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CALCIUM NUTRITIONAL EFFECTS ON SUPPRESSION OF BASAL STEM ROT (BSR) DISEASE IN OIL PALM SEEDLINGS IN NURSERY AND FIELD TRIALS

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ABSTRACT

The oil palm industry is significantly affected by basal stem rot (BSR) caused by Ganoderma boninense. *Nutrients are frequently used in soil fertilisers to protect plants from various stresses. Manipulating plant nutrients, particularly calcium (Ca), offers a promising strategy to prevent BSR disease in oil palm. This study evaluates the effect of Ca-based formulations on the suppression of* G. boninense *infection in oil palm seedlings using the root-sitting technique in three-month old oil palm seedlings. The study was further conducted in the field to test the disease incidence of BSR using the seedling baiting technique. Oil palm seedlings were pre-treated with fertiliser containing 1,000 ppm Ca as $CaSO_4$. They were then exposed to* G. boninense *PER 17 using two methods; in a 12-month nursery trial, colonised rubber woodblocks were attached with seedling roots to simulate infection, while in a 21-month field trial, seedlings were planted in soil naturally infested with the fungus to test real-world conditions. In both trials, fertiliser with 1,000 ppm Ca reduced BSR incidence by 53% in the nursery and 81% in the field. This suggests Ca supplementation as an effective alternative for preventing BSR in oil palm plantations.*

Keywords: basal stem rot, calcium, *Ganoderma boninense*, oil palm, soil fertiliser.

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INTRODUCTION

Basal stem rot (BSR), caused by the soil-borne fungus *Ganoderma boninense*, poses a major threat to the oil palm industry in Southeast Asia, particularly in Malaysia and Indonesia (Jazuli et al., 2022). The disease is also reported in Southern Thailand, Papua New Guinea, Africa, and Colombia (Rebitanim et al., 2020). In heavily infected regions, up to 70%-80% of oil palm trees over 15 years old experience significant yield decline, leading to economic losses in the hundreds of millions annually (Jazuli et al., 2022; Supramani et al., 2022). BSR compromises productivity by degrading root and vascular systems, reducing water and nutrient uptake, which results in stunted growth, lower fruit yield and premature tree death (Idris et al., 2006).

In Indonesia, BSR leads to palm mortality rates of up to 80.0%, while even a 1.0% infection can cause annual losses of USD38 million (Supramani et al., 2022). In Malaysia, it affects 7.4% of estate palms (221,000 ha) and 9.2% of smallholder plantations (3,450 ha), resulting in RM1.5 billion in losses annually (Idris et al., 2019). BSR reduces productive palms and fruit bunch weight, leading to substantial economic losses.

Conventional methods for controlling BSR in oil palms, including physical, biological and chemical approaches, often have limited effectiveness. This

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is because treatments are usually applied after the disease is well-established, reducing their impact. Some commercialised products, like GanoCare® (Rebitanim et al., 2020), show promise in managing *G. boninense,* but their success depends on factors such as timing, environmental conditions and disease severity. These challenges emphasise the need for integrated management strategies and earlier detection to improve outcomes. Current detection methods for *G. boninense* include visual symptom assessment, but these are typically too late for effective intervention. Hence, molecular techniques such as polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) and hyperspectral imaging are increasingly utilised for earlier and more precise diagnosis. Ground-penetrating radar (GPR) and electronic noses also offer non-destructive detection capabilities at earlier infection stages (Jazuli et al., 2022; Supramani et al., 2022). In addition to nutrient enhancement, the role of biocontrol agents such as *Trichoderma* spp., *Pseudomonas aeruginosa* and *Arbuscular mycorrhizal* fungi (AMF) has gained attention for their antagonistic activity against *G. boninense*, further supporting a sustainable and integrated disease management approach (Sundram et al., 2014). Modification of fertiliser nutrients has historically enhanced disease resistance and increased plant yields. Applying soil nutrients like nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) helps boost plant disease defenses (Tripathi et al., 2022). Rebitanim et al. (2020) highlighted that GanoCare®, a nutrient-based treatment, significantly improved oil palm growth and resistance against *G. boninense* in both nursery and field trials.

Calcium (Ca) is essential for plant structural strength, stress tolerance and disease resistance, making it a valuable nutrient for managing BSR in oil palm. It reinforces cell walls, limiting pathogen entry (Miedes et al., 2014), and activates defense enzymes like peroxidases (POD) and laccases, which promote lignification — A key defense against fungal infections (Bhuiyan et al., 2009; Wang et al., 2013). Calcium (Ca) supplementation boosts lignin production, strengthening plant resistance (Bivi et al., 2016; Nur Sabrina et al., 2012), while also improving soil health by regulating pH and enhancing microbial balance, indirectly reducing *Ganoderma* colonisation (Zakaria *et al*., 2023). Study shows that Ca treatments can reduce BSR incidence by up to 71.7% in oil palm seedlings (Nur Sabrina et al., 2012) and applying calcium nitrate ($Ca(NO_3)_2$) before infection further lowers disease risks (Sariah et al., 1997). Compared to chemical fungicides, Ca is cost-effective and environmentally friendly and promotes long-term plant health, making it a sustainable and effective strategy for BSR management.

However, the role of Ca nutrition in influencing the occurrence of BSR in oil palms remains poorly understood, highlighting the need for further investigation. To address this gap, the study expanded its scope from nursery trials using the root-sitting technique to field trials employing the seedling baiting technique in areas identified as infected. Different rates of Ca-based formulations were applied to evaluate their potential in managing BSR disease and serving as alternative inhibitors of disease incidence (DI). The findings revealed the efficacy of Ca-based treatments in suppressing *G. boninense* infections in oil palm seedlings. By broadening the research from controlled nursery conditions to real-world field applications, the study provided valuable insights into the role of Ca nutrition in mitigating BSR disease. This approach not only aims to reduce the prevalence of BSR but also seeks to optimise fertiliser use, improve palm oil quality, and support sustainable agricultural practices for enhanced productivity. This study demonstrated that pre-treating oil palm seedlings with 1,000 ppm Ca in the nursery for 12 months reduced BSR infection by up to 81% at 21 months after planting in the field, providing novel evidence for the effectiveness of Ca-based pretreatment as a preventive strategy under real plantation conditions.

MATERIALS AND METHODS

Nursery Trial

The investigation was executed in a shaded nursery in the Malaysian Palm Oil Board (MPOB) Research Station, Bangi (2°55′39.40″N, 101°45′54.30″E). Three-months-old seedlings (*Dura* × *Pisifera*) from MPOB Research Station, Kluang, were planted in small polybags (25 cm height × 20 cm diameter; 6 kg of soil) filled with 'Munchong' series soils comprising 3:1 v/v topsoil:sand. In this experiment, three groups of seedlings (NT1, NT2 and NT3) were studied to assess the effects of Ca pre-treatment, with NT1 serving as the untreated control. The main difference between NT2 and NT3 is the application of Ca treatment. NT2 (positive control) consisted of seedlings artificially infected with *G. boninense* but without Ca supplementation, whereas NT3 (Ca treatment) included infected seedlings that received Ca supplementation. For the treated groups (NT2 and NT3), Ca was applied in the form of calcium sulphate ($CaSO_4$) at a rate of 20.4 g per application, providing an equivalent of 1,000 ppm of Ca ions (Ca^{2+}) per month. This treatment was administered to 6 kg of soil per application and repeated monthly for three months, ensuring a sustained and consistent supply of Ca throughout the pre-treatment period. Seedlings were watered twice daily, and one rate of N, P and

K fertilisers as basic soil fertilisers was added to the experiment. The selection of 1,000 ppm Ca was based on previous findings (Mayzaitul-Azwa et al., 2022) and further supported by Bivi et al. (2016), who reported that similar Ca levels significantly enhanced disease suppression in oil palm seedlings challenged with *Ganoderma* spp. The current study extends this work, investigating its potential to suppress *G. boninense* in both nursery and field conditions.

Preparation of* G. boninense *inoculum and inoculation. The 54 rubber wood blocks (RWB) were prepared according to MPOB's general inoculum method (Idris, 1999). The RWB (6 × 6 cm) produced from fresh rubber *(Hevea brasiliensis*) wood blocks were autoclaved at 121°C, 103.4 kPa psi for 30 min, then oven-dried at 80°C for 30 min. This sterilisation was done twice. After placing the RWB in a heatproof polypropylene bag (10 × 32 cm), 60 mL of malt extract agar (MEA) was added to the block, autoclaved again and cooled within the specified conditions. The RWB were rotated at 360° during the cooling process, so the MEA completely covered the whole block surface before being solidified. After the cooling process, the RWB were inoculated with a 7th to 10th-day-old pure *G. boninense* PER 71 culture grown on potato dextrose agar (PDA). To prevent contamination, the inoculated RWB were sealed in a polypropylene bag with an elastic band. After three months of dark incubation at 27°C, the *G. boninense* mycelium (PER 71) exhibited colonisation both internally and externally.

After three months, seedlings (*Dura* × *Pisifera*) were transferred into larger polybags (38 cm height × 46 cm diameter; 18 kg soil). The nursery study followed a randomised complete block design (RCBD) with three blocks, each containing three blocks and 20 sub-replications across four treatments, resulting in a total of 240 experimental units (*Table 1*). Artificial infection was conducted using the root sitting technique, where seedlings were carefully uprooted and the root balls were placed in direct contact with actively colonised RWB containing *G. boninense* mycelia (*Figure 1a*). This method allows for uniform exposure of the roots to the pathogen and has been widely used for pathogenicity assays in nursery trials**.** The roots of the NT2 and NT3 seedlings were placed directly on top of the RWB inoculum in a polybag filled with a soil mixture in the same ratio as previously mentioned. Most of the oil palm seedlings were covered with soil when they were in place. The NT1 seedlings (negative control) are planted in polybags without *G. boninense*. The nursery study was performed for 12 months. The application of basic fertiliser was mentioned in the nursery trial.

TABLE 1. OIL PALM SEEDLING TREATMENTS IN NURSERY STUDY

Treatment	Description
NT1	Seedlings untreated and uninfected (negative control) and BF
NT2	Seedlings untreated and artificially infected with *G. boninense* (positive control) and BF
NT3	Seedlings treated with Ca and artificially infected with *G. boninense* and BF + 61.2 g $CaSO_4$ (1,000 ppm of Ca^{2+}) /one month /18 kg of soil

Note: BF - basic fertiliser: Urea (N, 46%), monopotassium phosphate (MKP) (P_2O_5, 52%), muriate of potash (MOP) (K_2O, 60%) and kieserite (MgO, 25%).

Assessment of pathological parameters. The severity of foliar symptoms (SFS) of BSR disease was assessed based on the proportion of affected leaves, categorised as Green (G) for healthy leaves

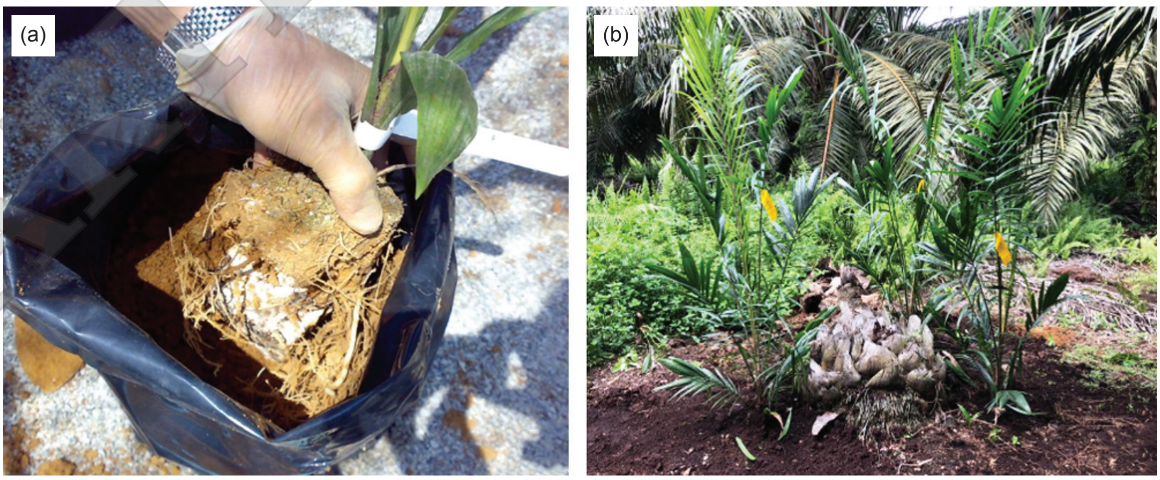


Figure 1. (a) Artificial infection of oil palm seedlings with Ganoderma boninense *PER 71 using the root sitting technique in the nursery and (b) field planting of pre-inoculated oil palm seedlings as baits around a* Ganoderma*-infested stump. Three pre-inoculated seedlings were positioned at three corners surrounding the infected stump.*

with no visible symptoms, Yellow (Y) for leaves showing yellowing or mild chlorosis, and Die (D) for completely wilted or necrotic leaves. The severity index was then calculated using the following Equation (1):

$$\text{BSR severity index (\%)} = \frac{(Y \times 1) + (D \times 2)}{G + Y + D} \times 100 \qquad (1)$$

This Equation (1) accounts for the proportion of symptomatic leaves, with higher weights assigned to more severe symptoms.

The disease development in oil palm seedlings was monitored at a three-month interval by assessing DI. The DI was applied to the number of palm seedlings displaying disease symptoms such as chlorosis and foliage necrosis, including those lacking white fungal mass or fruiting bodies. The DI was expressed in conformity with the Equation (2) published by Idris *et al*. (2006):

$$\text{DI (\%)} = \left[\left(\frac{\text{Number of seedling infected}}{\text{Total number of seedlings assessed}} \right) \times 100 \right] \qquad (2)$$

The development of the disease was determined in accordance with Campbell and Madden (1990), utilising the area under the disease progressive curve (AUDPC) using Equation (3).

$$\text{AUDPC} = \sum_{i}^{n-1} \left[\left(\frac{Y_i - Y_{i+1}}{2} \right) (t_{i+1} - t_i) \right] \qquad (3)$$

where, n = assessment time number, Y = disease incidence (DI) and t = observation time.

The disease reduction (DR) was calculated based on the AUDPC using the Equation (4):

$$\text{DR (\%)} = \left[\frac{(\text{AUDPC control - AUDPC treatment})}{\text{AUDPC control}} \right] \times 100 \qquad (4)$$

In oil palm seedlings, the progression of BSR disease was assessed using the disease severity index (DSI). According to Kranz (1998), the DSI quantifies the extent of plant tissue disorders. The evaluation considered both external and internal indicators of disease severity in the seedlings. The DSI of foliar (DSIF) was calculated depending on foliar manifestation at a 0-4 scale (Nur Sabrina et al., 2012), 0 = Healthy; 1 = Yellowing of lower leaves and growth of a rhizomorph at base of bole; 2 = Necrosis of lower leaves and the appearance of button-like sporophore at the base of bole; 3 = >50% necrosis of leaves and creation of sporophore at the base of bole; and 4 = Total necrosis and production of sporophore at the base of bole. The *Ganoderma* selective medium (GSM) was used on oil palm tissue to confirm the presence of *Ganoderma* species, including *G. boninense*. However, GSM alone does not distinguish between different *Ganoderma* species. Additional molecular or morphological identification methods are required for species-level confirmation (Ariffin & Idris, 1992).

Oil palm seedlings were processed destructively by being cautiously planted, lengthwise cut and visually inspected for internal signs after the 12-month experimentation period where *G. boninense* PER 71 has artificially inoculated. The evaluation of the root DSI of root (DSIR) and the DSI of bole (DSIB) was done with minor adjustments from Nur Sabrina et al. (2012); 0 = Healthy; 1= Up to 20% decaying of bole or root tissues; 2 = From 20%-50% decaying of bole or root tissues; 3 = Over 50% decaying of bole or root tissues and 4 = Over 90% decaying of bole or root tissues. The following Equation (5) from Liu et al. (1995) was utilised for the assessment of DSIF, DSIB and DSIR.

$$\text{DSIF / DSIB / DSIR} = \left[\left(\frac{\text{Number of seedlings in the rating} \times \text{rating number}}{\text{Total number of seedlings assessed} \times \text{highest rating}} \right) \times 100 \right] \qquad (5)$$

Bait Seedling Trial in the Field

Field trials were conducted in plots identified as high-risk zones for BSR disease to meet the objectives of this investigation, fulfilling the requirements for selection as the study location. The study was conducted in a 25-year-old oil palm plantation area in Block 2A1 (3°49′09.0″N, 100°58′58.7″E) belonging to MPOB Research Station, Teluk Intan, Perak, Malaysia. The experimental method used in this study followed a completely randomised design (CRD) and involved two key procedures: (1) A field survey to identify and select oil palms severely infected with *G. boninense* based on visible disease symptoms, such as multiple spear leaves, trunk-leaf skirting, canopy collapse and the presence of fruiting bodies at the palm base; and (2) the planting of pre-treated oil palm seedlings around these infected palms or stumps to assess disease progression under natural field conditions *Figure 1b* (*Table 2*). Each procedure was repeated twice, with 27 seedlings per repetition. This method ensured that the study accurately represented real-world DI while evaluating the effects of Ca treatment on BSR suppression.

TABLE 2. CALCIUM (Ca) TREATMENT IN PLANTATION FIELD TRIAL

Treatment	Description
FT1	BF (positive control)
FT2	BF + 40.8 g of $CaSO_4$ (1,000 ppm of Ca^{2+}) per month for 12 kg of soil

Note: BF - basic fertiliser: Urea (N, 46%), monopotassium phosphate (MKP) (P_2O_5, 52%), muriate of potash (K_2O, 60%) and kieserite (MgO, 25%).

Pre-treatment was carried out at the MPOB Research Station, Seksyen 15, Bandar Baru Bangi, Selangor, Malaysia where six-month-old oil palm seedlings (*Dura* × *Pisifera*) were treated with Ca for 12 months, following the same procedure outlined in the nursery trial pre-treatment process. Subsequently, all the seedlings were transplanted in an oil palm plantation. The field trial lasted 21 months. Fertiliser application and regular operation were carried out according to conventional, approved field practices.

The seedling bait study was performed on a field with a high occurrence of BSR disease. Selection was made based on the severity of BSR infection on the palm. The baiting of seedlings begins by selecting oil palms affected by *G. boninense* based on visible physical signs, including multiple spear leaves, trunk-leaf skirting, a collapsing canopy and the presence of pathogenic fruiting bodies at the base of the palm. Oil palms severely infected by *G. boninense*, exhibiting three to four or more fruiting bodies, were chosen as the experimental palms (Sundram et al., 2014). The tissues collected from the fruiting body were placed directly onto GSM for confirmation of the disease.

The seedlings were planted next to the selected infected palm or stump, following the randomly chosen process. Each infected palm or stump represented a treatment with three pre-treated seedlings receiving the respective treatments. The seedlings were planted 0.4-0.6 m away from the infected palms or stump, and every planting hole had a depth of around 0.3-0.5 m, respectively. In the field trial, the infected palm or stump served as the natural inoculum, with seedlings planted at 0.4-0.6 m from the infected stump.

Pathological Parameters Assessment in Field Trial

The external disease assessment was executed in the same way as defined in the nursery trial. All observable symptoms and data of the seedlings were recorded at a three-month gap up to 21 months post-field planting.

Statistical Analysis

The details have been assessed statistically using SAS 9.2 software's analysis of variance (ANOVA). Separation of means was determined by the least significant difference (LSD) at a 5% confidence level.

RESULTS AND DISCUSSION

Nursery Trial

Severity of foliar symptoms (SFS) and disease incidence (DI). There was a significant difference in the SFS between the interaction of months and treatment application at $p \leq 0.05$. The NT2 seedlings showed the most severe foliar symptoms three months after inoculation and were significantly different from the other treatments (*Table 3*). The severity of foliar symptoms for the NT2 and NT3 seedlings became more pronounced as the disease progressed. Three months after inoculation, no foliar symptoms were observed in the NT3 seedlings, suggesting a potential delay in the progression of BSR disease. As expected, no signs of *G. boninense* were observed in the NT1 seedlings, as they were not inoculated. However, the SFS in NT2 seedlings progressively increased in number from three to 12 months after inoculation. At six months, foliar symptoms started to appear in NT3, but the symptoms were more pronounced in NT2. At nine months, the foliar symptoms in NT2 were higher than in NT3 and NT1. After 12 months of *Ganoderma* infection, the SFS for all treatments NT1, NT2 and NT3 seedlings was significantly different at $p \leq 0.05$. It is worth noting that the highest SFS observed during the experiment was identified 12 months after inoculation on NT2 seedlings compared to NT3 and NT1.

A significant difference in DI was observed between treatments and months at $p \leq 0.05$. In NT3 seedlings treated with Ca, the DI progressed significantly slower compared to NT2 seedlings (*Table 3*). As predicted, no indications of *G. boninense* could be observed in NT1 seedlings since they were not inoculated. A lower DI indicated disease suppression. No DI was detected for three months following inoculation with *G. boninense*. This finding proposed that the seedlings had partly suppressed BSR disease during NT3. However, the appearance of disease symptoms in NT3 was reported after six months of evaluation. At six and nine months, DI appeared more in NT2 than NT3 seedlings. The signs of disease in oil palm seedlings for NT3 rose steadily after 12 months of observation with a DI of 45.6%. These findings indicated that the

TABLE 3. SEVERITY OF FOLIAR SYMPTOMS (SFS) AND DISEASE INCIDENCE (DI) OF OIL PALM SEEDLINGS AT 12 MONTHS AFTER INFECTED WITH *G. boninense* PER 71

Months	SFS (%)			DI (%)		
	NT1	NT2	NT3	NT1	NT2	NT3
0 MAI	0.0^{a}	0.0^{a}	0.0^{a}	0.00^{a}	0.00^{a}	0.0^{a}
3 MAI	0.0^{b}	26.6^{a}	0.0^{b}	0.00^{b}	57.8^{a}	0.0^{b}
6 MAI	0.0^{c}	39.5^{a}	6.8^{b}	0.00^{c}	61.1^{a}	37.8^{b}
9 MAI	1.6^{c}	40.5^{a}	13.4^{b}	0.00^{c}	63.3^{a}	43.3^{b}
12 MAI	3.2^{c}	40.7^{a}	20.1^{b}	0.00^{c}	75.6^{a}	45.6^{b}

Note: MAI - months after inoculation; NT1 - negative control + non-inoculated; NT2 - positive control + inoculated with *G. boninense*; NT3 - Ca + inoculated with *G. boninense.* Means with the same letter in a row are not significantly different by using LSD ($p\leq0.05$).

addition of Ca to the NT3 seedlings resulted in an acceptable degree of disease suppression. Disease signs emerged even sooner in the NT2 seedlings after three months of *G. boninense* artificial infection. As predicted, the NT2 untreated control of oil palm seedlings gave the highest DI of 75.6% at 12 months of analysis. The NT2 seedlings showed a DI of 39.7% more than NT3 seedlings. Nonetheless, the damages in the roots and the bole area cannot be reported by foliar symptoms, although the symptoms were pronounced.

The area under disease progress curve (AUDPC) and disease reduction (DR). The AUDPC measures BSR disease severity in oil palms over time, helping compare treatment effectiveness or plant susceptibility (*Table 4*). A higher AUDPC value indicates a faster or more severe progression of BSR disease. Meanwhile, the lower AUDPC value suggests the efficacy of this procedure in disease suppression (Campbell & Madden, 1990). NT3 seedlings exhibited a slightly lower AUDPC (311.67 unit^2) following Ca treatment in the nursery, 12 months after artificial infection with *G. boninense*. However, NT2 had the highest AUDPC of 660.00 unit^2. In NT2 seedlings, the highest AUDPC value showed increased sensitivity to the disease. In contrast, the AUDPC indicated that the NT3 treatment was the most effective in slowing down BSR. The DR in oil palm seedlings was estimated at 52.8%.

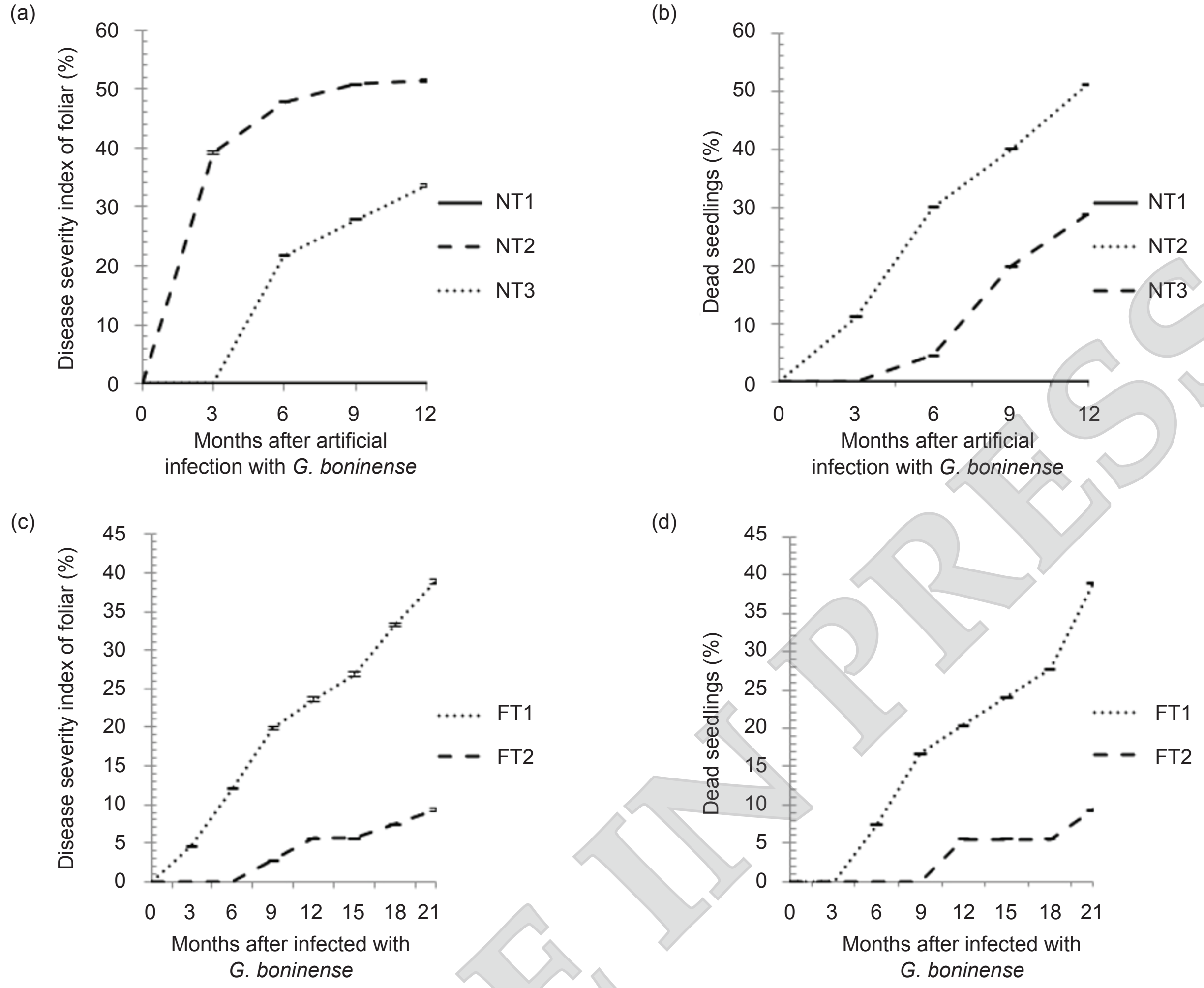
TABLE 4. THE AREA UNDER DISEASE PROGRESS CURVE (AUDPC) AND DISEASE REDUCTION (DR) OF OIL PALM SEEDLINGS AT 12 MONTHS AFTER ARTIFICIALLY INFECTED WITH *G. boninense* PER 71

Treatment	NT1	NT2	NT3
AUDPC (unit^2)	0.00	660.00	311.67
DR (%)	-	-	52.8

Note: NT1 - negative control + non-inoculated; NT2 - positive control + inoculated with *G. boninense*; NT3 - Ca + inoculated with *G. boninense.*

Disease severity index of foliar (DSIF). The DSIF between months and the interaction of treatment application at $p\leq0.05$ was significantly different. Nursery results showed that seedlings treated with Ca (NT3) remained symptom-free, with 0.0% infection observed three months after inoculation with *G. boninense*. In contrast, NT2 seedlings were affected by the pathogen, with a DSI of up to 39.2% (*Figure 2a*). Between six and nine months after inoculation, NT2 seedlings exhibited the highest DSIF values (47.8% and 50.8%), while NT3 seedlings showed the lowest DSIF values (21.7% and 27.8%). A lower DSIF indicates that the emergence of BSR infection diseases in oil palm seedlings has been successfully suppressed. At 12 months, the DSIF percentage observed for NT2 was 34.6% higher than the NT3 seedlings.

Disease severity index of root (DSIR) and bole (DSIB). The *G. boninense* infection starts by attaching the filamentous cells of hyphae to the root surface. Once the fungus penetrates the root tissues, it spreads along the roots and eventually reaches the boles. Thus, the destructive sample was carried out at the final phase of the experiment to evaluate how much internal damage was induced in the root and bole regions. Our findings indicate that the NT1, NT2 and NT3 seedlings in the DSIR and DSIB procedure are different *(Table 5*). For NT2, the oil palm seedlings showed the highest root rot rate and this led to an elevated level of DSIR with brown discoloration of 52.2% of the primary roots compared to 33.6% of NT3 seedlings. In the meantime, the measurement of bole degradation with respect to DI shows a substantial change from the DSIB of 49.4% showed by the NT2 seedlings with NT3 of oil palm seedlings with an addition of Ca with the lowest DSIB of 25.8% (*Table 5*). A brown lesion with an irregular and dark zone will distinguish the longitudinal portions of the infected bole. The findings indicate that NT3 treatment minimised the infiltration and proliferation of *G. boninense* to the seedlings' vascular system.



Note: NT1 - negative control + non-inoculated; NT2 - positive control + inoculated with *G. boninense*; NT3 - Ca^+ inoculated with *G. boninense*; FT1 - positive control + infected with *G. boninense*; FT2 - Ca^+ infected with *G. boninense.*

Figure 2. Disease severity and dead oil palm seedlings under (a,b) nursery and (c,d) field conditions following G. boninense *infection. Data are means ± standard error using LSD* (p<0.05). *Error bars indicate LSD levels between treatments.*

TABLE 5. DISEASE SEVERITY INDEX OF ROOT (DSIR) AND BOLE (DSIB) OF OIL PALM SEEDLINGS AT 12 MONTHS AFTER ARTIFICIALLY INFECTED WITH *G. boninense* PER 71

Treatment	DSIR (%)	DSIB (%)
NT1	0.0[c]	0.00[c]
NT2	52.2[a]	49.4[a]
NT3	33.6[b]	25.8[b]

Note: NT1 - negative + non-inoculated; NT2 - positive control + inoculated with *G. boninense;* NT3 Ca + inoculated with *G. boninense*. Means with the same letter in a column are not statistically different using ($p \leq 0.05$).

Dead seedlings (DS). There was an interaction in the DS between months and treatment applications during the study's duration at $p \leq 0.05$. Treatment seedlings NT3 were healthy at three months since inoculation with *G. boninense*, while NT2 (positive control) revealed a substantial 11.1% rate of death (*Figure 2b*). No signs of mortality can be found in NT1 (absolute control) seedlings, as expected, since they were not inoculated. After 12 months of inoculation, treatment NT3 seedlings exhibited the lowest DS of 28.9%, followed by treatment NT2 seedlings (51.1%) with a significant difference between the treatments. Thus, the application of Ca was considered useful in suppressing BSR disease since the DS of treatment NT3 oil palm seedlings was significantly reduced.

The current study examined Ca's effect when challenged with *G. boninense* PER 71 in oil palm seedlings, BSR's causal agent. SFS, DI, AUDPC, DSIF, DSIR, DSIB and DS with greater value of DR in the seedlings cured with 1,000 ppm of Ca (NT3) compared to NT2 (positive control) has shown that improvements in nutrients by soil fertilisation is vital for handling *Ganoderma* disease in oil palm seedlings. Balanced nutrition plays a significant role in enhancing plant resistance or susceptibility to diseases (Sariah & Zakaria, 2000). The nursery trial demonstrated significant reductions in DI and DSI with Ca supplementation. For instance, the DI reduction in this study (45.6%) aligns with findings by Nur Sabrina et al. (2012), who reported a 71.7% decrease in BSR severity in seedlings supplemented with Ca and copper (Cu). Additionally, the AUDPC values in this study are comparable to those observed in treatments incorporating integrated nutrient management strategies (Raut et al., 2022). These results underscore Ca's vital role in improving disease resistance and plant health.

Calcium (Ca) is vital in plants. The structure of cells and the physical barriers that protect against pathogens are supported by cell walls and membranes. Ca-deficient plants have weaker barriers, making them more sensitive to pathogens (Dayod et al., 2010). Calcium (Ca) is beneficial for plants' resistance against BSR disease in external applications in soil fertiliser. According to the nursery trial results, the application of 1,000 ppm Ca (equivalent to 60.4 g of $CaSO_4$) in soil showed promising potential in suppressing BSR disease. However, since this was the only concentration tested in this study, further analysis is needed to determine whether other Ca levels may provide similar or better effects. Future studies should explore a range of Ca concentrations to validate and optimise its effectiveness in disease suppression. Consistent with the findings of this study, Sariah et al. (1997) showed that adding 1.425 g of Ca (equivalent to 7.5 g of $Ca[NO_3]_2$) to soil one month before inoculating with *Ganoderma*-infected RWB reduced foliar symptoms, root lesions, and bole infections in oil palm seedlings. The study emphasised that Ca strengthened the seedlings by forming Ca pectate, which fortified cell walls and blocked pathogen enzymes. These findings highlight the vital role of Ca in enhancing structural defense against *Ganoderma* infection.

Interestingly, this study indicated that the rate of 1,000 ppm of Ca within a 12-month period was found to be the best supplementation treatment to slow the onset of BSR diseases incidence in nursery trial. The analysis findings suggested that the mechanism involved may be reinforcing the cell wall, which is the first line of plant protection by protecting against pathogen penetration by the lignification process (Bhuiyan et al., 2009). Ca supplementation is essential for strengthening plant defenses by promoting the lignification process. Lignification involves the production of lignin, a key component that reinforces cell walls (Miedes et al., 2014). Ca enhances the activity of two important enzymes, POD and laccase, which are responsible for lignin formation and deposition in cell walls, leading to increased lignin levels (Wang et al., 2013). Lignin acts as a protective shield, strengthening cell walls and preventing cellulose fibres from breaking down or being damaged by chemicals or pathogens (Bhuiyan et al., 2009). This strong barrier slows down pathogen penetration, making it more difficult for fungi like *Ganoderma* to invade plant tissues (Miedes et al., 2014). Ca also indirectly reduces *Ganoderma* attachment to plant roots. Improving soil structure creates an environment less suitable for pathogen growth and spread (Larkin, 2015). Additionally, Ca supports cell membrane integrity, helping plants maintain their natural defenses (Nur Sabrina et al., 2012). Ca supplementation enhances lignin production, limits pathogen attachment and supports soil and cell health, playing a vital role in protecting plants from *Ganoderma* infections.

Numerous studies have demonstrated that exogenous Ca treatments significantly influence secondary metabolic synthesis in plants, particularly lignin formation. For instance, exposure to $CaCl_2$ in pear (*Pyrus* spp. L.) leaves activated key genes involved in secondary metabolic pathways. Similarly, exogenous Ca modulated the phenylalanine pathway in the inflorescence stems of peonies (*Paeonia lactiflora* P.), promoted stem thickness in roses (*Rosa* spp.), and enhanced stem rigidity in chrysanthemums (*Chrysanthemum morifolium* R.) (Etemadi et al., 2012; Lee & Nam, 2011; Zhao et al., 2019). These effects are likely linked to lignin development and the cross-linking of pectin molecules, resulting in thicker cell walls due to compositional changes. However, while Ca is thought to influence lignification, the exact mechanism by which exogenous Ca affects lignin production remains unclear. Field studies are therefore essential to investigate the efficacy of Ca supplementation in managing *G. boninense* infections, particularly in vulnerable soil areas. Further examination will provide valuable insights into the potential of Ca treatments in combating *Ganoderma*-related diseases under field conditions.

Field Trial

Severity of foliar symptoms (SFS) and disease incidence (DI). In the bait seedling test in the field, there was a significant interaction between months and treatment application in the SFS at $p \leq 0.05$. The SFS for FT1 and FT2 seedlings became more pronounced as the seedlings became older. Treatment with FT1 had more serious foliar effects three months after being infected with *G. boninense* than FT2 seedlings (*Table 6*). This result suggests the ability of FT2 to slow down the development of BSR disease. However, the SFS in the treatment of FT1 seedlings progressively increased in number three to 21 months after being infected. At 21 months after *Ganoderma* infection, the SFS for FT1 and FT2 seedlings was significantly different at $p \leq 0.05$. It is important to note that the highest SFS was observed at 21 months after infection on FT1 seedlings treatment during the experiment.

A similar trend was recorded in the DI of oil palm seedlings after being infected with *G. boninense* (*Table 6*). In the DI, at $p \leq 0.05$, there was an interaction between treatment and months. The DI based on SFS increased more slowly in FT2 seedlings treated with Ca than in FT1 seedlings. As expected, no DI was observed in FT2 seedlings at three and six months after infection with *G. boninense*, indicating partial inhibition of BSR disease. However, disease symptoms began to appear in FT2 seedlings at nine months. At 21 months, the DI reached 9.3%, showing that disease symptoms in the oil palm seedlings were increasing. This suggests that adding Ca to FT2 seedlings provided effective disease suppression. In contrast, FT1 seedlings showed disease symptoms slightly earlier, just three months after *G. boninense* infection. The highest DI of 38.9% was recorded in FT1-treated oil palm seedlings after 21 months of evaluation. The DI in FT1 seedlings was 76.1% higher than in FT2 seedlings. However, root and bole damage could not be fully assessed based on foliar symptoms alone, despite the visible severity of external signs.

TABLE 6. BASAL STEM ROT (BSR) SEVERITY OF FOLIAR SYMPTOMS (SFS) AND DISEASE INCIDENCE (DI) IN OIL PALM SEEDLINGS 21 MONTHS AFTER INFECTION WITH *G. boninense*

Months	SFS (%)		DI (%)	
	FT1	FT2	FT1	FT2
0 MAI	3.2^{a}	6.1^{b}	0.0^{a}	0.0^{a}
3 MAI	23.2^{a}	16.4^{b}	7.4^{a}	0.0^{b}
6 MAI	27.4^{a}	14.5^{b}	16.7^{a}	0.0^{b}
9 MAI	32.5^{a}	15.7^{b}	24.1^{a}	5.6^{b}
12 MAI	39.0^{a}	19.9^{b}	22.6^{a}	5.6^{b}
15 MAI	40.2^{a}	19.9^{b}	32.7^{a}	5.6^{b}
18 MAI	42.8^{a}	19.1^{b}	38.9^{a}	9.3^{b}
21 MAI	48.3^{a}	21.4^{b}	38.9^{a}	9.3^{b}

Note: MAI - months after infected; FT1 - positive control + infected with *G. boninense;* FT2 - Ca^{+} infected with *G. boninense.* Means with the same letter in a row are not significantly different by using LSD ($p \leq 0.05$).

The area under disease progress curve (AUDPC) and disease reduction (DR). In the study of the bait seedling trial in the field, FT2 seedlings exhibited significantly lower (91.71 unit^2) AUDPC after being treated with Ca at 21 months after being infected with *G. boninense* (*Table 7*). However, the positive control seedlings (FT1) had the highest AUDPC of 486.11 unit^2. FT1 oil palm seedlings showed the highest AUDPC value, indicating increased susceptibility to the disease. It was also obvious from the AUDPC that FT2 was the most appropriate treatment for the slowdown of BSR. As predicted, 81.1% of palm seedlings experienced DR.

TABLE 7. AREA UNDER THE DISEASE PROGRESS CURVE (AUDPC) AND DISEASE REDUCTION (DR) IN OIL PALM SEEDLINGS 21 MONTHS AFTER INFECTION WITH *G. boninense*

Treatment	FT1	FT2
AUDPC (unit^2)	486.11	91.71
DR (%)	-	81.1

Note: FT1 - positive control + infected with *G. boninense*; FT2 - Ca + infected with *G. boninense.*

Disease severity index of foliar (DSIF). There was an interaction in the DSIF between months and treatment application interactions at $p \leq 0.05$. The oil palm's DSI evaluated based on external foliar signs decreased in FT2 seedlings (*Figure 2c*). Lower DSIF has shown that the growth of BSR infection disease in the palm seedlings has been successfully suppressed. In contrast with the FT1 seedlings, the DSIF percentage observed during treatment was lower. As expected, no DSIF was found in FT2 seedlings at three months of observation. However, exterior disease symptoms developed far sooner in FT1 seedlings at three months of evaluation. The DSIF of FT2 seedlings only appeared after six months of planting in the field. Subsequently, the disease recurrence in oil palm seedlings of FT1 and FT2 rapidly progressed, with a DSIF of 38.9% and 9.3%, respectively, at 21 months of observation. At 21 months, the DSIF in FT1 seedlings showed 76.2% higher than in FT2 seedlings. This finding indicated that the supplementation of Ca would accomplish a successful BSR elimination.

Dead seedlings (DS). There was a significant difference in DS between months and treatment application interactions at $p \leq 0.05$ (*Figure 2d*). All treated FT2 seedlings were alive at six and eight months after planting in the field, while FT1 showed 7.4% and 16.7%, death incidence respectively. DS were found in FT2 just nine months after field planting. At 21 months after planting, dead incidense FT1 seedlings was 76.1% more than that of FT2 seedlings. All seedlings were evaluated based on the SFS (*Figure 3*) and development of the fruiting body in the field trial only and the occurrence of white mycelia and basidiomata development was minimal.

In addition to *G. boninense* inoculation, continual Ca supplementation may improve oil palm disease

Figure 3. Bait seedlings approach in the field: (a, b) Ca-treated seedlings with no apparent symptoms of basal stem rot disease at 21 months after planting, (c, d) chlorotic leaves due to G. boninense *infection, and (e, f) dead seedlings due to* G. boninense *infection.*

resistance. This study showed that seedlings treated with 1,000 ppm of Ca had lower SFS, DI, AUDPC, DSIF, and DS with more DR (FT2) than the positive control (FT1), suggesting that nutrients helped former seedlings achieve *G. boninense*-induced physical damage tolerance. In the field trial, Ca-treated seedlings showed an 81.1% reduction in DI, significantly higher than the 52.8% reduction observed in the nursery trial. This aligns with findings by Zakaria et al. (2023), suggesting the field environment may enhance the effects of Ca supplementation due to improved soil interactions and microbial activity. However, this improvement highlights the need for further exploration into optimising nutrient delivery systems in different planting conditions.

Calcium (Ca) addition in soil fertilisation is the best strategy for managing BSR outbreaks within oil palm seedlings (Sariah & Zakaria, 2000). Nur Sabrina et al. (2012) found that BSR disease was 71.7% lower in seedlings supplemented with Ca and Cu six months after *Ganoderma*-challenged inoculation. While our initial investigation suggests that prolonged Ca supplementation with other soil fertilisers may help delay *G. boninense*-induced BSR disease, further finding is needed to confirm its specific effects on soil structure, pH, and nutrient availability. The nutrient application can trigger disease level conditions by influencing, (1) plant micro-climate, which may affect pathogenic outbreaks and sporulation pathogen; (2) tissue, cell walls, and biochemical structure; (3) seedling growth rate, which may help them avoid infections at their most vulnerable point and (4) soil conditions, which affect pathogen behaviour and spread (Larkin, 2015). Fertilisation changes plant structure, morphology, growth patterns and chemical characteristics, affecting disease.

According to this study, Ca and other macro-nutrients may decrease BSR disease in oil palm seedlings. Zakaria et al. (2023) found that N fertiliser reduced BSR disease in oil palms in certain soil conditions. By improving oil palm physiological resilience, P fertilisation reduces BSR disease (Hendarjanti & Sukorini, 2022). For K fertilisation, it thickens oil palm epidermal cell walls, reducing pathogen infection (Sahebi et al., 2018). Micro-nutrients may enhance oil palm BSR disease control. A recent study indicated that BSR disease suppression was better with the double combination (boron and manganese; Cu and manganese) (Tengoua, 2014). Thus, controlling oil palm seedling nutrition delivery is crucial.

Calcium (Ca) is a structural component of plant cell walls essential to their integrity and function. The investigation showed that 1,000 ppm Ca in $CaSO_4$ was substantially linked with the initial stage of lignification in oil palm seedling roots as supported by studies like Miedes et al. (2014), Nur Sabrina et al. (2012) and Wang et al. (2013) but further biochemical or histological analysis is needed to confirm lignification involvement. Oil palm tissues with high Ca accumulation effectively withstand *G. boninense* disease. This study employed two theories to explain how Ca gives oil palm seedlings resistance. The first hypothesis is that Ca acts as a physical barrier to fungal invasion. This study suggests that root epidermal cell wall lignification protects oil palm seedlings from *G. boninense*. Shuhada et al. (2016) found that insufficient Ca nutrients in oil palm plantations altered the lignin composition and defense mechanism and favoured pathogen invasion. Bivi et al. (2016) found that extra Ca, Cu and SA in oil palm seedlings thicken root secondary cell walls and middle lamella, facilitating cell wall lignification. The altered cell wall tissues of treated seedlings may also explain why they are more resistant to *G. boninense* pathogen penetration and less vulnerable to cell wall-destroying enzymes (Wang et al., 2013). Other investigations found that lignification limits fungus growth and proliferation (Bhuiyan et al., 2009).

The second hypothesis suggested that Ca supplementation suppressed BSR disease by activating and accumulating defense mechanisms, such as lignin enzymes, POD, laccase and physical barrier changes, ending in lignin formation. Nur Sabrina et al. (2012) found that H_2O_2, POD and laccases in oil palm seedling roots treated with Ca and Cu boost this plant defense mechanism. POD may be accountable for the substantial amounts of reactive oxygen species alerting plant cells and creating a harmful pathogen environment. POD also participates in lignin and suberin depositions that strengthen oil palm root cell walls by mechanically blocking pathogens. Thus, this study showed that Ca is a structural component of plant cell walls essential to their integrity and function.

Pathological investigations of nursery and field trials revealed that Ca fertiliser suppressed BSR, with control seedlings being the most affected. Both results showed that seedlings treated with Ca fertiliser had the lowest BSR incidence in the nursery and field trials, 45.6% and 9.3%, resulting in significant DR of 52.8% and 81.1%. Oil palm seedlings in the field trial had 34.9% higher DR than those in the nursery trial. The nursery had a lower DR than the field experiments due to polybag space constraints, which hindered oil palm seedling growth.

This study aligns with Malaysia's National Agrofood Policy, which emphasises sustainable agricultural practices and contributes to Sustainable Development Goals (SDGs) 2 (Zero Hunger) and 12 (Responsible Consumption and Production) (Rahmann et al., 2021). By reducing the incidence of BSR disease through effective Ca-based nutrient management, the study enhances crop yield and resilience, directly supporting food security while minimising chemical inputs. The findings suggest that Ca supplementation could play a transformative role in ensuring the long-term sustainability of the oil palm industry by enhancing yield stability and reducing reliance on less sustainable chemical controls. This aligns with global market demands for environmentally friendly practices and ensures the industry's economic viability. Alternative methods of Ca delivery, such as foliar applications or enhanced bioavailability soil amendments, offer targeted and environmentally conscious approaches to nutrient application, potentially reducing wastage. Furthermore, integrating biofertilisers with Ca supplementation could amplify disease suppression through improved soil microbial diversity and root health, offering a synergistic approach to managing *G. boninense*. Future analysis should explore the compatibility and efficacy of such integrated strategies to further enhance the oil palm industry's resilience and sustainability (Rahmann et al., 2021).

The "No Palm Oil" movement aims to ban palm oil due to concerns over deforestation, biodiversity loss and carbon emissions. However, replacing palm oil with alternative vegetable oils like soybean or sunflower would require significantly more land, potentially worsening environmental damage. Instead of banning palm oil, promoting sustainable practices is a better solution. This study highlights how nutrient management, particularly Ca supplementation, can improve oil palm health and reduce DI (BSR), leading to more sustainable production. By integrating better agronomic practices, such as disease-resistant planting materials, balanced fertilisation and responsible land management, the industry can improve yields without expanding plantation areas. Rather than banning palm oil, global efforts should focus on supporting certified sustainable palm oil (CSPO), improving plantation management and investing in eco-friendly disease control strategies like those explored in this study. Sustainable production, rather than elimination, is the key to balancing economic, environmental and social needs in the palm oil industry.

The findings of this study contribute significantly to advancing Sustainable Development Goal 13 (Climate Action). By enhancing disease resistance in oil palm through Ca nutrient supplementation, this approach promotes improved crop productivity and reduces dependency on synthetic fungicides, aligning with environmentally responsible agricultural practices. Furthermore, minimising the impact of *G. boninense* through nutrient-based strategies supports SDG 13 (Climate Action) by reducing the carbon footprint associated with replanting and disease-related losses.

In the context of local and international policies, this study aligns with Malaysia's commitment to sustainable palm oil production through compliance with the Malaysian Sustainable Palm

Oil (MSPO) and Roundtable on Sustainable Palm Oil (RSPO) certification standards. Both schemes emphasise environmentally sound, socially responsible and economically viable practices. The integration of nutrient enhancement techniques, such as Ca application demonstrated in this study, can support planters in meeting MSPO and RSPO principles related to soil and crop health management, continuous improvement and sustainable productivity. As Malaysia strengthens its stance in the global palm oil market, such research provides practical and policy-relevant contributions to maintaining the sustainability and reputation of the oil palm industry.

CONCLUSION

This study further demonstrated that the addition of 1,000 ppm Ca to soil nutrients effectively reduced and delayed BSR disease progression, with control seedlings exhibiting the most severe symptoms. The findings indicate that 1,000 ppm Ca was the most effective treatment in suppressing BSR disease in both nursery and field trials, as evidenced by lower values of SFS, DI, AUDPC, DR, DSIF, DSIB, DSIR and DS. It is proposed that Ca fertiliser may help manage BSR disease in oil palms by shifting physiological components, including plant defense mechanisms. *Ganoderma* infection decreased due to the tolerance of the treated seedlings. Considering the positive outcomes of this study, it is imperative to investigate the impact of Ca on lignin synthesis to mitigate BSR disease in oil palm seedlings.

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