UNIVERSITI PUTRA MALAYSIA

BASAL STEM ROT OF OIL PALM : SCREENING SYSTEM AND BIOEFFICACY OF STEROL BIOSYNTHESIS INHIBITORS AGAINST GANODERMA BONINENSE

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By

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Studies were designed to develop a practical, quick and reliable screening system for basal stem rot (BSR) disease of oil palm using rubber wood inoculum block as an infection foci. Inoculation was performed under non-sterile conditions. 100% success on establishment of disease was achieved on seedlings and immature palms in a relatively short period. *G. boninense* was able to invade and colonise the oil palm roots without mechanical wounding.

Close contact between *G. boninense* and oil palm seedlings was established in a specially designed plastic container. Disease associated-symptoms were observed within two to three months after inoculation. The typical symptoms observed were the progressive yellowing and desiccation of leaves from the oldest to the youngest and the presence of basidioma. The extend of foliar symptoms reflected the disease severity of the roots and bole. The foliar symptoms were more severe when basidioma were detected. Mean severity of foliar symptoms was 42%, 33% and 16% on seedlings with fully developed basidioma, basidioma primodia and

without basidioma, respectively. Arrested growth and development were also observed on the seedlings. This was later followed by the death of the seedlings.

By placing the inoculum block in close contact with three roots and the bole of the one year old oil palm in the field, 15-25% infection of the bole tissues was achieved nine months after inoculation. The BSR associated symptoms observed were desiccation of the fronds and retardation of growth. BSR development tends to concentrate at the centre of the bole. Infected palms in the field were usually free from visible foliar symptoms until considerable damage had occurred in the bole region. Although all inoculated palms were infected, only 25% of the infected palms exhibited foliar symptoms 22 months after inoculation.

In vitro study showed that triazoles were significantly more effective than morpholines, with an EC_{50} values of 0.008-0.121 ppm a.i. and 1.939-2.944 ppm a.i., respectively. Total inhibition of mycelial growth by triazoles occurred at 1-5 ppm a.i. as compared to 250 ppm a.i. with morpholines. Difenoconazole appeared to be the most active fungicide to the mycelial growth of *G. boninense*. However, the morpholines generally exhibited better vapour phase activity than the triazoles against mycelial growth of *Ganoderma*. Penconazole and tridemorph showed good vapour phase activity and uniform colony inhibition.

Uptake and efficacy of fungicides were detected by the bioassay on oil palm seedlings. Apparently penconazole had greater systemic activity than propiconazole and difenoconazole. Fungicides also have phytotoxicity in the order penconazole > propiconazole > difenoconazole. The greenhouse fungicide screening study on the bioefficacy indicated the potential curative activity of penconazole (0.5 g a.i., preliminary experiment) and both difenoconazole and propiconazole (1.5 g a.i., repeat experiment) against BSR during early infection stage. However, activity decreased with increase in degree of colonisation, especially on seedlings which have already produced basidioma.

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PENYAKIT REPUT PANGKAL BATANG PADA KELAPA SAWIT: SISTEM PENYARINGAN DAN KEBERKESANAN PERENCAT STEROL BIOSINTESIS TERHADAP *Ganoderma boninense*

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Kajian dijalankan untuk mendapatkan satu sistem penyaringan bagi penyakit reput pangkal batang (BSR) kelapa sawit yang praktikal, cepat dan berkesan dengan penggunaan inokulum blok kayu getah sebagai sumber jangkitan. Penginokulatan dilakukan dalam keadaan yang tidak steril. Dalam tempoh yang singkat, 100% kejayaan jangkitan telah tercatat pada anak benih kelapa sawit dan kelapa sawit yang belum matang . Cederaan tidak perlu untuk kemasukkan dan pengkolonian *G. boninense* pada akar kelapa sawit.

Sentuhan rapat antara *G. boninense* and anak benih kelapa sawit telah diwujudkan di dalam sebuah kotak plastik yang direka khas. Simptom yang berkaitan dengan penyakit dikesan dalam jangka masa dua hingga tiga bulan selepas inokulasi. Simptom yang biasa kelihatan adalah kekuningan dan pengeringan daun secara progresif bermula daripada daun peringkat tua ke muda dan kehadiran basidioma. Lanjutan simptom foliar memberi gambaran keterukan penyakit pada akar dan

pangkal batang pokok. Simptom tersebut bertambah teruk apabila terdapat basidioma. Anak beni yang memiliki basidioma, primodia basidioma dan tanpa basidioma masing-masing menunjukkan purata keterukan simptom foliar 42%, 33% dan 16%. Sekatan pertumbuhan dan perkembangan juga berlaku diikuti dengan kematian anak benih.

Dengan penempatan blok inokulum bersentuh rapat dengan tiga batang akar dan pangkal batang kelapa sawit yang berusia satu tahun, telah menghasilkan 15-25% jangkitan pada tisu-tisu pangkal batang sembilan bulan selepas penginokulatan. Simptom berkaitan dengan penyakit BSR yang dikesan adalah pengeringan pelepah daun dan pertumbuhan yang bantut. Perkembangan BSR lebih tertumpu kepada bahagian tengah pangkal batang pokok. Di ladang, pokok yang terjangkit selalunya bebas daripada simptom foliar yang nyata sehingga kerosakkan menular ke dalam pangkal batang. Walaupun keseluruhan pohon yang diinokulat terjangkit, hanya 25% daripadanya menunjukkan simptom foliar 22 bulan selepas penginokulatan.

Data *In vitro* menunjukkan bahawa triazole lebih berkesan secara bermakna daripada morpholine, dengan nilai EC_{50} 0.008-0.121 bsj b.a. dan 1.939-2.944 bsj b.a., masing-masing. Untuk triazole, perencatan pertumbuhan miselium sepenuhnya berlaku pada 1-5 bsj b.a. berbanding dengan morpholine pada 250 bsj b.a. Difenoconazole merupakan racun kulat yang paling aktif terhadap pertumbuhan miselium *G. boninense*. Akan tetapi, morpholine pada umunnya menunjukkan aktiviti fasa wapan yang lebih aktif daripada triazole merencat pertumbuhan miselium *Ganoderma.* Penconazole dan tridemorph menunjukkan aktiviti fasa wap yang baik dan perencatan koloni yang seragam.

Penyerapan dan keberkesanan racun kulat telah dikesan melalui bioasai anak benih kelapa sawit. Penconazole mempunyai aktiviti sistemik yang lebih tinggi daripada propiconazole dan difenoconazole. Racun kulat juga menghasilkan ketoksikan ke atas pokok dalam susunan penconazole > propiconazole > difenoconazole. Kajian keberkesanan racun kulat di rumah kaca menujukkan pada peringkat awal jangkitan, penconazole (0.5 g b.a., eksperimen awalan) dan kedua-dua difenoconazole dan propiconazole (1.5 g b.a., eksperimen ulangan) berpotensi menghasilkan aktiviti pembaik pulih daripada penyakit BSR, tetapi aktiviti ini berkurangan dengan pertambahan tahap pengkolonian, terutama ke atas anak benih kelapa sawit yang telah menghasilkan basidioma.

CHAPTER I

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) was introduced to Malaysia in 1930. However, interest to cultivate and expand this world's most productive oil crop only picked up in the early 1960's. This change of interest was the result of the agricultural diversification programme implemented by the Malaysian Government. In a period of thirty years, the area under oil palm has increased twenty-fold to almost two million hectares. Palm oil is now Malaysia's major economic contributor to the agricultural sector. The mid to long-term price forecastings are stimulating the plantation groups to expand their oil palm area at the expanse of rubber and cocoa. It is anticipated that the area under oil palm will further advance to 2.6 million hectares by the year 2000.

Recently the plantation sectors are facing numerous critical challenges. Areas of concern include shortage of human resources, cost of production, yield potential, rival oils and/or fats, competition from neighbouring countries and pest problems. Realising the importance of these potential predicaments, the Government and the plantation institutions are actively investigating feasible solutions. For instance, the Palm Oil Research Institute Malaysia (PORIM), Universiti Pertanian Malaysia (UPM) and Oil Palm Growers' Association have formed an Action Committee to address the problem of basal stem rot (BSR) of oil palm, a serious fungal malady in this region.

BSR of oil palm, caused by *Ganoderma* spp., is recognised to be one of the most important diseases of oil palm for sustainable economic production. This BSR disease was initially thought to be unimportant, and frequently associated with old ageing palms in the coastal areas. Presently, this disease is frequently found in 10-15 year-old fields, especially those located around the coastal region of lower Perak and at sites previously planted with coconut (Singh, 1991; Khairudin, 1990; Turner, 1981). A further shift in disease distribution was recorded recently. Occurrence of BSR was found on young palms of the second or third generation and in peat and inland areas (Benjamin, 1993; Singh, 1991; Ariffin *et al.*, 1989a). Widya *et al.* (1991) reported that the incidence of BSR in Indonesia is more prominent on palms of the second and third generation.

The planting techniques and/or previous crop appear to have close relationship on the speed of BSR appearance and disease incidence (Chung *et al.*, 1994; Khairudin, 1994; Singh, 1991). Higher incidence of BSR on young plantings before reaching their most productive period is anticipated. This is alarming as between 1995-2000 about 0.4 million hectares of oil palm will be replanted and cultivated under large monoculture systems. Most of these plantings will be the second and/or third generation cultivation. Besides, the adoption of the cost-effective under-planting technique of oil palm, will further contribute to the spread of BSR disease in immature fields.

The potential occurrence of disease in immature fields certainly is a threat to the industry. Until now, early diagnosis of the disease is impossible. Confirmation of BSR is based on the presence of basidiomata. This is inadequate as usually immature palms seldom produce basidiomata, even at the advanced stage of disease development (Benjamin, 1993). However, when above ground symptoms are noticeable, extensive damage has already taken place at the palm base. This scenario can lead to large vacant fields as currently experienced by some of the coastal and/or riverine estates.

Currently, there is no effective control of BSR palm in the field. The existing practice of the industry emphasising on preventive measure through clean clearing.

Complete removal of the infected tissues and root masses is impossible in the field, thus, the immature palms remain at risk to BSR. Besides, *Ganoderma* appears to produce resting structures in infected woody remains (Ariffin *et al.*, 1989b).

There is an urgent need to control BSR. Nevertheless, to conduct field trials on this disease is not an easy task. Critical constraints include the selection of uniform trial plots, slow progress of disease and inconsistency of trial methodology are still existing. It is extremely important to have a system where a preliminary test can be concluded in a short period under semi-control condition. Hence, the present studies were conducted with the following objectives:

- To develop a fast and reliable screening system of BSR on seedlings and immature oil palms using a rubber wood inoculum block

- To evaluate *in vitro* bioefficacy of sterol-biosynthesis inhibitors on *G. boninense* with direct contact and their vapour phase activity

- To investigate the *in vivo* behaviour and curative activity of triazoles against BSR on inoculated oil palm seedlings

CHAPTER II

LITERATURE REVIEW

Status of *Ganoderma* spp. as a Pathogen of Basal Stem Rot (BSR) of Oil Palm

Basal stem rot of oil palm was first reported in Malaya towards the end of 1927 and was initially associated with termites attack. Later, bracket-like fructifications emerged and were identified as *G. lucidum* (Leys) Karst (Thompson, 1931). In 1935, Thompson considered *G. lucidum* as pathogen of oil palms causing stem rot mainly of leaning palms (Thompson, 1935). It was described as basal stem rot by Voelcker (1950-51). BSR of oil palm was regarded a pathogen of some important when a patch of palms over 20 year-old was found infected (Heath, 1956). Initially, this malady did not arouse immediate attention since it had been viewed as an old age problem. Wijbrans (1955) considered that *G. lucidum* was a facultative parasite which would cause damage only on palms weakened by injuries and unfavourable environmental conditions. Turner believed that the old palms were susceptible because of the break-down in immunity barriers (Turner, 1981). Incidence of BSR on eight-year-old palms and subsequently on younger palms were observed for the first time in 1957 (Heath, 1957).

Several reports on BSR distribution in the country indicated close association of the disease incidence with areas previously planted with coconut (Turner, 1965a and 1965b; Navaratnam, 1964). There were signs that stumps left in the ground behaved as infection foci. In the 1980's, large hectarage of oil palm infected with BSR, particularly in the coastal areas and at sites formerly planted with coconut were reported. The disease reached endemic levels on 10 to 15-year-old plantings (Singh, 1991; Turner, 1981). Of late, the incidence of BSR has been recorded on peat areas, inland areas and on young palms of the second and/or third generation (Benjamin, 1993; Singh, 1991; Ariffin *et al*., 1989a). The fungus colonised young palms in a short period of time causing toppling over and/or die-standing of palms, even before reaching the optimum production stage. This leads to substantial economic loss which are due to low stand per unit cultivated area and reduced yield on diseased palms (Khairudin, 1994 and Singh, 1991).

Although much is known about the incidence of BSR, the confusion with regards to the identity of the pathogen has yet to be solved. In Malaysia, it was first identified as *G. lucidum* Karst by Thompson (1931). Varghese (1965) and Turner and Bull (1967) suggested that a complex of species might be involved. Identification made by Steyaert (1967) on morphological characteristics of sporophores indicated that more than one species of *Ganoderma* were associated with BSR in Malaysia. Based on the association with BSR from various parts of the world, Turner (1981) recorded at least 15 species of *Ganoderma* as likely pathogens of oil palm. However, based on associations and morphological studies of basidioma collected from various location in Peninsular Malaysia, Ho and Nawawi (1985) concluded that *G. boninense* was the sole pathogen, however, possible differential pathogenicity of the fungus was not confirmed. Thus, numerous problems on species or strains are yet to be resolved.

In the nineties, a significant progress to confirm the pathogenicity of *Ganoderma* was attained. With the development of a reliable inoculation technique (Ariffin *et al*.,1995; Sariah *et al*., 1994 and Khairudin *et al*., 1991) consistently high rates of BSR establishment on oil palm seedlings were achieved. Khairudin (1991) also showed that *G. boninense* was pathogenic to oil palm, whilst *G. philippi* and *G. lucidum* were not. Ariffin *et al*. (1991a) using selective media consistently isolated *G. boninense* from diseased palms, with and without visual symptoms. *G. boninense* is now established as the causal agent of BSR.

Infection of *G. boninense* takes place via root contact between healthy palms and the inoculum sources in the field, particularly infected dead stump tissues. The pathogen then colonises the roots and spread to the other tissues. Hitherto, the role of basidiospores of *G. boninense* in disease development is not clear. Inoculation of spores onto the cut frond bases (Turner, 1965c) and oil palm seedlings failed to yield infection (Ramasamy, 1972; Yeong, 1972). Turner (1991) believed that spores are responsible for spread into new planting areas. The possible role of insects in transmitting spores to healthy palms could not be ruled out.

Symptoms of Basal Stem Rot on Oil Palm

Foliar Symptoms

Inoculated Seedlings

Typical foliar symptoms of infection observed are progressive yellowing and desiccation of leaves from the oldest to the youngest. Desiccation of a leaf usually starts at the tip followed by rapid yellowing and drying of the entire lamina (Khairudin, 1990). Symptoms of yellowing and desiccation of leaves only appear in cases where infection has reached the bole, and generally appear on the same side of the inoculated root (Ariffin *et al.*, 1995). The extent of foliar symptoms is not indicative of the damage to the roots and bole region (Sariah *et al.*, 1994). Contrary to this, Khairudin (1990) reported that the severity of rot in the roots and/or bole was reflected by the foliar symptoms. Seedlings showed arrested growth and development, and later foliage dried-up and death of seedlings occurred (Khairudin, 1990).

Immature Palms

A typical early symptom is the occurrence of frond marginal necrosis starting from the lower whorl and later extending upwards. This is followed by chlorosis or mottling and desiccation. One-sided foliar symptoms are most common (Benjamin, 1993; Singh, 1991). This may serve as an indication of the site of infection. The newly unfolded leaves are shorter and chlorotic, and the tips may be necrotic. As the disease progresses, the palms take on a pale appearance, produce a number of unopened spears and growth becomes retarded (Singh, 1991).

Mature Palms

The symptoms resemble palms suffering from water deficiency. Excessive production of unopened spears are common. Subsequently, desiccation and wilting of fronds occurs. It usually begins with the oldest fronds and extends progressively to the younger ones and later to the crown. General nutrient deficiency symptoms may also appear. Desiccated fronds droop or break at the rachis and encircle the trunk. Often, when foliar symptoms are observed, more than 50% of the cross-sectional area of the palm base is necrotic. Eventually, palms die standing, and may remains erect or topple-over (Khairudin, 1994; Turner, 1981).

Basidioma

Inoculated Seedlings

Around the bole region, profuse vegetative growth of white masses rhizomorph can occur three to five weeks after inoculation, and later develop into a cushion-like stroma. This mycelia cushion rapidly becomes button-like (basidioma primodia) and is later followed by the apparent development of stipe. Primodia of basidioma develop into bracket shape basidioma within two weeks (Khairudin, 1990). Sariah *et al.* (1994) reported that typical white margin, bracket shape basidioma are formed within 12 to 16 weeks after inoculation. The position of the basidioma indicates the rough position of diseased area in the bole.

Immature and Mature Palms

On mature palms, basidioma are produced as the decay advances and usually develop at the base of the stem or on diseased primary roots. They may form before and/or after foliar symptoms. They begin as white basidioma primodia, but soon expand and become bracket-like. The mature basidioma vary in shape, size and colour. The shiny lacquered finish upper surface can be light to dark red brown, with a swollen white margin. The lower surface is greyish white in colour and has numerous minute pores. Basidioma are seldom seen on infected young palms. As the bole tissue of young palm is soft, it decays rapidly and palms usually die before the fruiting-bodies are produced (Turner, 1966; Singh, 1991).

Symptoms in the Roots and Stem Region (Bole)

Inoculated Seedlings

Severe root rotting has been reported in seedlings which had displayed progressive desiccation of the foliage. The cortex and epidermis of infected roots were readily separated from the stele (Khairudin, 1990). The fungus was detected on all root tissues, but was more pronounced in the vascular system (Sariah *et al.*, 1994). Ariffin *et al.* (1995) when screening for resistance revealed that the average movement of *G. boninense* within infected roots for all progenies tested was 10.22 mm/month.

On diseased tissues of the bole, progressive lesions were noted which later extended into the stem region as the fungus colonised further (Sariah *et al.*, 1994).

Immature and Mature Palms

In infected palms, the diseased roots are friable and brittle. The cortex becomes brown and the stele black. In the older roots, the fungus may be present as a whitish skin-like layer on the inner surface of the exodermis. The dry rotten tissues of the bole and stem tissues appear light brown. Interspersed within the decaying tissues are narrow dark lines or bands, which contain chlamydospore-like structures (Ariffin *et al.*, 1989b). Immediately above the advancing margin of the lesion, a lemon-yellow zone, presumably the result of host-parasite reaction is present (Singh, 1991; Turner, 1981). White mycelial of the fungus can be seen inside numerous small cavities of the light brown diseased tissues (Turner, 1981).

Establishment of *Ganoderma* Infection by Inoculation

Several techniques were used to establish the pathogenicity of *Ganoderma* on oil palm. Navaratnam (1961) carried out inoculation with a pure culture of *Ganoderma lucidum* (grew on sterilised sand and maize-meal medium) on the roots and stem tissues of old palms. The rate of success infection was 5% and 15% for root and stem inoculation, respectively. He ascribed the limited success rate to faulty technique and poor establishment of inoculum. Nevertheless, this observation has revealed that both oil palm root and stem were susceptible to *G. lucidum* infection.

Subsequently, successful inoculations of palm seedlings using large infected tissues were also reported (Navaratnam and Chee, 1965; Peries, 1974). However, Ramasamy (1972) and Ariffin *et al.* (1993) failed to obtain infection with this method. Inoculation of spores to different parts of oil palm also failed to yield infection (Turner, 1965c; Ramasamy, 1972; Yeong, 1972). Turner (1981) suggested that spores have inadequate inoculum potential for direct infection of a living palm.

Success of inoculation of *Ganoderma* was also reported on other crops. Adaskaveg and Gilbertson (1987) obtained a high success rate of infection by placing *G. lucidum* infected wooden blocks adjacent to wounded or unwounded roots of grapevines. Further inoculation on trunk tissues of field grapevine with infected grape wood chips was also successful. Sampath Kumar and Nambiar (1990) obtained positive pathogenicity of *Ganoderma spp.* on arecanut using stem insertion methods and planting of disease stump around healthy stem of arecanut.

Recently, a significant progress on disease establishment with pure culture of *G. boninense* on oil palm seedlings was demonstrated. Apparently, there are some similarity among the approaches. Firstly, pure cultures of *G. boninense* produced in the laboratory were used as inoculum. Secondly, inocula used were in a vigorous state and thirdly, close contact between the pathogen and host was made.

Okky *et al.* (1990) inoculated oil palm plantlets and calli *in vitro* with *G. boninense*. This technique had provided a contamination free environment to initiate the host-pathogen relationship. The mortality rate of the plantlets from those with wounded basal stems, wounded roots and unwounded plantlets were 90%, 35% and 45% respectively. They concluded that inoculation on wounded basal stem of plantlet was the best method.

Khairudin (1990) used mesocarp fibre of oil palm and rubber wood blocks as a substrate to culture *G. boninense* for the pathogenicity test on oil palm seedlings. Three to four leaf stage seedlings were placed on five-week-old inoculum in polypropylene bags and covered with sterilised soil. At nine months after inoculation, only two out of the 18 inoculated seedlings of the mesocarp fibre inoculum were infected. In the case where *G. boninense* was cultured on rubber wood blocks and placed in close contact with the roots of oil palm seedlings, excellent success infection rate was reported. Foliar symptoms were noticeable at seven months after inoculation, and by 11 months after inoculation all inoculated seedlings were infected with two dead. He concluded that the rubber wood block is an excellent substrate for the establishment of *G. boninense* inoculum. He further described that rubber wood, being solid, would enhanced the survival of the pathogen. Subsequent studies of Khairudin (1994) and Sariah *et al.* (1994) with rubber wood inoculum block had repeatedly yielded consistent and high success rates on oil palm seedlings. There appear to be a positive correlation existed between the size of inoculum and the degree of disease incidence (Khairudin, 1994). At 11 months, the incidence of disease for the 54, 108, 216 and 432 cm^3 rubber wood block inoculum were 5.9%, 5.9%, 17.6% and 82.4%, respectively.

Ariffin and Idris (1993) reported that their earlier attempts to artificially inoculate oil palm seedlings and field palms with pure cultures of *G. boninense* were unsuccessful due to contamination. Therefore, they later developed a single root inoculation technique with pure culture of *G. boninense*. During inoculation, a primary root was selected, surface sterilised, and then the distal end of the root was