Screening for Plant Extract, Antagonistic Microorganism and Fungicides to Control *Ganoderma Boninense* Caused Stem Rot of Oil Palm in Vitro

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The basal stem rot of oil palm caused by *Ganoderma boninense* has been reported as the severe disease of oil palm in Indonesia and Malaysia (Susanto *et al*., 2005) Africa, Papua New Guinea (Turner, 1981), Colombia (Nieto, 1995). In Thailand, a few studies of oil palm diseases was be reported on surveying by Limsriwilai *et al.* (1984) and Pornsuriya *et al* (2013). Basal stem rot occurred most serious of all diseases affecting the oil palm in southern Thailand. The pathogen attacks the palm at the stem base, the external symptoms do not observe at the early stage when the disease symptom appear, the plant cannot respond to treatment (Najmie *et al*., 2011). The alternative control of disease is various fungical treatment control (Idris *et al*., 2002).and more report of biological control thorough

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Trichoderma spp. (Abdullah et al., 1999; Ilias, 2000; Sariah et al., 2005; Susanto et al., 2005 and Sariah et al., 2005). However, several effective fungicides that high efficient control in vitro was often low or ineffective in field application (Idris et al., 2002 and Susanto et al., 2005). This research was trialed in vitro screening antagonistic microorganism, plant extract and fungicides, to control Ganoderma boninense caused stem rot of oil palm before bring to test on plant at greenhouse condition.

Materials and methods

Screening for plant extract to control Ganoderma boninense in vitro

Pathogen, Ganoderma boninense was isolated from basidiocarp collected on best stem of oil palm growing at surathani province. It was cultured on PDA for 5 days before brought to test with crude plant extract. Plants used in this test were extract from leaves of Antigonon leptopus and Carica papaya; from rhizome of Zingiber montanum, Curcuma longa and Zingiber officinale; and the latex of Carica papaya. Plant leaves and rhizomes of plant above were blended and macerated in sterile distilled water at ratio 500 g of fresh sample/ 500 ml (1 g/ml). After 24 hours, crude extract was filtrated using microfilter micron. Poison m

In vitro screening for antagonistic microorganism control Ganoderma boninense

Soil at rhizosphere of oil palm was collected and isolated by soil surface dilution plate. Different colony of fungi and bacteria were collected to test with pathogen by dual culture technique. Mycelial inhibition percentage were calculated to compare the control efficacy against Ganoderma boninense.

In vitro screening for fungicides to control Ganoderma boninense

Several fungicides distributed at local market was tested at recommendation dosage for efficacy in vitro included azoxystrobine (62.5µg/ml), carbendazim (150µg/ml), chlorothalonil (500µg/ml), copper oxychloride (1275µg/ml), cyproconazole (75µg/ml), dimethomorph+propiconazole (67.5µg/ml), dimethomorph (67.5µg/ml), fluopyrum (100µg/ml), fosetylaluminium (2000µg/ml), hexaconazole (62.5µg/ml), kresoxim methyl (100µg/ml), metalaxyl (150µg/ml),
myclobutanil (50µg/ml), prochloraz (112.5µg/ml), streptomycin (500µg/ml), thianosan (800µg/ml), and tridermorph (562.5µg/ml). PDA poison medium was used to test for mycelial inhibition of this pathogen. Growth inhibition percentage was calculated for control efficacy of those fungicides.

**Screening for plant extract to control Ganoderma boninense in vitro**

Fungal isolation

Plant crude extract from several herb tested on poison medium, *Carica papaya, Antigonon leptopus* crude extract and latex of *Carica papaya* showed a trend to control, but the control efficiency was low mycelium inhibition of 41.26, 24.44 and 9.63 % respectively. While *Allium sativum, Zingiber montanum, Curcuma longa* and *Zingiber officinale* did not affect to mycelium growth (Table 1). Previously research, several plant extract in this test have been reported as the high efficiency control fungal pathogen (Jamkratoke *et al.*, 2004 (Herger and Klingauf, 1990; To-anan, 1985; Tsinis *et al.*, 2006). Suvichayanon (2009) convinced that *Curcuma longa* and inhibited *Pythium aphanidermatum* in vitro. All plant extract in this research showed high efficient control *Pseudoidium nephelii* causing agent of powdery mildew of rambutan *in vitro* test (Srijan and Preecha, 2012), but only crude extract of *Carica papaya* express a trend of control effective against *Ganoderma boninense* in this test.

<table>
<thead>
<tr>
<th>Plant crude extract</th>
<th>mycelium inhibition percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allium sativum</em></td>
<td>0.00d</td>
</tr>
<tr>
<td><em>Antigonon leptopus</em></td>
<td>24.44b</td>
</tr>
<tr>
<td><em>Carica papaya</em></td>
<td>41.26a</td>
</tr>
<tr>
<td><em>Zingiber montanum</em></td>
<td>0.00d</td>
</tr>
<tr>
<td>Latex of <em>Carica papaya</em></td>
<td>0.00d</td>
</tr>
<tr>
<td><em>Curcuma longa</em></td>
<td>9.63c</td>
</tr>
<tr>
<td><em>Zingiber officinale</em></td>
<td>0.00d</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
</tr>
</tbody>
</table>

1/ Means with the same letter are not significantly different at 0.05 DMRT mean comparison (CV 30.35)

**In vitro screening for antagonistic microorganism to control Ganoderma boninense**

Indigenous bacterial from rhizosphere tested by dual culture was founded that only 2 isolates, B001, B002 and B003 was the highest efficacy to inhibit mycelium grow of this pathogen 77.35, 75.13 and 65.93 % respectively (Table 2). They were only 3 out of 22 isolates in this screening which likely control basal stem rot. For the fungi, Isolate T003 showed fair
control efficacy with 51.67 mycelium growth inhibition, T002 and T001 was lightly control this pathogen with low percentage mycelium growth inhibition of 36.11 and 45.56 % (Table3). Three isolates, B001, B002 and B003 showed the potential to be used as biological agent as well as the various antagonist previous reported to be control G. boninense included Trichoderma spp., Aspergillus spp., and Penicillium spp. (Bruce and Highley, 1991; Badalyan et al., 2004).

**Screening for plant extract to control Ganoderma boninense in vitro**

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Table 2 Screening antagonistic bacteria for high potential control *Ganoderma boninense* caused stem rot of oil palm *in vitro*

<table>
<thead>
<tr>
<th>Isolate antagonistic bacterium</th>
<th>Mycelium inhibition percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>B001</td>
<td>77.35&lt;sup&gt;d&lt;/sup&gt;a</td>
</tr>
<tr>
<td>B002</td>
<td>75.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B003</td>
<td>65.93&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>B004</td>
<td>20.63&lt;sup&gt;bcde&lt;/sup&gt;</td>
</tr>
<tr>
<td>BN</td>
<td>17.50&lt;sup&gt;bcde&lt;/sup&gt;</td>
</tr>
<tr>
<td>T001</td>
<td>0.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>T002</td>
<td>0.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>B005</td>
<td>33.57&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B006</td>
<td>26.38&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B007</td>
<td>16.51&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B008</td>
<td>20.54&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B009</td>
<td>30.64&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B010</td>
<td>8.81&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>B011</td>
<td>0.76&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>B012</td>
<td>20.96&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B013</td>
<td>19.80&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B014</td>
<td>10.66&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B015</td>
<td>11.40&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>B016</td>
<td>15.87&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B017</td>
<td>43.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B018</td>
<td>8.81&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>B019</td>
<td>0.76&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>B020</td>
<td>20.96&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B021</td>
<td>19.80&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>B022</td>
<td>10.66&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> = Means with the same letter are not significantly different at 0.05 DMRT mean comparison (CV 47.37%)

Table 3 Screening antagonistic fungi for high potential control *Ganoderma boninense* caused stem rot of oil palm *in vitro*

<table>
<thead>
<tr>
<th>Isolate antagonistic bacterium</th>
<th>Mycelium inhibition percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T001</td>
<td>36.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T002</td>
<td>45.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T003</td>
<td>51.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>1</sup> = Means with the same letter are not significantly different at 0.05 DMRT mean comparison (CV 16.84)

**In vitro screening for fungicides to control *Ganoderma boninense***

Several fungicides sold in the local market was tested *in vitro* by poison medium at the recommend dosage against this pathogen. The result revealed that prochloraz (112.5µg/ml) was excellent control, it inhibited mycelium growth of 96.22%. For the second group were kresaxim methyl (100µg/ml) and chlorothalonil (500µg/ml), with high control efficiency to inhibit mycelium growth of 88.89 and 86.44 %. For difenoconazole (62.5µg/ml) and difenoconazole (62.5µg/ml) were good control efficacy but
they were lower than those fungicides mention above with inhibited mycelium growth of 81.11 and 78.67 % (Table 4). Fungicides in vitro tested in this research, procilaz was the highest control efficency which significant distinguishes from the other. It was reported for the high efficiency to control in postharvest disease of mango (Prusky et al, 2006), avocado (Muirhead et al., 1982; Mavuso and Niekerk, 2014) and papaya (Diczbalis et al., 2014), soil born disease of Phellinus noxius (brown root rot) (Ann et al., 2002) and Fusarium oxysporum f.sp. lycopersici (wilt of tomato) (Amini and Sidovich, 2010).

Table 4 Efficiency of fungicides to control Ganoderma boninense caused stem rot of oil palm in vitro

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Mycelia Inhibition percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoxystrobin (62.5µg/ml)</td>
<td>56.89^a</td>
</tr>
<tr>
<td>Carbenazim (150µg/ml)</td>
<td>62.59^a</td>
</tr>
<tr>
<td>Chlorothalonil (500µg/ml)</td>
<td>86.44^b</td>
</tr>
<tr>
<td>Copper oxychloride (1275µg/ml)</td>
<td>50.00^d</td>
</tr>
<tr>
<td>Cyproflocazole (75µg/ml)</td>
<td>53.33^b</td>
</tr>
<tr>
<td>Difenoconazole (62.5µg/ml)</td>
<td>81.11^f</td>
</tr>
<tr>
<td>Dimethomorph-propiconazole (67.5µg/ml)</td>
<td>50.00^d</td>
</tr>
<tr>
<td>Fosetyl aluminium (2000µg/ml)</td>
<td>50.00^d</td>
</tr>
<tr>
<td>Hexaconazole (62.5µg/ml)</td>
<td>50.00^d</td>
</tr>
<tr>
<td>Kresaxin methyl (100µg/ml)</td>
<td>88.89^b</td>
</tr>
<tr>
<td>Metalaxyl (150µg/ml)</td>
<td>63.15^e</td>
</tr>
<tr>
<td>Myclobutanil (50µg/ml)</td>
<td>50.89^h</td>
</tr>
<tr>
<td>Prochloraz (122.5µg/ml)</td>
<td>96.22^i</td>
</tr>
<tr>
<td>Thianosan (800µg/ml)</td>
<td>58.99^h</td>
</tr>
<tr>
<td>Streptomycin (500µg/ml)</td>
<td>60.93^g</td>
</tr>
<tr>
<td>Tridermorph (562.5µg/ml)</td>
<td>70.56^d</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
</tr>
</tbody>
</table>

^a =Means with the same letter are not significantly different at 0.05 DMRT mean comparison (CV 2.79%)

References


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