

Nursery and Field Evaluation of *Streptomyces nigrogriseolus* GanoSA1 to Control Basal Stem Rot in Oil Palm Seedlings

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Basal stem rot (BSR) disease caused by Ganoderma species is a threat to the oil palm industry. In our initial study, rhizosphere actinomycetes identified as Streptomyces nigrogriseolus GanoSA1 (Streptomyces GanoSA1) possess competent biological control activity in the growth of Ganoderma in vitro. This study was carried out to evaluate whether Streptomyces GanoSA1 formulated in the vermiculite-bio charcoal powder can reduce disease incidence caused by G. boninense PER71, and promote oil palm growth through nursery and field trial. Mixing of Streptomyces GanoSA1 powder at 10⁸ CFU (colony-forming unit) per gramme in soil resulted in the strain establishment in the applied soil and increased oil palm seedlings height with no observed adverse effect on seedlings growth. The seedlings treated with the powder formulation resulted in a reduced percentage of disease incidence (DI, %) by 51.1 per cent and disease severity index (DSI, %) by 35.0 per cent compared to untreated seedlings and seedlings inoculated with G. boninense PER71 alone (93.3% DI and 75.83 % DSI, respectively). The field trial indicated that, after 36 months of planting, only 6.6 per cent of oil palm treated with the Streptomyces GanoSA1 powder showed symptoms of BSR disease and death due to Ganoderma infection compared to the untreated oil palm at 75.0 per cent. These trials highlight the potential of the Streptomyces GanoSA1 powder to reduce BSR disease in oil palm and promote oil palm growth.

Keywords: Streptomyces, Ganoderma, artificial inoculation, seedling baiting technique.

Basal stem rot (BSR) disease (Figure 1) caused by *Ganoderma* species is a serious threat to oil palm industries in Southeast Asia (SEA), especially in Malaysia and Indonesia. Significant economic losses resulted from this devastating disease (Kushairi *et al.*, 2019) through to reduction of yield production. In 2017, the incidence of BSR disease in Malaysia was 7.4 per cent with 221 000 hectares (ha) affected (Idris, 2019). Singh (1991) reported a reduction of fresh fruit bunch (FFB) yield around 26-46 per cent resulted from 31 per cent to 67 per cent of disease incidence. Assis *et al.* (2016) estimated potential yield would

decrease by 43.32 per cent due to the disease. Extensive effort to control BSR disease was carried out in Malaysia and Indonesia. Many strategies have been recommended and applied to control BSR disease in oil palm, as well as to sustain the economic lifespan. These strategies include cultural practices, sanitation, chemical fungicides and biological control (Idris *et al.*, 2016). One of the methods used is the application of chemical fungicide such as hexaconazole (Idris *et al.*, 2004). However, due to environmental concerns, an alternative to chemical fungicides should be considered. Integrated *Ganoderma* Management (IGM)

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Figure 1 Oil palm planted in the field; a) mature and healthy, b) mature and showing symptoms of *Ganoderma* infection, c) fruiting bodies of *Ganoderma* as observed on the trunk of mature oil palm, d) rotting oil palm bole due to *Ganoderma* infection, e) immature and healthy, and f) immature and showing symptoms of *Ganoderma* infection

which incorporates the biological control using microbial antagonists has been introduced to tackle the disease (Idris, 2019). Adoption of biological control is a green technology approach for management of plant disease. It offers safer management strategy and contributes to the enrichment of biodiversity in environmental friendly manners. Exposure of antagonist microorganism to rhizosphere area will manipulate the soil's indigenous microorganism communities, leading to the suppression of soil-borne pathogens and interference of survivability or disease-producing activities by the phytopathogens. It was reported that many species of beneficial microorganisms including actinomycetes, bacteria and fungi can effectively suppress plant diseases. The use of actinomycetes as biological control agents of soil-borne root disease is of interest due to its characteristic and vast potential. The actinomycetes, especially *Streptomyces*, are prolific producers of secondary metabolites, and are being used as a biological control agent in controlling soil-borne diseases in plants (Mun *et al.*, 2020; Oubaha *et al.*, 2019; Wonglom *et al.*, 2019; Dias *et al.*, 2017; Alekhya & Gopalakrishnan, 2017; Chen *et al.*, 2016). Several studies showed that actinomycetes were able to inhibit the growth of *Ganoderma* *in vitro* (Azura *et al.*, 2016; Shariffah-Muzaimah *et al.*, 2015; Tan *et al.*, 2002) and *in vivo* (Shariffah-Muzaimah *et al.*, 2018). This paper reports a study conducted to evaluate the potential of vermiculite-bio charcoal powder containing *Streptomyces* GanoSA1 in reducing *Ganoderma* infection of oil palm in nursery and field trials.

MATERIALS AND METHODS

Sampling, *in vitro* screening and identification of potential biocontrol agent were performed

as a separate experiment (Shariffah-Muzaimah *et al.*, 2020) and were not discussed in this paper. *Streptomyces* GanoSA1 was isolated from the rhizosphere area of healthy oil palm, surrounded by infected oil palm. This strain showed strong antagonistic inhibition activity against *G. boninense* PER71.

Effects of various powder carriers on *Streptomyces* GanoSA1 viability and activity against *G. boninense* PER71

In this experiment, the effects of other types of carriers such as rice bran (RB), rice husk charcoal (RHC), charcoal (C) and empty fruit bunch (EFB) as a combination to vermiculite (V) in the viability of *Streptomyces* GanoSA1 and antagonistic activity against *G. boninense* PER71 *in vitro* were studied. All of the said carrier was added individually to vermiculite at 1:1 ratio and sterilised at 121°C for 30 minutes for two consecutive days.

Spore and mycelial suspensions of *Streptomyces* GanoSA1 were maintained at -80°C in 20 per cent (v/v) glycerol. Working cultures were obtained by streaking one loopful of the stock suspension onto a yeast extract-malt extract (YME) agar and incubated at 28°C until a well-sporulated mature colony was obtained. The preparation of *Streptomyces* GanoSA1 inoculum and powder formulation using vermiculite was as previously reported (Shariffah-Muzaimah *et al.*, 2012). The viability of *Streptomyces* GanoSA1 in each substrate mixture was evaluated at monthly intervals over a 12-month period and expressed as colony-forming unit per gramme (CFU/g) based on the standard dilution plate count method. The *in vitro* efficacy test of *Streptomyces* GanoSA1 in each substrate mixture were assessed by adding 0.1 g of formulation to the centre of a potato dextrose agar (PDA) plate

and challenged with *G. boninense* PER71 (Sabaratnam & Traquair, 2002). The powder carrier with the longest viability of *Streptomyces* GanoSA1 and highest percentage inhibition of radial growth (PIRG) was selected for nursery assessment in oil palm seedlings.

Nursery trials

Application of Streptomyces GanoSA1 and inoculation of G. boninense PER71

The nursery trials to study the biocontrol effects of *Streptomyces* GanoSA1 powder in reducing *Ganoderma* disease in oil palm were carried out under glasshouse conditions in a shaded nursery with 30 per cent light absorbance. Seedlings were artificially infected with *G. boninense* PER71 through an artificial inoculation using 2-month-old *G. boninense* PER71 inoculum cultured on rubberwood block (RWB). The preparation of *G. boninense* PER71 inoculum on RWB and artificial inoculation technique was done as described by Idris *et al.* (2006). Fifty-gramme of the *Streptomyces* GanoSA1 powder at 10^8 CFU per gramme were mixed in the soil. Another 50 g of the powder was mixed in soil with the artificial inoculation of *G. boninense* PER71 using the RWB sitting technique 14 days after the application of the first biological control agent (BCA). All seedlings used in this trial were maintained in the nursery according to standard nursery practices (Esnan *et al.*, 2001). All the polybags containing the seedlings were arranged in a completely randomised design (CRD) in each treatment with three replicates. Each replicate contained six seedlings. The experiment was repeated twice in the same condition.

Disease assessments

Data on external symptoms of BSR disease

were taken on a monthly basis for 8 months, which includes the number of leaves produced (number of green, yellow or wilting) and the appearance of *Ganoderma* either in mycelia, white button or basidiocarps form. The assessments on the effect of BCA were measured quantitatively as Disease Incidence (DI), Severity of Foliar Symptom (SFS, %) and Disease Severity of Foliar Index (DSFI). The percentage of SFS and DI were obtained using the formula described by Sariah and Zakaria (2000) and Madden and Campbell (1990). Whereas for DSFI, seedlings were classified using an index scoring ranging from 0 to 4 based on the severity as described by Tengoua *et al.*, (2014) and calculated based on the disease class values using the following formula by Tengoua *et al.* (2014);

$$DSI = \frac{\text{number of seedlings in rating} \times \text{rating scale} \times 100}{\text{total number of seedlings assessed} \times \text{highest rating scale}}$$

A disease progression curve was developed based on the DSFI data. The Area under Disease Progression Curve (AUDPC) was calculated to indicate the disease progression in each treatment over time and calculated by the formula (Campbell & Madden, 1990);

$$AUDPC = \sum_{i=1}^{n-1} \frac{(Y_i + Y_{i+1})}{2} (t_{i+1} - t_i)$$

where, n is the number of assessment time, Y is the disease incidence or severity of foliar symptoms and t is the observation time. Lower AUDPC value shows the effectiveness of the treatment. Disease reduction caused by *Streptomyces* GanoSA1 powder was determined by comparing the AUDPC value based on the formula (Madden & Campbell, 1990):

$$DR = \frac{AUDPC_{\text{control}} - AUDPC_{\text{treatment}}}{AUDPC_{\text{control}}} \times 100$$

All percentage data (DI, SFS and DS) values were transformed by arcsine

transformed (Gomes & Gomes, 1984), analysis of the data was done using ANOVA, and the means were compared using least significant difference (LSD) at $P \leq 0.05$. The presence of *Ganoderma* was confirmed by re-isolating the fruiting body or infected tissues on freshly prepared *Ganoderma* Selective Medium (GSM) (Ariffin & Idris, 1991).

Internal disease severity

At the end of the experiment, the boles of each seedling were dissected into two longitudinal cuts to observe for BSR internal symptoms. The internal DS caused by *G. boninense* PER71 infection were visually observed based on the proportion of decayed bole tissue. The measurement of the rotted area was assessed by using a grid and the percentage of the rotted area was calculated as:

$$\text{Rotted area (\%)} = \frac{\text{rotted area}}{\text{total area}} \times 100$$

The disease class was determined according to the scale described by Breton *et al.* (2006) with modifications, 0=Healthy, no internal rot; 1=20 per cent rotting of bole tissues; 2=20 to 50 per cent rotting of bole tissues; 3=>50 per cent rotting of bole tissues, and 4=>90 per cent rotting of bole tissues. The value of disease severity of bole index (DSBI) was calculated by using the DS formula as described earlier.

Field trials

The efficacy of *Streptomyces* GanoSA1 powder on BSR disease incidence and severity of foliar symptoms were evaluated in a 25-year-old oil palm field located in Teluk Intan, Perak, Malaysia. The selected field were reported to be severely infected with *Ganoderma* and the soil type was classified as shallow peat (Tayeb, 2005).

Selection of experimental plot

The selection of experimental plot started with the selection of *Ganoderma* infected palm obtained through a ground survey. The ground survey was carried out by referring to the method described in Idris *et al.* (2016). Palms with observable external symptoms of *Ganoderma* and having more than three fruiting bodies at the base area were chosen as the experimental palm (Sundram *et al.*, 2015). Confirmation of *Ganoderma* infection was done by plating of the fruiting bodies on *Ganoderma* Selective Medium (GSM) (Ariffin & Idris, 1991).

Two types of treatments were applied; seedlings were either treated or not-treated with *Streptomyces* GanoSA1 powder. Each treatment consisted of 60 experimental palm planted with one pretreated seedling per palm respectively.

Preparation of seedlings and application of *Streptomyces* GanoSA1 powder

Oil palm seedlings with *Streptomyces* GanoSA1 treatment were treated with *Streptomyces* GanoSA1 powder at 4, 6 and 9 months-old through soil mixing. Seedlings were maintained in a nursery for 12 months before being planted in the planting hole in the field study. Two-hundred and fifty grammes of *Streptomyces* GanoSA1 powder was applied into a planting hole (size; 45 x 45 x 60 cm) approximately 35 cm away from the infected stump. The pretreated palms were planted in the planting hole acting as bait. Meanwhile, the infected palms represented natural *Ganoderma* inoculum. Fertiliser application and general field maintenance were done using conventional cultural and pest management practices for oil palm (Esnan *et al.*, 2001).

Disease assessment

Data collections were carried out at 3-monthly intervals for a period of 36 months. The assessment was done based on the observation of disease symptoms and the presence or absence of *Ganoderma*. The dead seedlings percentage for each treatment was calculated using the following formula (Campbell & Madden, 1990);

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

Estimation of *Streptomyces* GanoSA1 in soil

The recovery of *Streptomyces* GanoSA1 was quantified using the serial dilution-spread plate technique. The determination of *Streptomyces* GanoSA1 was done according to Naher *et al.* (2012) with modifications. Soil samples from seedlings in both, nursery and field application were collected at 15 cm depth from the surface at 1, 3, 6 and 9 months after inoculation (MAI). The soil samples were oven-dried at 30°C for 7 days before being ground and sieved.

One gramme of each sample was mixed into 10 ml of sterile distilled water by shaking it in an orbital shaker at 100 rpm for 15 minutes. A 10-fold serial dilution (up to 10⁻⁶) was performed and 100 µL from dilutions 10⁻⁴, 10⁻⁵ and 10⁻⁶ were plated onto YME supplemented with 50 ppm of nystatin, 50 ppm of cycloheximide and 20 ppm of nalidixic acid with three replicates for each dilution. All plates were incubated upside down to reduce evaporation in an incubator set at 28°C. *Streptomyces* GanoSA1 from the powder formulation was also serially-diluted and spread on YME plates. This plate was used to compare with other actinomycetes from soil samples with the BCA. Actinomycete with similar appearances of the strain from the soil

sample plate and control plate were counted. The CFU per gramme was determined at 7 days after incubation. From this value, the log₁₀ CFU per gramme was calculated.

Statistical analysis

All experiments were laid out in a completely randomised design. The nursery and field trials were repeated twice. Data from two experiments were analysed for experiment x treatment interactions. Given that there was no experiment x treatment interaction, data were pooled for statistical analysis (Student's *t*-test). Data that failed a normality test were analysed using the Mann-Whitney rank sum test.

RESULTS

***In vitro* survival of *Streptomyces* GanoSA1 in various powder carriers and activity against *G. boninense* PER71**

Streptomyces GanoSA1 (Figure 2) was isolated from the rhizosphere area of healthy oil palm and had been identified as *Streptomyces nigrogriseolus* GanoSA1 (Shariffah Muzaimah *et al.*, 2020). The growth of *Streptomyces* GanoSA1 in various substrates was evaluated based on the survivability, the effectiveness of the strain against *G. boninense* PER71 and the presence of contamination. The CFU per gramme of the strain in each of the powder carriers with the initial densities of 10⁸ CFU per gramme is shown in Table 1. Based on the data recorded, there were differences in the viability count of this strain in various powder carriers studied. The populations of *Streptomyces* GanoSA1 in the formulation vermiculite:charcoal (V+C) and vermiculite:empty fruit bunch (V+EFB) had significantly higher survivability throughout the



Figure 2 Pure culture of *Streptomyces GanoSA1* after seven days of incubation on yeast malt extract agar

storage period compared to the others.

Effective antagonism activity of *Streptomyces GanoSA1* in each powder formulation against *G. boninense* PER71 was evaluated *in vitro*. Based on the observation, all of the formulations showed inhibition with a PIRG of more than 80 per cent. However, the *in vitro* antagonism activity of each formulation showed a parallel correlation with the strain viability count. The formulation with viable densities of 10^8 - 10^6 CFU per gramme showed *G. boninense* PER71 inhibition of 80 per cent to 100 per cent, while viability below 10^5 CFU per gramme gave a PIRG lower than 50 per cent. The growth and morphological appearance of *G. boninense* PER71 on assay plates were also assessed and compared. *G. boninense* PER71 on control plates in this assay were observed as compact and cottony white mycelial mats with darkened undulating

TABLE 1
EFFECTS OF SOLID SUBSTRATE ON THE GROWTH OF *STREPTOMYCES NIGROGRISEOLUS GANOSA1* AND ITS ANTAGONISTIC EFFECT TOWARDS *GANODERMA BONINENSE* PER71

Solid substrate *	Months/ <i>Streptomyces</i> growth (log CFU/g)				Months/PIRG (%)					
	0	3	6	9	12	0	3	6	9	12
V+RB	8.05±0.02 ^b	4.06±0.03 ^d	2.62±0.02 ^c	0±0.0 ^b	0±0.0 ^b	91.25±0.64 ^b	50±4.02 ^c	46.15±2.28 ^c	0±0.0 ^b	0±0.0 ^b
V+RHC	8.23±0.03 ^a	6.34±0.01 ^c	4.08±0.02 ^c	0±0.0 ^b	0±0.0 ^b	91.67±0.87 ^b	89.47±1.41 ^b	50±1.52 ^c	0±0.0 ^b	0±0.0 ^b
V+C	8.12±0.04 ^b	7.00±0.02 ^b	6.39±0.01 ^a	6.02±0.02 ^a	4.08±0.02 ^a	100±0.0 ^a	100±0.0 ^a	86.36±1.96 ^a	75.61±2.38 ^a	50±3.21 ^a
V+EFB	8.25±0.01 ^a	7.29±0.01 ^a	5.34±0.01 ^b	3.08±0.03 ^b	0±0.0 ^b	100±0.0 ^a	94.74±0.15 ^{ab}	74.19±2.94 ^b	48.64±1.36 ^b	0±0.0 ^b

Notes: V+RB: vermiculite+rice bran; V+RHC: vermiculite+rice husk charcoal; V+C: vermiculite+ charcoal and V+EFB: vermiculite+empty fruit bunch. PIRG : percentage inhibition of radial growth. Values are the means of three replications. Values in a column followed by the same letter are not significantly different ($P < 0.05$) using Least Significance Difference (LSD).

brownish pigmentation. On the other hand, stunted growth of *G. boninense* PER71 was observed on plates with the powder formulation showing a significant inhibition activity (Figure 3).

Biological control effect of *Streptomyces* GanoSA1 on BSR disease development in nursery trial

Two months after inoculation with *G. boninense* PER71, 15.82 per cent of the SFS were observed in untreated seedlings and were significantly different from seedlings treated with *Streptomyces* GanoSA1 (3.06%). At this time, external signs and symptoms of *Ganoderma* infection, which is the yellowing of the lower leaves followed by desiccation of the oldest leaves were observed (Figure 4). The same trend was observed until the end of the experiment whereby the treated seedlings gave the highest SFS of 87.23 per cent (Figure 5a). In some seedlings, the white mycelial mass of *G. boninense* PER71 can be seen at the basal area of the seedlings. The white-coloured

hyphal mass continued to develop into a white button-like fruiting body. After this stage, the lower leaves of the seedling became progressively yellow, followed by desiccation which later led to the drying of the entire lamina. The larger brownish-coloured fruiting body was also observed. Based on the *G. boninense* PER71 appearance in seedlings, the untreated seedlings showed a significantly higher DI percentage compared to the treated seedlings (Figure 5b). By the end of the 8-month observation, the DI in treated seedlings was 50 per cent, which was significantly lower compared to the control (93.33%). No symptom of *Ganoderma* infection was observed in seedlings uninoculated with *G. boninense* PER71. The DSFI of external symptoms and DSBI of internal symptoms in seedlings treated with *Streptomyces* GanoSA1 were lower compared to the untreated seedlings. The disease progression curve based on DSFI and DSBI was developed to express the *Ganoderma* disease reduction caused by *Streptomyces* GanoSA1 (Figure 6). Seedlings treated with

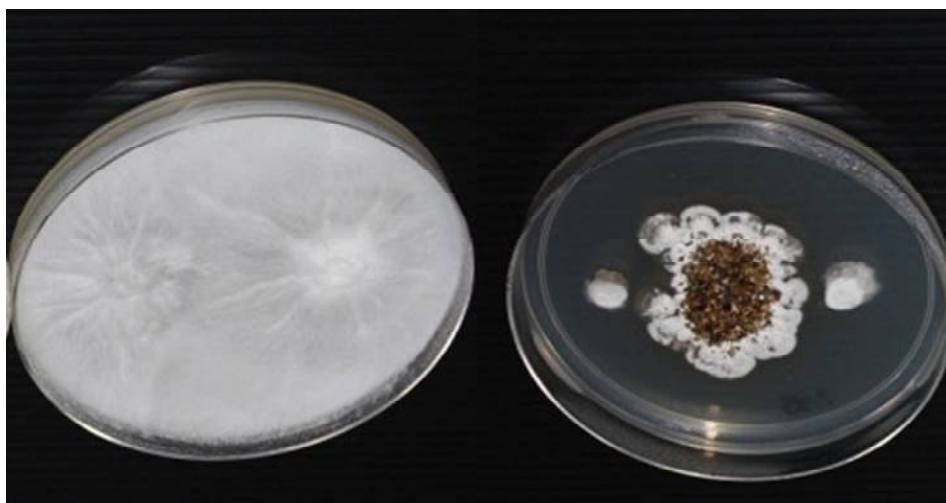


Figure 3 Effect of powder formulation towards the growth of *Ganoderma boninense* PER71 on potato dextrose agar (PDA)

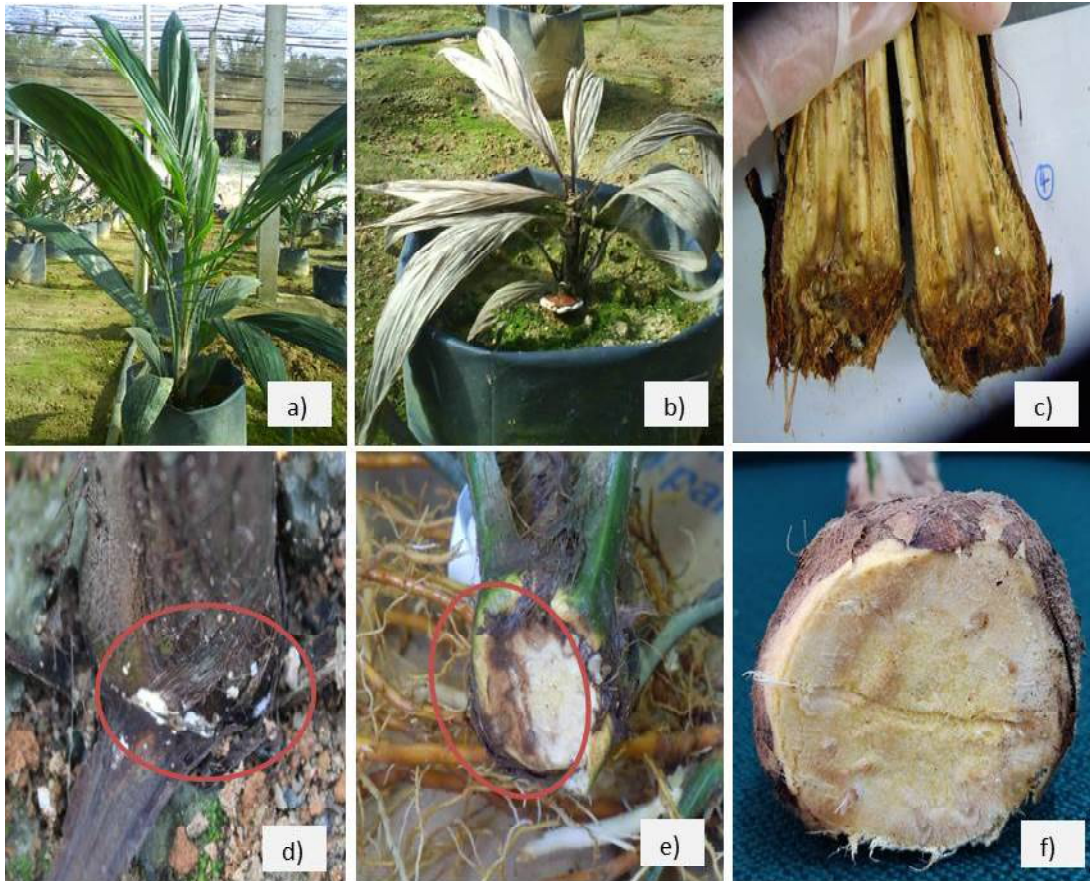


Figure 4 Effect of *Ganoderma* infection on oil palm seedlings; a) healthy seedlings, b) seedlings showing scale 4 of disease severity index (DSI) due to infection, c) bole showing scale 4 of internal DSI due to the infection, d) white mycelia (circled) observed on the bole area of oil palm seedling, e) cross-section of seedling showing lesion (circled) on the bole area due to *Ganoderma*, and f) cross-section of healthy seedling bole area

the powder gave a significantly lower AUDPC compared to the control. Based on this value, the use of *Streptomyces* GanoSA1 powder showed effectiveness in reducing BSR disease development by 68.5 per cent (Table 2).

Monthly observation on the vegetative growth indicated that *Streptomyces* GanoSA1 significantly increased the plant height, stem diameter and relative leaf chlorophyll (Chl) content over those of the control seedlings. At

8 months after inoculation, the plant height and number of frond production of seedlings treated with the powder (147.3 cm; 15 fronds) were significantly higher compared to untreated seedlings (132.8 cm; 13 fronds). The relative leaf chlorophyll content was lower in the untreated control seedlings (50.2 SPAD) compared to the seedlings treated with *Streptomyces* GanoSA1 powder (57.2 SPAD) over 8 months of treatment.

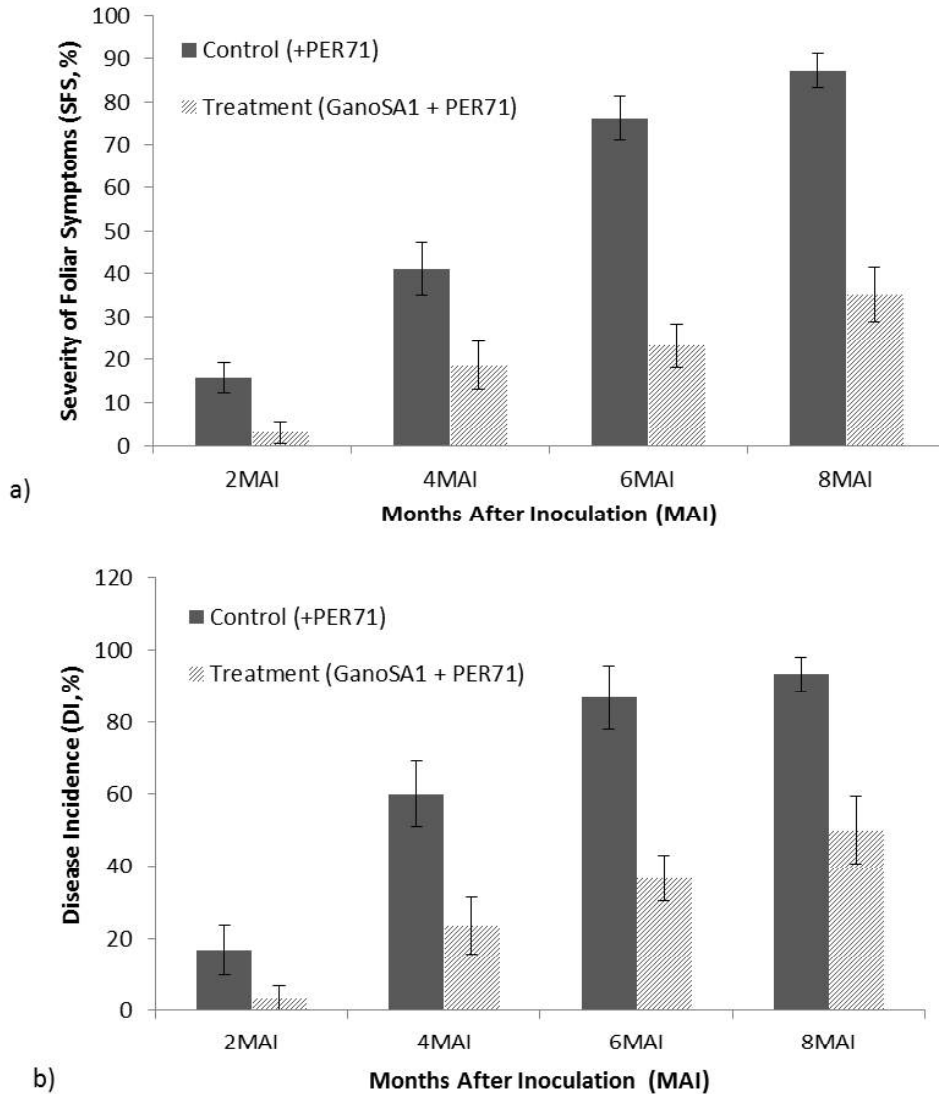


Figure 5 Effects of *Streptomyces GanoSA1* on: a) severity of foliar symptoms (SFS) and b) disease incidence (DI) due to *Ganoderma* infection in oil palm seedlings at 2, 4, 6 and 8 months after inoculation (MAI) with *Ganoderma boninense* PER71

Effect of *Streptomyces GanoSA1* powder application on BSR incidence in field trials

Under field conditions using seedling baiting technique, planted bait seedlings showed susceptibility towards BSR disease. The

application of *Streptomyces GanoSA1* powder controlled the *Ganoderma* infection. Treatment with the powder resulted in less BSR disease incidence compared to the untreated control oil palm. BSR disease on palms can be detected by the presence of the

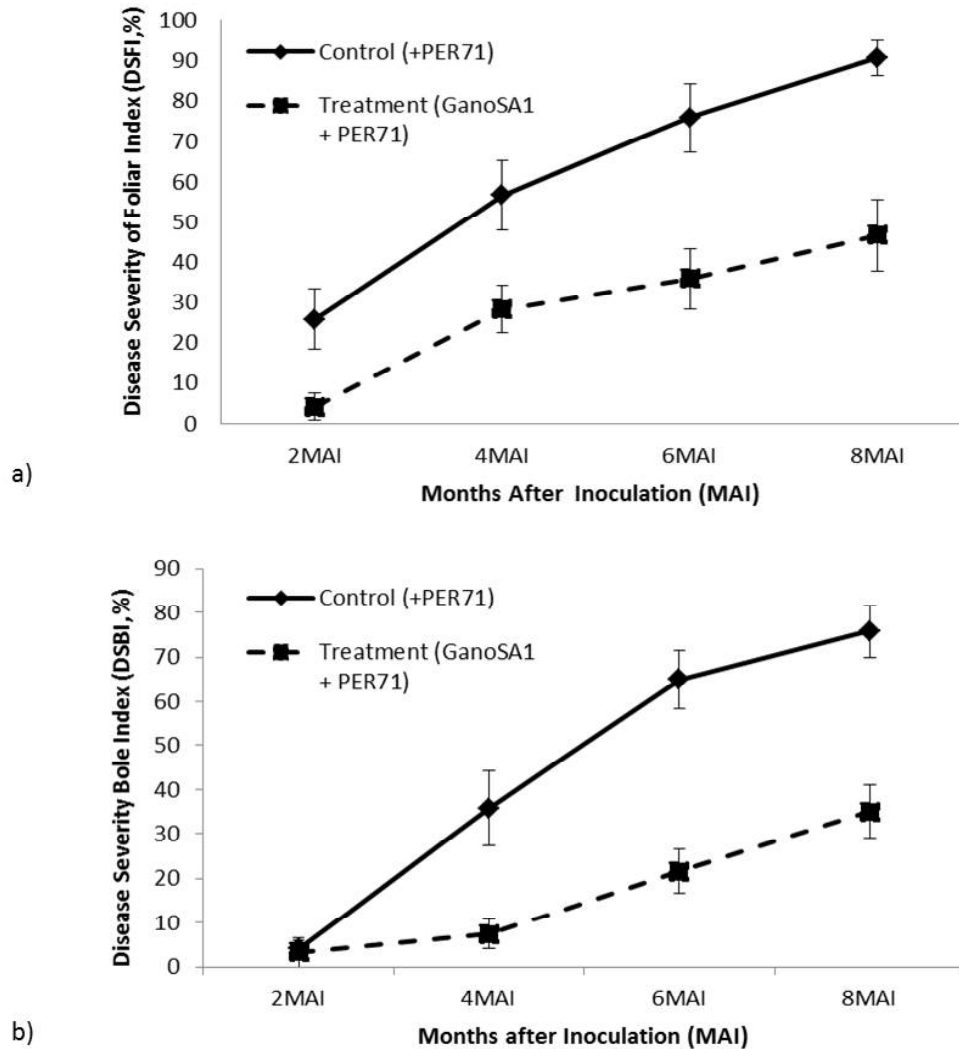


Figure 6 Effects of *Streptomyces nigrogriseolus* GanoSA1 on *Ganoderma* disease progression in oil palm seedlings at 2, 4, 6 and 8 months after artificial inoculation with *Ganoderma boninense* PER71: a) disease severity of foliar index (DSFI) and b) disease severity of bole index (DSBI)

Ganoderma fungus basidiomata or the fruiting body on the oil palm trunk at the bole. The formation of basidiomata was initially observed as a small white button which later developed into a large brownish bracket-shape. These structures may be present before the foliar symptoms were observable. Foliar symptoms

such as one-sided yellowing of leaves or mottling of the lower fronds followed by necrosis can be seen on severely infected oil palm below 3 years old. As the disease progresses, the oil palm will enter the advanced stage of infection whereby the leaves will be completely necrotic and eventually dies

TABLE 2
DISEASE SEVERITY, AREA UNDER THE DISEASE PROGRESS CURVE (AUDPC) AND DISEASE REDUCTION (DR) IN OIL PALM SEEDLINGS PRE-TREATED WITH *STREPTOMYCES NIGROGRISEOLUS* GANOSA1 EIGHT MONTHS AFTER BEING INOCULATED WITH *GANODERMA BONINENSE* PER71 (NURSERY TRIAL)

Treatment	Disease severity of foliar index (DSFI)		Disease severity of Bole Index (DSBI)	
	AUDPC	DR	AUDPC	DR
Untreated seedlings, inoculated with <i>G. boninense</i> PER71 (control)	251.98 ^a	-	299.17 ^b	-
Seedlings treated with <i>S. nigrogriseolus</i> GanoSA1 powder and inoculated with <i>G. boninense</i> PER71	73.46 ^b	68.5	177.5 ^b	59.68

Notes: AUDPC : Area under disease progression curve; DR : disease reduction. Values in a column followed by the same letter are not significantly different ($P < 0.05$) using Least Square Significance Difference (LSD).

(Figure 7). Typical BSR symptoms for immature palms were observed on oil palms infected with *G. boninense*. This was confirmed by planting pieces of the infected oil palm tissues and the fruiting body on the *Ganoderma* Selective Medium (GSM).

Oil palm seedlings treated with *Streptomyces* GanoSA1 powder had the lowest DI compared to the untreated oil palm seedlings (Table 3). After 36 months of planting, only 6.6 per cent of oil palm seedlings treated with *Streptomyces* GanoSA1 powder showed BSR symptoms and were dead due to *Ganoderma* infection. About 75 per cent (45 out of 60 palms) of the control untreated seedlings were dead due to *Ganoderma* infection. Cross sectioning of infected oil palm seedlings showed internal rotting of the bole area which indicates the severity of the infection. The base of the oil palm seedlings that was severely infected was completely rotten.

Recovery of *Streptomyces* GanoSA1 from treated soil

The initial amount of *Streptomyces* GanoSA1 in the vermiculite:biochar powder was 1×10^8 CFU per gramme. The CFU count of *Streptomyces* GanoSA1 in the treated soil after 3 months of application was at 1×10^6 before being reduced and maintained at 1×10^5 for 8 months. Higher recovery rates of *Streptomyces* GanoSA1 were recorded in the oil palm roots and rhizosphere-area soil treated with the powder compared to the untreated. However, no significant difference was observed for *Streptomyces* GanoSA1 recovery in the treated and untreated palm seedlings in the field.

DISCUSSION

The use of biological control agents in managing plant diseases, in particular, soil-borne diseases,



Figure 7 The performance of *Streptomyces GanoSA1* against *Ganoderma* disease in field-planted oil palm: a) palm treated with *Streptomyces GanoSA1* powder; b) palm untreated (control) showing one-sided yellowing of the leaves symptoms and c) palm untreated (control) showing necrotic leaves 12 months after planting (MAP) using the seedling baiting technique

TABLE 3
EFFECTS OF *STREPTOMYCES NIGROGRISEOLUS* GANOSA1 IN CONTROLLING *GANODERMA* DISEASE IN OIL PALM SEEDLINGS AFTER PLANTING (USING SEEDLING BAITING TECHNIQUE, FIELD TRIAL)

Treatments	Palms dead due to <i>Ganoderma</i> infection (%)		
	12 MAP	24 MAP	36 MAP
T1- Untreated seedlings (control)	8.33 ^a	20 ^a	75.0 ^a
T2- Seedlings treated with <i>S. nigrogriseolus</i> GanoSA1 powder	0 ^b	3.33 ^b	6.67 ^b

*MAP: Months after planting; **Columns with the same letter indicates no significant difference at p<0.05 using Tukey's Studentised Range.

has been a continuing area of active research for numerous decades (Mazzola & Freilich, 2016). Although it has long been a goal in sustainable agriculture, examples of successful application in commercial field-based crop production are limited. Since BCA is a viable microorganism, the commercial production needs to be carried out carefully to ensure the effectiveness, the desired impact achieved (Shaikh & Sayyed, 2015) and to be able to survive for a long time after being applied to soil (Shen *et al.*, 2016). Work on the novel formulation and delivery strategies will provide

enhanced biocontrol and plant growth-promoting products for sustainable agriculture (Amaesan *et al.*, 2018). Selection of a carrier substrate is an important role in enhancing the antagonist response and in maintaining the survivability of the antagonist when applied in a natural environment. The substrate type, particle size and moisture level are among the important factors for microbial growth and activity. The present trials studied the effects of rice bran, rice husk charcoal, charcoal and empty fruit bunch (EFB) on *Streptomyces GanoSA1* viability and efficacy towards

G. boninense in combination with vermiculite at a 1:1 ratio. According to Pandey (1991), smaller substrate particles would provide a larger surface area for microbial attack. Smaller sized substrate particles may interfere with microbial respiration, which will result in poor growth. On the other hand, larger particles provide better respiration or aeration due to increased interparticle space. However, larger particles provide a limited surface for microbial attack (El-Naggar *et al.*, 2009). In this study, a combination of vermiculite with charcoal (0.2-0.3 cm) from oil palm kernel shell provided the longest viability and the highest efficacy against *G. boninense* PER71 *in vitro* when tested under room temperature for 12 months. The EFB (0.1-0.2 cm), rice husk charcoal (0.1-0.2 cm), and rice bran (0.4-0.5 cm) showed the lowest *Streptomyces* GanoSA1 viability in descending order. When applied to unsterilised soil using the vermiculite:charcoal formulation, the strain established itself in the rhizosphere of oil palm seedlings at significant levels (10^5 CFU/g of soil), and it can be reisolated after eight months of inoculation. The survivability may strongly correlate with the C:N ratios of biochar used. Findings by several researchers indicate that biochar application will influence soil C:N ratio and will have an effect on soil bacteria (Hale *et al.*, 2015; Hogberg *et al.*, 2007; Muhammad *et al.*, 2014). However, this effect may be inconsistent across the different soil types.

In Malaysia, different strategies for the management of BSR disease have been introduced. However, to date, there is still no method for eliminating the pathogen effectively. This study aimed to investigate the potential biological control activity of *Streptomyces* GanoSA1 against *Ganoderma* disease and also act as a plant growth promoter in oil palm. The application of *Streptomyces* GanoSA1 in

oil palm roots is effective against *G. boninense* and has apparently delayed the appearance of BSR disease before the leaf symptoms and white mycelia were observed in some of the seedlings. This provided preliminary evidence that the strain has the ability to be a potential biocontrol agent. In both, the nursery and bait seedling field trials, *Streptomyces* GanoSA1 reduced the development of BSR disease. Disease development was slower in seedlings treated with *Streptomyces* GanoSA1 powder compared to the untreated seedlings. Seedlings treated with the powder gave a lower value of DI (%) and SFS (%) throughout the experiment compared to untreated seedlings, indicating a promising biological control potential. Under a nursery condition, *Streptomyces* GanoSA1 showed great potential in reducing BSR disease incidence in oil palm seedlings based on the percentage of DI (%), SFS (%) and DS (%).

In general, the addition of BCA through soil mixing may contribute to the plant protection *via* the reduction of pathogen density. This bio-protection by actinomycetes can occur *via* several mechanisms, such as the competition, or the direct antagonism resulting from the production of antimicrobial metabolites (Wonglom *et al.*, 2020; Lim *et al.*, 2018), siderophores (Zeng *et al.*, 2018; Jog *et al.*, 2012), volatile organic compounds (Cordovez *et al.*, 2015; Li *et al.*, 2010; Wan *et al.*, 2008) and secretion of cell-wall-degrading enzymes, such as chitinase, laminarase, peptidase and glucanase laminarase (Mun *et al.*, 2020; Wonglom *et al.*, 2019; Jog *et al.*, 2012), abiotic stress management (Amaresan *et al.*, 2018; Gopalakrishnan *et al.*, 2015) or activation of resistance pathways in the plant (Ansari *et al.*, 2020; Dias *et al.*, 2017; Awla *et al.*, 2017; Senthilraja, 2016; Zhou *et al.*, 2012). The efficacy of biocontrol activity by BCA is also

affected by the temperature, soil moisture and soil type (Spadaro & Gullino, 2005), given that the nutritional condition of the soil may enhance the production of lytic enzyme by BCA (Kucuk & Kivanc, 2008). Various reports on *Streptomyces* spp. potential in producing nutrient enhancer substances that can influence soil fertility have been published. They were also known to promote plant growth which may be affected by the production of indole acetic acid (IAA) and able to solubilise phosphate (Amaresan *et al.*, 2018; Tamreihao *et al.*, 2016). Apart from their potential to produce IAA and solubilising phosphate, they are also known to produce various enzymes such as amylase, cellulase, lipase, keratinase, peroxidase, pectinase, protease and xylanase. These enzymes play important roles in the catabolism of complex nutrients into simple mineral forms. This nutrient cycling capacity makes them ideal candidates as natural fertilisers (Jog *et al.*, 2016). Based on this study, the disease development in seedlings treated with *Streptomyces* GanoSA1 powder was significantly reduced in both the nursery and field trials. This finding indicates that the introduction of *Streptomyces* GanoSA1 in soil has promoted the growth of oil palm seedlings and reduced the incidence of *Ganoderma* disease in both nursery and field conditions. The effectiveness of the strains and its formulation should be further evaluated under different conditions of soil, soil moisture and temperature levels over a longer duration and/or with repeated application (oil palm is a 25-year-old or more perennial crop). Additionally, the movement of *Streptomyces* GanoSA1 through the soil after application is also an interesting aspect to be studied for a broader understanding on its distribution and fate after application.

ACKNOWLEDGEMENTS

The authors thank the Director-General of MPOB for being very supportive and for permission to publish this article and the staff of MPOB for their support in conducting this study. The authors would also like to thank Pascal Biotech Sdn Bhd, Shah Alam, Selangor, Malaysia for providing the *Streptomyces* GanoSA1 powder that was used throughout this study.

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