A Study of Integrated Diseases Management in Northern Part of Thailand

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Introduction of Royal Project Foundation
The Royal Project Foundation (RPF)

- Initiated by His Majesty, King Bhumipol Adulyadej in 1996.
- The aim of solving the problems on deforestation, poverty and opium production by promoting alternation crops.
Aims of Royal Project Foundation

To help the hill tribes for humanitarianism.

To help the nation by reducing the destruction of natural resources in terms of forest and watershed.

To stop opium poppy cultivation.
Setting up Royal Project Development Centers (RPDCs):

In 6 northern provinces of Thailand: Chiang Mai, Chiang Rai, Mae Hong Son, Lamphun, Phayao and Tak

- 4 Royal Agricultural Stations (RASs)
- 35 RPDCs
Activity Plans of RPF

To conserve soil and make proper use of land, that is to avoid the encroachment of cultivated fields upon forest areas.

To produce cash crops for the benefit of the Thai economy.
Test
- Variety
- Cultivation technology
- Disease & pest control
- Harvesting and packaging
RPF’s crop production systems:

• GAP = Good Agricultural Practice
• Global GAP = GAP at the world standard
• Organic = No chemicals use in the whole crop production system
Results of the RPF’s activity

solving the problems of poverty and opium production by promoting alternation crops.

“It was the world’s first project to replace drug-crops with legal crops and is one of the most successful project of this type.”
Fruits production at the northern part of Thailand

Temperate fruits

Tropical and sub tropical fruits

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Pitaya is an important crop fruit tree growth in many province in northern part such as Chiang Mai, Chiang Rai and Phayao provinces.
Pitaya planting plots at the northern part of Thailand

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At fruiting stage

10,000-15,000 kg/rai
Or
62.5-93.73 tons/hec

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Major diseases of Pitaya

- Suhi-Aromna (2015) reported that there were 3 major diseases of pitaya in Thailand such as:
  - **anthracnose** caused by *C. gloeosporioides* and *C. truncatun*, the pathogens were infected stems and fruits,
  - **fruit rot** caused by *Bipolaris cactivora* (Oeurn et al., 2015), the damage were found on stems and fruits, and
  - **brown spot** or stem canker caused by *Neoscytalidium dimidiatum* (Thongkam and Soytong, 2016) was found giving severe damage pitaya crop production.

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• In Thailand, Chemical pesticides are commonly used to control diseases in the planting plot such as copper oxychloride, azoxystrobin, difenoconazole, propiconazole, procloraz and mancozeb (Sudhi-Aromna, 2015).

• At present, the consumers in the whole world realized the danger of pesticide residues in agricultural produces. The farmers must try to was less chemical pesticides for safety to the consumers, themselves and the environment.

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Integrated Pest Management (IPM)

- IPM is the best way to control pests and diseases in the crop productions.
- IPM is the main axis of all production systems (GAP, GlobalGAP, and Organic), soil, water, seeds and seedlings must be well managed in advance to prevent the plants from getting diseases, keeping the planting plots clean, frequently observe the pests and saving natural enemies are included in IPM.
The objectives of this study

• to introduce the control techniques on pitaya diseases
• in the field: Stem rot caused by *Botryosphaeria dothidea* and
• post-harvest disease (fruit rot caused by *Bipolaris cactivora*)
• by using bio-pesticide and substitute chemicals in northern part of Thailand.
Materials and methods

1. Survey and collecting diseased samples
2. Experiment of fruit rot disease
3. Experiment of stem rot disease
4. IPM method in the fields
1. Survey and collecting plant diseased

A survey of major diseases of pitaya fruit at major planting areas which are in the highland of the northern part was conducted in 2014-2015. The infected plants were collected from 3 provinces including Chiang Mai, Chiang Rai and Phayao provinces.
In this research focus on fruit rot and stem rot diseases, to use chemicals substitute and bio-pesticides for controlling both diseases.
2. Experiment of fruit rot disease

2.1 Collecting, Isolation and Pathogenicity test

2.2 Inoculation method

2.3 Efficacy test: Effect of wood vinegar on mycelium inhibition and spore germination of fruit rot pathogen

2.4 Effect of wood vinegar on control of fruit rot disease in pitaya fruit
2. Experiment of fruit rot disease (continued)

2.1 Collecting samples, Isolation and Pathogenicity test

A fruit rot disease observed in white-fleshed pitaya which collected from the fresh market in Chiang Mai city.
Morphology of \textit{B. cactovora} causal agent of pitaya fruit rot: (A) disease symptom on fruit, (B) colonies of conidia on fruit, (C) characteristic of pathogen colony on PDA at 7 days, (D) mycelia and conidia, and (E and F) of pathogen under light microscope at 400x magnification.

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2.2 Inoculation method and Pathogenicity test

Using 3 methods:

1. **Spraying** with spore suspension of *B. cactivora on the fruit*.

2. **Dropping** with spore suspension of *B. cactivora on the fruit*.

3. **Placing** with culture disc of *B. cactivora* on the fruit with wound and no wound on fruit.
The fruit rot disease revealed on the fruit which **sprayed with spore suspension of** *B. cactivora* **at 4 days after inoculation**: (C1) control treatment: no wound and sprayed with sterile water, (C2) wound and sprayed with sterile water, (T1) no wound and sprayed with spore suspension and (T2) wound and sprayed with spore suspension.

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The fruit rot disease revealed on the fruit which dropping with spore suspension of *B. cactivora* at 4 days after inoculation: (C1) control treatment: no lesion and sprayed with sterile water, and (T2) wound and sprayed with spore suspension.

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The fruit rot disease revealed on the fruit which placed culture disc of *B. cactivora* at 4 days after inoculation: (C1) control treatment: placed PDA disc on fruit, and (T2) placed culture disc on fruit.

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2.3 Efficacy test: Effect of wood vinegar on mycelium inhibition and spore germination of fruit rot pathogen

- wood vinegar from para rubber wood and eucalyptus wood.

- Poison media prepared with mixed each concentrations of wood vinegar in PDA at 4 concentrations as 0.5, 1.0, 1.5, and 2.0% (v/v).

- Mycelia discs (0.5 mm diameters) from the peripheral region of a 7 day *B. cactovora* culture which grown on PDA, and transferred onto the poison plates (9 cm) and PDA plate (control treatment)
2.3 Efficacy test

Table 1 Effect of wood vinegar on mycelia inhibition of fruit rot pathogen

<table>
<thead>
<tr>
<th>Kinds of wood vinegar</th>
<th>Isolates of fungi</th>
<th>Percentage of mycelia inhibition(^1)/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 %</td>
</tr>
<tr>
<td>Para rubber</td>
<td>BcDFW_2</td>
<td>42.78fghi</td>
</tr>
<tr>
<td></td>
<td>BcDFW_3</td>
<td>39.26hij</td>
</tr>
<tr>
<td></td>
<td>BcDFW_4</td>
<td>36.67hijk</td>
</tr>
<tr>
<td></td>
<td>BcDFW_5</td>
<td>36.67hijk</td>
</tr>
<tr>
<td>eucalyptus</td>
<td>BcDFW_2</td>
<td>34.44ijk</td>
</tr>
<tr>
<td></td>
<td>BcDFW_3</td>
<td>30.37jkl</td>
</tr>
<tr>
<td></td>
<td>BcDFW_4</td>
<td>27.78kl</td>
</tr>
<tr>
<td></td>
<td>BcDFW_5</td>
<td>22.96l</td>
</tr>
</tbody>
</table>

LSD0.05 10.54

%CV 9.68

\(^1\) 3 replications/treatment
\(^2\) Means followed by the same letter in each column are not statistically different by LSD (\(p=0.05\))
### Para rubber wood vinegar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>0.5 %</th>
<th>1.0 %</th>
<th>1.5 %</th>
<th>2.0 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BcDFW_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BcDFW_3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BcDFW_4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BcDFW_5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sutasinee Nontajak (Ph.D)
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<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.5 %</th>
<th>1.0 %</th>
<th>1.5 %</th>
<th>2.0 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BcDFW_2</td>
<td><img src="BcDFW_2" alt="Image" /></td>
<td><img src="BcDFW_2" alt="Image" /></td>
<td><img src="BcDFW_2" alt="Image" /></td>
<td><img src="BcDFW_2" alt="Image" /></td>
<td><img src="BcDFW_2" alt="Image" /></td>
</tr>
<tr>
<td>BcDFW_3</td>
<td><img src="BcDFW_3" alt="Image" /></td>
<td><img src="BcDFW_3" alt="Image" /></td>
<td><img src="BcDFW_3" alt="Image" /></td>
<td><img src="BcDFW_3" alt="Image" /></td>
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<tr>
<td>BcDFW_4</td>
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<tr>
<td>BcDFW_5</td>
<td><img src="BcDFW_5" alt="Image" /></td>
<td><img src="BcDFW_5" alt="Image" /></td>
<td><img src="BcDFW_5" alt="Image" /></td>
<td><img src="BcDFW_5" alt="Image" /></td>
<td><img src="BcDFW_5" alt="Image" /></td>
</tr>
</tbody>
</table>

**Eucalyptus wood vinegar**

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Table 2  Effect of wood vinegar from para rubber wood and eucalyptus wood vinegar on inhibition of spore germination of *B. cactivora*, on PDA at 7 days after incubation.

<table>
<thead>
<tr>
<th>Kinds of wood vinegar</th>
<th>Concentration of wood vinegar</th>
<th>Percentage of inhibition (^1) (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Para rubber</td>
<td>0.5</td>
<td>100a(^2)</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100a</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>100a</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>100a</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>0.5</td>
<td>46.47b</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100a</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>100a</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>100a</td>
</tr>
<tr>
<td>LSD0.05</td>
<td></td>
<td>18.92</td>
</tr>
<tr>
<td>%CV</td>
<td></td>
<td>13.89</td>
</tr>
</tbody>
</table>

\(^1\) 3 replications/treatment

\(^2\) Means followed by the same letter in each column are not statistically different by LSD (\(p=0.05\))
Table 3  Effect of wood vinegar from para rubber wood and eucalyptus wood vinegar on inhibition of germ tube of *B. cactivora*, on PDA at 3, 6, 9, 12 and 24 hr after incubation.

<table>
<thead>
<tr>
<th>Kind of wood vinegar</th>
<th>Concentration of wood vinegar (%)</th>
<th>Time to measurement of germ tube (µm)$^{1/}$</th>
<th>3 hr</th>
<th>6 hr</th>
<th>9 hr</th>
<th>12 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>23.84a$^2/$</td>
<td>92.63a</td>
<td>358.17a</td>
<td>610.25a</td>
<td>∞$^3/$</td>
</tr>
<tr>
<td>Para rubber</td>
<td>0.5</td>
<td></td>
<td>0.00c</td>
<td>0.00d</td>
<td>43.87d</td>
<td>99.16d</td>
<td>230.33</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td></td>
<td>0.00c</td>
<td>0.00d</td>
<td>0.00f</td>
<td>0.00f</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td></td>
<td>0.00c</td>
<td>0.00d</td>
<td>0.00f</td>
<td>0.00f</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td></td>
<td>0.00c</td>
<td>0.00d</td>
<td>0.00f</td>
<td>0.00f</td>
<td>0.00</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>0.5</td>
<td></td>
<td>20.88b</td>
<td>88.25b</td>
<td>258.69b</td>
<td>284.47b</td>
<td>∞</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td></td>
<td>0.00c</td>
<td>34.2c</td>
<td>94.71c</td>
<td>124.27c</td>
<td>200.86</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td></td>
<td>0.00c</td>
<td>0.00d</td>
<td>26.63e</td>
<td>43.84e</td>
<td>71.68</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td></td>
<td>0.00c</td>
<td>0.00d</td>
<td>0.00f</td>
<td>0.00f</td>
<td>0.00</td>
</tr>
<tr>
<td>LSD0.05</td>
<td></td>
<td></td>
<td>0.43</td>
<td>0.64</td>
<td>1.30</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>%CV</td>
<td></td>
<td></td>
<td>6.01</td>
<td>1.86</td>
<td>1.03</td>
<td>0.56</td>
<td></td>
</tr>
</tbody>
</table>

1/ average of 3 replications/treatment 
2/ Means followed by the different letter in each column are significant different by LSD ($p=0.05$) 
3/ ∞ Length of germ tube cannot measurement
2.4 Effect of wood vinegar on controlling fruit rot disease in pitaya fruit

The healthy pitaya fruit was sterilized by surface disinfectant with 70% ethanol and air-dried before application. The experiment was done by 10 treatments as showed in the table 4.

Table 4 Conditions of an effect on wood vinegar on control fruit rot disease at laboratory

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Soaked in sterile water</td>
</tr>
<tr>
<td>C2</td>
<td>Soaked in sterile water + placed pathogen</td>
</tr>
<tr>
<td>C3</td>
<td>Soaked in 1.0% of wood vinegar for 1 min</td>
</tr>
<tr>
<td>C4</td>
<td>Soaked in 1.0% of wood vinegar for 3 min</td>
</tr>
<tr>
<td>C5</td>
<td>Soaked in 1.5% of wood vinegar for 1 min</td>
</tr>
<tr>
<td>C6</td>
<td>Soaked in 1.3% of wood vinegar for 3 min</td>
</tr>
<tr>
<td>T1</td>
<td>Soaked in 1.0% of wood vinegar for 1 min + placed pathogen</td>
</tr>
<tr>
<td>T2</td>
<td>Soaked in 1.0% of wood vinegar for 3 min + placed pathogen</td>
</tr>
<tr>
<td>T3</td>
<td>Soaked in 1.5% of wood vinegar for 1 min + placed pathogen</td>
</tr>
<tr>
<td>T4</td>
<td>Soaked in 1.5% of wood vinegar for 3 min + placed pathogen</td>
</tr>
</tbody>
</table>

1/4 replications/treatment

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2.4 Effect of wood vinegar on control fruit rot disease in pitaya fruit (continued)

Table 5 Effect on para rubber wood vinegar at concentration of 1.0 and 1.5% (v/v) fruit for 1 and 3 min on controlling fruit rot disease in pitaya fruit.

<table>
<thead>
<tr>
<th>Concentration of wood vinegar</th>
<th>Soaking time</th>
<th>Percentage of inhibition1/</th>
<th>BcDFW_2</th>
<th>BcDFW_2</th>
<th>BcDFW_2</th>
<th>BcDFW_2</th>
<th>Average3/</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1</td>
<td>100a 2/</td>
<td>68.75a</td>
<td>92.86a</td>
<td>79.17a</td>
<td>85.20a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>94.53ab</td>
<td>91.67a</td>
<td>89.29a</td>
<td>93.06a</td>
<td>92.14a</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>1</td>
<td>100a</td>
<td>77.08a</td>
<td>78.57a</td>
<td>93.06a</td>
<td>87.18a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>84.37b</td>
<td>100a</td>
<td>71.43a</td>
<td>75.70a</td>
<td>82.88a</td>
<td></td>
</tr>
<tr>
<td>LSD0.05</td>
<td></td>
<td>15.02</td>
<td>35.45</td>
<td>46.14</td>
<td>50.84</td>
<td>16.95</td>
<td></td>
</tr>
<tr>
<td>%CV</td>
<td></td>
<td>10.29</td>
<td>27.27</td>
<td>36.07</td>
<td>38.71</td>
<td>12.67</td>
<td></td>
</tr>
</tbody>
</table>

1/4 replications/treatment
2/ Means followed by the same letter in each column are not statistically different by LSD (p=0.05)
3/Average of 4 isolates of the B. cactivora
Fruit rot diseases on fruits which soaked in para rubber wood vinegar at concentration of 1 and 1.5% for 1 and 3 minutes respectively at 7 day after inoculation with *Bipolaris cactivora* (C1) Control treatment (soaked in sterile water and no inoculation) and (C2) Control Treatment (soaked in sterile water and inoculation)

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3. Experiment of stem rot disease

3.1 Collecting and Isolation
3.2 Dual culture test

Using dual culture technique: Antagonistic activity of SOL-G3 (*Bacillus amyloliquefaciens* subsp. *plantarum*), bio-product was obtain from Plant Protection Center (PPC) research project supported by TaiwanICDF (2014-2019), to test on efficacy of mycelial inhibition.
Fig 6  Antagonistic activity of SOL-G3 (*Bacillus amyloliquefaciens* subsp. *plantarum*) against *Botryosphaeria dothidea* causal agent of pitaya stem rot disease on PDA plate at 6 days after incubation.
4. IPM method in the fields

- Choosing disease free cuttings to grow the plant.
- Soil improvement with compost, adjust pH, and using antagonistic fungi: *Trichoderma* sp. to mix with soil and spraying spore suspension to the plants.

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Training on IPM: Soil management

The farmers who grow fruit crops attended the training on how to prepare Trichoderma in the compost mixed with rice bran and molasses.

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Training on IPM: Making medicinal plant extract

The farmers attended the training on how to prepare medicinal herbs extract fermented (PP1 = protect plants diseases and killer sucking insects); herbs mixed with rice bran and molasses.

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IPM: crop management

- Planting together with other crops
IPM: crop management (continued)

- Using coconut peel cover the ground stem base to protect loss of soil moisture
Using wood vinegar for chasing insect pests and controlling diseases

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Conclusions

• Using substitute chemicals as pyroligneous acid or wood vinegar at concentrations soaking the fruits in wood vinegar solution at concentration of 1% (v/v) for 3 minutes was effective for reduction of fruit rot disease of at 92.14% which is significantly at 95% level (LSD p=0.05).

• Using the antagonistic bacterium, SOL-G3 (Bacillus amyloliquefaciens subsp. plantarum) inhibited growth rate of Botryosphaeria dothidea caused of stem rot on laboratory condition.
Conclusions

• For this research, the Integrated Pest Management (IPM) was used for controlling pests and diseases in the planting plots and the use of bio-pesticides and substitute chemicals to reduce chemical pesticides was also practiced.

• Using wood vinegar to be used in IPM for control of plant pests and diseases in field, it was found that the treated plants showed less damages (still running experiment)
Any question!
Thank you for your attentions!