Evaluation of *Chaetomium* - Biological Fungicide to Control *Phytophthora* Stem and Root Rot of Durian

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**ABSTRACT**

Chaetomium-biological fungicide was proved to be effective against *Phytophthora palmivora* causing stem and root rot of Durian (*Durio zibethenus* L.). Bi-culture antagonistic tests proved that *C. cupreum* strain CC6 and *C. globosum* strain CG7 could significantly inhibit the growth of *P. palmivora*. Greenhouse test showed that the mixtures of *C. cupreum* CC6 and *C. globosum* CG7 as biological fungicide completely prevented *P. palmivora* which infested soil planted to durian seedlings which resulting to the reduction of pathogen inoculum and disease incidence. But those seedlings treated with metalaxyl showed lower disease incidence than the non-treated check seedlings. Results of 2-years field experiments proved practical successfully of the integrated biological control of Stem and Root Rot of Durian which introduced to an epidemic area of infested field-soil with *P. palmivora* planted to Durian. It was showed that biofungicide application at every 4 months and followed cultural practices such as organic amendments, changes in soil acidity and removal of diseased plant parts gave the most effective for suppressing *Phytophthora* rot under field condition and significantly higher reduction of the pathogen inoculum and disease incidence than metalaxyl treatment which served as controls.

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1. **Introduction**

Durian (*Durio zibethenus* L.) is one of the tropical fruit trees and the most economically important fruits in Thailand (Economic News, 1995). The big problem have been encountered by durian plantation owners is the lower quality of yield because it is due to a disease epidemic which is caused by *Phytophthora palmivora* (Butler). In the past several decades, the strategies for controlling the disease have been concerned with screening for disease resistance, cultural practices and application of chemical fungicides, which reduced the disease level below economic damage. But for about 40 years of continuous application of chemical fungicides in those epidemic areas, the pathogen, *P. palmivora* has become resistant to the chemical
fungicides (Waterhouse, 1963). Moreover, the hazardous use of heavy chemical fungicides also caused environmental pollution and build up of chemical residues in the air, soil, water and agricultural products. Recently, however, the biological control of plant pathogens has been taking place and it has therefore served as a new strategy for disease control (Soytong, 1995). This successful biological control measure particularly reduce the chemical usage has improved agroecosystem for sustainable agriculture and has maintained ecological balance.

There are many reports about the use of potential microbial antagonists to control plant pathogens. It was found that using the specific strain of C. globosum Kunze could control many plant pathogens, for example seed coating with spores of C. globosum prevented seedling blight of corn caused by Fusarium roseum f. sp.cerealis ’graminearum’ (Chang and Kommedahl, 1968) C. globosum and C. cochlioides were shown to be antagonistic to Fusarium spp. and Helminthosporium spp. (Tveit and Moore, 1954) C. globosum reduced the disease incidence of apple scab caused by Venturia inequalis (Heye and Andrews, 1983; Cullen et al., 1984; Cullen and Andrews, 1984 and Boudreau and Andrews, 1987). Moreover, it was also reported that C. globosum could produce certain antibiotics and release these to suppress the damping-off of sugar-beet caused by Pythium ultimum (Di Pietro et al., 1991) and was antagonistic to Alternaria brassicicola (Vannacci and Harman, 1987), and reduced the inoculum of Botrytis cinerea (Kohl et al., 1995).

The advanced research in biological control of plant pathogens using Chaetomium spp. in Thailand had been conducted since 1989. (Soytong, 1993) Several reports indicated that the specific strains of C. globosum and C. cupreum could significantly reduce the pathogen inoculum and disease level in many economic plants like rice blast (Pyricularia oryzae) (Soytong, 1989), basal stem rot of corn and tomato (Sclerotium rolfsii) and leaf spot of corn (Curvularia lunata). It had also been reported that C. cupreum can control P. oryzae (Soytong, 1995) and F. oxysporum f.sp.lycopersici (Soytong, 1994). Moreover, in many years of research work, it has been noticed that several other strains of C. globosum and C. cupreum have been screened and found to control other economically important plant pathogens like P. palmivora, P. parasitica, and Colletotrichum gloeosporioides(Soytong, 1991).

Undoubtedly, these specific Chaetomium spp. are known for their biological control on some plant pathogens with using spore suspension. Subsequently, this research aimed at evaluating in the field, the effectively of bioproducts formulated as in alginate pellets of Chaetomium spp. to control Phytophthora rot of durian trees.
2. Materials and methods

Isolation of the pathogen and pathogenicity test.

The stem with canker and gummosis, and rotting roots of durian var. Chanee were collected and brought to laboratory for isolation of the pathogen. Thus, root and stem tissue pieces 1-2 cm. long were cut from the advancing edge of lesions, washed and surface-disinfected for 30 seconds in 10 % sodium hypochlorite, followed by three washings in sterilized distilled water. Stem pieces were again cut into thin disk with a sterilized scalpel. Disks were then blotted dry on sterilize paper towels and transferred to Petri dish containing potato dextrose agar (PDA) mixed with BNPR which consisted of Benomyl 10 ug/ml, Nystain 50 ug/ml, Pentachloronitrobenzene 25 ug/ml, Rifampin 10 ug/ml and Amplicillin 500 ug/ml. The mycelia growing out of the stem and root tissues were transferred to PDA and cornmeal agar (CMA) plates and incubated at room temperature (25-27 C) for 10 days. Pathogenicity test was conducted to determine the isolated fungus on 15-months of durian seedling var. Chanee. Sporangial suspension (3x10⁵ sporagia/ml) of P. palmivora isolate was prepared and inoculated to the soil and basal stem of the test plants at the amount of 10 ml/plant. Pathogenicity on the other parts of the plants was also done by inoculation the 0.5 cm. diameter of culture agar plugs into the detached leaves, twigs and fruits. The non-inoculated ones treated with sterile distilled water served as controls. Each was replicated four times. Percentage of disease incidence was measured as number of infected plants/ total number of tested plants x 100, and disease ratings was evaluated as 0= healthy plants, and 3= seriously infected plants.

Bi-culture tests

Specific strains of C. cupreum CC6 and C. globosum CG7 were isolated and screened in previous works (Soytong,1993). Each isolate was individually tested for its ability to inhibit mycelial growth of P. palmivora. Tests were done in vitro using bi-culture antagonistic test that had been described (Soytong, 1989). The percent inhibition of radial growth (PIRG) was calculated for each fungus after incubation of the plates at 27-30 C for 10 days. There were five replications for each fungus combination, with controls consisting of fungus alone on PDA. The experiments were arranged in completely randomized design and the experiment was repeated four times. Analysis of variance was computed on colony diameter data and treatment means were compared using DMRT test at P=0.05 and P=0.01.

Greenhouse test for mycofungicide

The biological products were formulated for testing suppression of basal stem and root rot of durian var. Chanee seedlings in greenhouse tests. The formulation was made from a mixture of C. cupreum
CC6 and *C. globosum* CG7. All durian seedlings used in these tests were inoculated with $3 \times 10^5$ sporagia/ml of *P. palmivora*. Treatments were applied into rhizosphere soil of one-year old durian seedlings in black polyethylene bags (8-12 inches). The treatments were biological products of *Chaetomium* at the rate of 5 g/plant; chemical fungicide, metalaxyl 5% G, 20 g/plant; and sterile distilled water for controls. The experiment was done using RCBD in four replications and repeated 2 times, with 20 seedlings per replication, all treated seedlings were kept and maintained in greenhouse conditions at 27-03°C with average humidity of 80% for 30 days. Percentage of leaf disks colonization, propagules population of *P. palmivora* in each plot, and disease severity data were subjected to analysis of variance to determine the effect of treatments, and treatments means were compared using DMRT test ($P=0.01$). Percentage of disease reduction was calculated as shown in Table 3.

**Field evaluation of mycofungicides**

The 12-year old durian plantation, seriously infested (epidemic area) with *P. palmivora*, was used to evaluate the effectiveness of *Chaetomium* – biological fungicide for 2 years. The total area tested was 2.88 hectares planted to durian trees with plant spacing of 8x8 m. The experiment was done with 144 trees for each treatment. The applied treatments were separately treated as the following: *Chaetomium* pellets, 40 g/plant; metalaxyl 5%G, 40 g/plant. Treatments were repeated at the same rate of application every 4 months. Before the treatments were applied other cultural practices which were integrated, such as weeding under the canopy, adjusting soil acidity (pH) by liming (CaCO$_3$); 5 kg/plant, adding organic compost; 10 kg/plant, pruning and removal of diseased plant parts and improving water drainage. After treating plants with *Chaetomium* and metalaxyl 5%G, they were mulched with organic material, i.e. rice straw etc. and soil moisture content was maintained. The disease incidence was determined before and after the treatment; disease rating is shown in Table 4. It should be noted that the chemical fungicide-treated ones served as control. The disease severity data in each experimental plot was assessed monthly for 2 years during 1994 – 1996. The propagules population of *P. palmivora* in each randomized experimental plot was assessed in three years. Leaf disks colonization of *P. palmivora* in randomized soil sample collection from each plot was also assessed as percentage of colonization. The data were subjected to analyses of variance for RCBD with four replications from two repeated times and to determine the effect of treatments in each period of collected data, and treatment means were compared using DMRT test ($P=0.01$). Percentage of disease reduction in each month was calculated.
Results

Isolation of the pathogen and pathogenicity test

The pathogen that caused stem and root rot of durian cv. Chanee was isolated and identified as *P. palmivora*. The fungal growth on PDA is 7.5 cm diameter within 7 days. Culture was uniformly grew and slightly radiate, star-like pattern. Hyphae were undulating, non-septate, hyline, branched, 2.5 um in width. Sporangiophores were thin, sporae were of various shapes, mostly elongated ellipsoid, ovoid, lemon shapes, 25.40-40.60x50.80-101.60 um, tapering slightly to the stalk, mostly 1-papillae, rarely 2-papilla. Chlamydospores were globose, 30.48 um x 43.18, terminal or intercalary. Durian isolate of *P. palmivora* was highly virulent to durian cv. Chanee, one -year old seedling after 30 days of inoculation with 10 ml/plant sporangial suspension (3x10^5 sporangia/ml). The isolate proved to infected the detached leaves, twigs and fruits.

Table 1. Antagonistic reaction of strains of *Chaetomium* spp. against *Phytophthora palmivora*.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Colony diameter of pathogen (cm) in bi-culture plate</th>
<th>PIRG&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Antagonistic reaction&lt;sup&gt;y&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. cupreum</em></td>
<td>3.17 a&lt;sup&gt;z&lt;/sup&gt;</td>
<td>64.77</td>
<td>3</td>
</tr>
<tr>
<td><em>C. globosum</em></td>
<td>3.30 a</td>
<td>63.33</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>x</sup>Percent inhibition of radial growth = R1 – R2 / R1 x 100, where R1 = colony diameter of pathogen in control plates and R2 = colony diameter of pathogen in bi-culture plate.

<sup>y</sup>Degree of antagonistic reaction, 1 = low antagonistic activity (<50PIRG), 2 = moderate antagonistic activity (50-60 PIRG), 3 = high antagonistic activity (61-75 PIRG) and very high antagonistic activity (>75 PIRG).

<sup>z</sup>Average of four replications, mean within a column having letters in common do not differ statistically (P = 0.05) according to Duncan’s multiple range test, CV = 5.32 %.

Table 2. Disease level in Durian seedlings applied with chemical fungicide and biological product against *Phytophthora palmivora* and its population in greenhouse test.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Disease level&lt;sup&gt;x&lt;/sup&gt;</th>
<th><em>P. palmivora</em></th>
<th>Disease reduction&lt;sup&gt;y&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Propagules (x 10&lt;sup&gt;2&lt;/sup&gt;/soil.g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Colonization (%)</td>
</tr>
<tr>
<td>Chaetomium</td>
<td>1.50 c&lt;sup&gt;z&lt;/sup&gt;</td>
<td>0.47 b</td>
<td>21.00 c</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>2.95 b</td>
<td>0.95 b</td>
<td>32.25 b</td>
</tr>
<tr>
<td>Non-treated</td>
<td>10.42 a</td>
<td>2.14 a</td>
<td>100.00 a</td>
</tr>
</tbody>
</table>
Disease level, 1 = healthy plants, green and waxy leaves, no-defoliation; 2 = green and no waxy leaves, no-defoliation; 3 = defoliation, no waxy and yellowing leaves 1-10 % of the canopy; 4 = defoliation, no waxy and yellowing leaves 11-25 % of the canopy; and 12 = yellowing leaves 100 % of the canopy, rotting roots, die back and plant dead.

Disease reduction (%) = disease level in control plants – disease level in treated plants/ disease level in control plants x 100.

Average of four replications, mean within a column having letters in common do not differ statistically ($P = 0.01$) according to Duncan’s multiple range test, CV = 7.36 %.

Table 3. Diseases level on 12 years old of Durian trees treated with chemical fungicide and biological products against *Phytophthora palmivora* rot and its population in field trials for 3 consecutive years.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Pathogen propagules ($\times 10^3$/soil.g$^{-1}$)</th>
<th>Diseases level$^x$</th>
<th>Disease reduction$^y$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1$^{\text{st}}$ year</td>
<td>2$^{\text{nd}}$ year</td>
<td>3$^{\text{rd}}$ year</td>
</tr>
<tr>
<td>Chaetomium</td>
<td>1.0 a</td>
<td>0.32 b</td>
<td>0.29 b</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>0.9 a</td>
<td>0.38 a</td>
<td>0.38 a</td>
</tr>
<tr>
<td>C.V. %</td>
<td>21.54</td>
<td>5.42</td>
<td>4.20</td>
</tr>
</tbody>
</table>

$^x$ Disease level, 1 = healthy plants, green and waxy leaves, no-defoliation; 2 = green and no waxy leaves, no-defoliation; 3 = defoliation, no waxy and yellowing leaves 1-10 % of the canopy; 4 = defoliation, no waxy and yellowing leaves 11-25 % of the canopy; and 12 = yellowing leaves 100 % of the canopy, rotting roots, die back and plant dead.

$^y$ Disease reduction (%) = disease level in control plants – disease level in treated plants/ disease level in control plants x 100. Chemical fungicide-treated trees served as controls also.

$^z$ Average of four replications, mean within a column having letters in common do not differ statistically ($P = 0.01$) according to Duncan’s multiple range test.
Bi-culture tests

The tested microbial antagonists, *C. globosum* CG and *C. cupreum* CC have been antagonistic to *P. palmivora*. *C. cupreum* CC6 and *C. globosum* CG7 with the inhibition of radial growth of 71.38, 64.77, and 63.33 %, respectively (Table 2).

Greenhouse tests for mycofungicides

Results showed that *Chaetomium* –biological fungicide were significantly reduction of the disease incidence of durian seedlings cv. Chanee inoculated with *P. palmivora* at concentration of 10 ml of sporangia/ml) within 30 d. The application rates of 5 g/plant, significantly reduced the disease incidence to 85.56 %. The treated plants with antagonists were vigorous seedlings with green leaves, without defoliation and no symptoms. However, the plants treated with 20 g/plant of metalaxyl 5%G were observed to be slightly infected with the pathogen, had yellowing leaves of 10 % of the canopy within 30 d. but disease reduction was 71.68 %. Similarly, control seedlings were seriously infected with yellowing leaves, root rotting, defoliation and die back of 26-50 % of the canopy (Table 3).

Field evaluation of mycofungicides

Comparatively for effecting of *Chaetomium* – biological fungicide and chemical metalaxyl 5%G, to control *P. palmivora* causing root rot and stem rot, yielded significantly differences (P=0.01) among treatments. The results were based on reading of disease incidence level of the trees shortly before treatment application, one year after treatments (treatment every 4 months) and after two years. As shown on Table 4, shortly before the treatments, the disease level among experimental plots (36 trees/replication), did not differ significantly. It was in the range of 6-7, that is, showing the yellowing of leaves 51-100 % of the canopy and root rotting. Then one year after treatments, disease level reduction percentage were *Chaetomium* –treated plants, 76.27 and for metalaxyl 5%G treated plants, 70.95. These disease level reduction still did not differ significantly (P=0.01) from previous reading (before treatments) and among each other. But, the second year, disease level reduction reading differ significantly (P=0.01) among treatments and within treatments. Thus, the disease reduction percentage were *Chaetomium* –treated plants, 81.04, significantly different from 76.27 and metalaxyl-treated plants, 70.25, and from 81.04, of *Chaetomium*-treated plants. Surprisingly, the disease level after the experiment, at every set of the experimental unit was lower than before the experiment. On the whole, all treatments on plants including integrated control like cultural practices and controlled soil acidity etc., resulted to weakly the pathogen and promote the growth of antagonistic fungi. The pathogen propagules were significantly reduced in mycofungicides treated plots, resulting to decreasing Phytophthora rot of durian.
3. Discussion

_Phytophthora_ spp. have not only seriously infected durian trees but also other host plants, like black pepper, citrus, longan, betel vine, mango and para rubber for at least 50 years in Thailand (Economic News, 1995). It may indicated that this soil-borne pathogen is the most serious of root disease in Thailand (Lee et al., 1988). The inhibition patterns _in vitro_ tested showed that _C. globosum_ CG, _C. cupreum_ act as slow-growing antagonists, but were also effectively antagonistic to _P. palmivora_.

However, in the slow-growing antagonists like _Chaetomium_ spp., in our study we observed _P. palmivora_ hyphae just in front of the hyphae of _Chaetomium_ spp. were deformed and affected by an antagonistic substance from the _Chaetomium_ hyphae. Other workers claimed that the antagonistic activity from the hyphae of _Chaetomium_ is effectively only when the hyphae of the pathogens were near (Kommedahl and Mew, 1975). This expression of antagonistic activities, implicated antibiotic as a mechanism of biological control. In terms of competitive growth, all tested microbial antagonists grew over the colony of _P. palmivora_ on bi-culture plates.

Greenhouse tests showed that durian seedlings treated with the pellets of _Chaetomium_ as microbial antagonists, were not significantly different in disease level. The treated seedlings were still vigorous and healthy. On the other hand, those seedlings treated with metalaxyl 5%G had significantly higher disease level than antagonist-treated seedlings. The significant difference between antagonist-treated and chemically treated seedlings showed that _Chaetomium_ spp. could suppress the pathogen, _P. palmivora_ in pot experiments which the chemical metalaxyl was unable to aptly control _P. palmivora_. This inability of the chemical to control the pathogen could possibly be due to the pathogen’s becoming resistant to the chemical fungicide. Parallel report of Hinton and Parry (1993) indicating that using _C. globosum_ which were antagonists _in vitro_ for glasshouse tests against eyespot disease of wheat seedlings, (_P. herpatrichoides_), reduced disease symptoms significantly.

Results in our field trials showed the same pattern as other tests. That is, _Chaetomium_ spp. could significantly reduced the pathogen inoculum in the soil and disease incidence in durian trees attacked by _P. palmivora_. However, the chemical metalaxyl 5%G at the allowable amount used, could not reduce disease incidence as effectively significant as that of _Chaetomium_. This means _Chaetomium_ –biological fungicide are better controls than the chemical metalaxyl, even in field conditions. It should be noted in this field trials that integrated cultural practices were combined with these biological fungicide and chemical fungicide treatments, and the seeming effect was an enhancement of the disease control capacity of all treatments. However, the reported works on _Chaetomium_ spp., especially _C. cupreum_ for biological control of plant pathogens had been fewer than _C. globosum_ whether in greenhouse trials and more rarely in the field tests. Among the fewer
reports are strains of *C. globosum* as effective to control the onion white rot caused by *Sclerotium cepivorum* (Harrison and Stewart, 1988) and to control *V. inequalis* (apple scab) (Hey and Andrew, 1983). Evidently from the results of this study and as substantiated with related literature, this study demonstrated the usefulness of a new disease control strategy, not only in greenhouse, but also in field conditions, for *P. palmivora* in potentially fruiting durian trees. This possibility happens through selected antagonistic strains of *Chaetomium* spp. with integrated cultural practices. It should also be noted that in this study, a unique control set was used (metalaxyl-treated group) instead of the usual controls of non-treated batch. This was because the durian plantation owner did not allow non-treated batch due to expensive risks involved, in case the non-treated trees would have very low crop yield.

### 4. References


