

SHORT COMMUNICATION

SCREENING THE POTENTIAL OF ENDOPHYTIC *TRICHODERMA ASPERELLUM* M103 AND *T. HARZIANUM* M108 AGAINST *GANODERMA* BASAL STEM ROT DISEASE IN OIL PALM SEEDLINGS BY SEED COATING TECHNIQUE

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ARTICLE DETAILS

ABSTRACT

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Ganoderma sp. causes serious disease, known as the Basal Stem Rot (BSR) to oil palm in Malaysia. Current practices for managing the BSR disease primarily involve sanitation measures, while the application of fungicides is less cost-effective. The application of biocontrol agents, especially *Trichoderma*-based products, has been widely explored and shows great potential, although maintaining their population over an extended period remains a challenge. Thus, the prospecting endophytic *Trichoderma* could be the new potential biocontrol agent against BSR disease. The aim of this study is to determine the effectiveness of endophytic *Trichoderma asperellum* M103 and *T. harzianum* M108 against BSR disease by using a seed coating technique at the nursery stage. At the end of the study, both endophytic *Trichoderma* isolates M108 and M103 showed the potential in suppressing the BSR disease at 67.78% and 71.11%, respectively of disease incidence (%DI), compared to the control at 87.78%. Overall, M103 demonstrated its effectiveness against BSR disease, as symptoms in inoculated oil palm seedlings appeared only after 4 months. Additionally, M103 achieved the lowest epidemic rate (ER) of 7.07 units month⁻¹ and the smallest area under the disease progress curve (AUDPC) of 236.25 unit² over 11 months. These findings underscore the potential of endophytic *Trichoderma* as a viable biocontrol agent against BSR disease, offering a promising avenue for the sustainable disease management in oil palm cultivation.

KEYWORDS

Biological Control Agents (BCA), Biofertilizer, Disease Assessment, Disease Incidence.

1. INTRODUCTION

Basal stem rot (BSR) disease by *Ganoderma* is the greatest threat to oil palm production in Southeast Asia and this disease causes severe losses in Malaysia (Idris et al., 2004). The total area of oil palm plantations infected by BSR disease has increased, with no cost-effective control measures. Chemical application shows minimal control measures and is not recommended due to its harmful and detrimental effect to the environment (Dias, 2012). Therefore, the manipulation of biological control agents using beneficial microbes is sought after as one of the green approaches for managing crop diseases, including BSR disease, by many agricultural industries throughout the world such as *Hendersonia* sp. and *Streptomyces* sp. GanoSA1 (Idris et al., 2014; Lee et al., 2016; Pandit et al., 2022; Idris et al., 2012).

The potential of *Trichoderma* sp. as a biocontrol agent for a broad range of pathogens in agriculture has been explored including BSR disease. Besides, a lot of *Trichoderma*-based products are economically important as biopesticides, bioprotectants, biostimulant and biofertiliser, for a broad range of pathogens in agriculture (Harman and Kubicek, 1998). The ability of *Trichoderma* as a good biocontrol agent is verified by its various characteristics, such as fast growing, mycoparasitism, able to produce volatile and non-volatile substances that are toxic to the pathogen, improve the host plant's growth, and induce the host plant's systemic resistance.

The application of *Trichoderma*-based products in managing BSR disease has been tried by several oil palm plantations, but its population in the oil palm rhizosphere was found decreasing over time. Due to that problem,

endophytic *Trichoderma* seems to be a suitable candidate for biological control agent, based on its special characteristics that are able to colonise and sustain itself in the plant root system. In addition, the application of the endophytic *Trichoderma* may further improve the biological control strategies for BSR disease, because they are buffered from the environmental changes which is an important criteria in rhizosphere competence that facilitates the colonisation within the roots of the host, without being affected by the environmental changes (Sundram, 2013).

Thus, the prospection of the endophytic *Trichoderma* as a biological control agent (BCA) in managing the BSR disease at the nursery stage particularly by using seed coating technique is important. *Trichoderma asperellum* (M103) and *Trichoderma harzianum* (M108) were selected, based on their potential in suppressing the growth of *Ganoderma boninense* (PER71) in the in-vitro screening (Elya Masya et al., 2022).

2. MATERIALS AND METHODS

2.1 *Ganoderma* Challenge Inoculation at the Nursery Stage

T. asperellum M103 and *T. harzianum* M108 were isolated from healthy oil palm roots (Elya Masya et al., 2022). Endophytic *Trichoderma* suspensions were made by harvesting the spores or conidia of M103 and M108 plate culture (Figure 1) with sterile distilled water (SDW). About 0.05% of carboxyl methyl cellulose (CMC) was added as a sticky agent prior seed coating technique, and the concentration was adjusted to the final concentration of 10⁷ spores/conidial mL⁻¹ using a Neubauer hemocytometer.

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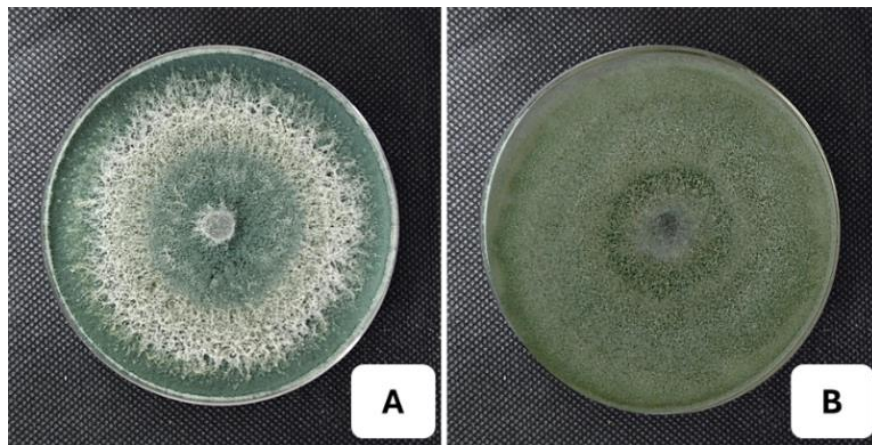


Figure 1: *T. asperellum* M103 (A) and *T. harzianum* M108 (B) culture plates.

Commercial oil palm germinated seeds were rinsed with SDW to remove any debris, chemical, or fungicide. The oil palm germinated seeds were then soaked separately in the M103 and M108 suspensions for 10 minutes. Then the treated oil palm germinated seeds were planted in pre-nursery polybags, sized 15cm × 23cm that filled with a soil mixture (1:2 volume / volume; sand: topsoil). Next, the planted germinated seeds were maintained at the pre-nursery stage for 3 months, watered and fertilised following the standard nursery practices.

The *Ganoderma* rubber wood block inoculum (sized 6cm × 6cm × 6cm) was prepared by using *G. boninense* PER71 culture (Idris et al., 2006). Once the oil palm seedlings have reached 3 months old, it was transplanted into the main nursery polybags (sized 38cm × 46cm) and challenged inoculation with *Ganoderma* RWB, using the sitting technique (Idris et al., 2006). Untreated oil palm seedlings were used as a control. The oil palm seedlings were watered and fertilised according to the nursery standard practice. The diagram illustrates the method used for testing seed coating application (Figure 2).

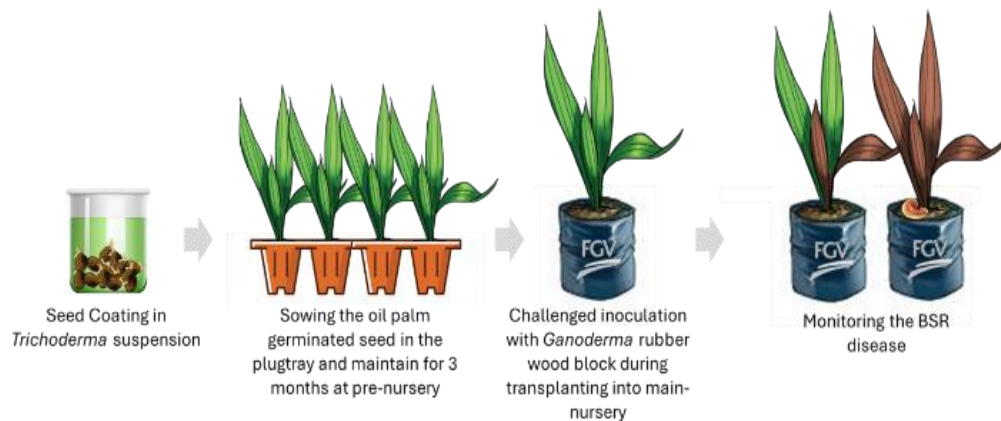


Figure 2. The diagram of seed coating application testing method

2.2 Disease Assessment

The development of BSR disease in the oil palm seedlings was monitored on the development of foliar symptoms. The infection of BSR disease was recorded and measured, based on the percentage of disease incidence (% DI), the severity of foliar symptoms (% SFS), disease severity (% DS), and dead seedlings (% Dead S) at monthly intervals. The % DI represents the number of infected oil palm seedlings, based on the visible necrosis of leaves and the production of fruiting body (Idris et al., 2006), calculated using the formula of Campbell and Madden 1990:

$$\% \text{ DI} = (\text{Number of infected seedlings} / \text{total no. of seedlings assessed}) \times 100$$

% SFS for each oil palm seedling was calculated by the formula derived by Sariah and Zakaria 2000;

$$\% \text{ SFS} = [(\text{Dry leaves} \times 1) + (\text{Yellow leaves} \times 0.5)] / \text{Total number of leaves} \times 100$$

Data on % DS of treated seedlings with M103 and M108 was computed according to the formula described by Abdullah et al., 2003 with disease scale with modification (Table 1);

$$\% \text{ DS} = (\sum ab / N.K) \times 100$$

Where,

$\sum ab$ = sum of the score scale of assessed seedlings

N = total number of assessed seedlings

K = highest score scale

Table 1: The BSR disease scales based on the external symptoms (Abdullah et al., 2003) with modifications.

Disease Scale	Symptom
0	Healthy without any appearance of fungal mycelium
1	>20% of SFS with the appearance of fungal mycelium/basidiocarp
2	>40% of SFS with the appearance of fungal mycelium/basidiocarp
3	>60% of SFS with the appearance of fungal mycelium/basidiocarp
4	>80% of SFS, the whole plant dry or dead, with the appearance of fungal mycelium/basidiocarp

$$\% \text{ Dead Seedling} = (\text{total number of dead seedlings} / \text{total number of seedlings assessed}) \times 100$$

Disease progress was estimated by calculating the area under the disease progress curve (AUDPC) for the severity of foliar symptoms value using a

formula (Campbell and Madden 1990);

$n-1$

$$AUDPC = \sum_i [(Y_i + Y_{i+1} + 1) / 2] (t_{i+1} - t_i)$$

Where;

N = number of assessment time

Y = disease severity of the foliar symptom

T = Observation time

The AUDPC indicates the amount of disease developed in each treatment over time. The lower the AUDPC value of a treatment, the more effective the treatment is in reducing the disease.

The percentage of disease reduction (%DR) of each treatment was deduced from the DS by the formula:

$$\% DR = [(\% DS \text{ Control} - \% DS \text{ treatment}) / \% DS \text{ Control}] \times 100$$

The epidemic rate (ER) for each treatment was calculated using the formula (Campbell and Madden,1990):

$Y = \ln [1/(1-x)]$ applied to a monocyclic epidemic model appropriate for

soil-borne like *G. boninense*.

Y = amount of disease at a given time

X = the disease incidence proportion

At the end of the experiment, the surviving oil palm seedlings were dissected to observe and confirm the internal symptoms of BSR infection.

2.3 Experimental Design and Statistical Analysis

The experiment was laid out in a randomised complete block design (RCBD). Each treatment involved 5 replications, containing 30 seedlings each. Data were analysed by ANOVA using SAS. The means were compared to the least significant difference (LSD) at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The nursery trial is the fastest way to screen for the potential of microbial candidates against the *Ganoderma* BSR disease. The treated oil palm seedlings were artificially challenged inoculation with *Ganoderma* by using the rubber wood blocks. Then, the development of BSR disease was assessed at the first month until month 11th of the experiment. The foliar symptoms such as yellowing and necrosis at the lower part of the oil palm leaves, could be observed at an earlier stage. The presence of the basidiocarp of *G. boninense* PER71, and dried leaves, would later lead to the death of the seedlings at the later stages of BSR disease (Figure 3).

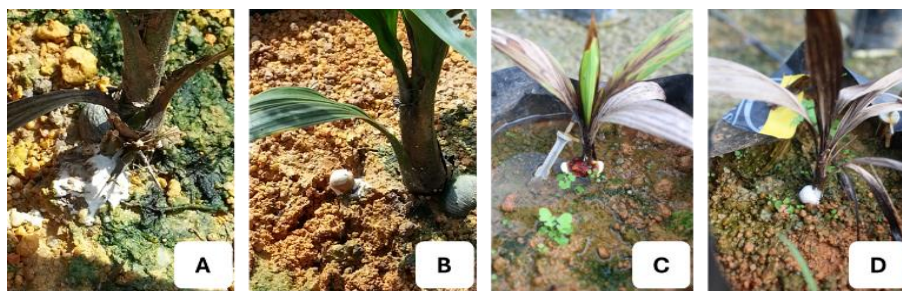


Figure 3: Development of foliar BSR disease symptoms at nursery stage, appearance of white mycelium (A); appearance of small white button (B); appearance of basidiocarp and 50% of necrosis leaves (C); dead oil palm seedling (D).

In this study, the first disease incidence was discovered in the oil palm seedlings in the control and M108 treatment, at three months after the BSR disease was introduced. Oil palm seedlings, treated with M103 were able to delay the *G. boninense* infection, at a month later than the control and M108 treatment, at month four. It showed that M103 provides protection to the oil palm seedlings, at least for 3 months by using the seed coating technique. However, at the end of the experiment, M108 has recorded the

lowest percentage of DI at 67.76%, followed by M103 at 71.11% compared to the control at 87.78% (Table 2). Further evaluation on the percentage of SFS and DS indicated that both M103 and M108 have suppressed *Ganoderma* BSR effectively in the oil palm nursery trials. M103 showed a slightly lower % DS at 60.83%, compared to M108 at 61.11%. Additionally, both treatments exhibited decreased seedling mortality compared to the control, with M103 recorded the lowest % of dead seedlings at 44.44%.

Table 2: Disease assessment of treated oil palm germinated seeds with endophytic *Trichoderma* suspension through seed coating technique after 11 months.

Treatment	Disease Incidence (%DI)	Severity of Foliar Symptom (%SFS)	Disease Severity (%DS)	Dead Seedlings (%)
Based on foliar symptoms				
<i>T. asperellum</i> M103	71.11 _{ab}	62.51 _a	60.83 _a	44.44 _a
<i>T. harzianum</i> M108	67.78 _b	64.07 _a	61.11 _a	47.78 _a

Means followed by the same letter are not significantly different at $p \leq 0.05$ LSD.

Examination of BSR internal symptoms at the bole of the oil palm seedlings were carried out by doing a cross section, to confirm the BSR infection. The infected bole exhibited a distinct rot and dry appearance, which

contrasted with the ivory to cream-colored tissues of the healthy roots (Figure 4). The result shows that M103 had produced the lowest %DI of internal symptom in the seedling. Some of the oil palm seedlings which looked healthy and without any foliar symptoms, however, were found positive with BSR after the internal symptom examination.



Figure 4: Development of internal BSR disease symptoms at nursery stage, rotten basal stem and root (A); browning and rotten of basal stem tissue (B); distinct rotten on basal stem tissue and dry appearance (C) and compared to healthy basal stem without any symptom (D).

Analysis of the Area Under Disease Progress Curve (AUDPC) revealed that M103 and M108 had produced lower cumulative disease development to the seedlings at 236.25 unit², followed by M108 at 252.92 unit² (Table 3). The lowest AUDPC value indicates the effectiveness of the endophytic candidates in reducing the disease (Sapak et al., 2008). The Epidemic Rate

(ER), which represents the speed of disease spread over time, was also lower for both M103 (7.07 unit month⁻¹) and M108 (7.50 unit month⁻¹) compared to the control (8.62 unit month⁻¹), indicating a slowdown in the progression of BSR.

Table 3: The effect of endophytic *Trichoderma* on BSR development in oil palm seedlings after 11 months.

Treatment	Disease Incidence of Internal Symptom (%DI)	Epidemic Rate (unit month ⁻¹)	AUDPC (units ²)	Disease Reduction (%)
<i>T. asperellum</i> M103	75.56 _a	7.07 _a	236.25 _a	18.28
<i>T. harzianum</i> M108	68.89 _a	7.50 _a	252.92 _a	17.90
CONTROL	88.89 _a	8.62 _a	293.61 _a	-

Means in columns followed by the same letter are not significantly different at $p \leq 0.05$ LSD

Overall, the results demonstrate that both of the endophytic *Trichoderma* (M103 and M108) have the ability as biocontrol agents for the oil palm seedlings against *Ganoderma* BSR, could be contributed through various mechanisms such as mycoparasitism and antibiosis activity that reported in in-vitro study (Elya Masya et al., 2022). A similar finding has been reported, whereby *T. asperellum* has the potential as the biocontrol agent for various diseases through the seed coating technique (Cotxarrera et al., 2022; Shores et al., 2005) but not effective enough against oil palm seedlings in pre-nursery stage (Jawak et al., 2018).

Despite the potential shown by both M103 and M108 in mitigating BSR disease in oil palm seedlings at nursery stage, there is a need for further improvement to enhance their effectiveness, particularly upon transplantation into *Ganoderma* infested plantation area. Seed coating technique is a cost-effective method for delivering microbial inoculants in protecting against various diseases and pests (Kthiri et al., 2020; O'Callaghan, 2016). It has emerged as a promising approach by applying small dosage of inoculant for inoculating various crop seeds (Rocha et al., 2019) especially short planting duration (Kubota et al., 2008). However, these techniques may not be as suitable for oil palm, due to several factors. Oil palm has a much longer planting duration compared to cash crops. Thus, there is a challenge to maintain the viability and effectiveness of the microbial inoculants (applied via seed coating) to protect the oil palm over such a prolonged period. Besides, the rhizosphere of the oil palm tree presents a unique environmental condition that may affect the survival and efficacy of the microbial inoculants applied via seed coating. Factors such as soil pH, moisture levels, and microbial competition in the rhizosphere may influence the establishment and activity of the introduced microbes. A booster application might be required to give a better protection on the oil palm seedlings, especially once they are transplanted to the infected field. This *Trichoderma* booster application is essential for managing the disease infection, because relying solely on the seed coating techniques may not provide sufficient protection against pathogens like *G. boninense*, particularly in oil palm cultivation. Several boosters applications of *Trichoderma* during the lifespan of the oil palm may help to replenish and reinforce the population of beneficial microbes in the rhizosphere, enhancing their ability to compete with other pathogens and suppress disease development. Additionally, by applying *Trichoderma* directly to the soil or foliage can target the pathogen hotspots more effectively, thereby complementing the protection provided by the initial seed coating. By combining the seed coating and booster applications should provide a multi-pronged approach to disease management, hence maximising the chances of success in controlling the BSR in oil palm.

Selecting the suitable endophytic *Trichoderma* candidates for product formulation is crucial. Notably, M103 grow faster than M108 and its ability producing a lot of spores, this distinction characteristic is crucial for efficient production processes, especially when formulating biocontrol products (Woo et al., 2023; Elya Masya et al., 2022). These strains can withstand formulation stresses, ensuring viability and efficacy in the final product. Properly formulated *Trichoderma* products would maintain the stability during storage and long shelf life, which are critical factor for commercial success.

Thus, continuous research is warranted to optimise the formulations, dosage, and application methods to enhance the efficacy and practicality in the agricultural settings (Nurzannah et al., 2022). The microbial inoculants are certainly having huge potential for the future agricultural

good practice. However, it is important to ensure that they are successfully applied to fulfil their role in sustainable agriculture.

4. CONCLUSION

The results suggest that both *Trichoderma* isolates have effectively reduced the disease incidence, severity, and seedling mortality compared to the untreated control. *T. asperellum* M103 has demonstrated a considerable efficacy as a biocontrol agent against BSR in oil palm seedlings, with a slightly superior performance in reducing foliar symptoms of BSR. These findings emphasise the potential application of these biocontrol agents in managing BSR in oil palm seedlings, contributing to the development of sustainable strategies in the oil palm industry. Further research and field trials would be valuable to validate and optimise the promising results in practical agricultural settings.

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