ENRICHED SILICON AND *Bacillus subtilis* ON *FUSARIUM* WILT DISEASE CONTROL AND THEIR EFFECTS ON GROWTH AND PHYSIO-BIOCHEMICAL CHANGES OF BANANA

By

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Musa acuminata (cv. Berangan) belonging to the Musaceae family are sensitive to the biotic stress condition affecting growth performance and physio-biochemical changes. *Fusarium* wilt disease caused by *Fusarium oxysporum* f.sp. cubense (FOC) is considered the most limiting factor in the vegetative and reproductive growth of the banana. The study's general objective was to investigate the potential effect of enriched silicon (Si) compounds, antagonistic bacteria and systemic fungicide on plant growth and physio-biochemical changes in controlling FOC. The treatments in the main plot for each greenhouse experiment were divided into healthy plants without FOC inoculation and diseased plants. For the treatments in the sub-plot, the soil planting media in Experiment 1A were drenched with a different nutrient formulation of enriched Si compound [T0 served as control, T1: 13% SiO_2 and 20% K_2O, T2: 26.6% SiO_2 and 13.4% K_2O and T3: 36.2% SiO_2 and 17% Na_2O]. Experiment 1B, only the best selected of enriched Si compounds applied at different application frequency [0, 7, 15, 30 days of interval (DI)]. Experiment 2A, the sub-plot treatments consisted of different species of antagonistic bacteria: 0B (without *Bacillus* application served as control), BS (*Bacillus subtilis*) and BT (*Bacillus thuringiensis*). For Experiment 2B, banana plants were treated with different application volume of selected enriched Si compound [0, 20, 40, 60 mL] before integrating with the best selected antagonistic bacteria Field assessment in the Experiment 3 was conducted in a *Fusarium* infected area and banana plants were subjected to treatment: Control (without any treatments), CBZ (Carbendazim), T3+BS (36.2% SiO_2 + 17% Na_2O + *Bacillus subtilis*) and CBZ+T3+BS (CBZ + 36.2% SiO_2 + 17% Na_2O + *Bacillus subtilis*). Results in the Experiment 1A showed that diseased banana plant significantly reduced plant height, pseudo-stem diameter, total leaf area (TLA) and total chlorophyll content (Chl_{a+b}) by 38.39%, 88.23%, 19.74% and 30.43%, respectively as compared to healthy plants. In the Experiment 1B, the best growth performance was observed

when infected banana clones applied with T3 at 15DI, significantly reduced disease incidence by 58.2% and proline content by 44.28%. Results in Experiment 2A showed the amount of TPC started to increase from 81.52 to 175.15 µg/g FW when the proline content of the banana clones started to increase from 23.79 to 51.34 µmol/g FW under different soil and plant health conditions. In Experiment 2B, the results showed exogenous application of enriched Si compounds with BS at 15DI on the soil and roots of banana clones significantly increased the electrolyte leakage (EL) by 2.94% (40 mL) and 11.27% (60 mL), but application 20 mL significantly reduced EL by 8.75% in relative to the control (0mL) by 53.67%. Based on field assessment in the Experiment 3, banana clones treated with CBZ and T3+BS significantly reduced the percent of dead plant by 46.43%, 17.86% and 60.71%, respectively as compared to control (69.64%). At harvest stage, banana plant treated with CBZ+T3+BS gained the highest harvested yield in term weight of fruit bunch by 24.72 kg, followed by CBZ (18.3 kg) and T3+BS (17.87 kg), while the control plant without treated by any treatments gained the lowest weight by 9.45 kg. In conclusion, integration treatments of CBZ+T3+BS at 15DI is recommended to enhance growth and physio-biochemical responses in controlling *Fusarium* wilt disease, thus produce better yield of banana.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SILIKON DIPERKAYA DAN *Bacillus subtilis* KEATAS KAWALAN PENYAKIT LAYU *FUSARIUM* DAN KESANNYA TERHADAP PERTUMBUHAN DAN PERUBAHAN FISIO-BIOKIMIA PISANG

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Musa acuminata (cv. Berangan) tergolong dalam keluarga Musaceae yang agak sensitif dengan keadaan ketegasan biotik yang mampu mempengaruhi prestasi pertumbuhan dan perubahan fisio-biokimia pokok pisang. Penyakit layu *Fusarium* telah dianggap sebagai faktor yang paling utama menghadkan pertumbuhan vegetatif yang disebabkan oleh *Fusarium oxysporum* f.sp. cubense (FOC). Objektif utama kajian adalah untuk mengkaji potensi kesan sebatian silicon (Si) yang diperkaya, bakteria antagonis dan racun kulat sistemik terhadap pertumbuhan tumbuhan dan perubahan fisio-biokimia dalam mengawal FOC. Rawatan dalam plot utama bagi setiap eksperimen rumah hijau dibahagikan kepada tanaman sihat tanpa inokulasi FOC dan tanaman berpenyakit. Untuk rawatan dalam sub-plot, media penanaman tanah dalam Eksperimen 1A dibasahi dengan formulasi nutrien berbeza bagi sebatian Si diperkaya [T0 berfungsi sebagai kawalan, T1: 13% SiO_2 dan 20% K_2O, T2: 26.6% SiO_2 dan 13.4% K_2O dan T3: 36.2% SiO2 dan 17% Na_2O]. Eksperimen 1B, hanya sebatian Si diperkaya terpilih dan terbaik digunakan pada kekerapan penggunaan yang berbeza [0, 7, 15, 30 hari selang (DI)]. Eksperimen 2A, rawatan sub-plot terdiri daripada spesies bakteria antagonis yang berbeza: 0B (tanpa aplikasi *Bacillus* berfungsi sebagai kawalan), BS (*Bacillus subtilis*) dan BT (*Bacillus thuringiensis*). Bagi eksperimen 2B, pokok pisang telah dirawat dengan isipadu penggunaan berbeza Si [0, 20, 40, 60 mL] sebelum disepadukan dengan bakteria antagonis terpilih terbaik. Penilaian lapangan dalam Eksperimen 3 telah dijalankan di kawasan yang dijangkiti *Fusarium* dan anak pokok pisang diberi rawatan: Kawalan (tanpa sebarang rawatan), CBZ (Carbendazim), T3+BS (36.2% SiO_2 + 17 % Na_2O + *Bacillus subtilis*) dan CBZ+T3+BS (CBZ + 36.2% SiO_2 + 17% Na_2O + *Bacillus subtilis*). Keputusan dalam Eksperimen 1A

menunjukkan bahawa pokok pisang berpenyakit mengalami pengurangan ketinggian, diameter pseudo-batang, jumlah luas daun (TLA) dan jumlah kandungan klorofil (Chl_{a+b}) dengan ketara sebanyak 38.39%, 88.23%, 19.74% dan 30.43% dibandingkan dengan anak pokok yang sihat. Dalam Eksperimen 1B, prestasi pertumbuhan terbaik yang diperhatikan apabila klon pisang yang dijangkiti dirawat dengan T3 pada 15DI, secara signifikan dapat mengurangkan kejadian penyakit sebanyak 58.2% dan kandungan prolin sebanyak 44.28%. Keputusan dalam Eksperimen 2A menunjukkan jumlah TPC mula meningkat daripada nilai 81.52 hingga 175.15 µg/g FW apabila kandungan prolin dalam klon pisang mula meningkat daripada 23.79 hingga 51.34 µmol/g FW bergantung kepada keadaan kesihatan tanah dan anak pokok yang berbeza. Dalam Eksperimen 2B, keputusan menunjukkan aplikasi sebatian Si diperkaya dengan BS pada 15DI ke atas tanah dan akar klon pisang telah meningkatkan kebocoran elektrolit (EL) dengan ketara sebanyak 2.94% (40 mL) dan 11.27% (60 mL), tetapi penggunaan 20 mL mengurangkan EL dengan ketara sebanyak 8.75. % berbanding kawalan (0mL) sebanyak 53.67%. Berdasarkan penilaian lapangan dalam Eksperimen 3, klon pisang yang dirawat dengan CBZ+T3+BS dapat mengurangkan peratus kematian pokok secara signifikan sebanyak 46.43%, 17.86% dan 60.71%, masing-masing berbanding kawalan (69.64%). Pada peringkat penuaian, pokok pisang yang dirawat dengan CBZ+T3+BS memperoleh hasil tuaian buah tertinggi sebanyak 24.72 kg, diikuti oleh CBZ (18.3 kg) dan T3+BS (17.87 kg), manakala pokok kawalan tanpa dirawat dengan mana-mana rawatan mendapat hasil tuaian buah berat terendah sebanyak 9.45 kg. Kesimpulannya, rawatan integrasi CBZ+T3+BS selang 15 hari (15DI) disyorkan untuk menggalakkan pertumbuhan dan tindak balas fisio-biokimia dalam mengawal penyakit layu *Fusarium*, sekaligus menghasilkan tuaian buah pisang yang lebih baik.

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%	Percentage
<	Less than
>	Greater than
*	Significantly difference at $p<0.05$
**	Significantly difference at $p<0.01$
***	Significantly difference at $p<0.001$
AA	Auto analyzer
AAS	Atomic absorption spectrometer
ANOVA	Analysis of variance
C	Carbon
Ca	Calcium
CO_2	Carbon dioxide
CFU	Colony forming unit
cm	Centimetre
cv.	Cultivar
℃	Degree celcius
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl_{a+b}	Total chlorophyll
DF	Degree of freedom
DW	Dry weight
et al.	And friends
EL	Electrolyte leakage
FW	Fresh weight

g	Gram
Ha	Hectare
H_2O	Water
IAA	Indole-3-acetic acid
Kg	Kilogram
L	Litre
LA	Leaf area
LSD	Least significant difference
m	Metre
MDA	Malonyldialdehyde
µg	Microgram
µmol	Micromole
mL	Millitre
mm	Millimetre
mmol	Millimole
NPK	Nitrogen phosphorus potassium
N	Number of respondence
NS	Not significant
pH	Measurement of acidity / alkalinity
R:S	Root to shoot ratio
RWC	Relative water content
RCBD	Randomized Complete Block Design
s	Second
Si	Silicon
SAS	Statistical Analysis System

CHAPTER 1

INTRODUCTION

Banana (*Musa spp*.) also known as Pisang in Malaysia, belonging to the family Musaceae is classified as non-seasonal fruit crop. However, it has a lot of potential with multi-uses products and plays an important role as a sustainable crop for household food security, income generation, and as an export revenue source worldwide. Banana is commercially cultivated in over 135 countries across the tropics and subtropics, serving as a staple food for more than 400 million peoples (Thangavelu et al., 2021). Meanwhile, the niche market demand for fresh bananas and increasing banana-based food production industry will be higher than present due to the increase in human population which is expected to reach 9 billion people by the year 2050 (Bahar et al., 2020). According to Godoy (2020), banana is one of the world's favourite fruit in terms of consumption quantity and among the largest global gross value in production after rice, wheat and maize. The largest banana exporter in the world is Ecuador, which accounts for 24.7% of the global exports market, followed by Belgium, Costa Rica, and Colombia. Martin et al. (2020) reported that Malaysia is one of the centers of origin for banana varieties in the world.

As a result of a changing agriculture business landscape, banana is the second most commonly grown fruit crops produced on larger scale for commercial production for export and local use. Among popular banana varieties available in Malaysia are Pisang Mas, Pisang Rastali, however Berangan is the most popular one in Malaysia for commercial purposes. Banana is rated highly consumed in Malaysia about 10 kg of fruit per capita (Tay et al., 2021). According to Sidhu and Zafar (2018), the consumption of banana fruits is known to be rich in carbohydrates, dietary fibres, certain vitamins, and minerals to promote general good health and lower the risk of numerous chronic diseases. Some farmers also planted banana as a cash crop or temporarily intercropped with oil palm, rubber and other perennial crops to generate their side income. Ishak et al. (2020) also reported that the most popular crops integrated with livestock are pineapple (29%) followed by banana (21%) and vegetables (9%). However, banana production in Malaysia has decreased recently due to an increasing threat of diseases, marketing issues, high labour costs, large amount of expensive fertilizer, and fungicide that unsafe to the environment when used extremely.

Fusarium wilt or well-known as Panama disease is considered the most limiting factor in the vegetative and reproductive growth of the banana in Malaysia. The devastating effect of *Fusarium* wilt disease caused by *Fusarium oxysporum* f.sp. cubense (FOC) on banana in Malaysia. Maryani et al. (2019) reported that the causative agent of FOC classified into three races: FOC 1, FOC 2 and FOC 4. However, tropical Race 4 (FOC-TR4) has spread drastically in the eastern hemisphere, especially in Malaysia, which has completely wiped-out banana plantations (Dong et al., 2019; Clement et al., 2019). Evans et al. (2020) also reported that the main disease that constitutes the primary threat to banana

production is *Fusarium* wilt disease (FOC-TR4), which can potentially to infect banana roots and eliminate many banana plantations worldwide. Dadrasnia et al. (2020) also stated that banana infected diseases spread up to 1.6 million ha by the year 2040 around the world. Therefore, multiple prevention actions are needed to reduce a major danger to banana productivity and food security as it ranks number eighth as the world's most important food crop. Paul et al. (2020) mentioned that the host, pathogen, and environment are the three main components in the "disease triangle" that determine an agriculture crop's potential risk. Most of the plant diseases are favoured by rains, high air humidity and soil moisture to establish and spread of disease-causing pathogens. Though, moisture is a commonly recognized environmental factor that controls the transmission of fungal entomopathogens (Biryol et al., 2021). Like other abiotic factors, humidity can also influence the germination of fungal spores, pathogen attack in the wounding site, disease development and helps in the completion of disease cycle.

Currently, applying chemical fungicide alone against pathogen is considered highly effective in controlling FOC, but the continuous application of chemical threating or polluting environment. However, banana growers are still facing problem with expensive cost and large amount of chemical fungicide and fertilizer are needed for their cultivation. Thus, it has drawn the attention of researchers to use biological Si-based nanoparticles compounds and antagonistic bacteria as natural remedies in minimizing FOC incidence to improve profitable agricultural yield as well as it is safe to the environment. Therefore, general objective of the study was aimed to investigate the effect of enriched silicon (Si) compounds, *Bacillus* genus (antagonistic bacteria) and Carbendazim (CBZ) on plant growth performance, physio-biochemical changes and yield of Berangan banana as well as in controlling *Fusarium* wilt disease. The specific objectives of the study undertaken was as follows;

1. to investigate the effects of different nutrient formulation of enriched Si compound as plant booster for growth promotion on morpho-physiological and biochemical changes.
2. to evaluate the best application frequency of selected enriched Si compound on growth, physio-biochemical changes and controlling *Fusarium* wilt disease.
3. to investigate the effects of enriched Si compound and antagonistic bacteria on growth performance and physio-biochemical changes in controlling *Fusarium* wilt disease.
4. to determine the optimum rate of enriched Si compound in term of application volume for enhancing plant growth performance and controlling *Fusarium* wilt disease at nursery stage.
5. to investigate the effects of the selected rate of enriched Si compound with antagonistic bacteria and CBZ fungicide for controlling *Fusarium* wilt disease in the banana grown in an open field.

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