On The Possibility of Using FTIR for Detection of *Ganoderma Boninense* in Infected Oil Palm Tree

Jedol Dayou, Arnnyitte Alexander, Coswald Stephen Sipaut, Chong Khim Phin, and Lee Ping Chin

Abstract*—Ganoderma boninense* is a basidiomycetes fungus that causes basal stem rot disease (BSR) in oil palm trees. In Malaysia alone, the loss caused by this disease was estimated between RM 225 Million to RM 1.5 Billion in 2011 by Malaysian Palm Oil Board. Unfortunately, many planters do not realize that their fields were infected with BSR until it is too late. Several methods have been proposed for early detection of *Ganoderma boninense* infection. In this paper, Fourier transform infrared spectroscopy (FTIR) is investigated as a tool to detect the presence of *Ganoderma boninense* in oil palm tree. It is shown that there are differences in the FTIR result from the infected and healthy oil palm tree that resembles the FTIR characteristics from pure *Ganoderma boninense*. The result presented in this paper shows the possibility of FTIR as a tool for detecting the infection of the fungi in oil palm tree.

Keywords*—Ganoderma boninense*, oil palm, Fourier transform infrared spectroscopy, basal stem rot.

I. Introduction

OIL palm (*Elaeis guineensis* Jacq.) has been known as a truly "golden crop of Malaysia" since it generated profitable export earnings for the country and truly nature's gifts for alleviating poverty in Malaysia [4]. However, the oil palm industry is being jeopardized with one major disease known as Basal Stem Rot (BSR) which is caused by *Ganoderma boninense*. This disease cause serious threat to the oil palm industry in the Southeast Asian countries, especially Malaysia and Indonesia, as which are of the major producers and exporters of palm oil in the world. Infection of this disease can cause numerous yield losses and ultimately result in the destruction of basal tissues hence death of disease palms. In Malaysia alone, it has been reported that the economic loss caused by *G. boninense* is between RM225 million to RM1.5 billion a year [2], [12]. Many methods have been taken to control this disease, but to date, no method gives good control of *Ganoderma* in established plantation. Some failed due to the late detection of infection. This is because the disease cannot be detected at the early stage, and when the disease symptoms do appear more than 50% of internal tissues are already rotten [14]. Usually young palms die within 1 or 2 years once the symptoms of the disease appears, while matured trees can survive for only 3 years or so [9]. Therefore, early detection and identification of *G. boninense* infection is very important in deciding the most suitable way for controlling and reducing the pathogen's severity, thereby minimizing economic losses.

Quite recently, utilization of IR spectroscopy in agriculture has been increasing and gaining momentum among researchers [11], [13]. Many studies have shown that it is possible to use Fourier Transform Infrared spectroscopy (FTIR) for detection, differentiation and identification of fungal phyto-pathogen at the level of isolates [10], [13]. In respect to this matter, this study was conducted to investigate the possibility of using FTIR spectroscopy technique for detection of *G. boninense* infection on infected oil palm tree.

II. Materials and Methods

A. Fungi

Pure culture of *Ganoderma boninense* was obtained from Genetic Laboratory of School of Science and Technology, Universiti Malaysia Sabah. The identity of *G. boninense* has been identified and confirmed using molecular technique [6]. The pure culture was subcultured on Potato Dextrose Agar (PDA) media and maintained at 27ºC until further use.

B. Samples collection

Healthy and infected tissues samples were collected from oil palm plantation in Sandakan, Sabah, Malaysia. Collection of trunk tissues was carried out following the method described by [7]. Precautions procedures to eliminate the possibility of contamination from unwanted saprophytes were

Jedol Dayou, Energy, Vibration and Sound Research Group (e-VIBS), School of Science and Technology, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia (corresponding author's phone: +60-88-320000 Fax: +60-88-435324; email: jed@ums.edu.my).

Arnnyitte Alexander, Energy, Vibration and Sound Research Group (e-VIBS), School of Science and Technology, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia (e-mail: aarnnyitte@yahoo.com).

Coswald Stephen Sipaut, Chemical Engineering Programme, School of Engineering and Information Technology, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia (e-mail: css@ums.edu.my).

Chong Khim Phin, Sustainable Palm Oil Research Unit (SPOR), School of Science and Technology, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia (e-mail: chongkp@ums.edu.my).

Lee Ping Chin, Biotechnology Pogramme, School of Science and Technology, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia (e-mail: leepc@ums.edu.my).

taken into consideration during trunk tissues collection using ethanol sterilization. Healthy tissues were confirmed free from *Ganoderma* based on ergosterol analysis [8] and *Ganoderma* Selective Media (GSM) [3]. Meanwhile, infected tissues were also confirmed with the detection of ergosterol content from the tissues and growth of *Ganoderma* fungi on GSM.

C.FTIR measurements

A Perkin Elmer 2000 Series Fourier transform infrared (FTIR) spectrometer was used for scanning. All samples (*G. boninense*, healthy and infected tissues) were dried and powdered before the measurement. The spectrum resolution was set at 4 cm^{-1} and the scanning range was selected from 650 to 4000 cm^{-1}. A small amount (~ 100 mg) of each sample was placed onto the FTIR sample holder and spectra were collected. Three independent replicate samples of either *G. boninense*, healthy and infected trunk tissues were measured.

III. RESULT AND DISCUSSION

The main objective in this study was to test and evaluate the potential of FTIR spectroscopy in detecting infected oil palm tissues and also differentiating between healthy and infected oil palm tissues. Figure 1 shows the FTIR absorption spectra of *G. boninense* pure culture, healthy and *Ganoderma*-infected oil palm tissues. In general, both healthy and infected oil palm trunk tissues have almost similar spectral pattern. However, obvious differences van be observed in certain range which is indicated as region I in Figure 1. Based on the spectral pattern of healthy tissues, there is a weak absorption in the region, whereas, a strong absorption is shown in the infected tissue in the same region. This is understood because of the *G. boninense* presence as the pathogen shows high absorption in this region (refer Figure 1). Therefore based on the spectral differences of healthy and infected tissue in region I, it is possible to distinguish between infected and healthy trunk tissue. This is in agreement with [5] which stated that infected biomass should result in a different IR 'fingerprint' compared to uninfected tissues due to the presence of fungi together with their metabolic products.

In this result, the presence of *Ganoderma* in the infected tissue was detected with the similar peak absorbance in region I between *G. boninense* and infected oil palm tissues. The prominent peak in this region seems to be specific to *Ganoderma* because they are missing in the healthy sample, thus, detection of this peak in oil palm tissues will gives early evidence of the pathogens infection. The C-O-C content which detected in this region (1300-1000 cm^{-1}) could possibly associated with *G. boninense* similar to the findings by [1]. This study shows that there are significant spectral biomarkers observed in the *Ganoderma*-infected tissues compared to the healthy tissues which resemble the spectral behaviour in *G. boninense*. Therefore, using this biomarker, it will be possible to discriminate between healthy and infected oil palm tree.

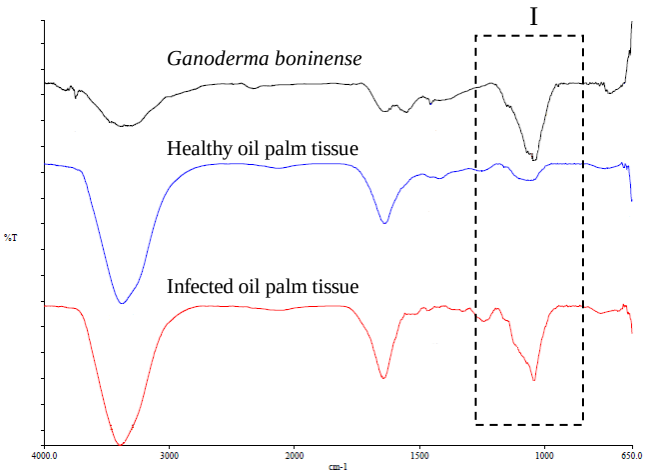

Fig. 1 FTIR spectra of *Ganoderma boninense*, healthy oil palm trunk tissues and infected oil palm trunk tissues.

IV. CONCLUSION

This paper presented an investigation on the use of FTIR spectroscopy as a possible method to detect the infection of *G. boninense* in oil palm tree. It was shown that there is an obvious and strong absorption of infrared radiation in certain range by the infected oil palm tree tissue which was not shown by the healthy tissue sample. Cross reference with pure *G. boninense* shows a resemblance in its absorption spectra. Therefore, this may serves as a biomarker to discriminate between healthy and infected oil palm tree. This finding shows that FTIR spectroscopy can be a useful tool in detecting the infection of *G. boninense* in oil palm tree.

ACKNOWLEDGMENT

The work presented in this paper is supported the Malaysian Ministry of Education under Fundamental Research Grant Scheme no FRG0342-ST-2/2013, and is greatly acknowledged.

REFERENCES

[1] A. H. Abdullah, A. Y. Md. Shakaff, A. H. Adom, M. N. Ahmad, A. Zakaria, S. A. Ghani, N. M. Samsudin, F. S. A. Saad, L. M. Kamaruddin, N. H. Hamid, and I. A. Seman, "Exploring MIP Sensor of Basal Stem Rot (BSR) Disease in Palm Oil Plantation". In *The 14th International Meeting on Chemical Sensors*. Nurembery, Germany, 20-23 May 2012.

[2] M. S. Arif, A. Roslan, and A. S. Idris, "Economics of OP pests and *Ganoderma* disease and yield losses", In *Proceedings of the Third MPOB-IOPRI International Seminar: Integrated OP Pests and Diseases Management*. Kuala Lumpur Convention Centre, 14 November 2011.

[3] D. Ariffin, and A, S. Idris, "The *Ganoderma* Selective Medium (GSM)" *PORIM Information Series no.8*. Persiaran Institusi, Kajang, Malaysia: Palm Oil Research Institute of Malaysia, 1992.

[4] Y. Basiron, "Palm oil production through sustainable plantations" *European Journal of Lipid Science and Technology*, 2007,vol 109,pp 289-295
http://dx.doi.org/10.1002/ejlt.200600223

[5] H. Brandl, "Detection of fungal infection in *Lolium perenne* by Fourier transform infrared spectroscopy". *Journal of Plant Ecology*,vol 4, 265-269, 2012.

[6] K. P. Chong, C. P. Foong, C. M .V. L. Wong, S. Rossall, and M. Atong, "First identification of *Ganoderma boninense* isolated from Sabah based on PCR and sequence homology" *African Journal of Biotechnology*, vol 10(66), pp. 14718-14723. 2011. http://dx.doi.org/10.5897/AJB11.1096

[7] K. P. Chong, "An evaluation of The *Ganoderma* Fungal Colonisation Using Ergosterol Analysis and Quantification", *The Planter*, Kuala Lumpur, vol 88(1034), pp.311-319, 2012.

[8] K. P. Chong, A. Markus and S. Rossall, "The susceptibility of different varieties of oil palm seedlings to *Ganoderma boninense* infection", *Pakistan Journal of Botany*, vol 44(6), pp. 2001-2004, 2012.

[9] R. H. V Corley and P. B. Tinker, "The Oil Palm", 4th edition. Blackwell Publishing. Oxford, UK. 2003. http://dx.doi.org/10.1002/9780470750971

[10] V. Erukhimovitch, L. Tsror, Hazanovsky, M. Talyshinsky, I. Mukmanov, Y. Souprun, and M. Huleihel, "Identification of fungal phyto-pathogens by Fourier-transfrom infrared (FTIR) microscopy". *Journal of Agricultural Technology*, vol 1(1),pp.145-152, 2005.

[11] R. Linker, and L. Tsror, "Discrimination of Soil-Borne Fungi Using Fourier Transform Infrared Attenuated Total Reflection Spectroscopy". *Applied Spectroscopy*, vol 62(3), pp. 302-305, 2008. http://dx.doi.org/10.1366/000370208783759678

[12] B. G. Ommelna, A. N. Jennifer, and K. P. Chong,"The potential of chitosan in suppressing *Ganoderma boninense* infection in oil-palm seedlings", *J Sustain Sci Manage*, vol 7(2),pp.186–192. 2012

[13] A. Salman, I. Lapidot, A. Pomerantz, L. Tsror, E, Shufan, R. Moreh, S. Mordechai, and M. Huleihel., "Identification of fungal phytopathogens using Fourier transform infrared-attenuated total reflection spectroscopy and advanced statistical methods", *Journal of Biomedical Optics*, vol 17(1), pp. 017002. 2012. http://dx.doi.org/10.1117/1.JBO.17.1.017002

[14] A. Kandan, R. Bhaskaran, and R. Samiyappan,"*Ganoderma*- a basal stem rot disease of coconut palm in South Asia and Asia Pacific regions", *Arch Phytopathol Plant Protect*,vol 43,pp. 1445–1149, 2010. http://dx.doi.org/10.1080/03235400802536527