Trichoderma AS A BIOCONTROL AGENT AGAINST *Ganoderma* IN OIL PALM

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Basal stem rot (BSR) caused by *Ganoderma* is a disease of economic importance to the oil palm industry in Malaysia. To date, the disease is managed through cultural practices such as sanitation during replanting which reduces the risk of encountering the disease at an early stage of growth. Studies using chemical control are still ongoing and needs further investigation. The most recent study on chemicals is the use of hexaconazole applied by a pressure injector which gave a lower mortality rate of the palms (Idris *et al.*, 2004). Using *Trichoderma* is an environmental-friendly approach towards managing the BSR problem. The fungus has some very unique mechanisms for controlling pathogens, such as mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress, solubilization and sequestration of inorganic nutrients, induced resistance of the host plant, and inactivation of the pathogen's enzymes (Chet, 1987). A number of *Trichoderma* spp., especially *T. harzianum*, has been commercialized as biological control agents of plant diseases, *e.g.* TRICHODEX™ used for the management of post-harvest rot of apple. *T. harzianum* is also combined with *T. polysporum* in the product BINAB-T™ which is applied against decay and wood rot (Ricard, 1983), giving good control over the diseases. At present, numerous researchers are investigating *Trichoderma* as a biological control agent (BCA) of *Ganoderma* and its modes of action. At MPOB, nursery trials and laboratory tests using *Trichoderma* spp. revealed good results in their use as biological control agents of BSR in oil palm.

IDENTIFICATION OF POTENTIAL *Trichoderma* ISOLATES

Potential *Trichoderma* isolates were selected through *in vitro* assessments. Isolation of the *Trichoderma* isolates was carried out from soils of oil palm plantations in Peninsular Malaysia. The isolated *Trichoderma* spp. were then identified to the genus level through slide cultures. More than 150 isolates of *Trichoderma* spp. were isolated. These isolates were eventually subjected to three types of antagonistic bioassays: dual culture, bilayer culture and poison agar (*Figure 1*). Only six isolates gave consistent results in controlling *G. boninense* (PER 71) in all the *in vitro* tests. All six isolates were subjected to *in vivo* assessment using four-month-old oil palm seedlings. The seedlings were artificially infected with *G. boninense* using the rubber woodblock (RWB) sitting technique (Shamala, 2005), and treated with a *Trichoderma* conidial suspension. *T. harzianum* Tri9 and *T. virens* Tri29 gave good control with a disease severity index (DSI) of 45 and 52.5 in the nursery trial, in comparison with infected and non-treated *Trichoderma* seedlings (*Figure 2*).

T. harzianum (Tri9) and *T. virens* (T29)

The *in vivo* assessment was repeated twice. It was carried out for a period of 24 weeks, and the disease symptoms were recorded according to the treatment given. Disease progression was described using the disease severity index (DSI) which depicts the severity of the disease based on the sequential progress of the disease. The symptoms were indexed along with the formula as follows (Shamala, 2005):

0 - for healthy plant;
1 - for appearance of 3 or more necrotic leaves;
2 - for appearance of white mycelial mat at the plant bole;
3 - for button-like sporophore appearance at the bole;
4 - for dead or dying plant.

$$\text{Formula: } \frac{\text{Number of seedlings x respective index}}{\text{n x highest index value}}$$

n – total number of plants in each treatment

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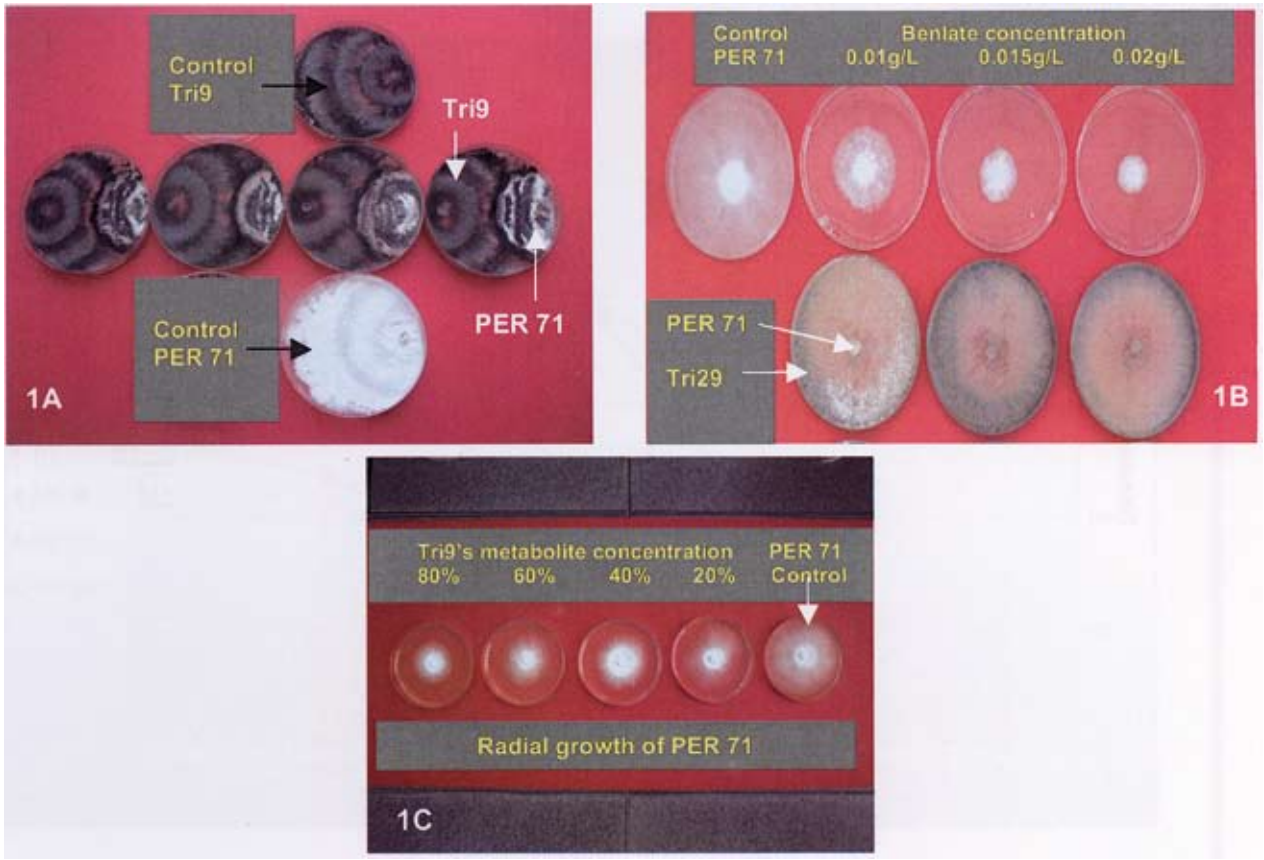

Figure 1. Effect of Trichoderma *on* Ganoderma – in vitro. *1A. Dual culture tests the physical characteristics of the biocontrol agent. Observe the greenish mycelia of Tri9 overgrowing the whitish mycelia of* Ganoderma *(PER 71). 1B. Bilayer culture tests the volatile compound properties of the biocontrol agent. Although benlate, a fungicide, reduced the mycelial growth of PER 71 in the control plates (top row), PER 71 was completely inhibited when grown on top of Tri29 (bottom row). 1C. Poison agar tests the effect of secondary metabolites against PER 71. Radial growth of PER 71 gradually decreased as the metabolite concentration of Tri9 incorporated in the agar increased.*

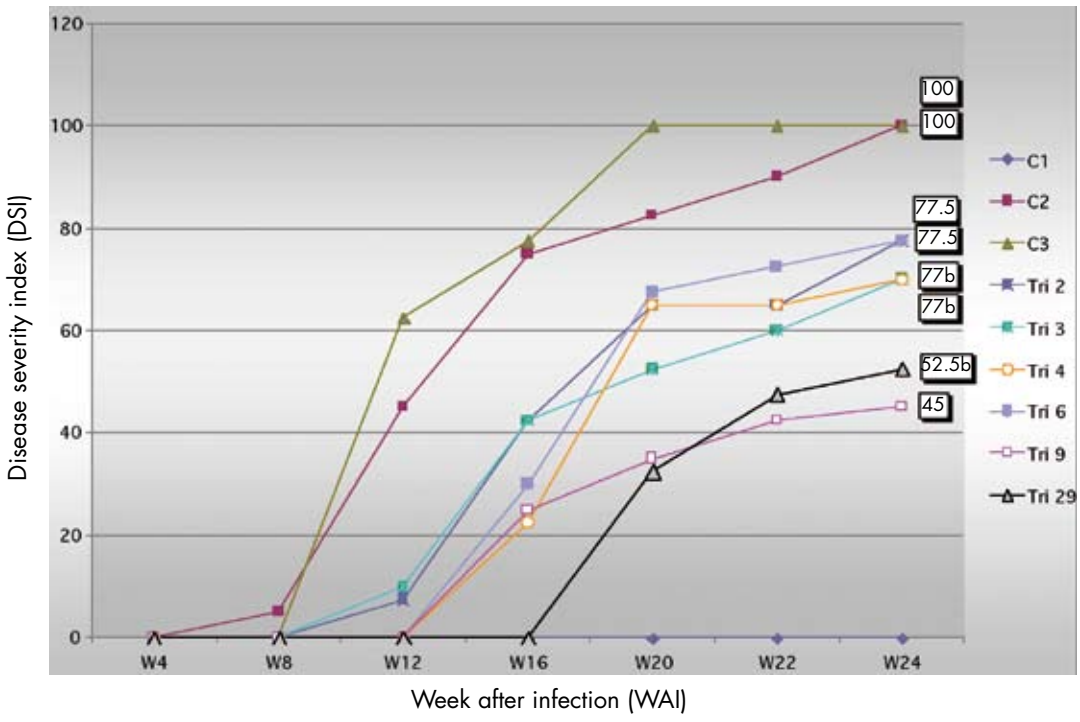

Figure 2. Disease progression of BSR symptoms throughout the 24 weeks of observation based on the disease severity index (DSI).

Notes: C1 = control seedlings without *G. boninense* infection (uninoculated);
C2 = control seedlings with *G. boninense* infection (6x6x6 RWB) and untreated;
C3 = control seedlings with *G. boninense* infection (6x6x12 RWB) and untreated;
Tri 2 = seedlings artificially infected with *G. boninense* and treated with Tri 2 (*T. harzianum*);
Tri 3 = seedlings artificially infected with *G. boninense* and treated with Tri 3 (*T. virens*);
Tri 4 = seedlings artificially infected with *G. boninense* and treated with Tri 4 (*T. harzianum*);
Tri 6 = seedlings artificially infected with *G. boninense* and treated with Tri 6 (*T. harzianum*);
Tri 9 = seedlings artificially infected with *G. boninense* and treated with Tri 9 (*T. harzianum*); and
Tri 29 = seedlings artificially infected with *G. boninense* and treated with Tri 29 (*T. virens*).

DSI values followed by the same alphabet are not significantly different at p=0.05 from one another according to Duncan's Multiple Range Test (n=10 sample plants; RWB=rubber wood block).

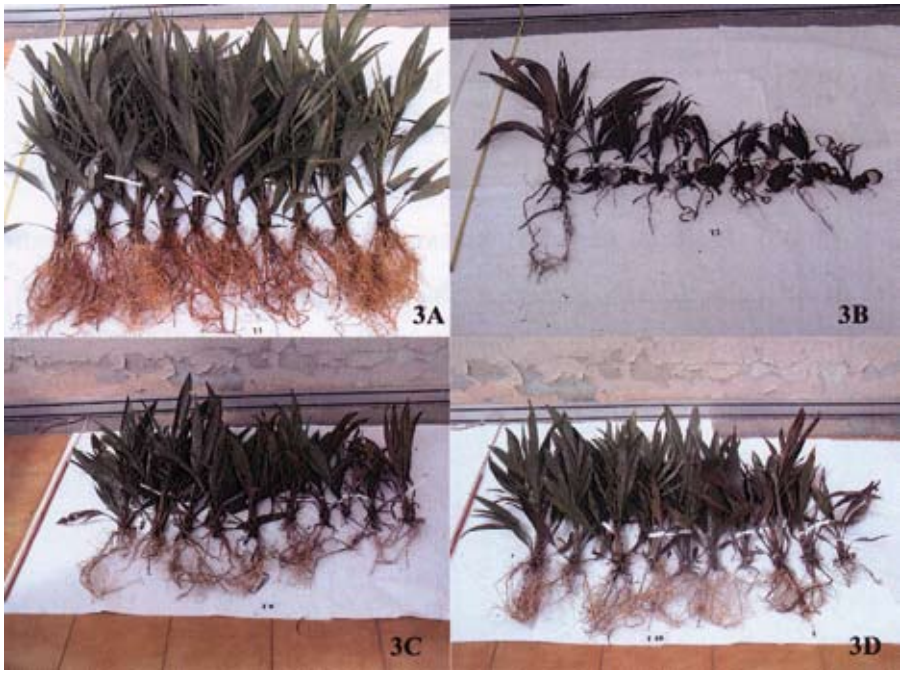

Figure 3. Effect of Trichoderma *on oil palm seedlings artificially infected with* Ganoderma – in vivo. ***3A****. Healthy seedlings.* ***3B****. None of the artificially infected seedlings without* Trichoderma *application survived.* ***3C****. Artificially infected seedlings treated with Tri9 resulted in 90% survival.* ***3D****. Artificially infected seedlings treated with Tri29 also resulted in 90% survival.*

Figure 2 shows the disease progression based on the DSI throughout the 24 weeks of evaluation. The *Trichoderma* carrier or food substrate for both nursery trials was palm press fibre (ppf). *Figure 3* demonstrates the control expressed by isolates *T. harzianum*, Tri9 and *T. virens*, Tri29 in oil palm seedings artificially infected with *G. boninense*.

CONCLUSION

Through the nursery trials, two species of *Trichoderma* (*T. harzianum*, isolate Tri9 and *T. virens*, isolate Tri29) were proven as potential as biological control agents against *G. boninense*. The nursery trials have been repeated twice to confirm the consistency of results produced by both *Trichoderma* isolates, Tri9 and Tri29. At present, formulation and mass production of both cultures using oil palm biowaste is ongoing.

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