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)
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*

**Basal stem rot disease development in oil palm seedlings treated with Trichoderma virens**

**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 biofertiliser producers and other agro chemical based entrepreneurs.
**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**


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