UNIVERSITI PUTRA MALAYSIA

SCREENING OF BACTERIAL ISOLATES AS POTENTIAL BIOCONTROL AGENTS AGAINST BOTRYTIS CINEREA IN CAUSING GRAY MOLD DISEASE OF TOMATO (SOLANUM LYCOPERSICUM L.)

ARAFAT HASHIM ALI

FP 2018 96

SCREENING OF BACTERIAL ISOLATES AS POTENTIAL BIOCONTROL AGENTS AGAINST *BOTRYTIS CINEREA* IN CAUSING GRAY MOLD DISEASE OF TOMATO (*SOLANUM LYCOPERSICUM* L.)

By

ARAFAT HASHIM ALI

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

August 2018

COPYRIGHT

DEDICATION

I dedicate this work to my family. A special feeling of gratefulness goes to my beloved husband, parents and to my faithful siblings. I also dedicate this work and indeed, give special thanks to my principal supervisor, Assoc. Prof. Dr. Khairulmazmi Ahmad

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

SCREENING OF BACTERIAL ISOLATES AS POTENTIAL BIOCONTROL AGENTS AGAINST *BOTRYTIS CINEREA* IN CAUSING GRAY MOLD DISEASE OF TOMATO (*SOLANUM LYCOPERSICUM* L)

By

ARAFAT HASHIM ALI

August 2018

Chairman: Assoc. Prof. Khairulmazmi Ahmad, PhD
Faculty : Agriculture

Gray mold disease, caused by the fungus *Botrytis cinerea*, is the most significant disease affecting tomato cultivation causing serious yield losses. In order to reduce the usage of synthetic pesticides in farm, an alternative control disease method is necessary for sustainable disease control. Biocontrol agents have been known to produce effective antimicrobial compounds to control phytopathogens. So that, this study was carried out (i) to isolate and screening bacteria against *B. cinerea*, (ii) to identify the most potential bacteria, determine the antimicrobial compounds produced by selected bacteria isolates and evaluate the efficacy of selected bacteria isolates against *B. cinerea in vivo*. A total of 43 bacterial isolates were obtained from tomato fruit. All the isolates were tested against *B. cinerea* by dual culture test on PDA and 12 isolates showed growth inhibition against *B. cinerea*. The best five isolates with more than 65% inhibition of radial growth (PIRG) were selected for the subsequent experiments. Findings in dual culture assay revealed that BM11 and BC4 were the most effective isolates, which have the PIRG of 82.5% and 71.8%, respectively. BM11 and BC4 isolates were then subjected to molecular identification using 16S rDNA gene region. The results revealed that BM11 and BC4 isolates were *Pseudomonas protegens* and *Brevibacterium casei*, respectively. Bioactive of volatile compounds from ethyl acetate crude extract were identified using gas chromatography-mass spectrometry (GCMS). Ten major bioactive compounds were successfully identified from both bacteria isolates and that possibly were responsible for the antifungal activity in this study. The minimum inhibitory concentration (MIC) showed that there were significantly differences ($P \leq 0.05$) against *B. cinerea* at different concentrations tested. *In vivo* study showed that the efficacy of the ethyl acetate crude extract of *P. protegens* and *B. casei* against *B. cinerea* by suppressing disease lesion on treated tomato fruit at 0.86 cm and

1.03 cm, respectively at 30 days after inoculation as compared to positive control (5.47 cm) at the same assessment period. These findings suggested that the extract manage to suppress fungal disease development effectively. Based on overall findings suggested that *P. protegens* and *B. casei* were the most promising biological control agent in controlling gray mold disease in tomato fruit.

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

SARINGAN ISOLAT BAKTERIA SEBAGAI AGEN KAWALAN BIOLOGI YANG BERPOTENSI TERHADAP *BOTRYTIS CINEREA* YANG MENYEBABKAN PENYAKIT KULAT KELABU PADA BUAH TOMATO (*SOLANUM LYCOPERSICUM* L.)

Oleh

ARAFAT HASHIM ALI

Ogos 2018

Pengerusi : Prof. Madya. Khairulmazmi Ahmad, PhD
Fakulti : Pertanian

Penyakit kulat kelabu disebabkan oleh *Botrytis cinerea* ada penyakit yang merbahaya yang menjejaskan penanaman tomato dengan menyebabkan kerugian yang serius. Bagi mengurangkan penggunaan racun sintetik di ladang, kawalan penyakit alternatif sangat diperlukan untuk menjamin kawalan penyakit secara lestari. Agen kawalan biologi diketahui menghasilkan sebatian antimikrobial yang berkesan untuk mengawal pathogen tumbuhan. Dengan itu, kajian ini dilakukan (i) untuk mengasingkan dan memilih bakteria baik menentang *B. cinerea*, (ii) untuk mengenalpasti bakteria yang paling berpotensi, menentukan sebatian antimikrobial dihasilkan oleh bakteria baik yang terpilih dan menilai keberkesanan bakteria yang dipilih menentang *B. cinerea* secara *in-vivo*. Sebanyak 43 pencilan bakteria diperolehi daripada buah tomato. Kesemua pencilan diuji menentang *B. cinerea* di dalam ujian kultur dual di atas PDA dan sebanyak 12 pencilan merencatkan pertumbuhan *B. cinerea*. Lima pencilan terbaik dengan lebih daripada 65% radial rencatan pertumbuhan (PIRG) dipilih untuk eksperimen seterusnya. Di dalam ujian kultur dual mendedahkan, pencilan BM11 dan BC4 adalah pencilan sangat berkesan, yang mana PIRG masing-masing adalah 82.5% dan 71.8%. Pencilan BM11 dan BC4 dikenal pasti melalui kaedah pengenalan molekul menggunakan daerah gen 16S rDNA. Hasilnya mendedahkan bahawa pencilan BM11 dan BC4 masing-masing adalah *Pseudomonas protegens* dan *Brevibacterium casei*. Sebatian aktif teruap daripada extrak mentah ethyl acetate telah dikenalpasti menggunakan gas chromatography-mass spectrometry (GCMS). Sebanyak 10 sebatian utama telah berjaya dikenalpasti daripada kedua-dua pencilan bakteria tersebut dan berkemungkinan bertanggungjawab kepada aktiviti antikulat dalam kajian ini. Kepekatan rencatan minimum (MIC) menunjukkan bahawa terdapat perbezaan ketara ($P \leq 0.05$) terhadap *B. cinerea* pada kepekatan berbeza yang telah diuji. Kajian *in-vivo* menunjukkan bahawa keberkesanan extrat mentah ethyl acetate daripada *P. protegens* and *B. casei* dengan menindas lesi penyakit pada buah tomato terawatt pada 0.86 cm

1.03 cm, masing-masing pada hari ke-30 berbanding berbanhding dengan kawalan positif (5.47cm) pada hari penilaian yang sama. Hasil temuan ini mencadangkan bahawa extrak tersebut telah berhasil menindas pembentukan penyakit kulat secara berkesan. Berdasar keseluruhan temuan mencadangkan bahawa *P. protegens* dan *B. casei* adalah agen kawalan biologi yang berpotensi untuk mengawal penyakit kulat kelabu pada buah tomato.

ACKNOWLEDGEMENT

All praises are to the Almighty Allah SWT, the Most Gracious, the Most Merciful who has given me power to complete this thesis and enable me to achieve the highest academic degree. My distinctive appreciation goes to my supervisor, Assoc. Prof. Dr. Khairulmazmi Ahmad for his constructive criticisms and advice in the writing of this thesis. I am truly appreciated all the period he spent on reading my thesis and offering suggestions for revisions that greatly developed the quality of this work.

I would like also to express big thanks Dr. Siti Izera Ismail and Dr. Idris Abu Seman who gave me a great deal of knowledge and helped me when in need. I would like to extend my thankfulness as well to the staff of Bacteriology Lab especially Ms. Junina Jaffar for her continuous cooperation and help in providing necessary tools to achieve the objectives of this thesis. Special thanks go to the unforgettable friends Mr. Wael Sultan for his kind assistance and advices during the journey of writing this thesis.

Gratitude and love goes to every member of my family; my husband, my parents, and my siblings. Thank you for your unconditioned love, encouragement and support.

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

Khairulmazmi Bin Ahmad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Siti Izera Ismail, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Idris Abu Seman, PhD
Senior Principle Research Officer
Head of Ganoderma and Disease Research for Oil Palm (Gano DROP) Unit,
Malaysia Palm Oil Board

_____
ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work
- quotations, illustrations and citations have been duly referenced
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No.: _____

Declaration by Members of Supervisory Committee

This to confirm that:

- The research conducted and the writing of tis thesis was under our supervision
- Supervision responsibilities as stated in the Rule 41in Rules 2003(Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman
of Supervisory
Committee: Associate Professor Dr. Khairulmazmi Ahmad

Signature: _____
Name of Member of Supervisory
Committee: Dr. Siti Izera Ismail

Signature: _____
Name of Member of Supervisory
Committee: Dr. Idris Abu Seman

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
℃	Degree Celsius
BCA	Biological Control Agents
Cm	Centimeter
CRD	Completely Randomized Design
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra actic acid
GC-MS	Gas Chromatography- Mass Spectrophotometer
g	Gram
h	Hour
LSD	Least significant differences
µl	Microliter
NA	Nutrient Agar
NB	Nutrient Broth
MIC	Minimum Inhibitory Concentration
mm	Millimeter
PDA	Potato dextrose agar
PIRG	percentage inhibition of radial growth
PCR	polymerase chain reaction

CHAPTER 1

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), along with pepper, potato, and eggplant, belong to the Solanaceae family and is considered the second most widely grown vegetable crop in the world after potato. The US National Agriculture Statistics Services reported that the value of fresh market tomatoes in 2005 was over US$1.6 billion (Sanders, 2004). Fruit quality is a combination of sensory factors such as taste, and acidity sugar, and visual stimuli like color, shape, and size (Clark 2006). Furthermore, the consumers' perception of quality is also heavily influenced by information and the products' appearance like transgenic, biological, and sun-ripe. Ripening is of interest to tomato breeders since ripening affects several quality traits, such as soluble solids content, flavor, and color. Another factor that is especially important for fresh-market tomatoes is their shelf-life. During ripening, several processes occur that negatively affect the storage of the fruit for example pathogens infection.

Plant diseases are a serious threat to food security production and global food. Diseased plants, vegetables, flowers, or a fruit, which are in less appealing and low quality to the consumer, is defined as a qualitative loss. With increased in disease incidences, the quantity of plants or healthy product is decreased. This is defined as the quantitative loss which leads to commercial losses as there are fewer yields to sell (Agarios, 2005). After harvest, vegetables and fruits suffer important losses from fungal infections (Sugar, 2002). Postharvest losses of fruit and vegetable are high, ranging from 1% to 40% reliant on the types (Spadaro, and Gullino, 2004). Fungal diseases are main concerns in commercial production of crops, vegetables, and fruits in the world. *Botrytis cinerea* is considered as one of the most serious postharvest diseases on numerous fruit including tomato. Disease infection can occur in the field during harvest or earlier, but the increase of the disease usually happens for the duration of the postharvest stage (Dıaz, *et al*., 2002).

Plant diseases need to be controlled to maintain the abundance and quality of food, fiber and feed produced by planters around the world. Diverse approaches can be used to control and prevent plant diseases. Beyond horticultural practices and good agronomic, farmers often depend greatly on chemical pesticides and fertilizers. In the past years, these inputs to agriculture have contributed significantly to enhancement in crop quality and productivity. However, pollution of the environment caused by misuse and excessive use of pesticides, as well as fear mongering by some oppositions of agrochemicals makers, has led to large changes in human attitudes towards the use of pesticides in agriculture (Ouda, 2014). Furthermore, the health awareness of the people coupled with the expansion of resistance to pathogens due to continued use of the agrochemicals has also contributed to the restricted use of pesticides in plants protection.

Biological control using bio-agents is an excellent alternative to chemicals and has been proven successful for controlling plant diseases in many countries. Biological control approaches have been studied as a practical and harmless alternative to compare to damaging chemicals as they have been confirmed to be effective, cheap and easily accessible. Various fungi from the genus *Gliocladium*, *Trichoderma*, *Aspergillus*, and *Paecilomyces*, and bacteria from the genus *Pseudomonas*, *Bacillus*, *Erwinia*, *Serratia*, *Paenibacillus* and *Rhizobium* are good examples. The beneficial effects of biological control agent's bacteria have been attributed to the production of metabolites, antifungals, and antibiotics.

The present study was carried out to determine the antifungal potentials of bacterial bioactive compound against infections of tomato by *B. cinerea*.

The objectives of the present study were:

a) to isolate ,screen , identify and determine the antimicrobial compounds produced by selected bacteria isolates.

b) to evaluate of selected bacteria isolates potential as a biological control agent against *B. cinerea in-vivo*.

REFERENCES

Agrios, G. N. (2005). Plant Pathology. 5th eds. Department of Plant Pathology. University of Florida. United States of America.

Al-Hakimi, A. M. A., & Alghalibi, S. M. (2007). Thiamin and salicylic acid as biological alternatives for controlling broad bean rot disease.

Arda-Pirincci, P., & Bolkent, S. (2011). The role of glucagon-like peptide-2 on apoptosis, cell proliferation, and oxidant–antioxidant system at a mouse model of intestinal injury induced by tumor necrosis factor-alpha/actinomycin D. Molecular and cellular biochemistry, 350(1-2), 13-

Barka, E. A., Gognies, S., Nowak, J., Audran, J. C., & Belarbi, A. (2002). Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. Biological Control, 24(2), 135-142.

Barkai-Golan, R. (2001). Postharvest diseases of fruits and vegetables: development and control. Elsevier.

Bartle, K. D., & Myers, P. (2002). History of gas chromatography. TrAC Trends in Analytical Chemistry, 21(9), 547-557.

Bartz, J. A., Sargent, S. A., & Mahovic, M. (2009). Guide to identifying and controlling postharvest tomato diseases in Florida. IFAS/extension HS, 866.

Beecher, G. R. (1998). Nutrient content of tomatoes and tomato products. *Proceedings of the Society for Experimental Biology and Medicine*, *218*(2), 98-100.

Berg, G., & Hallmann, J. (2006). Control of plant pathogenic fungi with bacterial endophytes. Microbial root endophytes, 53-69.

Biedermann, M., & Grob, K. (2009). Comprehensive two-dimensional GC after HPLC preseparation for the characterization of aromatic hydrocarbons of mineral oil origin in contaminated sunflower oil. Journal of Separation Science, 32(21), 3726-3737.

Borges, Á. V., Saraiva, R. M., & Maffia, L. A. (2015). Biocontrol of gray mold in tomato plants by *Clonostachys rosea*. Tropical Plant Pathology, 40(2), 71-76.

Bononi, M., & Tateo, F. (2009). Determination of furan by headspace solid-phase microextraction–gas chromatography–mass spectrometry in balsamic vinegars of Modena (Italy). Journal of Food Composition and Analysis, 22(1), 79-82.

Chan, Z., Qin, G., Xu, X., Li, B. and Tian, S. (2007). Proteome approach to characterize proteins induced by antagonist yeast and salicylic acid in peach fruit. J. Proteome Res, 6(5): 1677-1688.

Chen, C., Song, F., Wang, Q., Abdel-Mageed, W. M., Guo, H., Fu, C., & Xie, F. (2012). A marine-derived Streptomyces sp. MS449 produces high yield of

actinomycin X2 and actinomycin D with potent anti-tuberculosis activity. Applied Microbiology and Biotechnology, 95(4), 919-927.

Chloudhury, N.K.; Cho, H.I. and Huffaker, R.C. (1993) Ascorbate induced zeaxanthin formation in wheat leaves and photoprotection of pigment and photochemical activities during aging of chloroplasts in light. J. Plant Physiol. 141: 551-556.

Chowdhury, E. A., Kolodzey, J., Olowolafe, J. O., Qiu, G., Katulka, G., Hits, D., & Unruh, K. M. (1997). Thermally oxidized AlN thin films for device insulators. Applied Physics Letters, 70(20), 2732-2734.

Clark, M. M. (2006). Biological control methods for damping-off of tomato seedlings caused by *Pythium myriotylum*.

Clark, C. A., & Lorbeer, J. W. (1976). Comparative Histopathology of *Botrytis* squamosa. Phytopathology, 66, 1279-1289.

Colmenares, A. J., Aleu, J., Duran-Patron, R., Collado, I. G., & Hernandez-Galan, R. (2002). The putative role of botrydial and related metabolites in the infection mechanism of *Botrytis cinerea*. Journal of chemical ecology, 28(5), 997-1005.

Compant, S., Duffy, B., Nowak, J., Clément, C., & Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied and environmental microbiology*, *71*(9), 4951-4959.

Cook, A. E., & Meyers, P. R. (2003). Rapid identification of filamentous actinomycetes to the genus level using genus-specific 16S rRNA gene restriction fragment patterns. *International journal of systematic and evolutionary microbiology*, *53*(6), 1907-1915

Dıaz, J., ten Have, A., & van Kan, J. A. (2002). The role of ethylene and wound signaling in resistance of tomato to *Botrytis cinerea*. Plant physiology, 129(3), 1341-1351

David MW (1998). Biologicol control of soilborne plant pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopathol., 26: 379-407

Donmez, M. F., Esitken, A., Yildiz, H., & Ercisli, S. (2011). Biocontrol of Botrytis cinerea on strawberry fruit by plant growth promoting bacteria. J. Anim. Plant Sci, 21(4), 758-763.

Doss, R. P., Potter, S. W., Soeldner, A. H., Christian, J. K., & Fukunaga, L. E. (1995). Adhesion of germlings of Botrytis cinerea. Applied and environmental microbiology, 61(1), 260-265.

Dufková, V., Čabala, R., & Ševčík, V. (2012). Determination of C 5–C 12 perfluoroalkyl carboxylic acids in river water samples in the Czech Republic by GC–MS after SPE preconcentration. Chemosphere, 87(5), 463-469.

Emmert, E. A., & Handelsman, J. (1999). Biocontrol of plant disease: a (Gram-) positive perspective. FEMS Microbiology Letters, 171(1), 1-9.

Elad, Y., Koehl, J., & Fokkema, N. J. (1994). Control of infection and sporulation of *Botrytis cinerea* on bean and tomato by saprophytic bacteria and fungi. European Journal of Plant Pathology, 100(5), 315-336.

El-Aal, H. A., Habib, E. E., & Mishrif, M. M. (2005). Wilms' tumor: The experience of the pediatric unit of kasr el-aini center of radiation oncology and nuclear medicine (nemrock). Egypt Natl. Cancer Inst, 17, 308-314.

El-Tarabily, K. A., & Sivasithamparam, K. (2006). Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Soil Biology and Biochemistry*, *38*(7), 1505-1520

Edwards, C. (1993). Isolation properties and applications of thermophilic actinomycetes. Appl. Biodiv. Biotech. 42, 161–179.

Enya, J., Shinohara, H., Yoshida, S., Tsukiboshi, T., Negishi, H., Suyama, K., & Tsushima, S. (2007). Culturable leaf-associated bacteria on tomato plants and their potential as biological control agents. Microbial Ecology, 53(4), 524-536.

FAO. (2004). The state of food and agriculture (Vol. 37). Food & Agriculture Organization of the UN (FAO).

Faretra, F., Antonacci, E., & Pollastro, S. (1988). Sexual behaviour and mating system of *Botryotinia fuckeliana*, teleomorph of *Botrytis cinerea*. Microbiology, 134(9), 2543-2550.

Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A., & Olsen, G. J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and environmental microbiology*, *74*(8), 2461-2470.

Frederikse, P. H., Farnsworth, P., & Zigler, J. S. (1999). Thiamine Deficiencyin VivoProduces Fiber Cell Degeneration in Mouse Lenses. Biochemical and Biophysical Research Communications, 258(3), 703-707.

Fu, F., & Wang, Q. (2011). Removal of heavy metal ions from wastewaters: a review. *Journal of environmental management*, *92*(3), 407-418.

Gadhi, C. A., Weber, M., Mory, F., Benharref, A., Lion, C., Jana, M., & Lozniewski, A. (1999). Antibacterial activity of Aristolochia paucinervis Pomel. *Journal of ethnopharmacology*, *67*(1), 87-92.

Geoffrey, S. K., Hillary, N. K., Antony, K. M., Mariam, M., & Mary, M. C. (2014). Challenges and strategies to improve tomato competitiveness along the tomato value chain in Kenya. International Journal of Business and Management, 9(9), 205.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol., 43, 205-227.

Gopi, M., Dhayanithi, N. B., Devi, K. N., & Kumar, T. T. A. (2014). Marine natural product, Pyrrolo [-a] pyrazine–dione, hexahydro-(C7H10N2O2) of antioxidant

properties from *Bacillus* species at Lakshadweep archipelago. J. Coastal Life Med, 2, 632-637.

Govrin, E. M., & Levine, A. (2000). The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. Current Biology, 10(13), 751-757.

Gunatilaka, A.A.L., 2006. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implications of their occurrence. J. Nat. Prod., 69: 509-526.

Guo, Y., Zheng, H., Yang, Y., & Wang, H. (2007). Characterization of *Pseudomonas corrugata* strain P94 isolated from soil in Beijing as a potential biocontrol agent. Current Microbiology, 55(3), 247-253.

Guo, L., Wu, J. Z., Han, T., Cao, T., Rahman, K., & Qin, L. P. (2008). Chemical composition, antifungal and antitumor properties of ether extracts of Scapania verrucosa Heeg. and its endophytic fungus *Chaetomium fusiforme*. Molecules, 13(9), 2114-2125.

Gustavsson, J., Cederberg, C., Sonesson, U., Van Otterdijk, R., & Meybeck, A. (2011). Global food losses and food waste (pp. 1-38). Rome: FAO.

Guzzon, R., Franciosi, E., & Larcher, R. (2014). A new resource from traditional wines: characterisation of the microbiota of "Vino Santo" grapes as a biocontrol agent against *Botrytis cinerea*. European Food Research and Technology, 239(1), 117-126.

Hamada, A.M. and Hashem, M. (2003) Thiamin and salicylic acid as biological alternatives for controlling wheat root-rot disease. Egypt. J. Agric. Res. NRC 1:369-385.

Hammami, I., Siala, R., Jridi, M., Ktari, N., Nasri, M., & Triki, M. A. (2013). Partial purification and characterization of chiIO8, a novel antifungal chitinase produced by *Bacillus cereus* IO8. Journal of Applied Microbiology, 115(2), 358-366.

Hengky, S. H. (2016). Competition Gaps of Tomatoes' Industries in Cameron Highland, Malaysia. *Business and Economic Research*, *6*(2), 368-378.

Hobson, G.E. & Grierson, D. (1993) Tomato. In: Biochemistry of Fruit Ripening (ed. G.B. Seymour, J.E. Taylor & G.A. Tucker). Chapman and Hall, London, UK

Hoh, K. S. (2014). Bumper crop leads to tomato glut in Cameron Highlands, The sun daily.

Retrieved from http://www.thesundaily.my/news/1032978

Howard, R. J., Garland, J. A., Seaman, W. L., & Grafius, E. J. (1996). Diseases and Pests of Vegetable Crops in Canada. Journal of Economic Entomology, 89(4), 1045-1045.

Islam, G. M. N., Arshad, F. M., Radam, A., & Alias, E. F. (2012). Good agricultural practices (GAP) of tomatoes in Malaysia: Evidences from Cameron Highlands. *African Journal of Business Management*, *6*(27), 7969-7976.

Jaffe, N., Paed, D., Traggis, D., Salian, S., & Cassady, J. R. (1976). Improved outlook for Ewing's sarcoma with combination chemotherapy (vincristine, actinomycin D and cyclophosphamide) and radiation therapy. Cancer, 38(5), 1925-1930.

Janisiewicz, W. J., & Jeffers, S. N. (1997). Efficacy of commercial formulation of two biofungicides for control of blue mold and gray mold of apples in cold storage. Crop Protection, 16(7), 629-633.

Janisiewicz, W. J., & Roitman, J. (1988). Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology, 78(12), 1697-1700.

Janisiewicz, W. J., & Korsten, L. (2002). Biological control of postharvest diseases of fruits. *Annual review of phytopathology*, *40*(1), 411-441.

Jarvis, W. (1977) Botrytinia and Botrytis species: taxonomy and pathogenicity. [Canada Department of Agriculture Monograph No. 15]. Agriculture Canada, Ottawa.

Jensen, L. and Shock, C. (2009). Common Tomato Varieties. US Dept. of Agric & Oregon State Ext. Serv. Oregon State Univ. (Online) http://www.cropinfo.net/CommonTomatoVarietiesApril2010.

Jog, R., Pandya, M., Nareshkumar, G., & Rajkumar, S. (2014). Mechanism of phosphate solubilization and antifungal activity of Streptomyces spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology, 160(4), 778-788.

Johnston, D. J., & Williamson, B. (1992). Purification and characterization of four polygalacturonases from Botrytis cinerea. Mycological Research, 96(5), 343-349.

Jones Jr, J. B. (2007). Tomato plant culture: in the field, greenhouse, and home garden. CRC press.

Kelley, W. T., Boyhan, G. E., Harrison, K. A., Sumner, P. E., Langston, D. B., Sparks, A. N., & Fonsah, E. G. (2010). Commercial Tomato Production Handbook.

Kannabiran, K. (2016). Bioactivity of Pyrrolo [1, 2-a] pyrazine-1, 4-dione, hexahydro-3-(phenylmethyl)-Extracted from Streptomyces sp. VITPK9 Isolated from the Salt Spring Habitat of Manipur, India. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm, 10(04).

Khatua, S., Nair, C. N., & Ghosh, K. (2004). Immune-mediated thrombocytopenia following dactinomycin therapy in a child with alveolar rhabdomyosarcoma: the unresolved issues. Journal of pediatric hematology/oncology, 26(11), 777-779.

Kim, P. I., Bai, H., Bai, D., Chae, H., Chung, S., Kim, Y. & Chi, Y. T. (2004). Purification and characterization of a lipopeptide produced by *Bacillus thuringiensis* CMB26. Journal of Applied Microbiology, 97(5), 942-949.

Kristiansen, P., & Merfield, C. (2006). O er iew of organic agriculture. Organic agriculture: A global perspective, 1.

Kumar, P. S., Al-Dhabi, N. A., Duraipandiyan, V., Balachandran, C., Kumar, P. P., & Ignacimuthu, S. (2014). *In vitro* antimicrobial, antioxidant and cytotoxic properties of *Streptomyces lavendulae* strain SCA5. BMC microbiology, 14(1), 291.

Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A., & Gobbetti, M. (2000). Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. Applied and Environmental Microbiology, 66(9), 4084-4090.

Lee, B. K., Khare, S., Naider, F., & Becker, J. M. (2001). Identification of residues of the Saccharomyces cerevisiae G protein-coupled receptor contributing to α-factor pheromone binding. Journal of Biological Chemistry, 276(41), 37950-37961.

Lee, J. P., Lee, S. W., Kim, C. S., Son, J. H., Song, J. H., Lee, K. Y., ... & Moon, B. J. (2006). Evaluation of formulations of *Bacillus licheniformis* for the biological control of tomato gray mold caused by Botrytis cinerea. Biological Control, 37(3), 329-337.

Leroux, P., Gredt, M., Leroch, M., & Walker, A. S. (2010). Exploring mechanisms of resistance to respiratory inhibitors in field strains of *Botrytis cinerea*, the causal agent of gray mold. Applied and Environmental Microbiology, 76(19), 6615-6630.

Li, H., Liu, L., Zhang, S., Cui, W., & Lv, J. (2012). Identification of antifungal compounds produced by *Lactobacillus casei* AST18. Current microbiology, 65(2), 156-161.

Liu, F., Hashim, N. A., Liu, Y., Abed, M. M., & Li, K. (2011). Progress in the production and modification of PVDF membranes. *Journal of membrane science*, *375*(1-2), 1-27.

Louis, C., Girard, M., Kuhl, G., & Lopez-Ferber, M. (1996). Persistence of *Botrytis cinerea* in its vector Drosophila melanogaster. Phytopathology, 86(9), 934-939.

Mari, M., Guizzardi, M., & Pratella, G. C. (1996). Biological control of gray mold in pears by antagonistic bacteria. Biological Control, 7(1), 30-37.

Matsumoto, I., & Kuhara, T. (1996). A new chemical diagnostic method for inborn errors of metabolism by mass spectrometry—rapid, practical, and simultaneous urinary metabolites analysis. Mass Spectrometry Reviews, 15(1), 43-57.

Meziane, H., Chernin, L., & Hofte, M. (2006). Use of *Serratia plymuthica* to control fungal pathogens in bean and tomato by induced resistance and direct antagonism. IOBC WPRS BULLETIN, 29(2), 101.

Montealegre, J. R., Reyes, R., Pérez, L. M., Herrera, R., Silva, P., & Besoain, X. (2003). Selection of bioantagonistic bacteria to be used in biological control of Rhizoctonia solani in tomato. *Electronic Journal of Biotechnology*, *6*(2), 115-127.

Moore-Landecker, E. (1996): Fundamentals of the fungi. San Fransisco, CA.: Benjamin-Cummings Pub Co. 397.

Morales-González, J. A. (2013). Oxidative stress and chronic degenerative diseases-a role for antioxidants. Rijeka: Croatia InTech, 500.

Mukerji, K. G. (Ed.). (2006). Allelochemicals: biological control of plant pathogens and diseases (Vol. 2). The Netherlands: Springer.

Nasir, B., & Nawawi, N. (2014). Fama rescues tomato farmers. New Straits Times. Retrieved

From http://www2.nst.com.my/opinion/columnist/fama-rescues-tomato-farmers-1 *(PDF) Competition Gaps of Tomatoes' Industries...*. Available from: https://www.researchgate.net/publication/311570604_Competition_Gaps_of_Tomatoes'_Industries_in_Cameron_Highland_Malaysia [accessed Aug 19 2018].

Neubauer, C. and Yamamota, H.Y. (1992) Mehler-peroxidase reaction mediated zeaxanthin formation and zeaxanthin-related florescence quenching in intact chloroplasts. Plant Physiol. 99: 1354-1361

Niku-Paavola, M. L., Laitila, A., Mattila-Sandholm, T., & Haikara, A. (1999). New types of antimicrobial compounds produced by *Lactobacillus plantarum*. Journal of Applied Microbiology, 86(1), 29-35.

Nobre, S. A., Maffia, L. A., Mizubuti, E. S., Cota, L. V., & Dias, A. P. S. (2005). Selection of *Clonostachys rosea* isolates from Brazilian ecosystems effective in controlling *Botrytis cinerea*. Biological Control, 34(2), 132-143.

Norris, D. and April, M. (1991) Method for inducing resistance in plants using environmentally safe antioxidants. U.S. Patent. 5: 004-493.

Nunes, C., Usall, J., Teixidó, N., Fons, E., & Viñas, I. (2002). Post-harvest biological control by *Pantoea agglomerans* (CPA-2) on Golden Delicious apples. Journal of Applied Microbiology, 92(2), 247-255.

Ofori, E. U. G. E. N. E. (2015). Influence of Grafting On the Growth, Yield, Quality and Shelf Life of Tomatoes (*Solanum Lycopersicum* L.) Grafted Onto Three *Solanum* Species (Doctoral dissertation, University of Ghana).Ogle, H. J., & Dale, M. L. (1997). Disease management: cultural practices. Plant Pathogens and Plant Diseases, 390-404.

On, A., Wong, F., Ko, Q., Tweddell, R. J., Antoun, H., & Avis, T. J. (2015). Antifungal effects of compost tea microorganisms on tomato pathogens. Biological Control, 80, 63-69.

Ouda, S. M. (2014). Biological control by microorganisms and ionizing radiation. International Journal of Advanced Research, 2(5), 314-356.

Pollak, F. C., & Berger, R. G. (1996). Geosmin and Related Volatiles in Bioreactor-Cultured *Streptomyces citreus* CBS 109.60. Applied and Environmental Microbiology, 62(4), 1295-1299.

Prusky, D. (1996). Pathogen quiescence in postharvest diseases. Annual Review of Phytopathology, 34(1), 413-434.

Ramette, A., Frapolli, M., Fischer-Le Saux, M., Gruffaz, C., Meyer, J. M., Défago, G.,& Moënne-Loccoz, Y. (2011). *Pseudomonas protegens* sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2, 4-diacetylphloroglucinol and pyoluteorin. Systematic and Applied Microbiology, 34(3), 180-188.

Relf, D., McDaniel, A. and Morse, R. (2009). Tomatoes. Virginia Cooperative Extension Publication 426-381.

Sanders, D. C. (2004). Vegetable crop guidelines for the Southeastern US.

Sarwar, M., & Frankenberger, W. (1995). Fate of L-phenylalanine in soil and its effect on plant growth. Soil Science Society of America Journal, 59(6), 1625-1630.

Sawabe, T., Ogura, Y., Matsumura, Y., Feng, G., Amin, A. R., Mino, S., & Satomi, M. (2013). Updating the Vibrio clades defined by multilocus sequence phylogeny: proposal of eight new clades, and the description of Vibrio tritonius sp. nov. Frontiers in Microbiology, 4.

Schoonbeek, H. J., Jacquat-Bovet, A. C., Mascher, F., & Métraux, J. P. (2007). Oxalate-degrading bacteria can protect Arabidopsis thaliana and crop plants against *Botrytis cinerea*. Molecular Plant-Microbe Interactions, 20(12), 1535-1544.

Silvera-Pérez, A. E., Valdebenito-Sanhueza, R. M., Duarte, V., Santos, H. P., & Felippeto, J. (2010). Control of gray mold with *Clonostachys rosea* in the production of fuchsia cuttings. Tropical Plant Pathology, 35(3), 163-169.

Slininger, P. J., Burkhead, K. D., & Schisler, D. A. (2004). Antifungal and sprout regulatory bioactivities of phenylacetic acid, indole-3-acetic acid, and tyrosol isolated from the potato dry rot suppressive bacterium Enterobacter cloacae S11: T: 07. Journal of Industrial Microbiology and Biotechnology, 31(11), 517-524.

Someya, N., Nakajima, M., Hirayae, K., Tadaaki, H. I. B. I., & Akutsu, K. (2001). Synergistic antifungal activity of chitinolytic enzymes and prodigiosin produced by biocontrol bacterium, *Serratia marcescens* strain B2 against gray

mold pathogen, *Botrytis cinerea*. Journal of General Plant Pathology, 67(4), 312-317.

Spadaro,D., & Gullino, M. L. (2004).State of the art and future prospects of the biological control of postharvest fruit diseases. *International journal of food microbiology*, *91*(2) 185-194.

Staats, M., van Baarlen, P., and van Kan, J.A.L. 2004. Molecular phylogeny of the plant pathogenic genus Botrytis and the evolution of Host Specificity. Molecular Biology Evolution 22: 333-346

Stashenko, E. E., & Martínez, J. R. (2012). GC-MS analysis of volatile plant secondary metabolites. In *Gas Chromatography in Plant Science, Wine Technology, Toxicology and Some Specific Applications*. InTech

Sudha, S., & Masilamani, S. M. (2012). Characterization of cytotoxic compound from marine sediment derived actinomycete *Streptomyces avidinii* strain SU4. Asian Pacific Journal of Tropical Biomedicine, 2(10), 770-773.

Sudirga, S. K., & Ginantra, I. K. Journal of Biological and Chemical Research.

Sugar, D. (2002). Management of postharvest diseases. Fruit Quality and Its Biological Basis, 225.

Sutton, J. C., Li, D. W., Peng, G., Yu, H., Zhang, P., & Valdebenito-Sanhueza, R. M. (1997). *Gliocladium roseum* a versatile adversary of *Botrytis cinerea* in crops. Plant Disease, 81(4), 316-328.

Tabei, Y., Kitade, S., Nishizawa, Y., Kikuchi, N., Kayano, T., Hibi, T., & Akutsu, K. (1998). Transgenic cucumber plants harboring a rice chitinase gene exhibit enhanced resistance to gray mold (*Botrytis cinerea*). Plant Cell Reports, 17(3), 159-164.

Uberti, E. M. H., do Carmo Fajardo, M., Ferreira, S. V. V. R., Pereira, M. V., Seger, R. C., Moreira, M. A. R., & Schmid, H. (2009). Reproductive outcome after discharge of patients with high-risk hydatidiform mole with or without use of one bolus dose of actinomycin D, as prophylactic chemotherapy, during the uterine evacuation of molar pregnancy. Gynecologic Oncology, 115(3), 476-481.

Urbanek, H., & Zalewska-Sobczak, J. (1975). Polygalacturonase of *Botrytis cinerea* e-200 pers. Biochimica et Biophysica Acta (BBA)-Enzymology, 377(2), 402-409.

van Dam, B., Goffau, M. D., van Lidt de Jeude, J., & Naika, S. (2005). Cultivation of tomato: production, processing and marketing.

Whetzel, H. H. (1945). A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate discomycetes. Mycologia, 37(6), 648-714.

Williamson, B., Tudzynski, B., Tudzynski, P., & van Kan, J. A. (2007). *Botrytis cinerea*: the cause of grey mould disease. Molecular Plant Pathology, 8(5), 561-580.

Wilson, M., & Backman, P. A. (1999). Biological control of plant pathogens. Handbook of Pest Management, 309-335.

Yang, C. J., Zhang, X. G., Shi, G. Y., Zhao, H. Y., Chen, L., Tao, K., & Hou, T. P. (2011). Isolation and identification of endophytic bacterium W4 against tomato *Botrytis cinerea* and antagonistic activity stability. African Journal of Microbiology Research, 5(2), 131-136.

Zhang, L. S., & Davies, S. S. (2016). Microbial metabolism of dietary components to bioactive metabolites: opportunities for new therapeutic interventions. Genome Medicine, 8(1), 46.

Zhu, Z., Zhang, Z., Qin, G., & Tian, S. (2010). Effects of brassinosteroids on postharvest disease and senescence of jujube fruit in storage. Postharvest Biology and Technology, 56(1), 50-55.

Zitter, T. A. (1986). *Botrytis* Gray Mold of Greenhouse and Field Tomatoes.

ZongQi, C. (2009). Postharvest Technologies for Fresh Leafy Vegetables in Yunnan, China. In Best practices in postharvest management of leafy vegetables in Greater Mekong Subregion countries: Proceedings of a GMS workshop, 25-27 October 2007, Hanoi, Vietnam. AVRDC Publication No. 09-731. AVRD