Occurrence of Canker Caused by *Xanthomonas axonopodis* pv. *citri* on Pummelo (*Citrus maxima* (Burm.) Merr.) Cultivar. Tabtimsiam in Nakhon Si Thammarat Province, Thailand and Screening Fungicides, Antibiotics and Antagonistic Bacteria against *X. a. pv. citri in Vitro*

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**Abstract**

Pummelo (*Citrus maxima* (Burm.) Merr.) cultivar Tabtimsiam is the new cultivar growing in Pak Panang Basin, Nakhon Si Thammarat, southern of Thailand. It is the one of the most acceptable tropical fruit of consumers. Canker caused by *Xanthomonas axonopodis* pv. *citri* is the most seriously damage especially on fruit. This research was conducted to monitor disease severity and trialed for screening fungicides, antibiotics, and antagonistic bacteria to solve this disease at farmer orchards. The severity of canker monitoring on Tabtimsiam pummelo at 3 - 5 and 6 - 8-year age plant, on leaf was high at 11.85% and 16.37% and mature fruit was 16.15% and 17.12%. The result for controlling agent screening indicated that tetracycline was the highest control of 1.12 cm inhibition zone. It higher than streptomycin and ampicillin of 1.02 and 0.95 cm inhibition zone and also distinguish higher than broad spectrum fungicide, copper hydroxide and copper oxychloride of 0.57 and 0.46 cm inhibition zone respectively. For antagonistic screening, only the one antagonistic bacterial, *Bacillus amyloliquefacien* KPS 46 expressed of secondary metabolite to inhibit *X. a. pv. citri* of 0.14 cm inhibition zone, while *Bacillus subtilis* and *Paenibacillus pabuli* SW01/4 did not express antagonism by produced secondary metabolite to inhibit this pathogen.
1. Introduction

Thailand is the land of plenty of tropical fruit crop. The most popular is rambutan, pummelo, mangosteen and durian. Pummelo is one of the most acceptable of consumers. Pummelo (Citrus maxima (Burm.) Merr.) var. Tabtimsiam is the new cultivar growing in Pak Panang Basin, Nakhon Si Thammarat, southern of Thailand. This cultivar was regarded as the premium quality both domestic and exporting market which sweet, soft red juice. Pummelo was susceptible as well as most citrus which it was severe infected by Xanthomonas axonopodis pv. citri causing agent of canker [1]. Citrus canker was the seriously damage on citrus growing area all over the world [2] [3]. Tabtimsiam Pummelo was grow in Pak Panang, Nakhon Si Thammarat, the tropical monsoon with high precipitation of 2000 - 2500 mm/year [4]. It was suitable condition of spreading of X. a. pv. citri from warm weather and dispersed by splashed rain and wind [5] [6] [7] [8]. The Tabtimsiam pummelo growing in this area was seriously damaged by canker disease. The fruit was infected and symptom appeared due to highly decreasing marketing quality especially in appearance performance. This research objected to study the severity of canker on leaf and mature fruit and screen fungicides, antibiotics and antagonistic bacteria against X. a. pv. citri.

2. Materials and Method

2.1. Occurrence of Canker Disease on Tabtimsiam Pummelo

Canker on Tabtimsiam pummelo growing at Pak Panang Basin, Nakhon Si Thammarat province was evaluated using visual estimation scale modified from Horsfall-Barratt and James [9] [10] [11]. The trees were category into 3 - 5 and 6 - 8-year age plant. Nine trees were sampled for each group to estimate canker severity. Canker disease severity on leaves and mature fruits. Disease severity score on leaf was measurement scale for visual estimation modified based on Horsfall-Barratt [9] [10] [11]. Disease severity was rated into 5 categories: 0, 5, 15, 25%, and 50% infection leaf area. Canker severity on fruit was evaluated using visual estimation scale modified from Domínguez et al. [12]. Fruit infection symptom was rated into 5 groups as well as leaf infected scale. Severity of canker between 3 - 5 and 6 - 8-year age plant compared analysis by Independent Two-Sample T-Test.

2.2. Screening Screen Fungicides, Antibiotics, and Antagonistic Bacteria against X. a. pv. citri in Vitro

Leaf appeared of canker was collected to isolate on Nutrient Agar for 24 h and collected to test pathogenicity by leaf detaching technique. Young lime leaf was
collected by cutting at the leaf stalk, clean with distill water and wrapped the leaf stalk with cotton and placed in petri dish, then dropped sterile water on cotton, *X. a. pv. citri* cultured on Nutrient Broth (NB) for 24 h on rotary checker was diluted to 105 CFU/ml was drop on the pricking leaf. Monitoring canker symptom was done after incubation 7 and 14 days. Pathogenic *X. a. pv. citri* isolate was picked to collect.

Antagonistic bacterial *Bacillus subtilis, Bacillus amyloliquefacien* KPS 46, and *Paenibacillus pabuli* SW01/4 was cultured in NB for 24 h on rotary checker. The suspension was diluted to 0.2 O.D. at 600 nm for using in agar diffusion test.

Fungicides and antibiotic was used at recommended dosage, including copper hydroxide (0.57 mg/ml), copper oxychloride (0.63 mg/ml), ampicillin (0.1 mg/ml), validamycin (0.03 mg/ml), streptomycin (0.1 mg/ml), and tetracycline (0.1 mg/ml).

Pathogen, *X. a. pv. citri* was cultured in NB for 24 h on rotary checker, the suspension was diluted to 0.2 O.D. at 600 nm. Melt NA (200 ml) was leaved until temperature decrease to 40 Celsius in water bath, then mixed with 1 ml *X. a. pv. citri* suspension and poured plate. After agar solidification, wells of 5-mm diameter were cut by cork borer.

Agar diffusion technique was used to screen fungicides, antibiotics, and antagonistic bacteria against *X. a. pv. citri*. Antagonistic bacterial *B. subtilis, B. amyloliquefacien* KPS 46, and *P. pabuli*, 30 ul of suspension prepared above was dropped on well of *X. a. pv. citri* culture prepared above. After incubation for 24 h, inhibition clear zone was measured to compare control efficiency.

Completely Randomized Design was statistical analysis of variance and Duncan’s New Multiple Range Test was mean compare analysis.

### 3. Result

#### 3.1. Occurrence of Canker Disease on Tabtimsiam Pummelo

Disease severity of canker observed on Tabtimsiam pummelo at 3 - 5 and 6 - 8-year age plant showed that severity on leaf was different between 3 - 5 and 6 - 8-year age plant. The severity on leaf at 3 - 5 year was 11.85%. It was lower than at 6 - 8 year of 16.37% (*Table 1*). While severity on fruit was not different between 3 - 5 and 6 - 8-year age plant which severity 16.15% and 17.12% respectively (*Table 1*).

#### 3.2. Screening Fungicides, Antibiotics, and Antagonistic Bacteria

Fungicides, antibiotics, and antagonistic bacteria were screened in vitro to select the high potential to control *X. a. pv. citri* causing agent of canker on Tabtimsiam pummelo by agar diffusion technique. The result indicated that tetracycline was the highest control of 1.12 cm inhibition zone. It was higher than streptomycin and ampicillin of 1.02 and 0.95 cm inhibition zone and also higher than broad spectrum fungicide, copper hydroxide and copper oxychloride of 0.57 and 0.46 cm inhibition zone respectively (*Table 2*). For antagonist screening,
Table 1. Occurrence of canker on leaves and mature fruit of the Tabtimsiam pummelo at 3 - 5 and 6 - 8-year age plant.

<table>
<thead>
<tr>
<th>Canker disease severity and incidence</th>
<th>Age of tree</th>
<th>3 - 5-year (%)</th>
<th>6 - 8-year (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf symptom</td>
<td></td>
<td>11.85b</td>
<td>16.37a1/</td>
</tr>
<tr>
<td>Fruit symptom</td>
<td></td>
<td>16.15</td>
<td>17.12</td>
</tr>
</tbody>
</table>

1/ = Same letters in the same row indicate that values are not significantly different (p > 0.05) with statistical analysis by Independent Two-Sample T-Test.

Table 2. Efficiency of fungicides, antibiotics and antagonistic bacteria to control Xanthomonas axonopodis pv. citri causing agent of canker on Tabtimsiam pummelo in vitro by agar diffusion.

<table>
<thead>
<tr>
<th>Fungicides, antibiotics and antagonistic bacteria</th>
<th>Inhibition diameter (cm)</th>
<th>Inhibition zone (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper hydroxide 0.57 mg/ml</td>
<td>1.07</td>
<td>0.57d1/</td>
</tr>
<tr>
<td>Copperoxychloride (0.63 mg/ml)</td>
<td>0.96</td>
<td>0.46e</td>
</tr>
<tr>
<td>Ampicillin (0.1 mg/ml)</td>
<td>1.45</td>
<td>0.95c</td>
</tr>
<tr>
<td>Validamycin (0.03 mg/ml)</td>
<td>0.50</td>
<td>0.00h</td>
</tr>
<tr>
<td>Streptomycin (0.1 mg/ml)</td>
<td>1.52</td>
<td>1.02b</td>
</tr>
<tr>
<td>Tetracycline (0.1 mg/ml)</td>
<td>1.62</td>
<td>1.12a</td>
</tr>
<tr>
<td>Bacillus subtilis (10^5 CFU)</td>
<td>0.50</td>
<td>0.00g</td>
</tr>
<tr>
<td>Bacillus amyloliquefacien KPS 46 (10^5 CFU)</td>
<td>0.64</td>
<td>0.14f</td>
</tr>
<tr>
<td>Paenibacillus pabuli SW01/4 (10^5 CFU)</td>
<td>0.50</td>
<td>0.00g</td>
</tr>
<tr>
<td>Control</td>
<td>0.50</td>
<td>0.00g</td>
</tr>
</tbody>
</table>

1/ = Same letters in the same column indicate that values are not significantly different (p > 0.05), mean compared by DMRT.

only the one antagonistic bacterial, B. amyloliquefacien KPS 46 expressed of secondary metabolite to inhibit X. a. pv. citri of 0.14 cm inhibition zone, while B. subtilis and P. pabuli SW01/4 did not express antagonism by produced secondary metabolite to inhibit this pathogen.

4. Discussion

Disease severity of canker of Tabtimsiam pummelo at 3 - 5 and 6 - 8-year age plant on leaf was high at 11.85% and 16.37% and mature fruit (Table 1) was 16.15% and 17.12% respectively (Table 1). The result convince that this cultivar was susceptible to X. a. pv. citri and environment was enhanced more severity, which high of precipitation and high temperature. It was higher than 2000 mm/year [4]. This pathogen X. axonopodis pv. citri was favor by high temperature and humidity [5] [7] [8] [13]. More factors to initiated disease severity were growing infected seedling and epidemic of leaf miner (Phyllocnistis citrella) [14] [15] [16]. In some case, it increased canker severity up to 50% [17].
Disease symptom on fruit was threatened for market acceptable both domestic and exporting. Canker scare fruit was accepted by cut fruit market only, but price dropped from 6 USD/fruit to 3 USD/fruit. It was not exporting. To reduce severe fruit damage copper compound was applied by farmers. It seemed was not reduce severity. Antibiotics, streptomycin and tetracycline were the highest efficient control this disease. In this screening in vitro revealed that both streptomycin and tetracycline were higher distinguishing than copper compound. It was consistent efficient control similar to previous trial [18] [19] [20] [21]. For sustainable control antagonistic bacterial B. amyloliquifaciens KPS 46 recovered by Kasem [22] was expressed to inhibit X. a. pv. citri. This bacterial antagonist was tested and developed to control both bacterial diseases and fungal diseases. It has been several reports of production secondary for inhibition mode and stimulate plant by induce systemic resistance and systemic acquire resistance [23] [24] [25]. However, both antibiotic and antagonist should be test in farmer or cardboard before transfer to the farmers in this case.

References


Agr Sci (Scientific Meeting), 13th December 2005. Hanoi Agricultural University (HAU), Hanoi.