Temporal Changes Analysis of Soil Properties Associated with *Ganoderma boninense* Pat. Infection in Oil Palm Seedlings in a Controlled Environment

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Abstract: Basal stem rot (BSR) disease of oil palm (*Elaeis guineensis* Jacq.) spreads through the contact of the plant roots with *Ganoderma boninense* (*G. boninense*) Pat. inoculum in the soil. The soil properties can be altered by growing seedlings with or without *G. boninense* inoculum. In the early stage of infection, the symptoms are difficult to detect. Therefore, an understanding of the environmental soil conditions of the plant is crucial in order to indicate the presence of the fungus. This paper presents an analysis of the temporal changes of the soil properties associated with the *G. boninense* infection in oil palm seedlings. A total of 40 seedlings aged five months were used in the study, comprising 20 inoculated (infected seedlings: IS) and 20 control (healthy seedlings: HS) seedlings. The seedlings were grown in a greenhouse for six months (24 weeks) under a controlled environmental temperature and humidity. The data of the soil moisture content (MC in %), electrical conductivity (EC in µS/cm), and temperature (T in °C) for each seedling were collected daily using three MEC10 soil sensors every hour and then transferred to the ThingSpeak cloud using a 3G Internet connection. Based on the results, the mean MC and EC showed a decreasing trend, while the mean T showed an increasing trend in both HS and IS during the six-month monitoring period. The overall mean in both the monthly and weekly analysis of MC, EC, and T was higher in HS than IS. However, in the monthly analysis, a Student’s t-test at a 5% significance level showed that only the soil MC and EC were significantly different between HS and IS, while in the weekly analysis, HS was significantly different from IS in all parameters. This study suggests that soil MC, EC, and T can be used as indicators of the *G. boninense* infection, especially for the weekly data.

Keywords: *Ganoderma boninense*; temporal changes; soil properties; soil moisture content; soil electrical conductivity; soil temperature

1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) is the most important commodity crop in Malaysia as it yields millions of tons of crude palm oil per year. Malaysia is the second-largest palm oil producer and exporter in the world (after Indonesia), with approximately 18 million tons exported per year. However, the palm oil industry in Malaysia is threatened by basal stem...
rot (BSR) disease caused by the wood-rotting fungus, *Ganoderma boninense* (*G. boninense*), which reduces oil palm production [1]. According to [2], the yearly loss in Malaysia due to this disease could be up to USD 360 million and it is considered as the biggest threat to the palm oil industry of the country [1].

*G. boninense* is a soil-borne fungus which inoculates the host through soil [3,4]. One of the primary routes of infection appears to be through root contact with inoculum sources in the soil [5–7]. The penetration of intact roots by *G. boninense* occurs before the infection gradually spreads towards the stem base (bole). The further developments of the disease will finally destroy the vascular system and cause symptoms of water and nutrient deficiency [8].

Generally, the survival of a soil-borne fungal infection is significantly related to soil moisture content [9–12], nutrient content [13,14] and temperature [15–17], either directly or indirectly [18]. A high soil moisture content of coastal soils might encourage *G. boninense* to grow [19]. Soils with a poor drainage and a high water retention capacity might favour BSR disease. However, this finding was contradicted in a report by Chang [12] that suggested flooding the infected area as a way to control the disease spread, based on the inability of *G. boninense* to survive in a condition of high soil moisture, which was tested under laboratory conditions. Therefore, it can be concluded that there might be a certain range of soil moisture content that favours the occurrence of *G. boninense* infection. Measuring the soil electrical conductivity (EC) is one of the common methods used to estimate the nutrient content and salinity of the soil. The EC of a soil sample is mostly influenced by the concentration and composition of nutrients in the form of ions [20–22]. EC levels that are too low indicate a low availability of nutrients, and EC levels that are too high indicate an excess of nutrients and/or other ions. Therefore, the EC levels need to be optimal to ensure a good soil fertility. Nutrient content is believed to be associated with the occurrence of the disease where a low soil nutrient might cause a greater abiotic stress to the oil palm seedlings, resulting in the early formation of the fruiting bodies of *G. boninense*, and a high disease incidence at 3 months after inoculation in a nursery [23]. This condition is associated with a low value of EC, while [24] proved that the supplementation of calcium, copper, and salicylic acid in oil palm seedlings inoculated with *G. boninense* inoculum was effective in suppressing the disease. Meanwhile, according to [25], for a plantation application in Malaysia, a non-saline soil (below 0.2 S/m) is most suitably used to plant the oil palm. A high salinity with a high water retention capacity is also associated with a higher BSR incidence of the mature trees in a plantation [26–28]. Under laboratory conditions, Nawawi and Ho [16] reported that *G. boninense* was found to grow at an optimum soil temperature between 27 to 30 °C, while Rees et al. [18] suggested that the infected seedlings grown under a shaded area showed symptoms of infection earlier than infected seedlings grown under direct sunlight. In general, the soil temperature is highly influenced by the intensity of sunlight on the soil surface [29,30].

A few studies involved 22 to 32 weeks of temporal monitoring associated with *G. boninense* infection in oil palm seedlings. Alexander et al. [31] and As’wad et al. [32] investigated the relationship between the rate of the disease spread and the ergosterol concentration in the plants. When a plant is infected, the plant defence mechanism starts to increase the ergosterol content to fight the infection. A higher amount of ergosterol in the first three months indicates that the plant defence mechanisms are activated, revealing that an early infection of the disease has occurred. Idris et al. [33], Rees et al. [7] and Breton et al. [34] observed the progress of the disease penetration through the roots which involved the effect of *G. boninense* inoculum size on the disease occurrence and the appearance of symptoms. The observation of the *G. boninense* invasion rate in the roots, as well as the effect of root injury in boosting the spread of the disease, can be achieved by using transmission electron microscopy (TEM).

Based on the literature, it can be concluded that an understanding of the soil properties of an infected plant might help in detecting the *G. boninense* occurrence at an early stage of infection. Temporal data could provide a further understanding of the progress and spread
of the disease over time [35]. Therefore, this study aimed to analyse the temporal changes of the soil properties associated with G. boninense infection in oil palm seedlings at a weekly and monthly time scale. The soil properties that might have a significant influence on the ecology of the soil fungi, i.e., soil moisture content, electrical conductivity, and temperature were considered. As these parameters generally have a significant effect on the ecology of soil fungi, the conditions of soil planted with Ganoderma-infected seedlings might indicate a slight difference from the soil conditions of uninfected seedlings.

2. Materials and Methods

2.1. Study Area

The study was carried out at the transgenic greenhouse in the Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia. The greenhouse was equipped with an air conditioner, humidifier, metal benches, a drip irrigation system, power supply, water tank, environmental sensor, and sunlight filter. All seedlings were placed on the benches in the greenhouse and grown under a controlled temperature and humidity. The temperature and humidity in the greenhouse were set to normal conditions, i.e., 28 to 32 °C for the environmental temperature and 60 to 90% for the environmental humidity. In total, 50 commercial Tenera (Dura × Pisifera) seedlings at the age of five months were used in the study. Twenty-five seedlings were inoculated with G. boninense inoculum (infected seedlings: IS), while another 25 seedlings were used as control (healthy seedlings: HS). However, only 40 seedlings (i.e., 20 HS and 20 IS) were used in the experiment and monitored as described in Section 2.3 below, while the remaining 10 seedlings were kept in the same greenhouse with the same management operation as a back-up if the seedlings used in the experiment died. The seedlings were infected with G. boninense using the technique proposed by Idris et al. [33] where the roots were attached to a Ganoderma rubber wood block (GRWB), while the roots of the control seedlings were attached to a rubber wood block without Ganoderma inoculum (RWB) using the sitting technique. The sitting technique involved positioning the seedling right on the upper part of the GRWB/RWB in a polybag (24 cm × 21 cm × 33 cm) as shown in Figure 1. GRWB is a rubber wood block colonised with G. boninense inoculum.

Figure 1. Sitting technique: positioning the seedling on the Ganoderma rubber wood block (GRWB)/Rubber wood block (RWB) during transplantation into a polybag.

During the inoculation process, the preparation of the inoculated (infected) and uninoculated (healthy) seedlings were completed in two separate areas. This was to ensure that there was no physical contact between the healthy seedlings with GRWB. After the inoculation procedure, the seedlings were transplanted into 24 cm × 21 cm × 33 cm polybags comprising a mixture of 90% topsoil and 10% sand. The topsoil was taken from the agricultural land of University Agricultural Park, Universiti Putra Malaysia. The soil
used was filtered using a soil sifter and dried under direct sunlight for three days. The seedlings were placed and arranged in the greenhouse based on a randomised complete block design (RCBD). The whole monitoring period of the study was six months (24 weeks). Two months after inoculation, two inoculated seedlings were taken from the back-up seedlings and were sent to the laboratory at the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, for a polymerase chain reaction (PCR) test to confirm the G. boninense infection. The samples were taken from the bole and roots of the seedlings. The specific primer of G. boninense designed by Utomo and Niepold [36]: Gan1: 5′-TTG ACT GGG TTG TAG CTG-3′ and Gan2: 5′-GCG TTA CAT CGC AAT ACA-3′ was used.

The seedlings in the greenhouse were watered using a drip irrigation system. The daily watering was scheduled from 9:10 am to 9:40 am every day with a total amount of 800 mL per seedling. Drippers were placed at three different points in the soil area of each seedling (Figure 2). The distance between each dripper was approximately 15 cm. This was to ensure that the water supplied to the soil surface was evenly distributed. The distance between the dripper and the stem of the seedlings was approximately 8 cm. Each seedling was fertilised every month at week 3, 8, 12, 16, and 21 using 50 g of solid nitrogen (N), phosphorus (P), and potassium (K) (NPK) fertiliser (15:15:15).

![Top view](image)

Figure 2. Position of drippers and MEC10 sensors.

2.2. Physical Growth Measurement

There were four physical parameters measured during the study, namely chlorophyll content, measured using a Chlorophyll Meter SPAD-502 (Konica Minolta, Osaka, Japan); frond count, using manual counting; girth, using digital Vernier callipers (Mitutoyo Digital Calliper, Kanagawa, Japan); and height, using a measuring tape. The measurements were conducted every two weeks over the study period, where the first measurement was conducted immediately after transplanting as the initial growth of the seedlings.

Chlorophyll content was calculated from the average of 6 random readings of the top, middle, and bottom of the fully expanded left and right leaflets of the third frond using a technique proposed by Naidu et al. [37] and Rakib et al. [38]. The frond count was obtained by manually counting the fronds in every seedling. Fronds with more than 50% browning leaflets were excluded from the counting and considered as dry fronds. Girth was the diameter of the seedling stem. The measurement was taken at 2 cm above soil level. The height of the seedlings was measured from 2 cm above soil level to the tip of the highest frond. The highest fronds were identified by gathering all fronds to the centre of the polybag using the hand, and the highest frond appeared taller than the other fronds.
2.3. MEC10 Soil Sensor

A MEC10 soil sensor (Dalian Endeavour Technology Co., Ltd., Dalian, China) was used in this study to measure three soil parameters at a time: soil moisture content in volumetric water content (VWC in %), soil electrical conductivity (EC in µS/cm), and soil temperature (T in °C). It consisted of three stainless steel probes with 7 cm length each. It was a non-corrosive probe and could be continuously embedded into the soil without corroding.

2.4. Data Collection

The data were collected after two weeks of inoculation. Each soil medium in the polybag planted with the seedlings was monitored using three soil sensors simultaneously. The position of the MEC10 sensors was as shown in Figure 3. The probes of the sensors were embedded in the soil at 8 cm depth (Figure 3). There was generally a high concentration of roots in this layer as this was where the plants obtained much of their vital nutrients. The roots of the oil palm are generally not deeply rooted in the soil [39]. The data were collected for each hour every day for 24 weeks. Thus, for every hour, the sensors collected and transferred 120 readings of soil moisture content, soil electrical conductivity, and soil temperature to the ThingSpeak cloud using a 3G Internet connection. Data stored in the cloud were later downloaded for further analysis.

![MEC10 probe sensors buried 8 cm deep in the soil.](image)

**Figure 3.** MEC10 probe sensors buried 8 cm deep in the soil.

2.5. Data Analysis

Comparison of the healthy and infected seedlings was conducted using a paired-sample Student’s t-test available in Microsoft 365 Excel (Version 2108, Microsoft Corp., Redmond, WA, USA). The t-value of interest was the ratio of the mean difference between healthy and infected seedlings, i.e., HS-IS, at either the level of week or month, compared to its standard error.

3. Results and Discussion

Figure 4 shows the condition of the inoculated samples that were sent for a PCR test. Both samples (P1 and P6) did not show any physical symptoms of infection (Figure 4a), such as yellowing leaves. However, the boles showed a brown discolouration (Figure 4b). Based on the result of the PCR test (Figure 4c), a specific band size of approximately 160 to 170 bp was observed from all tested roots after gel electrophoresis, which indicated that the roots were infected with *G. boninense*. 

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*References*:
3.1. Analysis of Physical Properties

Overall, as shown in Figure 5, there was a trend between HS and IS for the (a) chlorophyll content, (b) frond count, (c) girth, and (d) height, where HS was higher most of the time, compared to IS seedlings. Many researchers agree that *G. boninense* infection may cause stunted oil palm seedlings in terms of chlorophyll content [38,40], frond count [37], girth [40,41], and height [41,42]. Stunted growth was typically due to nutrient deficiencies [43] caused by root damage. Other than damaging the roots, the infection also corrupted the xylem pathways and caused water stress.

![Figure 4](attachment:image.png)

**Figure 4.** Sample of infected seedlings: (a) physical condition, (b) brown discoloration at the bole of the seedlings, (c) PCR amplification using specific primer of *G. boninense*.

![Figure 5](attachment:image.png)

**Figure 5.** Cont.

- Chlorophyll content (SPAD)
Figure 5. Comparison of (a) chlorophyll content, (b) frond count, (c) girth, and (d) height between healthy seedlings (HS) and infected seedlings (IS) for every two weeks of monitoring.
3.2. Monthly Analysis

Figure 6 illustrates the changes in the mean of soil MC, EC, and T over six months for both HS and IS. From the graph, MC and EC showed a decreasing trend over six months. However, the trend of the soil T between HS and IS was slightly different. In HS, the soil T showed an increasing trend until the fourth month and slightly decreased at the fifth and sixth month, while, for IS, the soil T showed an increasing trend until the fifth month and slightly decreased in the sixth month.

Figure 6. Mean of soil (a) moisture content (MC), (b) electrical conductivity (EC), and (c) temperature (T) of healthy seedlings (HS) and infected seedlings (IS) over six months.
The decreasing trend in MC indicated that the amount of moisture content in the seedlings decreased as the seedlings aged. Generally, as a seedling or plant grows, the water absorption rate of the larger seedling or plant is higher due to the longer length of the roots [44] and larger root surface area [45], which causes a reduction in soil MC over the course of a month. In addition to the root surface area, the increased leaf surface area and, therefore, the increased transpiration rate also decreases the soil moisture content. In this study, the monitoring process started with 5-month-old seedlings. Therefore, an 11-month-old seedling would consume more water than a 5-month-old seedling. This condition also occurred for the soil EC. The decreased value of EC over the months could also be due to the increasing age of the seedlings. Generally, as a seedling or plant grows older, the rate of nutrient uptake is higher due to the wider spatial distribution of the roots [46] and an older root system age [47], which reduces soil EC over a month. In contrast to T, as the seedlings get older, the temperature increased over a month, except for the last two months in HS and the final month in IS. The increasing trend of soil T could be associated with a decreasing trend of soil MC over a month. Generally, as the MC in the soil decreased, the soil T increased [48]. The decreasing trend in the last two months in HS and in the final month in IS could be due to the shade effects of the canopy of the seedlings. As the seedlings age, the canopy size would become larger and cause the soil surface to be shaded by the canopy, thus resulting in a decrease in soil T. Figure 5 also shows that the MC and EC of HS were slightly higher than IS throughout the whole six months of monitoring. This result agrees with the research findings of Chang [12]. Meanwhile, the lower value of EC in IS could be due to the presence of \textit{G. boninense} inoculum where generally the existence of fungus was reported as having the ability to utilise nutrients that may be available in the soil [49–52]. For soil T, the mean of HS was higher than IS only for the first four months, while in the last two months the mean of IS was higher than HS.

Table 1 shows the mean and \( p \)-value for each of the soil properties obtained using a Student’s \( t \)-test tested at a 5% significant level. Overall, average mean of MC, EC, and T in HS (MC = 30.74%; EC = 142.34 \( \mu \)S/cm; \( T = 30.82 \) °C) was higher than IS (MC = 29.96%; EC = 122.55 \( \mu \)S/cm; \( T = 30.28 \) °C). Based on the \( p \)-value in Table 1, the monthly analysis revealed that only MC and EC data had a significant difference between HS and IS. Therefore, a further analysis (weekly analysis) was undertaken to check if the weekly data could provide a better indicator in differentiating between HS and IS.

Table 1. Mean and \( p \)-value of monthly analysis.

| Parameter | Condition | Mean   | \( p \)-Value |
|-----------|-----------|--------|--------------|
| MC        | HS        | 30.74  | 0.0010 *     |
|           | IS        | 29.96  |              |
| EC        | HS        | 142.34 | 0.0195 *     |
|           | IS        | 122.55 |              |
| T         | HS        | 30.82  | 0.2884       |
|           | IS        | 30.28  |              |

\* Significant at 0.05.

3.3. Weekly Analysis

Figure 7 shows the mean of the soil MC, EC, and T for every week. In general, MC showed a decreasing trend over 24 weeks. In this study, the data for week 9 was not available due to a technical error. Therefore, the data at week 9 was not analysed and mentioned throughout the manuscript. The mean of MC for both HS and IS was higher at the beginning of the monitoring week compared with the end of the monitoring week. The trend of the fluctuation of both HS and IS was similar for the whole 24 weeks of monitoring, where the MC showed an increasing trend from week 1 till week 4 and sharply decreased at week 5. The reason behind this sharp decrease is unknown. This could be due to the old age of the seedlings. It is also believed not to be due to the environmental conditions because the values of the environmental temperature and humidity data did not show a significant difference between weeks 4, 5, and 6. Starting from week 5 onwards, the MC
gradually decreased, although there was slight upward trend particularly in weeks 7, 8, 13, 20, and 21. Overall, the MC for HS was higher than IS for the whole of the monitoring period.

Figure 7. Mean of soil (a) moisture content (MC), (b) electrical conductivity (EC) and (c) temperature (T) of healthy seedlings (HS) and infected seedlings (IS) over 24 weeks.
In general, EC showed a decreasing trend over 24 weeks. However, the mean of the EC values fluctuated five times with the peak values occurring at weeks 4, 10, 13, 17, and 21. The increasing value of EC could be due to the fertiliser application activity completed at weeks 3, 8, 12, 16, and 21. Therefore, the mean of the EC values began to increase on and/or after the weeks in which the fertiliser was applied. An increase in the mean of the EC values was observed in HS and IS in response to the fertiliser application.

During the first 15 weeks of monitoring, the T of HS was significantly higher than IS, while for subsequent weeks, i.e., at week-16 to week-23, the mean of IS was significantly higher than HS. The trend of T for both HS and IS over the 24 weeks was almost similar. In the first 6 weeks of monitoring, the T gradually decreased until it began to rise again at week 7 and continuously increase until week 18. After week 18, the T started to decrease until it reached the temperature values of 30.44 °C and 30.29 °C for HS and IS, respectively. The environmental conditions of the study area were normal, where a high temperature and low humidity occurred during the day and a low temperature and high humidity occurred at night and in the early morning. Based on the ANOVA test, there were no significant differences in the environmental temperature across all of the weeks. Therefore, the rise of the soil temperature after week 11 might not be significantly influenced by the environmental temperature.

Table 2 shows the mean and p-value for each soil property. It is shown that the average mean of MC, EC, and T in HS (MC = 30.72%; EC = 148.18 µS/cm; T = 30.66 °C) was higher than IS (MC = 29.93%; EC = 125.99 µS/cm; T = 30.15 °C). Based on the p-value in Table 2, the weekly analysis revealed that all soil properties had a significant difference between HS and IS.

| Parameter | Condition | Mean  | p-Value   |
|-----------|-----------|-------|-----------|
| MC        | HS        | 30.72 | <0.0001 * |
|           | IS        | 29.93 |           |
| EC        | HS        | 148.18| 0.0004 *  |
|           | IS        | 125.99|           |
| T         | HS        | 30.66 | 0.0338 *  |
|           | IS        | 30.15 |           |

* Significant at 0.05.

4. Conclusions

Generally, for every two weeks of measurement, the physical growth of HS in terms of chlorophyll content, frond count, girth, and height was higher than IS, while the trend of a higher soil MC and EC in HS was mostly consistent during the whole period of the monitoring, except for soil T where the IS was higher during the last third of the monthly and weekly monitoring. Soil MC and EC in both HS and IS showed decreasing trends over the months and weeks. This could because, as the age of the seedlings increased, the rate of water and nutrient uptake was higher, resulting in a decreased mean of MC and EC, respectively. In contrast, the soil T showed an increasing trend over the months and weeks. This could be associated with the decreasing trend of the soil MC where, in general, as the MC in the soil decreased, the soil T increased. Weekly analysis could provide a more detailed analysis especially concerning the effect of the fertiliser application at certain weeks. Based on the p-value, the soil MC and EC between HS and IS were significantly different in both the monthly and weekly analyses, while the soil T between HS and IS was only significantly different in the weekly analysis. In conclusion, this study provided the initial information to reveal the potential use of soil properties as an indicator of BSR occurrence. Moreover, the significant results of detection using weekly data gave promising opportunities for early detection even if there were no visual symptoms appearing on the infected plant. Although the proposed method showed promising results of detection, there is room for improvement. The current method uses three sensors per polybag and the data were collected every hour for every day. Therefore, an abundant amount of data was
used, which was not economical. Thus, for the application under a controlled environment, the possibility of reducing the amount of data should be explored in the future. For an open-environment application, since there were many more uncontrolled variables, other variables that may influence the disease infection needed to be measured, such as soil pH, environmental temperature and humidity. Optimisation techniques could be used to identify significant input variables for disease detection using machine learning.

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