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

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Abstract The basal stem rot of oil palm caused by *Ganoderma boninense* has been reported as the severe disease in growing area, a few studies of this disease was reported in Thailand. This research was carried out to screen plant extract, antagonistic microorganism and fungicides for control *Ganoderma boninense* caused stem rot of oil palm in vitro. Plant extract from leaves of *Antigonon leptopus* and *Carica papaya*; rhizome of *Zingiber montanum*, *Curcuma longa* and *Zingiber officinale*; and latex of *Carica papaya* was test by poison media technique. The result showed that all of plant crude extract were not the high effective control of this pathogen. The highest was *Carica papaya* with mycelium growth inhibition 41.26 %. The indigenous antagonistic microorganism isolated from rhizosphere showed the high efficiency to control this pathogen was antagonistic bacterial isolate B001, B002 and B003 and antagonistic fungus isolate T003. For fungicides, in vitro screening, prochloraz, kresoxim methyl and chlorotalonil was the high efficacy control *Ganoderma boninense* with mycelium growth inhibition percentage of 96.22, 98.96 and 88.44 % respectively.

Keywords: basal stem rot, plant extract, antagonistic microorganism, fungicides

Introduction

The basal stem rot 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 fungicidal 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 medium was used to test for mycelial inhibition of this pathogen. Plant crude extract was added 10 ul/ml to PDA before inoculation with 5 mm diameter of pathogen mycelium. Mycelial growth diameter was measured after 5 days incubation at room temperature and inhibition percentage was calculated.

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), hexoconazole (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 1. Efficacy of plant extract at 10 ml/100ml PDA to control *Ganoderma boninense* in vitro

Plant crude extract	mycelium inhibition percentage
<i>Allium sativum</i>	0.00 ^{d1/}
<i>Antigonon leptopus</i>	24.44 ^b
<i>Carica papaya</i>	41.26 ^a
<i>Zingiber montanum</i>	0.00 ^d
Latex of <i>Carica papaya</i>	0.00 ^d
<i>Curcuma longa</i>	9.63 ^c
<i>Zingiber officinale</i>	0.00 ^d
Control	-

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 % (Table 3). Three isolates, B001, B002 and B003 showed the potential to be used as biological agent as well as the various antagonist previously reported to be control *G. boninense* included *Trichoderma* spp., *Aspergillus* spp., and *Penicillium* spp. (Bruce and Highley, 1991; Badalyan *et al.*, 2004).

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

Isolate antagonistic bacterium	Mycelium inhibition percentage
B001	77.35 ^{a1/}
B002	75.13 ^a
B003	65.93 ^{ab}
B004	20.63 ^{cde}
BN	17.50 ^{cde}
T001	0.00 ^e
T002	0.00 ^e
B005	33.57 ^{cd}
B006	26.38 ^{cde}
B007	16.51 ^{cde}
B008	20.54 ^{cde}
B009	30.64 ^{cd}
B010	8.81 ^{de}
B011	0.76 ^e
B012	20.96 ^{cde}
B013	19.80 ^{cde}
B014	10.66 ^{de}
B015	11.40 ^{de}
B016	15.87 ^{cde}
B017	43.20 ^{bc}
B018	8.81 ^{de}
B019	0.76 ^e
B020	20.96 ^{cde}
B021	19.80 ^{cde}
B022	10.66 ^{de}

^{1/} =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*

Isolate antagonistic bacterium	Mycelium inhibition percentage
T001	36.11 ^{b1/}
T002	45.56 ^{ab}
T003	51.67 ^a
Control	-

^{1/} =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, prochloraz was the highest control efficacy 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. *lycopercici* (wilt of tomato) (Amini and Sidovich, 2010).

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

Fungicide	Mycelia Inhibition percentage
Azoxystrobin (62.5µg/ml)	56.89 ^g
Carbendazim(150µg/ml),	62.59 ^e
Chlorothalonil(500µg/ml)	86.44 ^b
Copper oxychloride(1275µg/ml)	50.00 ⁱ
Cyproconazole(75µg/ml)	53.33 ^h
Difenoconazole(62.5µg/ml)	81.11 ^c
Dimethomorph+ propiconazole(67.5µg/ml)	50.00 ⁱ
Difenoconazole(62.5µg/ml)	78.67 ^c
Fosetyl aluminium(2000µg/ml)	50.00 ⁱ
Hexoconazole(62.5µg/ml)	50.00 ⁱ
Kresaxin methyl(100µg/ml)	88.89 ^b
Metalaxyl(150µg/ml)	63.15 ^e
Myclobutanil(50µg/ml)	50.89 ^{hi}
Prochloraz(112.5µg/ml)	96.22 ^{ai/}
Thianosan(800µg/ml)	58.89 ^{gh}
Streptomycin(500µg/ml)	60.93 ^{eg}
Tridemorph(562.5µg/ml)	70.56 ^d
Control	-

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

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