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Influence of pH levels on disease development in oil palm seedling roots infected with *Ganoderma boninensis*

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ABSTRACT

In a quest to find a solution to the problem of Basal Stem Rot (BSR) disease development in oil palm caused by *G. boninensis*, this study hypothesizes that soil pH levels have the potential to suppress the development of *G. boninense* in oil palm seedlings. Three different soil samples were collected; forest area, BSR infected, and uninfected soils from the oil palm plantation areas. Sixty rubber woodblocks were prepared with *G. boninense* inoculum. Calcium carbonate (CaCO3) was used to adjust pH levels into control (pH 4.5), pH 5, 6, and 7, and the results revealed that all treated seedlings showed peak disease incidences at fourth month and severity from weeks 8–12, with pH treated seedlings having fewer infection rates compared to control. Therefore, pH 6 was the most effective to suppress BSR disease development in *Ganoderma* infected roots of oil palm seedlings.

It is a well-known fact that soil pH can affect a range of soil parameters required by plants to healthily and strongly grow and develop thereby given the expected yields (Lie, 1969; Edwards and Scott, 1974; Smiley, 1974). Soil pH can affect disease development, root development, soil microbial activity, the availability of many essential nutrients for plants uptake, chlorophyll, and rate of photosynthesis in plants (Sewards, 2014). The variation in pH can influence the plant's ability to resist pathogen attacks and physiological development. As a soil-plant pathogen, *Ganoderma boninensis* development in oil palm seedlings is affected by soil pH levels (Chong et al., 2017). Oil palm seedlings are highly susceptible to *G.* b*oninensis* growth with high seedlings death and eventually economy loss (Idris, 2009). The *G. boninensis* is a causative agent for Basal Stem Rot (BSR) in oil palm (Idris, 2009). Low pH has been linked with suppression of BSR disease incidence in oil palm for decades (Chong et al., 2017). However, the high pH of soil series is associated with lower BSR incidences (Parthiban et al., 2016). *G. boninensis* has been shown to grow at an optimal pH between 3.7 to 5.0 and temperature between 27 - 30 °C under laboratory conditions (Tan et al., 2016). Suitable pH and light are the ultimate requirements for *Ganoderma* mycelium growth. pH between 6.0 to 7.0 increases most soil microbial activities (Sewards, 2014). However, an optimal pH for oil palms is between 6.5 to 6.8, but can moderately tolerate soil acidity up to a pH level of 4.3 (Akpo et al., 2014). It is indicated that oil palm seedlings grow on a soil pH level between 6.2 and 7.6 (Rosenani et al., 2016). Increasing the soil pH is one of the common methods of controlling the effect of soil acidity on plant and soil. To achieve this, Soil pH is increased by the application of liming materials on the soil surface (Li et al., 2019). The most used liming materials comprise calcium carbonates, hydroxides, and oxides. These liming materials have been established to increase soil pH, decrease soil acidity, increase microbial activities, and availability of nutrients (Nawarathna et al., 2018). Soils with 7.5–8.3 pH levels have high CaCO*3* content, which applies buffers on soil against pH variation by neutralizing the acidity (Li et al., 2019). Pathogenic fungi are sensitive to pH variations specifically those microbes in the root rhizosphere (Alexander et al., 2019). Roots anchored plants into the soil, which are directly involved in the discharge and transport of water and nutrients to other plant segments.

The existing literature has not reported suppression of the ability of pH on *G. boninense* infection through calcium on soil amendments. Following increasing incidences of BSR in Malaysia and its economic implication (Idris, 2009; Paterson, 2019), there is a need to provide effective and feasible measures to suppress their growth and development. Change in pH has the potential to influence the disease development in oil palm seedlings infected with *G. boninensis* (Tan et al., 2016). It can be hypothesized that soil pH levels have the potential to suppress the development of *G. boninense* in oil palm seedlings. Thus, the purpose of this study is to evaluate the influence of soil pH levels under agronomic liming application on suppression of *Ganoderma* development on the roots of oil palm seedlings. This study is not an attempt to determine the mechanism of *G. boninense* infection, but it

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Table 1
Treatment descriptions.

Treatments	Description
PH 4.5 G. b.	Control with *Ganoderma*
PH 4.5 G-	Control without *Ganoderma*
pH 5 G. b.	1 t/ha $CaCO_3$ with *Ganoderma*
pH 5 G-	1 t/ha $CaCO_3$ without *Ganoderma*
pH 6 G. b.	2.25 t/ha $CaCO_3$ with *Ganoderma*
pH 6 G-	2.25 t/ha $CaCO_3$ without *Ganoderma*
pH 7 G. b.	5 t/ha $CaCO_3$ with *Ganoderma*
pH 7 G-	5 t/ha $CaCO_3$ without *Ganoderma*

Table 2
Influence of *Ganoderma* inoculated seedlings on the development of oil palm seedlings roots after three months.

pH Levels	Treatments	Root Length (cm)	Surface area (cm^2)	Root volume (cm^3)	Root tip numbers
4.5	G. b.	443.49^{k}	307.43^{k}	5.73^{gf}	4393.4^{c}
	G-	2454.77^{e}	669.06^{g}	12.33 ^{gf}	15442.8^{abc}
5	G. b.	1384.76^{i}	514.23^{h}	11.52^{g}	15063.6^{abc}
	G-	2054.06^{g}	835.54^{e}	26.00^{b}	17113.2^{ab}
6	G. b.	2355.57^{f}	744.39^{f}	15.28^{e}	13508^{abc}
	G-	3225.70^{a}	1265.99^{a}	17.60^{d}	19825.2^{a}
7	G. b.	1043.96^{k}	307.54^{k}	12.35^{fg}	8385ab^{c}
	G-	2856.38^{b}	969.61^{c}	30.24^{a}	19957.6^{a}

G. b. = inoculated with *Ganoderma*, G- = Non-inoculated with *Ganoderma*. Means with the same letters in a column are not significantly different at $P \leq 0.05$.

focuses on the potential effect of pH on the *Ganoderma* disease development in oil palm and suppression of the spread of BSR in the seedlings.

To test the hypothesis, soil samples were collected from three areas at the United Malacca Berhad in Macap, Malacca, Malaysia; i) forest area, ii) BSR infected and iii) uninfected oil palm plantation area. The areas were located at “N 02° 0.35987 102° 0.2847 E, N 2°.37641 102°.25616 E, and N 02°. 36909 102°.27588 E, respectively. Sixty rubberwood blocks ($6.0 \times 6.0 \times 12.0\ cm^3$) were prepared according to Khairudin (1991).

Soil pH was determined using the glass electrode pH meter (pH M210, 65IR072, France). To adjust the pH, calcium carbonate ($CaCO_3$) was added at 0, 1, 2.5 and 5 t/ha (Table 1) into the soil with or without *Ganoderma.* The effect of different pH levels (control, pH 5, 6, and 7) on the growth of *Ganoderma boninense* and the incidence of BSR disease in oil palm seedlings were tested. The treated pots were thoroughly mixed 15 days before transplanting.

Sixty oil palm seedlings of three months old, commercial GH500 variant (*Dura* × *Pisifera*) placed in trays following normal nursery practices. The seedlings were then placed in a nursery. Watering was done twice daily. After two weeks, the seedlings were transplanted into polyethylene bags (polybags) 30 cm × 38 cm with a thickness of 500 gauges (0.125 mm) bags containing 5 kg soils of either i) BSR infected soil, ii) uninfected soil or iii) forest soil. A factorial randomized complete block design (CRBD) experiment was used with five replications.

The BSR disease development was examined based on quantitative valuation measured as disease incidence (DI) percentage at four-week intervals. The DI denoted the number of seedlings visually evaluated as disease type (leaves necrosis & chlorosis, with or without sporophore production) as described by Campbell and Madden (1990):

$$DI = (\text{Number of infected seedlings/total number of assessed seedlings}) \times 100.$$

The monomolecular model (Monit) was used to obtain the slopes of the curves of DI data transformation (Campbell and Madden, 1990).

The progress of the disease in the seedlings was assessed by disease severity index (DSI). The symptoms were indexed by the following formula, as described by Rakib et al. (2019):

0 = healthy seedlings; 1 = appearance of three necrotic leaves; 2 = appearance of more than three necrotic leaves; 3 = appearance of the fruiting body at the bowl; 4 = dying/dead seedling.

The DSI was calculated at the end of the study (120 days) using the following formula:

$$\text{Disease severity index (DSI)} = \frac{\sum (\text{A} \times \text{B}) \times 100}{\sum \text{B} \times 4}$$

Where: A: disease class (0, 1, 2, 3 or 4).

B: number of seedlings showing disease class per treatment.

The results show significant influence of agronomic application of $CaCO_3$ at 0, 1, 2.5 and 5 t/ha in adjusting soil pH and its alleviating effect on Ganoderma development in oil palm seedlings roots. Overall, the results revealed that the oil palm seedling plants infected with BSR but subjected to pH treatments demonstrated positive response toward root development in seedlings (Table 2). The pH levels significantly ($P < 0.001$) influence the growth and spread of *Ganoderma* disease in oil palm seedlings (Fig. 1). Although, those seedlings uninfected (G-) showed slight better root performance under influence pH as compared to those infected (G. b.). This indicated that pH levels have an influence on the root development of BSR infected oil palm seedlings, since infected seedlings almost catch-up with those healthy seedlings after both were subjected to pH treatments. This result signified that pH treatment improved the ability of non-inoculated (G-) seedlings to withstand the *Ganoderma* spread through the root system (Fig. 1) under agronomic practice. This result is consistent with the previous findings, which indicated that *G. boninense* as a causative pathogen of BSR disease is spread through oil palm root appears as a white rot basidiomycetous fungus (Alexander et al., 2019). The pH levels inhibited the

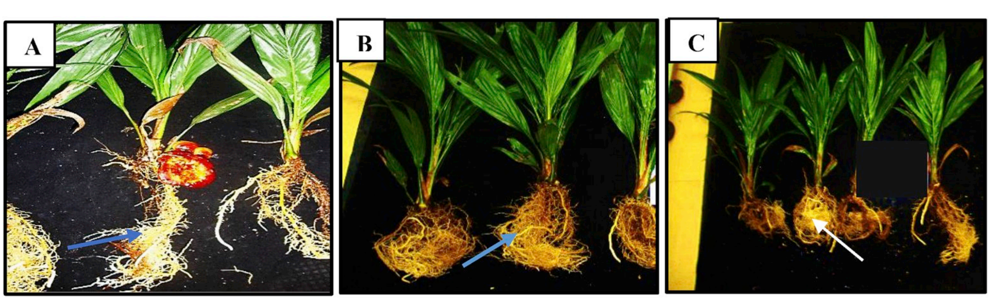

Fig. 1. (A) Roots of inoculated seedlings of BSR infected soil. The picture shows the presence of *Ganoderma* sporocarp and the developed roots after inoculation. The “blue” arrows showed sporocarp attached to the seedling at the base. (B) Roots of inoculated seedlings of uninfected soil. The “white” arrows showed the volume of root development after the inoculation of seedling planted in uninfected soil. (C) Roots of inoculated seedlings of forest soil. The “blue” arrows showed the volume of root development after inoculation. All the three plants in each picture are replicates of the same treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

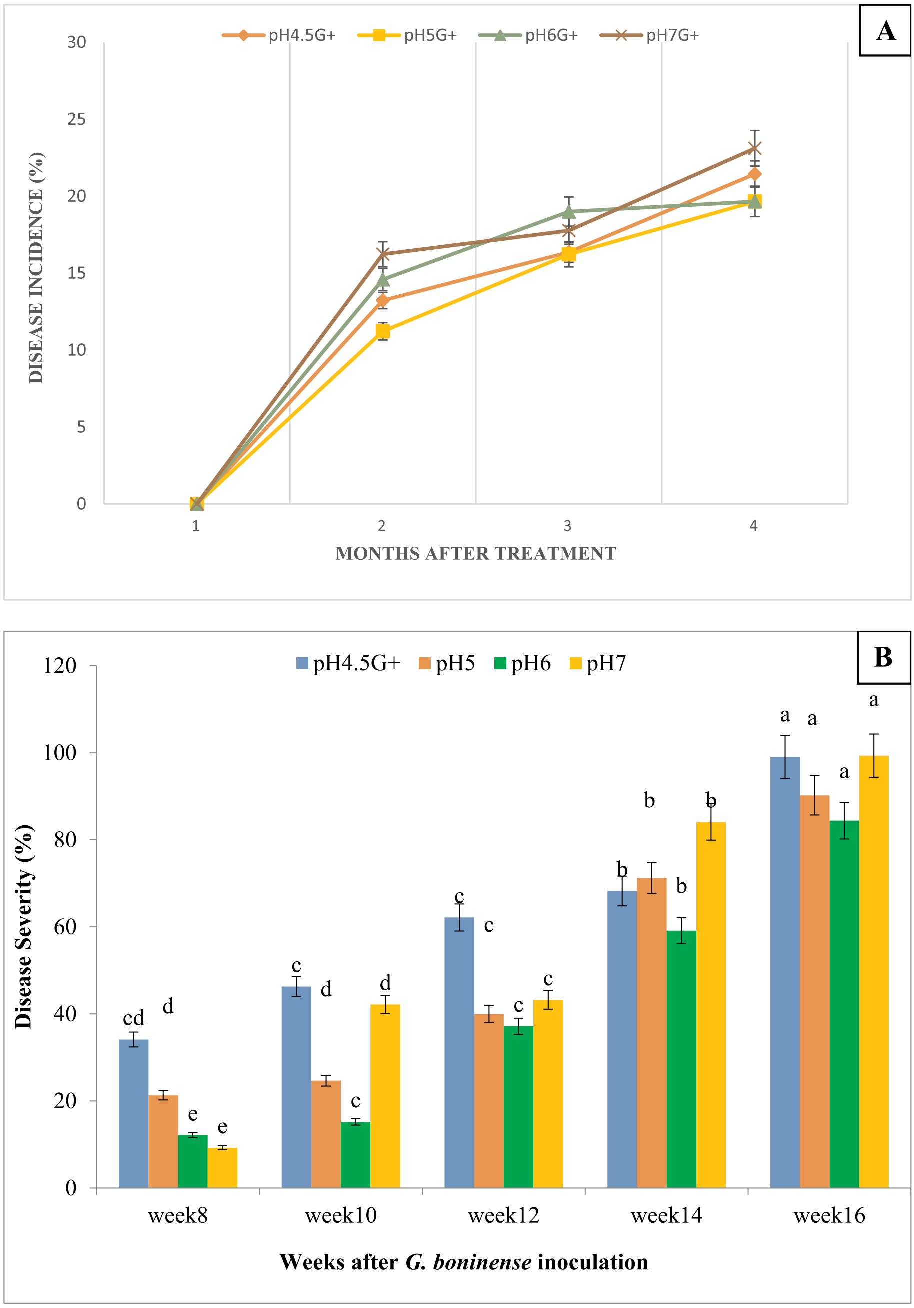

Fig. 2. (A) influence of soil pH levels on disease incidence in *Ganoderma boninense* inoculated oil palm seedlings. (B) soil pH levels on disease severity of *G. boninense* inoculated oil palm seedlings. All means without common superscripts differed significantly at $P \leq 0.05$; n = 5.

transmission process by reducing the spread of infection on the roots and improve the roots biomass yield, this is particularly noted in pH 6 and pH 7 treated seedlings. The pH 6 treated seedlings demonstrated higher improve in root length, surface area, root volume, and root tip numbers in response to *Ganoderma* infection (Table 2). This improvement may be due to reduced infection in pH 6 treated seedlings as a result of the increase in soil microbial activities at this pH level. According to Sewards (2014), root controls the microbial activities that influence plant growth.

However, reduction in root development in pH 5 treated seedlings, especially the inoculated group could be a result of the reduction in microbial activities. Since the pH was moving toward acidic which less favorable microbial activities that breakdown organic matters and make the nutrient available for the seedlings or plants update. This growth reduction effect of pH 5 might be ascribed to the roots-microbial ratio as affected by chemistry at the plant-soil interface (Raaijmakers, 2016), which is one of the common processes and effects of soil pathogenic infection. The pathogens commonly infect oil palm primarily through its root systems (Alexander et al., 2019).

The disease incidence is an indicator of *Ganoderma* infection in oil palms under agronomic practice. The results indicated that there was a significant increase in disease incidence in the first month after seedling planting (Fig. 2). Within the second to third months, all treated seedlings showed no further increase but maintained the attained levels of incidence. However, pH 6 showed a decrease in disease incidence from third to fourth months compared to other pH levels. Preceding this, all the seedlings showed symptoms two weeks after planting; over 95% were infected. The percentage of disease incidence developed much slower in the seedlings treated with pH compared to untreated seedlings (such as control plants). After two months of inoculation and planting, the *Ganoderma* incidence further progresses beyond the level already attained until the fourth month in all pH levels except for pH 6 that showed a decline in the fourth month. Naher et al. (2013) have previously reported the progress of *Ganoderma* incidence after months of infection. Oil palm seedlings die from *Ganoderma* infection a few months after the incidence of the disease (Supramaniam, 2016).

Overall, among all pH treated oil palm seedlings, a narrow window around pH 6 was the most practical agronomic pH for reducing BSR disease development, while maintaining oil palm growth. In the current pilot study, we have successfully shown that pH levels have a significant positive influence on the development of BSR disease caused by *G. boninensis*.

Declaration of competing interest

The authors declare that they have no competing interests.

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