Pycnoporus sanguineus AS BIODEGRADER OF OIL PALM TRUNK AND ROOT MASS FOR *Ganoderma* CONTROL

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Due to environmental concerns, the practice of land clearing by the clean burn method has been substituted by the non-burn regime (zero-burning) in the Malaysian oil palm plantation since 1993. Other benefits of zero burning practices include nutrient recycling, soil improvement, faster plantation establishment and lower costs (Hashim *et al.*, 1993). Despite of these benefits, disposal and management of oil palm biomass is a huge challenge under the zero-burning policy. The cost of removing old stand is expensive, and this practice is not feasible for independent estates and oil palm small holders, particularly those who cultivate oil palms on hills or deep peat soils. Normally, the old and diseased oil palm stands were felled, chipped or pulverised and evenly distributed between the planting rows for natural degradation (Idris, 2011; Hasan and Turner, 1998). Nevertheless, this unattended biomass (chipped oil palm trunk and root mass) requires approximately two years for complete degradation due to its high lignin and polysaccharides content (Murai *et al.*, 2009). In addition, the biomass becomes a medium for the breeding of pests such as the rhinoceros beetle (*Oryctes rhinoceros*) and pathogens like *Ganoderma*, which becomes a sources of inoculum for basal stem rot (BSR) infection in the healthy palms at replanting. Numerous methods have been developed for the management of basal stem rot (BSR) disease in oil palm plantations, including cultural, mechanical, chemical techniques and biological control, however, with little success (Idris, 2011). The use of biological control agents as stump and trunk treatments on oil palm infected with *Ganoderma* spp. as a part of basal stem rot (BSR) and oil palm generated waste management has been poorly investigated. The antagonistic potential of *Pycnoporus sanguineus* fungus that naturally occurs on oil palm trunks against the BSR pathogen *Ganoderma* spp. has been established. However, this study elucidates not only the efficacy of *P. sanguineus* in colonising, and minimising the survival rate of *Ganoderma* inoculum on oil palm trunks but also on reducing the duration of oil palm trunk biodegradation.

NOVELTY OF TECHNOLOGY

White-rot hymenomycetes, a naturally occurring *Pycnoporus sanguineus* (*Figure 1a*) on oil palm trunks, has been reported to have antagonistic characteristic against *Ganoderma boninense* (Naidu *et al.*, 2015). The present hymenomycetes is also known as promising wood degrader with the ability to simultaneously produce lignocellulolytic enzymes after subjected to an *in vitro* biodegradation assay (*Figure 1b*). Interestingly, the production of lignocellulolytic enzymes (laccase, manganese peroxidase, lignin peroxidase, CMCase, xylanase and amylase) was triggered under the solid state cultivation (SSC) of agro-industrial waste with *P. sanguineus*. The SSC formulation containing *P. sanguineus*, a biodegrader fungus in empty fruit bunches (EFB) and rice bran substrates, supplemented with C and N sources was developed (*Figures 1c* and *1d*). The SSC formulation of *P. sanguineus* was patented (PI 2015702850). Artificially induced fruiting body of *P. sanguineus* into fresh oil palm trunk was demonstrated (*Figure 1e*).

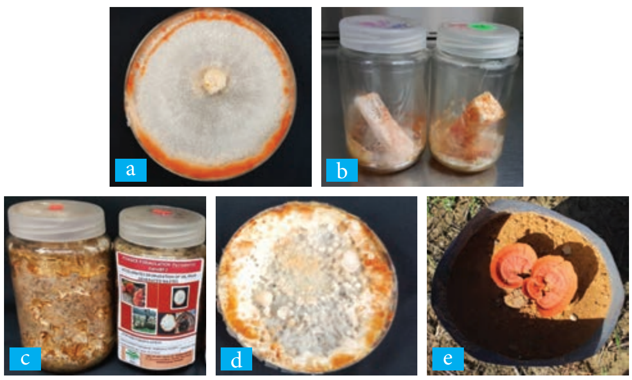

Figure 1. P. sanguineus *white-rot hymenomycetes. (a) Mycelia growth of* P. sanguineus *on PDA medium, (b) in vitro biodegradation assay and (c and d) SSC formulation of* P. sanguineus *and (e) artificial induced fruiting body of* P. sanguineus *on fresh oil palm trunk.*

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DEGRADATION MECHANISM OF *Pycnoporus sanguineus* ON OIL PALM TRUNK

Scanning electron microscopy (SEM) revealed the ingression and colonisation of fungal mycelium with clamp connections (*Figure 2a*) within the wood vessels and the parencymatic tissues (*Figure 2b*) of oil palm blocks (Naidu *et al*., 2017). The formation of the bore holes was clearly evident in the parencymatic tissue and appeared as round spots, causing loosening and forming large cavities on the ray parenchymal cells (*Figure 2c*). Towards the end of the decay periods (120 days), some of the cell wall components of the respective blocks had completely degraded and the absence of the fungal hyphae was seen at this stage (*Figure 2d*).

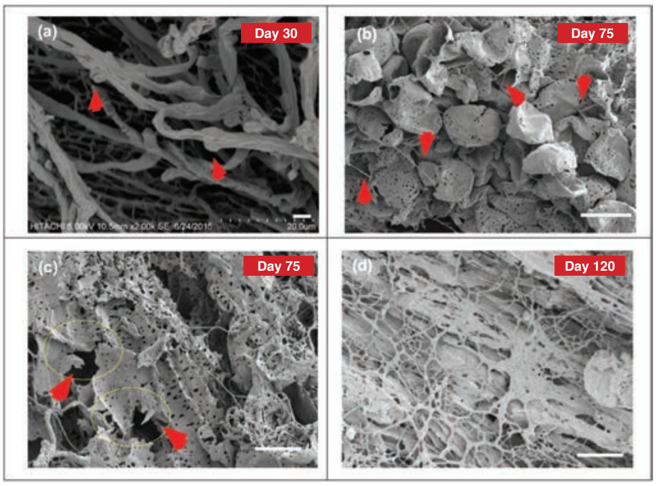


Figure 2. Scanning electron micrograph of cross sections of oil palm trunk blocks (infected tissues) decayed by P. sanguineus. *(a) Blocks showing fungal mycelium with clamp connections (arrowheads) penetrating and colonising within the parenchymal tissue by Day 30, (b) the fungal hyphae passing through the boreholes into adjacent cells (arrowheads) by Day 75, (c) loosening and formation of large cavities on the ray parenchymal cells (arrowheads) by Day 75, (d) complete degradation of other cell wall components and absence of the fungal hyphae by Day 120. Scale bars a- represent 20 µm and b-d represents 200 µm.*

PATHOGENICITY AND TOXICOLOGY ASSESSMENTS

The pathogenicity of *P. sanguineus* and its effect on the vegetative growth of oil palm seedlings were investigated under nursery condition. The fronds and boles of the seedlings inoculated with *P. sanguineus* were symptomless until the end of the 18-months study without compromising the plant growth. In contrast, the seedlings inoculated with *G. boninense* were severely infected and the infection had spread throughout the seedlings, causing BSR after nine months (Naidu *et al*., 2018). In addition, *P. sanguineus* was graded as non-toxic based on the acute oral toxicity test in rats (SIRIM Report No. R576/15/B19/27).

Note: Each rack can accommodate 32 bags of the solid state cultivation (SSC) formulation. SSC formulation containing *P. sanguineus* in each bag weighed about 500 g.

Figure 3. Mass production solid state cultivation formulation containing P. sanguineus *for field evaluation.*

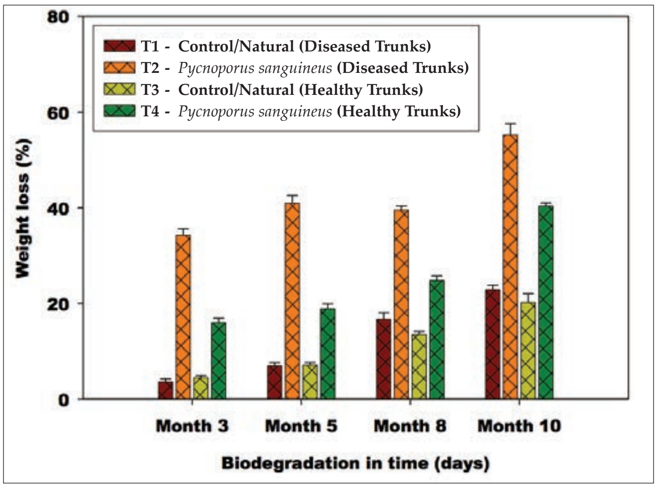


Figure 4. Biodegradation of oil palm trunks in terms of weight loss, 10 months after treatments application.

FIELD EVALUATION OF *Pycnoporus sanguineus* ON DEGRADATION OF INFECTED OIL PALM TRUNKS

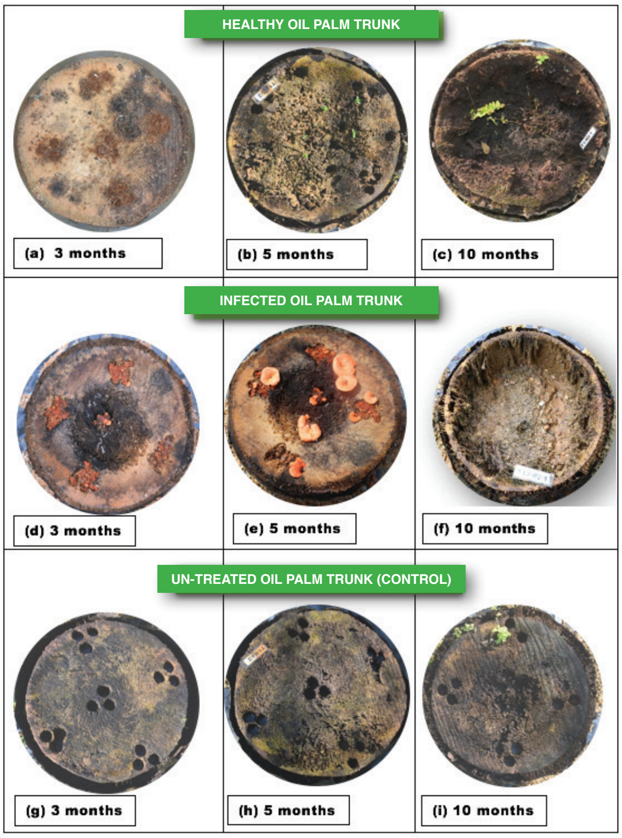
The efficacy of *P. sanguineus* in degrading infected oil palm trunks and minimising the survival of *Ganoderma* inoculum was reported. About 500 g of the SSC formulation containing *P. sanguineus* was inoculated into each of the healthy and infected oil palm trunk. The decay rate in term of mass loss in infected trunk tissues was significantly higher, 10 months after the application of SSC formulation containing *P. sanguineus* (T1) about 55% (*Figures 3* and *4*). A similar trend was observed in healthy trunk tissues with mass loss of about 40%. The lowest mass loss (20%) was noted in the healthy trunk (T3) in comparison to that of infected trunk (23%), both of which were naturally degraded (*Figure 4*). Degradation pattern was much faster in the infected trunks as illustrated in *Figures 5 e-f*. The degradation pattern was clearly distinguished between the treated and untreated trunks (*Figures 5 g-i*) using the developed SSC formulations (Naidu, 2018).

The lowest recovery of *Ganoderma* spp. was reported in infected trunks treated with *P. sanguineus*, T1 (33% ± 2.2) after 8 months, whereas no recovery of *Ganoderma* was noticeable at 10 months of application (*Table 1*). Conversely, after 10 months, the *Ganoderma* recovery on trunks treated with EFB alone (T2) was about 39% ± 0.8. Apparently, no *Ganoderma* was detected in healthy trunks till the end of experiment.

This study provides a great insight into the mechanisms and processes of *P. sanguineus*. Additionally, an alternative biotechnological approach has been identified for the degradation of infected oil palm trunks thus, reducing *Ganoderma* inoculum pressure in an eco-friendly manner.

BENEFITS OF THE TECHNOLOGY

- Cost-effective and eco-friendly management practices of *Ganoderma* disease and other major pests, such as rhinoceros beetle and rats.
- Sustainable ways to accelerate root mass, stumps and trunks (biomass) waste recycling.
- Added nutrients value to the soil.
- Minimise the infestation pressure caused by *Ganoderma* fungus and other pests – as an Integrated Pests Management (IPM) strategy, especially at replanting.



Note: The trunk size (palm age < 20 years) = 30 cm diameter x 35 cm height. Application rate – 500 g / trunk.

Figure 5. Field degradation of oil palm trunks by P. sanguineus. *(a, b and c) - Application method of SSC formulation of* P. sanguineus *into drilled holes of the infected oil palm trunk and partially degraded healthy oil palm trunks. (d, e and f) - Establishment and colonisation of* P. sanguineus *on oil palm trunk and completely degraded / hollow of the infected oil palm trunk. (g, h and i) - An un-treated oil palm trunk (as control) without degradation is observed.*

TABLE 1. PERCENTAGE RECOVERY OF *Ganoderma* ON GSM, 10 MONTHS AFTER TREATMENT APPLICATION (on infected oil palm trunk)

Treatment	Percentage recovery of *Ganoderma* spp.			
	Month 3	Month 5	Month 8	Month 10
T1 - Infected oil palm trunk treated with *P. sanguineus*	54b ± 1.8	41b ± 1.3	33b ± 2.2	n.d
T2 - Infected oil palm trunk treated with EFB alone	67a ± 2.1	51a ± 1.8	43a ± 1.7	39a ± 0.8
T3 - Infected oil palm trunk (Un-inoculated / natural)	*(used as control to calculate the % recovery of* Ganoderma *spp)*			

Note: Means with the same letter within the same column are not significantly different at ($P \leq 0.05$) according to Fisher's protected least significant difference (LSD) test. Each value represents the mean of five replicates.

ECONOMIC ANALYSIS

The estimated investment cost for the production of SSC formulation of *P. sanguineus* is approximately RM 2.0 million, which is based on the capacity of 412 000 kg per year. The estimated expenditure and other economic analyses in producing SSC formulation of *P. sanguineus* are listed in *Table 2*.

TABLE 2. ECONOMIC ANALYSIS OF SSC FORMULATION OF *P. sanguineus* AS OIL PALM TRUNK DEGRADER

Economic analysis	Value
Net present value (NPV), RM	3.7 million
Internal rate of return (IRR), %	36.25
Payback period, years	5.5
Benefit cost ratio (B:C)	1.27

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