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Co-infection of two Ganoderma boninense strains on oil palm seedlings

Infeksi ganda dua strain Ganoderma boninense pada bibit kelapa sawit

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ABSTRACT

Ganoderma boninense is the basal stem rot disease (BSR) pathogen that devastates oil palm plantations. Disease infection generally occurs by a single strain of *G. boninense,* or co-infection of two strains arises as revealed by somatic incompatibility. This study aimed to determine the effects of co-infection of two somatically incompatible *G. boninense* strains on the BSR disease of oil palm seedlings. Two strains of *G. boninense* were from 2 oil palm plantations and had different aggressiveness. Co-infection of two *G. boninense* strains was performed by inoculating the *Ganoderma* rubber wood blocks to the 3-month-old oil palm seedling and examined for 7 months. The results showed that co-infection with two *G. boninense* strains had similar disease symptoms, decreased disease severity (score 1.5 compared to 2.0 for a single aggressive strain), and similar seedling growth retardation by the single aggressive strain. Higher fungal colonization (92%) of oil palm roots was exhibited in the co-infection compared to 85–86% colonization of a single strain infection. This study revealed that co-infection with two somatically incompatible strains might favor host colonization by *G. boninense*.

ABSTRAK

Ganoderma boninense merupakan patogen penyakit busuk pangkal batang (BPB) yang menyerang tanaman kelapa sawit. Infeksi penyakit umumnya terjadi oleh strain tunggal *G. boninense* tetapi terjadi juga infeksi ganda oleh dua strain pada satu tanaman kelapa sawit yang dibuktikan dari kajian kelompok somatik. Penelitian ini bertujuan mendeterminasi pengaruh infeksi ganda dua isolat *Ganoderma* yang berbeda kelompok somatik terhadap penyakit BPB pada bibit kelapa sawit. Dua strain *G. boninense* berasal dari 2 perkebunan kelapa sawit dan memiliki agresifitas berbeda. Infeksi ganda dikaji dengan menginokulasi balok kayu karet yang dikoloni *Ganoderma* pada bibit kelapa sawit umur 3 bulan dan diuji selama 7 bulan. Hasil penelitian menunjukkan bahwa infeksi ganda 2 strain *G. boninense* menyebabkan gejala yang sama, penurunan keparahan penyakit (skor 1.5 dibandingkan skor 2 dari strain agresif), hambatan pertumbuhan bibit yang setara dengan infeksi strain agresif. Infeksi ganda tersebut menghasilkan kolonisasi akar 92% yang lebih tinggi dibandingkan 85-86% kolonisasi oleh strain tunggal. Penelitian ini mengungkapkan bahwa infeksi ganda dengan strain yang berbeda kelompok somatik dapat mendukung kolonisasi inang oleh *G. boninense*.

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INTRODUCTION

Basal stem rot (BSR) is a major disease in oil palm plantations in Indonesia (Goh et al., 2014; Wong et al., 2021). The diseases caused by the fungus *Ganoderma boninense* have been reported to have a high incidence at all growing stages of oil palms (Santoso, 2020), with a high attack rate can reach 60% (Bharudin et al., 2022). The disease cannot be optimally controlled because it is symptomless in the early period of infection and non-specific. Specific symptoms will appear after the advanced stage of the disease and for a long time from the initial infection (Suryantini & Wulandari, 2018). Symptoms of the infection in nurseries are indicated by young leaves that are abnormal in number or do not even develop, withered leaf midrib from the bottom leaf, advanced rotted at the base of the stem, and followed by the growth of the fruiting body of the fungus *G. boninense* (Paterson, 2019).

Symptoms of *G. boninense* infection are difficult to detect early because the mechanism of fungal infection involves several complex developmental processes. In addition, the infection mechanism is closely related to the diversity of basidiospores that develop into numerous heterokaryon mycelia (Rakib et al., 2017). A heterokaryon group with genetic similarity characterized by the ability of the mycelium to anastomose completely is called a somatic group or one somatic individual (Miller et al., 1999). Infection of *G. boninense* generally occurs by a single strain, but some co-infection of two strains arises in the field as revealed by somatic incompatibility assay (Miller et al., 1999; Rakib et al., 2020). The impact of co-infection of *G. boninense* strains on disease is unknown. This study aimed to determine the effects of co-infection with two somatically incompatible *G. boninense* strains on the BSR disease of oil palm seedlings.

MATERIALS AND METHODS

Pathogen strains and inocula

Ganoderma boninense strains used in this study were obtained from oil palm *Ganoderma* infected diseased in West Sumatra (Gb-A) and South Sumatra (Gb-B). Both strains had been identified based on morphology and the ITS sequences (Ayundra, 2022; Suwandi et al., 2022). The two strains were somatically incompatible, indicated by a demarcation line between paired colonies in a Petri plate (Figure 1) as tested following Castillo et al. (2022).

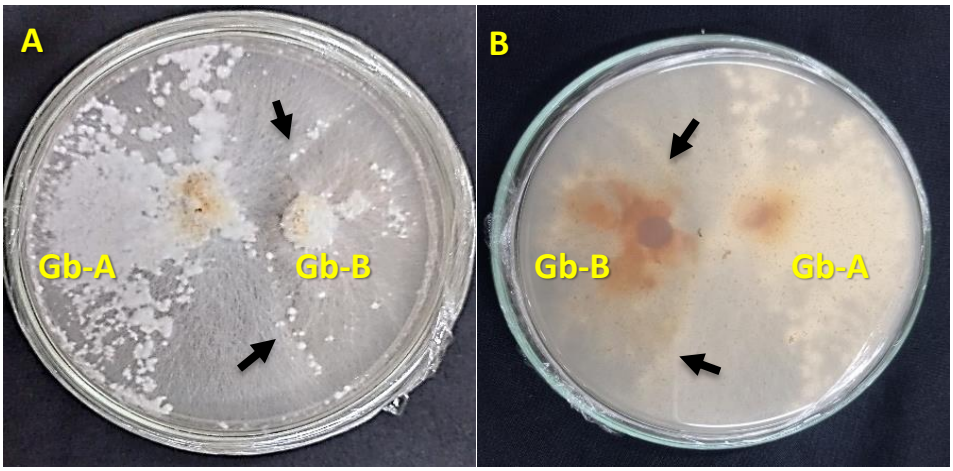

Figure 1. Somatic incompatibility was shown by a slight demarcation line (arrow) between the colony of *Ganoderma boninense* strain Gb-A dan Gb-B on malt extract agar. A. Top view, and B. Bottom view.

Rubber wood blocks (RWB) as an inoculum medium for *G. boninense* were prepared according to Suwandi et al. (2022). Rubber wood with a diameter of 7 cm and a length of 15 cm was first soaked in water for 24 hours, then autoclaved for 2 hours, and allowed to stand for 24 hours. Next, the RWB in a plastic bag was filled with 20 ml of 2% malt extract and autoclaved again. Finally, RWB was inoculated separately with 7-day-old MEA cultures of Gb-A or Gb-B and incubated for one month in the dark at room temperature.

Oil palm seedling and plant inoculation

The study used oil palm seedlings from sprouted seeds D×P Simalungun provided by the Indonesian Oil Palm Research Institute, Medan, North Sumatera, Indonesia. Sprouted seeds were pregrown for 3 months until having 2 or 3 leaves. The experiment was arranged in a completely randomized design with four treatments (single strain infection of Gb-A, single strain infection of Gb-B, co-infection of Gb-A + Gb-B, and non-inoculated control) and 15 replications. Each replication consisted of one oil palm seedling. Infection was performed by inoculating and binding the primary roots of 3-month-old oil palm seedlings using a parafilm to one RWB for a single infection treatment (Suwandi et al., 2022). In the multiple infection treatment, one seedling was tied with two half-sized RWBs of each Gb-A and Gb-B RWB. For non-inoculated control, the roots were tied with RWB without *G. boninense*. Inoculated seedlings were planted in a 5 L pot filled with sand media and arranged at 90 cm spacing in a greenhouse of the Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, South Sumatra, Indonesia. Seedlings were fertilized with 1% NPK 16-16-16 once a month, watered daily, and sanitized from the weeds

Ganoderma disease parameters

The observed *Ganoderma* infections variables were disease symptoms, incidence, severity, and root colonization. Disease symptoms were monitored every month. The incidence of basal stem rot symptomatic plant was calculated 7 months after inoculation as the number of symptomatic out of 15 inoculated plants multiplied by 100. Disease severity was observed every two months for seven months and measured using a severity score of 0–5 (0: healthy plant; 1: early symptoms on the first leaf; 2: first leaf died; 3: first two leaves died; 4: more than three first leaves died; and 5: total dead plants) (Suwandi et al., 2022). Root colonization by *G. boninense* was assessed following the procedure of Rees et al. (2009) at the end of the study (7 months after inoculation). Primary roots were cut into 12 segments (2 cm in length) for each plant and plated on *Ganoderma* selective media (GSM). The fungal colonization rate (%) was expressed as the percentage of the number of colonized root fragments from which the *Ganoderma* mycelia emerged on GSM out of 12 root segments.

Oil palm seedling growth parameters

The measured oil palm growth variables were plant height and leaf area, recorded at 1-, 3-, 5-, and 7 months post-inoculation. Plant height was measured using a ruler. The leaf area was calculated by measuring the width × the length of the leaf × 0.55 (Gromikora et al., 2014).

Data analysis

The disease severity, plant height, and leaf area data were subjected to a one-way analysis of variance. Tukey's HSD (honestly significant difference) test was performed to compare the mean values to see if there was a significant effect. The percentage of disease incidence and number of colonized root fragments between inoculation treatments were compared according to Fisher's exact test of independence by applying the Bonferroni corrected alpha level. Data were analyzed using the RStudio version 2022.02.2-485.

RESULTS AND DISCUSSION

Disease symptoms

The symptoms of infected oil palm seedlings were first observed after three months of *Ganoderma* infection treatment. The oldest bifid leaves were chlorosis, necrotic from the leaf tip, and thoroughly drying (Figure 2A and 2B). After two to three months from the first symptoms, the symptom continued to the second leaf. Eventually, all the leaves withered, and the seedlings died (Figure 2C). Diseased seedlings experienced retardation of growth with rotted primary roots colonized by the fungus mycelium (Figure 2D to 2F). Similar disease symptoms were shown in oil palm seedlings when infected by a single strain (Gb-A or Gb-B) and co-infection of Gb-A + Gb-B treatment. Disease incidence was not significantly different ($P>0.05$) between all infection treatments. However, disease incidence caused by single Gb-A was 40%, while Gb-B or co-infection of Gb-A + Gb-B was 20% (Table 1).

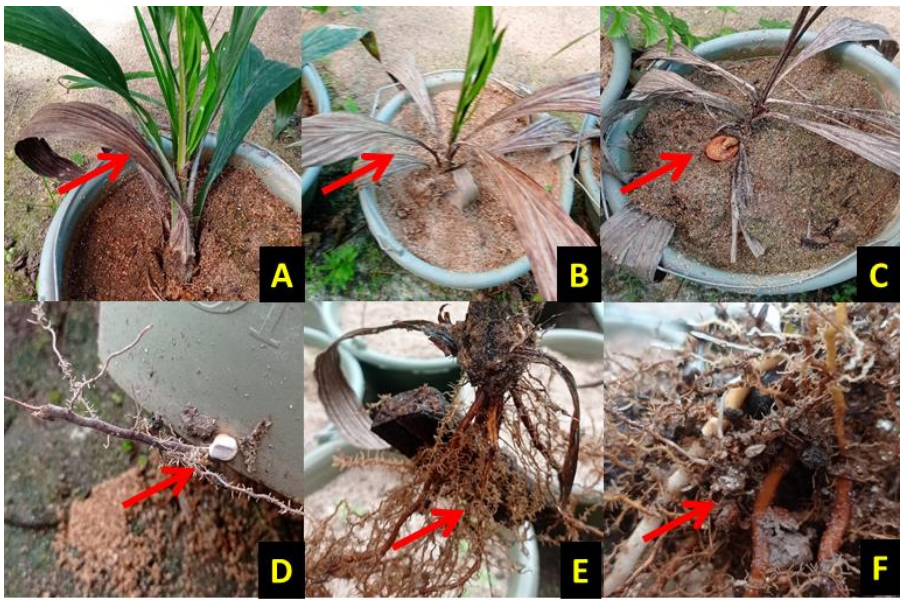

Figure 2. Symptoms of *Ganoderma* infection in oil palm seedlings after seven months of inoculation, A. Dead on the first leaf (score 2), B. Dead on more than three first leaves (score 4), C. Total plant dead with basidiocarp formation (score 5), D. Dense mycelium on an infected root, E. Light mycelium colonization on a rotted root, and F. A heavy mycelium colonization on rotted roots.

Disease severity

The disease severity increased every month for each infection treatment, but the severity score was not significantly different between the treatments. Non-inoculated control did not produce any disease symptoms (severity score 0). Single Gb-A infection showed to cause more severe disease than infection of Gb-B or co-infection of Gb-A + Gb-B. Co-infection of two strains (Gb-A + Gb-B) after 3, 5, and 7 months of inoculation resulted in lower disease severity but was not significantly different compared to inoculation with single Gb-A. The disease severity of the co-infected strains was 1.5 at 7 months after inoculation, which was equivalent to the severity average (score 1.6) by infection of both single strains (Figure 3).

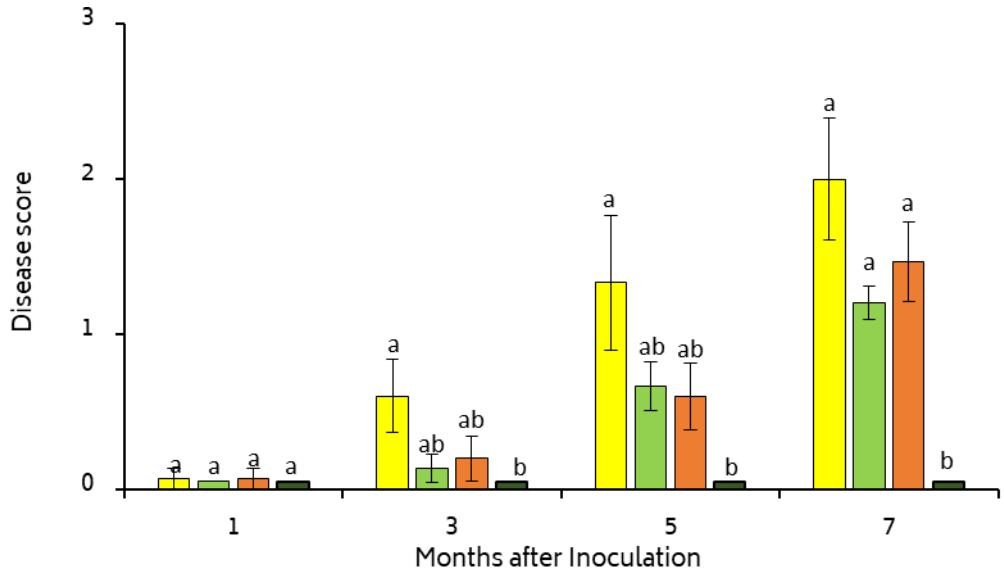

Figure 3. The disease severity of oil palm seedlings inoculated with a single (Gb-A given in yellow or Gb-B given in green) and multiple somatic individuals *Ganoderma boninense* (Gb-A + Gb-B given in brown). Error bars denote the standard error of means (SEM). For each month, values followed by different letters are significantly different (HSD test). Non-inoculated given in black.

Ganoderma colonization on roots

Re-isolation of *Ganoderma* colonized roots of oil palm seedling plated on GSM selective media resulted in fungal colonies that were uniform in color and shape, all of which changed the color of the culture media to reddish-brown. In addition, the intensity of the discoloration of the medium was also uniform (Figure 4). Differences were found in the number of colonized root fragments from which the *Ganoderma* mycelia emerged on GSM. Fungal colonization on an infected root resulted in a profuse growth with dense *Ganoderma* mycelia recovered from the root segment plated on GSM (Figure 4). Root colonization by *Ganoderma* was not significantly different ($P \geq 0.05$) between inoculation treatments, but co-infection treatment (Gb-A + Gb-B) resulted in the highest percentage of root colonization (92%), followed the colonization by Gb-A (86%) and Gb-B (85%) (Table 1).

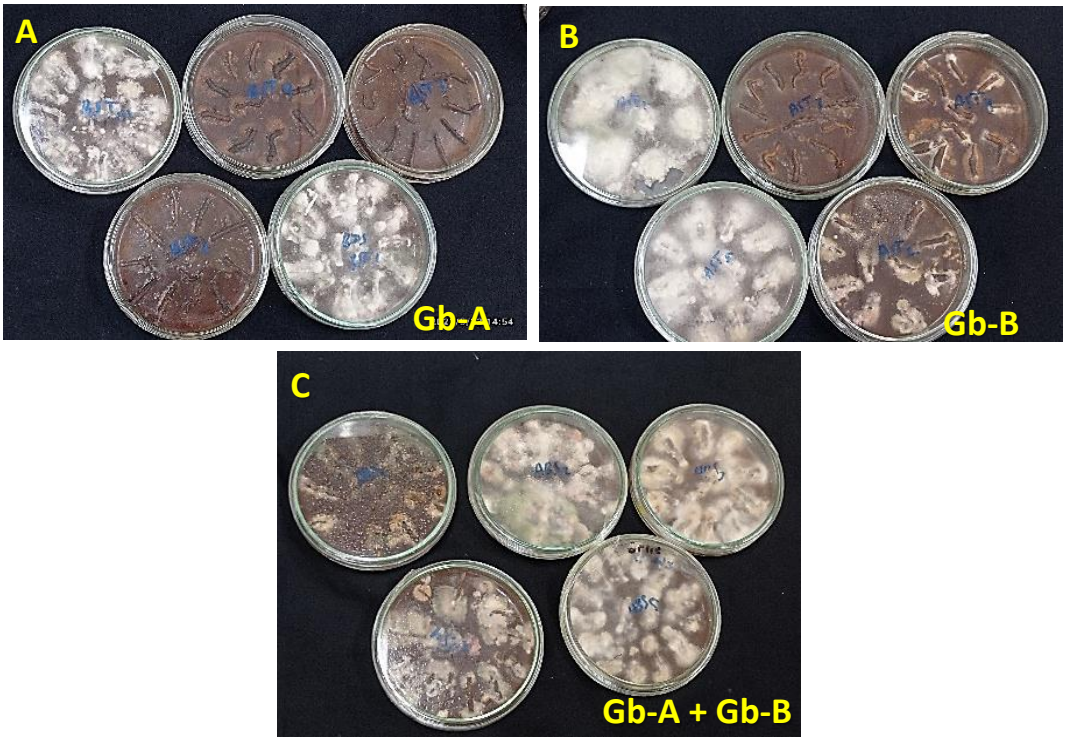

Figure 4. Colonization of *Ganoderma boninense* on the oil palm roots as confirmed after re-isolation on the GSM medium. Recovery of *Ganoderma* mycelium from root segments infected with Gb-A single strain (A), with Gb-B single strain (B), and with Gb-A and Gb-B (C).

Table 1. Effect of single or co-infection with *Ganoderma boninense* strains on the incidence of basal stem rot and percentage number of colonized root fragments by *Ganoderma* mycelia

Infection treatment	Disease incidence as the number of symptomatic out of inoculated plants (percent)	Percentage number of colonized root fragments as recovered on GSM medium
Single infection of Gb-A	6/15 (40%) a	86% a
Single infection of Gb-B	3/15 (20%) a	86% a
Co-infection of Gb-A + Gb-B	3/15 (20%) a	92% a
Non-inoculated control	0/15 (0%) a	0% b

Percentage values labeled by the same letter are not significantly different according to Fisher's exact test of independence by applying the Bonferroni corrected alpha level

Seedling growth of oil palm

The height of oil palm seedlings at 1, 5, and 7 months after inoculation was not significantly affected (P≥0.05) by the infection treatment of *G. boninense*. Gb-A infected seedlings had a significantly lower (P<0.05) height compared to that of Gb-B at 3 months after inoculation (Figure 5 A). The seedling height of co-infected *G. boninense* strains was 198.78 cm at 7 months after inoculation, which was within the range values of the infected seedlings by single strain Gb-A (194.49 cm) and Gb-B (213.07 cm).

The leaf area was significantly inhibited by treatment of Gb-A after three months of pathogen inoculation. The inhibition is more significant with the increasing seedling age. Gb-B single infection did not affect the leaf area, which was not significantly different (P≥0.05) from the uninoculated control plants. Co-infection of two strains (Gb-A + Gb-B) resulted in a relatively inhibited leaf area but was not significantly different (P≥0.05) from the uninoculated control (Figure 5 B).

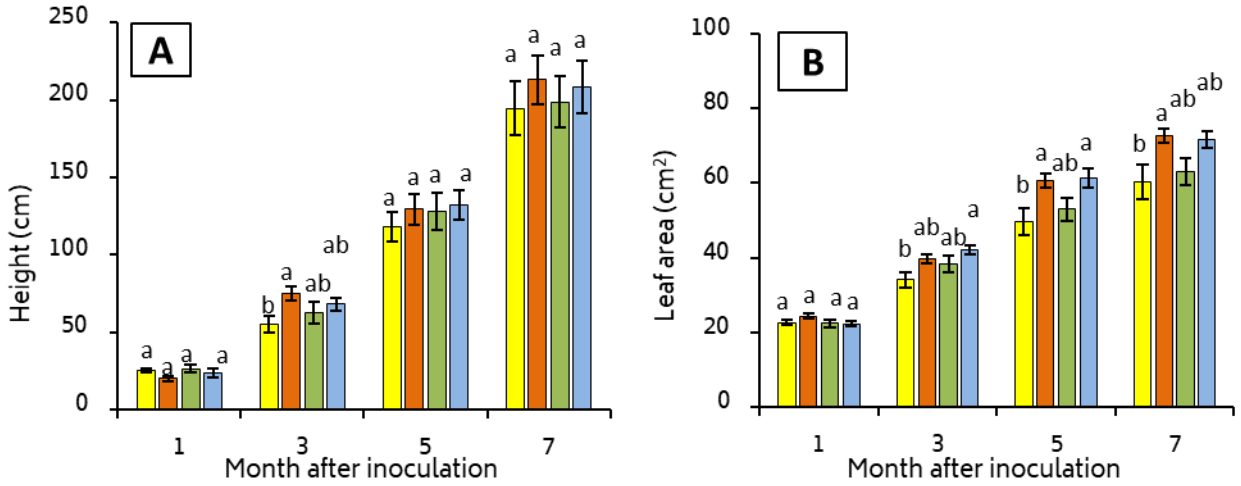

Figure 5. Height (A) and leaf area (B) of oil seedlings inoculated with a single (Gb-A given in yellow or Gb-B given in red) and two *Ganoderma boninense* strains (Gb-A + Gb-B given in green). Error bars denote the standard error of means (SEM). For each month, values followed by different letters are significantly different (HSD test). Non inoculated given in blue.

Co-infection of two somatically incompatible strains of *G. boninense* with different aggressiveness promoted more extensive colonization of oil palm seedling root by the fungus but caused less severe disease without growth retardation than a single infection of aggressive individuals. Reduced disease in this co-infection study was different from the co-inoculation test of white root fungus (*Rigidoporus microporus*) on rubber seedlings. Co-inoculation of two *R. microporus* strains with different aggressiveness, even between non-aggressive strains, led to increased disease severity (Suwandi, 2007). The difference in response to co-infection is thought to be related to the efficiency of *Ganoderma* infection by the inoculum size. *Rigidoporus* can infect rapidly using small volumes of RWB as inoculum, but *Ganoderma* needs a larger inoculum size. *Ganoderma boninense* is a hemibiotroph pathogen that can switch its colonization style between biotrophic and necrotrophic phases, depending on the environment and its conditions (Rees et al., 2009). The fungus exhibits a biotrophic phase during the initial stages of infection when hyphae colonize the root tissues without any symptoms. The transition to the necrotrophic phase, which involves extensive cell wall degradation, occurs once the pathogen overcomes the host defenses (Rees et al., 2009; Bahari et al., 2018). Symptoms and growth retardation were expressed following the necrotrophic phase (Faizah et al., 2022). Larger inoculum sizes could rapidly switch between the biotrophic and necrotrophic phases of *G. boninense* (Rees et al., 2009). The half size of RWBs as inocula for co-infection in this study was likely to provide good root colonization. Still, it could not support the extensive degradation of the root transport vessel network. The co-infection study with a larger inoculum size needs further investigation.

Co-infection of two pathogen strains to a single host can lead to competition for host space (within-host competition) (Bose et al., 2016). Two fungi in the same species (intra-species) or different species (inter-species) in different somatic compatibility or somatic deficiency could antagonize each other, resulting in reproductive parasitism (Grum-Grzhimaylo et al., 2021). Antagonism between somatically incompatible strains of *G. boninense* might induce separate root colonization and therefore promote the extensive root colonization by the fungus.

The within-host competition between pathogen strains may lead to the domination of an aggressive with higher fitness strain over a non-aggressive with lower fitness strain (Bose et al., 2016). The dominance of a single strain in the single diseased oil palm could explain the nature of the low incidence of multiple *Ganoderma* strains infections in the field. The incidence of multiple strain infections in the field, according to Miller et al. (1999), was 1 out of 256 plants or 0.4%. A low frequency of multiple strain infections of *G. boninense* was demonstrated by Rakib et al. (2020), finding one co-infection out of 17 diseased oil palms. *Ganoderma* is a fungus that produces abundant basidiospores and hence harbors abundant somatically incompatible strains (Bharudin et al., 2022). Still, few have reported the incidence of co-infection of somatically incompatible *G. boninense* strains in the field.

CONCLUSIONS

Co-infection of two somatically incompatible strains of *G. boninense* with different aggressiveness resulted in similar disease symptoms, decreased disease severity and incidence, and similar seedling growth retardation but caused higher fungal colonization of oil palm roots compared to those of a single aggressive strain.

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