AGING EFFECT TO ACCUMULATION OF RISHITIN IN TOMATO AFTER ELICITED BY ABIOTIC ELICITOR : SILVER NITRATE

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ABSTRACT

An accumulation of major phytoalexin in tomato leaves (*Lycopersicum esculeuntum*) rishitin were quantified after elicited by abiotic elicitor Silver nitrate ($AgNO_3$) at different stages of age week three, five, ten, fifteen and twenty using different value of optical density . Rishitin was spotted on Thin Layer Chromatography as a purple fluorescence band at Rf value 0.72 under UV light 364nm. No rishitin was detected in control plants at all plant ages. Rishitin were quantified by spectrophotometer at maximum absorbance 500nm and optical density value was taken as a value to compare the presence of rishitin in different ages. The value increased as the plants grew older from three to twenty weeks. The leaves week twenty of tomato showed the highest mean of rishitin value 0.4567 and the lowest was in week three with 0.0067. The data analysis showed that there was a significant correlation effect between the accumulation of rishitin with plant ages.

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ABSTRAK

Akumulasi fitoaleksin utama pada daun tomato (*Lycopersicon esculentum* (L.) Mill) yang dikenali sebagai rishitin dijumpai apabila dikenakan kepada agen abiotik iaitu argentum nitrat ($AgN0_3$) pada pokok pelbagai usia iaitu tiga, lima, sepuluh, lima belas dan dua puluh minggu. Kehadiran rishitin yang kelihatan berwarna ungu dikenalpasti dengan mengunakan kaedah Thin Layer Chomotografy. Rishitin dijumpai mempunyai Rf value bersamaan dengan 0.72 di bawah cahaya UV 364nm. Rishitin tidak dijumpai pada komotogram yang mengandungi ekstrak daun dari rawatan kawalan iaitu daun yang disembur dengan air suling. Analysis kuantiti rishitin dilakukan dengan menggunakan spektrofotometer pada nilai serapan maximum 500nm. Penyerapan dijadikan sebagai nilai ukuran untuk membandingkan kuantiti rishitin bagi ekstrak daun tomato berbeza umur. Ekstrak daun pada umur dua puluh mingggu menunjukkan jumlah penyerapan yang paling tinggi dengan nilai min 0.4567 manakala ekstrak daun umur 3 minggu menunjukkan nilai paling rendah dengan nilai min 0.0067. Keputusan analisis menunjukkn umur tumbuhan menberi kesan yang signifikasi pada akumulasi rishitin dalam daun tomato.

UMS
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CONTENTS

Page Number

UMS
UNIVERSITI MALAYSIA SABAH

UMS UNIVERSITI MALAYSIA SABAH

LIST OF FIGURE

UMS
UNIVERSITI MALAYSIA SABAH

Figure **Page**

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF APPENDIXES

UMS
UNIVERSITI MALAYSIA SABAH

ABBREVIATIONS

HR	Hypersensitive Reaction
$AgNO_3$	Silver Nitrate
OD	Optical Density
Rf	Retardation Factor
TLC	Thin Layer Chomotography
UV	Ultraviolet
SPSS	Statistical Package for Social Science

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SYMBOLS

%	Percentage
β	Beta
ml	Millimeter
<	Less than
>	more than
/	per

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CHAPTER 1

INTRODUCTION

1.1 Preface

Almost 31-42% of agricultural world production losses because of destructive and destroy by disease, insect and weeds. From this average almost 36.5 % of total losses was due to disease 14%, insect 10 % and weeds 12%. Disease shows the most percentage and major widespread losses and it alone was reported can cause until $ 220 billion of losses in agricultural production (Agrios, 2005). In developed countries losses typically lower in potential yield production than in developing countries (Bent and Yu, 1999). Losing of agricultural production contribute to many kinds of social problems to the worldwide especially in poor countries that their economic fully hope in agricultural industry. Furthermore with an increase of human population day by days, year by year around the world is afraid to say that some of the people still facing with starvation, lack of malnutrition because limited source of food (Agrios, 2005).

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The terms of disease development in plant can be defined as eventually outcome of any interaction will depend upon ability of the plant to prevent development of the pathogen versus the ability for pathogen to overcome the resistance of the host (Smith, 1996). Disease can cause by many kinds of factors that commonly can be divided into two which are biotic factor and abiotic factor. Biotic factor always refer as pathogen such as viruses, bacteria, fungi, nematodes, mollicutes and protozoa. They usually cause disease in plant by disturbing the metabolism of plant cell through enzymes, toxic, growth regulators and other substances they secrete and by absorbing food stuffs from the host cells for their own use and some of them may also cause disease by growing and multiplying in the xylem or phloem vessels of plants, thereby blocking the upward transportation of water or the downward move of sugar (Agrios, 2005).

Abiotic typically refer to environmental factors such as temperature, moisture, mineral, nutrient and pollutants that exist at levels above or below a certain ranged tolerate by plant. These two kinds of factors and insect or pest can infect and attack plant at any of their life span. They can infect seedling, young or mature plant or even senescence. Moreover the infection also can be at different part of plants such as stem, stolen, leaves or even seed. However plant change in their reaction susceptibility or resistance to disease with age (Agrios, 2005). Some of the plants become susceptible only during growth period and become resistant during adults such as *Pythium* damping off and root roots, downy mildew, peach curl and bacterial blight. Some of them will become resistant while young but become more susceptible in growth period and resistant again when they are mature. For example, rust disease and viral infection.

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Besides that there are also plant that have a stage of juvenile susceptibility during the growth period of the plant. Susceptibility continued by a period of relative resistance in the early adult stage and following susceptibility after maturity such as potato blight caused by *Phytophora infestans* and tomato blight cause by *Alterinaria solani* (Agrios, 2005).

Although plants are easily expose to an existence of a various number of potential pathogens in their life and vast of environmental stress including pollutant and toxicity, most plants can live free from the effect of this unfavorable condition. This is due to suitable and effective mechanism in their own protection (Smith, 1996). In order to survive, grow and reproductive, they protect themselves by an array of resistance mechanism. Generally, this mechanisms are formed by combination of physical and chemical barriers. In physical barriers they protect themselves by inhibit the pathogen from gaining entrance and spreading through the plant Whereas in chemical barriers, biochemical reaction which take place in the cells and tissues of the plant and produce substance that are either toxic to the pathogen or create condition that inhibit growth of the pathogen in the plant (Agrios, 2005).

One of the chemical resistance that found to be a potential antimicrobial compound due to resistance in plant disease called phytoalexin. Phytoalexin is toxic antimicrobial substances produced in appreciable amounts in plants only after stimulation by various type of elicitor either biotic or abiotic elicitor (Agrious, 2005). These compound was successfully isolated in many types of plant species such as camalexin in

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Arabidopsis thaliana, phaseollin in bean, furocoumarin in celery, lubumin in potato and rishitin in tomato (Strange, 2003). Phytoalexin can be a potential compound to inhibit the pathogen growth. Previous studied by Johnson *et al.* (1976) on leaves of sugarbeet (*Beta vulgaris*) found that, betavulgarin posses strong antifungal activity and play important role in the resistance of sugarbeet to *Cerespora beticiola.* Realizing the important roles of phytoalexin, many of the researcher were interested in finding out the factors that can contribute to the accumulation of this compound in plant species such as types of elicitor, of host resistance such as susceptible or resistant plant, part of plant and age of plant.

Tomato (*Lycopersicon esculentum* (L.) Mill) is one of the important crop in the world. Tomato have per capita fresh market consumption that continues to increase around the world. Tomato products are found in a great variety of processed food (Benton, 1999). In Malaysia, tomato is the third important vegetables planted in Highlands among 25 types of other vegetables. However, the production of tomatoes are limited contribute with many types of diseases. One of the serious diseases in highlands is late blight that cause by the fungus *Phytophora infestans*. This disease can destroy tomato leaves and can kill them (Nordin *et al*., 1986).

Similar disease was reported to the 50% of losses in the production of tomato in Ontario, Southern Georgia and Florida around 1970. Whereas phorma rot disease cause by *Phoma destructive* plowright occurs in most growing areas of the world especially in

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the Southern United States and India with contributed losses until 28% in early 1990. This fungus can infected tomato leaves, stem and fruit (Sherf and Macnab, 1986).

Other disease such as common mosaic by strain of tomato mosaic virus , Cinton that contribute the big losses especially in British greenhouse. Besides that, early blight by *Alternaria solani* can cause death of plant. This disease also can infect different part include seed, leaves, stem, branches, roots and fruit. Some of this disease can infect at various stages of plant development such as early growth and fruit development for example bacteria canker in tomato cause by *Corynebacterium michiganense* that can be affected at any stage and development (Sherf and Macnab, 1986).

To control the disease and pest insect among tomato plants, applying toxic chemicals which known as pesticides and fungicides are always be an option. However an extensive use of pesticides to overcome this problem can cause many other problem such as pollutant and toxicity to other organism such as soil microorganism, animals and human. Besides, it can effect the price of tomato fruit in the market when consumer are prefer to buy less use agricultural chemical tomato fruit (Benton, 1999).

There are other significant developments have been made to develop cultivars are resistant or tolerant to the occurring disease, insects and nematodes. One of this approach is breeding disease resistant cultivar and varieties of plant like tomato such as verticillium and fusarium wilt and tobacco mosaic virus. Some cultivar of tomatoes are resistant to tomato disease such as Venus, Saturn, Kewado and Rosita Mountain

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Supreme. These cultivar resistant to Bacteria wilt diseases (Peet, 1996). Another ways to protect tomato is by practicing crop rotation, sterilize seed, applying a good agriculture practice such as take care a good sanitary (Benton, 1999).

In tomato secondary metabolites including phenolic compounds, phytoalexins, protease inhibitors and glycoalkoloids play an important role in disease resistance. These metabolite play an important role to protect against adverse effect of host of predators such as fungi, bacteria, viruses and insect or even environmental stress (Friedman, 2002). Rishitin have been found to be the major phytoalexin in tomato (De Wit and Flach, 1979) and also can be found in other family Solanaceae such as potato (Harris and Dennis, 1976).

Since the founding of a major phytoalexin , rishitin is bring a new hope for a new mechanism of resistance in tomato. Rishitin was isolated in leaves (De Wit and Flach, 1979), stem (Harrison and Beckman, 1987) and fruit (Glazener and Wouters, 1981). The accumulation of rishitin due to the elicitation of either biotic or abiotic elicitor. Rishitin play an important roles in plant disease resistance in tomato (Hutson and Beckman, 1987). Rishitin can inhibit the early stages of *fusarium* infection from the region of the initial infection in stem vascular tissue. Similar result indicated that accumulation of rishitin and other phytolaexin such as solavetivone response to microbial infection and show antifungal activity against the majority of fungal *P. infestans* in potato tuber (Harris and Dennis, 1976).

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Although many studies have been done due to accumulation of rishitin in tomato but there are fewer studies on the this factor due to the effect of plant age. Thus, in this study tomato leaves tissue will be elicited by abiotic elicitor $AgNO_3$ and rishitin will be quantify among age of tomato plants.

1.1 Research Objective

The objective is :

- To quantify the accumulation of rishitin in tomato leaves at three, five, ten, fifteen and twenty weeks old tomato plant after elicited by abiotic elicitor $AgNO_3$

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CHAPTER 2

LITERATURE REVIEW

2.1 Tomato

2.1.1 Background

Tomato, *Lycopersicon esculentum* (L.) Mill. is the most solanaceous vegetable. The Aztecs of Central America cultivated the tomato plant, which they called *xitomatl* at around 700 A.D. Spanish conquistadors named it *tomate*. Tomato seeds were transported from the Andes to Spain and from there to other European countries around 1520. In Italy, tomatoes were named *pompodoro*or "golden apple" and in France, "pomme d'amour" or apple of love (Benton, 1999). The widespread use of tomatoes in the United States as a food began during the second half of the 19th century. In 1993 world tomato production 70 million metric tons were reported (Wien, 1999). Tomatoes are used in many processed foods such as canned and sun-dried tomatoes, juices, ketchup, pastes, purees, salads, sauces and soups (Rubatzky and Yamaguchi, 1997).

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2.1.2 Botany

Tomatoes are usually annuals in temperate region or short-lived perennials in the tropics. Tomato seed is small and hairy. Germination is epigeal which the seeds germinate with cotyledon appearing above the surface of the ground (Messiaen, 1992). Plants grow from 0.5-2.0 tall, with solid and thick stem but some dwarf cultivars, grown as novelties, are less than 30 cm tall. Growth habit can vary from erect to semi prostrate and some also exhibit substantial vining. Taproots usually are strong and deep, some occasionally reach depths of 3 m. Small glandular hairs that appear on stem, leaves and peduncles have a noticeable odor (Rubatzky and Yamaguchi, 1997).

Once the two foliaceous, oval cotyledons have unfolded, the seedling develops 7-14 composite leaves, bearing more and more leaflets, before the first inflorescence is formed Leaves are compound pinnate, coarsely toothed and often curled but also can be smooth. Tomato axillary buds can develop into stalks, appear in each leaf axil and sometimes at the tip of the inflorescence (Messiaen, 1992). Plant growth characteristics range from indeterminate to highly determinate. Inflorescence are borne opposite and between leaves. Flowers are perfect about 2 cm in diameter and often pendent with yellow star-shaped corolla: yellow anthers are united to form a tube. Tomatoes have pedicels that have abscission zone about midlength (Rubatzky and Yamaguchi, 1997).

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2.1.3 Plant Growth

Tomatoes are grown successfully on a wide range of soil types from sandy to fine textured clays, as well as in soils of high organic content. A soil pH range from 5.5 to 7 is usually suitable for tomato. Plants grow better when provided with uniform moisture and well- drained soils. They are intolerant of water logging, especially shortly after germination and at the period of fruit maturation (Benton, 1999). Excessive moisture is often conducive to damping off and root rot diseases. To minimize diseases problem soil commonly were sterilized (Sherf and Macnab, 1986). Usually, water is given about 25-30mm weekly and on a hot dry day, evapotranspiration can exceed 10 mm (Rubatzky and Yamaguchi, 1997).

Tomatoes can be grown in most open-field locations where there is a minimum of 3-4 months of warm, frost-free weather, which an average temperature 16 °C but if the temperature less than 12°C it can cause chilling injury. Days temperature of 25 – 30 °C with night temperatures between 16°C and 20°C are optimal for growth and flowering. Whereas for seed germination is need a temperature about 10°C and the maximum is 35°C. Between 25°C and 30°C, seedling emergence occurs within 6-9 days. Nitrogen is very important for vegetative growth. Whereas phosphorus also important for development and flowering, potassium and calcium for cell wall development. Commonly a starter fertilizer is applied prior to or at planting. Both phosphorus and potassium are commonly applied pre plant with a portion of the total nitrogen (Rubatzky and Yamaguchi, 1997).

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