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BIOPESTICIDE POTENTIAL OF PHYTOCHEMICAL EXTRACTS FROM THE NUT OF ARECA CATECHU L. AGAINST MANGO FRUIT ANTHRACNOSE

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By

AIZAD IZHA AHMAD RUSDAN

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

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DEDICATION

This thesis is dedicated to my loving family and friends who have constantly support me.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Agricultural Science

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October 2014

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Anthracnose is an important disease that attacks mangoes in Malaysia and many other regions. It is caused by a fungal known as *Colletotrichum gloeosporioides*. The disease has caused devastating effects on the post-harvest where mangoes are already infected. The application of fungicides is a common method use in controlling the infection of this disease; however, the usage brings negative effects on our environment and may affect the health of consumers as well. One of the potential alternatives to replace or reduce the use of fungicides is by applying natural extracts from plants. The nut of *Areca catechu,* which is also known as betel nut, is a potential to be explored as a natural antifungal agent. This exotic plant is native to the Southeast Asia region where it is available abundantly. The nut of *A.catechu* contains stimulant that was traditionally used (or chewed) with betel leaves during past-time especially by people in certain parts in Asia. The nut was also traditionally used as medicine to treat diseases in many cultures in Asia Pacific. For the purpose of exploring the potential of the nut as a natural antifungal agent, this study was conducted by applying its natural extracts to treat anthracnose disease on mangoes. Six extracts (namely, hexane, chloroform and methanol extracts) from ripe and unripe nuts of *A.catechu* were tested in antifungal screening tests against mycelium growth and spores germination of *C.gloeosporioides*. Among all the extracts, the chloroform extract from the unripe nuts (at the concentration of 10.0 mg/mL) gave best antifungal reaction by inhibiting about 52% of mycelium growth and 100% of spore germination. Compound screening carried out on that particular extract revealed that it contained both alkaloids and phenolics which are important and most studied substances in the nut of *A. catechu*. Both substances could cause antifungal activities and might be responsible in controlling the growth

development of *C. gloeosporioides* in the study. Based on the results in antifungal screening tests, the chloroform extract from the unripe nut at the concentration of 10 mg/mL was selected to be applied on mango in *in vivo* test with the purpose to treat anthracnose disease. Thus, this particular extract was selected to treat the fruit against anthracnose in two different ways, namely, by dipping them in the extract solution at 27^{o}C for one hour (normal dip) and also at 52^{o}C for five minutes (hot dip). Meanwhile, control and benomyl solutions (each applied in both dipping methods) were used as comparisons. The testproved that the treatment using the extract reduced 34% of disease infection and 27% of disease rate from control. However, the treatment using benomyl was slightly effective compared tousing the extract, reducing around 47% of disease infection and 38% of disease rate from control. Hence, results from test also proved that the treatment applied at 52^{o}C was more effective than those conducted at 27^{o}C,wheretreatment at 52^{o}C reduced 51% of disease infection and 35% of disease rate from treatment applied at 27^{o}C. Based on the results in *in vivo* test, post-harvest quality of fruit treated by dipping inside extract solution at 52^{o}C for five minutes was analysed.Results from the analysis showed that the treatment increased shelf life by slowing down the ripening process. The parameters analysed included physical characteristics of the fruits (weight loss, firmness, and external peel colour), whereas the parameters for the chemical characteristics analysed were titratable acidity, pH, soluble solid concentration, and ascorbic acid concentration. It is hoped that the extract can be a potential alternative for chemical fungicides to control plant diseases and improve the shelf life of fruit.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

POTENSI EKSTRAK FITOKIMIA DARI BIJI *ARECA CATECHU* L. DALAM MENGAWAL ANTRAKNOS PADA BUAH MANGGA

Oleh

AIZAD IZHA AHMAD RUSDAN

Oktober 2014

Pengerusi : Profesor Madya Jugah Kadir, PhD

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Antraknos ialah penyakit utama yang menyerang mangga di Malaysia dan di kebanyakan kawasan lain. Ia berpunca daripada jangkitan kulat yang dikenali sebagai *Colletotrichum gloeosporioides*. Penyakit ini memberikan kesan teruk sewaktu lepas tuai, di mana ketika itu buah sudah pun dijangkiti. Penggunaan racun kulat adalah kaedah biasa dalam mengawal jangkitan penyakit ini tetapi ia memberi kesan negatif kepada alam sekitar, serta mungkinmenjejaskan kesihatan pengguna. Salah satu antara langkah-langkah alternatif yang berpotensi untuk menggantikan atau mengurangkan penggunaan racun kulat ialah dengan menggunakan ekstrak semulajadi daripada tumbuhan. Biji *Areca catechu,* yang juga dikenali sebagai biji pinang berpotensi untuk diteroka sebagai bahan antikulat semulajadi. Tumbuhan eksotik ini berasal dari Asia Tenggara di mana ia didapati dengan banyak. Biji *A.catechu* mengandungi bahan peransang yang tradisinya dikunyah dengan daun sirih pada waktu lapang terutama sekali oleh mereka di beberapa kawasan di Asia. Biji tersebut juga digunakan sebagai ubatan tradisional oleh suku-suku kaum di Asia Pasifik bagi mengubati pelbagai penyakit. Bertujuan untuk mengetahui potensi biji pinang sebagai agen antikulat semulajadi, kajian telah dijalankan dengan menggunakan ekstrak-ekstrak semulajadi daripada biji tersebut bagi merawat mangga daripada jangkitan antraknos. Enam ekstrak (iaitu ekstrak heksana, kloroform dan metanol) daripada biji *A.catechu* yang ranum dan tidak ranum telah diuji dalam ujian saringan antikulat terhadap pertumbuhan miselia dan percambahan spora*C.gloeosporioides*. Daripada kesemua ekstrak, ekstrak klorofom daripada biji tidak ranum (pada kepekatan 10.0 mg/mL) telah memberikan kesan antikulat terbaik dengan merencat 52% pertumbuhan miselia dan 100% percambahan spora.Saringan komponen yang dijalankan pada ekstrak tersebut menunjukkan ia mengandungi kedua-dua alkaloid dan fenolik iaitu komponen-komponenen utama yang paling banyak dikaji terdapat dalam biji *A.catechu*. Dalam kajian

yang telah dijalankan,kedua-dua bahan tesebut telah didapati menyebabkan kesan antikulat dan berkemungkinan telah mengawal pertumbuhan *C.gloeosporioides* dalam kajian yang dijalankan. Berdasarkan daripada keputusan ujian saringan antikulat, ekstrak kloroform daripada biji tidak ranum pada kepekatan 10 mg/mL telah dipilih dan diuji untuk merawat mangga daripada antraknos dalam ujian *in-vivo*. Ekstrak ini telah dipilih bagi merawat jangkitan antraknos melalui dua kaedah berbeza, iaitu, dengan merendam buah ke dalam larutan ekstrak pada suhu 27^{o}C selama satu jam (kaedah rendaman biasa) dan juga pada suhu 52^{o}C selama lima minit (kaedah rendaman hangat). Sementara itu, larutan kawalan dan larutan benomyl (turut diaplikasikan dalam setiap kaedah) digunakan sebagai perbandingan. Ujian menunjukkan kaedah rawatan menggunakan ekstrak mengurangkan 34% jangkitan penyakit dan 27% kadar jangkitan daripada rawatan kawalan. Walaubagaimanapun, kaedah rawatan menggunakan benomil sedikit lebih berkesan berbanding ekstrak, mengurangkan sekitar 47% jangkitan penyakit dan 38% kadar jangkitan daripada rawatan kawalan. Keputusan ujian juga menunjukkan kaedah rawatan pada suhu 52^{o}C lebih berkesan berbanding kaedah rawatan pada suhu 27^{o}C, di mana kaedah rawatan pada 52^{o}C telah mengurangkan jangkitan penyakit sebanyak 51% dan kadar jangkitan sebanyak 35% daripada kaedah rawatan pada 27^{o}C. Berdasarkan daripada keputusan daripada ujian *in vivo*, kualiti lepas tuai buah yang dirawat dengan rendaman di dalam larutan ekstrak pada suhu 52^{o}C selama lima minit telah dianalisis. Keputusan analisis menunjukkan rawatan tersebut telah melanjutkan hayat rak buah dengan melambatkan proses peranuman.Parameter-parameter yang dianalisis termasuklah ciri-ciri fizik buah (kehilangan berat, kekerasan, dan warna luar kulit buah) manakala ciri-ciri kimia yang dianalisis termasuklah asid tertitrat, pH, kepekatan pepejal terlarut dan kepekatan asid askorbik. Menjadi harapan agar ekstrak tersebut berpotensi menjadi alternatif kepada racun kulat bagi mengawal penyakit-penyakit tumbuhan dan turut menambah baik hayat rak buah.

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I certify that a Thesis Examination Committee has met on 9 October 2014 to conduct the final examination of Aizad Izha Ahmad Rusdan on his thesis entitled "Biopesticide Potential of Phytochemical Extracts from The Nut of *Areca catechu* L. Against Mango Fruit Anthracnose" in accordance with 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 Agricultural Science.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AUDPC	The Area Under the Disease Progress Curve
^{o}C	Celsius
C*	Chroma
CRD	Completely Randomized Design
cm	Centimetre
cm^2	Square centimetre
DMRT	Duncan Multiple Range Test
DMSO	Dimethylsulfoxide
EU	European Union
h^o	Hue angle
g	Gram
L*	Lightness
L	Litre
LCB	Lactophenol cotton blue
M	Molar
m	Metre
min	Minute
mg	Milligram
mL	Millilitre
mm	Millimetre
N	Newton
NaOH	Sodium hydroxide
PDA	Potato Dextrose Agar
ppm	Part per million
RH	Relative humidity
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

Mango is a popular tropical fruit that is highly demanded in international markets (Krishna and Singh, 2007). It originated from the Indo-Burma regions (Lim and Khoo, 1985). The popularity of mango can be related to the fruit's attractive bright colour, taste, and nutritional values (Kim *et al*., 2007). It is also a nutritional source of vitamin C and carotenoids, which are beneficial for health (Djioua *et al*., 2009). Anthracnose is an important disease that infects mangoes and considered as the most serious disease in South East Asia and other mango growing regions with high rainfall (Pordesimo *et al.*, 1984). In most regions, *Colletotrichum gloeosporioides* has been identified as the most important pathogen that causes anthracnose post-harvest diseases (Prior *et al*., 1992). The fungus brings devastating effects to mangoes by killing the inflorescences and causing spots on leaves and common dark brown to black decaying spots on ripening fruit (Agrios, 2005).

Anthracnose on mangoes is mainly controlled by applying chemical fungicides in planting areas and as a post-harvest treatment.However, the use of fungicides has brought about a lot of negative effects including environmental pollution (Vivekananthan *et al*., 2004), health risks to the public (Janisiewicz and Korsten, 2002) and development of resistance in pathogen (Widmer and Laurent, 2006). One potential alternative to replace or reduce the usage of chemical fungicides is by applying natural extracts from plants to control plant diseases.

Intensive research and development on natural compounds from plants as antifungal substances have a great potential to be developed in Malaysia due to the country's rich biodiversity and abundant natural resources. One of potential plants to be explored is *Areca catechu*. The nut or the seed of the fruit, which is also known as betel nut, is chewed with betel leaves by people during free and working times (Hesse, 2002). It is also used by people of various cultures in the Asian and Pacific regions, and it often used in ceremonies and gatherings (Benegal *et al*., 2008). The nut is also applied as a traditional medicine, especially in rural areas of certain parts of the Pacific region. The nut is known to contain alkaloids and phenolics (Wang *et al*., 1997), which can potentially be antifungal substances. Although Malaysia is one of the main countries producing this fruit (Gracy *et al*., n.d.), no research has been conducted in the country so far to study the potential of the nut as a natural antifungal agent, which if successful, will optimize its usage.

The following are the objectives of this study:

1) To isolate a virulent *C. gloeosporioides* from mango.
2) To study the potential of the nut extract of *A. catechu* for suppressing anthracnose on mango fruit.
3) To analyse the post-harvest quality aspects of mango fruit treated with the nut extract of *A. catechu.*

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