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Detection and Control of *Ganoderma boninense* in Oil Palm Crop

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Preface

Oil palm is the world's highest oil crops' producer with potential yield capacity 10–15 times higher compared to other oil crops planted on the same size of land. Increases in global demand for edible oil and biofuel, driven by the increasing population, remain the main factors driving up the expansion of oil palm cultivation in South East Asia (SEA) and other regions of the world. Currently, Malaysia and Indonesia are the two countries that contribute up to 90% of world's palm oil export. Unfortunately, the oil palm industry in SEA is under threat of a devastating disease. This disease is known as basal stem rot (BSR) which is caused by a fungus known as *Ganoderma boninense.* With no known remedy at present, BSR disease continues to erode the profitability of the oil palm industry and created a significant concern globally.

This book is a joint effort by the authors who are currently working actively on finding suitable detection and management methods for BSR disease in oil palm. With immense experience in the field, this book provides good information with backup data covering both detection and management strategies of *Ganoderma.* The six chapters in this book address many current issues in tackling the pathogen and the development of sustainable disease management programs of BSR. There are including; an introduction to the oil palm industry in global perspective and its future potential (Chap. 1), The pathogenic nature of *Ganoderma* (Chap. 2), Some of the current detection methods of *G. boninense* (Chap. 3), Control methods of the pathogen, which cover cultural practices, chemical control, development of disease resistance and biological control (Chap. 4) and more in-depth review of latter subject in control of *G. boninense* using combination of biocontrol agents (Chap. 5). This chapter gives an in-depth information on the use of biological approaches in controlling *G. boninense* to meet the current oil palm–environmental dilemmas and demands for more eco-friendly practices in the field. The last chapter (Chap. 6) concludes the contents of this book and summarizes the discussed matters as well as suggests several recommendations for future research or further improvements.

This book serves as an exclusive source of information on BSR caused by *G. boninense.* This book will make an essential contribution to the oil palm industry and will be a valuable reference and guide for planters, agricultural students,

agronomists, and all those working in the oil palm industry. The authors believe that this book will complement the existing books on different approaches in the similar field as it discussed in-depth details and guidance on controlling the BSR disease using biological means, which is the unique feature of the book itself.

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Chapter 1
Introduction

Abstract Oil palm is a tropical tree mainly grown for the production of palm oil. Palm oil is an edible oil extracted from the mesocarp of the oil palms fruit of African oil palm *Elaeis guineensis* Jacq. Besides edible usage, palm oil also possesses suitable materials for non-edible properties used in soap making, candles, cosmetic products and in rubber processing. This crop is a highly valuable and profitable crop as even its waste materials can be utilized. An attractive approach in utilizing oil palm wastes in the production of eco-friendly biofuel has increase the value of oil palm crop, hence, making this crop more efficient than other oil crops. Currently, oil palm has become the world's leading vegetable oil crop. The total land area for oil palm cultivation has increased over the years, where most of the cultivated area are in South East Asia countries.

1.1 World Oil Palm Industry

Oil palm which is scientifically known as *Elaeis guineensis* Jacq. is originally native to West and South Africa. It grows wildly along the coastal swampland and freshwater riverines. It belongs to the palm family, Palmae, order Spadiciflorae and is grouped with Cocos (the coconut) and other genera in the subfamily of Cocosoidae. *Elaies* is derived from the Greek word elaion, meaning oil. Meanwhile, the word *guineensis* is refering to its place of origin. Oil palm is a tropical crop which can grow in a tropical climate with plenty of sunshine and rain with year round temperature approximately 25–28 °C. It also grows on a wide variety of soils ranging from sandy soils to lateritic red and yellow podzols, young volcanic soils, alluvial clays and peat soils (Obire and Putheti 2010).

Oil palm begins to produce its fruits at a very early stage. Normal oil palm tree usually starts to bear fruits after 30 months of planting and continues to be productive for the next 20–30 years, thus ensuring a consistent supply of oil. An oil palm tree also produces between 8 and 12 bunches of fruits in a year, thus, there are several harvests per year. Oil palm fruits are like large reddish plums and clustered in large bunches weighing 10–40 kg and contain 1000–3000 fruitless which are

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rich with oils. There are two distinct types of oil that can be extracted from the oil palm trees, which are palm oil and palm kernel oil. Crushing and extracting the oil from the oil palm fruits produce palm oil. Meanwhile, palm kernel oil is obtained by crushing and extracting the oil from the seed or kernel of the fruit. Apart from oil, almost every part of the palm can be utilised. Its byproducts include vast array of consumable and non-consumable products. It is also becoming more popular as a biofuel. Laws that encourage the use of biofuels are adding to demand. The international trade of oil palm sparked during europe industrial revolution in the 19th century when demand soared due to the use of its oil as lubricant in steam engines and other machineries and soap. Since then, the oil palm cultivation expanded to other places in the world.

Currently, oil palm plantation worldwide has taken up more than 14 million hectares where most of it are grown in the South East Asia countries. With that figure, the total global production of palm oil has increased to 62.6 million tonnes in 2015, where this volume was mainly produced by Indonesia and Malaysia (see Fig. 1.1). Alongside the two giant producers from South-East Asia, significant crop expansion is also occurring in other countries such as Thailand, Papua New Guinea, Costa Rica, Colombia, Ecuador, Cameroon and the Democratic Republic of Congo. Currently, the palm oil production has dominate over 31% of the world oil and fats production (see Fig. 1.2). The oil palm production has been steadily increased over the years, exceeding the production volume of the second biggest oilseed crop by more than 15 million tonnes. Palm oil offers many advantages over other competing vegetable oils. It is the most efficient oil bearing crop in the world, requiring only 0.26 ha of land to produce one tonne of oil while soybean, sunflower and rapeseed

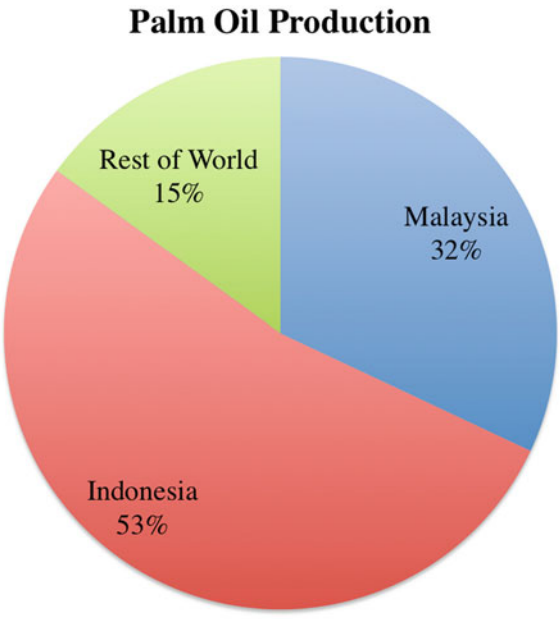

Fig. 1.1 World palm oil production in 2015 where almost half of it was grown in Indonesia (53%), with Malaysia contributing 32 and 15% from other places over the world. *Source* European Palm Oil Alliance

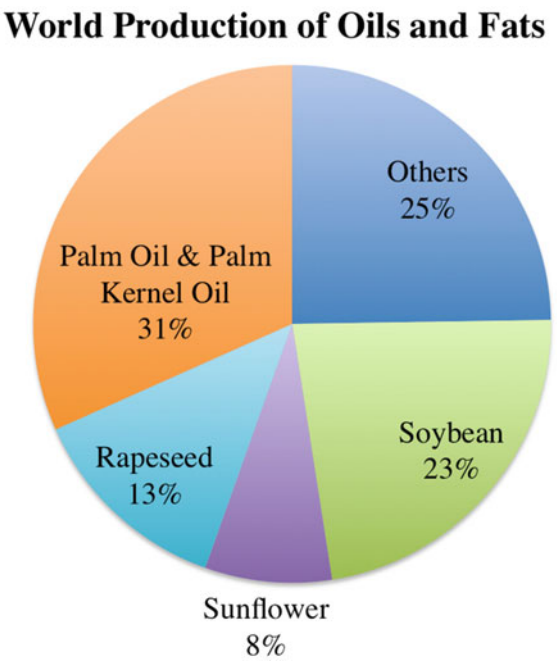

Fig. 1.2 World production of oils and fats in 2015 with the total production of 204 million metric tonnes. *Source* Oil World

require 2.22, 2 and 1.52 ha, respectively, to produce the same amount of oil. Although oil palm accounts for the smallest percentage (5.5%) of all the cultivated land globally, but it produces the largest percentage (31%) of total output (see Figs. 1.2 and 1.3).

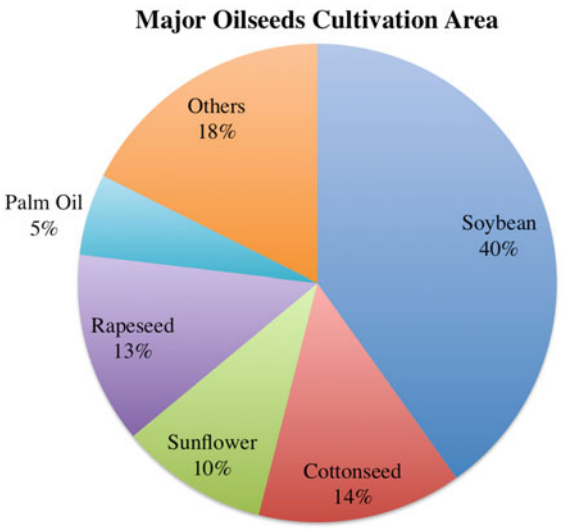

Fig. 1.3 Area of major oilseeds crop cultivated globally with the total of 258.9 million ha. *Source* Oil World

Among the major oils and fats, palm oil was the highest consumed oil in 2015, reaching almost three billion people in 150 countries and accounting 61.57 million metric tonnes of global consumption, making it the most consumed oil in the world. The main consumers countries are including China, India, Indonesia and European Union. In line with the world demand, palm oil is the most traded oil in the world, accounting almost 90% of the global oil production traded. Since its first trades in the 1980s, the global trade has increased from 3.78 million tonnes to 47.8 million tonnes in 2015 (Oil World). Indonesia and Malaysia dominated the palm oil market and represented 90.7% of the 2015 exports, accounting 54.2 and 36.5% respectively. Meanwhile, far, far behind in third place lied Papua New Guinea with only 1.1% of palm oil exported in 2015. With the growth in demands, world cultivation and production of palm oil should continue in the upcoming years, to satisfy food and biofuels requirements.

References

European Palm Oil Alliance (ND) Palm Oil Production. Retrieved online 17 Oct 2016 from http://www.palmoilandfood.eu/en/palm-oil-production

Obire O, Putheti RR (2010) The oil palm tree: a renewable energy in poverty eradication oping countries. Drug Interv Today 2:34–41

Oil World (2015) Annual Report 2015

Chapter 2
Pathogenic Nature of *Ganoderma boninense* and Basal Stem Rot Disease

Abstract The oil palm industry is under threat of a prevailing incurable disease called Basal Root Stem (BSR), which is caused by a white rot fungi, known as *Ganoderma boninense*. With no current remedy at present, BSR is the major disease in oil palm plantations of SEA and, therefore, of great economic importance to the world oil palm industry, especially to Malaysia and Indonesia, which are oil palm major producers and exporters. The disease is highly associated with the decay of lower stem, leading to severe symptoms such as unopened and flattening spear leaves. There are numerous mode of infection associated with the epidemiology of *G. boninense* in oil palm plantation, including in-contact roots with nearby diseased palms and through airborne basidiospores. Deep insight on the route of infection and mycological pathogenicity behaviour of the pathogen is the greatest priority in order to successfully develop effective management practices for disease control.

2.1 Biology and Epidemiology of *G. boninense*

Ganoderma boninense is a polyporoid fungus which grows on wood. It belongs to the family of Ganodermataceae and Classed under Basidiomycetes (Idris 2009). It is a lignolitic fungus which commonly belongs to white rot fungi and known for their capability in degrading the lignin component of wood while leaving the white cellulose exposed (Paterson 2007). Therefore, this fungus is more active in degrading lignin compared to other groups.

In early reports, a number of *Ganoderma* species have been reported to be associated with the basal stem rot of oil palm (Turner 1981). Among them are *G. applanatum, G. boninense, G. chalceum*, *G. lucidum*, *G. miniatocinctum*, *G. pseudoferreu* and *G. tornatum*. Based on morphology of basidiomata and basidiospores collected from oil palm fields or induced in vitro, Khairudin (1990) concluded that two species namely *G. boninense* and *G. tornatum* are the causal pathogen of BSR. However, in later reports, after isolation of real pathogenic isolates of *Ganoderma*, it was identified that *G. boninense, G. zonatum* and

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G. miniatocinctum to be associated with BSR disease. After the general consensus, it was apparent that *G. boninense* is the main species pathogenic to the oil palm (Moncalvo 2000). In addition to this report, *G. boninense* was also found to be the most common and virulent species than the other two (*G. zonatum* and *G. miniatocinctum*) in several estates with high incidence of BSR disease (Idris 1999). While, *G. tornatum, G. applanatum, G. lucidum, G. pfeifferi* and *G. philippi* were not pathogenic (Idris et al. 2000).

Unfortunately, until today, studies have shown there are some uncertainties regarding the identity of the species of *Ganoderma* causing BSR in different countries leading to confusion in the identification process, thus, leading to inefficient disease management. Miller et al. (2000) also reported a great deal of variability between strains of *G. boninense*. This species also appears to have broad host specificity and there is conflicting information on its characteristics and also its relationship to species associated with previous cropping or vegetation. Higher incidences of BSR were observed in oil palm planted in ex coconut planting compared to ex rubber plantings (Turner 1981). This conflict has lead to the fungal variability related to the capacity of the species to adapt and survive in different substrate for a long time.

Over the last few decades, several studies have been conducted on the molecular aspect of these *Ganoderma* spp. Studies have shown high level of genetic variation among monokaryons of *G. boninense*, suggesting *G. boninense* is genetically heterogenous which could be caused by different geographical locations or outcrossing of the isolates over generations (Miller et al. 2000; Pilotti et al. 2003) whereby the pathogen could originated from the same species containing wide genetic variation or from closely related species (Zakaria et al. 2005). Pilotti (2005) has revealed *G. boninense* is heterothallic with a tetrapolar mating system and multiple alleles at both mating type loci; this favours out-breeding within a population (Sanderson and Pilloti 1997; Pilotti 2005). Thus, this out-crossing ability is responsible for the wide genetic variation found in the *G. boninense* population on oil palm.

Although the morphology of the genus *Ganoderma* remains confusing (Miller et al. 1999), it is now seems to be accepted (Ho and Nawawi 1985; Khairudin et al. 1991) that *G. boninense* is the predominant species responsible for BSR disease occurrence and development. Lim and Fong (2005) also suggested that BSR infection on oil palm tree could possibly be from different strains of the same *Ganoderma* species. Evidently, the identity of the pathogen is crucial in deciding the most efficient and economic disease management, hence, more studies on the identification of *Ganoderma* spp. should be conducted.

Based on in vitro study reported by Idris et al. (2000), the colonies of *G. boniense* was characterized morphologically as white in colour on the surface and the reverse was darkened (pigmented). Cultures of *G. boninense* had an undulating surface in the darkened regions that buckled the agar. *Ganoderma boninense* can grow at pH 3–8.5 at the optimum temperature of 30 °C, critically hindered at 15 and 35 °C, and unable to grow at 40 °C. Idris et al. (2000) also demonstrated when basidiomata of *G. boninense* was artificially induced on rubber

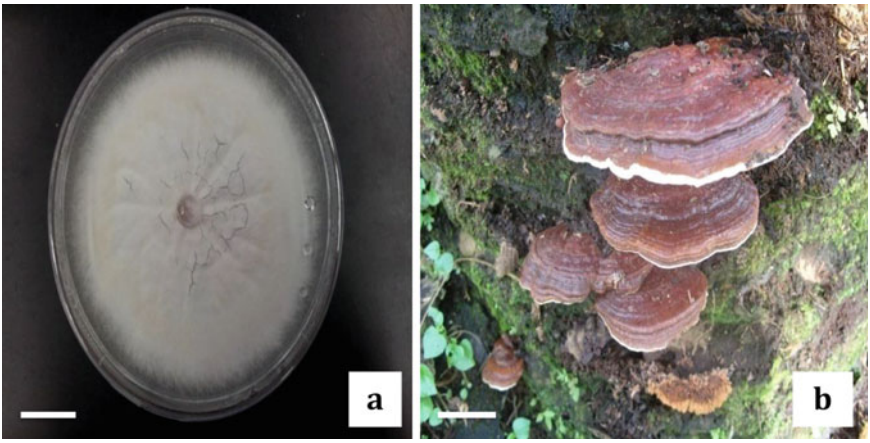

Fig. 2.1 Morphology of different stages of *G. boninense*. **a** Mycelial of *G. boninense* grown on potato dextrose agar (PDA), after 14 days of incubation at 28 °C. **b** Basidiocarps of *Ganoderma* on the trunk of infected oil palm tree. Bar scale: **a** 2 cm; **b** 5 cm

wood block (RWB), its formation was first identified by the appearance of a white mycelium after one to three weeks of incubation on RWB, which then developed into small, white, button-like structure. The apical end began expanding rapidly giving rise to bracket-like structures which were generally white when first formed, but as their length and width increased rapidly, the upper surface developed various yellowish-brown colour with concentric zonations (Idris 2009). *Ganoderma* are characterized by their large, perennial and woody basidiocarps (Fig. 2.1). Their fruiting bodies typically grow in a fan-like manner on the trunks of infected palms, with double-walled, truncate spores with yellow to brown ornamented inner layers.

2.2 Basal Stem Rot Disease

Oil palm is one of the most efficient and important crops in the world. Oil palm has contributed in uplifting the quality of life of people and has helped alleviate poverty among landless farmers. However, the never-ending problem of BSR disease has affected the production of oil palm and burden planters especially smallholders and farmers. BSR was once only found to be infecting older plants, but recently it has been found in seedlings (Sanderson 2005) and younger plants where symptoms appear earlier and are more severe, leading to greater replanting (Susanto et al. 2005). Losses begin to have a financial effect once the disease affects more than 10% of the stand (Hasan and Turner 1998). Losses due to BSR can occur not only through the direct reduction in oil palm numbers in the stand, but also through the reduction in the number and weight of fruit bunches from infected palms and those with subclinical infection (Flood et al. 2000). On average there is a decline of the

yield of the fresh fruit bunch (FFB) of 0.16 tonne per hectare for every palm lost, and when the stand had declined by 50% the average FFB yield reduction was 35% (Subagio and Foster 2003). Malaysia has recorded a yearly losses up to RM 1.5 billion (500 million USD) due to BSR.

In new oil palm planted area such as jungle or conversion from ex-rubber planting, BSR incidence of 25% has been recorded after 25 years of planting, while in ex-coconut planted area, an incidence of 60% occurred after 16 years (Singh 1991) whereas oil palm to oil palm under planting has resulted in 33% infection after 15 years. High incidence of BSR occurs in Malaysia and Indonesia with lower incidences recorded in Africa, Papua New Guinea and Thailand (Idris et al. 2004). A survey by Subagio and Foster (2003) on some oil palm plantations in Indonesia indicated the BSR infection rate was 70% in certain areas of a second planting cycle after 15 years of growing. Similar infection rates were also reported in Malaysia (Ariffin et al. 1996; Singh 1991). In Malaysia, high incidence of BSR disease was recorded when replanting to be placed in coastal clay soil area (Hasan and Turner 1998). This disease seemed to remain confined to the coastal areas, indicated the nature of the soil and its water retentions may have bearing on the disease development. Soils in coastal areas are mainly clays, silty clays or clay loams with poor internal drainage and high water retention capacity. However, BSR was also reported in peat soils, which were once thought to be non-conducive for the BSR disease (Cooper et al. 2011), serious incidences of the BSR disease have been also reported in these areas (Ariffin et al. 1989; Rao 1990).

2.3 Symptoms and Disease Development

Infection of *G. boninense* progresses slowly without any symptoms, thus, making it difficult to be recognized at the early stage. However, when the infection progresses to 60–70%, the symptoms begin to emerge. The earliest external symptoms of basal stem rot of oil palms occur in the foliage, generally after at least half of the cross-sectional area of the stem base has been destroyed. Decay leads to a restriction of water and nutrient supply to the aerial parts, causing symptoms resembling to those of water stress and malnutrition (Turner and Gillbanks 1974).

In young palms, the external symptoms of BSR usually comprise a one-sided yellowing or mottling of the lower frond, followed by necrosis (Singh 1991). Young unfolded leaves will become chlorotic and may be reduced in length, sometimes with necrotic on the tips. Similar symptoms also observed in mature palms, with multiple unopened spears, flattening of the crown, generally pale leaf canopy and production of basidiocarpsbasidiocarps (Turner 1981). Basidiomata may develops at the stem base, leaf base, or infected root; the location provides a guide to the diseases area inside the palms (Paterson 2007). In severe cases, affected palms will die and fall over. Severe infection by *G. boninense* may lead fracture at the base of oil palm and make it collapses, leaving diseased bole tissues on the

Fig. 2.2 Symptoms of Basal stem rot **a** Fallen oil palm tree due to rotten bole tissue, which weakens the tree making it susceptible to wind damage. **b** Typical basidiocarps of *G. boninense* on a BSR affected palm. **c** Unopened spears of oil palm tree. **d** Decaying in oil palm bole tissues leaving the trunk in hollow

ground. All of these symptoms can occur as combination and there is no fixed pattern or progression of symptoms (Fig. 2.2).

G. boninense is a soil-borne pathogen and there are three possible ways in which this fungi can spread directly to the host plants; root-to-root contact, basidiospore, and free secondary inoculum in the soil. Numerous infection trials using oil palm seedlings and often using large *Ganoderma*-colonized rubber-wood blocks have provided data supporting this view (Sariah et al. 1994; Breton et al. 2006). Rees et al. (2007) showed that by attaching infested wood blocks to roots, much smaller inoculum can be used, allowing infection to occur through unwounded roots and

progression and rate of invasion to be followed. Successful root infection was also reported by Chong et al. (2012) using direct spray of *Ganoderma* mycelia suspension onto seedling roots. *Ganoderma* species pathogenic to oil palm has wide host range (Turner 1981). The large inoculum left by coconut probably caused the high incidence of BSR on oil palm. It has been proven beyond doubt that root's contact with infected debris is an important method of infection and the dead diseased stumps were responsible for spread of BSR in the plantation.

Pilotti et al. (2003) and Sanderson (2005) have also reported basidiospores are implicated as the main mode of dispersal of *G. boninense* and grow in the non-living tissues. Mature basidiocarp produces thousands of basidiospores, and could well be good sources of inoculums for new infections, as dispersed by air movement and strong winds. These spores may colonise new substrate, enter wounds caused by shedding of branches and become new infection foci. However, Cooper et al. (2011), showed pruning can bring in spores as deep as 10 cm into the oil palm in which may accelerate further infection. However, very unlikely the basidiospore is the cause of the infection, as *Ganoderma* basidiocarps in field usually produce monokaryotic basidiospores. Monokaryotic mycelium from basidiospores can colonise palm wood but is non-infective (Hasan and Flood 2003; Rees et al. 2007); anastomosis with a compatible mating type is required to form the potentially invasive and faster growing heterokaryon. However, Hasan and Flood (2003) showed that single germinated basidiospores producing monokaryotic mycelia could colonise rubber wood and oil palm tissues.

Generally, initiation of BSR on oil palm by *G. boninense* established from infected debris that enters and gets in contact with roots and wounded part, which then progresses mainly through the inner, thin-walled cortex in plant. Rees et al. (2009) suggested that *Ganoderma* colonization appears to involve developmental switches which are biotrophic and necrotrphic phase. Initial infection starts with biotrophic phase where colonization in root cortex and stem base occur, involving largely intercellular colonization by wide hyphae of host cells with fully intact cell walls. This phase then followed by necrotrophic phase which associated extensive cell wall degradation. The formation of melanised mycelium might be considered as a third which is probably indicative of the oxidative breakdown of lignin and the white rot status ascribed to *G. boninense* (Adaskaveg et al. 1990).

Plant cell wall comprises of several components which are cellulose, hemicellulose, pectin and lignin. Thus, for successful colonization, production of an array of cell wall degrading enzymes (CWDE) (Cooper 1984) is likely to be required to penetrate and degrades these components in plant outermost tissues. *Ganoderma* produces several CWDEs such as amylase, oxidase, invertase, coagulase, protease, renetase, pectinase, and cellulosic enzyme. Based on Transmission Electron Microscopy (TEM) observation of infected roots and bole tissues, Rees et al. (2009) also suggested CWDEs are important in the extensive degradation of oil palm cell walls during the pathogenesis by *G. boninense* and are likely to be pathogenicity factors for this interaction. Development of holes through cell wall layers due to *G. boninense* attack is indication of simultaneous wood decay with the ability of *G. boninense* to produce enzymes that can attack all cells' wall layers.

References

Adaskaveg JE, Gilberston RL, Blanchette RA (1990) Comparative studies of delignification caused by *Ganoderma* species. Appl Environ Microbiol 56:1932–1943

Ariffin D, Singh G, Lim TK (1989) *Ganoderma* in Malaysia-current status and research strategy. In: PORIM international palm oil development conference. Palm Oil Research Institute of Malaysia, 5–9 Sept 1989

Ariffin D, Idris AS, Marzuki A (1996) Spread of *Ganoderma boninense* and vegetative compatibility studies of a single field palm isolates. In: PORIM International Palm Oil Congress (Agriculture), Bangi Selangor, Malaysia, Sept 1996

Breton F, Hasan Y, Hariadi, Lubis Z, de Franqueville H (2006) Characterization of parameters for the development of an early screening test for basal stem rot tolerance in oil palm progenies. J Oil Palm Res (Special Issue—April):24–36

Chong KP, Atong M, Rossall S (2012) The role of syringic acid in the interaction between oil palm and *Ganoderma boninense*, the causal agent of Basal stem rot. Plant Pathol 61(5):1365–3059

Cooper RM (1984) The role of cell wall-degrading enzymes in infection and damage. In: Wood RKS, Jellis GJ (eds) Plant diseases: infection, damage and loss. Blackwell Scientific Publications, Oxford, UK, pp 13–27

Cooper RM, Flood J, Rees RW (2011) *Ganoderma boninense* in oil palm plantations: current thinking on epidemiology, resistance and pathology. The Planter 87(1024):515–526

Flood J, Bridge PD, Holderness M (2000) *Ganoderma* diseases of perennial crops. CABI Publishing, Wallingford, UK

Hasan Y, Turner PD (1998) The comparative importance of different oil palm tissues as infection sources for basal stem rot in replanting. The Planter 74:119–135

Hasan Y, Flood J (2003) Colonization of rubber wood and oil palm blocks by monokaryons and dikaryons of *Ganoderma boninense*-implications to infection in the field. The Planter 79:31–38

Ho YW, Nawawi A (1985) *Ganoderma boninense* Pat. from basal stem rot of oil palm (*Elaies guineensis*) in Peninsular Malaysia. Pertanika 8:425–428

Idris AS (1999) Basal Stem Rot (BSR) of oil palm (*Elaeis guineensis* Jacq.) in Malaysia: factors associated with variation in disease severity. Dissertation, University of London

Idris AS (2009) Basal Stem Rot in Malaysia-Biology, economic importance, epidemiology, detection and control. In: Proceeding of the international workshop on awareness, detection and control of oil palm devastating diseases. Kuala Lumpur Convention Centre, Malaysia

Idris AS, Ariffin D, Swinburne TR, Watt TA (2000) The identity of *Ganoderma* species responsible for BSR disease of oil palm in Malaysia-Pathogenicity test. MPOB Information No. 77

Idris AS, Ismail S, Ariffin D, Ahmad H (2004) Prolonging the productive life of *Ganoderma*-infected palms with Hexaconazole. MPOB Information Series No. 214

Khairudin H (1990) Basal stem rot of oil palm: Incidence, etiology and control. MSc (Agriculture) thesis. Universiti Pertanian Sabah

Khairudin H, Lim TK, Abdul RAR (1991) Pathogenicity of *Ganoderma boninense* Pat. on oil palm seedlings. In: PORIM International Congress (Agriculture). Palm Oil Research Institute of Malaysia, Kuala Lumpur, Malaysia

Lim HP, Fong YK (2005) Research on basal stem rot (BSR) of ornamental palms caused by basidiospores from *Ganoderma boninense*. Mycopathol 159:171–179

Miller RNG, Holderness M, Bridge PD, Chung GF, Zakaria MH (1999) Genetic diversity of *Ganoderma* in oil palm plantings. Plant Pathol 48:595–603

Miller RNG, Holderness M, Bridge PD, Chung GF, Zakaria MH (2000) Genetic diversity of *Ganoderma* in oil palm plantings. Plant Pathol 48:595–603

Moncalvo JM (2000) Systematics of *Ganoderma*. In: Flood J, Bridge PD, Holderness M (eds) *Ganoderma* diseases of perennial crops. CABI Publishing, Wallingford, pp 23–45

Paterson RRM (2007) *Ganoderma* disease of oil palm-a white rot prespective necessary for integrated control. Crop Protection 26:1369–1376

Pilotti CA, Sanderson FR, Aitken EAB (2003) Genetic structure of a population of *Ganoderma boninense* on oil palm. Plant Pathol 52:455–463

Pilotti CA (2005) Stem rots of oil palm caused by *Ganoderma boninense*: pathogen biology and epidemiology. Mycopathologia 159:129–137

Rao AK (1990) Basal stem rot (*Ganoderma*) in oil palm small holdings-IADP Johore Barat experience. In: Proceedings of Ganoderma workshop. Palm Oil Research Institute of Malaysia, Malaysia, 11 Sept 1990

Rees RW, Flood J, Hasan Y, Cooper RM (2007) Effects of inoculums potential, shading and soil temperature on root infection of oil palm seedlings by the basal stem rot pathogen *Ganoderma boninense*. Plant Pathol 56:862–870

Rees RW, Flood J, Hasan Y, Potter U, Cooper M (2009) Basal stem rot of oil palm (*Elaeis guineensis*); mode of root infection and lower stem invasion by *Ganoderma boninense*. Plant Pathol 58:982–989

Sanderson, FR Pilloti CA (1997) *Ganoderma* basal stem rot: an enigma or just time to rethink the problem. The Planter 73:489–493

Sanderson FR (2005) An insight into spore dispersal of *Ganoderma boninense* in oil palm. Mycopathology 159:139–141

Sariah M, Hussin MZ, Miller RNG, Holderness M (1994) Pathogenicity of *Ganoderma boninense* tested by inoculation of oil palm seedlings. Plant Pathol 43:507–510

Singh G (1991) *Ganoderma*—the scourge of oil palm in the coastal areas. The Planter 67:421–444

Subagio A, Foster HL (2003) Implications of *Ganoderma* disease on loss in stand and yield production of oil palm in North Sumatra. In: Proceedings of the MAPPS conference, Kuala Lumpur, Aug 2003

Susanto A, Sudharto PS, Purba RY (2005) Enhancing biological control of basal stem rot disease (*Ganoderma boninense*) in oil palm plantations. Mycopathology 159:153–157

Turner PD, Gillbanks RA (1974) Oil palm cultivation and management. The Incoorporated Society of Planters, Kuala Lumpur

Turner PD (1981) Oil palm disease and disorders. Oxford University Press, United Kingdom

Zakaria L, Kulaveraasingham H, Tan SG, Abdullah F, Ho YW (2005) Random amplified polymorphic DNA (RAPD) and random amplified microsatellite (RAMS) of *Ganoderma* from infected oil palm and coconut stumps in Malaysia. Asian Pacific J Mol Biol Biotechnol 13:23–34

Chapter 3
Current Detection Methods of *G. boninense* Infection in Oil Palm

Abstract Basal Stem Rot (BSR) disease has been spreading fast due to the inability to detect infection at the early stage in the field. The detection of the disease is challenging because the external symptoms are only visible when it is at the critical stage. During this stage, any treatment or management methods are no longer effective. Therefore, many viable and effective methods to detect the presence of *G. boninense* has been developed to diagnose the disease at early stage. This chapter elaborates established methods in detecting *G. boninense* including molecular analysis, biochemical assay, field detection methods using devices and some others emerging methods currently utilized.

3.1 Lab-Based Detection Methods

3.1.1 Molecular Analysis

i. Internal Transcribed Spacer (ITS)

Polymerase chain reaction (PCR) technology has revolutionised the field of plant pathology in diagnosing various plant pathogens (Henson and French 1993), and have been widely used to identify *Ganoderma* species (Idris et al. 2003; Chong et al. 2011). The internal transcribed spacer (ITS) regions of ribosomal RNA gene (rDNA) have been selected as specific targets for the detection of *Ganoderma* (Utomo and Niepold 2000). This method mostly used in isolating *Ganoderma* following positive result from other detection methods.

ii. Random Amplified Polymorphic DNA (RAPD)

The use of Random Amplified Polymorphic DNA (RAPD)-PCR and ITS had shown different results of identification whereby RAPD was found to be suitable for systematic of lower taxonomy level that cannot be resolved by using ITS sequence

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data. Several arbitrary short primers are used with a long genomic DNA template with the expectation that some of them will amplify and provide a profile of the template (Williams et al. 1993). The RAPD analysis showed variations despite the high degree of similarity for *G. boninense* isolates (Zakaria et al. 2009). Thus, RAPD can be used to differentiate different isolates of *Ganoderma* spp. that have identical ITS sequences (Hseu et al. 1996). However, accurate identification of *Ganoderma* spp. is still not possible via RAPD analysis.

iii. ITS—Restriction Fragment Length Polymorphism (RFLP)

The use of Restriction Fragment Length Polymorphism (RFLP) using ITS 1 and ITS 4 primers to amplify Internal Transcribed Spacer (ITS) region reported by Nusaibah et al. (2011). The use of RFLP has the advantage of combining highly conserved sequences in the Internal transcribed spacer (ITS) 5.8S-ITS4 rDNA regions with variable sequences in the ITS regions at species level (Moritz et al. 2000), whereby, the ITS shows a high inter-specific variability and an extremely low intra-specific variability. ITS-PCR-RFLP is a power tool that has been proven to facilitate in genetic variation studies among *Ganoderma*. This method has been reported to be both more accurate and less sensitive to contamination than RAPD technique. A similar tool was used by Moncalvo et al. (1995), Utomo and Niepold (2000), and Latiffah (2001) in their *Ganoderma* population work. PCR-RFLP on ITS1-5.8S-ITS4 region is a reliable technique to discriminate the *Ganoderma* species, but not down to *G. boninense* strain.

iv. Multiplex PCR (MPCR)

A novel technique developed recently, known as Multiplex PCR (MPCR), has the ability to differentiate *Ganoderma* species in a single reaction (Wong et al. 2012). This technique utilizes Dual Priming Oligonucleotide (DPO) which can block non-specific priming, thus, increases sensitivity and specificity of PCR reactions. In addition, this technique is also able to eliminate template activity of contaminating DNAs and identify samples which may contain substances that can interfere with the PCR amplification reaction (Idris et al. 2012). However, this technique requires longer time before the final result is obtained.

v. DNA-microarray

DNA-microarray is another interesting technique using an electrochemical DNA biosensor which was designated for detection of *G. boninense* (Dutse et al. 2013) by utilizing newly synthesized ruthenium $[Ru(phen)_2(qtpy)]^{2+}$ complex as hybridization indicator, incorporated with the use of gold electrode. A specific sequence of a *Ganoderma boninense* DNA probe has been immobilized on the modified electrode. Although it is specifically designed for the pathogen, sample preparation and DNA extraction has been a major challenge.

3.1.2 Biochemical Analysis

i. *Ganoderma* Selective Media (GSM)

Detection of *Ganoderma*-infected tissues using *Ganoderma* Selective Media (GSM) (Ariffin and Idris 1991) is one of the earliest methods in detecting the disease. GSM provides a useful tool for isolating *Ganoderma* free from other contaminants. The content of fungicide and antibiotics in GSM eliminates growth of bacteria and other contaminating fungi, while allowing *Ganoderma* to thrive. Although isolation of *Ganoderma* using GSM had some merit for early detection of infected palms but it was inconclusive as other basidiomycetes may also grow on this media. Application of this method is usually supported with other detection methods.

ii. Ergosterol analysis

In addition to GSM, another method known as ergosterol analysis is also currently applied in detecting *Ganoderma*. Ergosterol is the cell membrane component in fungal and is not found in plants (Toh Choon et al. 2011). The use of ergosterol to detect invasion of *G. boninense* on oil palm tree has been first reported by Chong et al. (2009). Several studies also demonstrated this compound can be employed in detecting and quantifying early infection of *Ganoderma* on oil palm (As'wad et al. 2011; Toh Choon et al. 2011; Chong 2012). Chong et al. (2009) suggested that ergosterol related directly to the growth of *G. boninense* and severity of the BSR disease in oil palm. The relationship between ergosterol concentration and *G. boninense* mycelial biomass revealed ergosterol increased proportionally to *Ganoderma* biomass (Paterson 2007). Furthermore, ergosterol was not detected in healthy palm, and only present in the infected tree with the concentration varied proportionately to the degree of infection (As'wad et al. 2011). Thus, this method may provide suitable quantification of *Ganoderma* colonization when compared with other molecular identification as ergosterol presents in all type of fungi, although not specific for *G. boninense*.

iii. Immunoassay

Development of monoclonal (MAb) and polyclonal (PAb) antibodies against the crude mycelia proteins of *Ganoderma* to serologically detect this fungus by applying Indirect Enzyme-linked Immunosorbent Assay (ELISA) and Dot Immunobinding Assay (DIBA) techniques have also been reported in detecting *Ganoderma* spp. However, the assay using ELISA-MAb and PAb showed cross reaction with other species of *Ganoderma* and saprophytic fungi which commonly found on diseased oil palm roots and trunk such as *Penicillium*, *Aspergillus* and *Trichoderma*, although the percentage of cross reaction were low (Sundram et al. 2006; Idris and Rafidah 2008). In addition, no distinction was found between pathogenic *Ganoderma* spp. isolated from oil palms and other pathogenic *Ganoderma* spp, therefore, indicating ELISA test cannot be used to distinguish the

difference among *Ganoderma* species (Utomo and Niepold 2000). However, immunoassay techniques offer greater simplicity and less equipment than those of DNA probe analysis such as PCR (Darmono 2000). ELISA offers advantages in terms of speed, ease of use and costs compared to PCR based assay (Utomo and Niepold 2000).

3.2 Field Detection Methods Using Devices

3.2.1 Tomography

In addition to the lab-based techniques, several methods for *Ganoderma* detection in the actual field were also developed. The development of PODITOO™ tomography to locate and identify infection and Geographical Information System (GIS) to detect possible sites and distribution of *G. boninense* infection in plantations also have been attempted (Idris et al. 2009). Decayed tissues due to *G. boninense* infection were detected based on the sound lines integration by the sound sensors in PODITOO™. These sound lines are calculated between the sound wave emitted in one sensor and time flight of the sound propagation from the emitter to the other sensor. These sound lines are then used to construct the tomography image of the stem, indirectly detecting the size of the decay. Fuzzy inference system is another brilliant detection method using tomography image to recognize *Ganoderma* infection lesion in the oil palm stem. Identification and selection of palms by expert were done to develop and establish tomography images of the *Ganoderma* infected and healthy oil palm trunk. The assigned tomography images from the selected palms were installed into the system and will enable user to perform automatic detection directly on the field. However, bias on the selection of palm prior to data installment into the system can be interfered due to human error.

3.2.2 Molecularly Imprinted Polymer (MIP) Sensors

Another work conducted, on utilising an electronic nose (E-nose) also had been reported in detecting BSR disease (Markon et al. 2008). Based on the difference of odour profiles, 32 different sensors in Cyranose 320 sensor are able to give individual palms odour fingerprints of healthy and infected trees with a high rate of accuracy. All sensors are conducting-based polymer and each sensor has different response specificity to a broad range of compounds. Three types of odour samples were selected for both healthy and infected oil palm trees, namely odour of the air surrounding the tree, odour of bored tree trunk and as well as odour of soil surrounding the base of the tree trunks. Each of these odours represents a different parameter of the tree odour, which will then become the benchmark of data

collection. This method provides a quick result on field, however, this method is not specifically designed to detect infection by *G. boninense* alone, it also generates odour profiles when palms are infected by other pathogens. Therefore, a fast, reliable and accurate detection and quantification of *Ganoderma* is still in demand.

3.2.3 Hyperspectral Reflectral Data

Hyperspectral imaging (e.g., satellite sensing) can help to identify severely infected oil palm without damaging the palm itself, although not able to detect early infection of BSR disease. This technique uses a statistical modeling to classify canopy spectra into *Ganoderma* infection severity (Lelong et al. 2010), and has been able to assess 4 levels of disease severity with more 94% accuracy. However, this technique is limited and not specifically modelled to detect *G. boninense* accurately.

3.2.4 Ergosterol Detection Using Thin Layer Chromatography

The detection of ergosterol in the field is now made possible with the assistance of Thin Layer Chromatography (TLC) (Chong and Alexander 2014). The TLC method may allow faster and cheaper detection in field without relying on sophisticated equipments. An estimation of the amount of ergosterol in different infected palms may also possible by comparing the intensity of ergosterol extracted from oil palm tissue with a serial of different concentrations of standard ergosterol spots on TLC plate under UV lamp. However, since ergosterol is not a specific component for *Ganoderma*, the tissue collection procedure for this TLC detection is very crucial to avoid collection of unwanted materials. This method will be a quick and reliable solution for planters or practioners in detecting and possibly quantifying *Ganoderma* colonization on their own if the collection of the oil palm tissues for the detection is carefully done and the ergosterol extracted from the oil palm tissues are originated from *Ganoderma* which infecting the oil palm.

3.3 Emerging Detection Methods

3.3.1 Infrared Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was used for the identification and discrimination of functional group for *G. boninense* which could possibly used as

an indicator in BSR early detection. Liaghat et al. (2013) found different stages of BSR development can be discriminated from oil palm leaves using mid-infrared. Dayou et al. (2014) also reported unique infrared derived signature from *G. boninense*, which cannot be found in healthy oil palm tissue using FTIR. Cross reference with *Ganoderma*-infected trunk tissue shows a resemblance in its absorption spectra. This unique finding has a future potential as a reference to discriminate between healthy and infected oil palm tree. Further research is required to establish this method especially for early detection.

3.3.2 Ultrasonic

A study on the ultrasonic transmission properties on the thickness of residual wall of oil palm trunk using a commercial ultrasonic instrument at the frquency of 54 kHz was reported by Najmie et al. (2011). The research found trunk density of *Ganoderma*-infected oil palms were reduced to 50% compared to healthy trunk. However, at this stage, the palm has reached its late stage where it can collapse at anytime. Later on, a study by Ishaq et al. (2014) reported that using a sonic tomography, oil palm trunk damages due to *G. boniense* can be detected at different stages. Supported with other detection method, this technique provide accuracy up to 96% in detecting BSR and 82% in detecting the disease severity level.

References

Ariffin D, Idris AS (1991) A selective medium for the isolation of *Ganoderma* from diseased tissues. In: International palm oil conference, progress, prospects and challenges towards the 21st century. PORIM, Bangi, 9–14 Sept 1991

As'wad AWM, Sariah RRM, Paterson MA, Abidin Z, Lima N (2011) Ergosterol analyses of oil palm seedlings and plants infected with *Ganoderma*. Crop Prot 30:1438–1442

Chong KP, Rossal S, Markus A (2009) A comparison on the susceptibility of varieties AVROS, Calabar and Ekona of oil palm seedlings to *Ganoderma boninense.* Int J Eng Technol, pp 461–167

Chong KP, Foong CP, Wong CMVL, Rossall S, Atong M (2011) First identification of *Ganoderma boninense* isolated from Sabah based on PCR and sequence homology. Afr J Biotechnol 10(66):14718–14723

Chong KP (2012) An evaluation of the *Ganoderma* fungal colonisation using ergosterol analysis and quantification. The Planter 88(1034):311–319

Chong KP, Alexander A (2014) Early detection of *Ganoderma* in oil palm: a field guide. Universiti Malaysia Sabah, Malaysia

Darmono TW (2000) *Ganoderma* in oil palm in Indonesia: current status and prospective use of antibodies for the detection of infection. In: Flood J, Bridge PD, Holderness M (eds) Diseases of perennial crops, CAB International, pp 249–266

Dayou J, Alexander A, Sipaut CS, Chong KP, Lee PC (2014) On the possibility of using FTIR for detection of *Ganoderma boninense* in infected oil palm tree. Int J Adv Agri Environ Eng 1(1):161–163

Dutse SW, Yusof NA, Ahmad H, Hussein MZ, Hushiarian R (2013) DNA-based biosensor for detection of ganoderma boninense, an oil palm pathogen utilizing newly synthesized ruthenium complex [Ru $(phen)_2(qtpy)]^{2+}$ based on a PEDOT-PSS/Ag nanoparticles modified electrode. Int J Electrochem Sci 8:11048–11057

Henson JM, French R (1993) The polymerase chain reaction and plant disease diagnosis. Ann Rev Phytopathol 31:81–109

Hseu RS, Wang HH, Wang HF, Moncalvo JM (1996) Differentiation and grouping of isolates of the *Ganoderma lucidum* complex by random amplified polymorphic DNA-PCR compared with grouping on the basis of internal transcribed spacer sequences. Appl Environ Microbiol 62:1354–1363

Idris AS, Yamaoka M, Hayakawa S, Basri MW, Noorhasimah I, Ariffin D (2003) PCR technique for detection of *Ganoderma.* MPOB Information No. 202

Idris AS, Rafidah AR (2008) Polyclonal antibody for detection of *Ganoderma.* MPOB Information No. 430

Idris AS, Mazliham MS, Madihah AZ (2009) Current technologies for detection of *Ganoderma* in oil palm. In: Proceedings of agriculture, biotechnology and sustainability conference. PIPOC, Kuala Lumpur, Malaysia

Idris AS, Rajinder S, Madihah AZ, Wahid MB (2012) Multiplex PCR-DNA kit for early detection and identification of *Ganoderma* species in oil palm. MPOB Information No. 531

Ishaq I, Alias MS, Kadir J, Kawawani I (2014) Detection of basal stem rot disease at oil palm plantations using sonic tomography. J Sustain Sci Manag 9(2):52–57

Latiffah Z (2001) Comparative studies on *Ganoderma* from infected oil palm and coconut stumps with special reference to their morphological, molecular and isozymecharacteristics. Dissertation, Universiti Putra Malaysia

Lelong CC, Roger J-M, Brégand S, Dubertret F, Lanore M, Sitorus N, Raharjo D, Caliman J-P (2010) Evaluation of oil-palm fungal disease infestation with canopy hyperspectral reflectance data. Sensors 10(1):734–747

Liaghat A, Mansor S, Ehsani R, Mohd Shafri HZ, Meon A, Sankaran S (2013) Mid-infrared spectroscopy for early detection of basal stem rot disease in oil palm. Comp Electron Agri 101:48–54

Markon MA, Shakaff AY, Adom AH, Ahmad MN, Abdullah AH (2008) The feasibility study of utilising electronic nose and ANN for plant malaise detection. In: Proceedings of MUCET Malaysian Universities Conferences on Engineering and Technology (MUCET), Putra Brasmana, Perlis, Malaysia, March 8–10

Moncalvo JM, Wang HH, Hseu RS (1995) Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia 87:223–238

Moritz G, Delker C, Paulsen M, Mound LA, Burgermeister W (2000) Modern methods for identification of Thysanoptera. EPPO Bulletin 30:591–593

Najmie MMK, Khalid K, Sidek AA, Jusoh MA (2011) Density and ultrasonic characterization of oil palm trunk infected by *Ganoderma boninense* disease. Meas Sci Rev 11(5):160–164

Nusaibah SA, Latiffah Z, Hassaaan AR (2011) ITS-PCR-RFLP analysis of *Ganoderma* sp. Infecting industrial crops. Pertanika J Agri Sci 34(1):1551–3701

Paterson RRM (2007) *Ganoderma* disease of oil palm-a white rot prespective necessary for integrated control. Crop Prot 26:1369–1376

Sundram S, Chris D, Sioban O, Idris AS (2006) Preliminary studies on the development of monoclonal antibodies against Mycelia of *Ganoderma boninense*, the causal pathogen of basal stem rot of oil palm. Malaysian J Microbiol 2(1):30–34

Toh Choon RL, Sariah M, Siti Mariam MN (2011) Ergosterol from the soilborne fungus *Ganoderma boninense*. J Basic Microbiol 51:1–5

Utomo C, Niepold F (2000) Development of diagnostic methods for detecting *Ganoderma* infected oil palms. J Phytopathol 148:507–514

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV (1993) Genetic analysis using random amplified polymorphic DNA markers. In: Wu R (ed) Methods in enzymology, vol 218. Academic Press, New York, pp 704–740

Wong LC, Bong CF, Idris AS (2012) *Ganoderma* species associated with basal stem rot disease of oil palm. American J Appl Sci 9(6):879–885

Zakaria L, Ali NS, Salleh B, Zakaria M (2009) Molecular analysis of *Ganoderma* species from different hosts in Peninsula Malaysia. J Biol Sci 9:12–20

Chapter 4
Control Methods of *G. boninense* in Oil Palm Industry

Abstract Basal Stem Rot (BSR) is a very important disease to the oil palm industry. When it was first recorded, BSR only affected mature palms. However after years, the disease had progressed to affecting younger palms and causing more losses. The disease continues to inflict considerable yield losses of infected palms and also direct loss of stand due to palm death. Attempts to control the disease has been taken to wider extent, including cultural practices (clean clearing, windrowing, mounding, surgery, digging trenches, sanitation), fungicide application, development of resistance variety and application of biological control agents. This chapter elaborates all possible managing strategies in combating *Ganoderma boninense.*

4.1 Cultural Practices

Good cultural practices are often advisable in the management of BSR in established oil palm plantations. Cultural measures usually involve eradicating and reducing the pathogen inoculums to avoid disease spread. These include clean clearing, windrowing, soil mounding, surgery, digging trenches and sanitation.

4.1.1 Clean Clearing

Clean clearing is a procedure of excision and removal of all remaining fragments within an infected palm area by digging pits with 1.5 m^2 and 60 cm deep from both untreated vacant points and diseases palm points (Singh 1990; Flood et al. 2000). Generally, all remaining fragments from the infected palm will be brought to the surface for subsequent removal or burned down. However, this method is costly, and open burning is forbidden in many palm oil producing countries and in Malaysia it is governed under Air Regulation Act of 1978. Therefore, a common practice is to shred all palm fragments and can either be scattered over the whole field or stacked in rows and covered with legume cover crop to facilitate decay.

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Although these practices generally result in lower BSR incidence in replanted oil palms, infection can still become established progressively due to debris left in the soil (Singh 1991).

4.1.2 Windrowing

Windrowing is a method where felled oil palm trunks and excised root tissues are laid, unshredded, along the old rows. This technique is less labour intensive than clean clearing and has shown to be almost effective in limiting losses in the subsequent crop as clean clearing. However, a comparative study by Hashim (1991) indicated clean clearing was the most effective in reducing BSR incidence (from 27.3% in the previous stand to 14% in the replanted stand after 15 years, followed by windrowed treatment (27.3–17.6%). This is because windrowed materials deal significant problems with regard to potential sources of inoculums (Flood et al. 2000).

4.1.3 Soil Mounding

Soil mounding is one of the most common practices in almost all plantations. Soil from adjacent areas is collected for heaping at the infected palm trunk to prevent the weakened boles from being toppled by wind. Studies by Ho and Khairudin (1997) and George et al. (2000) showed the benefit in term of cost effectiveness of soil mounding on BSR. However, this method only helps to prolong the economic life of yielding palms affected by BSR and it did not retard *Ganoderma* spread.

4.1.4 Curative Surgery

Other less common cultural practices such as conducting surgery by excision of diseased tissues using hand held chisel (Turner 1981) or mechanically using the blade of a back-hoe (Singh 1991) is also practiced in some plantations. Some enhanced survival and better yields were reported from palms after surgery (Hasan and Turner 1994). However, in most cases, it failed due to late detection, large disease lesion and lesion extended below ground including the infected root masses (Chung 2011) causing treated palms to collapse at the end. Furthermore, surgery often requires repetition, as infection normally resurges if lesions are not completely removed. In addition to this technique, sealing the freshly cut palm tissues also had been reported as a procedure to control spores to come in contact with the wound (Panchal and Bridge 2005).

4.1.5 Digging Trenches

Digging trenches to avoid contact from palm to palm also have been integrated in cultural practices. It may be a better option than open clearing and windrowing. The area for trenching is 2 m × 2 m to adequately isolate the diseased palm, and the trench is 0.5 m in wide and in 1 m deep (Hasan and Turner 1998; Lim and Udin 2010). Isolation of trenches around old stumps on field trial by Lim and Udin (2010) showed this method is effective in preventing BSR infection up to 14 years for oil palm planted in between the old stands. However, this practice has generally proved to be ineffective because the trench depths were insufficient to remove all infected root material (Hasan and Turner 1998), this control measure also need to be maintained continuously since there will be partial filling up due to erosion of the trench edges. Generally this measure is not widely practiced.

4.1.6 Sanitation

Diseased stumps are the most infectious tissues, when suitable disease inoculum present. *Ganoderma* inoculums in these stumps will attack oil palm when there is a contact. Therefore, field sanitation and elimination of diseased tissues is very important to keep the plantation free from sources of the pathogen. A large hole of 2 m × 2 m × 1 m deep is dug in this sanitation operation (Idris et al. 2004a). The diseased materials are dug out, shredded into small pieces and left in the interrow areas for natural decay. This method is commonly practiced at the time of replanting in most large plantations. Trial results up to 15 years have shown that proper sanitation at replanting can achieve low BSR incidence, and in contrast, high percentage of BSR was recorded in poor sanitation in which diseased inoculums had been deliberately left behind (Chung 2011).

4.2 Chemical Control

In some early diagnosed palms which treated with fungicides, palms showed a reduction in disease intensity after several months of application. Correct amount and technique for application of fungicides helped to reduce the progress of the disease in the palm. Studies conducted by Palm Oil Research Institute of Malaysia (PORIM) (1997) and Idris et al. (2002) on the use of systemic fungicides such as triadimefon, carboxin, carbendazim, methfuroxam and hexaconazole to control BSR in the lab and field had shown inconclusive results, although some systemic fungicides and soil fumigants seemed to be promising. Application of fungicide through trunk injection technique was found to be significant in reduction of BSR incidence (George et al. 1996). However, over-use of systemic fungicides can lead

to accumulation of fungicide in the root tissues causing negative impact to some beneficial soil microbes. Roots of fungicide treated plants are not susceptible to colonization by mycorrhizal fungi up to 3 weeks after the application of systemic fungicide.

A study conducted by Soepena et al. (2000) demonstrated field control of BSR by contact chemicals treatment have not been very successful even with those in vitro screened effective against the fungus. This might be due to the fact that both visibly infected and subclinical palms may already have the disease established severely by the time treatment is applied (Sapak et al. 2008). Therefore, chemical fungicides are not a preferable method for long term solution in oil palm health management. This is because the necessity for repeated applications, residue problems, health and environmental hazards and development of fungicide resistance in the pathogen are the major problems associated with the use and over dependent on chemical fungicides (Mukhopadhyay and Mukherjee 1996; Suryanto et al. 2012). Growing concerns about the negative impact of chemical usage have encourage planters and researchers to seek alternatives that are more environmentally friendly such as pathogen-resistant cultivars and the use of biocontrol agents.

4.3 Development of Disease Resistance Variety

Development of host resistance to *Ganoderma* has been widely investigated. For decades, pure Deli material has been emphasised in breeding material resistance to *Ganoderma* due to its high relativity on susceptible towards BSR. Screening and breeding are the fundamental methods in developing BSR resistance of oil palm. Turnbull et al. (2014) has investigated the potential of 115 Deli progenies and 1847 crosses which consequently found Deli SIB crosses and the la Mé selected origins to be highly resistant compared to other breeding materials. Field observations in North Sumatra, Indonesia revealed *Elaeis guineensis* of deli origin from Malaysia and Indonesia was more susceptible than African material (Durand-Gasselin et al. 2005), and other trials have revealed differences in susceptibility, indicating possible genetic resistance within host populations (Idris et al. 2004b; Breton et al. 2006). In another work, Idris et al. (2004a) has performed a study to evaluate 23 oil palm seedlings progenies and found out that the most susceptible progeny was D×D [Deli (Elmina) × Deli (Elmina)], whilst a partially resistant progeny was D×P (Congo × Cameroon). In addition, several potential resistant planting material against BSR was also reported as summarized in Tables 4.1 and 4.2 respectively.

There is no *Ganoderma*-free variety and given the biology of the pathogen itself, it would be advisable to seek partial resistance (Mc Donald and Linde 2002) despite of their slow disease progresses. The genetics and nature of resistance or tolerance

Table 4.1 Susceptibility of different parent materials against *Ganoderma* (*Source* Durand-Gasselin et al. 2005)

Origin	No. crosses tested	Percentage of BSR (%)
Deli	5	72.2–93.6
Lame	5	12.4–40.9
Yangambi	2	17.1–24.4
Deli mean	5	79.6
Deli + Yangambi mean	7	23.5

Notes BSR = Basal Stem Rot

Table 4.2 Tolerant progeny of oil palm against *Ganoderma* (*Source* Ariffin et al. 1999; Idris et al. 2004a)

Tolerant progeny (<20% dead)	Susceptible progeny (>70% dead)
Zaire × Cameroon (D×P)	Ulu Remis Selfs (D×D)
Cameroon × Cameroon (T×T)	Dumpy Selfs (D×D)
Cameroon × AVROS (T×P)	Chemara Selfs (D×D)
Nigeria × Nigeria (T×T)	Ulu Remis × Elmina (D×D)

Notes D = Dura; P = Pisifera; T = Tenera

shown by palms to *Ganoderma* is not known and will await the availability of palm lines with effective resistance (Cooper et al. 2011). Development of resistant progenies may provide a long-term solution to BSR and successful component in preventing and controlling this disease as this approach is inexpensive, biologically safe and convenient. However, development of a resistance progenies requires tedious work and is a long-term activity.

4.4 Biological Control

In plant pathology, the term biological control leads to the introduction of microbial antagonists or host specific pathogens to suppress disease and population of one or more plant pathogens (Pal and Gardener 2006). It is environmentally safe, cheaper in cost and in some cases is the only option available to protect plants against pathogens. The most effective biocontrol active microorganisms studied appear to antagonize plant pathogen employing several modes of action which are classified as: mycoparasitism, antibiosis, metabolite production, competition, lytic enzymes and induced resistance (Pal and Gardener 2006). Recent control measures to overcome BSR are now focused on the use of biological control agents (BCA). In most studies dealing with the biological control of plant pathogens, only one BCA is applied.

4.4.1 *Bacteria as Biocontrol Agent Against* G. boninense

Some established endophytic bacteria such as *Burkholderia* spp., *Pseudomonas* spp., *Bacillus* spp. and *Serratia* spp. have been reported to show positive results in controlling *Ganoderma boninense* pathogen (Sapak et al. 2008; Azadeh et al. 2010). They were found to be capable in inducing systemic resistance in plants and showed biological traits like antimicrobial activity and lysis against *G. boninense*. These bacteria are considered to be endophytic as they are found mostly in the vascular systems adjacent to the phloem and xylem vessels and are uniform throughout the cortex, which suggests they may play a role in inhibiting penetration by *Ganoderma* and their development to the vascular systems (Bivi et al. 2010). Their ability to colonize internal plant tissues may cause them to compete within the vascular system limiting *Ganoderma* for both nutrients and space for its proliferation. Although endophytic bacteria live in the plant tissues, they caused no substantive harm or gaining benefit other than residency to the plant (Kobayashi and Palumbo 2000; Sapak et al. 2008). Numerous modes of action have been postulated and demonstrated for the antagonistic effects of *Bacillus* spp. in controlling *G. boninense*; these include synergistic effects observed on the fungal pathogens with a combination of antifungal compounds, competition for nutrients, production of cell wall lytic enzymes and induced systemic resistance (Nandakumar et al. 2001; Karthikeyan et al. 2006). Study concluded by Suryanto et al. (2012) showed *Bacillus* spp. was able to degrade cell wall of *G. boninense* component in vitro to some extent results in hyphal abnormality. Ability of *Bacillus* spp. to produce mycolytic enzymes such as chitinase and ß-1, 3-glucanase that lyse fungal cells (Patel et al. 2007; Gohel et al. 2006) and through parasitic mechanism had been reported to be responsible in degrading the pathogen cell wall components and inhibiting its growth (Alabouvette et al. 2006).

4.4.2 *Fungal as Biocontrol Agent Against* G. boninense

Several saprophytic fungi such as *Trichoderma* (Sariah 2003) and *Penicillium* (Dharmaputra et al. 1989) have shown antagonistic activity against *G. boninense.* Studies by Ilias (2000), Sariah et al. (2005) and Susanto et al. (2005) found biocontrol using *Trichoderma* spp. showed a high efficacy in controlling growth and infection by *G. boninense* in plant house trials and under field condition. Several *Trichoderma* species such as *Trichoderma virens, Trichoderma viride* and above all, *Trichoderma harzianum* have been reported to be antagonistic against *G. boninense*. Zainudin and Abdullah (2008) were successful in plant house trials using *T. harzianum*, and showed the isolate has good antagonist properties against *G. boninense* and is effective in controlling disease development.

Different mechanisms have been suggested as being responsible for the effects of *Trichodema* genus as BCA; which include the production of a wide range of board

spectrum antifungal metabolites, mycoparasitism, competition with the pathogen for nutrients and space, induced resistance, production of protease and fungal cell wall degrading enzymes (CWDE) (Elad 2000; Perelló et al. 2003). Production of CWDEs by *T. harzianum, T. viride* and also *Penicllium citrinum* had been reported (Dharmaputra et al. 1989; Susanto et al. 2005) which not only repressed the growth of *G. boninense* in vitro but also caused lysis of the hyphae, and the colony was totally overgrown by the antagonists. In addition to these mechanisms, the success of *Trichoderma* strains as BCAs is also probably associated to their high reproductive capacity, ability to survive under very unfavourable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi, and efficiency in promoting plant growth and defense mechanisms (Benítez et al. 2004).

4.4.3 *Yeast as Biocontrol Agent Against* G. boninense

Among soil microorganisms, yeasts have received little attention as biocontrol agents of soil borne fungal plant pathogens in comparison to bacteria, actinomycetes, and filamentous fungal antagonists. The ability of certain species of yeast to multiply rapidly, to produce antibiotics and cell wall-degrading enzymes, to induce resistance of host tissues, and to produce plant growth regulators indicates the potential to exploit yeasts as biocontrol agents (Suprapta 2012). Although there is no study yet on the use of yeast against *G. boninense* pathogen, several commonly found yeast species have been proven to be good BCA against plant pathogens. For example, *Saccharomyces cerevisiae* has been studied to be a new promising biocontrol agent due to their ability to induce plant resistance against different diseases (Suprapta 2012). Protection to plant may be due to the increment of certain enzymes such as peroxidase, chitinase and pathogensis-related protein which were activated by *S. cerevisiae* (El-Sayed 2000).

4.5 Multi-biological Agent Concept in Controlling *G. boninense*

The survival and effectiveness of single BCAs were highly affected by biotic and abiotic conditions. The growth and rate of survival of BCAs applied were important to activate the microbial antagonistic activity in the soil, hence inhibiting *Ganoderma* to thrive. Therefore, in recent years, more emphasis is laid on the use of combined biocontrol agents with different mechanisms of disease control, for improved disease control and also to overcome the inconsistent performance of the introduced biocontrol agents. Sapak et al. (2008) suggested that application of more than one BCA might be needed to prevent *G. boninense* infection and to reduce its

sporulation. In other studies, application of several BCAs simultaneously was attempted to improve control efficacy under diverse environments (Guetsky et al. 2001). Attempts to apply more than one BCA have been made for several reasons and are discussed in more depth in Chap. 5.

References

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant diseases: the European situation. Eur J Plant Pathol 114:329–341

Ariffin D, Idris AS, Kushairi A, Watt TA, Swinburne TR (1999) Screening of oil palm for resistance to *Ganoderma*. Paper presented at the Colloquium on Advances in Oil Palm Research under IRPA-funded programmes, PORIM, Bangi, 1–3 November 1999

Azadeh BF, Sariah M, Wong MY (2010) Characterization of *Burkholderia cepacia* genomovar I as a potential biocontrol agent of *Ganoderma boninense* in oil palm. Afr J Biotechnol 24:3542–3548

Benítez T, Rincón AM, Limón MC, Codon AC (2004) Bioncontrol mechanisms of *Trichoderma* strains. Int Microbiol 7(4):249–260

Bivi MR, Farhana MSN, Khairulmazmi A, Idris A (2010) Control of *Ganoderma boninense*: a causal agent of basal stem rot disease in oil palm with endophyte bacteria in vitro. Int J Agri Biol 12:833–839

Breton F, Hasan Y, Hariadi, Lubis Z, de Franqueville H (2006) Characterization of parameters for the development of an early screening test for basal stem rot tolerance in oil palm progenies. J Oil Palm Res 24–36

Chung GF (2011) Managment of *Ganoderma* diseases in oil palm plantations. The Planter 87 (1022):325–339

Cooper RM, Flood J, Rees RW (2011) *Ganoderma boninense* in oil palm plantations: current thinking on epidemiology, resistance and pathology. The Planter 87(1024):515–526

Dharmaputra OS, Tjitrosomo HS, Abadi AL (1989) Antagonistic effect of four fungal isolates to *Ganoderma boninense*, the causal agent of basal stem rot of oil palm. J Biotrop 3:41–49

Durand-Gasselin T, Asmady H, Flori A, Jacquemard JC, Hayun Z, Breton F, de Franqueville H (2005) Possible sources of genetic resistance in oil palm (*Elaeis guineensis* Jacq.) to basal stem rot caused by *Ganoderma boninense*-prospects for future breeding. Mycopathol 159:93–100

Elad Y (2000) Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Prot 19:709–714

El-Sayed S (2000) Microbial agents as a plant growth promoting and roots protector. In: 10th Microbiology conference, Cairo, Egypt, 12–14 November 2000

Flood J, Bridge PD, Holderness M (2000) *Ganoderma* diseases of perennial crops. CABI Publishing, Wallingford

George ST, Chung GF, Zakaria K (1996) Updated results (1990–1995) on trunk injection of fungicides for the control of *Ganoderma* basal stem rot. In: Ariffin et al (eds) Proceedings of the 1996 PORIM International Palm oil Congress (Agricultue), September 1996. Palm oil Research Institute of Malaysia, Bangi, Selangor, Malaysia, pp 508–5151

George ST, Chung GF, Zakaria K (2000) Benefits of soil mounding tall palms in a high *Ganoderma* incidence areas in Lower Perak. In: Pushparajah E (ed) Plantation tree crops in the new millenium. The Incorporated Society of Planters, Kuala Lumpur

Gohel V, Singh A, Vimal M, Ashwini D, Chhatpar HS (2006) Bioprospecting and antifungal potential of chitinolytic microorganism. Afr J Biotechnol 5:54–72

Guetsky R, Shtienberg D, Elad Y, Dinoor A (2001) Combining biocontrol agents to reduce the variability of biological control. Phytopathology 91:621–627

Hasan Y, Turner PD (1994) Research at BAH LIAS Research Station on basal stem rot of oil palm. In: Holderness M (ed) Proceedings of the 1st International Workshop on Perennial Crop Diseases caused by *Ganoderma,* UPM Serdang, Selangor, Malaysia, 28 November–3 December 1994

Hasan Y, Turner PD (1998) The comparative importance of different oil palm tissues as infection sources for basal stem rot in replanting. The Planter 74:119–135

Hashim KB (1991) Results of four trials on *Ganoderma* basal stem rot of oil palm in Golden Hope Estate. In: Proceedings of the *Ganoderma* Workshop, PORIM, Selangor, Malaysia, September 1990

Ho CT, Khairudin H (1997) Usefulness of soil mounding treatments in prolonging productivity of prime-aged Ganoderma infected palms. The Planters 73:239–244

Idris AS, Ismail S, Ariffin D, Ahmad H (2002) Control of *Ganoderma* infected palm-development of pressure injection and field applications. MPOB Information No. 131

Idris AS, Kushairi A, Ismail S, Ariffin D (2004a) Selection for partial resistance in oil palm to *Ganoderma* basal stem rot. J Oil Palm Res 16(2):12–18

Idris AS, Ismail S, Ariffin D, Ahmad H (2004b) Prolonging the productive life of *Ganoderma*-infected palms with Hexaconazole. MPOB Information Series No. 214

Ilias GNM (2000) *Trichoderma* and its efficacy as a bio-control agent of basal stem rot of oil palm (*Elaeis guineensis* Jacq.). Dissertation, Universiti Putra Malaysia

Karthikeyan M, Radhika K, Bhasakaran R, Mathiyazhagan S, Samiyappan R, Velazhahan R (2006) Rapid detection of *Ganoderma* disease of coconut and assessment of inhibition effect of various control measures by immunoassay and PCR. Plant Prot Sci 42(2):49–57

Kobayashi DY, Palumbo JD (2000) Bacteria endophytes and their effect on plant and uses in agriculture. In: Baco CW Jr, White JF (eds) Microbial endophytes. Marcel Dekker, Inc., New York, pp 3–29

Lim KH, Udin W (2010) Management of *Ganoderma* in peat soil in Indonesia. In: Second International Seminar Oil Palm Diseases: Advances in Ganoderma Research and Management. Sheraton Hotel Yogyakarta, Indonesia, 31 May 2010

Mc Donald BA, Linde C (2002) Pathogen population genetics, evolutionary potential and durable resistance. Ann Rev Phytopathol 40:349–379

Mukhopadhyay AN, Mukherjee PK (1996) Fungi, as fungicides. Ind J Trop Plant Disc 14:1–17

Nandakumar R, Babu S, Viswanathan R, Raguchander T, Samiyappan R (2001) Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. Soil Biol Biochem 33:603–612

Pal KK, Gardener BM (2006) Biological control of plant pathogens. The Plant Health Instructor, 25. doi:10.1094/PHI-A-2006-1117-02

Panchal G, Bridge PD (2005) Following basal stem rot in young oil palm plantings. Mycopathology 159:123–127

Patel B, Gohel V, Raoil B (2007) Statistical optimization of medium components for chitinase production by *Paenibacillus Sabina* strain JD2. Ann Microbiol 57:589–597

Perelló A, Monaco C, Simond MR, Sisterna M, Bello GD (2003) Biocontrol efficacy of *Trichoderma* isolates for tan spot of wheat in Argentina. J Crop Prot 22:1099–1106

PORIM, Annual Research Review (1997) Plant pathology and weed science. An Annual Report of The Palm Oil Research Institute of Malaysia, p 10

Sapak Z, Meon S, Ahmad ZAM (2008) Effect of Endophytic bacteria on growth and suppression of *Ganoderma* infection in oil palm. Int J Agri Biol 10:127–132

Sariah M (2003) The potential of biological management of basal stem rot of oil palm seedlings by calcium nitrate. The Planter 73:359–361

Sariah M, Choo CW, Zakaria H, Norihan MS (2005) Quantification and characterization of *Trichoderma* spp. from different ecosystems. Mycopathology 159:113–117

Singh G (1990) Palm disease in Malaysia. J Malaysian Agri 16:113–360

Singh G (1991) *Ganoderma*—the scourge of oil palm in the coastal areas. The Planter 67:421–444

Soepena H, Purba RY, Pawisukarto S (2000) A control strategy for basal stem rot (*Ganoderma*) on oil palm. In: Flood J, Bridge PD, Holderness M (eds) *Ganoderma* diseases of perennial crops. CAB International, Wallingford, pp 83–88

Suprapta DN (2012) Potential of microbial antagonists as biocontrol agents against plant fungal pathogens. J Int Soc Southeast Asian Agri Sci 18(2):1–8

Suryanto D, Wibowo RH, Siregar EB, Munir E (2012) A possibility of chitinolytic bacteria utilization to control basal stem disease caused by *Ganoderma boninense* in oil palm seedling. Afr J Microbiol Res 6(9):2053–2059

Susanto A, Sudharto PS, Purba RY (2005) Enhancing biological control of basal stem rot disease (*Ganoderma boninense*) in oil palm plantations. Mycopathology 159:153–157

Turnbull N, de Franqueville H, Breton F, Durand-Gasselin T (2014) Breeding methodology to select oil palm planting material partially resistance to *Ganoderma boninense*. In: 5th quadrennial International Oil Palm Conference, Indonesia, June 2014

Turner PD (1981) Oil palm disease and disorders. Oxford University Press

Zainudin NAI, Abdullah F (2008) Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum*. Plant Prot Sci 44:101–107

Chapter 5
Control of *G. boninense* Using Multi-biological Agents

Abstract Available control measures in managing Basal Stem Rot (BSR) disease has given unsatisfactory result. Alternative control measure to overcome this disease are now focused on the use of Biological Control Agents (BCAs). Currently introduction of Multiple Biological Agents (Multi-BCAs) to expand their mode of action has been applied in managing plant pathogen. Selecting the right combination of antagonists is necessary not only to avoid competition among the BCAs but also complementing each other. In addition, a different ecological requirement of BCAs would broaden the range of environmental conditions and made the biological control more feasible. Most combinations are only involving two organisms, but few involved combinations of three or more organisms. In this chapter, the efficacy of microbes using combinations of microbes which are solely Multi-BCAs and Multi-BCAs with additive against *Ganoderma boninense* are discussed.

5.1 Multi-biological Control Agents (Multi-BCAs) Without Addition of Additives

Bacillus spp. and *Trichoderma* spp. are the most utilized BCAs used against plant pathogens. These two genus have been known for long for their ability to fight with plant pathogen, and many researchers have studied their antimicrobial effect against *Ganoderma boninense*. *Bacillus* spp. and *Trichoderma* spp. are very common microbes in the soil, hence, their rate of establishment and survival in the soil ecosystem post-treatment application are likely high. Combination of *Bacillus* spp. and *Trichoderma* spp. in a single application was reported by Alexander et al. (2015). The in vitro study showed that the microbial combination suppressed the growth of *G. boninense* up to 70% (Fig. 5.1). Observation under Scanning Electron Microscopy (SEM) of *G. boninense* excised from the interaction zones of the antagonistic assay showed severe morphological abnormalities in hyphal structures compared with the control (*G. boninense* without Multi-BCAs treatment) (Fig. 5.2).

K.P. Chong et al., *Detection and Control of Ganoderma boninense in Oil Palm Crop*, SpringerBriefs in Agriculture, DOI 10.1007/978-3-319-54969-9_5

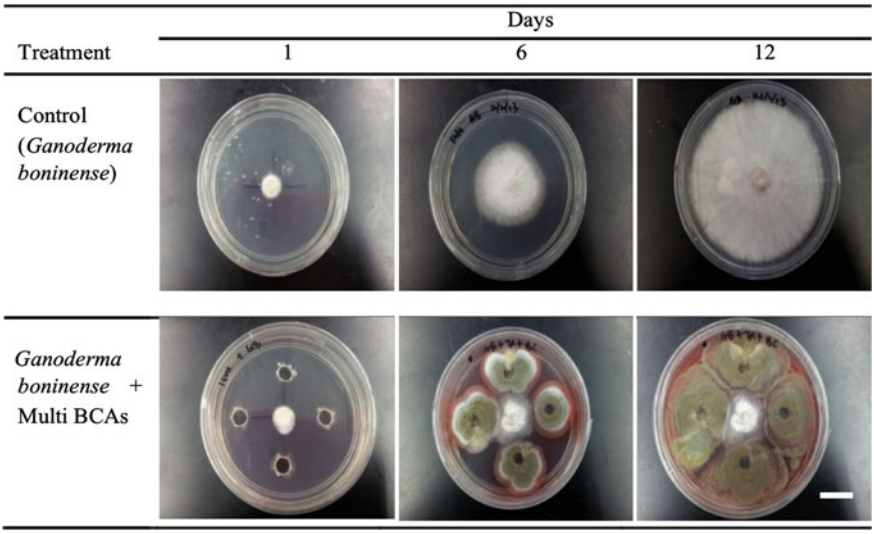

Fig. 5.1 Antagonistic assay of *G. boninense* on Potato Dextrose Agar (PDA) medium with or without the presence of Multi-BCAs which consist of *Bacillus* spp. and *Trichoderma* spp. at 1, 6 and 12 days of incubation. Growth of *G. boninense* was hindered compared to the control. *Bar size* 2 cm (*source* Alexander et al. 2015)

This result has shown a positive relationship between the Multi-BCAs in producing a synergistic effect against *G. boninense.*

Based on the SEM observation in Fig. 5.2, the spores of *Trichoderma* were adhered to the fungi hyphae. This is followed by germination of the fungal mycoparasite's spores and establishment of a successful parasitic interaction (Kubicek and Harman 1998). When spores germinated, they utilized the contents of the host hyphae as nutrient source and parasitized the host. Lectin present in the cell wall of the host are suggested to play a major role in the recognition of host hyphae by *Trichoderma* spp. to its host (Motlagh and Samimi 2013), suggesting that lectin could also be involved in the spores attachment.

The inhibition of *G. boninense* growth on the plate antagonized by Multi-BCAs may have resulted from the production of inhibitory metabolites by microbes in Multi-BCAs which diffused into the agar medium. It is also suggested the production of secondary metabolites from Multi-BCAs act synergistically with the production of cell wall degrading enzymes from both genus such as chitinses, glucanase and proteinase that break down polysaccharide, thereby destroying the pathogen cell wall integrity (Elad 2000; Devaki et al. 1992). This could also probably explain the observation of severe morphological abnormalities, with shrivelling and distortion of the fungal hyphae under SEM in Fig. 5.2.

Bacillus spp. and *Trichoderma* spp. combination was further evaluated for control of the *G. boninense* colonization in oil palm under nursery and field environment (Alexander and Chong 2014). The nursery evaluation was done in two

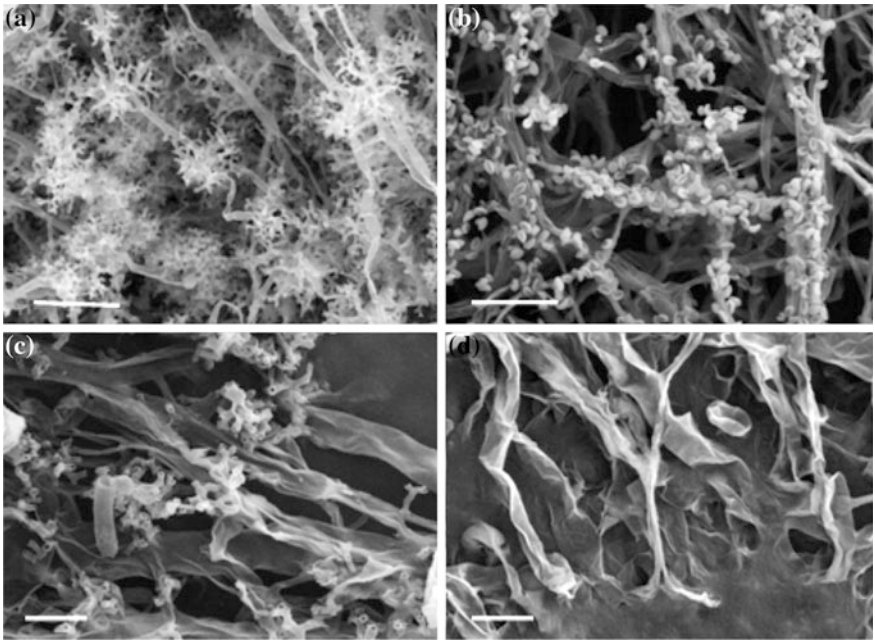

Fig. 5.2 **a** (control plate) shows a healthy, dense and branched mycelium of *G. boninense* which is free from any abnormality or disruption. However, the hyphal of *G. boninense* when challenged with Multi-BCAs were entirely covered and colonized by spores of *Trichoderma* spp. (Fig. 5.2b). The Multi-BCAs treatment caused the hyphal structure of the pathogen to become highly disrupted, disaggregated, flattened and shrivelled (Fig. 5.2c, d). The damage formed on the mycelium structure will eventually inhibit the growth of *G. boninense*. *Scale bars* **a**–**b**: 10 µm. **c**–**d**: 2 µm (*source* Alexander et al. 2015)

ways, prevention and reduction of *Ganoderma* colonization assessment. In prevention assesment, oil palm seedlings were artifically inoculated with *G. boninense* using a method as described by Chong et al. (2012), after pre-treated with multi-BCAs. Disease infection was assessed by determination of the concentration of *Ganoderma*-derived ergosterol in root tissues. These findings were substantiated by a parallel calculation of the percentage of root systems from which the pathogen could be cultured on *Ganoderma* Selective Media (GSM). The results from the GSM test were in accordance to ergosterol content detected in infected roots. All oil palm seedlings inoculated with *Ganoderma* were infected as indicated with the presence of ergosterol in their roots and growth of fungi on GSM. The result obtained from roots sampled after two and four months of *Ganoderma* inoculation in seedlings as based on ergosterol content and GSM test is shown in Fig. 5.3.

Based on Fig. 5.3, in control seedlings inoculated with *Ganoderma*, all seedlings were found to be infected, with a high concentration of fungal sterol of 3.53 µg/g in the extracted roots. Meanwhile, the sterol concentration in seedlings which had been treated with the multi-BCAs was significantly reduced to 1.99 µg/g and only

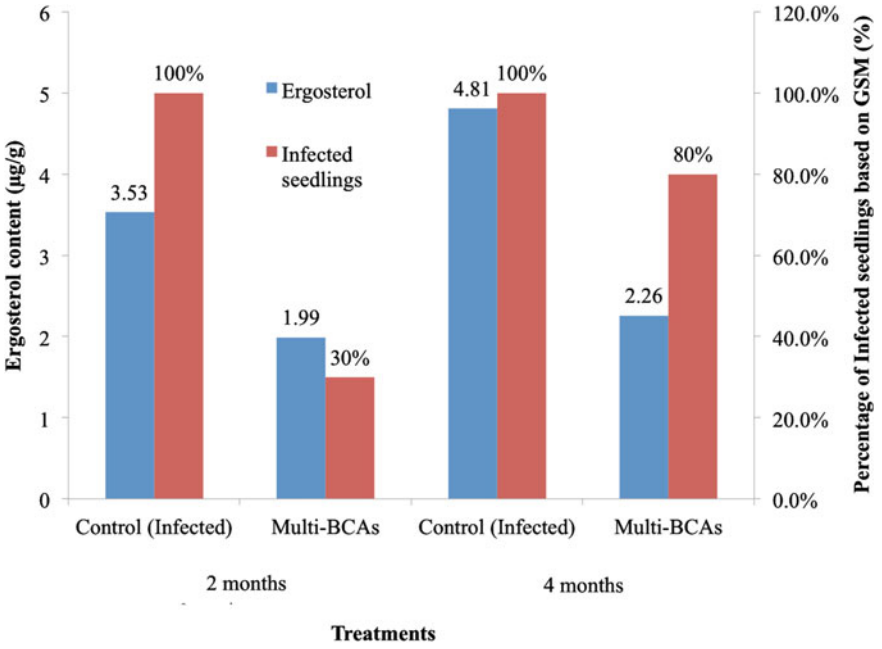

Fig. 5.3 Quantification of percentage of infected seedlings and ergosterol content in prevention of *Ganoderma* colonization in nursery. No ergosterol was detected in healthy (control) palms (Data not shown). *Notes* BCAs = Biological Control Agents; GSM = *Ganoderma* Selective Media

30% of infected seedlings. After four months of incubation, the percentage of oil palm seedlings infected by *Ganoderma* increased to 80%, but the ergosterol concentration is low compared to control.

Meanwhile in the reduction assessment, seedlings were first inoculated with *Ganoderma.* After the assessments described previously were made, BCAs were applied and results obtained are shown in Fig. 5.4. The microbial combination showed the greatest reduction of infected seedlings and ergosterol concentration of 20% and 1.66 µg/g respectively after two months, however increased to 80% and 2.39 µg/g after four months of treatments. However, the percentage of infection in treated seedlings was lower compared to untreated seedlings (Control) based on the lower ergosterol content detected in root tissues.

Ganoderma-infected oil palms were screened and selected from the field using ergosterol analysis and plating on GSM technique. Selected palms were treated with multi-BCAs for six months consecutively. The result in Fig. 5.5 reveals that treated palms showed a significant recovery, from 100% infection, down to only 12% found infected at the end of observation. This treatment also effectively reduced the amount of ergosterol by 81% of that found before treatment. The concentration of ergosterol in infected, non-treated palms more than doubled.

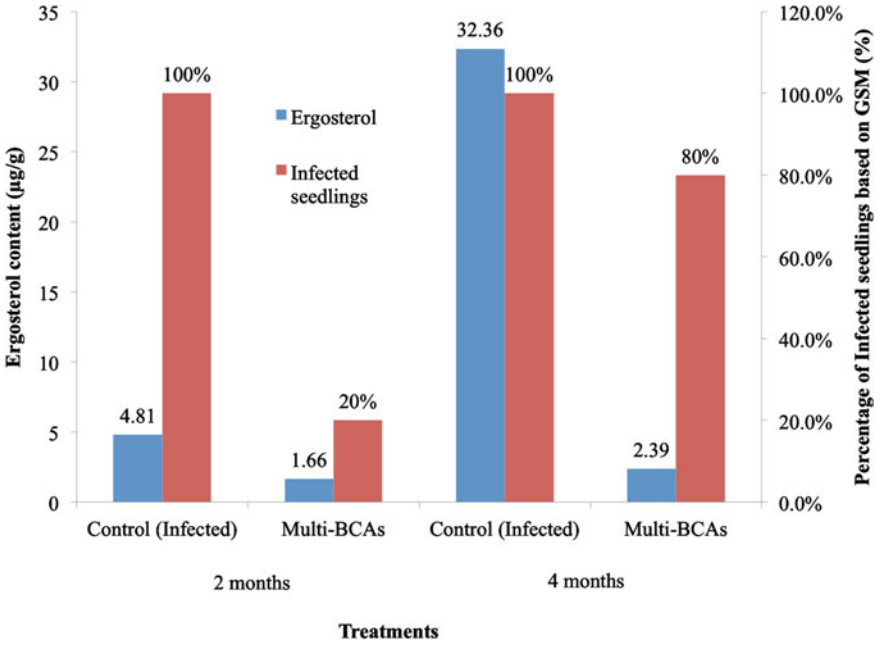

Fig. 5.4 Quantification of percentage of infected seedlings and ergosterol content in reduction of *Ganoderma* colonization in nursery. No ergosterol was detected in healthy (control) palms (Data not shown). *Notes* BCAs = Biological Control Agents; GSM = *Ganoderma* Selective Media

Based on the results presented it is revealed that the combination of *Bacillus* spp. and *Trichoderma* spp. was effective in controlling the colonization of *Ganoderma* in both nursery and field trials (Alexander and Chong 2014). These results may attributed to the synergistic effect between the combinations of the two genera in the treatment. The mechanism of *Trichoderma* and *Bacillus* on *G. boninense* may be complex and what has been defined as biocontrol is the final result of the different mechanisms acting synergistically by achieve disease control (Howell 2003). Different species of *Trichoderma* have different mechanisms and ability, thus their indirect effects may also vary. Some possible mechanisms of antagonism employed by *Trichoderma* spp. include competition of nutrients and space or as a result of the ability of *Trichoderma* to produce inhibitory compounds or by modifying the rhizospere to a condition where *G. boninense* cannot strive. Biocontrol results of *Trichoderma* spp. may also be due to direct interaction between these BCAs themselves and *Ganoderma*, such as in mycoparasitsm. The destructive stage in mycoparasitic process, which involves the degradation of the host cell wall, is mediated by the production of lytic enzymes such as glucosidases and chitinases (Chérif and Benhamou 1990; Benítez et al. 2004). Their ability to colonize plant root surface also stimulate plants to produce their own antimicrobial compounds that prevent further infection of plant pathogens, but may need further

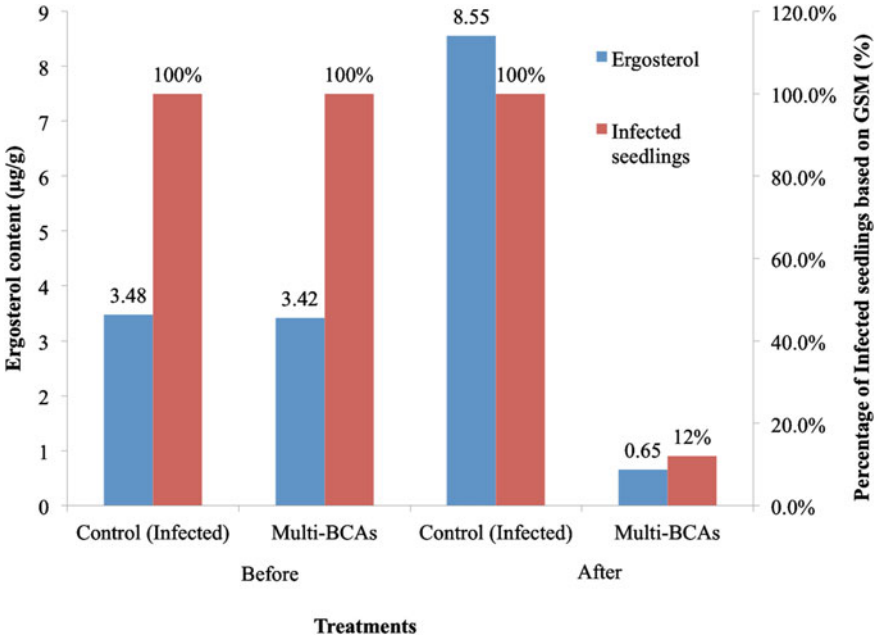

Fig. 5.5 Quantification of percentage of infected palms and ergosterol content in field trial before and after BCA treatments. No ergosterol was detected in healthy (control) palms (Data not shown). *Notes* BCAs = Biological Control Agents; GSM = *Ganoderma* Selective Media

investigation. However, a study by Vinale et al. (2008) showed several metabolites such as 6-pentyl-a-pyrone, harzianopyridone and harzianolide isolated from *Trichoderma* spp. involved in activation of plant defense mechanisms and plant growth regulation in pea, tomato and canola.

Bacillus spp. are natural inhabitant of rhizosphere. They are considered as endophytic, and capable in colonizing the internal host tissues and plant's vascular system without doing any substantive harm or gaining as residency (Sapak et al. 2008). The reduction of ergosterol content in infected oil palm treated with *Bacillus* spp and *Trichoderma* spp. combination suggests that *Bacillus* spp. in the formulation plays a significant role in inhibiting the penetration of *G. boninense* into vascular system. Their ability to compete within the vascular system may limit *Ganoderma* for both nutrients and space during its proliferation. Competition for nutrients, especially carbon, is assumed to be responsible for the well-known phenomenon fungistasis, characterizing the inhibition of *Ganoderma* spore germination (Alabouvette et al. 2006). Their inhibitory activity against *G. boninense* may also be due to their antagonistic effect mainly by producing antifungal lipopeptides such as surfactins, iturins and fengycins (Ongena and Jacques 2008). In addition to this antifungal activity, production of lipopeptides also suggested to stimulate plant defence response known as induced systemic resistance

(ISR) (Ongena et al. 2007) upon pathogen attack, leading to an enhanced resistance to *G. boninense* encountered.

These results are in agreement with an observation by Guetsky et al. (2002) which suggested that a combination of BCAs with different mechanisms of disease control will have an additive or synergistic effect and results in enhanced disease control compared to their individual application. Guetsky et al. (2001) postulated that as long as BCAs have different ecological requirements, combinations of agents with different requirement will likely increase reliability and decrease variability of biocontrol.

5.2 Organic Additives (Non-living Biostimulant)

5.2.1 Fulvic Acid and Humic Acid

Fulvic acids (FAs) and Humic acids (HAs) are important components of organic matter in soil. They play a vital role in enhancing soil fertility and plant nutrition, interacting with plant growth and microorganism activity, therefore are always applied onto soil as fertilizer. Both FAs and HAs are complementing each other, most research on FAs, are focusing on their antioxidant activities (Rodriguez et al. 2011), while HAs exhibited many biological activities and are well-known for their capability to induce plant development, especially root systems (Canellas et al. 2002, 2010). Crecchio and Stotzky (1998) also reported that beside HAs themselves demonstrate biological activity, some active microbes could also bound to HAs, thus, the substances could show antimicrobial activity. This figure supported current reports on combination of these substances with multi-BCAs against soil pathogen, particularly *Ganoderma.* Researchers believes that healthy soil improves plant protection against soil pathogens. Interesting study by Wu et al. (2016) also reported that soil treated with HAs and FAs show inhibition against phytopathogenic fungi under greenhouse condition. Application of HAs and FAs gives the soil the nature to resist soil borne diseases, able to inhibit many soil-pathogenic fungi. Therefore application of HAs and FAs as a biostimulant alongside with active microbes could benefits the soil environment and promotes the growth of beneficial microbes in the root zone, subsequently lessen the emerging of soil-borne diseases.

5.2.2 Chitin

Chitin is a polysaccharide of animal origin found abundantly in nature and is absence in plant. Chitin has been investigated as an antimicrobial material against wide range of pathogens. This component is believed to play an important role in

the activation of defense signalling pathways in plant. During fungal infection, plant cells secrete chitinases that release chitin fragments (chitooligosaccharides or chitin oligomers) from fungal cell walls that can act as an elicitor to induce plant innate immunity against the invading pathogen (Boller 1995; Passarinho and de Vries 2002). Consequently pre-treatment of plants with chitin fragments to increase expression of chitinase enhances plant resistance against various pathogens. Cretoiu et al. (2013) reported application of chitin onto soil suppress the emerging of soilborne pathogens and has indirectly increased the abundance of total soil microbial population. This scenario might due to the changes in the structure and/or activity of the microbiota in soil, which thus confers suppression of plant pathogens (Weller et al. 2002). Therefore, in response to application of chitin, chitinolytic microorganisms are increased in numbers and/or activites which capable of hydrolyzing the chitinous hyphae of pathogenic soil fungi.

5.2.3 Amino Acid

Amino acids are the building blocks for proteins which are important in the metabolic process and play keyroles in plant development, homeostasis and growth. Previous studies revealed the involvement of amino acids in plants systemic resistance against phytopathogenic fungi. Hasabi et al. (2014) reported that application of amino acid on canker infected-lime plant significantly increased the plant induced resistance as well as decreased the severity of disease by reducing necrotic lesion size. Study on the application of amino acid such as glutamate (Glu) also shown to induce rice disease resistance against rice blast in leaves (Kadotani et al. 2016) merely by inducing salicylic acid (SA)-responsive genes, rather than jasmonic acid (JA) or ethylene (ET)-responsive genes. Another study by Scheideler et al. (2002) also reported the expression of *avrRpt2* gene in *Arabidopsis thaliana*, inoculated with avirulent *Pseudomonas syringae pv. tomato* (Pto) which activates the transcription of genes involved in amino acid biosynthesis. Metabolic profiling has also shown that inoculation with virulent or avirulent pathogens alters amino acid contents in *Arabidopsis* (Ward et al. 2010).

5.3 Multi-biological Control Agents with Organic Additives

Other posible control methods were also reported on combination of selected multi-BCAs with other organic additives (Alexander and Chong 2014). The combination of multi-BCAs is different from the previous research. Combination of BCAs in each treatment was found to be compatible with organic additives against *Ganoderma*.

5.4 Multi-biological Control Agents with Humic and Fulvic Acid Additives

Most researches on biological control of different plant disease are focused primarily using bacteria, actinomycetes and filamentous fungi. The applications of yeast as biocontrol agents are made possible as a new trend against different pathogens. The ability of certain taxa of yeast to multiply rapidly, to produce antifungal metabolites and cell wall degrading enzymes and to induce resistance of host tissues indicates their potential to be exploited as biocontrol agents (El-Tarabily and Sivasithamparam 2006). In this treatment, combination of *Lactobacillus*, *Nattobacillus* and *Saccharomyces cerevisiae* incorporated with humic and fulvic acids worked synergistically against *G. boninense* and led to the success of the combination in reducing the pathogen colonization both in nursery and field trials (Alexander and Chong 2014). This was in agreement with the finding of Moustafa and Mohamed (2008), who reported that application of *S. cerevisiae* as biocontrol agents have reduced the infection of soil-borne fungal plant pathogen, *Fusarium oxysporum* causing damping-off symptoms in sugar beet seedlings. *S. cerevisiae* was used as biocontrol agent and inducer of plant systemic resistance mechanisms which are associated with increasing certain enzymes such as peroxidise, chitinase and pathogensis-related protein (El-Sayed 2000). Application of *Lactobacillus*, a Lactic Acid Bacterium (LAB) as a biocontrol agent has been reported against some pathogens such as *Xanthomonas campestris*, *Erwinia carotovora* and *Staphylococcus aureus* (Jalali et al. 2012; Anas et al. 2008). These bacteria are known to have the ability to convert Lactose and other similar carbohydrates into lactic acid which inhibit the growth of *Ganoderma*. Ström et al. (2002) identified 3-phenyl lactic acid produced by *Lactobacillus plantarum* MiLAB 393 and *Lactobacillus Coryniformis* Si3 has an inhibitory effect against *Aspergillus nidulans*. The antagonist effects of *Lactobacillus* are due to the production of antimicrobial agents such as organic acids, hydrogen peroxide and bacteriocin or related substances (Kalalou et al. 2010). The addition of *Nattobacillus* in this treatment is to help in activating, strengthening and enhancing *Lactobacillus* in producing metabolic compound such as lactic acid, producing strong protein degradation enzymes and starch degradation enzyme which is against *Ganoderma* growth (Hosoi et al. 2000).

Similar with the previous multi-BCAs combination, this combination was also tested both in nursery and field. Based on Fig. 5.6, percentage of infected seedlings which had been treated with the multi-BCAs with humic and fulvic acid additives was significantly reduced to only 60% and 1.99 µg/g of ergosterol concentration, compared to the untreated control. The percentage of oil palm seedlings infected by *Ganoderma* after four months incubation remained the same with 3.54 µg/g of ergosterol concentration. Meanwhile for the assessment on the reduction of colonization of *Ganoderma* (Fig. 5.7), the treatment showed great reduction of infected seedlings down to 20% and 1.62 µg/g of ergosterol concentration. However the

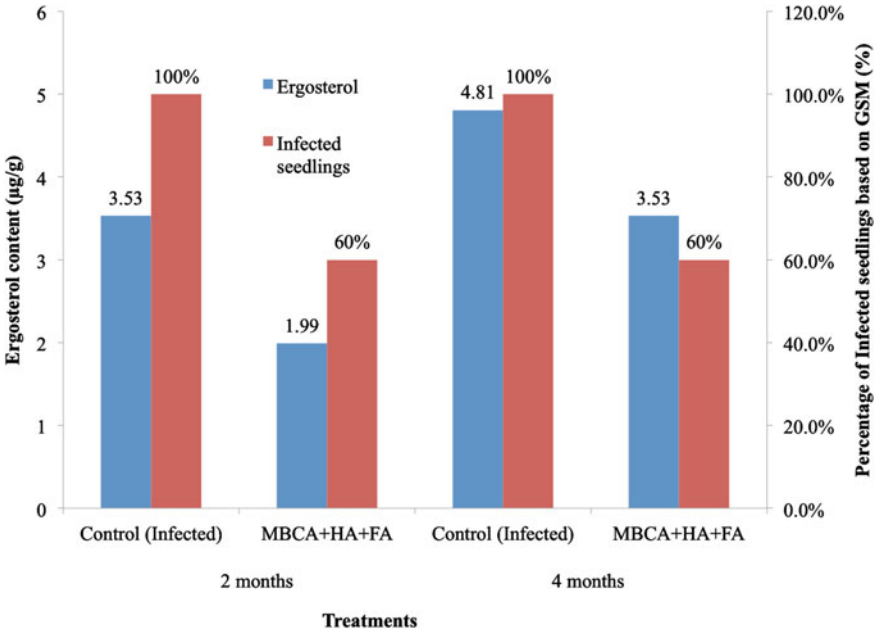

Fig. 5.6 Quantification of percentage of infected seedlings and ergosterol content in prevention of *Ganoderma* colonization in nursery. No ergosterol was detected in healthy (control) palms (Data not shown). *Notes* MBCAs = Multi-Biological Control Agents; GSM = *Ganoderma* Selective Media; HA = Humic Acid; FA = Fulvic Acid

percentage of infection increased to 70%, four months after, with 6.99 µg/g of ergosterol concentration.

Following the nursery trial, assessment on the multi-BCAs with humic and fulvic acid additives on real field were also tested. The result in Fig. 5.8 shows that the treatment was successfully reduced the percentage of fungal colonization to 24%, with a parallel reduction in ergosterol to 47% of the concentration at the outset. The concentration of ergosterol in infected, non-treated palms more than doubled.

Humic and fulvic acids are both organic matter substances formed during the decay of plant and animal residues. They are considered to be the most chemically active organic compounds in the soil which contain many nutritional phytochemical groups such as fatty acids, polyphenols, ketones, natural sterols, quinones, etc. Humic and fulvic acids may stimulate plant growth and microorganismc present in the soil (Ramya et al. 2014). Addition of humic and fulvic acids help to 'correct' the soil condition providing a better environment for the microbes. Past research found these substances enhanced the soil condition not only beneficial for plants, but also enhanced soil microbial population and microbial activity (Cooper et al. 1998). Study by Yigit and Dikilitas (2008) also found the application of these organic

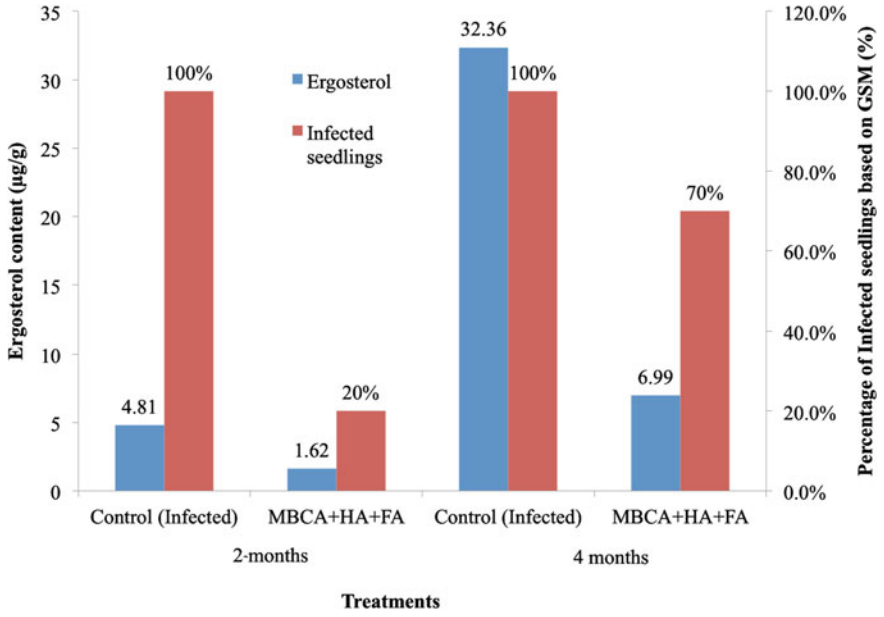

Fig. 5.7 Quantification of percentage of infected seedlings and ergosterol content in reduction of *Ganoderma* colonization in nursery. No ergosterol was detected in healthy (control) palms (Data not shown). *Notes* MBCAs = Multi-Biological Control Agents; GSM = *Ganoderma* Selective Media; HA = Humic Acid; FA = Fulvic Acid

substances helped plants less susceptible to the root rot pathogens. Combination of humic and fulvic acids with multi-biological agents will stimulate and help the BCAs survival in the soil ecosystem.

5.4.1 Multi-biological Control Agents with Chitin and Amino Acid Additives

Combination of multi-biological agents comprises of *Bacillus* spp., *Pseudomonas* spp. and *Aspergillus* spp. also has the potential to control *G. boninense* (Alexander and Chong 2014). Its ability to control *G. boninense* was associated with the presence of chitin and amino acids. Synergistic effects of *Bacillus* spp. and *Pseudomonas* spp. also have been reported in the control of *Fusarium* wilt of chickpea (Karimi et al. 2012). *Pseudomonas* is known to restrict pathogens by producing metabolites with antimicrobial activity (Chen et al. 2000). Their ability to produce secondary metabolites such as siderosphores, an iron chelator, has been known to suppress *Ganoderma* growth by limiting the iron availability in soil. In addition to these mechanisms, there is also the release of volatile non-specific

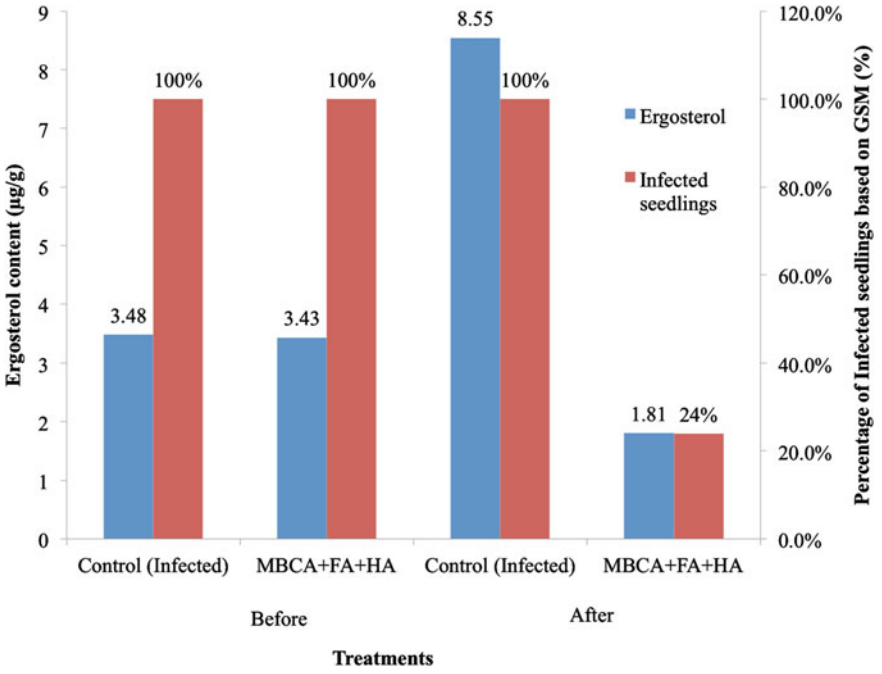

Fig. 5.8 Quantification of percentage of infected palms and ergosterol content in field trial before and after BCA treatments. No ergosterol was detected in healthy (control) palms (Data not shown). *Notes* MBCAs = Multi-Biological Control Agents; GSM = *Ganoderma* Selective Media; HA = Humic Acid; FA = Fulvic Acid

inhibitors, such as hydrogen cyanide (HCN), enzymes and phytohormones (Schippers et al. 1991; Gupta et al. 2001), which could hamper the activity of *G. boninense*. Eventhough *Aspergillus* species are usually considered free-living saprophytes in soil, several researches have shown *Aspergillus* species has the potential to be BCA against wood decay fungi and *Ganoderma* of oil palm (Ariffin et al. 2000; Tiwari et al. 2011). Major mechanisms involved in the biocontrol activity of *Aspergillus* spp. are competition for space and potential plant infection sites and production of numerous hydrolytic enzymes, including chitinase, protease and ß-1,3-glucanase (Szilágyi et al. 2012). These hydrolytic enzymes could partially degrade *Ganoderma* cell wall and lead to its parasitisation. Productions of hyrolytic enzymes are improved with the addition of chitin additives. Chitin are utilized by the microbes as a sole carbon and energy source resulting in secretion of hydrolytic enzymes, which are antagonist to pathogen (Herrera-Estrella and Chet 1998). Not only that, hydrolyzed chitin will produce chitosan compound which stimulates the plant to induce its growth hormon. In this sense, BCAs of soil-borne disease has been correlated with chitinase production. Meanwhile, amino acid significantly increased the plant induced resistance as well as decreased the severity of disease by reducing necrotic lesion size (Hasabi et al. 2014). Previous studies

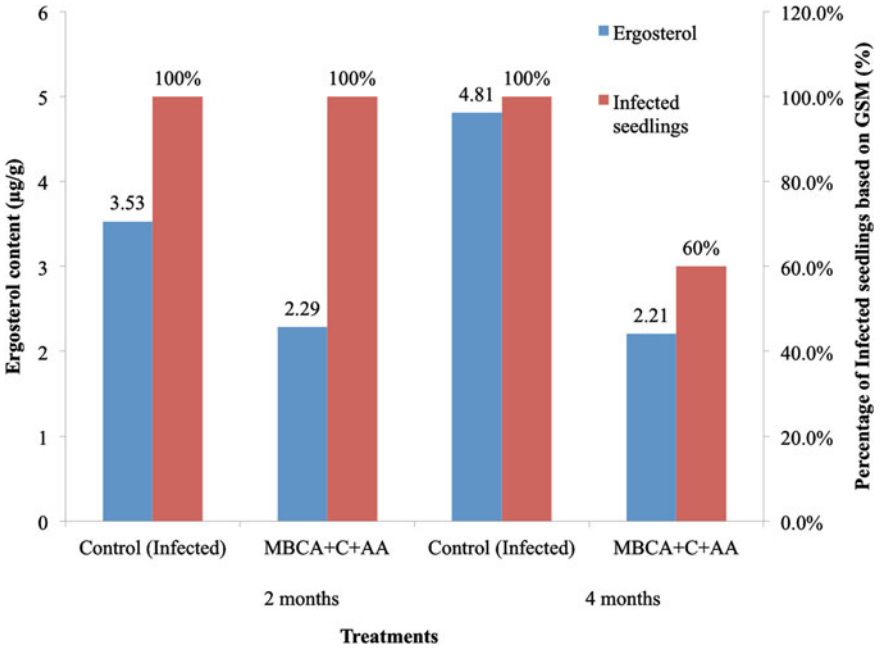

Fig. 5.9 Quantification of percentage of infected seedlings and ergosterol content in prevention of *Ganoderma* colonization in nursery. No ergosterol was detected in healthy (control) palms (Data not shown). *Notes* MBCAs = Multi-Biological Control Agents; GSM = *Ganoderma* Selective Media; C = Chitin; AA = Amino Acid

also have shown that the phenomena of synergism among BCAs, chitin and amino acids combination can fasten the killing of the pathogenic fungi compare to individual element contributions (Lorito et al. 1993).

The combination of multi-BCAs with chitin and amino acid additives was also tested in nursery. Based on Fig. 5.9, infected seedlings which had been treated with the treatment reduced the *Ganoderma*-associated ergosterol content, but there was no reduction of percentage of infected seedlings compared to control. However after four months of interval, this treatment succeed in reducing the percentage of infected seedlings down to 60% compared to the previous two months of incubation. This suggests the treatment may help in preventing *Ganoderma* colonization in the nursery provided the product was applied earlier, as the combinations of microbes in this treatment need longer time to be effective. Meanwhile assessment on the reduction of *Ganoderma* colonization shows that multi-biological control agents with chitin and amino acid additives has a great capacity to reduce the infection down to 10% with 2.29 µg/g of ergosterol content. However, the percentage of seedlings infected increased to 70%, four months later but with lower ergosterol content (Fig. 5.10).

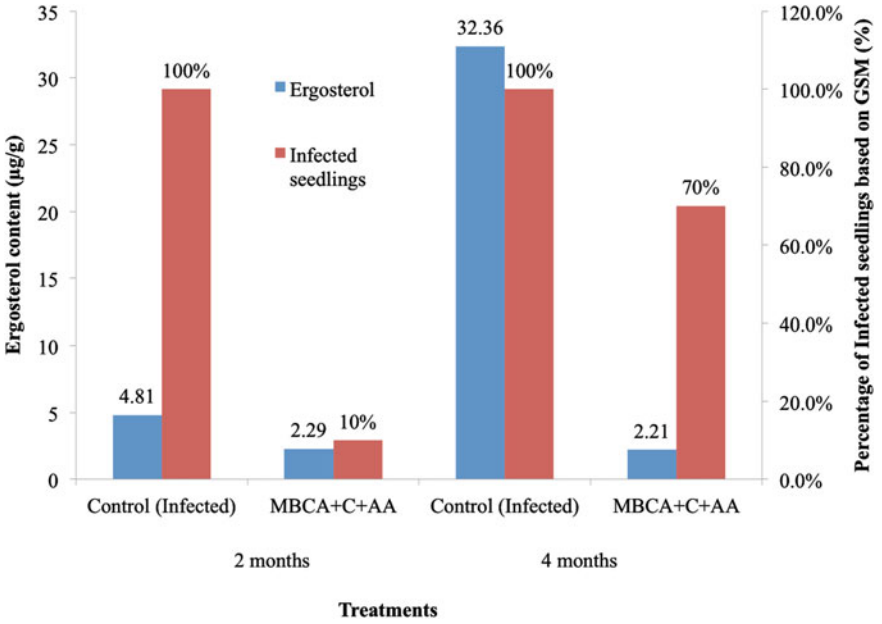

Fig. 5.10 Quantification of percentage of infected seedlings and ergosterol content in reduction of *Ganoderma* colonization in nursery. No ergosterol was detected in healthy (control) palms (Data not shown). *Notes* MBCAs = Multi-Biological Control Agents; GSM = *Ganoderma* Selective Media; C = Chitin; AA = Amino Acid

References

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant diseases: the European situation. Eur J Plant Pathol 114:329–341

Alexander A, Chong KP (2014) Combination of biological agents in suppressing colonization of *Ganoderma boninense* of basal stem rot. Am Eur J Sust Agri 8(7):1–7

Alexander A, Dayou J, Chong KP (2015) Morphological changes of *Ganoderma boninense* mycelia after challenged by *Trichoderma* and *Bacillus*. In: Proceedings of the 23rd Scientific Conference of Microscopy Society Malaysia. Tronoh, Malaysia, December 2014

Anas M, Jamal EH, Mebrouk K (2008) Antimicrobial activity of *Lacticbacillus* isolated from Algerian raw goats milk against *Staphylococcus aureus*. World J Dairy Food Sci 3(2):39–49

Ariffin D, Idris AS, Singh G (2000) Status of *Ganoderma* in oil palm. In: Flood J, Bridge PD, Holderness M (eds) *Ganoderma* diseases of perennial crops. CABI Publishing, Wallingford, pp 49–68

Benítez T, Rincón AM, Limón MC, Codon AC (2004) Bioncontrol mechanisms of *Trichoderma* strains. Int Microbiol 7(4):249–260

Boller T (1995) Chemoperception of microbial signals in plant cells. Ann Rev Plant Physiol Plant Mol Biol 46:114–189

Canellas LP, Okorokova-Façanha A, Olivares FL, Façanha AR (2002) Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H^+-ATPase activity in maize roots. Plant Physiol 130:1951–1957

Canellas LP, Piccolo A, Dobbss LB, Spaccini R, Olivares FL, Zandonadi DB, Façanha AR (2010) Chemical composition and bioactivity properties of size-fractions separated from a vermicompost humic acid. Chemosphere 78:457–466

Chen C, Belanger RR, Benhamou N, Paulitz TC (2000) Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. J Physiol Mol Plant Pathol 56:13–23

Chérif M, Benhamou N (1990) Cytochemical aspects of chitin breakdown during the parasitic action of a *Trichoderma* sp. on *Fusarium oxysporum* f. sp. *radicislycopersici*. Phytopathology 80:1406–1414

Chong KP, Atong M, Rossall S (2012) The role of syringic acid in the interaction between oil palm and *Ganoderma boninense*, the causal agent of basal stem rot. Plant Pathol 1365–3059

Cooper RI, Liu C, Fisher DS (1998) Influence of humic substances on rooting and nutrient content of creeping bent grass. Crop Sci 38:1639–1644

Crecchio C, Stotzky G (1998) Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil. Soil Biol Biochem 30:463–470

Cretoiu MS, Korthals G, Visser J, van Elsas JD (2013) Chitin amendment raises the suppressiveness of soil towards plant pathogens and modulates the actinobacteriaceal communities in an experimental agricultural field. Appl Environ Microbiol 79(17):5291–5301

Devaki NS, Bhat SS, Bhat SG, Manjunatha KR (1992) Antagonistic activities of *Trichoderma harzianum* against *Pythium aphanidermatum* and *Pythium myriotylum* on tobacco. J Phytopathol 136:82–87

Elad Y (2000) Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Prot 19:709–714

El-Sayed S (2000) Microbial agents as a plant growth promoting and roots protector. In: 10th Microbiology conference, Cairo, Egypt, 12–14 November 2000

El-Tarabily KA, Sivasithamparam K (2006) Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Mycoscience 47:25–35

Guetsky R, Stienberg D, Elad Y, Dinoor A (2001) Combining biocontrol agents to reduce the variability of biological control. Phytopathology 91:621–627

Guetsky R, Steinberg D, Elad Y, Fischer E, Dinoor A (2002) Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. Phytopathology 92:976–985

Gupta CP, Dubey RC, Kang SC, Maheswari DK (2001) Antibiosis mediated necrotrophic effect of *Pseudomonas* GRC2 against two fungal plant pathogens. Curr Sci 81:91–94

Hasabi V, Askari H, Alavi SM, Zamanizadeh H (2014) Effect of amino acid application on induced resistance against citrus canker disease in lime plant. J Plant Prot Res 54(2):144–149

Herrera-Estrella A, Chet I (1998) Biocontrol of bacteria and phytopathogenic fungi. In: Arie A (ed) Agricultural biotechnology. Marcel Dekkere, New York, pp 263–282

Hosoi T, Ametani A, Kiuchi K, Kaminogawa S (2000) Improved growth and viability of lactobacilli in the presence of *Bacillus sbutilis* (natto), catalase, or subtilisin. Can J Microbiol 46(10):892–897

Howell CR (2003) Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Discov 87:4–10

Jalali MD, Khosro I, Mohammad Faezi G, Tabrizi SSK (2012) Antagonism of *Lactobacillius* species against *Xanthomonas campestris* isolated from different plants. J Appl Biol Sci 2(9):480–484

Kadotani N, Akagi A, Takatsuji H, Miwa T, Igarashi D (2016) Exogenous proteinogenic amino acids induce systemic resistance in rice. BMC Plant Biol 16(60):1–10

Kalalou I, Zerdani I, Faid M (2010) Antagonistic action of biopreservative *Lactobacilluc plantarum* strain on pathogenic *E. coli 0157:H7* in fresh camel meat stored at 10 °C. World J Dairy Food Sci 5(91):7–13

Karimi K, Amini J, Harighil B, Bahramnejad B (2012) Evaluation of biocontrol potential of *Pseudomonas* and *Bacillus* spp. against *Fusarium* wilt of chickpea. Agri J Crop Sci 6(4): 695–703

Kubicek CP, Harman GE (1998) Trichoderma and Gliocladium. vol 1. Basic biology, taxonomy and genetics. Taylor & Francis, London

Lorito M, Harman GE, Hayes CK, Broadway RM, Tronsmo A, Woo SL, Di-Pietro A (1993) Chitinolytic enzymes produced by *Trichoderma harzianum*: antifungal activity of purified endochitinase and chitobiosidase. Phytopathology 83:302–307

Motlagh MRS, Samimi Z (2013) Evaluation of *Trichoderma* spp., as biological agents in some of plant pathogens. Ann Biol Res 4(3):173–179

Moustafa ES, Mohamed FEN (2008) Application of *Saccharomyces cerevisiae* as biocontrol agent against *Fusarium* infection of sugar beet plants. Acta Biol Szeged 52(2):271–275

Ongena M, Jacques P (2008) *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol 16(3):115–125

Ongena M, Jourdan E, Adam A, Paquot M, Brans A (2007) Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. Environ Microbiol 9:1084–1090

Passarinho P, de Vries SC (2002) Arabidopsis chitinase: a genomic survey. In: Somerville CR, Meyerowitz EM (eds) The Arabidopsis Book. American Society of Plant Biologists, Rockville MD, USA

Ramya DE, Pushparani A, Rajemdran P, Raju S, Sriranjani S, Sruthi I (2014) Humic acid, protein hydrolysate & microorganisms as a mixed consortium in plant growth. Indian J Appl Microbiol 17(1):23–31

Rodriguez NC, Urrutia EC, Gertrudis BH, Chaverri JP, Mejia GB (2011) Antioxidant activity of fulvic acid: a living matter-derived bioactive compound. J Food Agri Environ 9:123–127

Sapak Z, Meon S, Ahmad ZAM (2008) Effect of endophytic bacteria on growth and suppression of *Ganoderma* infection in oil palm. Int J Agri Biol 10:127–132

Scheideler M, Schlaich NL, Fellenberg K, Beissbarth T, Hauser NC, Vingron M, Slusarenko AJ, Hoheisel JD (2002) Monitoring the switch from housekeeping to pathogen defense metabolism in *Arabidopsis thaliana* using cDNA arrays. J Biol Chem 277:10555–10561

Schippers B, Bakker AW, Bakker PAHM, Van Peer R (1991) Beneficial and deleterious effects of HCN-producing *Pseudomonas* on rhizosphere interactions. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer, Dordrecht, pp 211–219

Ström K, Sjögren J, Broberg A, Schnürer J (2002) *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(l-Phe-l-Pro) and cyclo(l-Phe-*trans*-4-OH-l-Pro) and 3-phenyllactic acid. Appl Environ Microbiol 68:4322–4327

Szilágyi M, Anton F, Forgács K, Yu JH, Pócsi I, Emri T (2012) Antifungal activity of extracellular hydrolases produced by autolysing *Aspergillus nidulans* cultures. J Microbiol 50(5):849–854

Tiwari CK, Jagrati Parihar RK, Verma RK (2011) Potential of *Aspergillus niger* and *Trichoderma viride* as biocontrol agents of wood decay fungi. J Indian Acad Wood Sci 8(2):169–172

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008) *Trichoderma*–plant– pathogen interactions. Soil Biol Biochem 40(1):1–10

Ward JL, Forcat S, Beckmann M, Bennett M, Miller SJ, Baker JM, Hawkins ND, Vermeer CP, Lu C, Lin W, Truman WM, Beale MH, Draper J, Mansfield JW, Grant M (2010) The metabolic transition during disease following infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv tomato. Plant J 63:443–457

Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Wu M, Song M, Liu M, Jiang C, Li Z (2016) Fungicidal activities of soil humic/fulvic asids as related to their chemical structures in greenhouse vegetable fields with cultivation chronosequence. Sci Rep 6:32858

Yigit F, Dikilitas M (2008) Effect of humic acid applications on the root-rot diseases caused by *Fusarium* spp. on tomato plants. Plant Pathol J 7:179–182

Chapter 6
General Conclusion

The BSR disease caused by *G. boninense* is well known as the most notorious threat to the oil palm industry in South East Asian countries. BSR disease has continuously reduces the profitability and jeopardises the growth of the oil palm industry. The most significant problem for BSR disease is that it cannot be detected at early infection. Although the tree is physiologically affected, there is no clear indication of BSR infection at early stage, despite the disease progresses through the oil palm from the base without any external symptoms. Current method of detection of BSR carried out based on the external symptoms, and BSR infection is mostly confirmed when fruiting body of *Ganoderma* appear, otherwise, infection status is uncertain. However, at this stage the infection might be too late for any remedy. This slow progression of disease makes early detection of *G. boninense* infection extremely difficult. Lab-based detection methods using molecular and other biochemical assay have been proven to be more accurate and convincing, however the issue of cost efficiency, larger sample volume and proper sampling remain a big challenge. In parallel, some field detection methods such as E-nose, tomography, hyperspectral, spectroscopy and ultrasonic have shown some promising results but with long way off providing a *Ganoderma*-specific detection.

Until now, no satisfactory method available to control this disease. Most of the control methods taken are only to prolong the palms life-span. Cultural practices combined with biological or chemical control have been considered as the best approach in controlling this disease. A more permanent control, research strategy to minimize as low as possible the source of pathogen inoculum in the soil should be implemented. *Ganoderma* in infected tissue in the soil has the ability to survive for long-term and disease outbreak could emerge at any time that suit the pathogen condition. Infection of some young palms is initiated by small leftover of diseased roots from the previous planting, hence any source of infected fragments plays an important role for BSR outbreak. Hence, proper choice of land-preparation methods before the replantation should be taken into consideration.

Attempts to control BSR disease using fungicides have been one of the methods used in plantation to control *G. boninense.* The public concern over the harmful

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effects of chemical pesticides on the environment and human health has encourages the search for safer and environmentally friendly control alternatives. Control of plant pathogens by the application of biological control is an alternative approach to the chemical fungicides and it is considered to be safer, low cost and ecofriendly method. Application of more than one BCA at a given time may have advantages of broad spectrum activity, enhancing the efficacy and reliability of the biological control and they communicate with each other to maximize biocontrol efficacy. Other than that, addition of organic compounds or non-living biostimulant in order to get a formulation would also enhance their effectiveness.

Soils in an established oil palm plantation may be exposed to long-term soil disturbance and chemical contamination from extensive agricultural practices. Environmental stresses brought by the contamination could be adduced for the low soil microbial population and diversity which greatly suppressed some of the potential antagonists. Without competing antagonists, pathogens such as *G. boninense* have an open road to the roots. Integration of biological strategies into agricultural system could assist planters to control *Ganoderma* pathogens in the real agricultural environment. Correspondingly, cultural practices such as reduced or minimum tillage or minimum soil disturbance should also be implemented along with BCAs application to restore the soil microbial ecosystem into active state, inhibiting *G. boninense* colonization as it is a weak competitor in an active state soil ecosystem. Finding a solution to completely eradicate BSR disease is not going to be an easy task. Continuous and intensive research on detection and managing *G. boninense* are urgently needed to ensure the economic benefits of the palm oil industry are to be sustained in South East Asia.

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