Trichoderma virens, AN EFFECTIVE BIOCONTROL AGENT AGAINST *Ganoderma boninense*

SHAMALA SUNDRAM; LEE PEI LEE ANGEL; BONNIE TAY YEN PING; NUR DIYANA ROSLAN; INTAN NUR AINNI MOHAMED AZNI and IDRIS ABU SEMAN

716

MPOB INFORMATION SERIES • ISSN 1511-7871 • JUNE 2016 MPOB TT No. 587

Trichoderma species are considered as soil fungi that colonise superficially on plant root surfaces, forming a symbiotic relationship. Recent studies have reported that *Trichoderma* species are also capable of colonising the internal tissues of plants and characterised as having an endophytic relationship (Brotman *et al.*, 2013). The critical characteristic of this association is the penetration of the plant's root system by *Trichoderma* and the persistent survival of the fungus within living plant tissues. Previous studies on *Theobroma cacao* showed that *Trichoderma* persists in endophytic associations within the host plant's roots and above ground tissues (Evans *et al.*, 2003; Bailey *et al.*, 2006; 2008). Wide range of soil borne *Trichoderma* isolates as biocontrol agents for oil palm have been investigated (Sariah *et al.*, 2005; Izzati and Abdullah, 2008; Sundram *et al.*, 2008). However, attempts to isolate *Trichoderma* species as an endophyte and its biocontrol potential have yet to be investigated. This technology describes the isolation of endophytic *Trichoderma* spp. from root tissues of the oil palm, its antagonistic activity and diseases suppression on *Ganoderma boninense*, the causal fungus of basal stem rot (BSR) disease of oil palm.

NOVELTY OF TECHNOLOGY

This is the first report of endophytic *Trichoderma* isolated from oil palm with biocontrol potential against *G. boninense*, the causal agent of BSR disease. The innovative approach for this formulation is the characteristic of the microbe itself. The microbe colonises the internal root system of the oil palm and moves progressively as the root system develops. This gives an added advantage of a one-time application of the microbe in nursery or at the replanting stage of the crop. Unlike conventional application of microbial formulation that requires more than a single application, this approach provides to be more cost effective, sustainable and environmental-friendly approach in managing the disease.

EFFECTS OF *Trichoderma* IN NURSERY AND FIELD TRIALS

Six healthy mature oil palms (16 years) were selected randomly and their leaf, rachis, stem and root sections sampled for isolation of endophytic *Trichoderma* according to Arnold *et al.* (2001) and Evans *et al.* (2003). Although some *Trichoderma* isolates gave higher inhibition *in vitro*, nursery results were superior in determining potential biocontrol candidates. Isolates 7b and 159c gave the best suppression against *G. boninense*; identified as *T. virens* using standard internal transcribed spacer (ITS) primers. The disease severity index (DSI) for *Trichoderma* isolates 7b and 159c was at 36% and 25%, respectively. The investigation was continued using seedling bait method. Field baiting requires *Ganoderma* infected palms to serve as inoculum source for infection to take place. The palms were cut using chainsaw at 1 m above ground. Four *Trichoderma* pre-treated seedlings (four months) were planted at four corners of the *Ganoderma*-infected stump. Seedlings that were pre-treated with *T. virens* 7b and 159c gave lower BSR disease development compared to untreated positive control. The two isolates were then subjected to various assays to decipher the antagonistic mechanisms involved in suppressing *G. boninense*.

BIOCONTROL MECHANISMS FOR SUCCESSFUL CONTROL OF *Ganoderma*

The classic example of biocontrol mechanism is mycoparasitism. The interactions by *Trichoderma* involved coiling and strangulation of a pathogenic fungi, which further limits the pathogen's growth. Dual culture assay was performed by placing a mycelia plug (6 mm diameter) of *G. boninense* against the mycelia plug of *T. virens* 159c at the opposite side of the petri dish (*Figure 1*).

Antibiosis is a defense mechanism which produces both secondary metabolites and enzymes responsible in the suppression of pathogens ranging from bacteria to fungi (Whipps, 1997)

ISSN 1511-7871
9 771511 787001

Malaysian Palm Oil Board, Ministry of Plantation Industries and Commodities, Malaysia
6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia. *Tel:* 03-8769 4400 *Fax:* 03-8925 9446 *Website:* www.mpob.gov.my

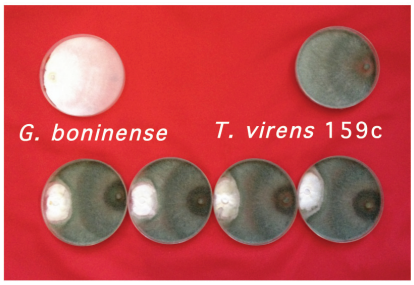

Figure 1. Dual culture assay subjected using Trichoderma *virens 159c against* Ganoderma boninense. *A total of four replicates are seen to limit the growth of* G. boninense *and slowly overgrow the pathogen.*

Morphological distortion of *G. boninense* hyphae were observed upon the contact of anti-fungal compounds released by *T. virens* 7b extracted in different solvents (*Figure 2*). Further investigation revealed that a novel discovery of anti-fungal compound was released by both *T. virens*.

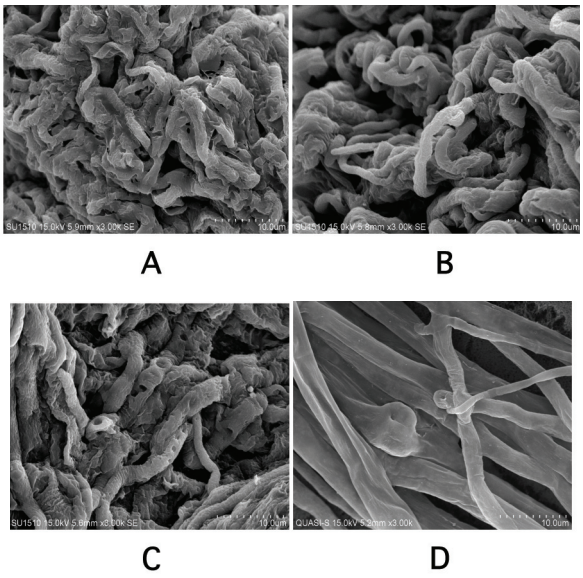

Figure 2. Destructive effects of the hexane extract (50 mg ml^{-1}) from Trichoderma virens *7b caused the hyphae of* Ganoderma boninense *to lyse and severely damaged (A). Similar effects were noted by using Benlate® (fungicide) (B), and the crude extract (C) of* Trichoderma *isolates. As a comparison, control* G. boninense *(D) showed a perfectly shaped mycelium with clamp connection.*

One of the key elements for the survival of microorganisms is iron (Fe), essential in metabolic and informational cellular pathways. Acquiring iron is crucial for both biological control agents (BCA) and pathogens (Symeonidis and Marangos, 2012). Siderophore chelation indicated superior acquisition of iron by *T. virens* compared to *G. boninense* (*Figure 3*).

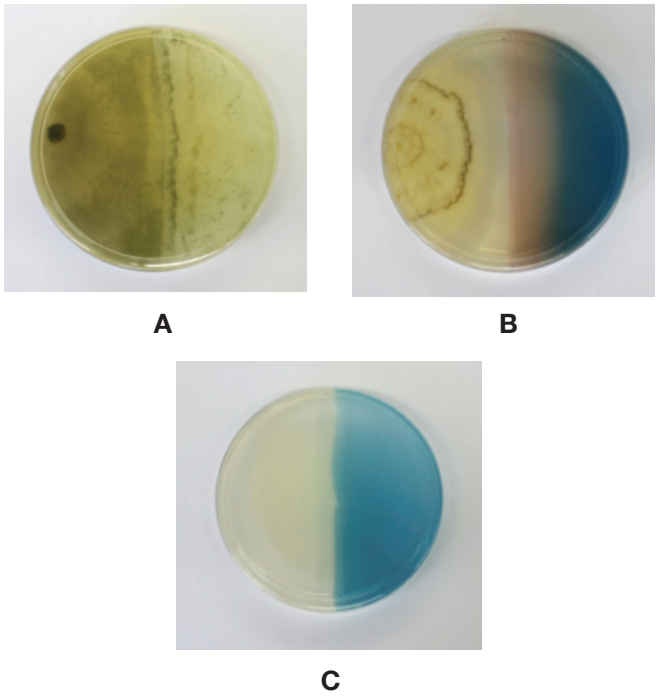

Figure 3. Siderophore production in (A) Trichoderma virens *159c, (B)* Ganoderma boninense, *(C) control. Discoloration of blue dye (Chrome Azurol S) occurs when active release of siderophore by the microorganism takes place to acquire the available iron in the blue agar.*

T. virens isolates tested via seedling bait found that BSR disease development in treated seedlings were slower (*Figure 4*). The DSI recorded for 7b and 159c isolates was at 36% and 25%, respectively (*Figure 5*).

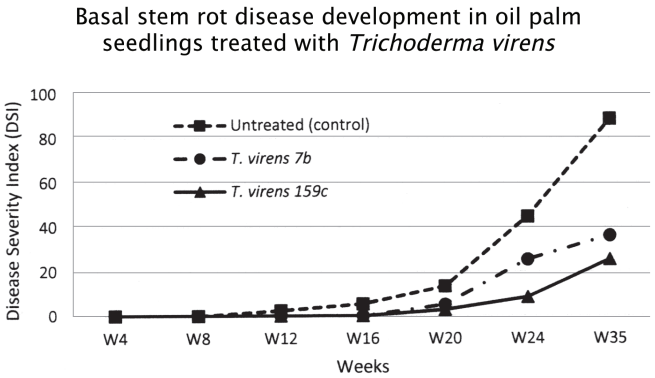

Figure 4. Basal stem rot disease development in oil palm seedlings treated with Trichoderma virens *7b and 159c assessed via disease severity index (DSI).*

POTENTIAL TAKERS

The technology is suitable to be adopted by bio-fertiliser producers and other agro chemical based entrepreneurs.

A B

Figure 5. Seedling bait trial. (A) Pre-treated seedlings with Trichoderma virens *7b and (B) untreated seedlings (control). Basal stem rot (BSR) disease stump is hidden by the vigorous growth of the pre-treated seedlings (A) compared to (B).*

BENEFITS TO THE INDUSTRY

Significant *Ganoderma* disease suppression was observed with the application of these endophytic *Trichoderma* species. It is recommended as prophylactic (preventive) treatment in nursery seedlings with a booster application at the replanting hole. Endophytic biocontrol agent play an important role in shielding the root system from potential soil borne pathogens. This would reduce any potential yield losses in oil palm cultivation.

REFERENCES

ARNOLD, A E; MAYNARD, Z and GILBERT, G S (2001). Fungal endophytes in dicotyledonous Neotropical trees: patterns of abundance and diversity. *Mycology Research, 105*: 1502-1507. DOI:10.1017/S0953756201004956.

BAILEY, B A; BAE, H; STREM, M D; CROZIER, J; THOMAS, S E; SAMUELS, G J; VINYARD, B T and HOLMES, K A (2008). Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biological Control, 46*: 24-35.

BAILEY, B A; BAE, H; STREM, M D; ROBERTS, D P; THOMAS, S E; CROZIER, J; SAMUELS, G J; CHOI, I-Y and HOLMES, K A (2006). Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. *Planta, 224*: 1449-1464.

BROTMAN, Y; LANDAU, U; CUADROS-INOSTROZA, A; TAKAYUKI, T; FERNIE, A R; CHET, I; VITERBO, A and WILLMITZER, L (2013). *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. *PLOS one, 9*: 1-15.

EVANS, H C; HOLMES, K A and THOMAS, S E (2003). Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. *Mycological Progress, 2*: 149-160.

IZZATI, M Z N A and ABDULLAH, F (2008). Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum*. *Plant Protecion Science Vol. 44 No. 3*: 101-107.

SARIAH, M; CHOO, C W; ZAKARIA, H and NORIHAN, M S (2005). Quantification and characterization of *Trichoderma* spp. from different ecosystems. *Mycopathologia, 159*: 113-117.

SYMEONIDIS, A and MARANGOS M (2012). Iron and microbial growth. *Insight and Control of Infectious Disease in Global Scenario* (Roy Priti ed.). p. 289 – 330.

SUNDRAM, S; ABDULLAH, F; ZAINAL, A M and UMI, K Y (2008). Efficacy of single and mixed treatments of *Trichoderma harzianum* as biocontrol agents of *Ganoderma* basal stem rot in oil palm. *J. Oil Palm Res. Vol. 20*: 470 - 483.

WHIPPS, J M (1997). Interactions between fungi and plant pathogens in soil and the rhizosphere. *Multitrophic Interactions in Terrestrial System* (A C Gange and V K Brown eds.). Oxford, UK: Blackwell Science Ltd. p. 47 – 59.

For more information, kindly contact:

Director-General
MPOB
6, Persiaran Institusi,
Bandar Baru Bangi,
43000 Kajang, Selangor,
Malaysia
Tel: 03-8769 4400
Fax: 03-8925 9446
www.mpob.gov.my