EFFECTS OF JASMONIC ACID IN REDUCING CUCUMBER MOSAIC VIRUS INFECTION AND IMPROVING GROWTH PERFORMANCE OF CHILI (Capsicum annuum L.)

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By

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April 2018

Chair: Prof. Mohd Razi Ismail, PhD
Institute: Institute of Tropical Agriculture and Food Security

The high demand for chili has made agriculture entirely dependent on application of chemical pesticide to control pests and diseases. Results demonstrated that plants treated with pesticide significantly affected the number of pests such as aphids, thrips and mites which reduced the percentage of disease incidence up to 80%. However, regarding the long-term effect of pesticide toxicity, many researchers have developed alternative approaches which could improve plant productivity and give benefits to human and environmental health. In this study, jasmonic acid (JA), a potential plant elicitor had been tested using different concentrations (0.6 mM and 1.0 mM) and spray frequencies (single, double and triple application) to evaluate the efficacy in promoting growth performances, physiological responses, improving yield and reducing disease incidence. Results demonstrated treatment of 0.6 mM JA with double spray showed the outstanding positive effect compared to others in lowering the disease incidence by 50%. Despite of insignificant in concentration and spray frequencies factors, concentration of JA at 0.6 mM with double spray succeeded in improving high fruit yield which produced similar results to plants treated with concentration 1.0 mM of JA in double and triple sprays. Therefore, the next study to evaluate the effect of JA on chili plant infected with Cucumber Mosaic Virus (CMV) was carried out using concentration of 0.6 mM with double spray. CMV was reported to be one of the most prevalent cucumovirus in Malaysia due to its large host range and insect vectors. The findings revealed that CMV-infected plant treated with JA had the highest inhibitory effect on CMV infection at 30 days post inoculation (dpi) compared to untreated and pesticide-treated plants that were severely damaged. Due to low percentage of disease severity in JA application, the chili yield increased up to 95% and significantly raised the dry matter accumulation in leaves, stems and roots. The elicitation of JA had a significant impact on activities of catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), ascorbic acid (AsA), a-tocopherol and carotenoids (CAR) in leaves and fruits. In the JA-treated plants a significant increase in AsA, CAT, APX and GPX activities showed that these

antioxidants were essential to detoxify reactive oxygen species (ROS) at 14 dpi (flowering stage) while AsA and α-tocopherol was suggested to become the selective protection against CMV infection at 30 dpi (fruiting stage). Among the enzymatic antioxidants, GPX had the highest activity in chili fruit of JA-treated plants followed by CAT and APX. These results suggested that exogenous application of JA could effectively reduce CMV infection by enhancing activities of enzymatic antioxidants and concentration of non-antioxidant to quench excessive ROS. So, it can be concluded that the optimum concentration and spray frequencies of JA can become a promising tool for reducing CMV disease thus improving the growth performances in chili plants as an alternative method to replace or reduce the pesticide use.

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

KESAN ASID JASMONIK DALAM MENGURANGKAN JANGKITAN VIRUS TIMUN MOZEK DAN MENINGKATKAN PRESTASI PERTUMBUHAN CILI (*Capsicum annuum L.*)

Oleh

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Permintaan yang tinggi untuk cili menyebabkan sektor pertanian bergantung sepenuhnya pada penggunaan racun perosak untuk mengawal serangga dan penyakit. Hasil kajian ini mendapati penggunaan racun perosak pada pokok telah memberi kesan yang signifikan terhadap bilangan serangga perosak seperti afid, kutu trip dan hamama yang telah mengurangkan sehingga 80% peratusan kadar penyakit. Walau bagaimanapun, disebabkan kesan jangka masa yang panjang penggunaan racun perosak, ramai pengkaji telah menghasilkan pendekatan alternatif untuk memperbanyakkan produktiviti dan memberi kesan yang baik kepada kesihatan manusia dan alam sekitar. Dalam kajian ini, asid jasmonik (JA) yang merupakan elisitor telah diujikaji dengan penggunaan kepekatan (0.6 mM dan 1.0 mM) dan kekerapan semburan (sekali, dua kali dan tiga kali) yang berbeza untuk mengkaji kesan dalam menggalakkan tumbesaran pokok, tindakbalas fisiologi, meningkatkan hasil dan mengurangkan peratusan penyakit. Hasil kajian menunjukkan bahawa rawatan menggunakan kepekatan 0.6 mM sebanyak dua kali semburan memberi kesan yang sangat positif berbanding rawatan yang lain dengan mengurangkan kejadian penyakit pada 50%. Walaupun faktor kepekatan dan kekerapan semburan tidak signifikan, namun kepekatan JA pada 0.6 mM sebanyak dua kali semburan berjaya meningkatkan hasil yang hampir sama seperti kepekatan JA pada 1.0 mM sebanyak dua dan tiga kali semburan. Oleh itu, kajian seterusnya untuk mengkaji kesan JA pada pokok yang dijangkiti dengan Virus Timun Mozek (CMV) telah dijalankan dengan menggunakan kepekatan 0.6 mM sebanyak dua kali semburan. CMV telah dilaporkan sebagai salah satu daripada cucumovirus yang sangat lazim di Malaysia berikutan kepelbagaian sel perumah dan vektor serangga. Hasil kajian mendapati bahawa pokok yang telah dijangkiti dengan CMV yang dirawat menggunakan JA menunjukkan kesan perencatan terhadap jangkitan CMV pada hari ke-30 inokulasi berbanding dengan kerosakan yang teruk pada pokok yang tidak dirawat dan dirawat dengan racun perosak. Disebabkan peratusan keterukan penyakit yang rendah dalam penggunaan JA, hasil cili telah meningkat sebanyak 95% dan menambahkan pengeluaran bahan kering dalam daun, dahan dan akar. Aplikasi JA juga memberi kesan yang ketara terhadap aktiviti katalase (CAT), askorbat peroksida (APX), guaiacol peroksida (GPX), asid askorbik

(AsA), α-tokoferol dan karotenoid (CAR) di dalam daun dan buah. Peningkatan yang signifikan dalam AsA dan aktivitit-aktiviti CAT, APX dan GPX pada pokok yang dirawat dengan JA menunjukkan antioksida ini sangat penting untuk menyahtoksidkan spesies oksigen reaktif (ROS) pada 14 hari selepas inokulasi (dpi) (peringkat berbunga) manakala AsA dan α-tokoferol terpilih untuk melindungi daripada jangkitan CMV pada 30 dpi (peringkat berbuah). Antara antioksida enzimatik, GPX mempunyai aktiviti yang tertinggi di dalam buah cili yang dirawat dengan JA, diikuti CAT and APX. Penggunaan JA juga telah meningkatkan jumlah AsA di dalam buah cili berbanding rawatan yang lain. Hasil kajian ini mencadangkan bahawa penggunaan JA secara luaran telah mengurangkan jangkitan CMV secara efektif dengan meningkatkan aktiviti antioksida enzimatik dan kandungan antioksida bukan enzimatik unntuk menghapuskan ROS. Oleh itu, secara kesimpulannya, kepekatan dan kekerapan semburan yang optima boleh menjadikannya sebagai satu cara untuk mengurangkan CMV sekaligus meningkatkan kadar tumbesaran pokok cili serta sebagai salah satu langkah alternatif untuk menggantikan atau mengurangkan penggunaan racun perosak.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

3T3	Mouse Embryonic Fibroblast Cell line
ABA	Abscisic Acid
ANOVA	Analysis of Variance
AP	Antibody-alkaline Phosphate
AOS	Allene Oxide Synthase
APX	Ascorbate Peroxidase
ArOU	Aryloxy radical
AsA	Ascorbic Acid
BSA	Bovine Serum Albumin
CAR	Carotenoid
CAT	Catalase
CCD	Coconut Coir Dust
CD	Cluster Designation
CEC	Cation Exchange Capacity
CLCV	Chili Leaf Curl Virus
CMV	Cucumber Mosaic Virus
CVMV	Chili Veinal Mottle Virus
DAS-ELISA	Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay
DAT	Day After Transplanting
DHA	Dehydroascorbate
DI	Disease Incidence
dpi	Days Post Inoculation
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
EC	Electrical Conductivity
ECE	Ethanol Crude-dried Extract
EDTA	Ethylenediaminetetraacetic Acid
EFB	Empty Fruit Bunch
F1	First Generation
GA	Gibberellic Acid
GGPP	Geranylgeranyl Diphosphate
GPX	Guaiacol Peroxidase
GR	Glutathione Reductase
GSH	Glutathione
H_2O_2	Hydrogen Peroxide
Ha	Hectare
HepG2	Hepatocellular Carcinoma Cell Line
hpi	Hours Post Inoculation
$HO^·$	Hydroxyl Radical
HOCl	Hypochlorous Acid
IAA	Indole Acetic Acid
IUE	Irrigation Use Deficiency
JA	Jasmonic Acid
LA	Linolenic Acid
LOX	Lipoxygenase
LSD	Least Significance Difference
MARDI	Malaysian Agricultural Research and Development Institute

MD	Monodehydroascorbate Radical
MDAR	Monodehydroascorbate Reductase
MEP	Methyerythritol 4-phosphate
MeJA	Methyl Jasmonate
MRL	Maximum Residue Level
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
n	Number of Plants
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
$NO^{\cdot}$	Nitric Oxide
NO_2	Nitrogen Dioxide
$^1O_2$	Singlet Oxygen
O_3	Ozone
O^{2-}	Superoxide Anion
OD	Optical Density
$ONOO^{\cdot}$	Peroxynitrite
OPDA	Cyclopentanone 12-oxophytodienoic Acid
PHI	Pre-harvested Interval
ppm	Parts Per Million
PPO	Polyphenol Oxidase
PPR	Portable Photosynthesis Rate
PS	Photosensitizer
PS II	Photosystem II
RCBD	Randomized Complete Block Design
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
RO_2.	Peroxyl
RO^-	Alkoxyl
ROS	Reactive Oxygen Species
RONS	Reactive Oxygen Nitrogen Species
Rpm	Rotation Per Minute
S.E	Standard Error
SA	Salicyclic Acid
SAS	Statistical Analysis System
SChE	Serum-cholinesterase
SOD	Superoxide Dismutase
TCA	Trichloroacetic Acid
TCV	Turnip Crinkle Virus
TMV	Tobacco Mosaic Virus
ULVC	Urdbean Leaf Crinkle Virus

CHAPTER 1

INTRODUCTION

Chili (*Capsicum annuum L.*) is one of the most valuable spice crops in Malaysia that occupied about 3523 ha area in major producing states such as Kelantan, Pahang, Johor and Perak in 2017 (DOA, 2017). Although local chili production reaches approximately 50,299 metric tons annually, the yield produced is deficient to meet domestic demands (2.0 kg/year per capita consumption) and therefore additional import dependency ratio (IDR) had raised from 38.7% to 52.9% in 2013 to 2014 (DOSM, 2017). Thailand becomes the leading exporter which represent 89% in fresh chili supply followed by China, India, Vietnam and Indonesia (APEDA, 2007). Besides limited cultivation areas, other predominant factor contributing to low chili production are the diseases infected by fungus, bacteria and virus. Among these, viral diseases are considered to be the major limiting factor causing yield losses based on surveys conducted by (Schreinemachers *et al.,* 2015) tropical and subtropical Asia.

Cucumber Mosaic Virus (CMV) is one of the important viral pathogens commonly found in chili plants in Malaysia which cause the severest damage and contribute to yield loss (Roff, 1992). According to field crop surveys, CMV was discovered to be the most prevalent on chili with higher disease incidence that can reach up to 80% (Iqbal *et al.*, 2012; Simón *et al.,* 2016). In addition, CMV damage on chili are influenced differently by time of infection as early and late infection cause yield losses at 10-15% and exceed 60% respectively (Rafidah *et al.*, 2016). The severely infected plants with mosaic disease produce less flowers and deformed fruits (Khan *et al.,* 2006) which result in drastic reduction in marketable yield and fruit quality. Consequently, chili being sold at higher prices due to low production and higher demands.

Regarding these problems, farmers are entirely relying on chemical pesticides application including insecticides, fungicides, molluscicide and weedicide to control the pest and diseases. About 78.4% of chili growers mentioned the input cost including pesticides and other chemicals greatly increase in order to reduce numbers of pest (Arumugam *et al.*, 2012). Besides the rising disease management cost, the inaccurate in handling the register pesticide and illegally use of unregistered pesticide can cause severe environmental consequences and produce carcinogenic chili (Mesnage *et al.,* 2014). In Malaysia, based on Food Safety Act 1983, pesticide can be applied by farmers without reaching the Maximum Residue Level (MRL), as overuse will cause harm to human health. Despite that, most of the farmers disobey the rule without considering the consequences of active ingredients that will remain in the fruits.

In order to overcome the disease, many researchers have developed eco-friendly approaches like cultural methods, use of chemical and biological fungicide and resistant cultivars (Than *et al.,* 2008). However, most of these options are still unable to control the diseases. Jasmonic acid (JA) was first reported by Farmer and Ryan (1992) to be a potential elicitor to induce disease resistance against insect and pathogen attack. The effectiveness of JA in defence is also proved in many crops such as alfalfa and tobacco

(Farmer and Ryan, 1992), arabidopsis (Turner *et al.*, 2002), grapes (Omer *et al.*, 2000), tomato (Cooper and Rieske, 2008) and chili (Awang *et al.*, 2013). The positive effects of JA on antioxidant capacity in many studies demonstrated that JA application are able to increase antioxidants thereby causing alleviation of oxidative stress in plants (Qiu *et al.*, 2014; Asghari and Hasanlooe, 2015). The reports regarding JA treatment succeeded in inhibiting CMV (Luo *et al.*, 2011) suggested JA can improve plant resistance and become capable applicator to give plant protection in fields (Shang *et al.*, 2011).

Although the use of pesticides facilitate the disease management strategy, it leads to the accumulation of toxic residues in crop which affect human health and the ecosystems (Williamson *et al.*, 2008; Zhou and Jin, 2009). So, in this study, we would like to develop an eco-friendly control strategy to manage virus diseases and improve yields in chili plants. Besides, this study can help the farmers to reduce the maintenance cost and make pesticide-free chili production. The main objective of this study is to evaluate the effect of jasmonic acid as elicitor to reduce the disease severity in CMV infection, increase antioxidants activities for plant defence which in turn improve the yield production of chili.

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

1) Cultivation of chili without pesticides application increase number of pest and diseases severely affect plant growth and cause greater yield loss.
2) Application of jasmonic acid with optimum concentration and spray frequencies enhance growth performance, chili yields, physiological responses and reduce disease incidence.
3) Selected concentration and spray frequencies of jasmonic acid reduce Cucumber Mosaic Virus (CMV) infection and improving growth performance by increasing biochemical responses.

The objectives of the present study were:

1) To evaluate the growth performances of chili plants with and without pesticide application.
2) To determine the concentrations and spray frequencies of jasmonic acid application in chili plants.
3) To study the effect of jasmonic acid on chili plant infected with Cucumber Mosaic Virus (CMV).

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