Journal of Oil Palm Research Vol. 23 August 2011 p. 1069-1077

DETECTION OF PHYTOSTEROLS IN *Ganoderma boninense*-INFECTED OIL PALM SEEDLINGS THROUGH GC-MS ANALYSIS

NUSAIBAH, S A*; SITI NOR AKMAR, A*; MOHAMAD PAUZI, Z‡; IDRIS, A S and SARIAH, M***

ABSTRACT

Ganoderma boninense *is a fungus known to be pathogenic to oil palm. It causes the basal stem rot (BSR) and upper stem rot (USR) diseases. This study shows that the interaction between* Ganoderma *and oil palm produced many secondary metabolites including sterol compounds revealed by gas chromatography-mass spectrometry (GC-MS) analysis. The roots of progenies were artificially infected with* G. boninense *and subjected to metabolite extraction. A total of 13 sterol compounds and two tocopherols were identified from the root extracts of both tolerant and susceptible oil palm seedlings. The main sterol compounds identified were sitosterol, stigmasterol, campesterol and ergostenol. The GC-MS library, namely NIST 08, Wiley 229 and comparison of fragmentation patterns of the mass spectra reported in literature made it possible to identify the sterol components present in the root extracts. The results indicate that the number and level of sterol compounds induced in infected palms were significantly higher than in uninfected seedlings. Variations in the type and level of compounds detected were also observed between infected tolerant and susceptible progenies. This study provides information that relates sterols and tocopherol (antioxidant) compounds to the oil palm defence mechanism against* G. boninense*.*

Keywords: oil palm, *Ganoderma boninense*, sterol compounds, GC-MS, defence mechanisms.

Date received: 7 July 2010; **Sent for revision:** 2 August 2010; **Received in final form:** 8 November 2010; **Accepted:** 19 April 2011.

INTRODUCTION

Oil palm is economically important to many countries such as Malaysia, Indonesia, Papua New Guinea, South America and several countries in the African continent. In order to increase and sustain the yield of this crop, disease control ought to be a significant aspect of its research. *Ganoderma boninense* has long been a devastating canker pathogen of oil palm (Pilotti, 2001; Nusaibah, 2007). Metabolomics can discover favourable applications to overcome an otherwise catastrophic plant disease caused by this fungus. Allwood *et al.* (2007) discussed how metabolomics can help to advance our understanding in metabolites that may functionally contribute to plant susceptibility and resistance to pathogens. Plant disease occurs when there is a compatible plant-pathogen interaction. As a result of this interaction, plants release secondary metabolites as defensive compounds which counter the effects of the chemical molecules released by the pathogen to injure and infect the plant tissues.

Secondary metabolites produced are in response to wounding, infection or stress, and these compounds serve as active and potent defensive mechanisms in plants. Plant phenolics, terpenes, sesquiterpenoids and sterols play an important role in plant defence against pathogenic bacteria, fungi or insects (Harborne, 1991). Plants accumulate sterols and triterpenes as anti-microbial glycosides (Simons, 2006). Plant sterols are amphiphilic and

* Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. E-mail: nus_ali@yahoo.com
** Malaysian Palm Oil Board, P. O. Box 10620, 50720 Kuala Lumpur, Malaysia.
‡ Faculty of Environmental Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

occur as membrane constituents. Apart from their role in maintaining adequate function of the plant cell membranes, plant sterols also act as precursors of plant growth factors (Piironen *et al*., 2000). Plant sterols like sitosterol, stigmasterol and campesterol are in a solid state when they appear in bulk, with melting temperatures ranging from 140°C-170°C (Piironen *et al*., 2000). Sterols become more hydrophobic when their side chains get larger (Stumpf *et al*., 1987). Sterol alkaloids act as precursors in the synthesis of steroidal glycoalkaloids (Simons *et al*., 2006). Sterols can induce defensive mechanisms as a result of hydrolysis of the sterol glycoalkaloids by pathogenic fungus (Bouarab *et al*., 2002). Steroidal glycoalkaloids, also referred to as saponins, have a broad range of properties enabling them to act as anti-microbial and anti-pest agents, and may also possess allelopathic activity which altogether contributes to plant defence (Bowyer *et al*., 1995; Hostettmann and Marston, 1995; Papadopoulou *et al*., 1999). Saponins have the ability to complex with sterols, permeabilize membranes and inhibit the growth of fungi (Hostettmann and Marston, 1995).

In this work, we used the metabolomic approach to study plant defence metabolites induced during plant-pathogen interaction between the oil palm and its pathogen, the *G. boninense* fungus. Accumulation of sterol compounds in both tolerant and susceptible progenies was observed to measure the level of resistance of these progenies against *G. boninense* infections. A study on the proportion of sterol compounds in both infected and non-infected seedlings was necessary to confirm the involvement of steroidal compounds as defence metabolites.

MATERIALS AND METHODS

Plant Materials

Four-month-old oil palm seedlings, comprising both progenies which were tolerant (Zaire × Cameroon) and susceptible (Dumpy Elmina × Nigeria) to *G. boninense* infection, were obtained from the Malaysian Palm Oil Board (MPOB). These materials were then infected with inocula from rubber wood-blocks fully colonised with *G. boninese* mycelia (T1). Seedlings treated with inocula from uncolonised rubber wood-blocks were used as the control (T2), while untreated plants make up the absolute control (T3).

Homogenisation of Root Samples

Five grams of fresh root samples from each treatment, together with an adequate amount of liquid nitrogen, were pounded to a fine powder with a mortar and pestle prior to metabolite extraction.

Metabolite Extraction

Roots samples (1000 mg) which had been crushed using liquid nitrogen were mixed with an adequate amount of anhydrous sodium sulphate (Merck) in a mortar, then placed in a falcon tube containing 5.0 ml of methanol (MeOH) and left for two days at 4°C. The mixtures were then filtered using 0.4-μm nylon syringe filters (Nalgene). The samples were subsequently subjected to one-step column chromatography through 5% deactivated silica gel for the purposes of cleaning up and water absorption. Later, the compounds were collected and subjected to evaporation until dryness using a rotary evaporator (BUCHI B-491) at a maximum temperature of 38°C to yield 600 mg per sample. Each dried sample was diluted with 300 μl HPLC grade methanol (MeOH) and again filtered using a 0.4-μm nylon syringe filter (Nalgene), and finally kept at 4°C prior to GC-MS analysis.

GC-MS Analysis

An Agilent Technologies 6890N Network GC system equipped with Agilent Technologist 5973 Network mass selective detector was used to carry out GC-MS analysis. Chromatography was carried out with a DB5 capillary column (30 m long, 0.25 mm I.D. 155 and a 0.25-μm 5%-phenyl-methylpolysiloxane column with an additional 10 m integrated guard column). A standard 10-μl injection needle was mounted onto the auto sampler, and each sample (2 μl) was injected in split less mode. The carrier gas, helium, was at a flow rate of 0.7 ml min^{-1}; column temperature, 5 min at 180°C, 180°C-260°C at 3°C min^{-1}, 5 min at 260°C, 260°C-280°C at 0.2°C min^{-1}, and finally 5 min at 280°C; injector temperature at 280°C; and detector temperature at 290°C. The MS operating parameters were as follows: ionisation potential, 70 eV; ion source temperature, 290°C; quadrupole, 100°C; solvent delay, 7.0 min; scan speed, 2000 amu/s; scan range, 30-600 amu; and EV voltage, 3000 volts.

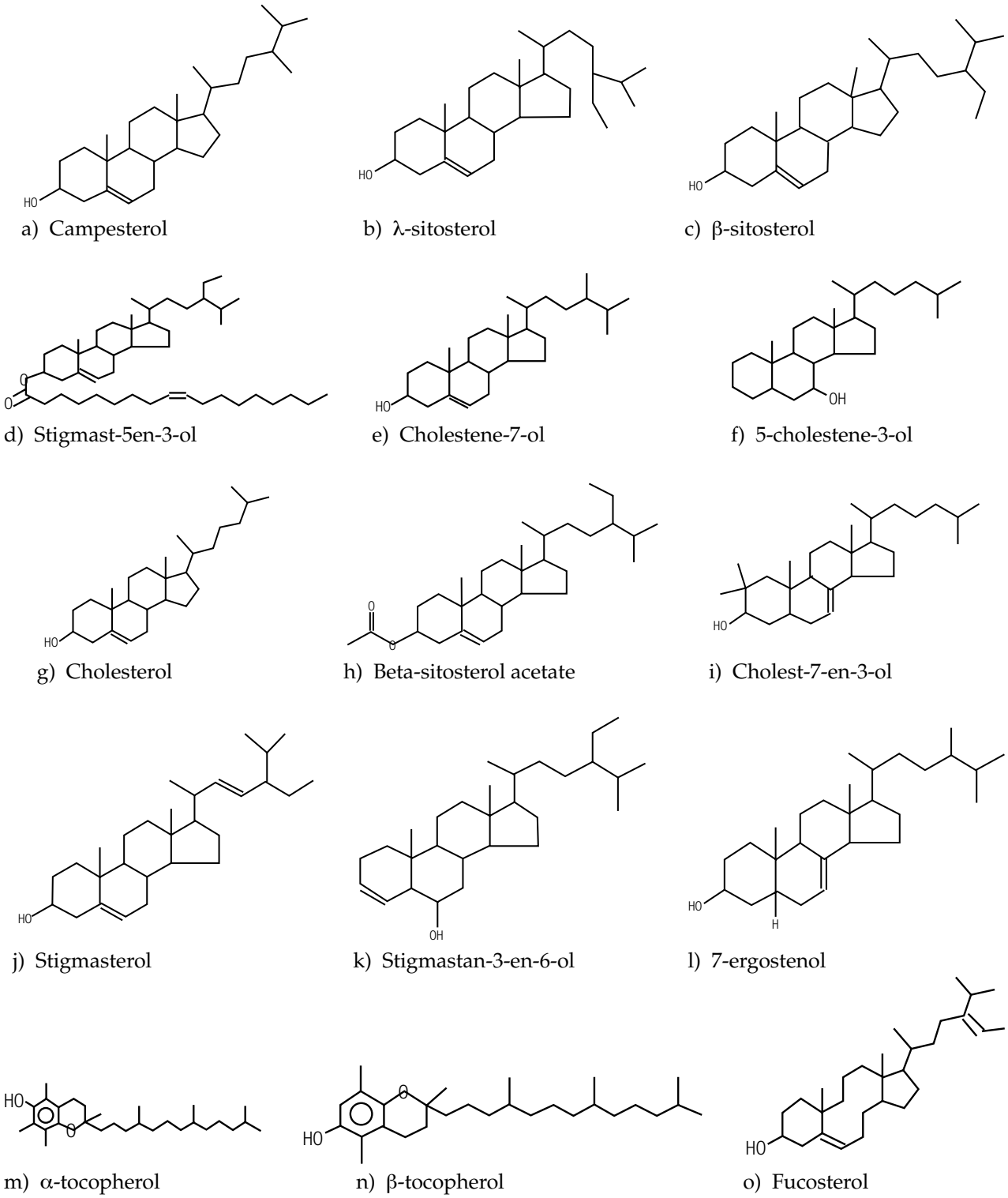
Identification of the Compounds

Identification of compounds present in the crude sample extracts was done by comparison of fragmentation patterns of mass spectra with those in reported literature (Massada, 1976; Adams, 2004) and computer matching with the Wiley 229, Nist 08 library data.

RESULTS AND DISCUSSION

The results of the GC-MS analysis on the treated oil palm seedling roots from susceptible and tolerant progenies leading to the identification of a number of sterol compounds are presented in *Table 1*.

A total of 13 sterol compounds were identified from the crude root extracts of both *Ganoderma*-inoculated tolerant and susceptible progenies. *Figure 1* illustrates structures of steroidal and tocopherols compounds detected in infected oil palm roots. The sterol compounds comprised about 30% of the total extracts. The β-sitosterol (*Figure 2*), γ-sitosterol and stigmasterol (*Figure 3*) were the major sterols present. These were followed by stigmast-5-en-3-ol, campesterol (*Figure 4*), cholestan, ergosterol (*Figure*



a) Campesterol b) λ-sitosterol c) β-sitosterol

d) Stigmast-5en-3-ol e) Cholestene-7-ol f) 5-cholestene-3-ol

g) Cholesterol h) Beta-sitosterol acetate i) Cholest-7-en-3-ol

j) Stigmasterol k) Stigmastan-3-en-6-ol l) 7-ergostenol

m) α-tocopherol n) β-tocopherol o) Fucosterol

Figure 1. Structures of sterol and tocopherol compounds present in Ganoderma boninense*-infected oil palm seedlings.*

TABLE 1. PHYTOSTEROLS PRESENT IN FRACTIONS OF MeOH EXTRACTS OF TREATED OIL PALM SEEDLING ROOTS

Compound	Retention time (min)	Amount[b] (%) T1	Amount[b] (%) T2	Amount[b] (%) T3	Molecular mass	Molecular formula
Phytosterols detected in root sample extracts from susceptible progeny						
Campesterol	16.952	1.65	1.05	NP	400	$C_{28}H_{48}O$
β-sitosterol	17.489	6.65	5.10	NP	414	$C_{29}H_{50}O$
Stigmasterol	17.484	7.86	NP	NP	412	$C_{29}H_{48}O$
γ-sitosterol	17.500	7.66	5.10	2.12	414	$C_{29}H_{50}O$
Fucosterol	17.605	NQ	NP	NP	412	$C_{29}H_{48}O$
Stigmast-5-en-3-ol	17.512	3.52	NP	NP	678	$C_{47}H_{82}O_2$
Cholestan	13.963	2.31	NP	NP	372	$C_{27}H_{48}$
7-ergostenol	16.946	2.04	NP	NP	400	$C_{28}H_{48}O$
α-tocopherol	14.399	1.55	0.82	0.46	430	$C_{29}H_{50}O_2$
β-tocopherol	15.780	0.92	0.59	0.48	416	$C_{29}H_{48}O_2$
Phytosterols detected in root sample extracts from tolerant progeny						
Campesterol	16.952	2.56	1.67	NP	400	$C_{28}H_{48}O$
β-sitosterol	17.489	7.31	6.67	NP	414	$C_{29}H_{50}O$
Stigmasterol	17.484	8.24	NP	NP	412	$C_{29}H_{48}O$
γ-sitosterol	17.500	8.12	5.39	3.11	414	$C_{29}H_{50}O$
Stigmast-5-en-3-ol	17.512	4.11	NP	NP	678	$C_{47}H_{82}O_2$
Cholestan	13.963	2.71	NP	NP	372	$C_{27}H_{48}$
7-ergostenol	16.946	1.95	NP	NP	400	$C_{28}H_{48}O$
Fucosterol	17.605	NQ	NP	NP	412	$C_{29}H_{48}O$
α-tocopherol	14.399	1.89	1.05	1.09	430	$C_{29}H_{50}O_2$
β-tocopherol	15.780	1.02	0.43	0.31	416	$C_{29}H_{48}O_2$

Note: NQ = detected but not at quantifiable levels.
[a]NP = not present.
[b]Determined by area normalization method.
T1: root samples inoculated with *G. boninense* mycelia colonising rubber wood-blocks.
T2: root samples inoculated with uncolonised rubber wood-block (control).
T3: uninoculated seedlings (absolute control).

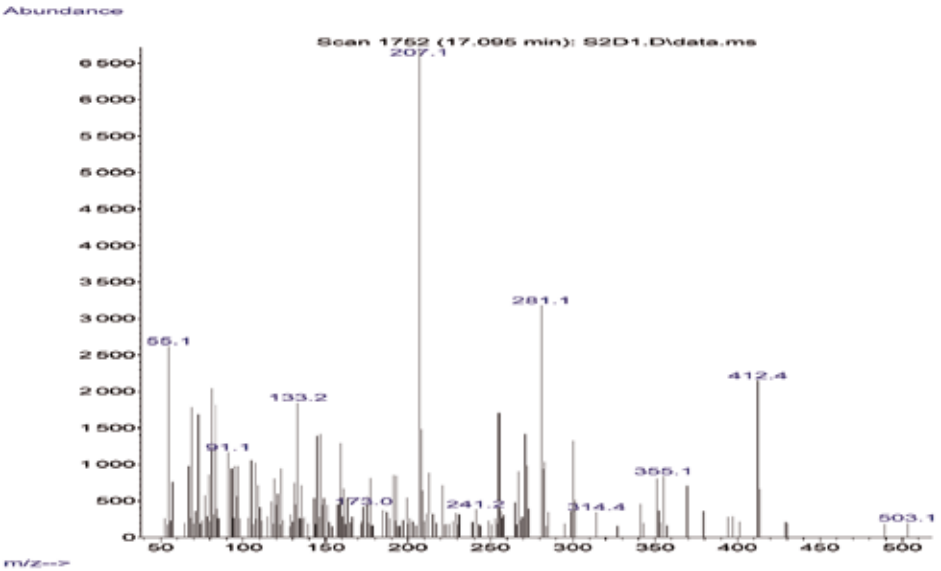


Figure 2. Mass spectrum of the compound eluting at 17.4 min, identified as sitosterol in Ganoderma boninense*-infected oil palm seedlings.*

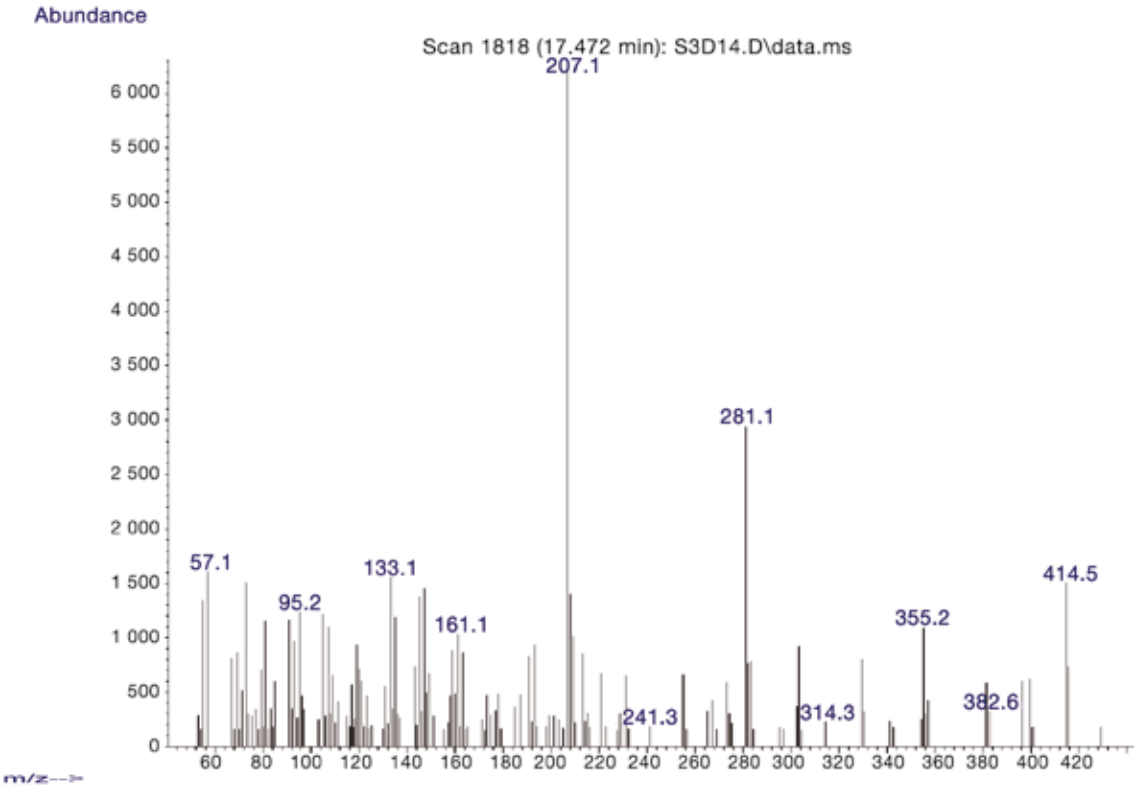


Figure 3. Mass spectrum of the compound eluting at 17.0 min, identified as stigmasterol in Ganoderma boninense*-infected oil palm seedlings.*

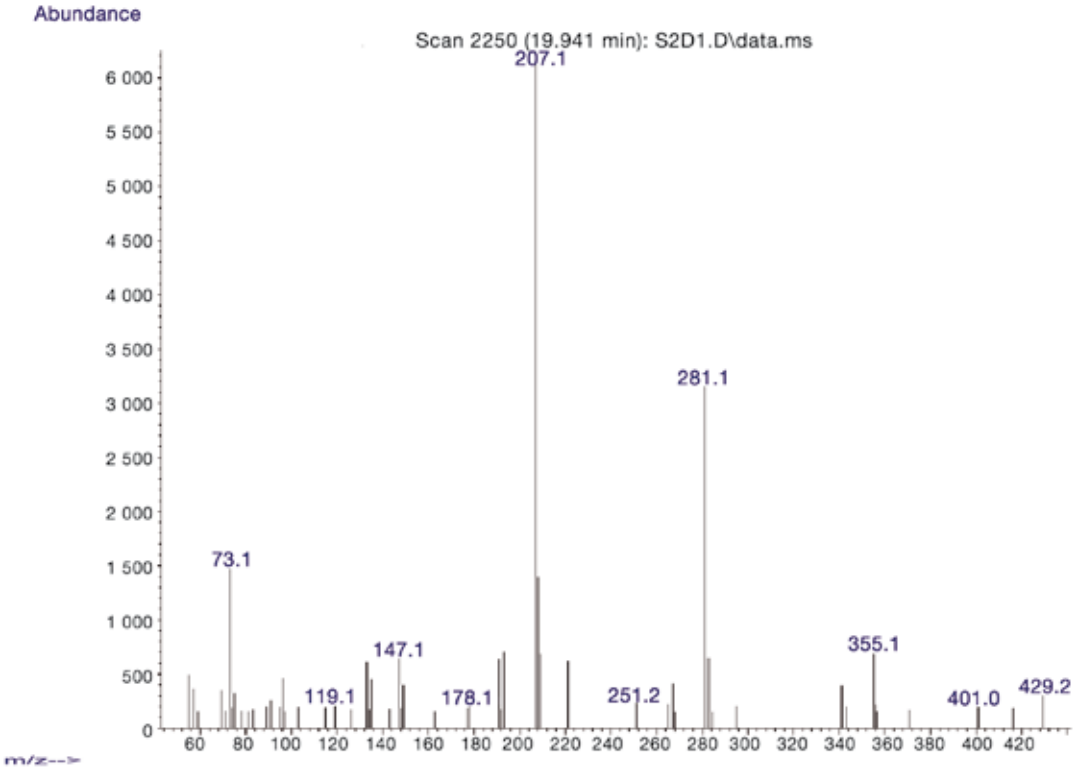


Figure 4. Mass spectrum of the compound eluting at 19.9 min, identified as campesterol in Ganoderma boninense*-infected oil palm seedlings.*

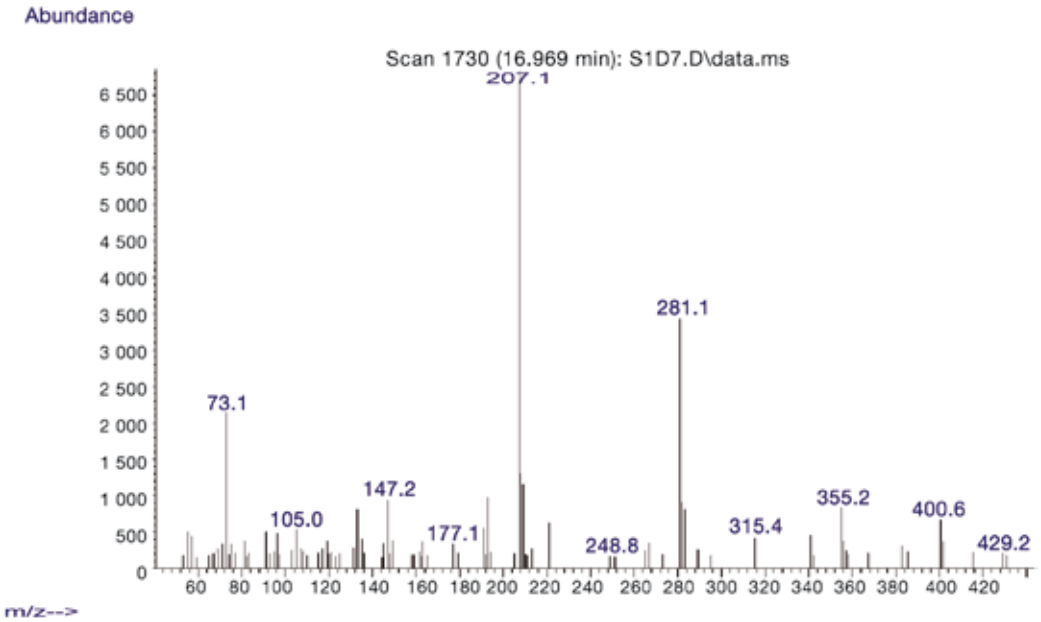


Figure 5. Mass spectrum of the compound eluting at 16.9 min, identified as 7-ergostenol in Ganoderma boninense*-infected oil palm seedlings.*

5), α-tocopherol and β-tocopherol. Sitosterols were the most abundant sterol compounds identified in both the tolerant (15.43%) and susceptible (14.31%) progenies. Sitosterol, stigmasterol and campesterol were classified as typical plant sterols. Most of the sterol compounds were found to have the ability in regulating membrane fluidity but with different efficiencies, while it has been reported that sitosterol and campesterol are the most efficient (Schuler *et al*., 1991).

Accumulation of sterol compounds in seedlings treated with *G. boninense* was higher in type and percentage (*Table 1*) of each compound compared to their accumulation in the control and absolute control. Compounds present in the extracts of both controls were γ-sitosterols, β-sitosterols and campesterol. The 7-ergostenol and fucosterol were only present in *G. boninense*-infected oil palm seedling roots. In this study, fucosterol was detected but was not at a quantifiable level. Gaulin *et al.* (2010) reported that they identified fucosterol as the major sterol through a sterol biosynthesis pathway in the legume pathogen *Aphanomyces euteiches*. Elliott (1977) also reported fucosterol as a common oomycete sterol. The reason why unquantifiable levels of fucosterols were detected in this study can be explained by the findings of Warner *et al*. (1982) who found that fucosterols are utilised efficiently by parasitic lower fungi, namely *Peronosporales* species, in producing 7-ergostenol through their sterol synthesis pathway.

Other sterol compounds present showed a higher level of accumulation in all root samples infected with *G. boninense* fungi. These findings suggest that steroidal compounds play an important role as plant defence metabolites apart from functioning as plant growth regulators (Faure *et al*., 2009) and regulating the fluidity of plant membranes for adaption to changes in temperature (Piironen *et al*., 2000). According to Delazar *et al*. (2010), to date the physiological roles of phytosterols as plant defence compounds are still not fully understood but they are known to possess properties such as being anti-fungal, insect-deterrent, mould-inhibiting and antimicrobial (Gus-Mayer *et al*., 1994; Morrisey and Osbourn, 1999). Piironen *et al*. (2000) reported that sterols also act as substrates for certain secondary metabolites such as glycoalkaloids, cardenolides and saponins. Osbourn (1996) stated that sterol saponins exhibit potent anti-fungal activity in monocotyledonous plants, and that they are present at high levels. According to Osbourn (1996), saponins can be divided into triterpenoid, steroid or steroidal glycoalkaloid depending on the structure of the aglycone. Steroidal saponins occur mainly in monocotyledonous plant families such as that of oil palm as well as *Liliaceae, Dioscoraceae* and *Agavaceae*, while triterpenoid saponins are found primarily in dicotyledonous plants and in some monocots.

Work by Osbourn (1996) on tomato and oats revealed the potential role of steroid saponins as defence metabolites against pathogenic fungi. Another study by Defago and Kern (1983) using tomato, on its resistance to the pathogenic *Fusarium solani*, showed that higher levels of resistance towards fungal attacks were observed in tomato with a high sterol content in its membrane composition compared to that with a low sterol content. Armah *et al.* (1999) suggested that aggregation of the saponin-sterol complexes in the membrane is probably

mediated by interactions between sugar residues. The saponin sugar chain attached to C-3 is crucial as a membrane permeabilizer and anti-fungal activator of saponins. Hydrolization of these sugar residues can result in the loss of the defence mechanism (Crombie *et al.*, 1986; Keukens *et al*., 1995; Wubben *et al*., 1996; Sandrock and van Etten, 1998; Armah *et al*., 1999). Some pathogenic fungi tend to detoxify complex steroidal saponins to decrease or even stop the anti-fungal activity of these compounds. This is done by producing glycosyl hydrolases that remove sugars from the sugar chain attached to C-3 of the saponin backbone, thus preventing the saponin from complexing with membrane sterols (Osbourn, 1996). Carter *et al.* (1999) observed that a collection of oat plants inhibiting fungi from different taxonomic groups showed the ability to hydrolyse sugar from the sterol-saponin complex, namely avenacins that function as antifungal compounds. The ability of pathogenic fungi to detoxify defence metabolites has always been the winning strategy of the pathogens in the plant-pathogen war.

The α-tocopherol and β-tocopherol were also detected in the GC-MS analysis of the extracted samples. Tocopherols are lipophilic antioxidants synthesised by all plants. Tocopherol reacts with the polysaturated acyl groups of lipids, stabilises membranes and scavenges various reactive oxygen species (ROS) and lipid-soluble compounds which are by-products of oxidative stress (Wang and Quinn, 2000). The percentage of the accumulated α-tocopherol and β-tocopherol in all samples was the lowest among all the steroidal compounds detected. However, both tocopherol percentages were higher in the infected samples compared to those of the control and absolute control. This was probably because tocopherols play a crucial role in the defence system as enzyme co-factors and antioxidants influencing higher plant growth and development by modifying processes from mitosis and cell elongation to senescence and death (Shao *et al*., 2007). Munne-Bosch and Algere (2003) stated that tocopherol levels increase in photosynthetic plant tissues in response to a variety of abiotic stresses. Findings by Munne-Bosch and Algere (2003) and Shao *et al*. (2008) supported the results obtained in this study which focused on biotic stress in that it produced the same observations as abiotic stresses.

CONCLUSION

GC-MS analysis has enabled the identification of compounds present in oil palm root extracts artificially infected by *G. boninense*, with sterol and tocopherol compounds being among the biggest fractions. The results suggest that sterol compounds and tocopherols may play a role in the plant's defence system. Tolerant progeny produced higher percentages of sterol compounds and tocopherols compared to susceptible progeny; thus, these compounds probably contributed to the higher level of resistance to *G. boninense* infection.

ACKNOWLEDGEMENTS

The authors thank Dr Sanimah Simoh of the Malaysian Agricultural Research and Development Institute (MARDI) for her guidance in the use of GC-MS equipment, and the Agro-Biotechnology Institute (ABI) for allowing us to use their GC-MS equipment. The authors also acknowledge the financial support from the Malaysian Palm Oil Board (MPOB) through a collaboration grant between MPOB and Universiti Putra Malaysia (UPM).

REFERENCES

ALLWOOD, J; ELLIS, D and GOODACRE, R (2007). Metabolomic technologies and their application to the study of plants and plant-host interactions. *Plant Physiology, 132*: 1111-1139.

ARMAH, C N; MACKIE, A R; ROY, C; PRICE, K; OSBOURN, A E; BOWYER, P and LADHA, S (1999). The membrane permeabilising effect of avenacin A-1 involves the reorganisation of bilayer cholesterol. *Biophysic J., 76*: 281-290.

ADAMS, R P (2004). *Identification of Essential Oil Component by Gas Chromatography/Quadrupole Mass Spectroscopy.* Illinois, USA.

BOUARAB, K R; MELTON, J; PEART, D; BAULCOMBE and OSBOURN, A E (2002). A saponin-detoxifying enzyme mediates suppression of plant defences. *Nature, 418*: 889-892.

BOWYER, P B R; CLARKE, P; LUNNESS, M J; DANIELS and OSBOURN, A E (1995). Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. *Science, 267*: 371-374.

CARTER, J P; SPINK, J; CANNON, P F; DANIELS, M J and OSBOURN, A E (1999). Isolation, characterization and avenacin sensitivity of a diverse collection of cereal-root-colonizing fungi. *Applied Environmental. Microbiology*, *65*: 3364-3372.

CROMBIE, W M L; CROMBIE, L; GREEN, J B and LUCAS, J A (1986). Pathogenicity of the take-all fungus to oats: its relationship to the concentration and detoxification of the four avenacins. *Phytochemistry, 25*: 2075-2083.

DEFAGO, G and KERN, H (1983). Induction of *Fusarium solani* mutants insensitive to tomatine, their pathogenicity and aggressiveness to tomato fruits and pea plants. *Physiological Plant Pathology, 22*: 29-37.

DELAZAR, A; NAZIFI, E; MOVAFEGHI, A; NAZEMIYEH, H; HEMMATI, S; NAHAR, L and SARKER, S D (2010). Analyses of phytosterols and free radical scavengers in the bulbs of *Ornithogalum cuspidatum* Bertol. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 9 (2)*: 87-92.

ELLIOTT, C G (1977). *Advance Microbiol Physiology, 15*: 121-173.

GAULIN, E; BOTTIN, A and DUMAS, B (2010). Sterol biosynthesis in oomycete pathogens. *Plant Signaling and Behavior, 5(3)*: 258-260.

GUS-MAYER, S; BRUNNER, H; SCHNEIDER-POETSCH, H A and RUDIGER, W (1994). *Avenacosidase* from oat: purification, sequence analysis and biochemical characterization of a new member of the BGA family of betaglucosidases. *Plant Molecular Biology, 26*: 909-921.

HARBORNE, J B (1991). Role of secondary metabolites in chemical defence mechanisms in plants. *Bioactive Compounds from Plants* (Chadwick, D J and Marsh J eds.). John Wiley and Sons, Chichester. p. 126-130.

HOSTETTMANN, K and MARSTON, A (1995). Saponins. *Chemistry and Pharmacology of Natural Products.* Cambridge University Press, Cambridge, United Kingdom.

KEUKENS, E A J; DE VRIJIE, T; VAN DEN BOOM, C; WAARD, D E; PLASMNA, P H H; THIEL, F; CHUPIN, V; JONGEN, W M F and DE KRUIJFF, B (1995). Molecular basis of glycoalkaloid induced membrane disruption. *Biochemical Biophysic. Acta, 1240*: 216-228.

MASSADA, Y (1976). *Analysis of Essential Oil by Gas Chromatography and Mass Spectrometry.* John Wiley and Sons, New York, USA.

MORRISSEY, J P and OSBOURN, A E (1999). Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiol Molecular Biology Review, 63*: 708-724.

MUNNE-BOSCH, S and ALGERE, L (2003). Drought-induced changes in the redox state of alpha-tocopherol, ascorbate, and the diterpene carnosic acid in chloroplasts of Labiatae species differing in carnosic acid contents. *Plant Physiology, 131*: 1816-1825.

NES, W R (1987). Multiple roles for plant sterols. *The Metabolism, Structure and Function of Plant Lipids* (Stumpf, P K; Mudd, B J and Nes, W R eds.). Plenum, New York. p. 3-9.

NUSAIBAH, S A (2007). *Morphological and Molecular Characterization of* Ganoderma *Species from Varies Host in Peninsular Malaysia.* M.Sc. thesis, Universiti Sains Malaysia, Minden, Pulau Pinang, Malaysia.

OSBOURN, A E (1996). Performed antimicrobial compounds and plant defense against fungal attack. *The Plant Cell*, *8*: 1821-1831.

PAPADOPOULOU, K R E; MELTON, M; LEGGETT, M J; DANIELS, M J and OSBOURN, A E (1999). Compromised disease resistance in saponin-deficient plants. *Proc. of the National Academy of Science USA, 96*: 12923-12928.

PILOTTI, C A (2001). *Genetic Studies of* Ganoderma *spp. Associated with Oil Palm in Papua New Guinea.* Ph.D thesis, Department of Botany, University of Queensland, Australia.

PIIRONEN, V; LINDSAY, D G; MIETTINEN, T A; TOIVO, J and LAMPI, A M (2000). Plant sterols: biosynthesis, biological function and their importance to human nutrition. *J. Science of Food and Agriculture, 80*: 939-966.

SANDROCK, R W and VAN ETTEN, H D (1998). Fungal sensitivity to and enzymatic degradation of the phytoanticipin a-tomatine. *Phytopathology, 88*: 137-143.

SHAO, H B; CHU, L Y; LU, Z H and KANG, C M (2008). Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. *International J. Biological Sciences, 4 (1)*: 8-14.

SCHULER, I; MILON, A; NAKATANI, Y; OURISSON, G; ALBRECHT, AM; BENVENISTE, P and HARTMANN, M A *(*1991*).* Differential effects of plant sterols on water permeability and on acyl chain ordering of soybean phosphatidylcholine bilayers. *Proc. of the National Academy of Science USA, 88*: 6926-6930.

SIMONS, V; MORRISSEY, J P; LATIJNHOUWERS, M; CSUKAI, M; CLEAVER, A; YARROW and OSBOURN, A E (2006). Dual effects of plant steroidal alkaloids on *saccharomyces cerevisiae. Antimicrobial Agents and Chemotherapy*, *50(80)*: 2732-2740.

WANG, X and QUINN, P J (2000). The location and function of vitamin E in membranes (review). *Molecular Membrane Biology*, *17*: 143-156.

WARNER, S A; EIERMAN, D F; SOVOCOOL, G W and DOMNAS, A J (1982). *Proc. of the National Academy of Science USA, 79*: 3769-3772.

WUBBEN, J P; PRICE, K R; DANIELS, M J and OSBOURN, A E (1996). Detoxification of oat leaf saponins by *Septoria avenae*. *Phytopathology, 86*: 986-992.