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EFFECT OF MICRONUTRIENTS-ENRICHED FERTILIZERS ON BASAL STEM ROT DISEASE INCIDENCE AND SEVERITY ON OIL PALM (*ELAEIS GUINEENSIS* JACQ.) SEEDLINGS

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ABSTRACT

Basal stem rot caused by *Ganoderma boninense* constitutes a serious threat to oil palm industry in Southeast Asia, especially in Malaysia and Indonesia and in Papua New Guinea and Pacific Islands. It is also expanding in some oil palm growing countries in Latin America and Africa and will soon become a worldwide concern to oil palm cultivation. To date, none of the various control measures developed and tested to control the disease since many decade gives entire satisfaction. An experiment was carried out to see whether incorporation of micronutrients, Copper (Cu), Boron (B) and Manganese (Mn) could reduce the incidence and severity of this disease on oil palm seedlings inoculated with *G. boninense*. The concentrations tested were 2 mg B/kg of soil, 2 mg Cu/kg of soil and 2 mg Mn/kg of soil incorporated into the basic fertilizer NPKMg 14-10-10-2. Treatments were applied in solution for three months before inoculation, followed by soil application for eight months after inoculation. The results showed that although no significant difference was detected among treatments, the double combinations of these micronutrients, B+Cu, B+Mn and Cu+Mn, performed better than the single nutrients in reducing the incidence and the severity of BSR, while their triple combination rather increased these pathological parameters. These double combinations could therefore be field-tested for their further integration in oil palm fertilization programme.

Keywords: Basal Stem Rot, *Ganoderma Boninense*, Oil Palm, Boron, Copper, Manganese

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1. INTRODUCTION

Ganoderma Basal Stem Rot (BSR) is the most devastating disease of oil palm (Ariffin *et al*., 2000; Idris, 2009; Susanto, 2009) and constitutes a permanent threat to oil palm in Southeast Asia (Pilotti, 2005; Utomo *et al*., 2005). Despite the slow progression of the disease, it can destroy thousands of hectares of oil palm. Serious yield reductions and death of palms due to *Ganoderma* attacks have been recorded especially in replanted areas, where the disease was endemic in the previous generation. Initially considered a disease of old palms and concentrated in coastal areas on peat soils (Singh, 1991), the BSR disease is now infecting oil palm at all ages starting from the first year of field planting. In Malaysia, a 3.7% incidence of BSR corresponding to 59,148 ha affected was reported in 2010 (Idris, 2011; Idris *et al*., 2011). It has spread rapidly to cover all oil palm cultivating areas and all types of soils (Idris, 1999; Khairudin and Tey, 2008), thereby becoming a great concern in Malaysia and all over the world (Turner and Gillbanks, 2003). In Sabah in the Kinabalu estate, about 30% of second generation under-planted palms were reported to be infected by *Ganoderma* with in one to two years after planting (Wan, 2007). In Sime Darby plantations, 1.4% BSR incidence, equivalent to about 580 ha, was recorded in 2007 (Khairudin and Tey, 2008). In Cameroon, losses as high as 53.2% of dead palms over 25 years old in a first generation plantation was estimated, mainly due to *Ganoderma* (Tengoua and Bakoume, 2005), while, 6.4% palm death was recorded in a 10-year-old replanting when palms were at their peak production age (Tengoua, 2005).

Recent efforts to tackle the problem have been focused on biological control agents, such as *Trichoderma* (Ilias, 2000; Shamala, 2005; Izzati and Faridah, 2008; Siddiquee *et al*., 2009), endophytic fungi and endophytic bacteria (Shamala *et al*., 2011), *Gliocladium* (Flood and Hasan, 2004), *Pseudomonas fluorescens* and *Bacillus* sp (Susanto *et al*., 2005). The use of a balanced chemical fertilizer, namely N, P and K (Mohd Tayeb and Hamdan, 1999; Mohd Tayeb *et al*., 2003) and manual application of calcium nitrate (Sariah and Zakaria, 2000; Flood and Hasan, 2004) have also been investigated.

Nutrient addition to soil as fertilizer is known to affect plant resistance/susceptibility to some diseases caused by fungal pathogens (Veresoglou *et al*., 2013). Despite their direct action on pathogenic agents through their biocidal properties and their indirect action through their involvement in a number of plant defence mechanisms, micronutrients have not been considered in BSR control strategies. Boron (Stangoulis and Graham, 2007), Cu (Evans *et al*., 2007) and Mn (Thompson and Huber, 2007) are reported to control many plant diseases. Copper, B and Mn are all intimately involved in phenol synthesis in plants and have major effects on host susceptibility to disease (Graham, 1983). This study was initiated to see whether incorporation of single and combined optimum concentrations of these micronutrients in a commonly used compound fertilizer grade NPKMg (14:10:10:2), can reduce BSR incidence and severity on oil palm seedlings.

2. MATERIALS AND METHODS

2.1. Plant and Fungal Materials

One-month-old commercial *Tenera* oil palm seedlings (*dura* × *pisifera*) were purchased from the Federal Land Development Authority (FELDA) Agricultural Services Sdn. Bhd. Sungai Tekam, Jerantut, Pahang, Malaysia. In the first phase of the experiment, seedlings were supplied for three months with different fertilizer treatments applied in solution. After two weeks acclimatization, kernels were removed to ensure that the response obtained will solely result from fertilizer treatments with minimum influence from endosperm nutrient reserves. Treatments were renewed every week. The composition of different fertilizer treatments used is as follows: T1 (Control 1, non-inoculated) = Basic fertilizer (BF); T2 (Control 2, inoculated) = Basic fertilizer (BF); T3 = BF +2 mg B/kg of soil; T4 = BF +2 mg Cu/kg of soil; T5 = BF +2 mg Mn/kg of soil; T6 = BF +2 mg B/kg of soil +2 mg Cu/kg of soil; T7 = BF +2 mg B/kg of soil +2 mg Mn/kg of soil; T8 = BF +2 mg Cu/kg of soil +2 mg Mn/kg of soil; T9 = BF +2 mg B/kg of soil +2 mg Cu/kg of soil +2 mg Mn/kg of soil. The Basic Fertilizer (BF) is the commonly used commercial fertilizer NPKMg (14:10:10:2), where N stands for % N (14%), P for % P_2O_5 (10%), K for % K_2O (10%) and Mg for % MgO (2%). The sources of micronutrients were borax pentahydrate [$Na_2B_4O_7$. $5H_2O$ (48% B_2O_3)] for B, copper sulphate pentahydrate [$CuSO_4$. $5H_2O$ (25% Cu)] for Cu and manganese sulphate monohydrate [$MnSO_4$. H_2O (31.8% Mn)] for Mn.

The pure culture of *Ganoderma boninense* PER 71 used was supplied by the *Ganoderma* and Disease Research for

Oil Palm (GanoDROP) Unit, Biology Division, Malaysian Palm Oil Board (MPOB), Bangi, Malaysia.

2.2. Inoculum Preparation and Inoculation of Oil Palm Seedlings with *G. Boninense*

The *Ganoderma* Rubber Wood Block (RWB) inoculum was prepared following the general method routinely used by MPOB and described by (Idris, 1999). Briefly, freshly cut RWBs (6×6×6 cm) were oven-dried at 80°C for 48 h and sterilized at 121°C for 30 min. The RWBs were then put in a heat resistant polyethylene bag (one RWB per bag) to which 60 mL of Malt Extract Agar (MEA) were added, autoclaved at 121°C for 30 min and left to cool over night, with rotation to allow the medium to evenly cover the RBWs before solidification. After cooling, the RWBs were inoculated with a 7 to 10-day-old pure culture of *G. boninense* PER 71. One plate divided into eight fragments was used to inoculate two RWBs, each receiving four fragments of *G. boninense* put on the four lateral surfaces. The inoculated RWBs were kept in the dark at 28°C for three months to allow external and internal colonization by *G. boninense*. At the end of three month incubation, prior to inoculation, fully covered RWBs (**Fig. 1A and B**) were tested on *Ganoderma*-Selective Medium (GSM) (Ariffin and Idris, 1992) to confirm the internal colonization. The positive test was confirmed by the development of *Ganoderma* mycelium from the fragment of wood taken from the centre of split RWBs (**Fig. 1C**) and by browning of the medium when observed from the bottom of the Petri dish (**Fig. 1D**).

The inoculation of oil palm seedlings with *Ganoderma* RWB inoculum (second phase of the experiment) consisted of a sitting technique, whereby the bulb and washed roots of each seedling were firmly put in contact with a RWB inoculum standing on one third of 5 kg of the Munchong series soil in a polybag (25×30 cm) and covered with the remaining soil. Soils of the Munchong series are characterized by their low fertility status. For each treatment, two extra seedlings were inoculated and kept aside for eventual replacement. Seedlings of treatments T1 (absolute control) were not inoculated with RWB inoculum but simply transplanted in polybags filled with 5 kg of soil.

2.3. Maintenance

Inoculated seedlings and the control received monthly fertilizer treatments (10 g/plant) with daily watering for eight months. On a weekly basis, the lower surface of the leaves was sprayed with water to wash off red spider mites (*Oligonychus* sp. and/or *Tetranychus piercei*). The use of chemicals was minimal so as not to compromise the infection by *G. boninense* (Naher *et al*., 2012). When extremely needed, insecticides (Rogor 40 (Dimethoate 38.0% w/v) and Decis (Deltamethrin 1.4% w/v)) were sprayed alternatively to control insect attacks.

2.4. Assessment of Pathological Parameters

2.4.1. External Symptoms

External symptoms of *G. boninense* infection were recorded every month starting two months after inoculation until the eighth month (end of experiment). These include the total number of leaves; the number of green, yellow and dry leaves; presence or absence of mycelium and/or white button or basidiocarps at the plant base and the disease class. From these data, the Severity of Foliar Symptoms (%SFS), the Disease Incidence (DI) and the Disease Severity Index (DSI) were assessed. The severity of foliar symptoms for each seedling was calculated by the following formula (Sariah and Zakaria, 2000):

$$\%SFS = \frac{\left[(D\times 1)+(Y\times 0.5)\right]}{T}\times 100$$

where, D is the number of desiccated (dry or dead) leaves, Y the number of yellow, chlorotic or wilted leaves, T the total number of leaves, the numerical value 1 represents the index for desiccated leaves and 0.5 the index for chlorotic leaves.

The Disease Incidence (DI) for each treatment in each replication and the Area Under Disease Progress Curve (AUDPC) for the severity of foliar symptoms and disease incidence were assessed following (Campbell and Madden, 1990):

$$DI(\%) = \frac{Number\ of\ seedlings\ infected}{Total\ number\ of\ seedlings\ assessed}\times 100$$

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

where, n is the number of assessment time, Y the disease incidence or the percentage severity of foliar symptoms and t the observation time. The AUDPC indicates the amount of disease developed in each treatment over

time. The lower the AUDPC value of a treatment, the more effective the treatment in reducing the disease. The percentage of Disease Reduction (DR) for each treatment was deduced from the AUDPC by the formula:

$$DR\ (\%) = \frac{AUDPCcontrol - AUDPCtreatment}{AUDPCcontrol} \times 100$$

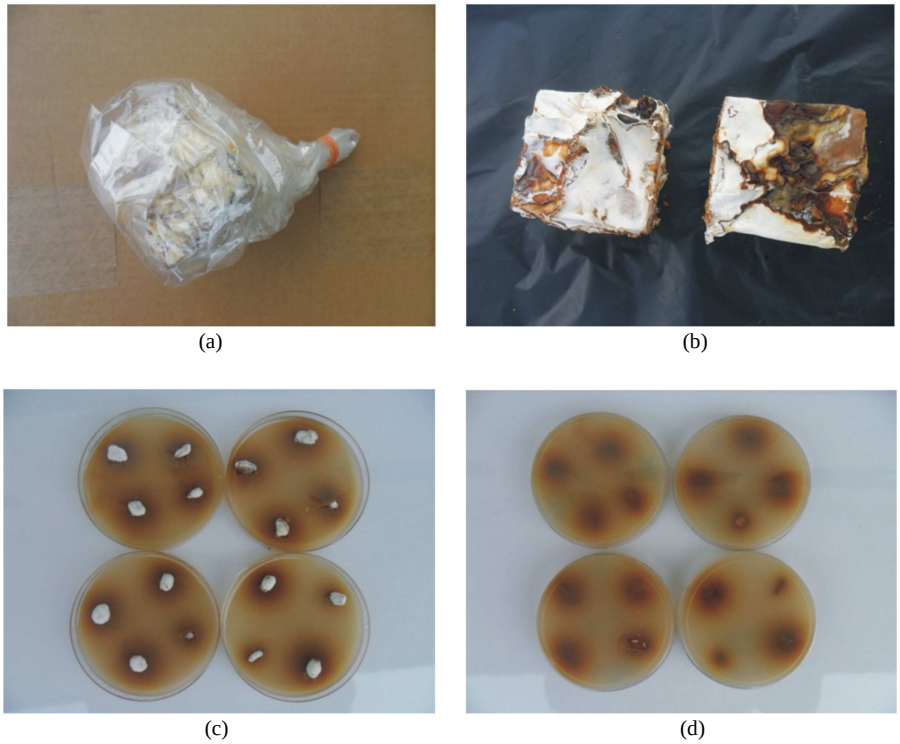
The control here is T2.

The Epidemic Rate (ER) for each treatment was calculated using the formula $y = \ln\left[\frac{1}{(1-x)}\right]$ applied to a monocyclic or monomolecular epidemic model (Campbell and Madden, 1990) appropriate for soil borne fungi like *G. boninense*, where y is the amount of disease at a given time and x the disease incidence in proportion, not in percentage. The value of the Epidemic Rate (ER) corresponds to the slope of the straight line $y = \ln\left[\frac{1}{(1-x)}\right]$ plotted against t, the observation time.

The Disease Severity Index (DSI) was calculated according to (Ilias, 2000; Abdullah *et al*., 2003) as follows:

$$DSI = \frac{\sum(A\,x\,B)}{\sum B\,x4} \times 100$$

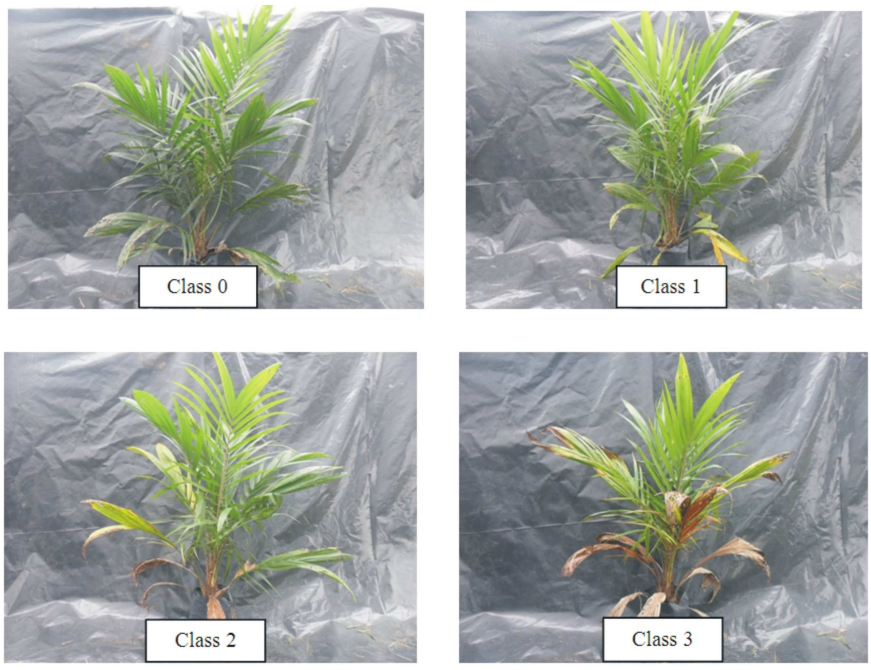
where, A is the disease class (0, 1, 2, 3, or 4), B the number of seedlings showing that disease class per treatment, $\sum B$ the total number of seedlings assessed and 4 the highest rating or disease class value. Since the basidiocarps (fruiting bodies) do not always develop in all *Ganoderma* screening tests (Sariah *et al*., 1994; Kandan *et al*., 2009), the description of disease scales (classes) (Ilias, 2000; Abdullah *et al*., 2003; Breton *et al*., 2006) that emphasized on the presence of a fruiting body as an important characteristic in classifying *Ganoderma* symptoms was modified to address this natural fact (**Table 1, Fig. 2 and 3**). These modifications rely on the observation that most of the infected plants passed across all the scales and died without any basidiocarp (**Fig. 4A**).



(a) (b) (c) (d)

Fig. 1. Three-month-old fully colonized Rubber Wood Block (RWB) by *G. boninense* PER 71 in heat resistant plastic (A), removed from the plastic for GSM testing (B); Note: The development of *G. boninense* from the RWB fragments (C) and the brown coloration of the medium observed from the inverted plates (D) at the 5th day of incubation at 28°C in the darkness

Fig. 2. Illustration of different classes (0-4) of *G. boninense* external symptoms on oil palm seedlings at an early growth stage (2-5 months after inoculation)



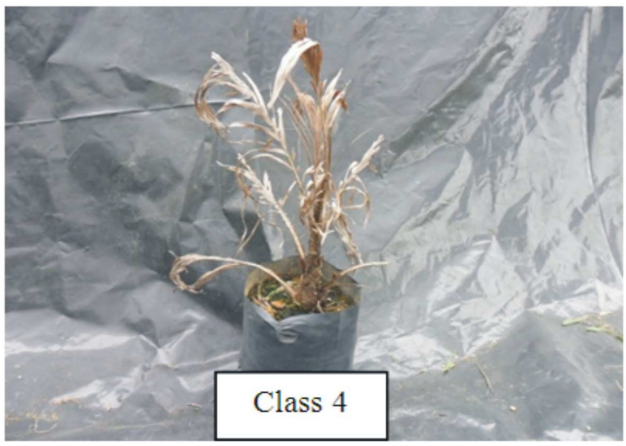


Fig. 3. Illustration of different classes of *G. boninense* external symptoms on oil palm seedlings at an advanced growth stage (8 months after inoculation)

(a) (b)

(c)

Fig. 4. Development of *G. boninense* white mycelium (A), white button (B) and formation of a full basidiocarp (C) on dead oil palm seedlings

Table 1. Description of disease classes of *Ganoderma* external symptoms

Disease class	Symptom description
0	Healthy plant with green leaves without the appearance of fungal mycelium on any part of the plant
1	Appearance of white fungal mass on any part of the plant, with or without chlorotic leaves
2	Chlorotic and/or necrotic leaves (1-3), with or without the appearance of basidiocarps on any part of the plant
3	Chlorotic and/or necrotic leaves (□3), ≥50% drying up or necrotic, with or without the formation of basidiocarps on any part of the plant
4	Total necrosis, whole plant dry or dead, with or without the formation of well-developed basidiocarps

Source: Adapted from Ilias (2000; Abdullah *et al*., 2003; Breton *et al*., 2006)

In all the cases observed, the white buttons, initiation or primordia of fruiting body development, appeared when the plants were already dead (class 4) (**Fig. 4B**) and progressed until the formation of a full basidiocarp on the dead plant (**Fig. 4C**).

2.4.2. Internal Symptoms

At the end of experiment, surviving seedlings were assessed for BSR internal symptoms. Number of rotted primary roots was recorded and the bulb was longitudinally dissected to observe and measure the extent of rotted area using a grid. Both the rotted and the total areas were measured to confidently transform in percentage the results expressed in surface unit and classify following the scale adapted from Nur Sabrina *et al*. (2012; Breton *et al*., 2006) (**Table 2 and Fig. 5**). The percentage of infected bulb area was calculated as followed:

$$\%\,infected\ bulb\ area\left(\%IBA\right)=\frac{Infected\ bulb\ area\left(IBA\right)}{Total\ bulb\ area\left(TBA\right)}\times 100$$

After determining the disease class from the %IBA, the Disease Severity Index (DSI) for internal symptoms, more specifically for bulb symptoms or bulb decay (DSIB) was then calculated as previously described for external symptoms:

$$DSIB\left(Internal\right)=\frac{\sum\left(A'\times B'\right)}{\sum B'\times 4}\times 100$$

where, A' is the rating number or class, B' the number of seedlings in that rating number, $\sum B'$ the total number of seedlings assessed and 4 the highest rating.

Similarly, the Disease Severity Index for Root infection (DSIR) was calculated according to the formula:

$$DSIR=\frac{\sum\left(A''\times B''\right)}{\sum B''\times 4}\times 100$$

where, A'' is the rating number, B'' the number of seedlings in that rating, $\sum B''$ the total number of seedlings assessed and 4 the highest rating. The classification or the rating of *Ganoderma* infection in the roots of oil palm seedlings was adapted from Nur Sabrina *et al*. (2012; Breton *et al*., 2006) as follows: Class 0 = healthy, no root infection; class 1 = Less than 25% of roots infected; class 2 = 25 to 50% of roots infected; class 3 = 51to 75% of roots infected; and class 4 = more than 75% of roots infected.

2.5. Experimental Design and Data Analysis

The experiment was laid out in a Randomized Complete Block Design (RCBD) with nine treatments and four replicates (blocks). Each treatment consisted of four oil palm seedlings (experimental unit). Data were analyzed by ANOVA using SAS 9.2. The disease incidence and disease severity of foliar symptoms were square root-transformed to reduce the standard error. Means comparison was performed by Duncan's Multiple Range Test (DMRT) at the 0.05 significance level.

3. RESULTS AND DISCUSSION

The pathological data (disease incidence and severity, percentage of dead seedlings) were recorded every month. For better visibility and clarity reasons, the results presented and discussed in this study concern the data of two, four, six and eight months after inoculation, i.e., two-month intervals.

3.1. Severity of Foliar Symptoms

Two months after inoculation, treatment T9 had the most severe foliar symptoms (13.15%) and was significantly different from the other treatments (**Fig. 6**). The inoculated control (T2) and the B + Cu treatment (T6) had the lowest Severity of Foliar Symptoms (SFS)

(0.39%). No foliar symptom was observed on treatments T4 (Cu) and T8 (Cu + Mn) at this stage, indicative of their ability to delay disease development. As expected, no sign of *Ganoderma* could be seen on T1 since it was not inoculated. Four months later, *Ganoderma* infection was not significantly different with all treatments but varied from SFS 23.6% with B + Mn (T7) to 34.85% with B alone (T3). At six months, treatments were not statistically different in SFS but B + Mn (T7) had the lowest SFS (58.10%) and Mn alone (T5) the highest (66.43%). The same trend was observed at eight months where T7 registered 67.69% SFS and the triple combination (T9) the highest SFS (87.71%). It is interesting to notice that throughout the experiment, the highest SFS was recorded either on T9 or on any treatment with a single micronutrient, while the lowest SFS was observed mostly on treatments supplemented with a combination of two micronutrients. This denotes the superiority of a double combination of micronutrients over the single and triple combination in reducing the SFS on inoculated oil palm seedlings.

3.2. Disease Severity Index for Foliar Symptoms

There were significant differences among treatments for the disease severity index for foliar symptoms (DSIF) (**Table 3**). At two months, seedlings receiving B, Cu and Mn (T9) were severely infected by *G. boninense* compared to other treatments with index as high as 14.06. Treatments Cu (T4), Mn (T5) and Cu +Mn (T8) had a DSIF = 0, showing they were not yet infected. From four months on, despite the increasing DSIF, the statistical analysis did not detect any significant difference among treatments; however, T9 remained the most severe throughout while, T6, T8 and T7 were less severe at four, six and eight months, respectively, supporting once more the superiority of double combinations of B, Cu and Mn over the triple combination in reducing *Ganoderma* infection.

3.3. Disease Incidence

Although there were no significant differences in Disease Incidence (DI) (**Fig. 7**), treatment T9 had the highest DI (18.7%) as early as two months after inoculation and was the most severely infected from the sixth month till the end of the experiment. With the exception of treatments T2, T3, T6 and T7 with the same incidence of 6.25%, treatments T4, T5 and T8 were not yet infected at two months. Four months after inoculation, T2 (NPK 14:10:10:2 without micronutrients) had the highest disease incidence (56.25%) compared to the treatments supplemented with B, Cu and Mn although none were significantly different. At eight months after inoculation, while T9 and T6 had the highest DI (87.50%), T2, T7 and T8 had the lowest DI (68.75%). This could indicate that the fertilizer formulation NPKMg 14:10:10:2 has the same capacity to reduce *Ganoderma* incidence as its B + Mn and Cu + Mn-supplemented formulations, whereas, the NPKMg 14:10:10:2 supplemented with either B, Cu and Mn alone, or B + Cu, or with the combination of the three micronutrients has low potential to reduce *Ganoderma* incidence.

3.4. Area under Disease Progress Curve, Disease Reduction and Epidemic Rate

Treatment T7, a combination of B + Mn, had the lowest Area Under the Disease Progress Curve (AUDPC) of 236 unit^2, with the corresponding highest disease reduction of 9.63% and the lowest epidemic rate of 0.19 unit month^{-1} (**Table 4**). This indicates the effectiveness of B added together with Mn to the basic fertilizer in reducing the severity of foliar symptoms of *G. boninense* on oil palm seedlings. Surprisingly, the inoculated control T2 had an AUDPC lower than some treatments with an Epidemic Rate (ER) of 0.21 unit month^{-1} and close to the most effective treatment T7. Treatments T3, T5 and T9 with higher AUDPCs than T2, had negative Disease Reductions (DR) of -7.67, -4.58 and -15.15%, respectively. This indicates that the addition of B, Mn, or B + Cu + Mn to NPK fertilizer increased the severity of foliar symptoms of *G. boninense* in oil palm seedlings. The negative effects of these nutrients are more intense with the triple combination which had an AUDPC of 300.70 unit^2, increased severity of foliar symptoms by 15.51% and the highest epidemic rate of 0.32 unit month^{-1}.

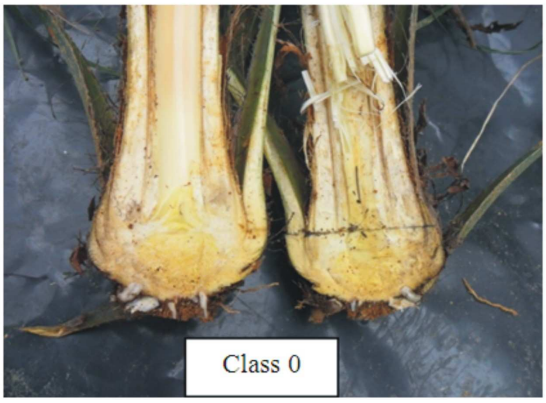
As far as the disease incidence is concerned, Cu + Mn (T8) had the lowest AUDPC (237.5 unit^2) which could mean that the combination of Cu + Mn with the basic fertilizer may be more effective than other treatments in reducing *Ganoderma* incidence in oil palm seedlings; however the treatment T9, with B + Cu + Mn had the highest AUDPC (356.25 unit^2) meaning that the triple combination not only failed to reduce but rather increased the incidence of disease by 14% which was the highest rate of 0.36 unit month^{-1}. Like treatment T9, T5 also had a higher AUDPC (318.75) than the control and increased disease incidence by 2%. On the other hand, B (treatment T3), which increased the severity of foliar

symptoms, decreased disease incidence by 8%. Combination of B + Mn (T7), with the second lowest AUDPC (262.45 unit^2) had the second highest DR (16.02%) suggesting that the double combination of B + Mn with NPK fertilizer may reduce *Ganoderma* incidence as well as Cu + Mn.

In general, all of the double combinations, B + Cu (T6), B + Mn (T7) and Cu + Mn (T8) reduced both *Ganoderma* severity of foliar symptom and *Ganoderma* incidence. Manganese alone and the triple combination of the selected micronutrients detrimentally increased *Ganoderma* incidence and severity. Copper alone reduced to a slight extent these two pathological parameters, while B alone has a variable effect to reduce *Ganoderma* incidence but increases its severity. The positive effect of Cu on *Ganoderma* reduction obtained in the current study is consistent with that of Nur Sabrina *et al*. (2012) who found that Cu supplementation reduced *Ganoderma* incidence by 51.52% and its severity by 60.00%; however, the disease reduction by Cu in our study (6% disease incidence and 1.06% disease severity) was not significant and low compared to these authors report. The discrepancy observed could be explained by two main reasons. Firstly, they inoculated six-month-old seedlings with *Ganoderma* after pre-treatment with fertilizers for three months. In contrast, in the present study, the oil palm seedlings were pre-treated for the same three months but starting at one-month-old and inoculated at four months. In this case, the seedlings might not have developed a strong enough defence system to withstand *Ganoderma*. Secondly, the present study used basic fertilizer NPKMg 14:10:10:2 whereas, Nur Sabrina *et al*. (2012) used NPK 15:6:4, i.e., with high N, low P and very low K. According to Graham (1983), excess N may encourage fungal pathogens, especially if P or K is low. Since a high N- and low K-fertilizer has been shown to increase *Ganoderma* incidence and severity as opposed to a low N- and high K-fertilizer (Mohd Tayeb *et al*., 2003), fertilizer used in this study may have contributed to the reduction of *Ganoderma* incidence and severity to mask the beneficial effects of micronutrients in this study. This is supported by the AUDPCs of some treatments that were higher than the inoculated control (T2) making it appear effective in disease reduction. The positive lesson to learn from this fact may be that in *Ganoderma* screening tests care should be taken to ensure that the formulation of basic fertilizer will not compromise the expected results, more specifically high N- and low K- or low P - fertilizer should be preferred in such tests.

3.5. Percentage of Dead Seedlings

As early as two months after inoculation (**Table 5**), the B + Cu + Mn Treatment (T9) already had 12.50% of dead seedlings and T2 only 6.25% due to *Ganoderma* infection. All of the inoculated seedlings were still alive in other treatments. Four months after inoculation, the percentage of dead seedlings increased in all treatments with T9 having the highest percent (31.25%). As the observation time proceeded, the number of dead seedlings increased with a little variation in the ranking without statistical differences until the end of the experiment (eight months after inoculation). At that time, the number of dead seedlings reached the highest level (81.25%) in T9. The lowest percentage was recorded with B + Mn (T7) at 56.25%, followed by Cu + Mn (T8) at 62.50%. For this pathological parameter, the trend toward the favourable effect of the double combination of the selected micronutrients in delaying BSR infection was still observed, even though T6 (combination of B + Cu) had 75% dead seedlings at the end of the experiment.



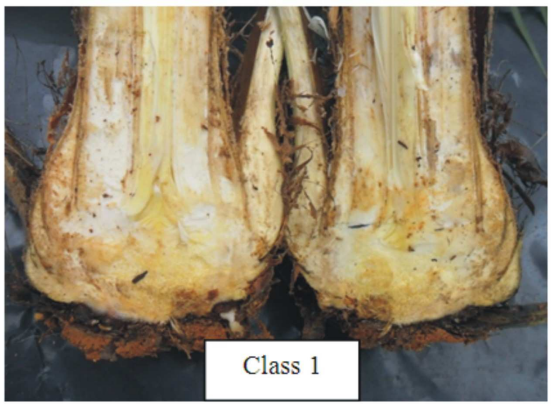


Fig. 5. Illustration of different classes of *G. boninense* internal symptoms (bulb infection) on oil palm seedlings

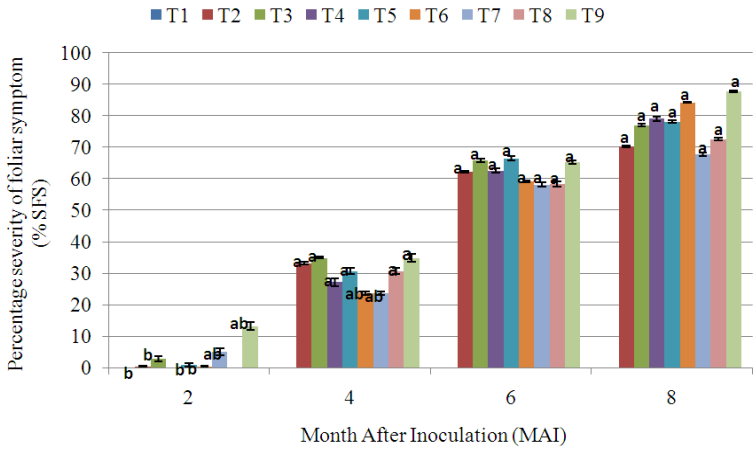


Fig. 6. Percentage severity of foliar symptoms of *G. boninense* on oil palm seedlings supplied with different micronutrient-supplemented fertilizers; Note: Means with the same letter at a given time period are not significantly different according to Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$; values are the means of four replicates. Bars represent standard errors. T1 (Absolute control) = BF without *Ganoderma*; T2 (Inoculated control) = BF + *Ganoderma*; T3 = BF + B + *Ganoderma*; T4 = BF + Cu + *Ganoderma*; T5 = BF + Mn + *Ganoderma*; T6 = BF + B + Cu + *Ganoderma*; T7 = BF + B + Mn + *Ganoderma*; T8 = BF + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*

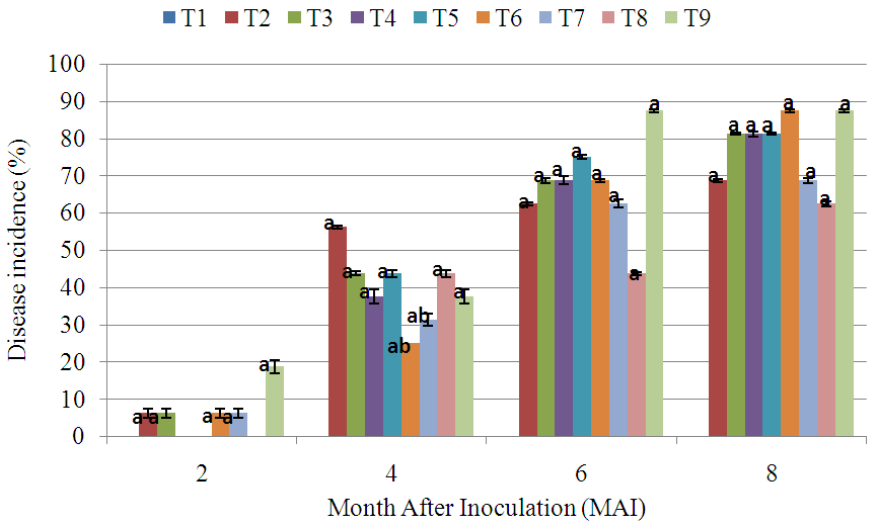


Fig. 7. *Ganoderma* basal stem rot incidence on oil palm seedlings supplied with different micronutrient-supplemented fertilizers; Note: Means with the same letter at a given time period are not significantly different according to Duncan's Multiple Range Test (DMRT) at α = 0.05; values are the means of four replicates. Bars represent standard errors. T1 (Absolute control) = BF without *Ganoderma*; T2 (Inoculated control) = BF + *Ganoderma*; T3 = BF + B + *Ganoderma*; T4 = BF + Cu + *Ganoderma*; T5 = BF + Mn + *Ganoderma*; T6 = BF + B + Cu + *Ganoderma*; v T7 = BF + B + Mn + *Ganoderma*; T8 = BF + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*

Table 2. Classification of *Ganoderma* infection of bulb tissues of oil palm seedlings

Disease class	Symptom description
0	Healthy: no rotting of the bulb tissues
1	Rotted area less than 25% of the bulb area
2	Rotted area between 25 and 50% of the bulb area
3	Rotted area between 51 and 75% of the bulb area
4	Rotted area greater than 75% of the bulb area or total rotting

Source: Adapted from Nur Sabrina *et al*. (2012; Breton *et al*., 2006)

Table 3. Disease severity index for foliar symptoms of different micronutrient-supplemented fertilizer treatments applied to oil palm seedlings inoculated with *G. boninense*

	Disease severity index for foliar symptoms (%)			
Treatment	2 MAI	4 MAI	6 MAI	8 MAI
T1	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.0^{b}	0.00±0.00^{b}
T2	1.56±1.56^{b}	34.38±6.50^{a}	59.38±5.98^{a}	65.63±5.98^{a}
T3	1.56±1.56^{b}	34.38±4.03^{a}	62.50±13.50^{a}	75.00±8.84^{a}
T4	0.00±0.00^{b}	25.00±14.21^{ab}	60.94±12.60^{a}	75.56±14.06^{a}
T5	0.00±0.00^{b}	31.25±12.76^{ab}	68.75±10.82^{a}	78.13±8.27^{a}
T6	1.56±1.56^{b}	18.75±3.61^{ab}	60.94±8.22^{a}	76.56±9.67^{a}
T7	6.25±6.25^{ab}	23.44±8.22^{ab}	57.81±12.85^{a}	60.94±12.60^{a}
T8	0.00±0.00^{b}	29.69±11.80^{ab}	56.25±11.97^{a}	67.19±10.63^{a}
T9	14.06±8.22^{a}	34.38±13.86^{a}	73.44±3.34^{a}	85.94±6.44^{a}

Note: Means with the same letter at a given time period are not significantly different according to Duncan's Multiple Range Test (DMRT) at α = 0.05; values are the means of four replicates ± standard error. T1 (Absolute control) = BF without *Ganoderma*; T2 = BF + *Ganoderma*; T3 = BF + B + *Ganoderma*; T4 = BF + Cu + *Ganoderma*; T5 = BF + Mn + *Ganoderma*; T6 = BF + B + Cu + *Ganoderma*; T7 = BF + B + Mn + *Ganoderma*; T8 = BF + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*.; MAI = month after inoculation

Table 4. Comparative Area under the disease progress curve, disease reduction and epidemic rate of different treatments for the severity of foliar symptoms and disease incidence eight months after inoculation

	Severity of foliar symptoms			Disease incidence		
Treatment	AUDPC	DR	ER	AUDPC	DR	ER
	Unit^2	%	Unit month^{-1}	Unit^2	%	Unit month^{-1}
T2	261.14	-	0.21	312.5	-	0.17
T3	281.18	-7.67	0.25	287.5	8	0.27
T4	258.38	1.06	0.27	293.75	6	0.28
T5	273.11	-4.58	0.26	318.75	-2	0.29
T6	250.27	4.16	0.31	281.25	10	0.34
T7	236	9.63	0.19	262.45	16.02	0.19
T8	250.64	4.02	0.22	237.5	24	0.15
T9	300.7	-15.15	0.32	356.25	-14	0.36

Note: AUDPC = Area under the disease progress curve; DR = Disease reduction; ER = Epidemic rate; T2 (Inoculated control) = BF + *Ganoderma*; T3 = BF + B + *Ganoderma*; T4 = BF + Cu + *Ganoderma*; T5 = BF + Mn + *Ganoderma*; T6 = BF + B + Cu + *Ganoderma*; T7 = BF + B + Mn + *Ganoderma*; T8 = BF + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*

Table 5. Percentage of dead oil palm seedlings recorded in different treatments

	Dead seedlings (%)			
Treatment	2 MAI	4 MAI	6 MAI	8 MAI
T1	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
T2	0.00±0.00^{b}	18.75±6.25^{ab}	56.25±6.25^{a}	62.50±7.22^{a}
T3	0.00±0.00^{b}	25.00±0.00^{ab}	56.25±15.73^{a}	68.75±11.97^{a}
T4	0.00±0.00^{b}	12.50±7.22^{ab}	50.00±10.21^{a}	75.00±10.21^{a}
T5	0.00±0.00^{b}	18.75±11.97^{ab}	62.50±12.50^{a}	68.75±11.97^{a}
T6	0.00±0.00^{b}	12.50±7.22^{ab}	43.75±11.97^{a}	75.00±0.00^{a}
T7	6.25±6.25^{a}	12.50±7.22^{ab}	50.00±10.21^{a}	56.25±11.91^{a}
T8	0.00±0.00^{b}	18.75±11.97^{ab}	50.00±17.68^{a}	62.50±7.22^{a}
T9	12.50±7.22^{ab}	31.25±11.97^{a}	56.25±11.97^{a}	81.25±6.25^{a}

Note: Means with the same letter at a given time period are not significantly different according to Duncan's Multiple Range Test (DMRT) at α = 0.05; values are the means of four replicates ± standard error. T1 (Absolute control)) = BF without *Ganoderma*; T2 (Inoculated control) = BF + *Ganoderma*; T3 = BF + B + *Ganoderma*; T4 = BF + Cu + *Ganoderma*; T5 = BF + Mn + *Ganoderma*; T6 = BF + B + Cu + *Ganoderma*; T7 = BF + B + Mn + *Ganoderma*; T8 = BF + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*; MAI = Month After Inoculation

Table 6. Percentage of infected roots and disease severity index for root symptoms of different treatments eight months after inoculation

Treatment	Percentage of infected roots	Disease severity index for root symptoms
T1	0.00±0.00^{b}	0.00±0.00^{b}
T2	60.92±8.77^{a}	62.50±9.20^{a}
T3	66.95±11.05^{a}	68.75±10.52^{a}
T4	75.31±15.23^{a}	76.56±14.06^{a}
T5	80.16±7.32^{a}	81.25±6.75^{a}
T6	84.26±5.96^{a}	84.38±5.98^{a}
T7	67.54±10.25^{a}	70.31±8.97^{a}
T8	69.47±10.79^{a}	73.44±9.67^{a}
T9	87.50±7.22^{a}	87.50±7.22^{a}

Note: Means with the same letter for a given parameter are not significantly different according to Duncan's Multiple Range Test (DMRT) at α = 0.05; values are the means of four replicates ± standard error. T1 (Absolute control) = BF without *Ganoderma*; T2 (Inoculated control) = BF + *Ganoderma*; T3 = BF + B + *Ganoderma*; T4 = BF + Cu + *Ganoderma*; T5 = BF + Mn + *Ganoderma*; T6 = BF + B + Cu + *Ganoderma*; T7 = BF + B + Mn + *Ganoderma*; T8 = BF + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*

Up to five months after inoculation, differences among treatments in DSIF and the percentage of dead seedlings were significant. Treatment T6 (B + Cu) had the lowest DSIF and the lowest percentage of dead plants and the inoculated control T2 the highest; however, from the seventh month on, there were fewer or no differences observed among treatments for almost all of the parameters evaluated. This could indicate that the micronutrients under study might have some positive effects in reducing *Ganoderma* infection; but that seedlings might have been too young (four-month-old) to withstand high inoculum pressure to suggest that, as mentioned earlier, three months pre-treatment might have not been enough time to allow the development of a strong defence system before inoculation.

3.6. Percentage of Infected Roots and Disease Severity Index for Root Symptoms

Variations in the percentage of infected roots were observed among different treatments (**Table 6**); however, there were no significant differences among the inoculated treatments. It is worth mentioning that the highest percentage of root infection (87.50%) was recorded in T9 and the lowest with T2 (60.90%). This indicates that the addition of B + Cu + Mn to the basic NPK fertilizer predisposed oil palm seedlings to *Ganoderma* infection to thereby cancel any beneficial effect of the individual micronutrient-supplemented fertilizers in mitigating BSR infection. Among the other micronutrient-supplemented treatments, T3 and T7 also resulted in a low percentage of root infection, close to T2, at 66.95 and 67.54%, respectively, suggesting less predisposition with B alone and B + Mn on root infection by *G. boninense*.

The B + Cu treatment (T6) had the highest percentage of bulb tissue decayed, followed by T9, with respectively, 75.83 and 74.92% of the bulb tissues infected. The lowest bulb infection was with B + Mn (T7) at 57.67%. This indicates that the combined B + Mn may predispose less to (or delay) the expansion of *Ganoderma* infection in the bulb while B + Cu, as well as B + Cu + Mn might predispose bulb tissues to easy maceration by this pathogen although none of the treatments were significantly different.

3.7. Bulb Area

The estimation of bulb area (**Table 7**) at the end of the experiment showed that *Ganoderma* infection drastically and significantly ($p \leq 0.05$) reduced the total bulb area compared to the non-inoculated control. The reduction rate was 66.38% between the most infected treatment T9 (6.34 cm^2) and the absolute control T1 (18.86 cm^2). Although the differences observed among inoculated treatments were not significant, treatments T8, T7 and T3 had higher values for total bulb area (8.90, 8.30 and 8.22 cm^2, respectively), stressing once more the positive contribution of combined Cu and Mn, combined B and Mn and B alone relative to BSR on oil palm seedlings. Treatment T9 again exhibited the worst performance (6.34 cm^2), close to that of T4 (6.37 cm^2).

3.8. Percentage of Infected Bulb Tissues and Disease Severity Index for Bulb Symptoms

There was no significant difference observed among inoculated treatments for the percentage of bulb tissues decayed (**Table 8**) but the B + Cu Treatment (T6) had the highest percentage of infected bulb tissues (75.83%), followed by B + Cu + Mn (T9) (74.92%). The treatment B + Mn (T7) had the lowest percentage of bulb tissues infected by *G. boninense* to suggest a delay in disease progress by B + Mn added to the basic fertilizer.

Differences in the Disease Severity Index for Bulb symptoms (DSIB) were not significant, but followed the same trend observed for the percentage of infected bulb tissues whereby the B + Cu treatment (T6) had the highest DSIB (82.81%) followed by T9 (79.69%), while T7 gave the lowest index (62.50%). The same indication of some potential of combined B + Mn to impair *Ganoderma* progress compared to combined B + Cu and B + Cu + Mn can be stated; however, this effect also may be from the B treatment alone. It could also be stated that Cu or Mn tended to increase disease severity.

A positive RWB can reflect the degree of inoculum pressure on oil palm seedlings. Thus, in spite of the high inoculum pressure exerted by *G. boninense* as indicated by almost 100% positive RWB, the B + Mn (T7), Cu + Mn (T8) and, to a slight extent, B + Cu (T6) had less mycelium and fruiting body development compared to the other treatments considered under lower pressure (**Table 9**). This could suggest that T7 (B + Mn) and T8 (Cu + Mn) suppress *G. boninense* better than all the other treatments; however, this was not reflected in significantly reduced disease severity (**Table 8**).

Overall, B, Cu and Mn were shown to influence *Ganoderma* BSR on oil palm seedlings. The effects observed are either synergistic when these elements are applied in double combinations, or antagonistic when they are supplied in triple combination. Taken individually, their effects are not as positive as their combinations.

Table 7. Effects of B, Cu and Mn-supplemented fertilizers on the bulb area of oil palm seedlings eight months after inoculation with *G. boninense*

Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9
Bulb area (cm^2)	18.86±1.53[a]	7.77±1.47[b]	8.22 ±3.37[b]	6.37±2.72[b]	7.74±1.88[b]	7.19±0.68[b]	8.30±1.16[b]	8.90±3.38[b]	6.34±0.93[b]

Note: Means with the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at α = 0.05; values are the means of four replicates ± standard error. T1 (Absolute control) = BF without *Ganoderma*; T2 (Inoculated control) = BF + *Ganoderma*; T3 = BF + B + *Ganoderma*; T4 = BF + Cu + *Ganoderma*; T5 = BF + Mn + *Ganoderma*; T6 = BF + B + Cu + *Ganoderma*; T7 = BF + B + Mn + *Ganoderma*; T8 = BF + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*

Table 8. Percentage of infected bulb tissues and disease severity index for bulb symptoms eight months after inoculation

Treatment	Percentage infected bulb tissue	Disease severity index for bulb symptoms
T1	0.00±0.00[b]	0.00±0.00[b]
T2	64.61±6.24[a]	65.63±5.98 [a]
T3	60.70±13.39 [a]	62.50±12.76 [a]
T4	70.70±15.07 [a]	73.44±14.96 [a]
T5	69.49±8.51 [a]	71.88±8.27 [a]
T6	75.83±5.10 [a]	82.81±5.34 [a]
T7	57.69±12.17 [a]	62.50±12.24 [a]
T8	63.26±11.69 [a]	65.63±11.55 [a]
T9	74.92±4.05 [a]	79.69±5.34[a]

Note: means with the same letter for a given parameter are not significantly different according to Duncan's Multiple Range Test (DMRT) at α = 0.05; values are the means of four replicates ± standard error. T1 (Absolute control) = BF without *Ganoderma*; T2 (Inoculated control) = BF + *Ganoderma*; T3 = BF + B + *Ganoderma*; T4 = BF + Cu + *Ganoderma*; T5 = BF + Mn + *Ganoderma*; T6 = BF + B + Cu + *Ganoderma*; T7 = BF + B + Mn + *Ganoderma*; T8 = BF + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*

Table 9. Summary of fungal structures observed on inoculated seedlings in different treatments and number of positive rubber wood blocks

	Treatment								
Fungal structure	T2	T3	T4	T5	T6	T7	T8	T9	Total
Mycelium	11	13	13	13	14	11	11	14	100 (78.12%)
White button/Basidiomata	6	7	5	7	4	4	5	6	44 (34.37%)
Positive RWB	14	12	14	15	16	15	15	15	116 (90.62%)

Note: Average of 16 oil palm seedlings for each treatment; T2 (Inoculated control) = BF + *Ganoderma*; T3 = BF + B + *Ganoderma*; T4 = BF + Cu + *Ganoderma*; T5 = BF + Mn + *Ganoderma*; T6 = BF + B + Cu + *Ganoderma*; T7 = BF + B + Mn + *Ganoderma*; T8 = BF + Cu + Mn + *Ganoderma*; T9 = BF + B + Cu + Mn + *Ganoderma*

These variations reflect the inconsistency of the effects of micronutrients on disease pointed out by Marschner (2012). The antagonism of the triple combination is quite difficult to explain and shows how complicated and interdependent the interrelationships are between nutrients in the plant system (Ranade-Malvi, 2011). This negative effect of B + Cu + Mn is somewhat similar to the results of Jones and Woltz (1970) who found that Fe + Mn + Zn or Fe + Mn did not nullify the positive effect of liming in reducing *Fusarium* wilt of tomato incited by *Fusarium oxysporum* f. sp. *lycopersici*, while Fe + Zn and Mn + Zn reversed it. They stated that why certain combinations of micronutrients encouraged wilt development whereas others did not is not known.

Boron, Cu and Mn use various mechanisms to control plant diseases. As pointed out by (Graham and Webb, 1991), there is no conclusive evidence which explains how B decreases diseases caused by vascular or soil-borne pathogens. That is, it is difficult to determine at which point in pathogenesis B has an effect. Lignified tissues constitute a physical barrier suggested as a mechanism used by B to inhibit invasion of xylem by pathogen (Lewis, 1980). Other mechanisms include retardation in the movement of fungal hyphae through the cortex (Graham and Webb, 1991), improvement of pectin synthesis or stabilization to strengthen middle lamella and make them suppressive or less conducive to hyphae penetration since many root-infecting pathogens synthesize pectinases (Marschner, 2012). Boron also

reduces some plant diseases thanks to its role in the production of antifungal phenolic/lignin metabolism (Graham and Webb, 1991). Another defence mechanism of B is the formation of papillae to block pathogen penetration. The papillae are said to contain both lignin and callose whose synthesis is influenced by B (Lewis, 1980; Shimomura, 1982; Marschner, 1986; Cadena-Gomez and Nicholson, 1987). In the present study, B in synergy with Mn or Cu may have reduced BSR most probably by retarding *G. boninense* hyphae progression in root cortex and bulb tissues rather than by lignin synthesis (Lewis, 1980) because *G. boninense* is an efficient lignin-degrader. Moreover, from the early results of this study, it appeared that lignin could not be a good criterion to differentiate susceptible and tolerant oil palm progenies.

Copper participates with B in lignification as a plant defence strategy against plant pathogens. Specific mechanisms employed by Cu to reduce plant diseases include direct toxicity to pathogens (Huber, 1989; Evans *et al*., 2007) and enhancement of plant resistance to disease as a regulator or a cofactor of many enzymes involved in plant defence (Graham, 1983; Graham and Webb, 1991). The effect of Cu on pathogen virulence might be the most important mechanism to scrutinise in the present study. Copper is an essential micronutrient for pathogens, plants and animals (Evans *et al*., 2007). White-rotting fungi such as *G. boninense* have Cu-containing enzymes, laccases, among their complex enzymatic system to degrade the wood. Laccases constitute an important virulence factor for many plant-pathogenic fungi which enables them to degrade physical barriers, such as lignin and detoxify phytoalexins and tannins in the infection site (Mayer and Staples, 2002). The detoxification of tannic acid in *Ganoderma* Selective Medium (GSM) is revealed by the brown coloration exploited to confirm the internal colonization of RWB by *G. boninense*. It is also conceivable that in the absence of B and Mn, Cu alone supplied to the soil for plant use was mostly diverted to meet *G. boninense* needs which may explain the poorer performance of treatment T4 (Cu) in this study. On the other hand, in the presence of B or Mn, Cu may have been able to express its defence potentials against *G. boninense*. For the reason mentioned earlier, lignin or lignification is less likely to be efficient to counteract *G. boninense*. In synergy with B or Mn, Cu may have improved the resistance of oil palm seedlings in different ways. The positive effect of Cu + Mn could be explained by synergistic action and improvement of Cu net uptake by Mn as reported by Fageria (2001). Being a component of polyphenol oxidase (Graham and Webb, 1991), Cu involvement in the synthesis of soluble phenols and in their oxidation to toxic quinones such as caffeic quinones to kill *G. boninense* is conceivable, but was not manifest in the RWB. Copper deficiency has a primary effect on the content of phenolic constituents of the cell walls (Robson *et al*., 1981). Phenolic compounds are known to be implicated especially in the resistance of plants to diseases of fungal origin (Graham, 1980). Terpenoids, such as sesquiterpenoids, are another group of secondary metabolites which accumulate in plants in response to Cu treatment (Chmielowska *et al*., 2010). This antimicrobial compound, together with soluble phenolics, should have contributed to reduced *G. boninense* effects on oil palm seedlings, but most parameters for disease were increased by Cu.

Besides its involvement, like B and Cu, in lignin biosynthesis as a defence mechanism, Mn can indirectly act on the pathogen by improving the production of toxic compounds (phenolics, phytoalexins), which inhibit pathogen growth, enzyme production, replication and sporulation (Huber and Wilhelm, 1988; Thompson and Huber, 2007). The oxidation of soluble phenols into oxidized forms toxic to fungi and involved in plant defence is under the control of Mn-containing enzymes (Graham, 1983). The enhancement of plant resistance indirectly through root exudates to modify the root environment, or the modification of important metabolic constituents required for pathogenic activity is also reported (Thompson and Huber, 2007). By activating important biochemical reactions in plant defence, such as the production of phenylalanine ammonia lyase, the first enzyme committed in the phenylpropanoid pathway, Mn increases the deposition of recalcitrant lignin to slow down fungal invasion (Burnell, 1988). The formation of lignitubers was found to halt the development of take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* (Huber and Wilhelm, 1988). The efficiency of lignin or lignitubers in halting fungal invasion could be explained by the incapacity of the pathogen to decompose the lignin component of the barrier. In the present research, this mechanism is less important. Manganese-peroxidase is part of the enzymatic system used by white rot fungi in their wood decaying activity. It is possible that individual Mn added to NPK fertilizer in its reduced form (Mn (II)) was oxidized by *Ganoderma* Mn-oxidizing enzymes, namely Mn-peroxidase, to Mn (IV),

to nullify or reduce its beneficial effect in reducing BSR on oil palm seedlings. The function of Mn oxidation in fungi is believed to primarily involve the depolymerisation of lignin, using Mn (III) as the final redox mediator in the breakup of randomly assembled, enzyme-resistant polyphenolic structures (Thompson and Huber, 2007). The oxidation of Mn is said to be the sole reaction performed by Mn-peroxidase (Thompson and Huber, 2007) and requires reduced Mn as a cofactor (Glenn *et al*., 1986). When Mn-peroxidase oxidizes Mn (II) to Mn (III), the Mn (III) complexed to lactate or other alpha-hydroxy acids is able to oxidize all of the compounds which are oxidized by the enzymatic system (Glenn *et al*., 1986). In this study, Mn in synergy with B or Cu may have been available for oxidation by *Ganoderma* Mn-oxidizing enzymes. From the lower Epidemic Rate (ER) indicative of slower disease development and lower percentage of infected bulb area, among other good performances, observed with B + Mn (T7) and Cu + Mn (T8), it can be postulated that in association with either B or Cu, Mn exerted its influence on host resistance to *G. boninense* by improving the defence system of oil palm seedlings. The direct effect of Mn on *G. boninense* is also possible as observed by (Mortvedt *et al*., 1961; 1963) who found that the amount of Mn absorbed by potato was not the control factor for scab caused by *Streptomyces scabies*, but the direct effect of water soluble soil Mn on the pathogen itself. Furthermore, Mortvedt *et al*. (1961) observed that adding from 2 to 20 mg Mn/kg to the soil surrounding potato tubers reduced scab; however, as shown for most of the treatments and parameters in the present study, they found that the difference required for significance was high but that there was a constant decrease in scab incidence as Mn levels were increased, with 20 mg kg^{-1} giving a scab incidence of zero.

In the previous experiments, Mn appeared to be toxic to oil palm seedlings at 5 mg L^{-1} in solution culture and negatively affected the growth of oil palm seedlings singly or in combination with B and Cu in solution culture at 2 mg L^{-1}. However, in soil, the 2 mg/kg in combination with B or Cu reduced *Ganoderma* infection better than the single micronutrients or their triple combination. Hence, it is believed that additional Mn might not be needed for oil palm with regard to the initial results and other reports (Corley and Tinker, 2003; Goh and Härdter, 2003). The present results showed that the addition of Mn + B or Mn + Cu to fertilizer applied to inoculated-oil palm seedlings may reduce *Ganoderma* incidence and severity slightly. The additional amount of Mn seldom leads to phytotoxicity since it is well known that under acidic and poor drainage conditions, oil palm is able to accumulate up to 800-1000 mg Mn/kg without visible signs of toxicity (Munevar, 2001).

4. CONCLUSION

The results of this study indicate that the double combinations of the selected micronutrients could perform better than the individual nutrients and their triple combination on pathological parameters although none were generally significantly different from the control. Among the double combinations, B + Mn (T7) and Cu + Mn (T8) generally gave better results than B + Cu (T6) for nearly all the parameters investigated. The only individual nutrient which performed well was B with a lower epidemic rate for the percentage or severity of foliar symptoms, lower percentage of infected roots and lower disease severity index for root symptoms, lower disease severity index for infected bulb tissues and higher total bulb area. Subject to confirmative field tests, B + Mn and Cu + Mn, should be considered as combinations to integrate into the oil palm fertilization programme to reduce *Ganoderma* BSR incidence and severity.

5. ACKNOWLEDGEMENT

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