UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ELICITORS IN IMPROVING MORPHOPHYSIOLOGICAL TRAITS AND BIOCHEMICAL ACTIVITIES IN CHILLI (Capsicum annuum L.) IN SOILLESS CULTURE

NOOR ASMA'A BINTI AWANG

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By

NOOR ASMA'A BINTI AWANG

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

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

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July 2013

Chairman: Mohd Razi bin Ismail, PhD
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Modern agriculture relies heavily on pesticides for the control of pests and diseases infestations in chilli plants. Without any effective control measures, plants disease could reduce chilli yields up to 95%. Concerning the negative impact of extensive pesticides used to human health and ecosystem, a number of recent researches are now focusing on alternative tools in an effort to reduce or eliminate yield losses and maintain high product quality. In the present study, attempts were made using natural plant elicitors like jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) at different concentration (0.5, 1.0 and 1.5mM). These elicitors were applied as foliar sprays aiming to enhance plant health by induce resistance to reduce the risk of pest entry in chilli plant. Results show that different concentrations of elicitors significantly improved dry matter accumulation in the leaves, stems and roots, and resulting in increased chilli yield. Applications of 0.5mM jasmonic acid (JA) or 1.5mM salicylic acid (SA) gave the strongest positive effect compared to other treatment combinations. Therefore, further experiment was carried out to determine the effects of both selected elicitors on the growth and yield of chilli. The result showed that jasmonic acid treated plants significantly enhance plant health by improving plant growth and performance, stem and canopy diameter, photosynthesis rate and yield production compared to salicylic acid treated plants. Jasmonic acid was the best elicitor although there was a significant decrease in yield compared with pesticide treated plants. This negative effect on yield may have been due to under dosage of jasmonic acid application and heavy rainfall that occurred shortly after treatments.

Another study was carried out to improve effectiveness of JA by increasing the frequency of application. The finding shows that jasmonic acid sprayed twice (JA2) had significantly in enhancing plant health by improved chilli plant growth and physiology in term of relative chlorophyll content, stomatal conductance, chlorophyll fluorescence and photosynthesis rate, thus enabling it to be more resistant to pests and diseases infestations which leads to improve chilli production. The mean values of peroxidase for

inducible enzymes which have a role in plant resistance at vegetative stage were significantly higher in chilli plants treated with jasmonic acid. Enzymatic activity (Guaicol Peroxidase, Ascorbate Peroxidase and Catalase) was lowest in the untreated plants, which corresponded with the lower plant growth and physiological responses of unhealthy plant. Thus, it could be concluded that jasmonic acid are importance in enhancing plant health with induces resistance thus leading to better plant growth and improved chilli production which could be replace application of pesticide.

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

KESAN ELISITOR DALAM MENINGKATKAN CIRI-CIRI MORFOFISIOLOGI DAN AKTIVITI BIOKIMIA DALAM TANAMAN CILI (*Capsicum annuum* L.) SECARA FERTIGASI

Oleh

NOOR ASMA'A BINTI AWANG

Julai, 2013

Pengerusi: Mohd Razi bin Ismail, PhD
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Pertanian moden sangat bergantung kepada penggunaan racun perosak untuk kawalan serangan serangga perosak dan penyakit tanaman cili. Tanpa langkah kawalan yang efektif, ia boleh mengurangkan hasil sehingga 95%. Berkaitan kesan negatif racun perosak kepada kesihatan manusia dan ekosistem, beberapa penyelidikan telah dibuat bertumpu kepada kaedah alternatif dalam usaha untuk mengurangkan kerugian hasil serta mengekalkan kualiti produk. Dalam kajian ini, percubaan telah dibuat menggunakan elisitor semulajadi seperti asid jasmonik (JA), asid salisilik (SA) and etilena (ET) dengan kepekatan berbeza (0.5, 1.0 dan 1.5mM). Ia telah digunakan sebagai cecair semburan bertujuan untuk meningkatkan kesihatan pokok dengan mendorong ketahanan bagi mengurangkan risiko serangan serangga perosak tanaman cili. Keputusan menunjukkan elisitor dengan kepekatan berbeza meningkatkan biojisim daun, batang dan akar, justeru meningkatkan hasil secara signifikan. Penggunaan 0.5mM asid jasmonik atau 1.5mM asid salisilik memberikan kesan positif berbanding dengan kombinasi rawatan yang lain. Penyelidikan lanjut telah dibuat kepada kedua-dua elisitor yang telah dipilih untuk menentukan kesan pertumbuhan dan hasil pengeluaran. Hasil kajian menunjukkan pokok yang disembur jasmonic asid meningkatkan kesihatan pokok secara signifikan dengan meningkatkan pertumbuhan, diameter batang dan kanopi, kadar fotosintesis dan pengeluaran hasil berbanding pokok yang disembur asid salisilik. Asid jasmonik adalah elisitor terbaik walaupun mengeluarkan hasil yang lebih rendah berbanding pokok yang disembur racun perosak. Ia mungkin disebabkan oleh hujan lebat sejurus selepas penyemburan dilakukan yang boleh mengurangkan kesan tersebut.

Penyelidikan lanjut telah dibuat untuk meningkatkan keberkesanan elisitor dengan meningkatkan kekerapan penyemburan. Dua kali penyemburan asid jasmonik (JA2) telah meningkatkan kesihatan pokok secara signifikan dengan meningkatkan pertumbuhan dan aktiviti fisiologi seperti kandungan klorofil relatif, konduktan stomata, fluoresen klorofil dan fotosintesis sekaligus membolehkan ia lebih tahan kepada serangan perosak dan penyakit yang membawa kepada peningkatkan hasil. Purata kandungan peroxidase yang berperanan dalam meningkatkan rintangan penyakit adalah

paling tinggi dalam pokok yang disembur asid jasmonik terutamanya pada peringkat vegetatif. Aktiviti enzim (GPX, APX dan CAT) adalah terendah dalam pokok yang tidak sihat iaitu yang tidak dirawat, sejajar dengan pertumbuhan dan tidak balas fisiologi yang rendah. Oleh itu, kesimpulan dibuat bahawa asid jasmonik adalah penting dalam meningkatkan kesihatan pokok cili dengan peningkatan rintangan yang mendorong kepada peningkatan pertumbuhan serta memaksimumkan pengeluaran hasil, yang boleh menggantikan penggunaan racun perosak.

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I certify that a Thesis Examination Committee will be met to conduct the final examination of Noor Asma'a Binti Awang on his thesis entitled "Effects of Elicitors in Improving Morphophysiological Traits and Biochemical Activities in Chilli (*Capsicum annuum* L.) in Soilless Culture" 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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which has been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NOOR ASMA'A BINTI AWANG

Date: 1 July 2013

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	Percentage
=	Equal to
>	Greater than
≤	Less than and equal to
µl	Microlitre
µmol	Micromole
ANOVA	Analysis of Variance
APX	Ascorbate Peroxidase
CAT	Catalase
CD	Coir Dust
cm	Centimetre
CMV	Chilli Mosaic Virus
CO_2	Carbon Dioxide
CVMV	Chilli Veinal Mottle Virus
DAT	Day After Transplanting
DMRT	Duncan Multiple Range Test
DNA	Deoxyribonucleic Acid
EFB	Empty Fruit Bunch
ET	Ethylene
et al.	And friends
F1	First Generation
g	Gram
GR	Glutathione Reductase
H_2O_2	Hydrogen Peroxide
ha	Hectare
HR	Hypersensitive Response
ISR	Induced Systemic Resistance
JA	Jasmonic Acid
L	Litre
LSD	Least Significant Difference
M	Molar
MARDI	Malaysian Agricultural Research and Development Institute
MDAR	Monodehydroascorbate Reductase
MeJA	Methyl Jasmonate
mg	Milligram
ml	Millilitre
mM	Millimolar
n	Number of Plants
nm	Nanometre
NPR1	Atrionatriuretic Peptide Receptor A
ns	Not Significant
O_2^-	Superoxide anion
ºC	Degree-Celsius
PAL	Phenylalanine Ammonia Lyase

pH	Measurement of Acidity / Alkalinity
Pn	Photosynthesis
POX	Peroxidase
PPO	Polyphenol oxidase
PR	Pathogenesis Related
PSII	Photosystem II
RCBD	Randomized Complete Block Design
RM	Ringgit Malaysia
ROS	Reactive Oxygen Species
rpm	Rotation per minute
s	Second
S.E	Standard Error
SA	Salicylic Acid
SAR	Systemic Acquired Resistance
SAS	Statistical Analysis System
SOD	Superoxide Dismutase
TMV	Tobacco Mosaic Virus
ULVC	Urdbean Leaf Crinkle Virus
USA	United State of America
v/v	Volume Per Volume
WCIMV	White Clover Mosaic Virus
Σ	Sum

CHAPTER 1

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the most popular vegetable planted among vegetable farmers in Malaysia. It is also considered as an important vegetable crop grown throughout the world. In Malaysia, cultivation area of chilli increased in the recent time but total production decreased due to its poor production and substantial imports are still needed to meet country's requirements (Department of Statistics Malaysia, 2011). Major problem of chilli cultivation is its susceptibility to plant damage by several pests and diseases. Different morphological, physiological and environmental factors are evolved to make a chilli plant susceptible to disease and pest infestation, which caused excessive yield losses, both in quality and quantity (Idris and Mohamad Roff, 1999; Tusiime *et al*., 2010). Some of diseases are caused by pathogens that can spread rapidly from one plant to another under optimum environmental conditions (Black *et al*., 1991). Insect attack is also an important factors leading to stunted plant growth, reduction crop productivity and affecting other agricultural problems (Ochoa-Alejo and Ramirez-Malagon, 2001).

Modern agriculture relies heavily on pesticides used for the control of pathogens and herbivores pests especially when they create risk in yield loss. Without any effective control measures, it is estimated that yield losses due to pests and diseases would be as much as double compared with its present level (Oerke and Dehne, 2004). Extensive use of pesticides has become a threat to pest resurgence and destruction of natural enemy fauna, and has the potential lead to presence of pesticide residue (David, 1991; Awasthi *et al*., 2001). Furthermore, with concern of existing pesticide residue to human poisonous and environmental hazard, and the promising in development of alternative strategies controlled practices using a mimic plant hormones as well as elicitors to increase plant health which act in the plant through induced systemic resistance that are generally considered to be eco-friendly and safe for use in agriculture setting, as these elicitors are not directly toxic (Stout *et al.*, 2002; Black *et al.*, 2003).

In Malaysia, chilli production reached 32.8 thousand tonnes per year in 2010 with an approximate 2.8 thousand hectares of areas under chilli cultivation (DOA, 2011). This chilli production remained the same during the period of 2005 – 2009, meanwhile the consumption increased up to 50 thousand tonnes per year. Due to insufficient amount of national production, the import dependency ratio of chilli rises up to 50% either in the form of fresh, dried or powdered. The high import dependency ratio showed that domestic production was still low and substantial imports are still needed to support the country's consumption (Department of Statistics Malaysia, 2011). To achieve desirable production of chilli, it is important to increase production, as well as cultivation area. Management of different disease and pests are also important for better crop production. Leaf curl disease, cucumber mosaic virus (CMV) and chilli veinal mottle virus (CVMV) are the common diseases in Malaysia (Shih *et al*., 2006; Arogundade *et al*., 2012), and some herbivores insect such as aphid, thrips and mites as a vector for the transport of plant viruses (Thaler *et al*., 1996).

As a general rule, most pests and diseases cannot be completely eradicated, but can be managed and controlled to minimize the collateral damage. Successful management of these potential problems require the development of alternative control strategies as part of integrated pest management strategy. The use of elicitors is a swift implementation of preventative methods to enhance plant health thus enabling it to be more resistant with induces plant responses by reducing herbivorous and diseases, and increase fruit production (Thaler, 1999).

Plants are able to defend themselves against pest attacks through constitutive defences as well as induction of defence compounds that can be activated by several metabolic ways. Application of elicitors to improve plant health by activates plant defences for controlling pest and disease problem in an emerging area could play an important role in chilli production. In other crop species, plant diseases or insect herbivores can be negatively affected through the use of different elicitors such as plant signalling molecules (Thaler *et al*., 1996). Three phytohormones such as jasmonic acid (JA), salicyclic acid (SA) and ethylene (ET) collectively termed abiotic elicitors, may have the potential to enhance plant health thus enabling it to control diseases and pests' infestation of chilli production through the activation of plant defence compounds. Each inducer activates a specific pathway and they act individually, synergistically or antagonistically, depending upon the pathogen involved. Besides, to local resistance these elicitors play a major role to induce defence responses against various pathogens and pests in systemic tissue (Thaler *et al*., 1996; Glazebrook, 2005; Broekaert *et al*., 2006; Loake and Grant, 2007; Balbi and Devoto, 2008).

Application of JA in tomato plants, resulted in induction of polyphenol oxidase and proteinase inhibitors, and in a decrease in the preference, performance and abundance of many common herbivores in the field, including *Myzus persicae* (aphids), *Frankliniella occidentalis* (thrips) and *Spodoptera exigua* (noctuid caterpillar) (Thaler *et al*., 1996). These pests are virus vectors that can be important of yield-reducing of tomato (Fan *et al*., 1997 and Koda, 1997).

Therefore, understanding of physiological responses to the plants treated with natural plant elicitors may allow growers to cultivate chilli in more conducive ways and develop productive plant with induction on plant yield, possibly by valuable pest management tools especially when there are high densities of herbivores through effective and safe methods. Work done by Thaler (1999) on the use of natural plant elicitors in tomato plant also highlights the potential roles of elicitors on the net effects of induction on seedling survivorship, phenology, plant biomass, fruit production, and total yield in the presence and absence of herbivores . Therefore, to use induced responses as an effective management tools, it is very important to evaluate the effects of induced responses on plant growth performance and yield in an agricultural setting.

The main objective of this study is to evaluate the effect of elicitors that enhance plant health as the option to reduce the risk of pest entry, lead to better plant growth and maximize the yield production of chilli plant.

The three main hypotheses to be tested in the present study were:

1. Cultivation of chilli without pesticide sprays severely increase plant damage, affect plant growth and reduce total fruit yield production.
2. Applications of natural plant elicitors with optimum concentration will reduce the risk of pest entry with improve plant health, plant performance and yield of *Capsicum annuum*.
3. Selected elicitors increasing plant growth, physiological process and biochemical activities with induce plant health thus enabling it to be more resistant to pest infestation.

Therefore, the aims of the present study were:

1. To quantify the plant damage and yield reduction on cultivation of chilli plants without pesticide sprays.
2. To determine the most potential of elicitors with optimum rate as a plant defence promoter in chilli plants.
3. To study the effects of elicitors on growth performance, physiological and biochemical activities, and yield of chilli.

In order to meet these objectives, three stages of research were carried out, hence:

1. Quantification of plant damage on chilli plants with and without pesticide application.
2. Study of various plant elicitors with different concentration on disease resistance and growth of chilli plants.
3. Effect of the selected elicitors on the plant health, physiological effects and biochemical activities in chilli plants.

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