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Interference of wood decay, growth, and infection of *Ganoderma boninense* by ligninolytic fungi from herbaceous plants

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Abstract. Basal stem rot (BSR) disease caused by *Ganoderma boninense* is the most destructive disease in oil palm plantations. Herbaceous plants such as arrowroot, cocoyam, and canna were reported to interfere with the *Ganoderma* disease of oil palm under mixed planting on non-sterilized soil. This study aimed to determine the role of the ligninolytic fungi isolated from herbaceous plants on wood decay, mycelial growth, and infection of *Ganoderma boninense*. A total of 24 ligninolytic fungal isolates were isolated from arrowroot, cocoyam, and canna plant and grouped into 6 types of wood decay interaction, namely (1) neutral, (2) negative interference to the herbaceous isolates, (3) negative interference to both fungi, (4) negative interference to *Ganoderma*, (5) negative interference to the herbaceous isolates and positive for *Ganoderma*, and (6) positive interference in both fungi. Ligninolytic fungi from cocoyam and canna plants were able to negatively interfere with the *Ganoderma* wood decay, inhibit the colony, and reduce the initial root infection of oil palm. Keywords: antagonistic *in vitro*, *Ganoderma boninense*, ligninolytic fungi, root necrosis, wood decay.

1 Introduction

Basal stem rot (BSR) caused by a wood-degrading fungus, *Ganoderma boninense*, is the most destructive disease in monoculture oil palm plantations [1, 2]. BSR had killed 31−67% of oil palm trees in some inland plantations of North Sumatra [3]. BSR was also responsible for 30−54% of plant mortality in some peatland oil palm plantations in Sumatra [4]. In Malaysia, BSR disease had infested 8.72%, 14.0%, 6.08%, and 27.7% of oil palm in inland, coastal, peat, and lateritic area out of 37 359.81 ha of smallholder plantations [5]. The fungus degrades the lignin compositions of cortex cell tissues and kills oil palm trees. *Ganoderma* produces ligninolytic enzymes such as laccase and manganese peroxidase that can degrade the lignin component of plant cell walls, causing nutrient leakage of the affected palm [6]. Ligninolytic enzymes and other cell wall degrading enzymes were the key enzymes during both the necrotrophic phase of *Ganoderma* pathogenesis and the saprophytic life cycle [7, 8].

Management of BSR is difficult and challenging, as the fungus produces a pseudo-sclerotia in the infested roots and stems [9] that survive for a long time in the soil. Infested

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planting may harbor many infested roots and is considered responsible for the disease increase over several plant generations [10]. Mixed planting of oil palm with herbaceous plants may reduce the disease and potential inoculum of *Ganoderma boninense*. *Ganoderma* disease interference by rhizomatous plants such as ginger and Java turmeric occurred directly through both antifungal plant exudates and infection interference [11, 12]. Taro plants interfered with *Ganoderma* disease through those above mechanisms [13] and also by increasing the decaying of Ganoderma wood inoculum [14]. Disease suppression by herbaceous plants such as cocoyam, arrowroot, and canna was shown to be affected by rhizosphere microorganisms as loss of the disease suppression after mixed planting on autoclaved soil medium [15]. The role of wood-decaying fungi colonizing the herbaceous plants on the *Ganoderma* wood decay, growth, and infection was not known. Naidu et al. [16] isolated 25 isolates of wood decay hymenomycetes and three species *Pycnoporus sanguineus*, *Trametes lactinea*, and *Grammothele fuligo* had shown a high antagonistic activity, but the interference with *Ganoderma* wood decay and infection was not elucidated. In other co-culture systems, positive ligninolytic interferences were reported in the co-culture of *Trametes versicolor*, *T. maxima*, and *Ganoderma* spp. with *Aspergillus niger* by increasing the activity of the ligninolytic enzyme [17]. This study aimed to determine the role of the ligninolytic fungi isolated from herbaceous plants on wood decay, mycelial growth, and infection of *Ganoderma boninense*.

2 Materials and methods

2.1 Isolation of ligninolytic fungi

Ligninolytic fungi were isolated from surface-sterilized cocoyam (*Xanthosoma sagittifolium*) tuber and rhizomes of arrowroot (*Maranta arundinacea)* and canna (*Canna indica*). Sections of tuber or rhizomes were soaked in 70% ethanol for 2 min, then soaked in 1% NaOCl for 2 min, and rinsed with autoclaved distilled water. The sections were plated on 2% MEA + 0.05% Remazol Brilliant Blue R (RBBR medium) or 2% MEA + 0.1% tannin (tannin medium). The fungal colony that decolorizes the RBBR or tannin medium was determined as ligninolytic fungi [18], and then re-isolated and maintained in an MEA slant at room temperature.

2.2 Ganoderma boninense isolate

Ganoderma boninense strains used in this study were obtained from BSR-infected oil palm and identified based on morphology and the ITS sequences [11].

2.3 Antagonistic activity of ligninolytic fungi

A dual culture test was performed to measure the percentage inhibition of radial growth (PIRG) of tested ligninolytic fungi against *G. boninense*. Five mm diameter culture disk of tested fungi and *G. boninense* were plated at a 3 cm distance on MEA in a 9 cm Petri plate. Triplicated plates were used for each dual culture. PIRG was measured 4 days after incubation and calculated as (the radius of the *Ganoderma* colony away from the test colony (R1) - the radius of the *Ganoderma* colony toward the test colony) / R1 × 100 [19].

2.4 Wood decay interference assay

Wood decay was assessed following the method described by Xu et al. [20] with some modifications. The rubber wood block (RWB) measuring 2.0 × 2.0 cm was oven dried at 105ºC for 24 hours to obtain initial dry weight. Five RWBs were placed into a 330 ml bottle, re-wetted with 2% malt extract, and then autoclaved for 1 hour at 121ºC. RWBs were inoculated with a 0.5 cm diameter of either tested fungi or *G. boninense* and incubated for 7 days at 25-27 ºC. The fungi colonized RWBs were buried 2 cm deep within autoclaved sand with a water content of 36% in a 300 ml polypropylene cup. The RWB of tested fungi was placed in contact parallel side by side with two *Ganoderma* RWBs. Non-inoculated RWBs were placed in the same manner between two *Ganoderma* RWBs for non-inference treatment. Five RWBs were treated for each tested fungus. The tested cups were covered, sealed, and incubated at 25-27 ºC. Following incubation for 60 days, colonized RWBs were removed from sand media, and the adhering mycelium was carefully cleaned. The RWBs were oven dried at 105ºC and weighed to obtain the final dry weight. Wood decay was determined as the percentage of dry weight losses following incubation for 60 days and calculated as (initial dry weight - final dry weight)/initial dry weight × 100 [21].

2.5 Infection interference assay

Three-month-old D×P oil palm seedlings were inoculated by binding the wounded primary roots using a parafilm [11] to two parallel RWBs, one colonized by the fungi and the other by the *G. boninense*. Control seedling was inoculated with two RWBs, one RWB without fungi and the other colonized by *G. boninense*. RWBs were prepared as in the section of wood decay interference assay. Inoculated seedlings were planted on a 1-L pot filled with autoclaved soil media (a mixture of field soil and sand). Two months after inoculation, seedlings were uprooted and the inoculated primary root was excised laterally to measure the length of brownish necrotic root tissue. Colonization of *G. boninense* on the necrotic root was confirmed after growing the necrotic section on the *Ganoderma* selective medium (GSM).

2.6 Data analyses

The percentage dry weight loss of paired ligninolytic fungi colonized wood block was compared to the unpaired colonized one using t-test. Dry weight loss of *G. boninense*, PIRG, and length of the necrotic root was analyzed using ANOVA and the difference to the control was compared with Dunnett's test. Data were analyzed using the RStudio version 2022.07.0.

3 Results

3.1 Morphological characteristics of ligninolytic fungi

Twenty-four fungal isolates were isolated from surface-sterilized cocoyam tuber (4 isolates) and rhizome of arrowroot (11 isolates) and canna (9 isolates). All isolates were determined to have the ligninolytic ability as able to decolorize RBBR media to be pinkish color and tannin medium to be dark brown. Isolates varied in morphological appearance in MEA culture.

3.2 Antagonistic activity of ligninolytic fungi

All isolates of ligninolytic fungi showed an antagonistic activity that inhibited radial growth of *G. boninense* colony in dual culture test on MEA or RBBR or Tannin medium. The RBBR or Tannin discoloration at the *Ganoderma* colony side was increased (negative interference) or decreased (positive interference) compared to the control *Ganoderma* colony. The *Ganoderma* tannin discoloration was decreased in dual culture with Gyt3e and Gr19b and increased with Gr28d, Gr3a, and TLTF8. For RBBR medium, Ganoderma side discoloration was reduced on dual culture with Gytf1. The PIRG value was varied ($P<0.0001$) between isolates, but had a similar manner in the three tested culture media. Three isolates, Gytf5, TLRF3, and TLTF8 were strong antagonists with more than 90% of PIRG. TLTF3, Gyrf4, Gr18d, Gr3a, Gr16b, and Gr11b which mostly originated from arrowroot showed a weak antagonistic activity with a PIRG of less than 26% (Figure 1C).

3.3 Wood decay interference

Ganoderma boninense colonized RWB and buried as single inoculated control in the sand medium causing 2.7% dry weight loss after 1 month and 18.8% dry weight loss (DWL) after 2 months of incubation. Most ligninolytic fungi showed less wood decay activity (< 10% dry weight loss for 2 months) compared to *G. boninense*, except for Gr21 which caused 19.7% dry weight loss and was not significantly different from *G. boninense.* Co-inoculation between RWBs colonized by *G. boninense* and ligninolytic fungi resulted in different wood decay interference of interacting fungi. The wood decay interference could be grouped into six types of interactions, namely (1) neutral ($A_0 \times G_0$), (2) negative interference to the herbaceous isolates ($A_- \times G_0$), (3) negative interference to both fungi ($A_- \times G_-$), (4) negative interference to *Ganoderma* ($A_0 \times G_-$), (5) negative interference to the herbaceous isolates and positive for *Ganoderma* ($A_- \times G_+$), and (6) positive interference in both fungi ($A_+ \times G_+$) (Figure 1A and 1B). There was no association between interference RWB decay and Tannin or RBBR discoloration in the dual culture.

3.4 Infection interference

A single inoculation of *G. boninense* or co-inoculation with the ligninolytic fungi resulted in root necrotic of oil palm seedlings at two months post-inoculation. Co-inoculation with nine isolates (TLTF3, Gr28d, Gyr1a, Gytf5, Gr11c, TLTF6, TLRF3, TLTF8, and Gyrf1) caused a significant ($P<0.05$) reduction in infection of *G. boninense* as co-inoculation induced a shorter length of root necrosis compared to single inoculation of *G. Boninense*. All of those *Ganoderma*-suppressive isolates had a weak decay activity (less than 10% DWL). Six isolates negatively interfered with the *Ganoderma* wood decay with interaction $A_- \times G_-$ and $A_0 \times G_-$ and one isolate Gyrf1, could induce the *Ganoderma* wood decay (interaction $A_- \times G_+$) (Figure 1D).

3.5 Correlation between wood decay interference, antagonism, and root necrosis suppression

PIRG in dual culture (antagonism) had a close relationship with the percentage of root necrosis inhibition. Correlation analysis showed that ligninolytic wood decay activity (DWL) was slightly associated ($r=0.3670$, $P=0.0777$) with antagonism *in vitro*. A positive correlation ($r=0.7763$, $P<0.0001$) and a linear relationship was observed between the antagonism and root necrosis. Higher antagonism activity *in vitro* (PIRG) produced a higher root necrosis inhibition.

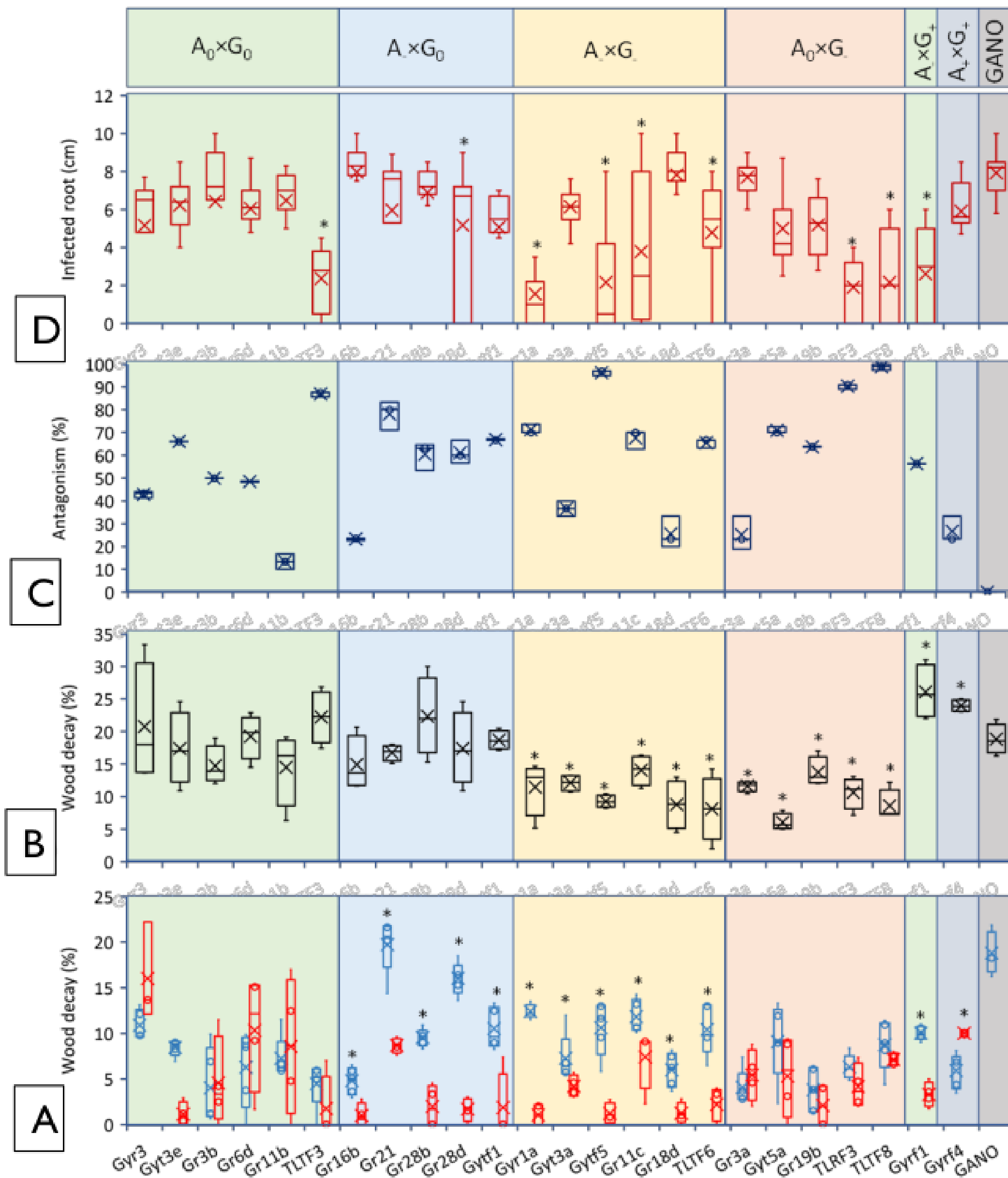

Fig. 1. Interference of wood decay under co-incubation of *Ganoderma boninense* colonized RWB (Gb) (B) and the ligninolytic fungi colonized RWB (B) based on dry weight loss (DWL) of the RWBs and its association with in vitro antagonism as measured by the percentage inhibition radial growth of Gb in the dual-culture assay (C) and the length of the infected root of oil palm seedling (D). Asterix (*) denotes significant differences (P<0.05) between the blue single inoculation box-plot relative to that of the red co-inoculation box-plot (A) or significant differences (P<0.05) with the single *Ganoderma* (GANO) treatment (B and D).

4 Discussion

Co-inoculation between two species of wood decay fungi, *G. boninense,* and ligninolytic fungi resulted in negative interference with decay activity (as measured by DWL) of one or both co-inoculated fungi. Only two (Gyrf1 and Gyrf4) out of 24 interactions had positive

decay interference. Six types of wood decay interaction were determined namely (1) neutral, (2) negative interference to the herbaceous isolates, (3) negative interference to both fungi, (4) negative interference to *Ganoderma*, (5) negative interference to the herbaceous isolates and positive for *Ganoderma*, and (6) positive interference in both fungi. Most ligninolytic fungi isolated from the tuber of cocoyam and rhizomes of arrowroot and canna showed less wood decay activity compared to *G. boninense* and easily negatively interfered or even lost in their ligninolytic activities when contact with *Ganoderma*. On the contrary, *G. boninense* had strong decaying activity and negatively interfered merely by some of both moderately and weakly ligninolytic fungi.

This study suggested that *G. boninense* is a strong ligninolytic and not easily interfered with by other ligninolytic fungi. *G. boninense* produces lignin-degrading enzymes such as laccases, manganese peroxidases (Mn-P), and lignin peroxidases (Li-P) to infect and degrade cell wall lignin of oil palm [22]. The decaying activity of *G. boninense is influenced* by different factors including nitrogen sources, hydrogen peroxide (H_2O_2), and phenolic compounds [6, 21]. Ligninolytic fungi could secrete (H_2O_2) and the secretion increased in an incompatible interaction (antagonism) between two fungal species [23,24]. Low centration of H_2O_2 is necessary for Mn-P dan Li-P activity but may inactive the enzymes at the high concentration [23].

Negative wood decay interference was determined in this study in most co-inoculation between two species of wood decay fungi, *G. boninense,* and ligninolytic fungi. This result was in contrast to other interactions between two species of fungi that demonstrated the induction of wood-decaying activity. Lira-Pérez et al. [17] reported that dual-cultures of *Trametes versicolor*, *T. maxima*, and *Ganoderma* spp. with *Aspergillus niger* increased 67 times Li-P and Mn-P activity. Copete-Pertuz et al. [24] demonstrated that the ligninolytic activity of wood decay *Leptosphaerulina* sp. was enhanced under dual culture with *Trichoderma viride* and *Aspergillus terreus* antagonists. Wiberth et al. [23] recorded the increasing activity of laccase and Mn-P of wood-decaying fungi, *Pycnoporus sanguineus* and *T. maxima* under co-culture with antagonistic fungi, *Purpureocillium lilacinum*, *Beauveria brongniartii*, *Metarhizium anisopliae*, dan *Trichoderma* sp. This study found no correlation between antagonistic activity *in vitro* and wood decay interference.

Nine isolates of ligninolytic fungi had suppressed *Ganoderma* root necrosis under the co-inoculation test and six isolates (Gyr1a, Gytf5, Gr11c, TLTF6, TLRF3, dan TLTF8) negatively interfered with *Ganoderma* wood decay. Gyr1a dan TLRF3 (sterile dark septate fungi), Gytf5 (*Penicillium* sp.), Gr11c (hyaline sterile hyphae), TLTF6 (*Penicillium* sp.), dan TLTF8 (*Trichoderma* sp.) were the ligninolytic fungi that inhibited the *Ganoderma* wood decay, *Ganoderma* mycelial growth in vitro, and necrosis in planta. All of the *Ganoderma* root necrosis suppressive isolates had high antagonistic activity *in vitro*. There was a higher correlation between the *Ganoderma* root necrosis suppressive isolates with Ganoderma mycelial inhibition *in vitro* compared with those of the *Ganoderma* wood decay suppressive isolates. In this study, the antagonistic activity is more likely to determine the inhibition of the fungi against the *Ganoderma* root necrosis rather than the wood decay interference.

5 Conclusion

Ligninolytic fungi from cocoyam and canna plants were able to negatively interfere with the *Ganoderma* wood decay, inhibit the colony, and reduce the initial root infection of oil palm.

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