayat menunjukkan ekstrak segar untuk semua tumbuh-tumbuhan memberikan keberkesanan

terbaik di bawah keadaan penyimpanan yang berbeza. Kajian jangka hayat ekstrak tumbuhan segar di 3000ppm belajar di bawah keadaan yang berbeza direkodkan perencatan tinggi dalam medium PDA selama 3 minggu apabila disimpan pada 4^{o}C, 1 minggu apabila disimpan pada 25 ^{o}C, dan kurang dari 1 minggu (3 hari) apabila sampel telah disimpan di luar pada ± 32^{o}C.Lima ekstrak tumbuhan mentah dari Pong-pong, Cili, Semambu, Serai dan Halia telah dipilih sebagai tumbuhan yang terbaik. Pong-pong dan cili menghalang jangkitan secara menyeluruh pada 4000 dan 5000 ppm dan meningkatkan jangka hayat buah-buahan selama lebih berbanding dengan buah-buahan tanpa rawatan tiga minggu pada suhu bilik. LC50 nilai di bawah 2μg/ml dianggap beracun dan tidak selamat untuk kegunaan manusia. Nila-nilai ekstrak tumbuhan LC50 adalah: Pong-pong 5μg/ml (rendah); cili -20 μg/ml (rendah); semambu -30 μg/ml (selamat); serai -473 μg / ml (selamat) dan halia-495 μg / ml (selamat). Massa Spektrometri analisis menunjukkan kesan sisa rendah dan tidak melebihi had sisa maksimum nilai MRL dalam buah dirawat dengan ekstrak terbaik selepas pertama, kedua dan minggu ketiga pada semburan. Pembalut dan buah-buahan yang disalut menunjukkan peningkatan jangka hayat dan penyusutan berat buah-buahan yang disimpan pada suhu bilik berbanding dengan buah-buahan tanpa rawatan.Cerebera menghasilkan keputusan yang terbaik dalam eksperimen dan ekstraknya telah dipilih untuk mengenalpasti bio-kompaun anti-kulat yang aktif daripada ekstrak eter. Ekstrak tumbuhan pong-pong digandingkan dengan buah-buahan salutan (chitosan, kanji dan minyak parafin) an penurunan berat badan dan jangka hayat yang meningkat untuk buah-buahan yang disimpan pada suhu bilik (25±oC) berbanding dengan buah-buahan yang tidak dirawat. Alpha glikosid (4, 6 Benzylidene -1O-Methyl -2O-(2346 Tetra -O- Acetyl- Betad-Glucosyl) sebatian baru daripada ekstrak daun *Cerebra odollam* L.untuk anti-kulat. Pembalut dan buah-buahan yang disalut menunjukkan peningkatan jangka hayat dan penyusutan berat buah-buahan yang disimpan pada suhu bilik berbanding dengan buah-buahan tanpa rawatan

Efficacy of plant extracts to control post-harvest fungal rot

ABSTRACT

Fungicides are widely used in conventional agriculture to control plant diseases. Prolonged usage often poses health problems as the modern society is becoming more health-conscious because of their harmful residual effects. The diseases are currently managed with synthetic fungicides but there is, however, a growing global interest on their replacement with other alternatives such as with environment–friendly biopesticides, such as use of botanicals or biological control. *Penicillium digitatum*, *Aspergillius niger and Fusarium Sp*, the causal agents of citrus green mold, black rot and brown rot, are important post-harvest pathogens that cause serious losses in citrus annually, besides affections other commercial fruits. The current study tends to the application of botanical as alternative to synthetic fungicides carrying out their antimicrobial screening, longevity study, toxicology, post-harvest study included spraying plant extracts, resident effect, wrapping and coating using different statistical analysis and ANOVA .The research also focus on the selected plant extracts in control post-harvest fruit rot besides the cost-effectiveness for commercial purpose .Then selection of the best anti-fungal plant extract on the isolation of its active anti-fungal compound using chromatography analysis. Ten Ethanol extracts(concentrations 500-5000 ppm) of *Cerbera odollam* L.(Pong-pong), *Capsicum frutescence* L.(Chili), *Azadirachta indica* L. (Neem), *Cymbopogon nardus* L. (Lemon grass), *Zingiber officinale* L. (Ginger), *Andrographis paniculata* L . (Green chirayta), *Curcuma longa* L. (Turmeric), *Syzygyum aromaticum* L. (Cloves), *Murraya koenigii* L. (Curry leaf), *Swietenia macrophyllai* L. (Mahogani), were tested for their anti-fungal activity for citrus green mold, black rot and brown rot and compared with the control fungicide (Guazatine). Longevity of crude plant extracts was studied depending on their anti-fungi activity under different storage conditions (Refrigerator, Room conditions, and Outside) for four weeks. The best plant extracts were selected of plants under study based their on anti-microbial activity showing more than 70-90% fungal inhibition and longer stability and efficacy under different storage conditions. The toxicity (LC50) of the best anti-fungal extracts was determined by the Brine Shrimp Test (BST). Chromatography analysis GC-MS was used to determine the residual effect in fruits that were sprayed with plant extracts. Efficacy of the selected best plant extract incooperated with biodegradable materials wrappers (Polylactic acid, pectin and newspaper) was determined during storage at room temperature (±25C^{o}) to control fruit-pathogen decay under modified atmosphere packaging (MAP) to increase shelf life and improve the fruit quality and an another experiment in cooperating coating (chitosan, starch and oil paraffin) with fruit wrappers to further increase the fruit quality . The plant extract that exhibited best result to increase the shelf life and fruit quality was subjected to biocompond analysis using chromatography LC-MS. Crude extracts from pong-pong and chili showed fungal inhibition zone in PDA medium at 3000ppm (c.100%), while neem, lemon grass, ginger recorded more than (70%) fungus inhibition; other plants showed low effect less than (50%) for the same concentration. Longevity study showed the fresh extract solution for all plants under study gave best effectiveness of crude plant extracts stored under different conditions. Longevity study of fresh plant extracts under different condition recorded high inhibition in PDA medium for 3 weeks when stored at 4^{o}C, 1 week when stored at 25 ^{o}C, and less than 1 week (3days) when samples were kept outside at ±32^{o}C. *In vivo*, the fruits sprayed with fresh plants extract of neem, pong-pong and chili completely prevented infection at 4000 and 5000 ppm and increased shelf-life of fruit compared with untreated fruit by three week at room temperature. Five crude plant extracts from pong- pong, chili, neem, lemon grass and Ginger were selected as the best plants based their on anti-microbial activity showing more than 70-90% fungal inhibition and longer stability and efficacy under different storage conditions. LC50 values below 2μg/ml are considered to be toxic and unsafe for human consumption. The LC50 values of the plant extracts were: pong-pong was 5μg/ml (low but safe); chili: 20 μg/ml (low but safe); neem: 30 μg/ml (safe); lemon grass: 473 μg/ml (safe) and ginger: 495 μg/ml (safe). Mass Spectrometry analyses show low residual effect and not exceed maximum residue limits MRL values in fruit treated with the best extracts after first, second and third week on the spray. Five plants extracts namely pong-pong, chili, neem, lemon grass and ginger incooperated with fruit wrappers fruit showed increased shelf life and decreased weigh loss for fruits stored at room temperature compared with untreated fruit. Plant extract of pong-pong incooperated with coating fruit (chitosan, starch and oil paraffin) showed decreased weigh loss and increased shelf life for fruits stored at room temperature (±25^{o}C) as compared with untreated fruit. *Cerebera* produced the best results in the experiments and its extract was selected for the identification of active anti-fungal biocompounds from. Alpha Glycoside (4, 6 Benzylidene -1O-Methyl -2O-(2346 Tetra-O-Acetyl-Betad -Glucosyl), a new compound from the leaf extract of *Cerebra odollam* L. was identified for its antifungal action.

CHPTER 1
INTRODUCTION

Citrus is considered to be one of the major fruit crops produced in the world. Citrus fruits such as orange, lemons, pummelos, grapefruits and others are grown widely as fresh fruit for commercial purposes throughout the world in fifty countries (Fig1.1), such as the America, Asia, Medial East region, Australia and Spain (Manuel and Fred, 2008; FAO, 2011). Citrus fruits contain a variety of vitamins (B6, E2 and C) minerals, fiber, and phytochemicals such as carotenoids, flavonoids, and limonoid, which appear to have biological activities and health benefits (Citrus Australia, 2010; Codoner-Franch, 2010).

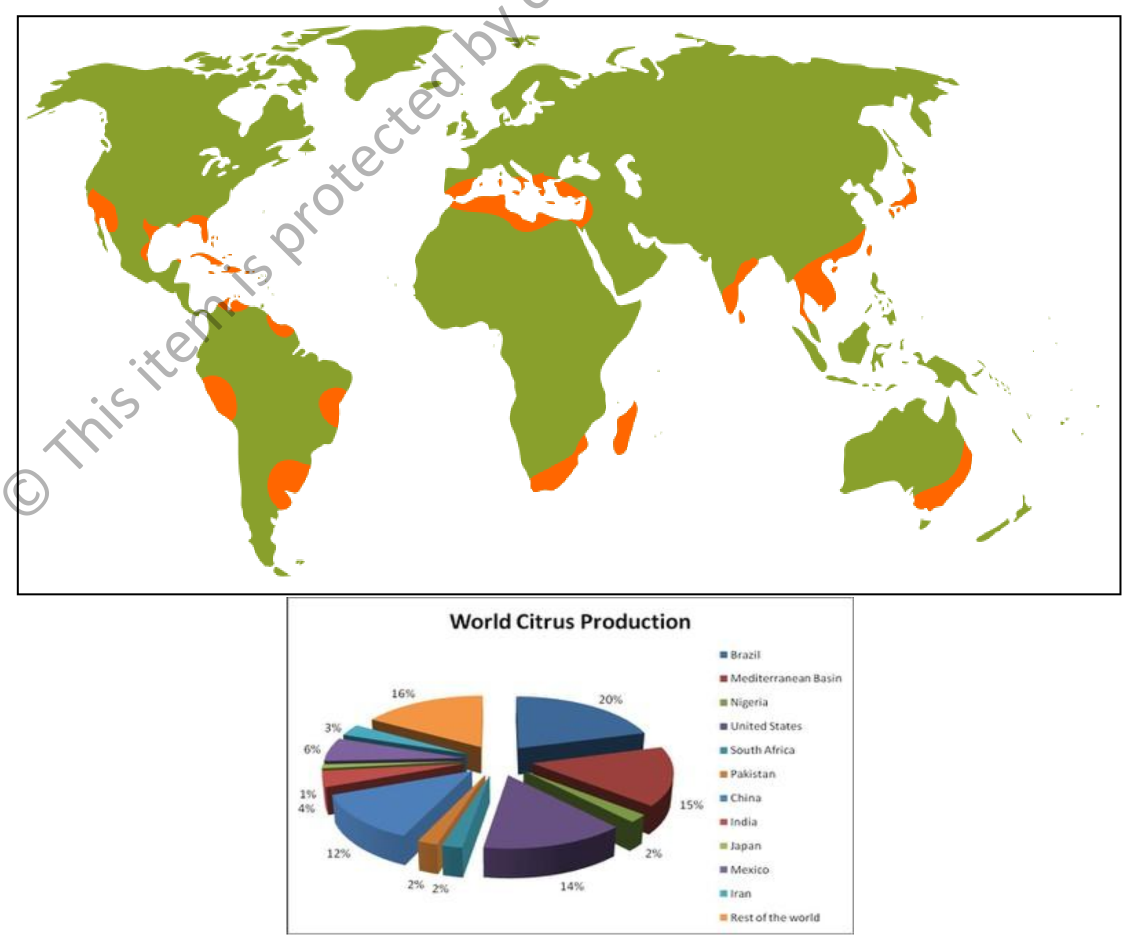

Fig1.1Major citrus growing regions in world (Thomas, 2010).

The citrus industry has its commercial importance due to its influence in generating jobs for millions of people through harvesting, handling, transportation, storage and marketing. The citrus fruit industry is rapidly growing due to population increase and improved economic conditions together with the advance of agricultural sciences and technology of by-products and increased awareness of the nutritious value of the fruit (Elyatem, 2008). However, although production and area in the Asia region are increasing compared to the Western countries, the post-harvest handling and processing practices coupled with high temperature ±30^{O}C and humidity 75-90% have contributed in limiting the postharvest life of citrus fruits (Pratibha *et al*, 2011). The softening of the fruits upon ripening and damage in the store undergo a series of physiological during transport and packaging. These factors also expose the fruits to post-harvest damage by pathogens. Post-harvest diseases attack the citrus fruits and can cause huge economic losses ranging between 15- 25% during transportation, storage and marketing in countries citrus-producing in Asia (Post-harvest technology in citrus, 2009). Ramu *et al*., (2011) reported 45-50% losses caused by fungal rot under poor storage after-harvest.

The citrus fruits can be attacked by many pathogens that can affect the fruits post-harvest. The most important pathogens affecting the citrus are: *Alteraria* spp., *Penicillium* spp., *Aspergillus* spp., *Rhizopus* and *Fusarium* sp. (Josepha *et al*., 2008).

Fungal post-harvest rot in citrus is currently being managed with synthetic fungicides chemicals, radiation, hot-water treatments (Cunningham, 2008). However, there is a growing global concern over the use of synthetic fungicides chemicals on food crops because of the continuous exposure of man to low levels of fungicides residue through his diet. In addition fungicides have on impact on the environment through accumulation in soil and water and effect on biological diversity (Behnaz *et al*., 2009).These aspects have lead to the implementation of more restrictive legislations regarding the maximum residue levels (MRL) of chemical residues in fruits exported particularly to world markets (Pal and McSpadden, 2006). The application of fungicides often leads to new fungicide-resistant strains as in *P. digitatum* (William, 2009). Keeping in view of these developments, the recent trend in disease management focus on using natural plant products as all an alternative to fungicides. The natural plant products (botanicals) are generally safer as they degenerate fast thus avoiding residue effect (Barkai-Golan, 2011). The bioactive pathogen-inhibiting compounds may include-compounds flavonoids, glycosides, phenols and terpenoids (Pramila *et al* ., 2008). The previous studies conducted by (Heam *et al*., 2009; Ibtesam *et al*., 2011; Adetunji *et al*., 2012; Maria *et al*., 2012) reported reduction in fungal inhibition zone in lab condition using plant extract of neem, lime, thyme, camphor Shiraz thyme, Aloe and garlic against Green and Blue mould caused by *Penicillium digitatum* and *Penicillium italicum* on citrus fruits.

1.1 Problem Statement

Losses in fruit industry due to post-harvest rot fungi may occur at any time during post-harvest handling, from harvest to consumption. When estimating post-harvest fungi rot losses, it is important to consider reductions in fruit quality, cost of harvesting, packaging and transport, and storage facilities. Beside economic considerations, it is important to be aware that diseased product poses a potential health risk, since a number of fungal genera such as *Pencillium*, *Alternaria* and *Fusarium* are known to produce mycotoxins under certain conditions (Pitt and Hocking, 2009). The use of synthetic fungicides effects to the environment such as their residue effect can be harmful to human health, besides increased accumulation in water, soil, and fruit besides the elimination of the natural enemies of the