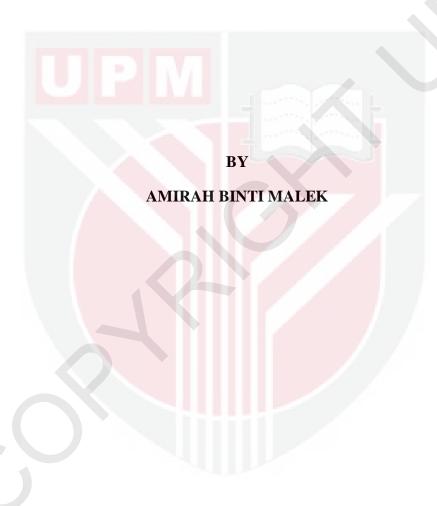


EVALUATING GINGER EXTRACT FOR CONTROLLING POSTHARVEST ANTHRACNOSE OF MANGO

AMIRAH MALEK

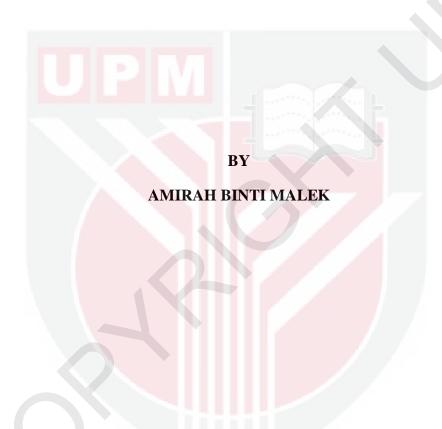
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Faculty of Agriculture
Universiti Putra Malaysia
2015/2016

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A project report submitted to Faculty Agriculture, Univesriti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science

Faculty of Agriculture
Universiti Putra Malaysia
2015/2016

CERTIFICATION

This project report entitled evaluating ginger extract for controlling postharvest anthracnose of mango is prepared by Amirah Binti Malek and submitted to the Faculty of Agriculture in fulfillment requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

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LIST OF ABBREVIATIONS

°C Degree celcius Gram g Mililiter mLRotation per minute rpm Milimeter mm Microliter μL Lactophenol cotton blue LCB ANOVA Analysis of variance

ABSTRAK

Colletotrichum gloeosporioides (Penz.) Sacc ialah agen penyebab penyakit bintik berpusar yang menyerang pada kebanyakan pokok buah-buahan terutama pokok buah mangga. Oleh itu, halia bentong (Zingiber officinale rosc) di pilih sebagai agen penghalang penyakit berdasarkan potensi halia mempunyai aktiviti antimikrob untuk menghalang pertumbuhan patogen. Penyelidikan ini, melihat aspek luaran (in vitro) aktiviti anti-kulat pada buah manga, Colletotrichum gloeosporioides dengan pengunaan ekstrak daripada halia. Tambahan, penggunaan bahan racun kulat kimia yang selalu digunakan di dalam pengurusan kawalan penyakit bintik berpusar telah menyebabkan penghasilan kulat yang tahan racun tersebut. Oleh itu, kaedah pengawalan biologi penyakit bintik berpusar yang berkesan dikaji menggunakan cara ekstrak mentah. Penyelidikan terhadap kesan ekstrak mentah yang daripada halia bentong melalui kaedah pengunaan etanol dan air suling telah di uji kaji pada C. gloeosporioides untuk melihat perencatan pertumbuhan kulat tersebut. Secara keseluruhannya, pengurangan yang signifikasikan terhadap pertumbuhan kulat C. gloeosporioides berhubung kait dengan kaedah ekstrak tumbuhan yang dikaji.

ABSTRACT

Colletotrichum gloeosporioides (Penz.) Sacc. (C. gloeosporioides) is a causal agent of anthracnose disease in many tropical fruit trees, especially mango trees. Therefore, ginger, (Zingiber officinale rosc) has been used as naturopathy due to their antimicrobial activity potential to inhibit the growth of different microbial pathogens. This study investigated the *in vitro* potential of antifungal activities of rhizome extract from Z. officinale rosc to control Colletotrichum gloeosporioides on post-harvest anthracnose of mango. In addition, the common use of chemical fungicides to manage anthracnose disease causes the development of fungal resistance. Hence, effective bio-control methods for managing anthracnose diseases were investigated. The effect of crude extracts obtained from Z. officinale rosc through ethanol and water extraction methods were tested against C. gloeosporioides. Both of these crude extracts were efficient in inhibiting the growth of C. gloeosporioides. However, ethanol was found to be most effective at concentration of 50%. Overall, a significant reduction in the growth of *C. gloeosporioides* was found to be associated with the plant tested. extract

CHAPTER 1

INTRODUCTION

Anthracnose is a group of diseases that has symptoms that resembles dark, sunken lesions on leaves, stems, flowers, and fruits. Anthracnose is common on many deciduous and evergreen trees and shrubs, and in some regions infects turf grass and post-harvest such as mango. This disease is very harmful and can cause spoilage and rotting of plants by resulting in low yield and poor quality of the fruits (Peraza-Sánchez et al. 2005).

These diseases have been controlled using chemical pesticides and are effective in reducing the loss of post-harvest products. They also use of chemical fungicides is the most common choice for management of anthracnose disease, but this also causes the development of fungal resistance (Brent and Hollomon, 1998). In addition, continuous and inappropriate use of chemical fungicides to manage anthracnose disease is not considered to be the long-term solution because this can increase the investment expenses, the risk of having high levels of toxic residues, and also the concerns in human health and environmental settings (Latha et al., 2009).

Due to these reasons, several attempts to search for an alternative measure to control the anthracnose especially on fruits were performed. One of the alternative controls is the use of bio pesticides. Bio pesticides are effective in controlling some post-harvest disease without leaving any harmful effect to environment and humans. Recently,

there are many medical plants extracts that gained attention as potential antimicrobial agents because they are assumed to be more acceptable and less hazardous than the synthetic compounds (Tripathi et al. 2008). In addition, the integration of number of practices aiming to reduce or eliminate negative side effects that is caused by chemical pesticides for controlling major mango diseases is the most realistic option for solving this problem (Chowdury and Rahim, 2009). Hence, the aimed of this study was to use ginger crude extract as an herbal plant for controlling postharvest anthracnose disease caused by *Colletotrichum gloeosporioides* on mango.

OBJECTIVE

The aimed of this study was to evaluate the *in vitro* antifungal activities of rhizome extract of ginger on *Colletotrichum gloeosporioides* growth.

REFERENCES

- Arauz, L. F. and González-Lobo, M. 1986. Fuentes de inóculo de algunas enfermedades de poscosecha del mango. Agron. Costarric, 10(1/2):217-220
- Ann, P. J. 1995. The sexual stage (*Glomerella cingulata*) of *Colletotrichum gloeosporioides* from mango, and effect of temperature and light on its reproduction. (Abstr.) Plant Pathol. Bull. (Taiwan), 4:173-179.
- Arauz, L. F. 2000. Mango anthracnose: Economic impact and current options for integrated management. Plant Disease, 84:600–611.
- Arora, S.D. and Kaur, G.J. 2007. Antibacterial activity of some Indian medicinal plants. Journal of Natural Medicine 61: 313-317.
- Anand T, Bhaskaran R. 2009. Exploitation of plant products and bio-agents for eco-friendly management of Chili fruit rot disease. J Plant Prot Res, 49: 195-203.
- Atai z, Atapour M and Mohseni M. 2009. Inhibitory effect of ginger extract on *Candida albicans*. American Journal of Applied Science, 6(6): 1067-2009.
- Abdalla M.A, Siham E., Suliman and Bakhiet A.O. 2010. Method for reducing contamination of indigenous cattle carcasses during slaughtering (With 2 table). Assiut Veterinary Medicine Journal, Vol. 56, No. 125. April.
- Ali Ghasemzadeh, Hawa Z E Jaafar, Asmah Rahmat. 2010. Identification and Concentration of Some Flavonoid Components in Malaysian Young Ginger (Zingiber officinale roscoe) Varieties by a High Performance Liquid

- Chromatography Method. 15, 62311-6243; doi: 10.3390/molecule15096231.
- Ashutosh Pandey, L.P. Yadava, R.K.M, Brajesh K.P and Mutukumar. 2012. Studies on the incident and pathogenesis of *Colletotrichum gloeosporioides* penz.

 Causes anthracnose ofmango, International Journal of Science and Nature,

 Vol.3 (2):220 -232.
- Ajay Kumar Gautam. 2014. *Colletotrichum gloeosporiodies*: Biology, pathogenicity and Management in India. Journal of plant physiology & pathology. 2:2.
- Arshad HR, Fahad MA and Slah MA. 2014. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities; 6 (2): 125-136.
- Collins, G.N. 1903. The mango in Puerto rico. U.S.D.A. Bur. Pl. Ind. Bull, pp. 28.
- Cardin, P.P. 1910. Annual report of the mango in Cuba. The Cuba Review 8(5), 28 -29.
- Cowan, M.M. 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews.12(4): 564 582.
- Cannon PF, Bridge PD, Monte E. 2000. Linking the past, present and future of Colletotrichum systematics. In: Prusky D, Freeman S, Dickman M (eds) Colletotrichum: host specificity, pathology, and host-pathogen interaction. APS Press, St Paul, pp 1–20.

- Chairgulprasert V, Prasertsongskun S and Wichaporn W. 2005. Chemical constituents of the essential oil and anti-bacterial activity of Zingiber wrayi var. halabala. Songklanakarin J. Sci. Technol, 279 (4): 813-818.
- Doidge, E.M. 1932. Black spot of mangoes. Farming South Africa 7, 89-91.
- Dodd, J.C., Jeffries, P. and Jeger M.J. 1989. Management strategies to control latent infection in tropical fruits. Asp. Appl. Biol. 20, 49–56.
- El-Mahmood, AM and Amey JM. 2007. *In vitro* antibacterial activity of *Parkia biglobosa* (*Jacq*) root bark extract against some microorganisms associated with urinary infections. Afr. J. Biotechnol.; 6 (11): 1272-1275.
- Fagan, H.J. 1979. Postbloom fruit drop a new disease of citrus associated with a form of *Colletotrichum gloeosporioides*. Annals Applied Biology 91, 13-20.
- Fitzell, R., D. and Peak, C.M. 1984. The epidemiology of anthracnose disease of mango; Inoculum sources, spore production and dispersal. Ann Appl. Biol., 104: 53-59.
- Freeman S, Pham M and Rodriguez RJ. 1993. Molecular genotyping of Colletotrichum species based on arbitrarily primed PCR, A+ T-rich DNA, and nuclear DNA analyses, 17:309.
- Farr DF, Aime MC, Rossman AY and Palm ME. 2006. Species of *Colletotrichum* on agavaceae. Mycol Res, 110: 1395-1408.

- Gordillo Quesada, L. and Hernández López, E. 1980. Forma sexual y medios de cultivo para Colletotrichum gloeosporioides, patógeno del mango en Cuba. Cienc. Agric. 7:11-17.
- Gantotti, B.V. and Davis, M.J. 1993. Pectic zymogram analysis for characterizing genetic diversity of mango anthracnose pathogen. Acta Horti, 341:353-359.
- Higgins, J.E. 1906. The mango in Hawaii. Agriculture Experimentation. Station Bulletin, pp.12.
- Hiremath SV, Hiremath PC and Hegde RK. 1993. Studies on cultural characters of *Colletotrichum gloeosporioides* a causal agent of Shisham blight. Karnataka J Agricul Sc.6: 30-32.
- Hasan HA, Raauf AMR, Abd Razik BM and Hassan BAR. 2012. Pharmaceut Anal Acta, 3: 2153 -2435.
- K. J. Brent and D. W. Hollomon. 1998. Fungicide resistance the assessment of risk, FRAC, Global Crop Protection Federation, vol. 2, pp. 1–48.
- Kumar, A., Reeja, S. T., Bhai, R. S. and Shiva, K. N. 2008 .Distribution of Pythium myriotylum Drechsler causing soft rot of ginger. J. Spices and Aromatic Crops, 17(1): 5-10.
- Lu G,Cannon PF,Reid A and Simmons CM .2004. Diversity and molecular relationship of endophytic *Colletotrichum* isolates from the Iwokrama Forest Reserve, Guyana, 108:53-56.

- Lee SO, Choi GJ, Jang KS, Lim HK, Cho KY. 2007. Antifungal activity of five plant essential oils as fumigant against post-harvest and soil borne plant pathogenic fungi. Plant Pathol J; 23: 97-102.
- Mpalantinos, M. A., de Moura, R. S., Parente, J. P. and Kuster, R. M. 1998.

 Biologicallyactive flavonoids and kava pyrones from the aqueous extract of

 Alpinia zerumbet .Phytotherapy Research, 12, pp. 442–444.
- M.N.A. Chowdhury & M.A. Rahim. 2009. Integrated crop management to control anthracnose (Colletotrichum gloeosporioides) of mango. Journal Agri Rural Dev 7 (1& 2), 115-120, June.
- Moss, MO. 2002. Mycotoxins review 1. Aspergillus and Penicillium. Mycologist, 16: 116-119.
- Mendonca-Filho RR. 2007. Bioactive phytocompounds: New approaches in the phytosciences. In: Ahmad I, Aqil F, Owais M, editors. 2006. Modern phytomedicine: Turning medicinal plants into drugs. Weinheim: Wiley VCH Verlag GmbH&Co.p.1–24.ch1.
- Malencic, D.; Popovic, M.; Mliladinovic, J.. Phenolic content and antioxidant properties of soybean (*Gleine max (L.*) Merr. Seeds. Molecules,12,pp 576 -581.
- Purkayastha RP, Sen Gupta M .1973 .Studies on conidial germination and appressoria formation in Colletotrichum gloeosporioides Penz. Causing anthracnose of

- jute (Corchorus olitorius L.). Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 80: 718-724.
- Ploetz, R.C. and Prakash, O. 1997. Foliar, floral and soilborne diseases. In: Litz, R.E. (ed.) The Mango:Botany, Production and Uses. CABI. pp. 281-326.
- P. Latha, T. Anand, N. Ragupathi, V. Prakasam, and R. Samiyappan. 2009.

 Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against Alternaria solani, Biological Control, vol. 50, no. 2, pp. 85–93.
- Ravindran, P.N. and K. Nirmal-Babu. 2005. Introduction. In: Ginger: the genus Zingiber. (Eds.):CRC Press, New York, pp 1-14.
- Rozanida, A.R., Nurul Izza, N., Mohd Helme, M.H. and Zanariah, H. 2006.

 Cosmaceutical Product from Species in the Family Zingiberaceae. In

 Harnessing Cures from Nature: Trends and Prospects; Mazura, M.P., Ed.;

 Forest Research Institute: Kepong, Selangor, Malaysia, pp. 31–36.
- Raghavendra, S.N., Rastogi, N.K., Raghavarao, K.S.M.S., Maya Prakash and Bhat, K.K. 2009. A process for the production of coconut beverage. Indian Patent. 283/DEL/09.
- Ravishanker, Kumar, S., Chatterjee, A., Baranwal, D. K. and Solankey, S.S. 2014.

 Genetic variability for yield and quality traits in ginger (Zingiber officinale Roscoe). The Bioscan.8(4): 1383-1386.

- Stevens, F.L. and Pierce, A.S. 1933. Fungi from Bombay. Indian Journal Agriculture Science 3,912-916.
- Sattar, A. and Malik, S.A. 1939. Some studies on anthracnose of mango caused by *Glomerella cingulate* (Stonem.) Spauld. Sch.(*Colletotrichum gloeosporioides Penz.*). India Journal Agriculture Science 1, 511-521.
- Sutton,B.C. 1992. The genus *Glomerella* and its anamorph *Colletotrichum*. In Colletotrichum Biology, Pathology and Control, edited by J.A. Bailey & M.J. Jeger. Wallingford, UK:CAB International. pp. 1-26.
- Shahi, S.K., Patra, M., Shukla, A.C. and Dikshit, A. 2003. Use of essential oil as bio pesticide against post-harvest spoilage in fruits, Malus pumilo. Bio-Control., 48 (2): 223-232.
- S. R. Peraza-Sánchez, E. O. Chan-Che, and E. Ruiz-Sánchez. 2005. Screening of Yucatecan plant extracts to control *Colletotrichum gloeosporioides* and isolation of a new pimarene from Acacia pennatula. Journal of Agricultural and Food Chemistry, vol. 53, no. 7, pp.2429-2432.
- Singhai A, Singour P K, Pawar RS and Patil U K. 2009. Pharmacological Activities of Chandrakhya Leaves. International Journal of Pharmaceutical Sciences and Drug Research, July-September, Vol 1, Issue 2 pp.107-109.
- Sharma S, Vijayvergia R and Singh T. 2010. Evaluation of antimicrobial efficacy of some medicinal plants. J. Chem. Pharm. Res., 2(1): pp.121-1124.

- Supreetha, S, S. M, Sqqueira PS, Jithesh Jain, Shreyas Tikare, Amint M. 2011.

 Anitfungal Activity of Ginger Extract on *Candida Albicans*: An *In-vitro* study, Vol. 2, Issue 2, September.
- Senhaji B, Ben Hmamou D, Salghi R. 2013. *Asteriscus imbricatus* extract: Antifungal activity and anticorrosion inhibition. Int J Electrochem Sci, 8:6033–6046.
- Taro, R.A. 1929. Plant disease notes from the Central Andes. Phytopathology 19, 969-974.
- Traub, H.T. and Robinson, T.T. 1938. Improvement of subtropical fruit other than citrus. USDA bull, 1589, pp. 77.
- Tarafdar, J. and Saha, N. 2007. Correlation study on population dynamics of ginger soft rot inciting pathogens under different organic amendments, disease incidence and its survival in Darjeeling hill soils. Proceedings of the 13th ISTRC Symposium, 165-169.
- Venkateswarlu, S et al. 2013. Nutrient utilization in buffalo bulls fed crop residue based rations. Online Journal of Animal and Feed Research, 3 (2), 101-105.
- Vidyalakshni A and Divya CV. 2013. New report of *Colletotrichum gloeosporioides* causing anthracnose of *Pisonia alba* in India. Arch Phytopathol Pl Prot 46: 201-204.
- Wester, P.J. 1911. The Phillippines Island, Bureau of Agriculture Bulletin No. 18, pp. 60.

Zakaria L,Juhari N.Z, Iskandar S.,Vijaya and Mohd Anuar I.S. 2015. Molecular Characterization of Colletotrichum Isolates Associated with Anthracnose of Mango Fruit, Sains Malaysia 44(5): 651-656.

