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In vitro and In vivo Biocontrol Performance of *Trichoderma harzianum* Rifai on *Ganoderma boninense* Pat. Related to Pathogenicity on Oil Palm (*Elaeis guineensis* Jacq.)

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***Ganoderma boninense*, causes basal stem rot disease in oil palm, which leads to severe losses to the palm oil industry. The antagonistic effect of *Trichoderma harzianum* on *G. boninense* (a causal pathogen for oil palm) was tested by *in vitro* and *in vivo* studies. *T. harzianum* inhibited the mycelia growth of *G. boninense* on *in vitro* plate assay experiment and *in vivo* on the glass house grown oil palm plants. During the *in vivo* experiment, at first time point after one month, *G. boninense* development was found in oil palm root tissues in control plants whereas in *T. harzianum* treated plants no disease symptoms were observed until at the end of the experiment. It is a broad spectrum approach to use *T. harzianum* to control *G. boninense* infection of oil palm.**

Key words: In vitro, In vivo, Biocontrol, *Trichoderma*, *Ganoderma*, Oil palm.

Ganoderma boninense is a highly pathogenic fungus for oil palm, causing a disease called basal stem rot (Susanto *et al.*, 2005). The disease is so destructive that it can affect thousands of hectares of oil palm plantation (Susanto *et al.*, 2005). The disease was first detected in 1915 the Belgian Congo (Democratic Republic of congo), west Africa (Wakefield, 1920). In Malaysia, the disease was first recorded in 1931 on 25 years old oil palm plant (Arrifin *et al.*, 2000). *Ganoderma* can infect in all stages of oil palm growth. The disease progress is slow but eventually every infected plant will die due to this disease. Usually, the disease spreads from root and then the fungus degrades specifically the lignin component of the wood then leaving the white cellulose exposed and finally collapse the plant (Sanderson *et al*., 2000).

The use of biological control agents such as fungi or bacteria is considered important because they control the disease without causing any negative effect to the plant and environment. *Trichoderma* spp. is one of the most versatile soil borne fungus belongs to the filamentous class deuteromycetes with most strains adapted to an asexual life cycle (Harman, 2004).

The rapidly growing fungus *Trichoderma* spp. has persistent conidia and broad spectrum substrate utilization. Therefore, it is very efficient competitors for nutrition and living space compared to other fungi, characteristic makes it is a good biocontrol agent (Hjeljord *et al*., 2000). Another characteristic 'mycoparasitism' also enhances its capability to become a good bicontrol

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agent. In the process of mycoparasitism, *Trichoderma* recognize other fungi, then grows straight towards them, and sequentially produce hydrolytic cell-wall degrading enzymes. Next, *Trichoderma* spp. grows on that fungus, coil its hyphae around it thus killing the host hyphae growth (Steyaert *et al*., 2003).

Trichoderma spp. also act as bioferlilizer as it enhance plant growth and root development and stimulate plant defence mechanisms (Harman, 2004). *Trichoderma* spp. has been shown to improve the growth of various plants such as lettuce, tomato, and pepper (Ahmed *et al*., 1999; Vinale *et al*., 2006). *Trichoderma* spp. not only acts as a biocontrol agent or biofertilizer but it also induces plant defence response genes such as chitinase, glucanases, and peroxidase to act against antagonistic microbes (De Meyer *et al*., 1998; Yedidia *et al*., 2003; Hanson and Howell, 2004). Cucumber plant roots treated with *T. harzianum* showed that the induction of pathogenesis-related proteins (PR) and hydrolytic enzyme (Yedidia *et al.*, 2003). It had been noted that *Trichoderma* spp. are avirulent symbionts, and when added to the rhizosphore in plants, not only protect the plants against various microbial pathogens, including bacteria, viruses, and fungi but also stimulate plant health (Harman, 2004). Today, *Trichoderma* spp. has attention for its abilities to control both soil borne and foliage pathogen by inducing the production of host plant enzymes which inhibit the growth of other fungi and enhance plant health (Cumagun, 2012). Thus, this current study was conducted on the in vitro biocontrol effects of *T. harzianum* on *G. boninense* in plate assay as well its in vivo effects on *G. boninense* infected oil palm seedlings. The destructive sampling of *Ganoderma* infected plants was also conducted to see that how early *G. boninense* could internally infect the oil palm without there being any external symptoms.

MATERIALS AND METHODS

Fungal isolation and culture

Fungal isolations of *Trichoderma harzianum* and *Ganoderma boninense* were carried as described by Naher *et al.* 2011. *T. harzianum* strain FA 1132 was obtained from the slant stock culture of the Mycology Laboratory, Department of Biology, Universiti Putra Malaysia (UPM). The fungal mycelium was transferred on potato dextrose agar (PDA) plate at room temperature. Wooly white colonies were observed within 2–3 days. Within 5–6 days, the culture plates were fully covered with green conidia.

G. boninense was obtained from the Malaysian Palm Oil Board (MPOB, Bangi, Malaysia). The fungal mycelium was transferred onto potato sucrose agar (PSA) plates at room temperature (28±2 °C). The culture plates were observed daily to check on the growth of mycelium and for any contamination by other fungi or bacteria. After 7 days, mycelium covered with crust culture (which is the characteristics of *Ganoderma*) was considered to be a good culture and was used to prepare *Ganoderma* wood block inocula for infecting oil palm plants.

In vitro effect of *Trichoderma* on *Ganoderma*

In vitro antagonistic activity was observed between *G. boninense* PER 71 and *T. harzianum* in a plate assay. Around 6 mm of diameter agar disc was taken from the edge of an actively growing pure culture of *Ganoderma* and placed 1 cm inside the edge of PSA contained plate. The samples were allowed to grow for 3 days, then a 6 mm diameter disc was taken from the *T. harzianum* and placed on the opposite side of Petridish containing *Ganoderma* sp. The control plates contained only *Ganoderma*. The experiment conducted for 6 days.

Provision of Gano-wood blocks and Tricho-mulch inoculants

The Gano-wood- blocks were prepared by using rubber wood. Briefly, rubber wood of 2 x 2 × 5 cm size blocks were cut and thoroughly washed with distilled water. The clean blocks were soaked in distilled water for overnight. After overnight incubation the blocks were autoclaved at 121 ºC for 45 minutes. Next, 100 ml of PSA media were transferred into the sterilized wood blocks and they were again autoclaved using the same condition. Then, the blocks were cooled on laminar flow and after cooling then 6 days of *G. boninense* cultures plate were cut into pieces and transferred into the wood block. Finally, they were kept in dark condition for 10-12 weeks for overall wood block inoculation.

Tricho-mulch was prepared by using oil palm empty fruit branches called palm press fiber

(PPF). The fibers were randomly washed with running tap water and then soaked in water overnight. The fibers were pulled from water after overnight incubation and left for some times to rinse the rest of the water. Then, they were transferred at 300 g/plastic bag and autoclaved at 121 ºC for 15 minutes. Upon cooling, a suspension of 6-day old cultures of *T. harzianum* with 10 ml of distilled water was added to the top of each bag containing the sterilized fibers. The plastic bags were tied and all bags were incubated in the dark at room temperature for 15 days to be fully covered with green conidia.

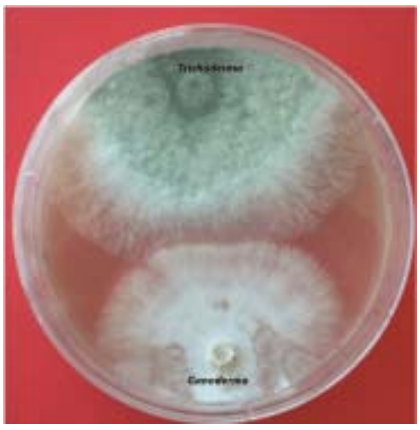
***G. boninense* and *T. harzianum* treatment on oil palm seedlings for in vivo activity**

Assessment of the *G. boninense* and *T. harzianum* in vivo activity was carried out in a glass house experiment. Five-month-old oil palm seedlings (Dura X Pisifera) obtained from (Sime Darby Seeds & Agricultural Services Sdn Bhd (Banting, Selangor, Malaysia) was used in this experiment. Before planting, the seedlings were carefully uprooted from the sand beds and the roots were washed with tap water. All experiments were conducted at the same time with three sampling time frames in three biological replicates. In the artificial inoculation of the oil palms, a Gano-wood block was attached around the roots of an oil palm seedling making sure there was close contact between the mycelia and the oil palm roots. (*Ganoderma* and *Trichoderma* together treated plant had a Gano-wood block attached around its roots and 600 g of Tricho-mulch placed on the surface of the soil it was growing in. Tricho-mulch was given every two weeks interval until the end of this experiment. Samplings were carried out at one, two, and four months post inoculation.

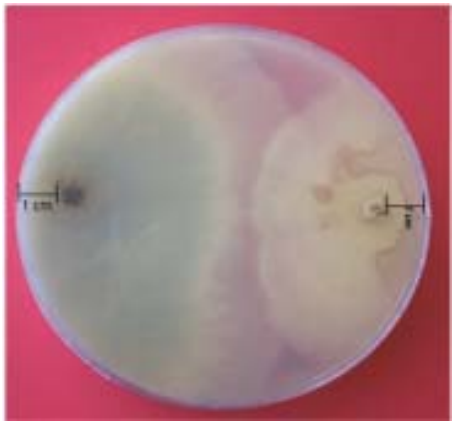
RESULTS AND DISCUSSION

In vitro action of *Trichoderma* against *Ganoderma*

Ganoderma was cultured on plate for 3 days then on opposite site *Trichoderma* was placed on the same plate. *Trichoderma* touched *Ganoderma* by day 2 and then by another 2 days *Trichoderma* was overgrown on *Ganoderma*. Hence, within 4 days *Trichoderma* showed over growth on *Ganoderma* (Fig. 1a and 1b). Usually, *Trichoderma* shows its antagonistic activity by the mycoparasitism process or by producing the volatile compounds. Fig. 1b shows the yellow colour formation from *Trichoderma* which indicate the production of some secondary metabolites in the process of antagonistic activity. 6-pentyl-α-pyrone (6PAP) is one of the secondary metabolite compounds which were first isolated from *T. viride* (Collins & Halim, 1972). This compound was also found in *T. harzianum* during its antagonistic with *Fusarium moniliforme* (El-Hasan *et al.*, 2007). This study did not identify the metabolic compounds from *Trichoderma* but the yellow zone showed that it was a strong antagonistic activity because *Ganoderma* also produced the yellow growth. Therefore, further study is needed to identify the compounds produced during the interaction between *Trichoderma* and *Ganoderma.*



1a



1b

Fig.1. Antagonistic activity of *T. harzianum* against *G. boninense* in plate assay. Fig 1a shows the interaction between *T. harzianuma* and *G. boninense*; Fig. 1b shows the inhibition of hyphal extension of *G. boneinese* and overgrowth of *T. harzianum*. Scale bar is equal to 1 cm.

In vivo action of *Trichoderma* against *Ganoderma*

Assessment of in vivo action of *Trichoderma* against *Ganoderma* was determined in a glass house trial study on oil palm seedlings. For in vivo action of *Trichoderma*, the first sampling were carried out after one month and then at two months and final samplings were at four months. After one month no external sign of disease symptoms appeared physically on the plants. However, when plants were revomed from the soil bed the mycelia of *Ganoderma* already grew into the root tissues (Fig. 2a). At 2 months in

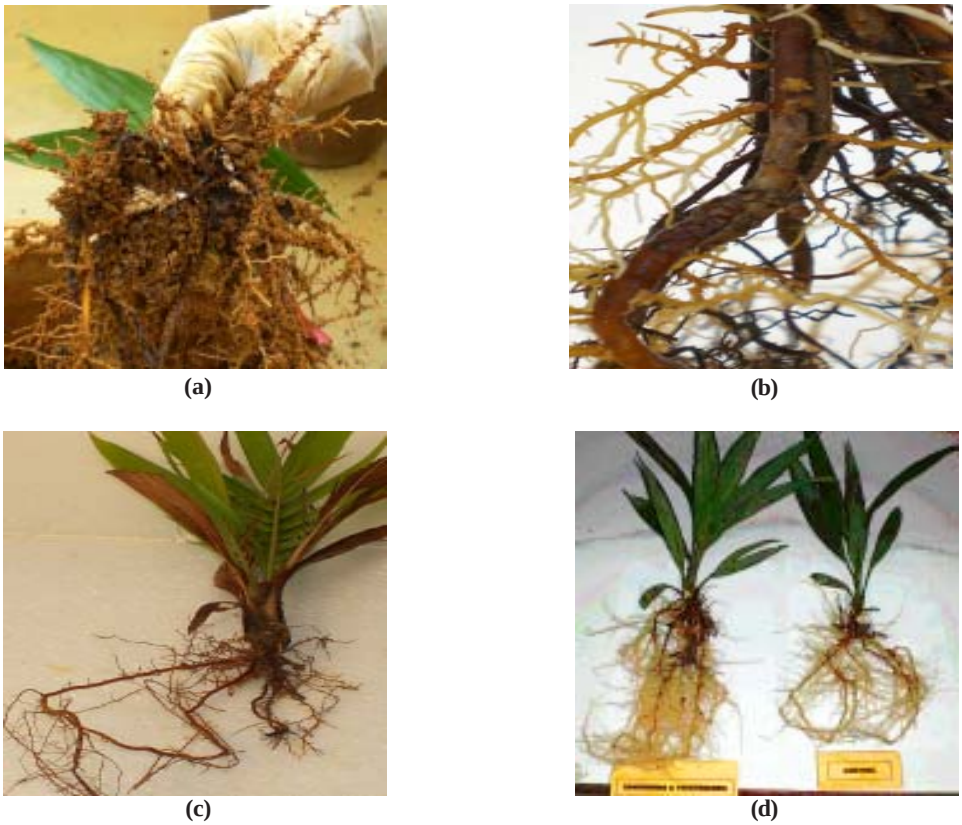


Fig. 2. Assessment of *G. boninense* infection in oil palm root tissues. Fig 1a, 1b, and 1c show the *G. boninense* hyphal development on roots, infections on roots and leaves at 1 month, 2 months, and 4 months, respectively. Fig 2d shows *in vivo* biocontrol activity of *T. harzianum* in the presence of *G. boninense* in oil palm

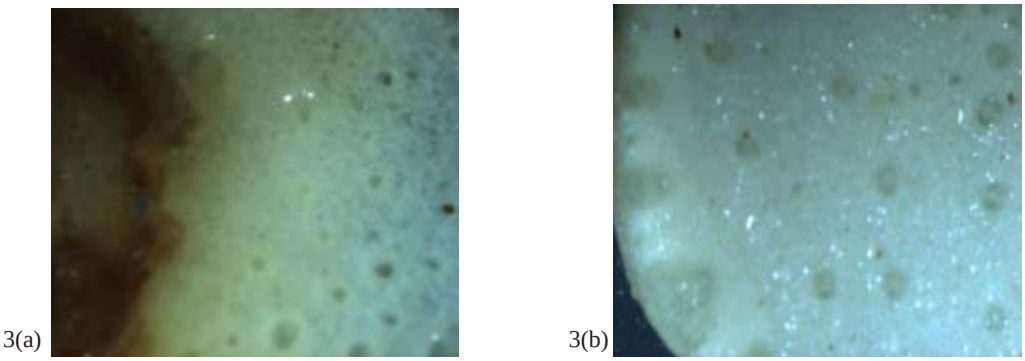


Fig. 3. Cross sectioning of basal tissue of *Ganoderma* singly infected and *Trichoderma* and *Ganoderma* together treated plant. Fig 3a shows the rotting infection by *Ganoderma* while in *Trichoderma* treated plant (Fig 3b) no rotting infection was apparent

Ganoderma alone treated plants signs infections were observed on the roots (Fig. 2b) and some bottom leaves were also necrotic. At 4 months, no new roots were observed while all the roots became very unhealthy in *Ganoderma* alone treated plants and some leaves were dead (Fig. 2c). In *Trichoderma* and *Ganoderma* together treated plant the mycelium of *Ganoderma* onto the roots and this feature was not observed at the all time points (Fig. 3). Moreover, the *Trichoderma* treated plants were healthier than the control and *Ganoderma* alone treated plant. As stated before mycoparasitism is the process that *Trichoderma* used to control the growth of other pathogenic fungi. At 4 months the basal tissues of *Ganoderma* alone treated plant were cut and examined under the light microscope during which infection was found in the basal cortex tissue. The dark brown zone showed that internally the plant was badly infected by *G. boninense* (Fig. 4) but no internal infection was observed in *Trichoderma* treated plants. *Trichoderma* is the fungus that not only controls pathogenic fungal growth but it also stimulates the plant defence response such as PR-proteins or stress tolerance gene (Harman, *et al.*, 2004; Shoresh and Harman, 2008; Brotman *et al.*, 2013). In a previous study we found that some chitinase gene expression was increased in oil palm root tissues of *Trichoderma* and *Ganoderma* together treated plants when compared to *Ganoderma* alone treated oil palm plants (Naher *et al.*, 2011).

CONCLUSION

The effect of *T. harzianum* on *G. boninense* both in vitro and in vivo showed that *T. harzianum* controlled *G. boninense* patogenicity on oil palm seedlings. The production of yellow zone during the antagonistic activity between the two fungal species in the plate assay needs molecular investigation.

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