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Potential of Plant Extracts for Controlling Citrus Canker of Lime

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ABSTRACT

Five plant extracts of *Hibiscus subdariffa* Linn., *Psidium guajava* Linn., *Punica granatum* Linn., *Spondias pinnata* (Linn.f.)Kurz, and *Tamarindus indica* Linn. were evaluated for control of canker disease on *Citrus aurantifolia* (lime) caused by *Xanthomonas axonopodis* pv. *citri* (XC) (synonym *X. campestris* pv. *citri*) under greenhouse condition. Aqueous extracts of *H. subdariffa*, *P. granatum*, *S. pinnata*, and *T. indica* exhibited an inhibitory effect against XC lime strain Xci12 by reducing canker incidence vary from 18% to 52%. The extracts from *T. indica* was the most effective control of citrus canker which disease incidence was 48% by one spray after leaf puncture inoculation whereas the control was 100 %. In field experiment with natural infection of XC on lime, the aqueous extract of *T. indica* reduced number of diseased leaves. There was significantly difference in mean disease incidence between sprayed (3.59%) or not sprayed (9.46%) lime ($P< 0.05$).

Key word: plant extract, citrus canker, control, *Xanthomonas axonopodis* pv. *citri*, lime, *Citrus aurantifolia*

INTRODUCTION

Citrus canker, caused by *Xanthomonas axonopodis* pv. *citri* (XC) (synonym *X. campestris* pv. *citri*), is a serious disease reducing the external quality of citrus fruits. It affects all types of citrus and severely infects on *Citrus aurantifolia* (lime). Canker occurs in all areas where lime grows in Thailand. Typical symptom on leaves is a raised necrotic lesions surrounding with yellow halo but on fruit and stem halo seldom occurs. Control of the disease requires integrated cultural practices and chemical sprays. Copper compound products are recommended for canker control. One major limitation of using chemical control agents is that phytopathogenic bacteria frequently develop a resistance to these compounds (Sigee,1993). In recent year much interest have been developed in the antimicrobial effects of medicinal plants for plant disease control. Some plant extracts were reported as effective inhibitors of phytopathogenic bacterial growth and XC was also suppressed by plant extracts (Leksomboon *et al*,1998; Leksomboon *et al*,2000; Garden *et al*,1978; Grainge and Alvarez,1987). Plant extracts are of interest as an alternative source of natural pesticide for controlling of plant pests. The objective of this investigation was to evaluate the potential control of lime canker by plant extracts.

MATERIALS AND METHODS

Plant extracts preparation

Extracts from five plants, *Hibiscus subdariffa* Linn., *Psidium guajava* Linn., *Punica granatum* Linn., *Spondias pinnata* (Linn.f.)Kurz, and

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Tamarindus indica Linn., were used in this study (Table1). An aqueous crude extract was prepared as follows: 200g of fresh leaves were ground in 1,000 ml of sterile distilled water with a homogenizer. The homogenate was passed through two layers of cheese-cloth and the filtrate was store at 4 C in refrigerator for inhibition assay. For other plant parts, 200g or 100g of fresh or dry samples, respectively were soaked in 1,000 ml of sterile distilled water for 6 hr and then passed through the sieve. The supernatant was used for assaying inhibition activity against bacterial citrus canker both under greenhouse and field experiment conditions.

Evaluation of plant extracts in a greenhouse

Seeds of *Citrus aurantifolia* (lime) were sown in sterile soil and each seedling was transplanted to a pot of 6 inches in diameter. The 6 months-old plants were sprayed with plant extract at 30 min after inoculation. The control treatment was done by inoculated with a suspension of *Xanthomonas axonopodis* pv. *citri* without treated with plant extract. To prepare the inoculum, the XC strain Xci12 isolated from lime was grown on nutrient glucose agar (NGA) at 28 °C for 24 hr and cells were suspended in sterile distilled water to a concentration of 10^8 CFU/ml by using Spectrophotometer. Lime leaves were inoculated by leaf puncture method. Immediately after pricking the leaves with sterile sharp needle, the inoculum of fresh XC was dropped approximately 5µl per spot. Two spots per each leaf side were positive and negative inoculations (Figure 1). Five leaves per plant and ten plants were used for each plant extract. The tested plants were grown in a greenhouse with the mean temperature of 30 °C. Percentage of disease incidence was determined at 14 days after inoculation. Difference in disease incidence was evaluated by analysis of variance and the means were compared for significance by the least significant difference (LSD).

Effect of *Tamarindus indica* extract on lime bacterial canker

T. indica aqueous extract used to investigate efficacy for control of lime bacterial canker under field conditions was selectively performed at Department of Plant Pathology, Kasetsart University, Kamphaengsaen campus, Nakhon Pathom province. The experiment was conducted on 3 year-old lime plant grown from seedlings. The *T. indica* extract was sprayed on lime plants at weekly interval for 6 times from 6 July to 20 August 2000 with a rate of 500 ml per plant and control was nontreated. Treated and nontreated controls were arranged in a completely randomized design with four replications. To determine efficacy of plant extract, leaves were collected at one week after the last application and assessed for disease incidence (percentage of diseased leaf or infected leaf showing canker symptoms).

Table 1 Medicinal plants used for extraction to control lime citrus canker (*Xanthomonas axonopodis* pv. *citri*) in this study.

Botanical name	Common name	Family	Plant part
Hibiscus subdariffa Linn.	Roselle	Malvaceae	Dry flower
Psidium guajava Linn.	Guava	Myrtaceae	Fresh leaf
Punica granatum Linn.	Pomegranate	Punicaceae	Dry fruit skin
Spondias pinnata (Linn.f.) Kurz	Hog plum	Anacardiaceae	Fresh leaf
Tamarindus indica Linn.	Tamarind	Leguminosae	Fruit pulp

RESULTS

Evaluation of plant extracts in a greenhouse

In greenhouse experiments, aqueous extracts of *H. subdariffa* , *P. granatum* , *S. pinnata* , and *T. indica* reduced canker disease incidence in lime when spray over inoculated lime leaf (Figure 1) whereas *P. guajava* produced no disease reduction occurred (Table 2). Leaves of lime grown in pots treated with *T. indica* extract showed the least canker disease with 48 % of disease incidence compare with the water control showed 100 % of disease incidence. Furthermore, a lesser disease occurred significantly in pots treated with plant extracts of *T. indica* than in pots treated with *H. subdariffa, P. granatum, S. pinnata* and *P.guajava* which was no effective in reducing disease symptom (Table 2).

Effect of *Tamarindus indica* extract on lime bacterial canker

Under field condition, lime plants (*Citrus aurantifolia*) at 3 year-old showed that the aqueous extract of *T. indica* reduced the disease incidence. Application of lime plants at 1-week interval with *T. indica* aqueous crude extract for 6 times was 3.59% disease incidence compared to 9.46 % in nontreated control (Table 3). These values were significantly different (P<0.05).

DISCUSSION

Investigation on efficacy of plant extracts for the effective control of citrus canker was conducted on lime plant both under greenhouse and field conditions. In greenhouse, the incidence of canker was determined 14 days after inoculation in which the inoculated leaf showed 100% infection showing typical canker symptom. The canker lesion number was recorded and analysis of the percentage of disease incidence between the treated with each plant extract and nontreated control. Among the plant extracts tested, *T. indica* was the most effective showing only 48 % disease incidence which was

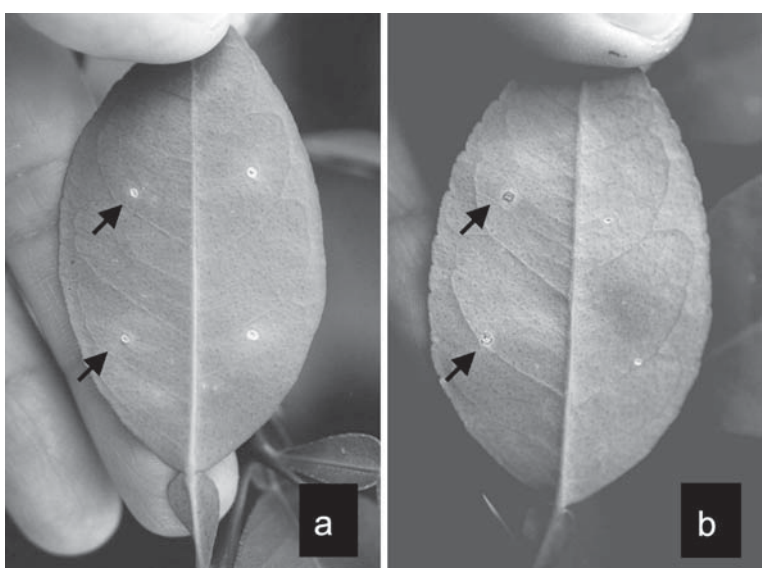

Figure 1 Inhibition of canker disease incidence by *Tamarindus in dica* extract on *Citrus aurantifolia* at 14 days after inoculation with *Xanthomonas axonopodis* pv. *citri* (XC) in greenhouse (a) and symptoms of canker disease in control (nontreated with plant extract) (b). C. *aurantifolia* was wound-inoculated with XC on one side of leaf (arrows) and water on opposite side.

Table 2 Effect of aqueous crude plant extracts on lime canker disease in greenhouse 14 days after inoculation.

Plant extract treatment	Disease incidence (%)[x]
Hibiscus subdariffa Linn.	78 b[y]
Psidium guajava Linn.	100 a
Punica granatum Linn.	81 b
Spondias pinnata (Linn.f.)Kurz	82 b
Tamarindus indica Linn.	48 c
Control (water)	100 a

x = % disease incidence = $\frac{\text{no. of diseased leaves X 100}}{\text{total leaves}}$

y = mean values within a column followed by the same letter do not differ significantly according to a least significant difference (LSD) at $P< 0.05$

Table 3 Suppression of canker disease of lime by spraying aqueous extract of *Tamarindus indica* Linn. to lime plants under field experiment at Kamphaeng Saen, 7 weeks after the first application.

Treatment	Number of diseased leaves/plant	Number of total leaves/plant	% disease incidence[x]
Tamarindus indica extract	222	6176	3.59 b[y]
Control (nontreated)	740	7825	9.46 a

x = %disease incidence = $\frac{\text{no. of diseased leaves X 100}}{\text{total leaves}}$

y = mean values within a column followed by the same letter do not differ by Student's t-test at $P< 0.05$

about one and two times lesser than other plant extracts and nontreated control, respectively. After preliminary screening in the greenhouse, the potential of *T. indica* extract for control of lime canker was assessed in the field under natural infection. The experiment was conducted in the rainy season in which the incidence of canker was severe because the rainstorms brought about disease epidemic in the presence of diseased leaves derived from primary infection and the infection was greatly enhanced by leaf miner occurring during rainy season. Before spraying with the plant extract, number of citrus canker symptom was observed approximately 1.6 % by random sampling of 60 leaves per plant. Application of *T. indica* extract was effectively reduced canker infection from 9.46% (nontreated control) to 3.59 % which was about 3 times better than the control.

One of the most commonly known chemical for controlling foliar bacterial diseases is through the use of copper compounds. Copper compounds are routinely used in commercial citrus orchard for control of citrus canker in Thailand. To date, some plant pathogenic bacteria have inherited a resistance to copper compounds. The resistant strains can still be controlled by alternating spray with other chemical (Marco and Stall, 1983). In tomato plant, leaflets treated with the copper and mancozeb combination had significantly lower copper-resistant populations of *Xanthomonas campestris*

pv. *vesicatoria* than treated with copper alone(Jones *et al*, 1991). These findings clearly demonstrated that *T. indica* extract could be used as chemical control of canker disease. Therefore, further development of *T. indica* extract can be applied as post-infection sprays for canker control in a disease management program.

Reduction of leaf infection when treated with *T. indica* extract was possibly caused by acidic solution of the extracts (pH 3.5). In this respect, Sand and McIntyre (1977) reported that aqueous solutions of citrate and tartrate (100mM, pH3.0) are bactericidal and tartrate also reduced the number of lesions on the cowpea leaves caused by *Pseudomonas syringae* (syn. *P. syringae* pv. *syringae*). However, the chemical compounds and mechanism of *T. indica* extract controlling canker remained to be determined. In addition, phytotoxicity and the environmental effects of the extract on epiphytic beneficial microorganisms need to be addressed before the extension of the plant extract to farmers are performed.

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