Leaf Nutrient Status in Relation to Severity of Ganoderma Infection in Oil Palm Seedlings Artificially Infected with Ganoderma boninense using Root Inoculation Technique

Nuranis, I*; Kamaruzaman, S**; Khairulmazmi, A*; Mohd Shukri, I*; Zulkifli, H* and Idris, A S*

ABSTRACT
Basal stem rot (BSR) caused by Ganoderma spp., a basidiomycete fungus, is a major devastating disease of oil palm, especially in Malaysia and Indonesia. Several factors were reported to influence the outbreak of BSR disease which include nutrient status, age of palm, types of soil, previous crop and replanting techniques. Elemental nutrient is known to have some beneficial effects on plant disease control. Over many years, macro- and micro-nutrient application has been totally overlooked in oil palm fertiliser programmes in relation to outbreak of BSR disease incidence. A study was conducted to determine leaf nutrient concentrations in relation to severity of Ganoderma infection in oil palm seedlings artificially inoculated with G. boninense using root inoculation technique. This study was conducted at Universiti Putra Malaysia (UPM), Serdang which involved a total of 210 Dura x Pisifera (DXP) oil palm seedlings. After inoculation, external and internal symptoms developing on seedlings and disease severity index (DSI, four disease classes of 0, 1, 2 and 3) were recorded. At 15 months of inoculation, leaf samples were collected and macro-nutrient such as Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) and micro-nutrient such as Copper (Cu), Zinc (Zn), Manganese (Mn), Iron (Fe) and Boron (B) were analysed. Analysis of variance (ANOVA) was performed to test the difference between leaf nutrient concentrations in relation to DSI, followed by comparison means using Least Significant Difference (LSD) test at 0.05 significant levels. A total of 25.0% of inoculated seedlings were dead due to G. boninense infection. Significant difference of leaf nutrient concentration in relation to DSI was observed. Leaf macro-nutrient concentration of N, K, and Ca showed significant difference (p<0.05) while P and Mg showed no significant difference in relation to BSR disease development. Meanwhile, leaf micro-nutrient concentration of Cu, Mn and B showed significant difference (p<0.05) while Zn and Fe showed no significant difference. Among significant nutrients, Ca and Cu were found higher in healthy seedlings (DSI - 0) compared to infected seedlings (DSI – 1, 2 or 3). This study suggested that formulation of fertiliser consists mainly of Ca and Cu are needed in order to reduce BSR disease incidence in oil palms.
INTRODUCTION

The oil palm (Elaeis guineensis) is an important perennial crop in Southeast Asia especially in Malaysia and Indonesia because of its production of palm oil and palm kernel oil. In 2003, the total area planted with oil palm in Malaysia was 5.2 million ha and now increase to 5.39 million ha in 2014 (MPOB, 2014). In Malaysia, basal stem rot (BSR) is the most destructive disease in oil palm where tremendous losses have been reported (Khairudin and Chong, 2008; Arif et al., 2011; Idris et al., 2011; Roslan and Idris, 2012). Various strategies have been recommended and subsequently applied to control the disease as well as to sustain the economic life-span of the palms. Several factors have been reported to influence the outbreak of BSR disease which include nutrient status, age of palm, types of soil, previous crop, and replanting techniques (Ariff et al., 2000; Idris, 2011). Nutrient application for controlling BSR frequently unrecognised but the nutrient has always be an important component in controlling plant diseases. Nutrient application has not been given due consideration in oil palm fertiliser programmes in relation to outbreak of the disease. Imbalanced nutrient application may have weakened the oil palm, leading to BSR disease outbreak in oil palm plantation. Investigation on nutritional elements in oil palm tissues is needed that can eventually be manipulated to control the BSR disease. Therefore, a study was carried out to determine leaf nutrient concentrations in relation to disease severity index (DSI) of BSR disease in oil palm seedlings artificially inoculated with Ganoderma boninense.

MATERIALS AND METHODS

This study was conducted at the Universiti Putra Malaysia (UPM), Serdang, Selangor. A total of 210 oil palm DxP seedlings (15 months old) used in this study were obtained from MPOB Kluang, Johor, where 42 seedlings were not inoculated and 168 seedlings were inoculated with G. boninense using root inoculation technique (RIT) described by Idris et al. (2006), with modification. A small incision was made at the side of the polybag revealing part of the root system. The exposed primary root was then pulled through the opening and washed with water to remove the adhering soil. The distal end of the root was excised and inserted into the rubber wood block (RWB, 3x3x6 cm) fully colonised with G. boninense. This procedure was carried out on two primary roots per seedling. All seedlings were watered daily and applied with NPK Blue fertiliser (12:12:17) monthly.

After 15 months of inoculation (30 months old seedling), all the seedlings were recorded for external and internal symptoms of G. boninense infection. Disease symptoms recorded were foliar symptoms, mycelium or fruiting body, dead palm, and bole/stem and root infection. The seedling was confirmed infected with G. boninense by plating root/stem tissues onto the GSM (Ariffin and Idris, 1992). Based on external and internal symptoms, the disease severity index (DSI of 0, 1, 2 and 3) were formed and detailed description of DSI is presented in Table 1. Each of the seedlings was grouped into four DSI (0, 1, 2 and 3). Fifteen seedlings (total of 60 out of 120 seedlings) for each of the DSI were randomly taken for determination of leaf nutrient concentrations (macronutrient – N, P, K, Ca and Mg and micronutrient - Cu, Zn, Mn, Fe and B). Leaf tissues were collected from frond number 3 or 2 (Roslan and Haniff, 2004; Mohd Tayeb et al., 2003). The samples were oven dried at 60° C for 3 days and then ground into powder and stored. The leaf samples and nutrients were analysed using the methods described by Zulkifli and Masnon (1993).

Statistical analysis was performed using Statistical Analysis System (SAS 9.2). Analysis of variance (ANOVA) was performed to test the...
The data relating external and internal symptoms for each of the seedlings were recorded after 15 months’ inoculation. Using the root inoculation technique, all the inoculated seedlings (168 seedlings) were confirmed infected with *G. boninense*. These were confirmed by plating roots or stems tissues onto the GSM and they yielded *G. boninense*. Therefore, the disease incidence (DI) was 100%. The uninoculated seedlings (42 seedlings) did not show any sign of disease symptom and *G. boninense* was not present. The results of external symptom of *G. boninense* infection in seedlings is presented in Table 2. This symptom included the progressive yellowing of the lower fronds, followed by necrosis, and the leaf then started to desiccate from oldest to the youngest leaves. As the disease progressed, seedlings appeared dry with retarded growth and later died. Formation of white mycelia at the basal stem part and which later developed into small white button and finally into fruiting body (bracket shape). A total of 77.4% of the inoculated seedlings were found producing fruiting bodies of *G. boninense* but none in uninoculated seedlings. The symptoms follow the typical pattern of infection which had previously been described by Khairuddin (1990), Sariah *et al.* (1994), and Idris (1999). The severity of foliar symptoms (SFS) which developed in the inoculated seedlings was 43.3% but none on the uninoculated seedlings. A total of 25.0% of the inoculated seedlings died due to *G. boninense* infection. Destructive sampling was carried at the end of experiment (i.e. 15 months after inoculation of seedlings with *G. boninense*) to determine the extent of decay in the root and bole regions. The roots (27.1%) and stem bulb (20.7%) rotted and decayed with extensive colonisation of fungal masses observed in inoculated seedlings and none in uninoculated seedlings (*Table 3*). The inoculated seedlings produced dry rot of internal tissues in the roots and the base of the stem part. Therefore, water and nutrient uptake was affected and caused limited seedling growth leading to symptoms of yellowing and wilted leaves. It was reported that the pathogen causes severe plugging in the vascular vessels, which may account for the symptoms of impairment of water uptake in infected palms (Ariffin *et al.* 1989; 1991).

### Leaf Nutrient Status in Relation to Severity of *Ganoderma* Infection in Oil Palm Seedlings Artificially Infected with *Ganoderma boninense* using Root Inoculation Technique

**TABLE 1. DESCRIPTION OF DISEASE SEVERITY INDEX (DSI) USED IN THIS STUDY**

<table>
<thead>
<tr>
<th>Disease severity index</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Healthy seedling)</td>
<td>Uninoculated seedlings with no BSR foliar symptom, no fungal mass (white mycelium/fruiting body) and healthy stem (no infection). GSM negative.</td>
</tr>
<tr>
<td>1 (Mild infection)</td>
<td>Inoculated (infected) seedlings with no or low BSR foliar symptom (&lt;10%), appearance of fungal mass (white mycelium/fruiting body) and no or slight stem infection (&lt;5%). GSM positive.</td>
</tr>
<tr>
<td>2 (Moderate infection)</td>
<td>Inoculated (infected) seedling with BSR foliar symptoms (10%-50%), appearance of fungal mass (white mycelium/fruiting body) and moderate stem infection (5%-30%). GSM positive.</td>
</tr>
<tr>
<td>3 (Severe infection)</td>
<td>Inoculated (infected) seedling with BSR foliar symptoms (&gt;50%), appearance of fungal mass (white mycelium/fruiting body) and severe stem infection (&gt;30%). GSM positive.</td>
</tr>
</tbody>
</table>

Note: BSR – basal stem rot; and GSM - *Ganoderma* selective medium.
Besides soil type, nutrient status and sources of nutrient also have a significant influence to the development of the disease (Ariffin et al., 2000). The use of fertiliser, muriate of potash (MOP) and phosphate rock was recorded to increase the disease incidence whereas urea reduced the effects (Singh, 1990). Increase in Ca level has reduced BSR infection as the disease severity was reduced (Nur Sabrina et al., 2012). Low Ca content in the plant tissues could increase the rate of breakdown of the cell wall by *Ganoderma* pathogen as it could attempt to breach and invade the plant cell. Therefore, leaf macronutrient concentration such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in relation to DSI of *G. boninense* infection in oil palm seedlings were investigated. The results are presented in Table 4.

Leaf nutrient concentration of Ca was the most significantly higher in healthy (DSI - 0) compared to mild (DSI - 1), moderate (DSI - 2) and severe (DSI - 3). Whereas for N and K, leaf nutrient concentrations were significantly higher in healthy (DSI - 0) and mild (DSI - 1) compared to moderate (DSI - 2) and severe (DSI - 3). No significant difference among four DSI in leaf nutrient concentration of P and Mg. Among N, P, and Ca, the most critical nutrient in relation to disease severity of *G. boninense* infection in oil palm was Ca. Sugimoto et al. (2008) reported that Ca significantly suppressed disease incidence and delayed the onset of Phytophthora stem rot in soybean. These results indicate that Ca rich areas may be more resistant to invasion by *P. sojae* and that calcium crystals may play an important role in Ca ion storage and its availability to allow the plant tissues to maintain long term field resistance. According to Akai and Fukutomi (1980), Ca may harden plant primary cell walls by cross linking of pectic polymers and confer resistance to pathogen attack. For lignification to be successful, the middle lamella and the cell wall corners must be rich in calcium pectate because these regions are the primary sites of lignification (Lewis and Sarkanen, 1999). Nur Sabrina et al. (2012) reported that Ca increased the production of lignin-related enzyme peroxidase (POD) and laccase and thus amplified lignin production in oil palm. Sufficient Ca content decreases the rate of breakdown in pathogenic disease so that when *G. boninense* attempts to break and invade the plant cell, the barrier reinforces the cell wall. The beneficial effects of Ca include the improvement of the structure of the soil, the

**TABLE 2. RESULTS OF EXTERNAL SYMPTOMS OF DXP SEEDLINGS DUE TO *G. boninense* INFECTION, 15 MONTHS AFTER INOCULATION**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seedlings with white mycelium/fruitsing body presence (%)</th>
<th>Disease incidence (DI)</th>
<th>Severity of foliar symptoms (SFS)</th>
<th>Dead seedlings (DS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXP seedlings inoculated with <em>G. boninense</em></td>
<td>77.4%</td>
<td>100%</td>
<td>4.3%</td>
<td>25.0%</td>
</tr>
<tr>
<td>DXP seedlings uninoculated with <em>G. boninense</em></td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**TABLE 3. RESULTS OF INTERNAL SYMPTOMS OF DXP SEEDLINGS DUE TO *G. boninense* INFECTION, 15 MONTHS AFTER INOCULATION**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Primary roots infected (%)</th>
<th>Stem bulb tissues infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXP seedlings inoculated with <em>G. boninense</em></td>
<td>27.1</td>
<td>20.7</td>
</tr>
<tr>
<td>DXP seedlings uninoculated with <em>G. boninense</em></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
stabilisation of the cell membranes, and an increase the pH of soil. These effects decrease the probability of *G. boninense* to penetrate the oil palm roots.

**Leaf Micro-nutrient Concentration in Relation to Disease Severity Index (DSI)**

Studies on leaf micronutrient such as copper (Cu), zinc (Zn), manganese (Mn), ferum (Fe) and boron (B) in oil palm seedlings in relation to disease severity were investigated. Result was presented in Table 5. Leaf nutrient concentration of Cu was most significantly higher in healthy (DSI - 0) compared to mild (DSI - 1), moderate (DSI - 2) and severe (DSI - 3). Whereas for Mn and B, leaf nutrient concentration was significantly higher in healthy (DSI - 0) and mild (DSI - 1) compared to moderate (DSI - 2) and severe (DSI - 3). No significant difference among four DSI in leaf nutrient concentration of Zn and Fe. Among Cu, Mn, and B, the most critical nutrient in relation to disease severity of *G. boninense* infection in oil palm is Cu. Pilon *et al.* (2006) and RosBarceló (1995) reported that Cu plays an essential role in photosynthesis, respiration, antioxidant activity, cell wall metabolism, hormone perception and the induction of peroxidase (POD). According to Passardi *et al.* (2005), POD is known to be induced by both abiotic and biotic stresses, including heavy metal stress and pathogen attack. POD may play several roles in the plant, such as the functions related to resistance to pathogen. In addition, POD can produce massive amounts of reactive oxygen species (oxidative burst) that are involved in plant cell signaling and that also create a highly toxic environment for pathogens. Moreover, POD is involved in the deposition of materials such as lignin and suberin, which strengthen the cell wall by forming a mechanical barrier against pathogenic agents. Another possible role for Cu-induced POD in plant defence is a direct and intrinsic antifungal activity, which has been reported for POD from several plant sources (Caruso *et al.*, 2001; Ghosh, 2006). Even though Cu is often used as an active ingredient in fungicide, there was no direct

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**TABLE 5. LEAF MACRO-NUTRIENT CONCENTRATIONS IN RELATION TO DISEASE SEVERITY INDEX (DSI) OF SEEDLINGS DUE TO *G. boninense* INFECTION**

<table>
<thead>
<tr>
<th>Disease severity index (DSI)</th>
<th>Leaf nutrient concentrations (%)</th>
<th>Nitrogen (N)</th>
<th>Phosphorus (P)</th>
<th>Potassium (K)</th>
<th>Calcium (Ca)</th>
<th>Magnesium (Mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Healthy seedling)</td>
<td></td>
<td>2.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 (Mild infection)</td>
<td></td>
<td>2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 (Moderate infection)</td>
<td></td>
<td>1.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 (Severe Infection)</td>
<td></td>
<td>1.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means with the same letter within the same column are not significantly different at p<0.05 using Least Significant Difference (LSD).
assessment made on the growth of *Ganoderma* as monitoring was carried out on disease development on inoculated plants. Tengoua *et al.* (2015) reported that combination of B with Mn and Cu in fertiliser programme can be used to reduce BSR incidence.

**CONCLUSION**

The root inoculation technique (RIT) where a rubber wood block (RWB) was used as a substrate for the inoculum, has produced the disease symptoms in oil palms seedlings, thus confirming the pathogenicity of *G. boninense* as the causal agent of the BSR disease. The technique is possible to study the pathogenicity of different *Ganoderma* isolates or species in order to evaluate resistance in planting materials.

The leaf nutrient concentration in relation to disease severity index of DxP seedlings due to infection by *G. boninense* was studied. It was showed that leaf nutrient concentrations of N, K, and Ca were higher in healthy and mildly infected seedlings compared to moderately and severely infected ones while P and Mg showed no significant different in relation to BSR disease. Meanwhile, for micronutrient leaf nutrient concentration of Cu, Mn and B showed significant difference while Zn and Fe showed no significant difference. These information is important in any consideration of BSR disease control strategies through fertiliser programme. Further nursery and field studies are needed to investigate the effect of these nutrients, especially Ca and Cu on development of BSR disease in oil palm and also to determine the best method of application for the management of BSR disease.

**REFERENCES**


