CHARACTERIZATION OF *Stagonosporopsis cucurbitacearum* AND FIELD EVALUATION OF A NEW CODED FUNGICIDE (XDE-659) EFFICACY AGAINST GUMMY STEM BLIGHT ON WATERMELON IN MALAYSIA

By

WAZIRI ABDUL AZIZ

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

June 2021

FP 2021 68

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DEDICATION

I dedicate this thesis to my beloved mother and wife, cute daughter, and loving brothers whom always wishful of my success. To the pillar of my strength, my wife, I am truly indebted for her endless attention, love and devotion in my life. I am really astonished by her patience in bearing my absence for more than two years. My heartfelt thoughts also go to my siblings, who are always honestly happy with my progress.

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

CHARACTERIZATION OF *Stagonosporopsis cucurbitacearum* AND FIELD EVALUATION OF A NEW CODED FUNGICIDE (XDE-659) EFFICACY AGAINST GUMMY STEM BLIGHT ON WATERMELON IN MALAYSIA

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Gummy stem blight (GSB) disease is caused by *Stagonosporopsis cucurbitacearum* fungus. It is the most destructive disease in most of the watermelon-producing areas of Malaysia. This study was aimed to characterize the *S. cucurbitacearum* fungus based on morphological and molecular characteristics, to assess the pathogenicity of *S. cucurbitacearum* isolates on watermelon *in vitro* and in planta assays as well as to assess the control efficacy of the new coded fungicides. Ten isolates of *S. cucurbitacearum* were obtained from infected watermelon leaves and stems from two different locations; Ladang 15, UPM and MARDI, Selangor, Malaysia. The isolates were identified based on morphological and molecular identification. The pathogenicity test (in vitro and in vivo) was carried out on Red Rocky watermelon seedlings. The efficacy of the new coded fungicides that developed by Dow AgroSciencesTM was evaluated in the field. For morphological characteristics, potato dextrose agar (PDA) and chickpea seed agar (CSA) media were used to assess these isolates. From the top view of petri dish, fungal colony appeared as white, gray and olivaceous mycelia while from bottom view of petri dish, the colony was initially white and later turned to olivaceous green or black with concentric circles. The conidial shapes of these isolates were mainly oblong round with hyaline and smooth growth. These spores were having average size of 5-14 µm (length) and 3-5 µm (width), therefore, the isolates were identified as *S. cucurbitacearum*. The molecular identification using internal transcribed spacer region (ITS1-5.8S-ITS2), and a partial sequence of the β-tubulin (TUB) gene have confirmed the ten isolates as *S. cucurbitacearum*. The ten isolates were selected for pathogenicity tests based on *in vitro* and *in planta* inoculation assays on healthy watermelon plants. The isolates M1, M2, M3, M4, M5, M6 and M7 that obtained from MARDI were significantly more virulent based on the size of the lesions formed after inoculation. While the isolates M8, F1 and L were found to be the least aggressive among the isolates tested by having small lesions on leaves and stems. For efficacy evaluation of the new coded fungicides, GF 3716, GF 3540, GF 3542 and Amistartop® (standard fungicide) were used to manage GSB. The results show that all the coded fungicides were found to be effective and have reduced

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the disease severity in watermelon plants as compared to the untreated control. Trt 1 (GF 3716/1500 mL/ha), Trt 5 (tank mix of GF3540/1500 mL/ha + GF3542/600 mL/ha) and Trt 6 (tank mix of GF3540/1000 mL/ha + GF3542/400 mL/ha) and Trt 10 (tank mix of GF3540/1000 mL/ha + Adsee C80W/ 250 mL/ha) were significantly ($P < 0.05$) effective in reducing the GSB disease severity where these fungicides provided higher control efficacy with 77.45, 79.41, 74.5 and 76.47%, respectively, as compared to the standard and untreated control. Moreover, Trt 2 (GF3716/1000 mL/ha), Trt 3 (GF3716/750 mL/ha) and Trt 7 (tank mix of GF3540/750 mL/ha + GF3542/300 mL/ha) showed a high control efficacy with 70.58, 68.62 and 66.66%, respectively, as compared to the rest of the treatments. Trt 4 (GF3716/500 mL/ha), Trt 8 (GF3540/500 mL/ha + GF3542/200 mL/ha) were not significantly different from standard fungicide. However, Trt 9 (GF3540/1000 mL/ha + GF3542/0.3 mL/ha) has the least control efficacy as compared to all coded treatments and standard except the untreated control treatment. In conclusion, the findings of morphology and molecular characterization have identified the pathogen as *S. cucurbitacearum*, more so, the products with XDE-659 as an active ingredient and a tank-mix of adjuvant with the highest application rate had a higher potential to control GSB disease caused by *S. cucurbitacearum* in watermelon farm.

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

PENCIRIAN *Stagonosporopsis cucurbitacearum* DAN PENILAIAN LAPANGAN KEBERKESANAN KOD RACUN KULAT BAHARU (XDE-659) TERHADAP PENYAKIT HAWAR BATANG BERGAM TEMBIKAI DI MALAYSIA

Oleh

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Penyakit hawar batang bergam (GSB) disebabkan oleh kulat *Stagonosporopsis cucurbitacearum,* merupakan penyakit yang paling utama di kawasan pengeluaran tembikai di Malaysia. Kajian ini bertujuan untuk mencirikan *S. cucurbitacearum* berdasarkan ciri morfologi dan molekul, menilai patogenik isolat *S. cucurbitacearum* pada tembikai secara in vitro dan in planta, serta menilai keberkesanan racun kulat baharu. Kajian ini meneliti sepuluh isolat *S. cucurbitacearum* yang diperolehi dari daun tembikai yang dijangkiti pada dua lokasi yang berbeza; Ladang 15, UPM dan MARDI, Selangor, Malaysia. Isolat dikenalpasti dengan menggunakan kaedah morfologi dan molekul. Ujian patogenisiti (in vitro dan in vivo) dilakukan pada anak benih tembikai Red Rocky. Keberkesanan racun kulat berkod baharu yang dibangunkan oleh Dow AgroSciencesTM dinilai di lapangan. Berdasarkan ciri morfologi yang dikulturkan pada media PDA dan CSA, pandangan dari atas piring petri mempamerkan miselium berwarna putih, kelabu dan zaitun, sementara pandangan dari bawah piring petri menunjukkan keputihan pada mulanya dan kemudian bertukar menjadi hijau atau hitam zaitun pada pusat bulatan. Bentuk isolat ini adalah bulat panjang dan halus. Spora berukuran di antara 5-14 µm (panjang) dan 3-5 µm (lebar). Oleh itu, isolate dikenalpasti sebagai *S. cucurbitacearum*. Pengenalpastian molekul menggunakan spacer transkripsi dalaman (ITS1-5.8S-ITS2) dan urutan separa gen β-tubulin (TUB) telah mengesahkan bahawa kesemua sepuluh isolat tersebut adalah *S. cucurbitacearum*. Kesemua sepuluh isolat tersebut dipilih untuk ujian patogenik berdasarkan pengujian inokulasi in planta pada tanaman tembikai yang sihat. Isolat M1, M2, M3, M4, M5, M6 dan M7 yang diperolehi dari MARDI adalah lebih virulen daripada isolat lain berdasarkan saiz luka yang terbentuk selepas inokulasi. Manakala isolat M8, F1 dan L didapati adalah paling kurang agresif di antara kesemua isolat yang diuji di mana luka pada daun dan batang adalah paling kecil. Hasil kajian penilaian keberkesanan racun kulat berkod baharu GF3716, GF3540, GF3542 dan Amistartop® sebagai racun kulat standard dalam pengurusan GSB telah menunjukkan bahawa kesemua racun kulat berkod baharu didapati berkesan dan telah mengurangkan keparahan penyakit pada tanaman tembikai

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berbanding dengan kawalan yang tidak dirawat pada pelbagai tahap peratusan. Trt 1 (GF 3716/1500 mL/ha), Trt 5 (campuran tangki GF3540/1500 mL/ha + GF3542/600 mL/ha) and Trt 6 (campuran tangki GF3540/1000 mL/ha + GF3542/400 mL/ha) and Trt 10 (campuran tangki GF3540/1000 mL/ha + Adsee C80W/ 250 mL/ha) telah mengurangkan keparahan penyakit GSB dengan berkesan ($P<0.05$) di mana keberkesanan kawalan masing-masing adalah setinggi 77.45, 79.41, 74.5 dan 76,47% berbanding dengan kawalan racun kulat standard dan yang tidak dirawat. Selain itu, Trt 2 (GF3716/1000 mL/ha), Trt 3 (GF3716/750 mL/ha) dan Trt 7 (campuran tangki GF3540/750 mL/ha + GF3542/300 mL/ha) menunjukkan keberkesanan kawalan yang tinggi masing-masing dengan 70.58, 68.62 dan 66.66% berbanding dengan rawatan yang lain. Manakala, keputusan kajian Trt 4 (GF3716/500 mL/ha), Trt 8 (GF3540/500 mL/ha + GF3542/200 mL/ha) tidak jauh berbeza dengan racun kulat standard. Akan tetapi, Trt 9 (GF3540/1000 mL/ha + GF3542/0.3 mL/ha) memberikan keberkesanan kawalan penyakit yang paling rendah di antara kesemua rawatan kecuali rawatan kawalan yang tidak dirawat. Sebagai kesimpulan, penemuan morfologi dan pencirian molekul telah mengenalpasti S. cucurbitacearum sebagai patogen, lebih-lebih lagi, produk yang mengandungi XDE-659 sebagai bahan aktif dan campuran tangki dengan adjuvan pada kadar aplikasi tertinggi mempunyai potensi yang lebih tinggi untuk mengawal penyakit GSB yang disebabkan oleh *Stagonosporopsis cucurbitacearum* di ladang tembikai.

ACKNOWLEDGEMENTS

First of all, I would like to thank and praise to almighty Allah, my parent and family for their support and encouragement during of my studies. I wish to express my sincerest gratitude to Dr. Siti Izera Ismail, my main supervisor for giving me opportunity to pursue my graduate studies in the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. Dr. Siti Izera Ismail taught me the skills of designing and performing scientific research work, conducting research and thesis writing. I appreciate her advice and guidance throughout my study. I also greatly appreciate my co-supervisor Dr. Dzarifah Mohamed Zulperi for her support in my master journey. From their invaluable guidance, I was able to obtain knowledge in plant pathology. I would also like to express my gratitude to the Higher Education Development Program (HEDP) and the Ministry of Higher Education of Afghanistan for providing me with the scholarship during my Master degree study. I also want to express my special thanks to staff of the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, especially Mr. Johari Mohd Sarikat and Mrs. Asmalina Abu Bakar for their excellent support. I am greatly indebted to all my laboratory mates for their kindness. I also would like to express my sincere thanks to Siti Fairuz Yusoff for the help.Last but not least, I am always grateful to my friends for their continuous support. Thank you to everyone who had contributed direct or indirectly in making this study successful.

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This thesis was submitted to the Senate of the 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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TABLE OF CONTENTS

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LIST OF TABLES

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LIST OF FIGURES

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LIST OF ABBREVIATIONS

AUDPC	Area under disease progress curve
AD	Anno Domini
CSA	Chickpea seed agar
DMI	Demethylation inhabitation
DNA	Deoxyribonucleic acid
DAI	Days after inoculation
DAA	Days after application
DSI	Disease severity index
DAT	Days after treatment
FRAC	Fungicide Resistance Action Committee
FAO	Food and Agriculture Organization
GSB	Gummy stem blight
GPS	Global positioning system
ITS	Internal transcribed spacer
LSU	Large sub unit
LSD	Least significant difference
MOA	Ministry of Agriculture and Food Industry
MEA	Malt extract agar
MEYEA	Malt extract yeast extract agar
NADH	Nicotinamide adenine dinucleotide
NCBI	National Centre for Biotechnology Information
PDA	Potato dextrose agar
PCR	Polymerase chain reaction
QoI	Quinone outside inhibiter
QPDA	Quarter strength potato dextrose agar

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RCBD	Randomize complete block design
SDHI	Succinate dehydrogenase inhibiter
TM	Methyl thiophanate
UV	Ultra violet

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CHAPTER 1

INTRODUCTION

Watermelon (*Citrullus lanatus*) that belong to the family of Cucurbitaceae is a non-seasonal crop and commonly grown in open-field system (Thomas, 2011; Popescu, 2012). In Malaysia, watermelon is among the top five tropical fruit crops with planting acreage of about 10,4303.59 ha which yield around 150,006.89 metric ton of fruit (DOA, 2018). In Malaysia, the state of Kelantan is the largest production region for watermelon. Gummy stem blight (GSB) that caused by *S. cucurbitacearum* is the most serious foliar disease affecting cucurbits such as watermelon, cantaloupe, cucumber and muskmelon (Thomas et al., 2014; Babu et al., 2015). GSB spread rapidly in warm and humid seasons and could cause significant loss in watermelon yield (Zhao et al., 2019; Newark et al., 2020).

GSB is a common disease of cucurbits in the United States and China (Babu et al., 2015; Newark et al., 2020). Meanwhile, GSB has been reported on watermelon in Turkey (Basim et al., 2016), cantaloupe in Thailand (Nuangmek et al., 2018), Taiwan (Huang and Lai, 2019) and India (Mahapatra et al., 2020). This disease could cause more than 60% leaves defoliation and fruit rot (Newark et al., 2020). Leaf spots on the plant are usually the first symptoms used to diagnose GSB disease. The spots on the host leaves start at the margins of the leaves or extend to the margins. When the lesion expand, the leaves become coalesce and the leaves became blighted (Keinath and Hansen, 2013). The foliar symptom of GSB started as necrotic and brownish lesions, followed by a yellow halo on the leaves. Meanwhile, the GSB symptoms on the stems appeared as water-soaked lesions, dried up, and cracked as the disease progressed (Kurowski et al., 2015; Basım et al., 2016).

Cultural, and chemical control are commonly recommended for controlling GSB in watermelon (Santos et al., 2016). Recently, genetic resistant varieties have been used to against this disease (Hassan et al., 2018), but cultivars that resistant to *S. cucurbitacearum* is not available in the market yet (Santos et al., 2019; Gimode et al., 2021). The most successful way of controlling GSB is through repeating spray of fungicides on the crops (Thomas et al., 2012). The chlorothalonil protective fungicide is effective in preventing GSB infection. Nevertheless, it should be used only in the early growing stage due to its phytotoxicity on mature watermelon fruit (Avenot et al., 2012).

There are many commercial systemic and contact fungicides have been developed to control GSB in cucurbits, for example, Quinone Outside Inhibitor (QoI), Succinate Dehydrogenase Inhibitor (SDHI), and Demethylation Inhibitor (DMI) (Thomas et al., 2014; Li et al., 2019). Unfortunately, the fungal pathogen of *S. cucurbitacearum* may develop resistance against these fungicides (Thomas et al., 2012; Keinath, 2015; Li et al., 2019). DMI fungicides such as difenoconazole, cyprodinil, and a mixture of cyprodinil and fludioxanil (phenylpyrrole) have been used by growers. However, repeated application of similar fungicides to control GSB may develop fungal resistance towards GSB populations in the field.

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In Malaysia, foliar and stem lesions were observed on watermelon plants in commercial planting fields, especially during the warm and rainy season in 2018-2019. However, there was no report on identification and characterization of the causal agent of GSB disease on watermelon plants in Malaysia. Many fungicides have been labeled for use in cucurbits against GSB, but it has a remarkable ability to adapt and become resistant to most fungicides developed to control it (Keinath and Hansen, 2013; Li et al., 2019). For examples, QoI (Azoxystrobin), SDHI (Pristine) and DMI (Tebuconazole) fungicides caused development of unpredictable fungicides resistance in crops. Therefore, a new coded fungicide containing new active ingredient (XDE-659) were tested on watermelon plants in field.

The objectives of this study were:

- To identify and characterize *S. cucurbitacearum* causing gummy stem blight disease on watermelon plant using morphological and molecular techniques.
- To assess the pathogenicity of *S. cucurbitacearum* isolates on watermelon using *in vitro* and *in planta* inoculation assay for watermelon plant.
- To evaluate the efficacy of a new coded fungicide, XDE-659 and tank-mix of XDE-659 and adjuvant, in controlling gummy stem blight disease on watermelon plant in the field.

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