THE ROLE OF LETTUCENIN A IN LETTUCE (*Lactuca sativa*) AGAINST PATHOGEN *Xanthomonas campestris* pv. *oryzae*

HONG CHIA YEAN

THIS DISSERTATION IS SUBMITTED AS PART OF THE REQUIREMENT TO OBTAIN BACHELOR OF SCIENCE IN HONOURS

PLANT TECHNOLOGY PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

April 2007

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: THE ROLE OF LETTUCENIN A IN LETTUCE (*Lactuca sativa*) AGAINST PATHOGEN *Xanthomonas campestris* pv. *oryzae*

Ijazah: BACHELOR OF SCIENCE IN HONOURS

SESI PENGAJIAN: 2007

Saya HONG CHIA YEAN

(HURUF BESAR)

mengaku membenarkan tesis (~~LPS~~/Sarjana/~~Doktor Falsafah~~)* ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (/)

[ ] SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)

[ ] TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

[✓] TIDAK TERHAD

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 242, TAMAN LAM SUN, JALAN TELOK WANJAH, 05200 ALOR STAR, KEDAH DARUL AMAN.

Nama Penyelia

Tarikh: 16-04-2007

Tarikh:

CATATAN: * Potong yang tidak berkenaan.

** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that this work in the thesis is my own except for quotations and summaries, which have been duly acknowledged.

10th MARCH 2007

HONG CHIA YEAN
HS2004-1237

EXAMINER APPROVAL

APPROVED BY

Signature

1. **SUPERVISOR**
   (MR. CHONG KHIM PHIN)

CHONG KHIM PHIN
Dip. Agric, BSc (UPM), MRes (London) DIC
Pensyarah Patologi Tumbuhan
Universiti Malaysia Sabah.

2. **EXAMINER**
   (ASSOC. PROF. DR. MARKUS ATONG)

PROF. MADYA DR. MARKUS ATO

3. **DEAN OF SCHOOL OF SCIENCE AND TECHNOLOGY**
   (SUPT/KS. ASSOC. PROF. DR. SHARIFF A. KADIR S. OMANG, *ADK*)

ACKNOWLEDGEMENT

First, deepest and sincere gratitude is expressed to my supervisor, Mr. Chong Khim Phin for his guidance and exceptional ideas when this project was conducted. I thank Mr. Chong for giving me advice and encouragement that kept me going. I am thankful for his friendliness and providing many journals and articles for this project.

I would like to express my appreciation to our lab assistant, Mr. Sanin for his assistance and guidance during the lab works. Warmness gratitude is addressed to Clament Chin for his comments, suggestions and advice for the improvement of this dissertation. Without his enthusiasm and help, this dissertation would not be completed.

I am thankful to my colleagues, Wen Dee, Sheau Yng, Desyi, Kee, Jason and others for sharing their knowledge and apparatus during this project.

Finally, my warmness and sincere thanks go to my parents and family. Thanks for the everlasting love and support which have been a constant of strength for me to continue and complete this project.

Thank you.

ABSTRACT

Lettucenin A is the major phytoalexin produced by lettuce after elicited by biotic or abiotic elicitors. The production of lettucenin A in leaves can be induced by 5% $CuSO_4$ (w/v) and 1% $AgNO_3$ (w/v). A clear inhibition zone where the fungus *Aspergillus niger* failed to grow on TLC bioassay after the chromatogram was developed in hexane: ethyl acetate (1:1, v/v) at R_f 0.45 was observed. Lettucenin A was detected at a retention time of approximately 5.3 min when analyzed with HPLC in isocratic solvent system with water: acetonitrile (60:40, v/v). *In vitro* antibacterial study with *Xanthomonas campestris* pv. *oryzae* showed that this pathogen had different sensitivity to all tested concentrations of lettucenin A. The bacterium was more sensitive to higher concentration of lettucenin A (333, 533 and 667 $\mu g\ ml^{-1}$) than in lower concentration such as 67 $\mu g\ ml^{-1}$. Thus, the relationship between the bacterial growth rate and lettucenin A concentration was negatively correlated. However, the bacterial growth rate was continuing to increase after two hours of incubation time. Hence, it is suggested that *X. campestris* pv. *oryzae* might had the ability to detoxify the lettucenin A. It is also suggested that the success or failure of *X. campestris* pv. *oryzae* to invade lettuce depends on a balance between accumulation and degradation of lettucenin A at the invading site of lettuce. In summary, lettucenin A may play an important role in the resistance of lettuce against microbial infection.

ABSTRAK

Letusenin A merupakan fitoalexin utama dari sayur "lettuce" atau salad selepas dirangsang dengan perangsang biotik dan abiotik. Penghasilan letusenin A dapat dirangsangkan dengan penggunaan bahan kimia seperti 5% $CuSO_4$ (w/v) dan 1% $AgNO_3$ (w/v). Satu kawasan kerintangan yang jelas di mana kegagalan kulat *Aspergillus niger* bertumbuh dapat diperhatikan pada ujian biologi TLC pada R_f 0.45 setelah kromatogram itu dicelupkan dalam hexane: ethyl acetate (1:1, v/v). Letusenin A telah dikesan pada masa penahanan minit ke-5.3 apabila dianalisis dengan HPLC dalam sistem pelarut isokratik, iaitu air: acetonitrile ialah 60:40 (v/v). Ujian *in vitro* antibakteria letusenin A terhadap *Xanthomonas campestris* pv. *oryzae* telah menunjukkan bahawa patogen ini mempunyai tahap kepekaan yang berbeza terhadap semua kepekatan letusenin A yang diuji. Bakteria ini lebih sensitif dalam kepekatan letusenin A yang lebih tinggi (333, 533 dan 677 $\mu g\ ml^{-1}$) berbanding dengan kepekatan letusenin A yang rendah, iaitu 67 $\mu g\ ml^{-1}$. Oleh yang demikian, kadar pertumbuhan bakteria adalah berkorelasi secara negatif dengan kepekatan letusenin A. Namun demikian, kadar pertumbuhan bakteria meningkat semula dua jam selepas inkubasi. Oleh itu, *X. campestris* pv. *oryzae* dicadangkan mempunyai kebolehan untuk mendegradasikan letusenin A. Terdapat juga cadangan bahawa kejayaan atau kegagalan bakteria dalam serangan ke atas sayur salad bergantung kepada keseimbangan antara penghasilan dan degradasi letusenin A pada kawasan serangan salad. Sebagai kesimpulan, letusenin A mungkin memainkan satu peranan yang penting dalam memberikan rintangan sayur salad ke atas serangan mikroorganisma.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATION

°C	degree of Celsius
kPa	kilo Pascal
>	more than
<	less than
%	percentage
pv.	pathovar
λ_{max}	maximum absorbance
μg	microgram
g	gram
ml	milliliter
nm	nanometer
μm	micrometer
mm	millimeter
cm	centimeter
w/v	weight/volume
v/v	volume/volume
rpm	round per minute
p	significant value
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
HR	hypersensitive resistance
UV	ultraviolet
$CuCl_2$	copper(II) chloride
$CuSO_4$	copper(II) sulfate
$AgNO_3$	silver nitrate
PDA	Potato Dextrose Agar
NA	Nutrient Agar
R_f	retention factor
R_t	retention time
SD	standard deviation
OD	optical density

CHAPTER 1

INTRODUCTION

1.1 Background

Throughout their life cycles, plants may be challenged by different of environmental conditions or numerous of pathogens such as viruses, bacteria, mollicutes, fungi, nematodes, insects and others. In lettuce, it is normally attacked by *Bremia lactucae*, which is a fungus that causes downy mildew of this plant. Lettuce is also infected by *Botrytis* sp. The symptom of this disease is the formation of whitish-gray mold on the leaves. Besides that, lettuce can be attacked by lettuce mosaic virus, which cause yellowing and necrosis on leaves. Lettuce infectious yellow virus or LIYV, one type of crinivirus can infect lettuce as well and cause the leaves become yellow and stunned. Most of the viruses that cause disease in lettuce are transmitted by insects such as whiteflies and aphids (Agrios, 2005). In addition, bacteria *Xanthomonas campestris* pv. *vitians* cause bacterial spots of lettuce (Barak *et al*., 2002).

In Sabah and most of the plantation areas in Malaysia, chemical pesticides are used to control the diseases of lettuce and other crops (Jipanin *et al*., 2001). These pesticides are toxic compounds and are also known as fungicides, bactericides, nematicides, herbicides and insecticides depending of the kind of pests they against. The public assumed that pesticides were only toxic to pests at which they were aimed. Therefore, large quantity of pesticides was applied liberally on fields, farm, vegetables and fruits. Even more powerful and toxic pesticides were produced annually to control the pests at lower concentration in a higher speed (Agrios, 2005; Jipanin *et al.*, 2001).

Public concern about the safety of chemical pesticides began at 1960s. Researchers and scientists found that one of the disadvantages arises from the frequently usage of pesticides is high residue contain in these vegetables or crop products. This is because low biodegradability and persistence in the environment of the pesticides. Bird, fish, insect and other animals' deaths because of these pesticides are being accumulated and concentrated through the food chain, and finally cause pollution problems which contaminant the soil, water and air. In addition, some of the species in insects had developed genetic resistance to these chemicals and could no longer be controlled with them. Pesticides can be hazardous to human health as well, in direct or indirect ways. As the results, pesticides containing mercury, chlorinated hydrocarbons such as DDT, aldrin, endrin, lindane, chlordane and carcinogenic compounds were banned (Dahama, 2003; Gossett, 2003; Young, 1987). Recently, the desirability of using fewer and safer pesticides and the demand of consumers for fruits and vegetables which are free of chemicals are increasing. So, there is a greater need to reduce the usage of chemical pesticides by screening alternative strategies or technologies in order to improve plant disease resistance and control the pathogens.

Lettuce or salad continues to increase in popularity and has bright future in Malaysia. It is grown for local market and export such as to Singapore. In Sabah, the total plantation area of lettuce increased from 39.1 hectares in 2002 to 49.0 hectares in 2003 (Department of Agriculture Sabah, 2003a, 2003b). For Malaysian and people in other countries, lettuce is grown for its leaves, which are eaten raw as salad or cooked as vegetable (Grubben & Sukprakarn, 1994). However, this species is always infected with bacterial spots caused by *Xanthomonas campestris* pv. *vitians* (Barak *et al.*, 2002). Plants attacked by this pathogen have the symptoms of spots on leaves and thus will decrease its commercial value in market. Farmers may use chemicals or other cultural practices to control this disease, thus increasing the expenses. These are economic and financial losses for both farmers and agricultural department (Jipanin *et al*., 2001).

The first step in any infection is the recognition of host by pathogen or in the opposite way – recognition of pathogen by host. Early recognition between host and pathogen can be explained by gene-for-gene concept. There are 4 possible gene combinations: a) host resistance gene recognizes pathogen elicitor molecules and triggers defense reactions; b) pathogen lacks elicitor and thus, host resistance gene can't recognize this elicitor and no defense reaction is activated; c) host lacks receptors to recognize pathogen elicitor and defense reaction is not activated; and d) host lacks the resistance gene while pathogen carries virulent gene thus defense mechanism is not activated (Agrios, 2005).

Phytoalexins are produced in plants after stimulation of various types of phytopathogenic microorganisms, chemical, mechanical injury or stress. Resistance occurs when their concentrations are sufficient to inhibit the growth of fungi, bacteria or other pathogens (Agrios, 2005; Cruickshank, 1963; Ebel, 1986; Hammerschmidt, 1999; Kuć, 1972, 1995; Mansfield, 2000; Nicholson & Hammerschmidt, 1992; VanEtten *et al*., 1989). Although phytoalexins have been studied in numerous years, but little is known with its direct effect to plant resistance. So, it is hoped that we can have much better view of it after the study in this field. Beside that, the level of phytoalexin accumulation after infection will be a good criterion to study plant-bacteria interaction.

There is a positive linear relationship between the amount or time of phytoalexin produced and the degree of disease resistance. This relationship is also known as quantitative relationship. In other words, accumulation of phytoalexin can be correlated with the degree of pathogen restriction. When there is a higher rate or quantity of phytoalexin accumulated, the smaller lesion size and so for the number of the bacterial cells is found in the host plant (Hammerschmidt, 1999). Therefore, it is important to determine whether the interaction between the concentration of lettucenin A and *Xanthomonas campestris* pv. *oryzae* follows the same pattern of expression.

In this study, abiotic elicitors – $CuSO_4$ and $AgNO_3$ are used for elicitation. Thin Layer Chromatography or TLC is used to recover lettucenin A from plant tissue extracts. The samples are then subjected to High Performance Liquid Chromatography or HPLC for quantification. Different concentrations of lettucenin A are tested on *Xanthomonas campestris* pv. *oryzae* to verify the antimicrobial effect of different concentration of lettucenin A to *X. campestris* pv. *oryzae.* The antifungal activities of lettucenin A have been assessed in terms of the bacteria growth compared with that in control without lettucenin A.

1.2 Research objectives

Objectives of the study are:

i) To carry out a preliminary TLC bioassay with lettucenin A.

ii) *In vitro* study the role of lettucenin A in different concentrations against pathogen *Xanthomonas campestris* pv. *oryzae*.

1.3 Hypothesis

i) Null Hypothesis, H_0: High concentration of lettucenin A is unable to inhibit the growth of pathogen *Xanthomonas campestris* pv. *oryzae*.

ii) Alternative Hypothesis, H_1: High concentration of lettucenin A is able to inhibit the growth of pathogen *Xanthomonas campestris* pv. *oryzae*.

CHAPTER 2

LITERATURE REVIEW

2.1 Lettuce

2.1.1 Origin and uses

Lettuce or *Lactuca sativa* is a member in Family of Compositae or Asteraceae. Compositae is one of the largest angiosperm or flowering families. This family contains over 25,000 species including dandelion, chicory and endives (Mabberley, 1997). Linnaeus named this plant as *Lactuca*, which derived from the word '*lac*', meaning 'milk' in Latin. This is because the sap of lettuce is milk-like. Our modern lettuce is believed to evolve in Mediterranean or the Middle East from the wild *Lactuca serriola*. Until now, lettuce is still popular and always cultivated as leafy vegetable and eaten raw as salad. It is grown well in temperate country, and also in subtropical and tropical lands (Grubben & Sukprakarn, 1994).

2.1.2 Descriptions and ecology

Lettuce is a very variable, glabrous annual or biennial herb. Leaves arrangement, size, shape, and colour always differ between cultivars. However, it usually forms a dense basal rosette and later with a tall, branched, flowering stem. The colour of leaves always comes with green, sometimes with red anthocyanin. Lettuce grows best at moderate day temperature at

15-20 °C and cool nights, like in the highlands. While in lowlands, it always cultivated during the coolest season. Lettuce adapts to soil with good structure, high fertility and water capacity. It prefers the sandy-loam soils with slightly alkaline and does not tolerate acid soil with pH < 6 (Grubben & Sukprakarn, 1994).

2.1.3 Types

There are 5 common types of lettuce which can be differentiated by leaf arrangement and structure. The first type is 'Butterhead' lettuce. The head contains overlapping leaves while the inner leaves are thin, oily and buttery in texture. This variety is the most popular variety in cool temperate country such as Europe. 'Crisp' lettuce, also known as Iceberg lettuce has dense, thick and crunchy leaves with prominent flabellate veins and midribs that resemble cabbage. 'Cos' lettuce has elongated leaves and forms a tall, loose and cylindrical head. While 'Bunching' lettuce has thin, broad and curled leaves in loose rosette stem. The last type of lettuce is 'Stem' lettuce. This type of lettuce has thick stem and is always used as celery stalk. Its crispy leaves are eaten as salad (Grubben & Sukprakarn, 1994; Simpson & Ogorzaly, 1995).

2.1.4 Diseases

Lettuce it is often attacked by *Bremia lactucae*, which are fungi that cause downy mildew in this species. Another fungal pathogen, *Botrytis* sp. causes disease which has the symptom of whitish-gray mold formation on the leaves. Besides that, lettuce can be attacked by lettuce mosaic virus and cause yellowing and necrosis on leaves. This virus is transmitted by aphids. Lettuce infectious yellow virus or LIYV can infect lettuce as well and cause the leaves become yellow and stunned. LIYV is one type of crinivirus and is transmitted by whiteflies (Agrios, 2005). While bacterial pathogen *X. campestris* pv. *vitians* cause bacterial spots of lettuce (Barak *et al*., 2002).

2.1.5 Prospects

Lettuce is one type of vegetables that becomes and more important recently in agriculture, both for local use and export. This is proved by estimated plantation area of salad (lettuce) in Sabah increased from year 2002 to year 2003 (Department of Agriculture, Sabah, 2003a, 2003b) and the increased for export from 1997 to 2000.

Table 2.1 Estimated plantation area of lettuce in Sabah of year 2002 and 2003 (Department of Agriculture, Sabah, 2003a, 2003b).

Area in Sabah	Year 2002 (hectares)	Year 2003 (hectares)
Tawau	2.0	2.0
West Coast	35.9	47.0
Interior Divisions	1.2	-
Total	**39.1**	**49.0**

Table 2.2 Export of lettuce in Sabah of year 1997-2000 (Jipanin *et al*., 2001).

Year	Tone	RM
1997	126	330 000
1998	123	328 000
1999	153	398 000
2000	170	434 000

2.1.6 Classification

Below is the taxonomy or scientific classification of lettuce (Mabberley, 1997):

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Asterales

Family: Compositae (Asteraceae)

Genus: *Lactuca*

Species: *sativa*

2.2 Mechanisms of defense in plants

Every species of plants will be facing a challenge throughout their life cycles by numerous of pathogens and microorganisms such as viruses, bacteria, mollicutes, fungi and nematodes. Generally, plants defense themselves from pathogens by a combination of these 2 actions: the physical barriers that limit or inhibit the pathogens from entering and spreading through the plants and secondly, the biochemical reactions which occur in the cells and tissues, producing substances that are toxic to the pathogens or creating the conditions that will inhibit the pathogens growth (Agrios, 2005).

Physical barriers can be divided into 2 groups: the preexisting defense structures and induced defense structures. Preexisting structures are first line of defense of plants against pathogens. These include waxes and cuticles on leaf surfaces, thickness and toughness of the epidermal cell walls, size, location and shapes of stomata and lenticels. Induced defense structures are cytoplasmic reactions, formation of cork layers, abscission layers, tyloses and deposition of gums (Agrios, 2005; Maor & Shirasu, 2005).

Biochemical reactions can be divided in 2 groups as well. Preexisting chemical defenses are exudation of some fungtitoxin compounds, phytoanticipins, phenolic compounds, tannins, dienes, lectins and others in leaves, young fruits or seeds which will inhibit development of pathogens. Another type is induced biochemical defenses including hypersensitive responses, production of pathogenesis-related proteins and secondary metabolites such as phenolic compounds and phytoalexins (Agrios, 2005; Grayer & Kokubun, 2001). Resistance or defense of plants against pathogens is controlled directly or indirectly by the genetic materials of the host and of the pathogen. According to this concept, there are 3 types of plant resistances.

2.2.1 Non-host resistance

This type of resistance is the most common form of plant resistance in nature and is expressed by every plant. A plant can defend itself from a pathogen because it is not a host for that pathogen. The reason is genetic makeup of that plant is different from the host plants. Thus, the interaction between plant and pathogen is unsuccessful (Agrios, 2005). Preformed or preexisting defenses are major components in this type of resistance (Heath, 2000). For example, destructive oomycete pathogen *Phytophthora infestans* do not cause disease and macroscopic symptoms in crucifer *Arabidopsis thaliana* because the interaction between them was non-host pathosystem. *A. thaliana* is not the host for *P. infestans*. However, localized hypersensitive response was observed in *A. thaliana* epidermal cells. Infections were observed in tomato after treated with *P. infestans* because tomato is the host for this pathogen (Huitema *et al.*, 2003; Kamoun, 2001). Other examples are non-host resistance of parsley and *Nicotiana* to *Phytophthora* spp. (Kamoun, 2001).

2.2.2 Race-non specific resistance

This type of resistance is also known as partial, quantitative or horizontal resistance. After attacked by certain pathogen, a plant may develop no disease, mild disease or severe disease. In other words, it is exhibited in varying degrees, from minimal to maximal each with a small contribution to the resistance. This is because the resistance depends on the specific genetic makeup of the plant itself and also the pathogen. Many minor genes are involved in protecting the plant from pathogens. Presence or formations of preexisting or induced defense structures depend on these genes. Thus, there will be different level of resistance against pathogen attacks (Agrios, 2005; Sadasivam & Thayumanavan, 2003).

2.2.3 Gene-for-gene interaction

It is also known as qualitative or horizontal resistance. In this type of resistance, defense of a host plant against its pathogens is through the presence of matching pairs of genes for disease between the host plant and the pathogens. Host resistance is specific and determined by gene-for-gene interaction between a resistance (*R*) gene in host and avirulence (*avr*) gene in pathogen. It is initiated by highly molecular recognition between these 2 genes. Recognition is accomplished by the detection of elicitors that originate from pathogen or degradation from plant cell walls. Race-specific elicitors are encoded by the pathogen *avr* gene while specific receptors are encoded by the host *R* gene. Absence of either determinant will lead to the breakdown of resistance. After recognition, signaling events become initiated and trigger some responses such as synthesis of reactive oxygen species, changes in gene transcription, hypersensitive responses and cell death, necrosis and lesions, formation of antimicrobial compounds such as phytoalexins and others. For examples, *Rpg1* gene confers resistance of barley against *Puccinina graminis* pv. *triticae*. *RRS-1R* gene of

REFERENCES

Abraham, K.J., Pierce, M.L. & Essenberg, M. 1999. The phytoalexins desoxyhemigossypol and hemigossypol are elicited by *Xanthomonas* in *Gossypium* cotyledons. *Phytochemistry* **52**: pp. 829-836.

Agrios, G.N. 2005. *Plant Pathology*. 5th Ed. Elsevier Academic Press, Amsterdam.

Banthorpe D.V. & Charlwood, B.V. 1980. The Isoprenoids: The terpenoids. In: Bell, E.A. & Charlwood B.V. (eds.). *Encyclopedia of Plant Physiology: Secondary Plant Products*, Volume **8**. Springer-Verlag Berlin Inc., Heidelberg, pp. 185-220.

Barak, J.D., Koike, S.T. & Gilbertson, R.L. 2002. Movement of *Xanthomonas campestris* pv. *vitians* in the stems of lettuce and seed contamination. *Plant Pathology* **51**: pp. 506-512.

Bennett, M.H., Gallagher, M.D.S., Bestwick, C.S., Rossiter, J.T. & Mansfield, J.W. 1994. The phytoalexin response of lettuce to challenge by *Botrytis cinerea*, *Bremia lactucae* and *Pseudomonas syringae* pv. *phaseolicola*. *Physiological and Molecular Plant Pathology* **44**: pp. 321-333.

Bennett, M.H., Mansfield J.W., Lewis, M.J. & Beale, M.H. 2002. Cloning and expression of sesquiterpene synthases gene from lettuce (*Lactuce sativa* L.). *Phytochemistry* **60**: pp. 255-261.

Brooks, C.J.W. & Watson, D.G. 1991. Terpenoid phytoalexins. *Natural Product Reports*: pp. 367-388.

Cruickshank, I.A.M. 1963. Phytoalexins. *Annual Review of Phytopathology* **1**: pp. 351-374.

Dahama, A.K. 2003. Management of Agricultural Pollution: Agrochemicals. In: *Organic Farming: For Sustainable Agriculture*. Agrobios, Jodhpur, pp. 59-90.

Department of Agriculture, Sabah, 2003a. *Keluasan Tanaman Mengikut Bahagian danJenis Tanaman, Sabah 2002*.
http://www.sabah.gov.my/tani/Perangkaan/Perangkaan2002.pdf

Department of Agriculture, Sabah, 2003b. *Keluasan Tanaman Mengikut Bahagian dan Jenis Tanaman, Sabah 2003*. http://www.sabah.gov.my/tani/Perangkaan/Perangkaan2003.pdf

Dillon, V.M., Overton, J., Grayer, R.J. & Harborne, J.B. 1997. Differences in phytoalexin response among rice cultivars of different resistance to blast. *Phytochemistry* **44**: pp. 599-603.

Dixon, R.A., Harrison, M.J. & Lamb, C.J. 1994. Early events in the activation of plant defense responses. *Annual Review of Phytopathology* **32**: pp. 479-501.

Dye, D.W. & Lelliott, R.A. 1974. Genus II: Xanthomonas Dowson. In Buchanan, R.E. & Gibbons, N.E. (eds.). *Bergey's manual of determinative bacteriology*. 8^{th} Ed. The Williams & Wilkins Company, Baltimore, pp. 243-249.

D'Silva, I. & Heath, M.C. 1997. Purification and characterization of two novel hypersensitive response-inducing specific elicitors produced by the cowpea rust fungus. *Journal of Biological and Chemistry* **272**: pp. 3924-3927.

Ebel, J. 1986. Phytoalexin synthesis: The biochemical analysis of the induction process. *Annual Review of Phytopathology* **24**: pp. 235-264.

Gibbs, R.D. (eds.). 1974. Terpenoids. In: *Chemotaxanomy of flowering plants*. McGill-Queen's University Press, Montreal, pp. 770-872.

Gossett, D.R. 2003. Pesticides. In: Ness, B.D. (eds.). *Magill's Encyclopedia of Science – Plant Life*. Salem Press Inc., Pasadena, pp. 783-786.

Grayer, R.J. & Harborne, J.B. 1994. A survey of antifungal compounds from higher plants, 1982-1993. *Phytochemistry* **37**: pp. 19-42.

Grayer, R.J. & Kokubun, T. 2001. Plant-fungal interaction: the search for phytoalexins and other antifungal compounds from higher plants. *Phytochemistry* **56**: pp. 253-263.

Grubben, G.J.H. & Sukprakarn, S. 1994. Alphabetical treatment of species: *Lactuca sativa*. In: Siemonsma, J.S. & Piluek, K. (eds.). *Plant resources of South-East Asia:Vegetables 8*. Prosea Foundation, Bogor, pp. 186-190.

Hammerschmidt, R. 1999. Phytoalexins: What have we learned after 60 years? *Annual Review of Phytopathology* **37**: pp. 285-306.

Harborne, J.B. 1999. The comparative biochemistry of phytoalexin induction in plants. *Biochemical Systematics and Ecology* **27**: pp. 335-367.

Hargreaves, J.A. & Bailey, J.A. 1978. Phytoalexin production by hypocotyls of *Phaseolus vulgaris* in response to constitutive metabolites released by damaged bean cells. *Physiological Plant Pathology* **13**: pp. 89-100.

Heath, M.C. 2000. Nonhost resistance and nonspecific plant defenses. *Current Opinion in Plant Biology* **3**: pp. 315-319.

Huitema, E., Vleeshouwers, V.G.A.A., Francis, D.M. & Kamoun, S. 2003. Active defense responses associated with non-host resistance of *Arabidopsis thaliana* to the oomycete pathogen *Phytophthora infestans*. *Molecular Plant Pathology* **4**: pp. 487-500.

Jipanin, J., Rahman, A.A., Jaimi, J.R. & Phua, P.K. 2001. Management of pesticide use on vegetable production: Role of Department of Agriculture Sabah. *Proceedings of the Sixth Sabah Inter-Agency Tropical Ecosystem (SITE) Research Seminar*, 13-14 September 2001, Kota Kinabalu, Sabah, pp. 63-85.

Kamoun, S. 2001. Nonhost resistance to *Phytophthora*: novel prospects for a classical problem. *Current Opinion in Plant Biology* **4**: pp. 295-300.

Kuć, J. 1972. Phytoalexins. *Annual Review of Phytopathology* **10**: pp. 207-232.

Kuć, J. 1995. Phytoalexins, stress metabolism, and disease resistance in plants. *Annual Review of Phytopathology* **33**: pp. 275-297.

Mabberley, D.J. 1997. *The plant-book: a portable dictionary of the vascular plants*. 2nd Ed. Cambridge University Press, United Kingdom.

Mahmoud, Z.F., Kassem, F.F., Abdel-Salam, N.A. & Zdero, C. 1986. Sesquiterpene lactones from *Lactuca sativa*. *Phytochemistry* **25**: pp. 747-748.

Mansfield, J.W. 1980. Mechanisms of resistance to *Botrytis*. In: Coley-Smith, J.R., Jarvis, W.R. & Verhoeff, K. (eds.). *The Biology of Botrytis*. Academic Press, London, pp. 181-218.

Mansfield, J.W. 2000. Antimicrobial compounds and resistance: the role of phytoalexins and phytoanticipins. In: Slusarenko, A.J., Fraser, R.S.S. & van Loon, L.C. (eds.). *Mechanisms of Resistance to Plant Diseases*. Kluwer Academic Publishers, Netherlands, pp. 325-370.

Maor, R. & Shirasu, K. 2005. The arm race continues: battle strategies between plants and fungal pathogens. *Current Opinion in Microbiology* **8**: pp. 399-404.

Marshall, P.S., Harborne, J.B. & King, G.S. 1987. A spiroketalenol ether phytoalexin from infected leaves and stems of *Coleostephus myconis*. *Phytochemistry* **26**: pp. 2493-2494.

Mew, T.W. & Swings, J. 2000. *Xanthomonas*. In: Lederberg, J. (eds.). *Encyclopedia of microbiology*. 2nd Ed. Academic Press, San Diego, pp. 921-920.

Monde, K., Takasugi, M., Katsui, N. & Masamune, T. 1990. Total synthesis of lettucenin A, a guaianolide phytoalexin from *Lactuca sativa* var. *capitata*. *Chemistry Letters*: pp. 1283-1286.

Nicholson, R.L. & Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology* **30**: pp. 369-389.

Purkayastha, R.P. 1995. Progress in phytoalexin research during the past 50 years. In: Daniel, M. and Purkayastha, R.P. (eds.). *Handbook of phytoalexin metabolism and action*. Marcel Dekker Inc., New York, pp. 1-39.

Rathjen, J.P. & Moffett, P. 2003. Early signal transduction events in specific plant disease resistance. *Current Opinion in Plant Biology* **6**: pp. 300-306.

Romeis, T. 2001. Protein kinases in the plant defense response. *Current Opinion in Plant Biology* **4**: pp. 407-414.

Rossall, S. & Mansfield, J.W. 1984. Relationships between germ-tube growth by *Botrytis cinerea* and *B. fabae* and their metabolism of the phytoalexins wyerone, wyerone acid and wyerone epoxide. *Transactions of the British Mycological Society* **82**: 571-576.

Rossall, S., Mansfield, J.W. & Hutson, R.A. 1980. Death of *Botrytis cinerea* and *B. fabae* following exposure to wyerone derivatives *in vitro* and during infection development in broad bean leaves. *Physiological Plant Pathology* **16**: pp. 135-146.

Rouxel, T., Sarniguet, A., Kollmann, A. & Bousquet, J.F. 1989. Accumulation of phytoalexin in *Brassica* spp in relation to a hypersensitive reaction to *Leptosphaeria maculans*. *Physiological and Molecular Plant Pathology* **34**: pp. 507-517.

Sadasivam, S. & Thayumanavan, B. 2003. *Molecular Host Plant Resistance to Pests*. Marcel Dekker Inc., New York.

Simpson, B.B. & Ogorzaly, M.C. 1995. Foods from leaves, stems and roots. In: *Economic botany: Plants in our world*. 2nd Ed. McGraw-Hill Inc., New York, pp. 229-277.

Soylu, S., Bennett, M.H. & Mansfields, J.W. 2002. Induction of phytoalexin accumulation in broad bean (*Vicia faba L.*) cotyledons following treatments with biotic and abiotic elicitors. *Turkish Journal of Agriculture and Forestry* **26**: pp. 343-348.

Tahara, S., Hanawa, F., Harada, Y. & Mizutani, J. 1988. A fungitoxin inducibly produced by dandelion leaves treated with cupric chloride. *Agricultural and Biological Chemistry* **52**: pp. 2947-2948.

Takasugi, M., Okinaka, S., Katsui, N., Masamune, T., Shirata, A. & Ohuchi, M. 1985. Isolation and structure of lettucenin A, a novel guaianolide phytoalexin from

Lactuca sativa var. *capitata* (Compositae). *Journal of the Chemical Society, Chemical Communications* **10**: pp. 621-622.

VanEtten, H.D., Matthews, D.E. & Matthews, P.S. 1989. Phytoalexin detoxification: Importance for pathogenicity and practical implications. *Annual Review of Phytopathology* **27**: pp. 143-164.

Vicente, J.G., Conway, J., Roberts, S.J. & Taylor, J.D. 2001. Identification and origin of *Xanthomonas campestris* pv. *campestris* races and related pathovars. *Phytopathology* **91**: pp. 492-500.

Young, A.L. 1987. Minimizing the Risk Associated with Pesticide Use: An Overview. In: Ragsdale, N.N. & Kuhr, R.J. (eds.). *Pesticides: Minimizing the Risks*. American Chemical Society, Washington D.C., pp. 1-11.