EXPLORING POST-HARVEST DEFENSE MECHANISMS IN PAPAYA FRUIT: A PROTEOMIC STUDY OF ITS RESPONSE TO *FUSARIUM EQUISETI* INFECTION

JEEVINDRAMOORTHY A /L KARUNAMOORTHY

SULTAN IDRIS EDUCATION UNIVERSITY

2025

EXPLORING POST-HARVEST DEFENSE MECHANISMS IN PAPAYA FRUIT: A PROTEOMIC STUDY OF ITS RESPONSE TO *FUSARIUM EQUISETI* INFECTION

JEEVINDRAMOORTHY A/L KARUNAMOORTHY

DISSERTATION PRESENTED TO QUALITY FOR A MASTERS IN SCIENCE
(RESEARCH MODE)

FACULTY OF SCIENCE AND MATHEMATICS
SULTAN IDRIS EDUCATION UNIVERSITY

2025

UPSI/IPS-3/BO 32
Pind : 00 m/s: 1/1

UNIVERSITI PENDIDIKAN SULTAN IDRIS
SULTAN IDRIS EDUCATION UNIVERSITY

Please tick (√)
Project Paper ☐
Masters by Research ☑
Master by Mixed Mode ☐
PhD ☐

INSTITUTE OF GRADUATE STUDIES

DECLARATION OF ORIGINAL WORK

This declaration is made on the 9 September 2025

i. Student's Declaration:

I, **JEEVINDRAMOORTHY A/L KARUNAMOORTHY (M20212003385) FACULTY OF SCIENCE AND MATHEMATICS** (PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled **EXPLORING POST-HARVEST DEFENSE MECHANISMS IN PAPAYA FRUIT: A PROTEOMIC STUDY OF ITS RESPONSE TO *FUSARIUM EQUISETI* INFECTION** is my original work. I have not copied from any other students' work or from any other sources exceptwhere due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

Signature of the student

ii. Supervisor's Declaration:

I **DR.JAMEEL RABEE JAMEEL AL-OBAIDI** (SUPERVISOR'S NAME) hereby certifies that the work entitled **EXPLORING POST-HARVEST DEFENSE MECHANISMS IN PAPAYA FRUIT: A PROTEOMIC STUDY OF ITS RESPONSE TO *FUSARIUM EQUISETI* INFECTION** (TITLE) was prepared by the above named student, and wassubmitted to the Institute of Graduate Studies as a * partial/full fulfillment for the conferment of **MASTERS IN SCIENCE** (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student'swork.

11/09/2025
Date

Signature of the Supervisor

UPSI/IPS-3/BO 31
Pind.: 01 m/s:1/1

UNIVERSITI PENDIDIKAN SULTAN IDRIS
SULTAN IDRIS EDUCATION UNIVERSITY

INSTITUT PENGAJIAN SISWAZAH /
INSTITUTE OF GRADUATE STUDIES

BORANG PENGESAHAN PENYERAHAN TESIS/DISERTASI/LAPORAN KERTAS PROJEK
DECLARATION OF THESIS/DISSERTATION/PROJECT PAPER FORM

Tajuk / *Title*: **EXPLORING POST-HARVEST DEFENSE MECHANISMS IN PAPAYA FRUIT: A PROTEOMIC STUDY OF ITS RESPONSE TO FUSARIUM EQUISETI INFECTION**

No. Matrik /*Matric's No.*: **M20212003385**

Saya / *I* : **JEEVINDRAMOORTHY A/L KARUNAMOORTHY**

(Nama pelajar / *Student's Name*)

mengaku membenarkan Tesis/Disertasi/Laporan Kertas Projek (Kedoktoran/Sarjana)* ini disimpan di Universiti Pendidikan Sultan Idris (Perpustakaan Tuanku Bainun) dengan syarat-syarat kegunaanseperti berikut:-
acknowledged that Universiti Pendidikan Sultan Idris (Tuanku Bainun Library) reserves the right as follows: -

1. Tesis/Disertasi/Laporan Kertas Projek ini adalah hak milik UPSI.
   The thesis is the property of Universiti Pendidikan Sultan Idris
2. Perpustakaan Tuanku Bainun dibenarkan membuat salinan untuk tujuan rujukan danpenyelidikan.
   Tuanku Bainun Library has the right to make copies for the purpose of reference and research.
3. Perpustakaan dibenarkan membuat salinan Tesis/Disertasi ini sebagai bahan pertukaranantara Institusi Pengajian Tinggi.
   The Library has the right to make copies of the thesis for academic exchange.
4. Sila tandakan (√) bagi pilihan kategori di bawah / *Please tick (√) for category below:-*

☐	**SULIT/*CONFIDENTIAL***	Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub dalam Akta Rahsia Rasmi 1972. / Contains confidential information under the Official Secret Act 1972
☐	**TERHAD/*RESTRICTED***	Mengandungi maklumat terhad yang telah ditentukan oleh organisasi/badan di mana penyelidikan ini dijalankan. / *Contains restircted information as specified by the organization where research was done*
☑	**TIDAK TERHAD/*OPEN ACCESS***	

Dr. Jameel R. Al-Obaidi
Senior Lecturer
Department of Biology
Faculty Science and Mathematics
Universiti Pendidikan Sultan Idris
35900 Tanjong Malim, Perak
Malaysia

(Tandatangan Pelajar/ Signature)

(Tandatangan Penyelia / *Signature of Supervisor*) & (Nama & Cop Rasmi / *Name & Official Stamp*)

Tarikh: 11/09/2025

Catatan: Jika Tesis/Disertasi ini **SULIT** @ **TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaandengan menyatakan sekali sebab dan tempoh laporan ini perlu dikelaskan sebagai **SULIT** dan **TERHAD**.
Notes: If the thesis is CONFIDENTAL or RESTRICTED, please attach with the letter from the organization with periodand reasons for confidentiality or restriction.

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere obligation to my supervisor, Dr. Jameel Rabee Jameel Al-Obaidi from faculty of Science and Mathematics by providing me a lot of beneficial knowledge, guidance, and support for my dissertation. I also had learned some information from him that are not in my course syllabus throughout my whole dissertation. His dedication and responsible attitude enable me to achieve my goal in this dissertation. Besides, I also would like to express my appreciation to National Institutes of Biotechnology Malaysia for allowing me to proceed my research project there without any hesitation. Special thanks to Ms. Liza who able to guide me throughout my research project by providing proper explanation and suggestions on my research project. I also like to thank my postgraduate friend who able to support me on this dissertation. On top of that, a very deep sense of thanks to my families and friends who always motivating and supporting me in completing my dissertation.

ABSTRACT

Papaya is one of the fruit crops cultivated in Malaysia and contributed to more than USD 2 million in gross production value in the year 2022 which also threatened by plant diseases. Recently, *Fusarium equiseti* has been observed to cause post-harvest papaya disease in Malaysia. To date, there is limited study on the molecular processes related to the papaya defense mechanism during fungal infection. The objectives of the study are to identify the causal agent causing postharvest disease in Papaya fruit and changes in protein abundance involved in the papaya's defense mechanism. Firstly, a fungal growth pattern was observed after incubating the samples on Potato Dextrose Agar (PDA), giving evidence of *Fusarium* involvement in the postharvest disease. *F. equiseti* on PDA forms fast-growing, woolly colonies with salmon to reddish-brown coloration. After successful amplification using PCR, producing distinct bands corresponding to approximately 583 bp and 576 bp for the first and second isolates, respectively. A BLAST analysis revealed a 100% sequence similarity between the first isolate *F. equiseti*, with GenBank accession no MN335223.1. Following the establishment of *F. equiseti* as the pathogenic agent, the study proceeded to investigate the proteomic responses of papaya fruits to *Fusarium* inoculation. A total of five protein spots were identified, including Oxysterol-binding protein-related protein 4B, Stroma 70kDa heat shock-related protein, Lipoxygenase,30S ribosomal protein, Linoleate 9S-lipoxygenase (spots 1324, 1002, 1923, 2138, 2701) respectively. Three proteins were down regulated (spots 1324, 1002, 2138) in response to *F. equiseti* inoculation, indicating significant in the plant's defense response. After protein identification using MALDI-TOF/TOF mass spectrometry, total of five protein spots were successfully identified, comprising these proteins with known functions with their differential expression levels in response to *F. equiseti* inoculation. Throughout string analysis, the heat map of proteins and Gene cooccurrence was used. Gene cooccurrence results showed that the proteins identified from papaya show similarity to *Arabidopsis thaliana*.

MENEROKA MEKANISME PERTAHANAN PASCA-TUAIAN DALAM BUAH BETIK: KAJIAN PROTEOMIK TERHADAP TINDAK BALASNYA TERHADAP JANGKITAN *FUSARIUM EQUISETI*

ABSTRAK

Betik adalah salah satu tanaman buah yang ditanam di Malaysia dan menyumbang lebih daripada USD 2 juta dalam nilai pengeluaran kasar pada tahun 2022, yang juga terancam oleh penyakit tanaman. Baru-baru ini, *Fusarium equiseti* telah diperhatikan menyebabkan penyakit betik pascapanen di Malaysia. Setakat ini, terdapat kajian terhad mengenai proses molekular yang berkaitan dengan mekanisme pertahanan betik semasa jangkitan kulat. Objektif kajian ini adalah untuk mengenal pasti agen penyebab penyakit selepas tuai pada buah betik dan perubahan dalam kelimpahan protein yang terlibat dalam mekanisme pertahanan betik. Pertama sekali, pola pertumbuhan kulat diperhatikan setelah menginkubasi sampel pada Potato Dextrose Agar (PDA), memberikan bukti penglibatan *Fusarium* dalam penyakit pascapanen. Setelah penguatan berjaya menggunakan PCR, menghasilkan jalur yang jelas yang masing-masing berkaitan dengan kira-kira 583 bp dan 576 bp untuk isolat pertama dan kedua. Analisis BLAST menunjukkan 100% kesamaan urutan antara isolat pertama *F. equiseti*, dengan no akses GenBank MN335223.1. Mengikuti penetapan *F. equiseti* sebagai agen patogen, kajian ini meneruskan penyelidikan terhadap respon proteomik buah betik terhadap inokulasi *Fusarium*. Sebanyak lima titik protein dikenalpasti, termasuk Protein berkaitan pengikat oksisterol 4B, Protein berkaitan tekanan haba 70kDa Stroma, Lipoksigenase, Protein ribosom 30S, dan Lipoksigenase 9S-linoleate (titik 1324, 1002, 1923, 2138, 2701) masing-masing. Tiga protein telah turun teratur (titik 1324, 1002, 2138) sebagai tindak balas terhadap inokulasi *F. equiseti*, menunjukkan kepentingan dalam respons pertahanan tanaman. Setelah pengenalan protein menggunakan spektrometri jisim MALDI-TOF/TOF, sejumlah lima titik protein berjaya dikenalpasti, yang terdiri daripada protein ini dengan fungsi yang diketahui dan tahap ekspresi yang berbeza sebagai tindak balas kepada inokulasi *F. equiseti*. Melalui analisis STRING, peta haba protein dan ko-berlaku gen digunakan. Hasil ko-berlaku gen menunjukkan bahawa protein yang dikenalpasti dari betik menunjukkan kesamaan dengan *Arabidopsis thaliana*. Oleh itu, kajian ini menjalankan penyelidikan proteomik untuk memahami interaksi molekular antara *F. equiseti* dan betik bagi mendapatkan wawasan mengenai faktor virulensi dan cara jangkitan yang digunakan oleh *F. equiseti*.

CONTENTS

CHAPTER 2 LITERATURE REVIEW

CHAPTER 3 EXPERIMENTAL

CHAPTER 4 RESULTS

CHAPTER 5 DISCUSSION

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2D DIGE	Two- Dimensional Difference Gel Electrophoresis
2-DE	Two-Dimensional Gel Electrophoresis
ANOVA	Analysis of Variance
BSA	Bovine Serum Albumin
DDT	Dichloro-diphenyl-trichloroethane
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylene Diamine Tetra Acetic Acid
HCL	Hydrochloric Acid
ICAT	Isotope-Coded Affinity Tag
IEF	Isoelectric Focusing
IPG	Immobilized pH Gradient
iTRAQ	Isobaric Tags for Relative and Absolute Quantitation
ITS	Internal Transcribed Spacer
JA	Jasmonic Acid
KCL	Potassium Chloride
LC-MS/MS	Liquid Chromatography Mass Spectrometry
LOX	Lipoxygenase
MS	Mass Spectrometry
MudPIT	Multi-Dimensional Protein Identification Technology
NaOCl	Sodium Hypochlorite
NIBM	National Institute of Biotechnology Malaysia
ORPS	Oxysterol-Binding Protein-Related Proteins

PDA	Potato Dextrose Agar
PI	Isoelectric Point
ROS	Reactive Oxygen Species
SDS-PAGE	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
SILAC	Stable Isotope Labeling with Amino Acids
SPP.	Species
STRING	Search Tool for the Retrieval of Interacting Genes

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

Crop plants are a valuable source of food as they support global food security. The cultivation of fruit crops such as papaya offers a source of livelihood for local communities and is economically significant in various countries. However, crop plants are threatened with various challenges, including diseases caused by pathogens, which have impacted crop productivity and yield. *Fusarium* sp. is an example of a fungal pathogen that has been extensively studied due to its devastating impacts on many crop plants.

1.1 Background Of the Study

1.1.1 *Fusarium equiseti*: An Indirect Threat to Papaya Production

Fusarium is a genus of fungi that belongs to the (Nikitin et al., 2023). *Fusarium spp*. are soil inhabitants (Rampersad, 2020) capable of infecting the host plant's below- and above-ground parts. Fusarium infects a wide host range (Azil et al., 2021; Cai et al., 2021; Kurt et al., 2020). The fungi are common pathogens of several tropical plants, including banana, mango, pineapple, avocado, and papaya (Zakaria, 2023a). Several diseases have been recorded on papaya caused by *Fusarium*. These include root and stem rot, post-harvest fruit rot, and stem end rot. The aetiology of root rot in papaya can be attributed to *Fusarium solani* (Erazo et al., 2021; Gibert et al., 2022) and *Fusarium falciforme* (Gupta et al., 2019). *Fusarium solani* causes stem rot and stem end rot on papaya. (Patel et al., 2020; Wanjiku et al., 2020). While fruit rot is caused by *Fusarium citrullicola* and *Fusarium melonis* (Khuna et al., 2022), *Fusarium equiseti* (Rahman et al., 2021; Wang et al., 2021), *Fusarium semitectum* (syn. F. incarnatum) (García-Estrada et al., 2020; Soylu et al., 2023; Wonglom & Sunpapao, 2020) and *Fusarium oxysporum* (Balasubramaniam et al., 2023). Recently, *F. equiseti* has been identified as another pathogen causing fruit rot on local Malaysian papaya (Karunamoorthy et al., 2023).

Fruit rot is an example of post-harvest disease, usually caused by improper handling during the transportation and storage of fruits after harvesting (Etefa et al., 2022). *Fusarium* is regarded as a secondary, opportunistic pathogen that requires other infection or injury on the fruit to infect and enable the establishment of its colony, further causing the fruit to become unsuitable for consumption (Zakaria, L. et al., 2012). Therefore, understanding the complex plant-pathogen interactions between *Fusarium* and its host at the molecular level is important to develop effective disease management strategies to overcome post-harvest diseases caused by *Fusarium*.

Papaya (*Carica papaya*) is a tropical fruit crop that is widely known for its nutritional benefits as it is rich in various nutritional elements and essential minerals. The annual global production of papaya reached 14 million metric in 2022, with India, the Dominican Republic, Mexico, and Brazil as the main papaya-producing countries (Food and Agriculture Organization, 2024). However, papaya crop production faces various challenges and can deteriorate due to pests and pathogen infections (Biratu, 2022).

Pathogen infections, such as infection by *Fusarium equiseti* have caused rotting of the papaya fruit. The symptom of infection was described as small, white lesions symptoms on the fruit surface (Karunamoorthy et al., 2023). Similar observations of *F. equiseti* infection on cantaloupe and watermelon were reported by (Nuangmek et al., 2019) and (Li et al., 2018), where white mycelia were observed surrounding the infected epidermal tissue. These symptoms reduce the quality of papaya fruit (Tan et al., 2023).

1.1.2 The Promise of Proteomics in Studying Plant-Pathogen Interactions

The improvement of plant-aspect interaction has enhanced the provision in molecular science, genomics, and proteomics (Balotf et al., 2022; Sarethy & Saharan, 2021). Of the many "omics" disciplines, proteomics has gained recognition as a powerful approach to understanding the complexities of plant-pathogen interaction (Jain et al., 2021). Proteomics allows the measurement of levels of protein expression post-translational modifications and interactions of proteins with other molecules toward understanding cellular processes at the protein level (Al-Amrani et al., 2021).

Proteomics study can be utilized to understand the plant-pathogen interactions involving *Fusarium equiseti* and papaya. Proteomics study offers insights for understanding the protein expression and abundance in papaya when infected by *F. equiseti* and papaya. This would reveal the virulence factors and mode of infection by *F. equiseti* and the defence strategies employed by papaya during infection.

1.1.3 Papaya Guard Instruments: Exploring papaya's protein response

Recognizing that plants are sessile organisms, defence against environmental stresses and disease-causing pathogens are primarily carried out by different mechanisms of various chemical compound synthesis (Desmedt et al., 2020; Divekar et al., 2022; Yeshi et al., 2022). The applications of proteomics provided insight into the papaya's defence mechanism to different forms of infection.

Host factors involved in the pathogen infection of papaya were described in prior studies; however, no data had been collected on proteome dynamics. For example, Siriwan et al. (2021) also showed that papaya-expressed interacting proteins are associated with signaling, transcription, and translation, whereas interacting protein partners that are significantly enriched during viral infection are involved in stress response. Papaya viral infection-induced protein expression is also known to promote photosynthesis, inhibit proteasomal activity, and reinstate cell walls (Soares et al., 2017).

Proteomics studies have also revealed the synthesis and the role of secondary metabolites in papaya's defence during pathogen infection. Secondary metabolites with various benefits, such as antioxidants, were synthesized by papaya as a response to the oxidative stress caused by viral infection (Md Saad et al., 2022). Although these studies provide information about the proteins expressed during viral infection, these studies are exemplary in exploring papaya defence mechanisms against *Fusarium equiseti*.

1.2 Problem Statement

Papaya is one of the fruit crops cultivated in Malaysia (Zainal-Abidin et al., 2021). The papaya industry in Malaysia was transformed with the introduction of Eksotika, a new papaya variety. Eksotika bears the desirable traits of Sunrise Solo and Subang papaya varieties, that are well-suited for both commercial market and export (Sekeli et al., 2018). The papaya industry in Malaysia has contributed to more than USD 2 million in gross production value in the year 2022 (Food and Agriculture Organization, 2024).

Despite contributing significantly to the economy, the papaya industry in Malaysia is threatened by plant diseases such as the papaya dieback disease (Mohd-Azhar et al., 2021). An outbreak of the papaya disease dieback in the year 2006 affected 800 hectares of papaya plantations and resulted in the loss of approximately 1 million trees nationwide. Papayas in Malaysia are also affected by anthracnose caused by *Colletotrichum* sp. Post-harvest fruit decay is believed to be caused by *Lasiodiplodia theobromae, Aspergillus niger,* and *Colletotrichum gloeosporioides* (Mendy et al., 2019). Recently, *Fusarium equiseti* has been observed to cause post-harvest papaya disease in Malaysia (Karunamoorthy et al., 2023). Post-harvest disease on fruits affects the quantity and quality of fruits, leaving them unsuitable for consumption, thus, causing loss to stakeholders in the papaya industry (Mendy et al., 2019).

Papaya (*Carica papaya*) is a highly perishable tropical fruit that suffers significant post-harvest losses due to fungal infections, particularly during storage, handling, and transportation. Studies report that post-harvest diseases can lead to 30–50% loss of papaya yield, especially in countries like Malaysia where high humidity favors fungal growth (MARDI, 2022). One of the common pathogens associated with papaya post-harvest decay is *F. equiseti*, which causes fruit rot and significantly reduces market value and shelf life. To control such infections, synthetic fungicides and pesticides are frequently used; however, their prolonged application can lead to fungal resistance, toxic residues, and adverse effects on human health and the environment. These concerns have prompted the need for safer, sustainable, and targeted disease management strategies.

The relationship between papaya and *F. equiseti* can be described as plant-pathogen and in this, a plant-pathosystem is defined as a plant-pathogen combination of the network of plant and pathogen molecules. Research on the plant-pathogen relationship has advanced the knowledge available concerning the plant health immune system as having the ability to sense and strengthen the plant defence system against various pathogens (Kaur et al., 2022). Hence, it is crucial to understand how pathogenic interaction at the molecular level would have paved the way for other management practices like growing disease-free crops and identifying the management strategies for future outbreaks. However, this present study, relying on proteomic analysis seeks to give insight on *F. equiseti*-papaya interaction and identify virulence factors of *F. equiseti*, and mode of infection of the pathogen in papaya. (Castro-moretti et al., 2020). This study leverages proteomic approaches to explore the *F. equiseti*-papaya.

In recent years, proteomics has emerged as a powerful approach to understand host-pathogen interactions at the molecular level and identify biomarkers or proteins involved in plant defense mechanisms. By analyzing protein expression in papaya infected by fungal pathogens like *F. equiseti*, researchers can uncover potential targets for breeding disease-resistant varieties or developing biocontrol-based treatments. However, there is a notable lack of proteomic studies focused specifically on *F. equiseti* in papaya. Most related proteomic work has centered around *Fusarium oxysporum* or *Colletotrichum gloeosporioides* in crops such as tomato and banana (Li et al., 2020; Zhang et al., 2019), leaving a critical research gap in understanding *F. equiseti*-papaya interactions. Addressing this gap could lead to the discovery of safer and more effective post-harvest disease management alternatives, reducing dependence on harmful chemical treatments and supporting sustainable agriculture interaction, to determine virulence factors of *F. equiseti* and its mode of infection in papaya.

During infection, the plant-pathogen interaction is a complex molecular process. Proteomics study has evolved as an important tool to understand the virulence factors of pathogens, as well as the way of infection of pathogens in host plants (Rauwane et al., 2020a). Proteomics provides an alternate view of understanding the host-pathogen interaction because proteomics studies the protein abundance and functions involved in several biological processes in the host during pathogen infection (Rustagi et al., 2018) The process of papaya defence mechanism has not been studied to date on the molecular level related to fungal infection. In order to better understand the molecular interaction between *F. equiseti* and papaya, the virulence factor and the mode of infection employed by *F. equiseti*, this study performed a proteomics investigation. The proteomics study also revealed how papaya protects itself when infected with the pathogen.

1.3 Objectives of The Study

The objectives of this study were:

1. To identify the causal agent causing postharvest disease in Papaya fruit.
2. To identify the changes in protein abundance involved in the papaya's defence mechanism.

1.4 Research Questions

1. Is there any difference in protein abundance between healthy and infected papaya fruits?
2. What are the proteins involved in the papaya fruit's defence mechanism during pathogen infection?

1.5 Research Hypothesis

1. Papaya fruit defence mechanisms involve a sequence of early and late molecular responses, reflecting distinct stages of infection progression.
2. Pathogenesis-related (PR) proteins play a critical role in triggering and sustaining papaya's defence responses against fungal infections, with specific induction during pathological conditions.
3. Unraveling the molecular pathways underpinning plant-pathogen interactions provides key insights for innovating targeted and sustainable strategies to combat *Fusarium equiseti* infections.

1.6 Operational Definition

There are several terms used specifically in this study. These terms are listed and defined below:

i. Proteomic Study

A proteomics study to a systematic investigation aimed at analyzing the complete set of proteins (proteome) expressed within a biological sample, specifically within the context of interaction between *Fusarium equiseti* and papaya. Proteomics study also consists of the usage of mass spectrometry and other methods to detect, quantify, and characterize the complete set of proteins within the *F. equiseti* and papaya samples.

ii. Plant-Pathogen Interaction

In this study, the plant-pathogen interaction specifically refers to the complex molecular interaction between *Fusarium equiseti* and papaya fruit. The interaction involves a sequence of molecular events, such as the detection of *F. equiseti* in the early stages of infection, the defence mechanism of papaya fruit, and the colonization of *F. equiseti* after overcoming papaya's defence mechanism.

iii. Virulence Factor

Virulence factor refers to proteins, enzymes, or molecules expressed by *Fusarium equiseti* that facilitate the pathogen's colonization, penetration, and pathogenicity in papaya, ultimately leading to post-harvest fruit rot.

iv. Mode Of Infection

The mode of infection refers to the specific mechanisms and strategies employed by *Fusarium equiseti* to infect and colonize the tissue of papaya fruit.

v. Papaya

Papaya, *Carica papaya*, denotes the host plant species which is susceptible to post-harvest diseases caused by fungal infection.

1.7 Significance Of Study

Agriculture is an important factor that contributes to the sustainability of global food security (Pawlak & Kołodziejczak, 2020). The cultivation of fruit crops like papaya plays a crucial role in ensuring a stable food supply, especially in tropical and subtropical regions. However, the advancement of the agricultural sector is constantly hampered by various biotic factors, including the susceptibility of crops to fungal pathogens (Fisher et al., 2020). Fungal infections mostly cause post-harvest plant diseases that reduce the quality and marketability of fruit crops (Simonato et al., 2021).

Fusarium equiseti is known as one of the pathogens impacting post-harvest papaya fruit. Therefore, the understanding of plant-pathogen interaction through proteomics study is important to elucidate the molecular interaction between the two organisms to prevent disease outbreaks that would be economically devastating to the papaya industry.

1.7.1 Preserving Papaya's Economic Significance

Papaya is a tropical fruit plant cultivated for its nutritional and medicinal properties (Koul et al., 2022). Papaya cultivation provides livelihood for local communities and significantly contributes to the economy (Kansiime et al., 2023). In 2022, total global production of papaya reached 14 million tonnes (FAO, 2024). However, papaya cultivation is threatened by a number of pathogens (Tan et al., 2022). *F. equiseti*, a fungal postharvest papaya fruit rot pathogen, is the subject of this study to examine the virulence factor and pathogenic mode of infection. Against this background, this study aims to understand *F. equiseti* in order to prevent the devastating post-harvest papaya fruit rot and to contribute to the economy through the trade of papaya.

1.7.2 Proteomics: A Transformative Tool in Plant Pathology

In this study, a proteomics study has been used to provide an overview of protein interactions, post-translational modifications, and changes in protein abundance during pathogen infection. This study has shown that proteomics is a very useful tool for plant pathology studies that enable a detailed analysis of complex plant-pathogen interactions (Rustagi et al., 2018). Understanding the virulence factor of *F. equiseti* is a critical part of this study. This virulence factor includes the synthesis of phytotoxic substances by *F. equiseti in* interaction with papaya, and the mechanisms of colonization of the host plant by *F. equiseti.* In addition, this study examined the defence mechanism used by papaya against *F. equiseti* infection. Since plants are sessile organisms, they developed some defence mechanisms to prevent pathogens, such as pathogen detection, activating defence signals, synthesising protection chemicals, reinforcing cell walls and inducing cell apoptosis. The defence mechanism would not be able to fight back the successful pathogens and subsequently, the plant would become infected. This study therefore decided to identify the complex mechanism and signals of *F. equiseti* papaya interaction by proteomic analysis, to determine the mode of infection of *F. equiseti.*

1.7.3 Sustainable Agriculture and Reduced Pesticide Dependence

Papaya (*Carica papaya*) is a valuable tropical fruit, yet its high perishability makes it especially vulnerable to fungal infections after harvest—an issue that is more pronounced in humid climates like Malaysia. Among the common pathogens, *F. equiseti* is known to cause significant damage, leading to post-harvest losses of up to 30–50%, which negatively impacts both domestic sales and export potential (MARDI, 2022). While chemical fungicides and pesticides are often employed to manage these infections, their prolonged use raises concerns about human health, environmental degradation, and the emergence of resistant fungal strains (Pimentel & Burgess, 2014). As an alternative, proteomic research offers a more sustainable approach by enabling the discovery of proteins involved in plant defense mechanisms. This knowledge can be used to develop eco-friendly, natural strategies for disease management (Agrawal et al., 2020). However, there is currently a lack of proteomic studies specifically examining *F. equiseti* infections in papaya, represents a significant gap in research. Addressing this gap could help reduce post-harvest losses and lessen the reliance on hazardous agrochemicals in fruit production.

Plant diseases caused by pathogens have been extensively studied using proteomics (Jain et al., 2021). Knowledge of how *F. equiseti* interacts with papaya during infection provides a means to develop several strategies to reduce papaya disease caused by the pathogen, which includes post-harvest disease.

The study of *F. equiseti*-papaya interaction by proteomics allowed for the study of decreasing dependence on chemical pesticides. Chemical pesticide overdependence can lead to environmental pollution, damage to non-target organisms, as well as generating pesticide-resistant strains (Tudi et al., 2021). That is why studies of proteome can lead to the identification of the mode of infection or virulence factor of *F. equiseti* to apply the means to manage diseases caused by *F. equiseti* in an environmentally friendly way and decrease the amount of chemical pesticides. The proteomic analysis offers an opportunity to identify protein abundance and changes in the naturally resistant papaya varieties to fruit rot caused by *F. equiseti* (Zainal-Abidin et al., 2021). The results of this finding will be used to develop strategies to prevent the devastating effects of *F. equiseti* infection on post-harvest papaya fruit.

1.8 Organization of the Thesis

This thesis was organized into six chapters. Each chapter focused on the different aspects of research related to the application of proteomics on *F. equiseti*-papaya interaction to reveal the virulence factors and mode of infection in papaya.

Chapter 1 (Introduction) provides the background of the study, highlighting the importance and significance of proteomics application to study *F. equiseti*-papaya interaction. This chapter outlines the problem statement, objectives of the study, research questions, operational definition, and significance of the study.

Chapter 2 (Literature review) gives insights into previous studies that have been conducted related to papaya, plant diseases, *F. equiseti,* and proteomics approaches.

Chapter 3 (Materials and methods) discusses the research design and methodology used in this study to achieve the study objectives and answer the research questions.

Chapter 4 (Results) presents the results of this study, which focuses on the identification of the pathogen, protein identification, and network interaction. The result presented in this chapter is related to the objectives of the study and research questions listed in Chapter 1. Chapter 5 (Discussion) presented the interpretation and discussion of the results based on the previous literature. Chapter 6 (Conclusions and Recommendations) summarizes the findings of this study. This chapter also provided recommendations for upcoming studies.

1.9 Study Limitation

1.9.1 Complex and Dynamic Nature of Plant-Pathogen Interactions

The connection among plant life forms consists of complex and dynamic processes that contain different molecular components and pathways. The interaction between *F. equiseti* and papaya is not an unusual example. Microorganisms use defence mechanisms with monitored responses, and the exchange of various proteins adds complexity to their collaboration. Focusing on complex interactions at the proteomic level can be challenging, as it may consist of showing a detailed picture of a particularly unique collaboration (Dubiella et al. 2017).

1.9.2 Protein Identification and Quantification Challenges

Proteomics studies rely on advanced mass spectrometry and bioinformatics tools to detect and measure proteins. Thus, these techniques contain inherent limitations. Proteins with low abundance or undergoing post-translational modifications can be particularly challenging to identify. The precise assessment of proteins is complicated by variations in the levels of protein expression across different tissues and under many conditions (Cho et al. 2007).

1.9.3 Variability In Collecting and Preparing Samples

Proteomic research is highly dependent on collecting and preparing samples. Sample handling variability, extraction method variability, and storage condition variability can introduce bias and compromise the reproducibility of these results. Consistent and high-quality samples from field conditions for *F. equiseti*-infected papaya are challenging to obtain because environmental factors and root health vary (Wang et al. 2018).

1.9.4 Proteome Coverage and Depth

Despite advancements in proteomic technologies, achieving comprehensive coverage of the proteome remains a challenge. Certain proteins with low abundance or those attached to cell membranes may go undetected, limiting the completeness of the dataset. In interactions between plants and microorganisms, proteins linked to specific reactions may be present in low concentrations, making it more challenging to detect them (Liu et al., 2019).

1.9.5 Functional Annotation and Validation

Assigning biological functions to identified proteins is a fundamental step in proteomic research. While bioinformatics devices give useful comments, exploratory approval is frequently required. Approving the elements of various proteins distinguished in a complicated collaboration like *F. equiseti* and papaya can be an asset serious and tedious (Carnielli, Winck et al. 2015).

1.9.6 Data Integration and Interpretation

Combining proteomic data with other omics information, such as genomics and transcriptomics, is crucial for gaining a comprehensive understanding of plant-microorganism interactions. However, challenges arise in integrating and interpreting data due to differences in types, scales, and formats of information. Moreover, drawing causation solely from proteomic data can be limited, necessitating complementary experiments. Also, inducing causality from proteomic information alone can be restricted, requiring integral trials (Jendoubi 2021).