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Influence of Oil Palm-Fungi Interactions on Soil Microfungal Community and Growth Profile of Plant

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The density of soil microfungal community and growth profile of oil palm (*Elaeis guineensis* Jacq.) seedlings were investigated using artificial inoculations of the pathogenic fungus *Ganoderma boninense* and the symbiotic fungi *Trichoderma harzianum* and *Glomus etunicatum*. Because *Trichoderma* have wide distribution, the isolation frequency of *T. harzianum* was measured in soil during interaction oil palm with fungi. Additionally, moisture content of the soil was determined. Densities of the soil microfungal community increased in oil palm inoculated with *T. harzianum* and *G. etunicatum*. While oil palm inoculated with *G. boninense* showed a decrease in the density of the soil microfungal community. Isolation frequency of *T. harzianum* was approximately constant until 21 days postinoculation (dpi) and thereafter decreased when physical symptoms appeared in *G. boninense* inoculated plants. The frequency of *T. harzianum* was 100% in *T. harzianum* inoculated treatment at 3 to 147 dpi, while in *G.etunicatum* inoculated treatments no significant differences in frequency of *T. harzianum* were observed. Our data revealed that interactions between oil palm with *T. harzianum* and *G.etunicatum* significantly improved the growth of palms. A suppressive influence on growth was observed in the interaction between oil palm and *G. boninense*. The moisture content of the soil increased significantly in the case of *T. harzianum* inoculated seedlings. This study clearly demonstrates that density of the soil microfungal community and the associated growth profile of oil palm respond differently depending on the type of interaction. Thus, the density of soil microfungal community could be a useful indicator for early detection and control of *Ganoderma* disease in oil palm.

Key words: *Elaeis guineensis, Ganoderma boninense*, *Glomus etunicatum*, Microfungal community, *Trichoderma harzianum*.

The soil environment is a very important natural resource that provides suitable growth medium for plants. Soil contains numerous microorganisms that directly influence plant growth[1]. Several lines of evidence support the significant role of microfungal flora in the improvement of the soil ecosystem. However, the soil microfungal communities can be greatly affected by environmental stress, and are therefore excellent indicators of soil ecosystem health[2-6]. Plant roots have the ability to exude various molecules into the soil rhizosphere during both biotic and abiotic interactions[3, 7]. Root exudates are a source of nutrients and signaling molecules, and also appear to be an important driving force for microbial colonization that stimulates growth[4]. Changes in microbial communities are dependent

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on specific morphological characteristics and secondary metabolism of the associated plant species[7, 8]. Recent reports demonstrated that the plant-arbuscular mycorrhizal fungi (AMF) interactions have an influence on the microbial community and various AMF have different effects on the microbial community[9,10]. Interaction between maize and plant growth promoting fungi/ AMF modified the microbial community in the rhizosphere of maize plants[9]. It has also been proven that *Glomus intraradices* BEG87 and *Clonostachys rosea* IK7 markedly influenced soil microbial communities and promoted growth of tomato plants[11].

Oil palm (*Elaeis guineensis* Jacq.) is an important and profitable oil-bearing plant in the world. The extracted oil is widely used for diverse industrial applications including food, cosmetics, oleochemicals and biofuel. Basal stem rot (BSR) caused by *Ganoderma boninense* Pat., is the most destructive disease of oil palm in South-East-Asia[12, 13]. The plant growth promoting fungus, *Trichoderma* is cosmopolitan in soils. This opportunistic and avirulent symbiont competes with other microorganisms for nutrients and space[14, 15]. *Glomus* is the largest genus of AMF. All species establish symbiotic relationships with plant roots[16]. Numerous reports describe how *Trichoderma* and mycorrhiza produced beneficial effects on plant growth[14, 15, 17, 18]. *Trichoderma* and *Glomus* species are plant growth promoters with potential for the biocontrol of BSR in oil palm[19, 20]. As *G. boninense* is the main causes of BSR disease of oil palm and *Trichoderma* and *Glomus* species are able to produce beneficial effects on oil palm, both pathogenic and symbiotic fungi are compatible for a joint study.

Visible symptoms of *Ganoderma* disease such as formation of basidiomata only become apparent at the very advanced stages of infection. Since the disease occurs as the result of interaction between plant roots and the biota in soil environment[21], understanding the influence of oil palm- *G. boninense* interaction on the soil microfungal community could be helpful in early detection and respective preventive measures. The aims of the present study were first to test whether plant-fungi interactions are able to change quantitatively the microfungal community (i.e., stimulate or suppress microfungal community); second to establish altered growth profile of plant (i.e., enhancing or suppressive influence on growth profiles). To do this, the effect of plant-fungi interactions on the relationship between soil microfungal community and growth profile of oil palm were investigated. Here we have explored the density of soil microfungal as well as the plant growth characteristics during pathogenic-symbiotic interactions between artificially inoculated *G. boninense, T. harzianum* and *G. etunicatum*. The density of soil microfungal community was analyzed over a time course of 147 days (until full infection achieved by *G. boninense*). *Trichoderma* spp. are among the most frequently isolated soil fungi[14]. Because of their wide distribution and their potential for controlling BSR disease, the isolation frequency of *T. harzianum* was measured in soil during oil palm-fungi interactions. We also explored the growth profiles of the oil palm, including plant height, stem diameter, and fresh and dry weights of shoots and roots. Additionally, moisture content of the soil was determined.

MATERIALS AND METHODS

Experimental design and statistical analysis

The treatment used for this experiment was shown in Table 1. The two-factorial experiment of 9 treatments (T) and 3 replications of plants or soils (where appropriate) (PS) was conducted in which the replications were nested within each T × PS combination. The statistical analysis, i.e., the analysis of variance (ANOVA) was performed by the following liner additive model (LAM):

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where inY_{ijk}= ijk$_{th}$ observations; α_i = i$_{th}$ treatment effects;β_j = j$_{th}$ plants or soils (where appropriate) effects; $(\alpha\beta)_{ij}$=*ij*$_{th}$ T × PS interactions effects; and μ_{ijk} = ijk$_{th}$ error term.

In the above LAM, ε_{ijk} was pooled error term of experimental and sampling errors. The experimental error was the biological replicate nested to T × PS combinations, while the sampling error was the technical replicates nested within biological replicate × T × PS. The protected least-significant difference (LSD) test was used for multiple mean comparisons. Statistical analysis was performed using SAS software (version 9.1; SAS

Institute Inc.). All experiments were repeated two times to compensate for possible errors. As the results of analysis obtained in the second repeat were the same as the first, results were only shown for the data of the first experiment. Three replications of plants and samples of 5 technical replicates for each treatment were used for assessment of plant biomass. The contrast procedure was used to compare between group means.

Fungal colorization was assessed at 0, 3, 7, 21, 42, 63, 84, 105, 126 and 147 days postinoculation (dpi). The growth profiles of the plant were examined at 0, 63 and 147 dpi. Twenty gram soil samples were taken aseptically at 5 and 15 cm soil depths at 0, 3, 7, 21, 42, 63, 84, 105, 126 and 147 dpi and directly assessed for density of the microfungal community, isolation frequency of *T. harzianum* and moisture content.

Host plant and fungal inoculum

Four month old oil palm (*Elaeis guineensis* Jacq. Dura × Pisifera) seedlings used as host plants were provided by Sime Darby, Golden Hope Plantation, Banting, Malaysia. Fungal cultures of *Ganoderma boninense* PER 71, *Trichoderma harzianum* FA 1132 and *G. etunicatum* (Becker and Gerdemann) isolated from oil palm were obtained from the stock collection of Mycology Laboratory, Biology Department, Faculty of Science, Universiti Putra Malaysia. Munchung series soil used in this experiment. *G. boninense* and *T. harzianum* inoculum preparation was carried out using the method described by Alizadeh *et al*.[22]. Briefly, *G. boninense* was cultivated in freshly made PDA (Difco Laboratories, Detroit, Michigan, USA) containing 20 g/l rubber (*Hevea brasiliensis*) sawdust, 5 g/l peptone and 100 mg/l streptomycin sulfate at 28 °C for 4 days in darkness. The carrier substrate, fresh rubber wood obtained from Huot Hing Factory in Semenyih, Selangor, Malaysia, was cut to 10 × 5 × 5 cm pieces (weighing approximately 220 g). Rehydrated rubber wood blocks (RWBs) were placed in heat-resistant polypropylene bags (12.5 cm × 30 cm × 0.05 mm thick) and autoclaved at 121 °C for 45 min. Molten non sterilized PDA (50 ml), sawdust and peptone were added. The RWBs were each inoculated with one plate of *G. boninense* culture and incubated at 28 ± 2 °C for 4 weeks in darkness. Uninoculated RWB carriers were used as negative control.

T. harzianum was cultivated on PDA with 100 mg/l streptomycin sulfate, at 28 °C for 7 days. The conidia were harvested after incubation by flooding cultures with 10 ml of ddH_2O and scraping with a sterilized L-shaped glass rod. The suspension was filtered and adjusted with ddH_2O to a final concentration of 1.9×10^9conidia/ml. *T. harzianum* inoculum carriers, the oil palm empty fruit bunches (EFBs), was obtained from Seri Ulu Langat Palm Oil Mill in Dengkil, Selangor, Malaysia. The sterile EFB (50 g) was inoculated with *T. harzianum* conidial suspension and incubated at 28 ± 2 °C for 2 weeks in darkness. Uninoculated EFB carriers were used as a negative control. The *G. etunicatum* inoculum used in the experiment contained 50 spores/g of soil.

Fungal inoculation and growth conditions

Inoculation of *G. boninense* and *T. harzianum* treatments were carried out as described in Alizadeh *et al*. [22]. Briefly, three roots of the oil palm seedlings were tied to RWBs. The seedlings with RWBs were planted in pots one-third filled with soil. Artificial inoculation for *Trichoderma* was performed by applying colonized *Trichoderma* carriers (250 g) on the pot surfaces. Fresh conidial suspension was added at 1 l/pot every 2 weeks during experiment. The *G. etunicatum* inoculum was a root-and-soil inoculum consisting of rhizosphere soil containing spores and colonized root fragments of oil palm in amounts of 50 g per pot, which were analyzed, showing a high potential to produce significant levels of root colonization. The *G. etunicatum* inoculum was applied in a layer at 2 cm below the surface of the soil, as well as around the roots. All treatments were provided in ceramic garden pots of 31.0 × 27.5 × 16.5 cm size and filled with 10 kg soil. Treatments with uninoculated carriers were used as negative control. The experiment was carried out on a plant house under normal temperatures and natural lighting, and seedlings were watered twice a day with 200 ml of tap water.

Assessment of the fungal infection

The signs and symptoms in response to pathogen including chlorotic leaves, formation of white fungal mass in oil palm basal stem, internal symptoms (necrotic lesion), production of basidiomata and drying up followed by death of infected plants are indicative of *G. boninense* infection. In order to determine the colonization of

the roots by *G. boninense,* internal fresh root segments (1 cm) were surface-disinfested and plated on *Ganoderma* selective medium (GSM). Untreated negative controls and *G. boninense* uninoculated treatments were used as a negative control. The percent root colonization by *G. boninense* was calculated as the number of root segments with *G. boninense* divided by the total number of root segments for each plant individually. Mycelium was isolated from surface disinfected basidiomata, plated on PDA and identified by morphological method using light microscope. The percentage of total root colonization by *Trichoderma,* which plated on PDA was measured as described for *Ganoderma*. Quantitative and qualitative analysis of *T. harzianum* were obtained via viable counting and slide culture techniques. *G.etunicatum* spores were collected and counted under microscope. For this purpose, soil cores (1.5×8 cm) were taken from pots. Spores and roots were separated by wet sieving and decanting through 123, 63, 45 µm sieves followed by centrifugation in 60% sucrose. Root segments (1 cm) were cleared with 10% KOH at 90°C for 2 h and rinsed 3 times with sterile water. The roots were then acidified with HCl and stained with 0.05% trypan blue at 80°C for 30 min. Percent mycorrhizal root colonization pursued for assessment of *G.etunicatum* inoculation in the roots[23].

Quantitative assessment of the density of soil microfungal community

The density of microfungal community was determined via viable counting technique. Ten gram of wet soil was mixed with 100 ml of sterile distilled water, shaken in an orbital shaker at 100 rpm for 10 min, diluted serially and poured onto streptomycin-rose bengal agar[24]. The Petri dishes were incubated at 28°C for 4 and again after 7 days, to allow for the development of slow growing fungal colonies. The Petri dishes used in the enumeration had 30 - 300 colony units. Average colony forming units (CFU) numbers from five replicates were calculated and expressed on an oven-dry weight basis according to Germida and de Freitas[25].

Isolation frequency of *T. harzianum*

The fungal colonies that were similar in appearance to *T. harzianum* were subcultured on PDA with streptomycin sulfate for better identification. *T. harzianum* was identified according to the taxonomy proposed by Watanable[26]. The isolation frequency of *T. harzianum* was calculated as described by Nesci *et al.*[27].

Growth profile of oil palm seedlings

In order to investigate the growth profiles of the oil palm during interaction with fungi, growth characteristics including plant height, stem diameter, and fresh and dry weights of shoots and roots were evaluated. The plant height and stem diameter was measured using a standard ruler. Oil palm plants were carefully removed from the pots and soil was removed in running water. The root system was separated from the shoots and the fresh weight of both roots and shoots was recorded. The shoot and root samples were oven-dried to constant weight at 60°C and the dry weights recorded. All growth characteristics were measured at the beginning (0 dpi) of each treatment, and repeated 63 dpi when physical symptoms in *G. boninense* infected seedlings appeared, and at the end of the experiment (147 dpi). Identification of differential growth was based on consistent fold

Table 1. Treatments for experiment.

Treatments	Description
T1 (G)	*G. boninense* PER 71 inoculated (+ plant + carrier + fungi)
T2 (GU)	*G. boninense* PER 71 uninoculated (+ plant + carrier – fungi)
T3 (GPC)	*G. boninense* PER 71 inoculated carriers without the plant (+ carrier + fungi – plant)
T4 (T)	*T. harzianum* FA 1132 inoculated (+ plant + carrier + fungi)
T5 (TU)	*T. harzianum* FA 1132 uninoculated (+ plant + carrier – fungi)
T6 (TPC)	*T. harzianum* FA 1132 inoculated carriers without the plant (+ carrier + fungi – plant)
T7 (Glo)	*G. etunicatum* (Becker and Gerdemann) inoculated (+ plant + fungi)
T8 (GloU)	*G. etunicatum* (Becker and Gerdemann) uninoculated (+ plant – fungi)
T9 (NC)	Untreated negative controls (absolute control)

change of growth profile of plant relative to untreated negative control plants. To ensure reliable results, the growth profile levels measured at 63 and 147 dpi were normalized to the levels determined at the start.

Evaluation of soil moisture content

Soil moisture content was determined mass basis from the 5 and 15 cm depth of soil[28].

RESULTS

Assessment of fungal colonization

Our results demonstrated that *G. boninense* induced infection in oil palm seedlings. Oil palm root colonization achieved by *G. boninense* was 3.33% ± 1.78 at 21 dpi and subsequently increased to 100% at 147 dpi (Table 2). Symptoms including chlorotic leaves and appearance of white fungal mass were observed at 42 dpi, with formation of basidiomata and necrotic lesion at 63 dpi, which continued until plants were dried up at 147 dpi (Fig. 1). No necrotic lesions were present in control plants. Within 3 dpi, the percentage of root colonization by *T. harzianum* dramatically increased to 68.33% ± 9.39 and continued to increase to 90% ± 3.27 at 7 dpi, and 100% at 21 until 147 dpi (Table 3). The population level of *T. harzianum* in inoculated treatment was $23.33 \pm 3.75 \times 10^3$ and $23.11 \pm 3.73 \times 10^3$ CFU/g at 5 and 15 cm soil depth, respectively (data not shown). There were no significant differences ($p > 0.0001$) between 5 and 15 cm soil depths in terms of population level of *T. harzianum* over a time course of 147 days. On the other hand, the percentage of plant root colonization by *G.etunicatum* was 58% ± 0.94 at 147 dpi. In contrast, the percentage of root colonization by *G.etunicatum* was 7.01% ± 0.18 and 7.06% ± 0.16 in uninoculated and untreated negative control plants, respectively at 147 dpi. This demonstrates successful colonization of plant roots by AMF (Table 4). The spore density of *G.etunicatum* per 10 g of soil for inoculated seedlings was 55 ± 0.38 and for uninoculated and untreated negative control was 4.2 ± 0.11 and 4.2 ± 0.20, respectively at 147 dpi; indicating that spore density of *G.etunicatum* was significantly ($p \leq 0.0001$) increased in *G.etunicatum* inoculated seedlings (Table 5).

Density of soil microfungal community

Our findings indicate that the densities of soil microfungal community displayed significant variation during oil palm-pathogen and symbiont interactions at 5 and 15 cm soil depths over a time course of 147 days (Fig. 2). On the other hand, there was no significant difference ($p > 0.0001$) in density of the microfungal community between the 5 and 15 cm soil depths. The oil palm-*G. boninense* interaction caused a significant ($p \leq 0.0001$) decrease in the density of soil microfungal community. In *G. boninense* inoculated treatments, the density of soil microfungal community started to decrease after 3 dpi, with a significant drop at between 21 and 42 dpi, and thereafter continued to decrease until negligible levels at 126 dpi (Fig. 2b). The density of soil microfungal community increased significantly ($p \leq 0.0001$) in the oil palm- *T. harzianum* treatment, from $10.21 \pm 0.42 \times 10^3$ at 0 dpito $145.54 \pm 3.26 \times 10^3$ CFU/g dry weight of soil at 147 dpi. In the *T. harzianum* positive control, there was a significant ($p \leq 0.0001$) increase in the density of soil microfungal community from $9.83 \pm 0.25 \times 10^3$ to $46.83 \pm 0.68 \times 10^3$ CFU/g at 3 dpi and thereafter was maintained at about the same level. The increase in the density of soil microfungal community in *G. etunicatum* inoculated treatments was gradual until 84 dpi, but there was a significant ($p \leq 0.0001$) increase at 105 dpi. Increase in density levels of soil microfungal community was observed in *T. harzianum* uninoculated, while *G. etunicatum* uninoculated did not show any difference in comparison to the untreated negative control. However, means comparisons on density of soil microfungal community, showed significant differences among all treatments at 42-147 dpi.

Differential isolation frequency of *T. harzianum* in soil

In the untreated negative control the isolation frequency of *T. harzianum* was about 37% throughout from 0 to 147 dpi. The isolation frequency of *T. harzianum* was significantly different at different time intervals during oil palm-*G. boninense* and *T. harzianum* interactions from 0 to 147 dpi in comparison to the untreated negative control (Fig. 3). No significant difference in terms of isolation frequency of *T. harzianum* was found between 5 and 15 cm soil depths. Means comparison revealed significant ($p \leq 0.0001$)

Table 2. Percentage of root colonization by *G. boninense* during interaction with oil palm (0-147dpi).

Treatments /Sampling period (dpi)	0	3	7	21	42	63	84	105	126	147
G	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 1.78	8.33 ± 2.82	20.00 ± 4.59	61.11 ± 8.20	83.33 ± 2.44	86.67 ± 3.02	100.00 ± 0.00
GU	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

G, *G. boninense* inoculated; GU, *G. boninense* uninoculated; NC, percentage of root colonization with *G. boninense* in untreated negative control.
Values are means ± S.E.

Table 3. Percentage of root colonization by *T. harzianum* during interaction with oil palm (0-147dpi).

Treatments /Sampling period (dpi)	0	3	7	21	42	63	84	105	126	147
T	6.67± 2.18^{a}	68.33 ± 9.39^{a}	90.00 ± 3.27^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
TU	3.33 ± 1.78^{a}	5.00 ± 1.78^{b}	5.00± 1.78^{b}	6.66 ± 2.18^{b}	10.00± 1.67^{b}	16.67 ± 00.00^{b}	16.67± 0.00^{b}	16.67 ± 0.00^{b}	25.00 ± 1.41^{b}	28.33 ± 1.09^{b}
NC	5.00± 1.78^{a}	5.00± 1.78^{b}	5.00± 1.78^{b}	3.33± 1.78^{b}	6.66 ± 2.18^{b}	5.00± 1.78^{c}	5.00± 1.78^{c}	5.00± 1.78^{c}	6.66±2.18^{c}	6.66 ± 2.18^{c}

T, *T. harzianum* inoculated; TU, *T. harzianum* uninoculated; NC, percentage of root colonization with *T. harzianum* in untreated negative control.
Values are means ± S.E. $^{a-c}$, means ± S.E in a column with different superscript differ significantly ($p \leq 0.0001$).

Table 4. Percentage of root colonization by *G. etunicatum* during interactions with oil palm (0-147dpi)

Treatments /Sampling period (dpi)	0	3	7	21	42	63	84	105	126	147
Glo	3.00 ± 0.34[a]	3.40± 0.13[a]	3.20 ± 0.31[a]	5.80 ± 0.66[a]	14.60 ± 1.36[a]	48.00± 0.70[a]	49.20 ± 1.53[a]	49.80 ± 1.54[a]	52.20± 1.56[a]	55.00± 0.38[a]
GloU	3.00 ± 0.24[a]	3.00± 0.17[a]	3.00 ± 0.24[a]	3.20 ± 0.41[b]	3.40 ± 0.27[b]	3.60± 0.21[b]	3.80 ± 0.39[b]	3.80 ± 0.36[b]	3.40± 0.27[b]	4.20 ± 0.11[b]
NC	2.60 ± 0.21[a]	3.40± 0.21[a]	3.00 ± 0.17[a]	2.80± 0.11[b]	3.60 ± 0.21[b]	3.60 ± 0.21[b]	3.60 ± 0.13[b]	3.40 ± 0.21[b]	3.00± 0.00[b]	4.20 ± 0.20[b]

Glo, *G. etunicatum* inoculated; GloU, *G. etunicatum* uninoculated; NC, percentage of root colonization with *G. etunicatum* in untreated negative control.
Values are means ± S.E. [a-b], means ± S.E in a column with different superscript differ significantly ($p \leq 0.0001$).

Table 5. Spore densities of *G. etunicatum* per 10 g fresh soil during interaction with oil palm (0-147 dpi)

Treatments /Sampling period (dpi)	0	3	7	21	42	63	84	105	126	147
Glo	6.23 ± 0.24[a]	6.64 ± 0.27[a]	6.14 ± 0.16[a]	13.52 ± 0.55[a]	24.43± 0.41[a]	42.15 ± 0.55[a]	43.73 ± 0.36[a]	51.69± 1.61[a]	54.54 ± 1.42[a]	58.00 ± 0.94[a]
GloU	6.02 ± 0.21[a]	6.06 ± 0.20[ab]	6.15 ± 0.01[b]	6.14± 0.16[b]	6.12 ± 0.23[b]	6.88 ± 0.19[b]	7.00 ± 0.32[b]	6.11 ± 0.21[b]	6.88 ± 0.27[b]	7.01 ± 0.18[b]
NC	5.95 ± 0.22[a]	5.96 ± 0.23[b]	6.04 ± 0.05[b]	6.14 ± 0.22[b]	6.02 ± 0.20[b]	6.90 ± 0.13[b]	7.06 ± 0.39[b]	6.01 ± 0.22[b]	6.94 ± 0.28[b]	7.06 ± 0.16[b]

Glo, *G. etunicatum* inoculated; GloU, *G. etunicatum* uninoculated; NC, untreated negative control.
Values are means ± S.E. [a-b], means ± S.E in a column with different superscript differ significantly ($p \leq 0.0001$).

differences in the isolation frequency of *T. harzianum* in *G. boninense* inoculated treatments at time intervals of 21 to 147 dpi. The frequency of *T. harzianum* was approximately 37% in *G. boninense* inoculated treatments at 0 time, which was maintained until 21 dpi, followed by a significant drop to about 9% at 42 dpi when physical symptoms appeared. The isolation frequency of *T. harzianum* was significantly ($p \leq 0.0001$) different in *T. harzianum* inoculated plant. The isolation frequency of *T. harzianum* was 100% in *T. harzianum* inoculated treatment at 3 to

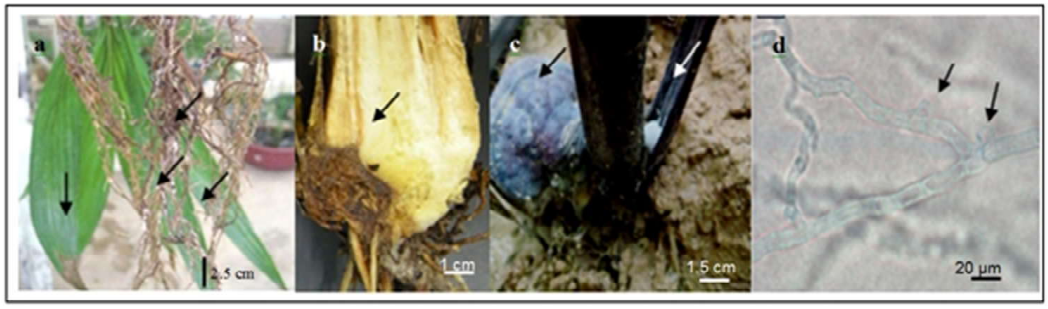

Fig. 1. Pathogen response in *G. boninense* inoculated oil palm. (a) *G. boninense* colonized root surface and produced chlorotic leave at 42 dpi. The black arrow marks the colonization of root and chlorotic leaves. (b) Necrotic lesion (black arrow) in stem base of infected plant at 84 dpi. (c) White fungal mass (white arrow) and basidiomata (black arrow) produced on oil palm stem base at 147 dpi. (d) Mycelium and clamp connections (black arrows) of *G. boninense* isolated from internal root of infected oil palm and basidiomata (Lactophenol cotton blue staining).

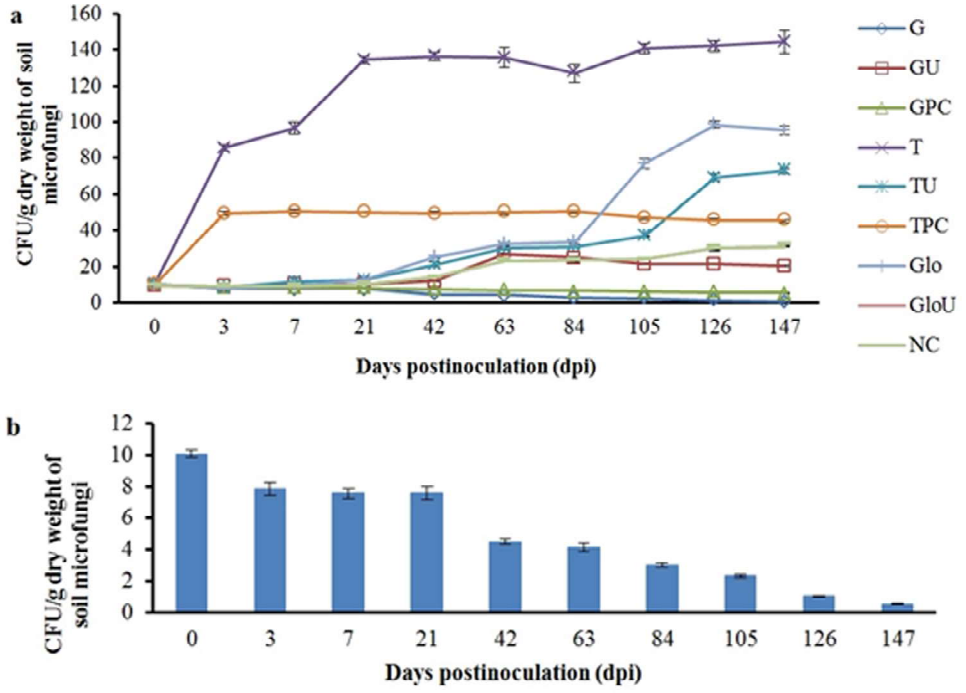

Fig. 2. Mean of CFU/g dry weight of soil microfungi × 10^3 at (a) 5 cm depth of soil during oil palm-fungi interactions from 0 to 147 dpi; (b) Mean of CFU/g dry weight of soil microfungi at 5 cm depth of soil in oil palm-*G. boninense* interactions at 0 to 147 dpi. G, *G. boninense* inoculated; GU, *G. boninense* uninoculated; GPC, *G. boninense* positive control; T: *T. harzianum* inoculated; TU, *T. harzianum* uninoculated; TPC, *T. harzianum* positive control; Glo, *G. etunicatum* inoculated; GloU, *G. etunicatum* uninoculated; NC, untreated negative control. Values are means ± S.E. The results for 15cm depth of soil were similar to those achieved with a 5 cm depth.

147 dpi. No significant differences were detected in the isolation frequency of *T. harzianum* during oil palm- *G. etunicatum* interaction.

Growth profile of oil palm in response to fungal inoculation

The plant growth characteristics, including height, stem diameter, and fresh and dry weights of shoots and roots were significantly affected by fungal inoculation (Fig. 4). Our data revealed that *G. boninense* inoculated seedlings decreased these variables; where as *T. harzianum* and *G.etunicatum* inoculated plants significantly ($p \leq 0.0001$) increased these growth parameters. The *T. harzianum* inoculated seedlings demonstrated the largest increase in the growth parameters. In *T. harzianum* inoculated seedlings, the plant growth increased by 1.52-fold for height, 2.08-fold for stem diameter, 1.74-fold for fresh shoot weight, 1.69-fold for fresh root weight, 1.67-fold for dry shoot weight and 1.76-fold for dry root weight at 147 dpi. The oil palm growth characteristics also increased significantly in *T. harzianum* uninoculated treatments.

Soil moisture content

The effect of different fungal treatments on the soil moisture content appeared to be maintained approximately at the same level in *G. boninense* and *G. etunicatum* treatments over the time course of 147 days. There was no significant difference between 5 and 15 cm soil depths on soil moisture content in all treatments. The soil moisture content in 5 cm soil depth at 0, 3, 7, 21, 42, 63, 84, 105, 126 and 147 dpi were 19.62 ± 0.08, 22.77 ± 0.13, 21.67 ± 0.19, 21.58 ± 0.09, 20.51 ± 0.18, 20.50 ± 0.17, 23.01 ± 0.22, 22.14 ± 0.15, 21.30 ± 0.30, 22.42 ± 0.18, respectively in *G. boninense* inoculated treatment. In the case of *T. harzianum* inoculated seedlings, moisture content increased significantly ($p \leq 0.0001$) at both 5 and 15 cm soil depths in comparison to the untreated negative control. The soil moisture content in 5 cm soil depth *T. harzianum* inoculated treatment increased 21.98 ± 0.04, 24.59 ± 0.04, 24.77 ± 0.08, 24.99 ± 0.11, 25.07 ± 0.07, 25.00 ± 0.11, 25.82 ± 0.15, 25.10 ± 0.02, 25.00 ± 0.05, 24.65 ± 0.10, respectively at 0-147 dpi. Increase in the soil moisture content was also

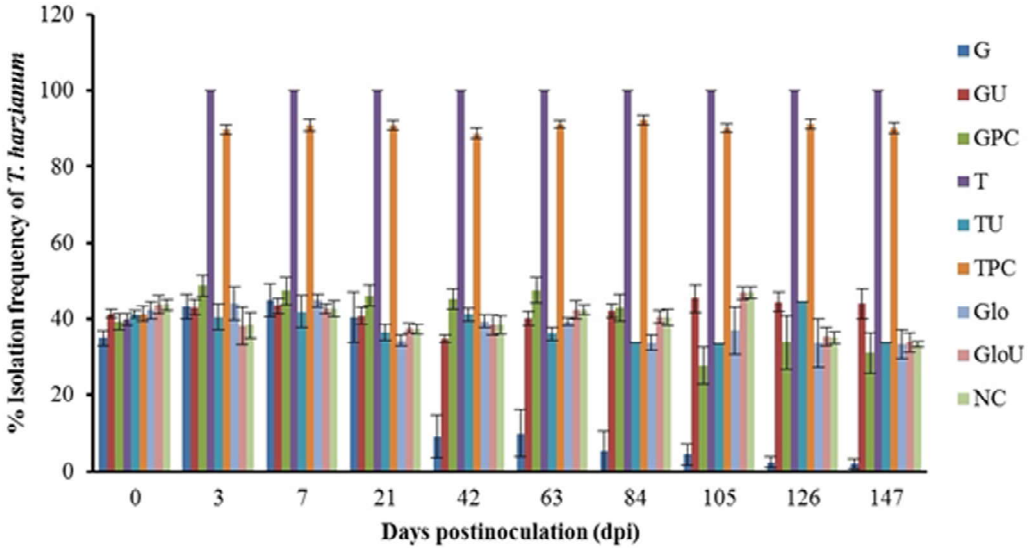

Fig. 3. Isolation frequency of *T. harzianum* at 5 cm soil depths during oil palm-fungi interactions from 0 to 147 dpi. G, *G. boninense* inoculated; GU, *G. boninense* uninoculated; GPC, *G. boninense* positive control; T: *T. harzianum* inoculated; TU, *T. harzianum* uninoculated; TPC, *T. harzianum* positive control; Glo: *G. etunicatum* inoculated; GloU, *G. etunicatum* uninoculated; NC, untreated negative control. Values are means ± S.E. The results for 15cm depth of soil were similar to those achieved with a 5 cm depth.

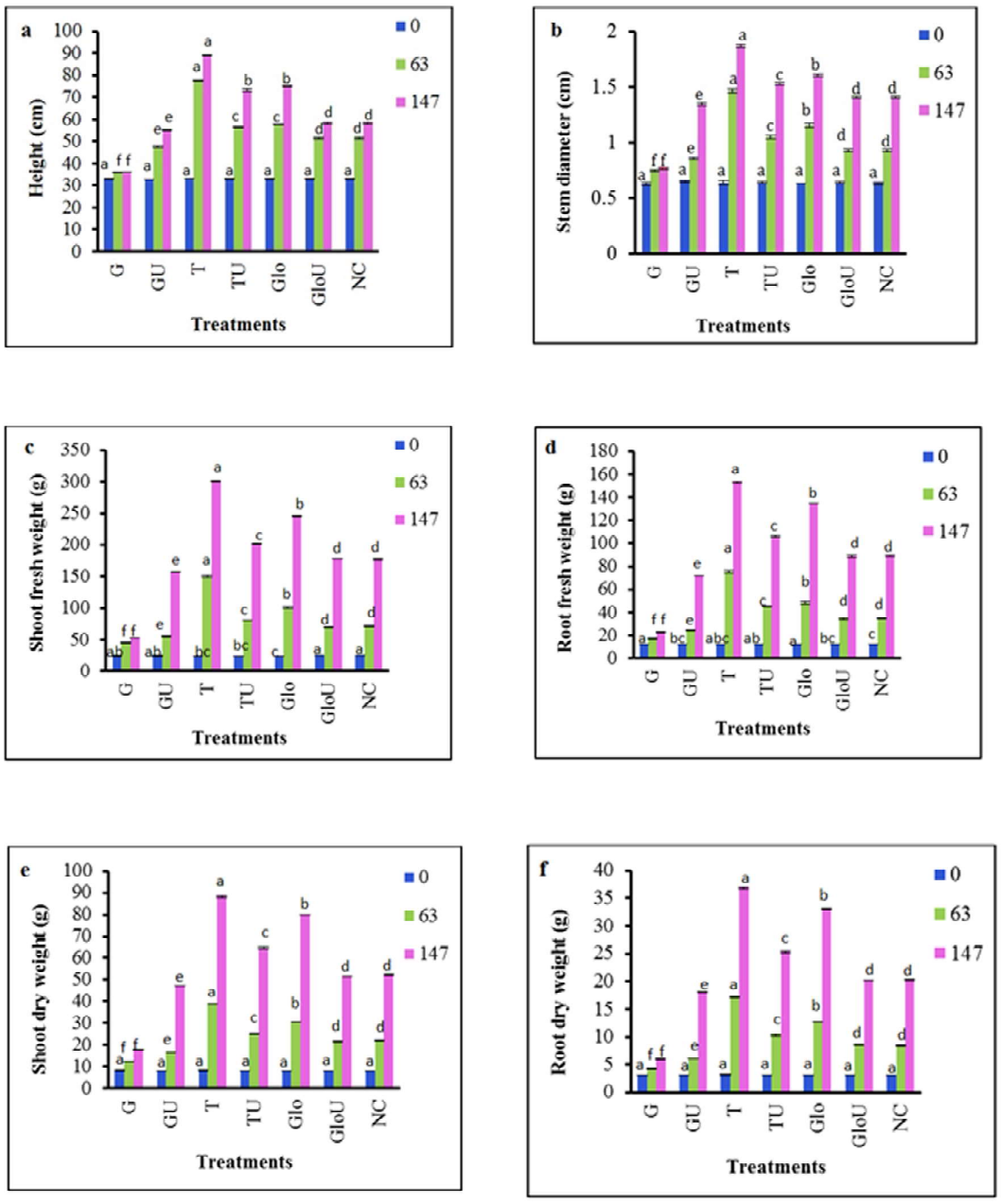

Fig. 4. Growth profile of oil palm in response to fungal inoculation. (a) Height; (b) stem diameter; (c) shoot fresh weight; (d) root fresh weight, (e) shoot dry weight; and (f) root dry weight of oil palm during interactions with fungi from 0 to 147 dpi. G, *G. boninense* inoculated; GU, *G. boninense* uninoculated; T, *T. harzianum* inoculated; TU, *T. harzianum* uninoculated; Glo, *G. etunicatum* inoculated; GloU, *G. etunicatum* uninoculated; NC, untreated negative control. Values are means ± S.E. Means with different letters indicate statistically significant differences at $p \leq 0.0001$.

observed in *T. harzianum* uninoculated treatment and *T. harzianum* positive control in comparison to the untreated negative control ($p \leq 0.0001$).

DISCUSSION

Changes in the soil microfungal community during plant-microbe interactions have previously been little explored. This work provides evidence on changes in the quantitative of microfungal community during interactions between oil palm and *G. boninense* (a pathogenic fungus); *T. harzianum* and *G. etunicatum* (symbiotic growth promoting fungi) based on the density of soil microfungal over a time course of 147 days. Feedback dynamics resulting from changes in the soil community are generated by the specific response in a particular plant-microbe interaction. Plant roots in the soil represent a rich source of diverse, abundant, and somewhat reliable substrates hence have a great influence on the soil microflora[3, 4, 29]. In fact, most bacteria and fungi in the rhizosphere are highly dependent on association with the host plants and they are also clearly regulated by root exudates. In addition, the plant growth response is strongly influenced by the soil microbial community[4, 30, 31].

Colonization of oil palm root by *G. boninense* decreased the soil microfungal community in *G. boninense* inoculated treatment. There is competition for nutrients, space or infection site between microfungi in the soil environment[32], and plant root exudates also have an effect on the interaction between roots and soil organisms[4]. *G. boninense* which degrades oil palm tissues provides favorable conditions for pathogen growth[13]. Moreover, infection of plants by pathogen alters secretions from roots and the chemotactic response[33]. Therefore, it may be suggested that increasing pathogen population in soil may lead to negative influence on the soil microfungal community. Clearly, colonization of oil palm roots by *G. boninense* decreased the frequency of *T. harzianum* in soil. These results are in agreement with those reported by Eastburn and Butler[34], who concluded that the environment has an effect on the distribution of *T. harzianum* in the field.

Oil palm-*T.harzianum* interaction strongly decreased population of other soil microfungal community such that only *T. harzianum* was isolated in *Trichoderma* inoculated seedlings. *Trichoderma* species could decrease the activity of deleterious microflora, by dominating the microfloral community on roots. *Trichoderma* species is the most common saprophytic fungi in the rhizosphere, and nearly all temperate and tropical soils contain 10^1-10^3 CFU/g of *Trichoderma*[14]. This species has the essential characteristics which enable it to be ubiquitously represented in any habitat and at high population densities[34]. *G.etunicatum* also has an effect on the density of microfungal community consistent with previous reports which demonstrated that AMF could change the density of soil microorganisms[35]. Both environmental factors and host plant genetics have been shown to affect the extent of mycorrhizal colonization of host plants[36]. *G. etunicatum* was able to increase oil palm root colonization at 21 dpi. Due to the biology of the oil palm root system, it is possible that the *G. etunicatum* penetrate into oil palm root gradually. Root exudation and rhizodeposition changed by AMF, are expected to have an influence on soil microflora. The chemotactic response involving organic and amino acids, soluble and non-soluble root exudates, border cells, large polysaccharide layer that surrounds roots, and electrical signals, all have an influence on the microflora community[4, 35].

The soil moisture content is also important for microorganisms because microbes require nutrients and supply of hydrogen/oxygen. Microbial activity and population proliferate best in soil moistures ranging from 15 to 25% [37]. In the present study, soil moisture content was found to be important in determining the distribution of *T. harzianum*, and was within the range of 20.97 to 25.87%. Our result demonstrated that the application of EFBs in *T. harzianum* treatments was able to cut down the evaporation of moisture from the soil.

Consistent with the findings of this study, Idris *et al.*[12] had also demonstrated *G. boninense* infection of 100% root colonization in oil palm at 180 dpi. The suppressive influence on growth observed during oil palm-*G. boninense* interaction could be accompanied by actual cell wall degradation of tissue xylem by ligninolytic enzymes, which may pose problems in water and nutrient distribution[13]. Similarly, suppression of

plant growth was observed in uninoculated *G. boninense* treatments. This may be attributed to RWB carriers producing stress in plants, thus resulting in suppression of growth[22]. The population level of *T. harzianum* was high with 100% potency to colonize the oil palm roots. *T. harzianum* also significantly increased the growth characteristics of the oil palm seedlings. The growth enhancing effects of *T. harzianum* observed was also reported in other plants. Ozbay and Newman[38] demonstrated that *T. harzianum* could enhance growth by 100% and 93% in tomato after application of isolate T22 and T95, respectively. *T. harzianum* was demonstrated to promote plant growth through production or control of hormones. It has a significant competitive advantage over other fungi due to its ability to increase root surface area for uptake and solubilization of nutrients from soil solution to the root cells[38,39]. *T. harzianum,* a rapid growing fungus, can produce or release a series of bioactive metabolites that have a role in both elicitation of defense reactions and growth regulation of plants[14,15]. Induction of plant growth was observed during oil palm- *G. etunicatum* interaction. The *G. etunicatum* was able to colonize roots in inoculated oil palm seedlings. There are numerous reports that describe the beneficial effects of mycorrhiza on plant growth[18,40]. The root mycorrhizal colonization increases the surface area for uptake of minerals and water from the soil, induces systemic resistance, offers protection from soil pathogens, improves resistance to environmental stress, and tolerance to pollution[18,40,41]. However, different plant species differed in the degree to which they respond to AMF species[42]. Limited published reports on the interaction of AMF and oil palm showed that inoculation of oil palm seedlings in the nursery resulted in a growth increase over uninoculated plants[20]. In comparison to *T. harzianum, G. etunicatum* was less effective in promoting growth in oil palm. Our findings are consistent with Nazir and Bareen[43] who reported that *T. pseudokoningii* was more effective than *G. fasciculatum* on *Heliathus annuus* growth promotion.

Based on the changes in the density of soil microfungal community occurring during interactions of oil palm with *G. boninense, T. harzianum,* and *G.etunicatum*, the present study showed that pathogen and symbiont inoculated seedlings influenced the density of soil microfungal community. The results of the study suggests that microfungi can be a useful indicator of the type of microbial species that is colonizing the plant root system with potential impact on the ecosystem health in general[3,4]. Our findings support the use of *T. harzianum,* and *G.etunicatum* as oil palm growth promoting biological agents. The results also suggest that the density of soil microfungal community is a useful indicator for the early detection of BSR and respective preventive measures.

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