Table 6 Comparison on recovery of *Xanthomonas smithii* subsp. *citri* (Xsc) strains on XSC medium with NA, FS and SX media[a].

Xsc strain	Mean no.colonies per plate[b]				Plating efficiency[c] (%)		
	NA	FS	SX	XSC	FS	SX	XSC
T1	149.0	124.0	107.0	72.0	83.3	71.9	48.2
T7	98.0	117.3	72.0	80.0	118.7	72.9	81.0
NT14	277.3	254.3	67.8	126.0	91.7	24.4	45.5
NT 18	40.0	49.0	21.8	39.8	122.5	54.4	99.4
NT22	84.3	29.0	35.8	33.8	34.4	42.4	40.1
1196	36.4	32.5	12.3	37.6	89.3	33.7	103.3
1201	27.8	25.3	10.0	31.3	91.0	36.0	112.6
1342	18.2	21.5	7.7	14.6	118.1	42.1	80.2
Average	91.5	81.6	41.8	54.4	93.6	47.2	76.3

[a]Media were NA = nutrient agar; FS = Fieldhouse and Sasser's semi-selective agar, SX = Schaad and White's semi-selective agar and XSC = new semi-selective agar for Xsc.

[b]Colonies per plate were averaged from four replications.

[c]Plating efficiency (%) = $\frac{\text{Mean number colonies on FS or SX or XSC}}{\text{Mean number colonies on NA}} \times 100$

Table 7 Colony diameter of *Xanthomonas smithii* subsp. *citri* (Xsc) strains on NA, FS, SX and XSC media[x] after incubation at 30°C.

Xsc strain	Colony diameter[y] (mm)			
	NA	FS	SX	XSC
T1	3.8	1.7	3	3.9
T7	4.2	1.8	1.95	4
NT14	3.9	1.2	1.15	2.6
NT 18	3.1	1	1.2	2.3
NT22	2.7	1	1.15	2.2
Mean	3.5 a[z]	1.3 b	1.7 b	3.0 a

[x]Media were NA = nutrient agar; FS = Fieldhouse and Sasser's semi-selective agar; SX = Schaad and White's semi-selective agar and XSC = new semi-selective agar for Xsc.

[y]Colony diameter(mm) was an average of three colonies.

[z]Means within row followed by the same letter were not significantly different at the $P = 0.05$ according to Duncan's multiple range test.

Table 8 Recovery of *Xanthomonas smithii* subsp. *citri* (Xsc) from citrus samples on NA, FS, SX and XSC media 5 days after incubation at 30°C.

Citrus sample	Number of Xsc colonies[a] on media[b]			
	NA	FS	SX	XSC
1. Lime leaf (*C. aurantifolia*)	37.8[c]	0.0	0.3	28.3
2. Lime leaf (*C. aurantifolia*)	11.3	0.0	0.0	5.0
3. Pummelo leaf (*C. grandis*)	0.8	0.0	0.0	0.3
4. Pummelo leaf (*C. grandis*)	23.5	0.8	0.0	24.0
5. Leach lime leaf (*C. hystrix*)	268.8	0.0	0.0	237.0
6. Lime twig (*C. aurantifolia*)	ND[d]	0.0	0.0	TC[e]
7. Lime twig (*C. aurantifolia*)	TC	0.0	0.0	TC

[a]Typical canker lesion from citrus samples of 6.0 mm from leaf or 1.0 g from twig were soaked in 2 ml of 0.85% NaCl. After soaking for 20 min, the suspension were diluted to 10^{-2} and 100 µl were spread on each medium for four replications.

[b]Media were NA = nutrient agar; FS = Fieldhouse and Sasser's semi-selective agar; SX = Schaad and White's semi-selective agar and XSC = new semi-selective agar for Xsc.

[c]Number of colonies = average from four replication

[d]ND = Plate was over grew by saprophytic bacteria.

[e]TC = Number of Xsc colonies were too much to count.

Specificity and sensitivity assay

A 354-bp expected fragment of PCR product was amplified with 354 primers from all 23 strains of Xsc (Fig. 5A, Table 9). No fragment of expected size was amplified with 354 primers from 34 strains of other xanthomonads that included 10 strains of *X. fuscans* subsp. *aurantifolii*, 2 strains of *X. alfalfae* subsp. *citrumelo*, 9 strains of *X. smithii* subsp. *smithii*, 1 strain of *X. fuscans* subsp. *fuscans*, 1 strain of *X. campestris* pv. *campestris* and 11 strains of *X. campestris* pv. *glycines* (Table 9).

Other primer pairs, VM3-VM4, KF-KR and 2-3 also amplified expected fragment of PCR product from all strains of Xsc (Table 9). However, VM3-VM4 primers still provided the expected fragment from 5 strains of *X. fuscans* subsp. *aurantifolii* including 1 strain of B-strain and 4 strains of C-strain, 9 strains of *X. smithii* subsp. *smithii* and 9 strains of *X. campestris* pv. *glycines*. The KF-KR primers were not cross-reacted to other xanthomonads. The 2-3 primers also gave expected fragment from 9 strains of *X. smithii* subsp. *smithii*.

Sensitivity of 354 primers for detection of viable cells of Xsc and purified total DNA of Xsc strain T7 were 70 cells per µl and 50 pg per µl (Fig. 6) respectively by PCR reaction and amplification program as shown in Table 2.

Southern blot hybridization

Representatives of each group of xanthomonad including 23 strains of *X. smithii* subsp. *citri*, 9 strains of *X. fuscans* subsp. *aurantifolii*, 2 strains of *X. alfalfae* subsp. *citrumelo*, 1 strain of *X. smithii* subsp. *smithii*, 1 strain of *X. fuscans* subsp. *fuscans*, 1 strain of *X. campestris* pv. *campestris* and 4 strains of *X. campestris* pv. *glycines* were detected by Southern blot hybridization with 354-bp probe (Fig. 5B). The amplified products from all strains of Xsc were hybridized with 354-bp probe but not with other xanthomonads.

Cloning and sequencing of PCR amplification product

The 354-bp expected PCR fragment from Xsc that was amplified by 354 primers was purified with Wizard® Plus SV Miniperps DNA Purification System (Promega®, Madison, WI) and ligated into pCR®8/GW/TOPO® vector (Invitrogen®). The mixture was transformed into *One Shot® Mach*1™*-T1*R *Chemically Competent E. coli* (Invitrogen®). The recombinant clones were screened by PCR amplification with 354 primers. Sequencing of PCR product was provided by BSU (Bioservice Unit) using GW1 forward primer/GW2 reverse primer that was designed from nucleotide sequence of pCR®8/GW/TOPO® vector at position 607-631 and 733-757, respectively. The nucleotide sequences of PCR product obtained from GW1-GW2 primers were analyzed by citing the map of pCR®8/GW/TOPO® vector. Similarity with the 354-bp expected product sequences were searched in Genbank database by using BLAST program provided by National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). Searching results showed that sequences of 354-bp of expected product were 99.7% similar to sequence of Xac strain 306 (Accession AE011881) (Fig. 7).

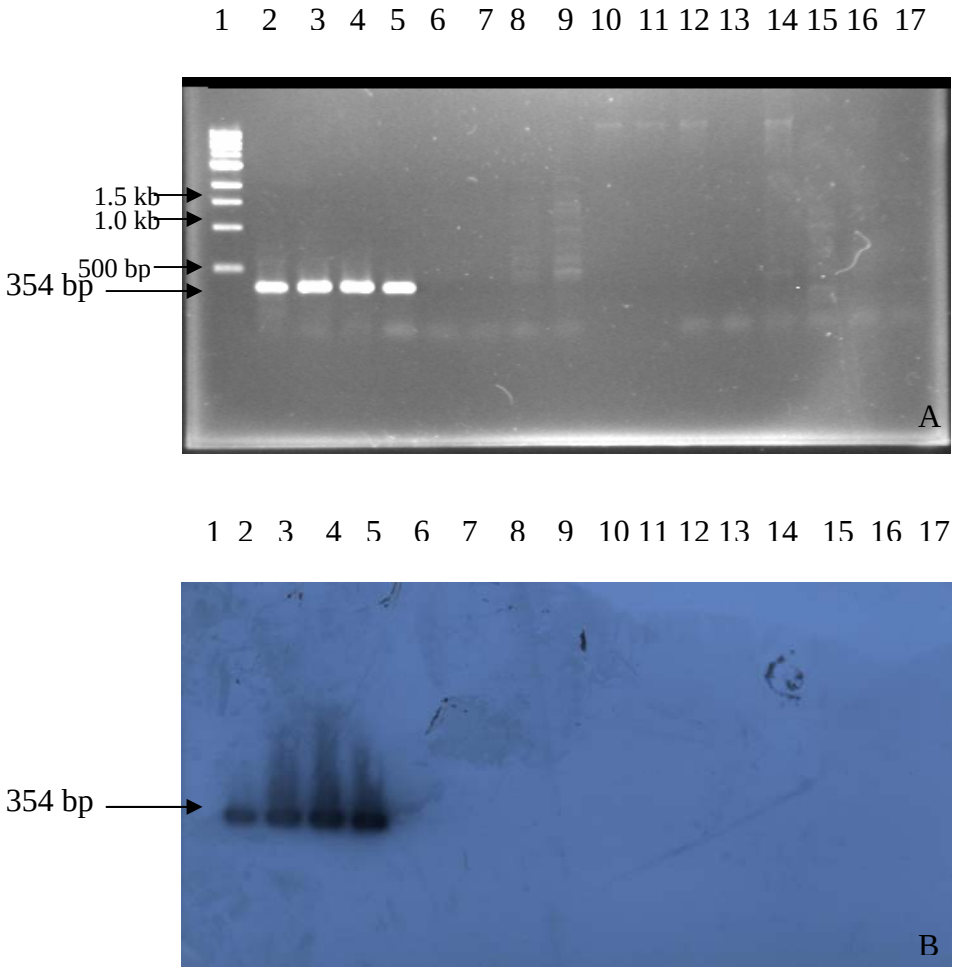

Figure 5 A) PCR amplification products of 354 primers on 1% agarose gel 0.5x TBE buffer. B) Southern blot hybridization with 354-bp probe of *Xanthomonas* species. Lane 1) DNA marker 1 kb (Biolab®); lanes 2-5) *X. smithii* subsp. *citri* strains: T7, J131, 1258, 1270; lanes 6-11) *X. fuscans* subsp. *aurantifolii* strains: 1415, 1416, 1419, 1420, 1360, 1361; lanes 12-13) *X. alfalfae* subsp. *citrumelo* strains: 1267, 1274; lane 14) *X. campestris* pv. *glycines* strain: NKR 21; lane 15) *X. smithii* subsp. *smithii* strain: 1318; lane 16) *X. fuscans* subsp. *fuscans* strain: 1316; and lane17) *X. campestris* pv. *campestris* strain: 657.

Table 9 Comparison on specific amplification for detection of *Xanthomonas* species by using new specific primer 354F-354R and other specific primers VM3-VM4, KF-KR and 2-3.

Strain	Classical PCR[a]			
	354F-354R	VM3-VM4	KF-KR	2-3
Xanthomonas smithii subsp. *citri*				
T1	+[b]	+	+	+
T3	+	+	+	+
T4	+	+	+	+
T5	+	+	+	+
T7	+	+	+	+
T8	+	+	+	+
T10	+	+	+	+
T13	+	+	+	+
NT14	+	+	+	+
NT18	+	+	+	+
NT20	+	+	+	+
NT22	+	+	+	+
NT25	+	+	+	+
OCr1.1	+	+	+	+
OCr1.2	+	+	+	+
LCp2.1	+	+	+	+
LCp2.2	+	+	+	+
SWRb	+	+	+	+
Fp1-2	+	+	+	+
J32	+	+	+	+
J131	+	+	+	+
1258	+	+	+	+
1270	+	+	+	+
X. fuscans subsp. *aurantifolii*				
1415	-	+	-	-

Table 10 (continued)

Strains	Classical PCR[a]			
	354F-354R	VM3-VM4	KF-KR	2-3
X. fuscans subsp. *aurantifolii*				
1416	-	-	-	-
1417	-	-	-	-
1418	-	+	-	-
1419	-	+	-	-
1420	-	+	-	-
1421	-	+	-	-
1360	-	-	-	-
1361	-	-	-	-
1363	-	-	-	-
X. alfalfae subsp. *citrumelo*				
1267	-	-	-	-
1274	-	-	-	-
X. smithii subsp. *smithii*				
1318	-	+	-	+
317	-	+	-	+
579	-	+	-	+
584	-	+	-	+
1034	-	+	-	+
1035	-	+	-	+
1037	-	+	-	+
1051	-	+	-	+
1232	-	+	-	+
X. fuscans subsp. *fuscans*				
1316	-	-	-	-

Table 10 (continued)

Strain	Classical PCR[a]			
	354F-354R	VM3-VM4	KF-KR	2-3
X. campestris pv. *campestris*				
657	-	-	-	-
X. campestris pv. *glycines*				
NKR21	-	+	-	-
CM 60-1	-	+	-	-
No. 21-1	-	+	-	-
RE 07	-	+	-	-
239	-	+	-	-
241	-	+	-	-
281	-	+	-	-
285	-	-	-	-
728	-	+	-	-
1204	-	+	-	-.
1324	-	-	-	-

[a]Classical PCR amplify with specific primer pairs for *X. smithii* subsp. *citri* including; 354F-354R = new specific primers designed from an unique fragment in chromosome from conserved hypothetical protein gene; VM3-VM4 = Mavrodieva *et al*. (2004) specific primers designed from the *pthA* gene family; KF-KR = Kingsley *et al*. (2001) specific primers designed from an unique fragment in chromosome; 2-3 = Hartung *et al*. (1993) specific primers designed from an unique fragment from plasmid DNA.

[b]Presence (+) or absence (-)of unique of predicted size after agarose gel electrophoresis.

Table 10 Sensitivity of classical PCR with 354 primers in detecting cells and purified DNA of *Xanthomonas smithii* subsp. *citri* (Xsc) strain T7

Dilution[a]	Cell/µl	PCR results[c]	DNA concentration[b]	PCR results[c]
0.1 $OD_{600\ nm}$	$7.0x10^4$	+	50 ng/µl	+
10^{-1}	$7.0x10^3$	+	5 ng/µl	+
10^{-2}	$7.0x10^2$	+	500 pg/µl	+
10^{-3}	7.0x10	+	50 pg/µl	+
10^{-4}	7.0	-	5 pg/µl	-
10^{-5}	0	-	500 fg/µl	-
			50 fg/µl	-

[a]Cell suspension of Xsc was adjusted the turbidity to 0.1 O.D.600nm gave $7.0x10^4$ cell/µl and serially ten-fold diluted to10^{-5}. The number of cell per microliter was counted by haemacytometer.

[b]DNA of Xsc was adjusted by ten-fold dilution from 50 ng to 50 fg.

[c]PCR specific amplification with 354 primer pair was performed following the reaction mix and amplification program of the Table 2. Presence (+) or absence (-) of unique of predicted size after agarose gel electrophoresis.

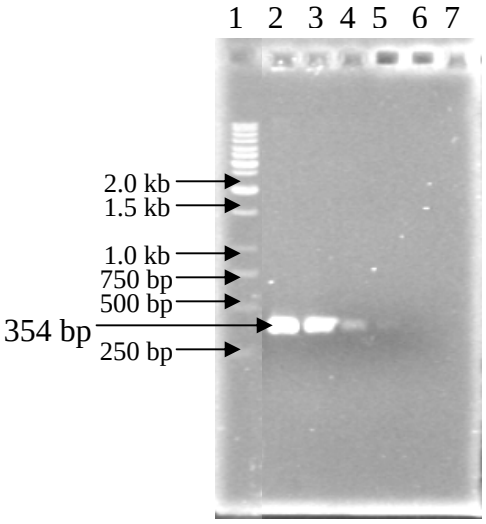

Figure 6 PCR amplification products of 354 primers on 1% agarose gel 0.5x TBE buffer. Lane 1) marker DNA 1 kb (Fermentas®); and lanes 2-7) chromosomal DNA of *Xanthomonas smithii* subsp. *citri* (Xsc) at concentration from 50 ng to 50 fg per microliter by ten-fold dilution.

```
Xsc(T7)          1  GACGGCGCGGCTCAGGATGCTGCTAAGGGAGCTGGACGCGCGAAAGGTAATCTGGAAGAC  60
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE011881.1    4675  GACGGCGCGGCTCAGGATGCTGCTAAGGGAGCTGGACGCGCGAAAGGTAATCTGGAAGAC  4734

Xsc(T7)         61  CAGCTGCGTGTTGCCAACGAGCTACTGCGTGGC*TTGCAAATCCTTGGCATTAGCGACGAA  120
                    |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
AE011881.1  4735  CAGCTGCGTGTTGCCAACGAGCTACTGCGTGGT*TTGCAAATCCTTGGCATTAGCGACGAA  4794

Xsc(T7)        121  GCCGAAGCGTTGGAGCAGGACCTCACCGGGATCTTAAATGCCTTTTCAAAGTCGATTCTG  180
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE011881.1    4795  GCCGAAGCGTTGGAGCAGGACCTCACCGGGATCTTAAATGCCTTTTCAAAGTCGATTCTG  4854

Xsc(T7)        181  CAAAGTGAAAGAGGGATCGCGACTGCTGAGGAGGCTAGACGCGAGCAGGCTCTCAATACG  240
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE011881.1    4855  CAAAGTGAAAGAGGGATCGCGACTGCTGAGGAGGCTAGACGCGAGCAGGCTCTCAATACG  4914

Xsc(T7)        241  CTTGTTGCATTTCTAATGAGCTTCGCGAGCCGAAGCGGCGTACGTGATCGACTGAACATC  300
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE011881.1    4915  CTTGTTGCATTTCTAATGAGCTTCGCGAGCCGAAGCGGCGTACGTGATCGACTGAACATC  4974

Xsc(T7)        301  TTTACCACCAACTATGACAGGCTAATCGAAGCTGGTGCTGAGTTGGCTGGGCTG        354
                    |||||||||||||||||||||||||||||||||||||||||||||||||||||
AE011881.1    4975  TTTACCACCAACTATGACAGGCTAATCGAAGCTGGTGCTGAGTTGGCTGGGCTG        5028
```

Figure 7 Comparison of nucleotide sequences of PCR product fragment of 354 F/R primers from *Xanthomonas smithii* subsp. *citri* (T7 strain) and *Xanthomonas smithii* subsp. *citri* strain 306 gene XAC 2443 (Accession AE011881) with BlastN program showed 99.7% similarity

* non-similar nucleotide

Control of citrus canker disease

Laboratory tests

Screening of chemicals for growth inhibition of Xsc *in vitro* under laboratory conditions showed that Phytomycin®, Thianosan® and Cupravit® inhibited growth whereas Canoron®, Masbrane® and Funguran® had no effect on the growth of Xsc similar to water control (Fig 8A, Fig. 8B and Table 11). Phytomycin® gave the highest inhibition zone ranging from 0.9-1.47 mm in diameter and inhibited 7 out of 8 tested strains (Table 11). The lesser growth inhibitors were Thianosan ® ranging from 0.07-0.77 mm for all 8 tested Xsc strains and Cupravit® ranging from 0.02- 0.05 mm for 2 tested strains. The Xsc strain NT14 and Fp1-2 were susceptible to the above 3 chemicals whereas strain NT22 was resistance to Phytomycin® and Cupravit® (Table 11). The results showed that 8 strains of Xsc isolated from mandarin, pummelo and sweet orange, were completely resistant to Funguran® (copper hydroxide) and were mostly resistant to Cupravit® (copper oxychloride) at the recommended rate.

Greenhouse tests

Efficacy test of six chemicals, Canoron® WP and FP, Thianosan®, Phytomycin®, Cupravit® and Masbrane® to inhibit disease incidence of Xsc strain NT22 in lime seedlings cv. Pan Rum Pai under greenhouse conditions resulted in an average disease incidence (%) of 0, 26, 48, 61, 89 and 99, respectively, while water control was 99 (Fig. 9). Citrus canker symptom development in lime leaves treated with Phytomycin®, Thianosan®, Canoron® FP and water control are shown in Figure 10 in which Canoron® FP had the least canker symptoms and severity.

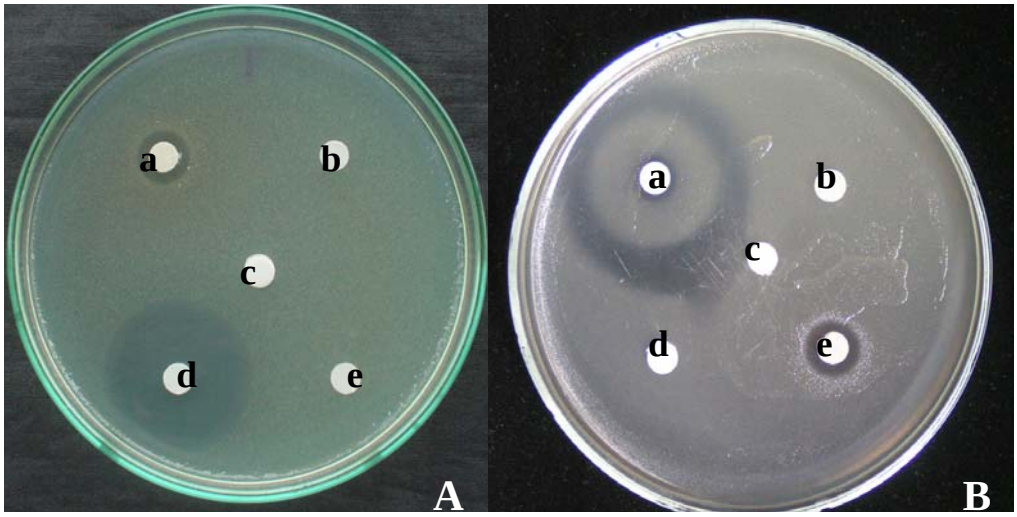

Figure 8 Efficacy of chemicals on growth inhibition of *Xanthomonas smithii* subsp. *citri* (Xsc) by paper disc diffusion assay in panel A: a) Cupravit®, b) Funguran®, c) control, d) Phytomycin®, e) Masbrane® and panel B: a) Thianosan® b) Canoron® FP, c) control, d) Canoron® WP, e) Cupravit®. Ten microliters of each chemical were added to sterile filter paper discs (6.0 mm diameter) and the discs were placed onto NA plates previously seeded with Xsc; the plates were incubated for 2 days and the zone of inhibition was measured. Each experiment was replicated 3 times.

Table 11 Effect of various chemicals on inhibition growth of 8 strains of *Xanthomonas smithii* subsp. *citri* (Xsc) by paper disc diffusion method.

Chemical[x]	Inhibition zone[y](cm.) of Xsc strains								Mean
	T1	T7	T8	NT14	NT22	NT25	Fp1-2	SWRb	
Canoron® (FP)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 a[z]
Canoron® (WP)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 a
Masbrane®	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 a
Funguran®	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 a
Cupravit®	0.00	0.00	0.00	0.02	0.00	0.00	0.25	0.00	0.03 a
Thianosan®	0.50	0.63	0.07	0.23	0.77	0.08	0.70	0.50	0.44 b
Phytomycin®	0.90	1.10	1.07	1.32	0.00	1.33	1.20	1.47	1.05 c
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 a

[x]Ten microliter of each chemical were added to sterile filter paper discs (6.0 mm diameter) and placed the discs onto NA plates previously seeded with Xsc, the plates were incubated for 2 days and the zone of inhibition was measured. Each experiment was replicated 3 times.

[y]Inhibition zone = diameter of inhibition zone – diameter of paper disc

[z]Means within column followed by the same letter were not significantly different at the $P = 0.05$ according to Duncan's multiple range test.

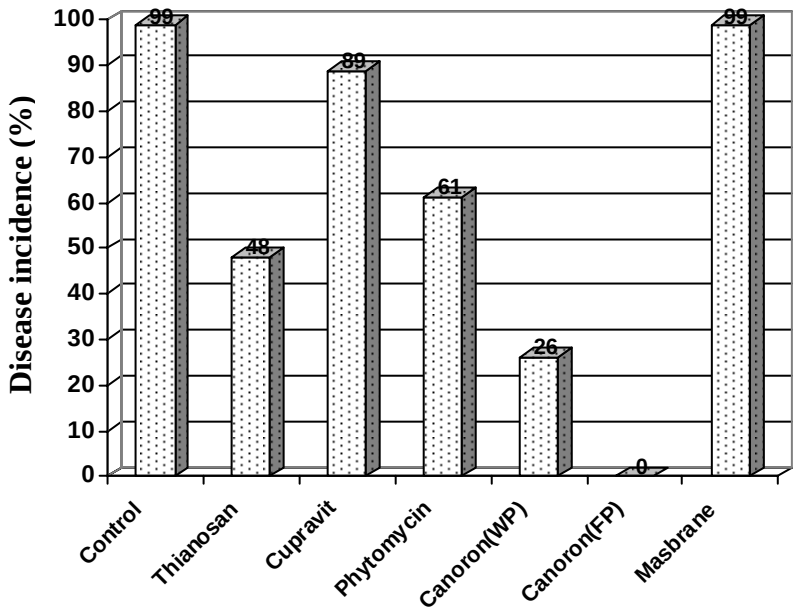

Figure 9 Effectiveness of chemicals to control citrus canker caused by *Xanthomonas smithii* subsp. *citri* (Xsc) on lime seedlings in greenhouse

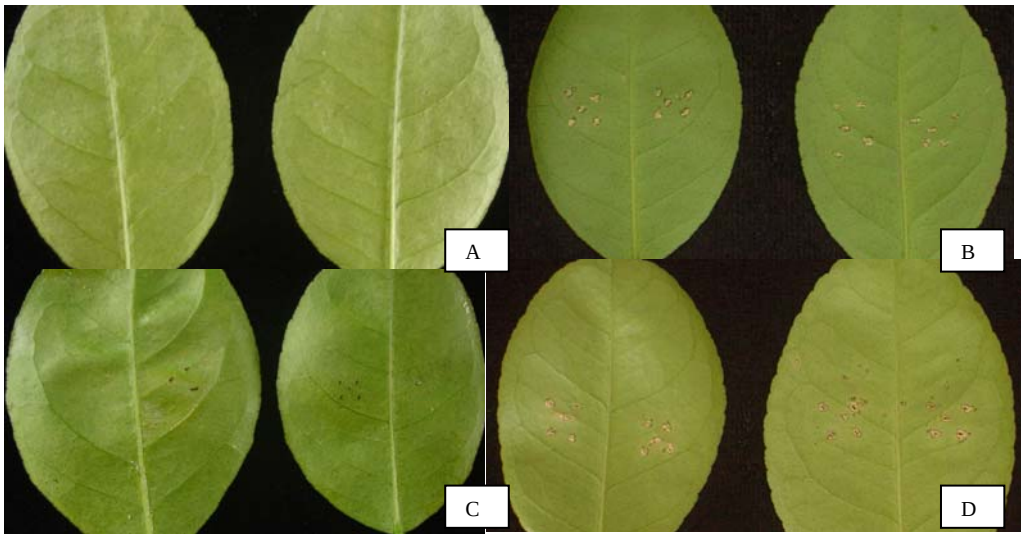

Figure 10 Severity of citrus canker symptoms on duplicated lime leaves inoculated with *Xanthomonas smithii* subsp. *citri* (Xsc) after treatments with chemicals A) Phytomycin®, B) Thianosan®, C) Canoron® (FP) and D) water control.

Field experiments

Experiment 1 The experiment was carried out during late rainy season from Oct. 7 – Nov. 17, 2004. Treatments were Canoron® (FP), Masbrane®, Cupravit® and water as control. Disease incidence (%) of all treatments was lower than water control treatment (Fig. 11-1a). Area under the disease progress curve (AUDPC) of disease incidence was significantly reduced by Canoron® (FP) (60.21) and Cupravit® (72.68) compared with Masbrane® (88.78) and control (137.18) (Table 12). However, disease incidence (%) in this experiment was rather high because the climate was suitable for citrus canker development due to a high volume of rain during the experiment (Fig. 11-1b, Table 14).

Experiment 2 The experiment was carried out during the dry season from Nov. 10 – Dec. 22, 2004. Treatments were the same as in Experiment 1. In this period disease incidence remained very low because the climate was not suitable for disease development due to the low volume of rain (3.9 mm), temperature (26^{o}) and relative humidity (69%) (Fig. 11-2b, Table 14). The AUDPC of all treatments were not significantly different (Table 12). However, Cupravit® showed the lowest disease incidence (2%) and AUDPC (27.4) when compared to all treatments (Fig. 11-2c, Table 11).

Experiment 3 The experiment was carried out during early rainy season from July 26 –Aug. 24, 2005. Treatments were Canoron® (WP), Phytomycin®, Cupravit® and water as control. Occurrence of the canker disease was medium rate (84.26). Statistical analysis of all treatments for an effective reduction of AUDPC was not significantly different among the treatments (Table 13). However, Phytomycin® gave the lowest disease incidence (8.9%) and AUDPC (59.7) when compared with all treatments (Fig. 12-1a, Table 14).

Experiment 4 The experiment was carried out during early dry season from Oct. 12 – Nov. 2, 2005. However, the weather was not normal in that rainstorms occurred and caused floods in the trial fields (Fig. 12-2b, Table 14). After collecting

the data only 3 times, the experiment had to be discontinued due to heavy rains flooding the entire citrus grove. Treatments were Canoron® (WP), *Bacillus subtilis* (CH6), Cupravit® and water as control. Development of disease incidence (%) occurred at high level (63.79) because of high volume of rain and relative humidity during the experiment (Fig. 12-2a, Table 14).

Table 12 Area under the disease progress curve (AUDPC) of Canoron® (FP), Masbrane® and Cupravit® for control of citrus canker on lime in Phetchaburi, Thailand, during late of rainy season (Oct. 7 – Nov. 17, 04) and dry seasons (Nov. 10 – Dec. 22, 04)

Treatment[y]	Experiment 1 Oct. 7 – Nov. 17, 04		Experiment 2 Nov. 10 – Dec. 22, 04	
	Disease incidence (%)	AUDPC	Disease incidence (%)	AUDPC
Canoron® (FP) 0.5 g a.i./liter	8.85	60.21 b[z]	3.34	34.96 a
Masbrane® 0.2%(vol./vol.)	16.32	88.78 ab	4.82	40.68 a
Cupravit® 1.27 g a.i./liter	11.01	72.68 b	2.01	27.42 a
Control (water)	32.16	137.18 a	4.90	40.78 a
%CV		37		60

[y]The treatments were applied 5 times at 1, 2, 4, 5 and 6 weeks after new flush of leaves by foliar spray at 2 L/min for a total of 7 L/plant . Disease incidence was recorded at weekly from the 3rd to 7th week of the first spray. The number of diseased leaves on each tagged branch was recorded and the results were calculated as percentage of disease incidence, and transformed to AUDPC.

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased leaves per branch}}{\text{Number of leaves per tagged branch}} \times 100$$

[z]Means within column followed by the same letter were not significantly different at the $P = 0.05$ according to Duncan's multiple range test.

Table 13 Area under the disease progress curve (AUDPC) of Canoron® (WP), Phytomycin®, Cupravit® and *B. subtilis* for control citrus canker on lime foliage in Phetchaburi, Thailand during early rainy season (Jul. 26 – Aug. 24, 05) and dry seasons (Oct. 12 – Nov. 2, 05)

Treatment[y]	Experiment 3 Jul. 26 – Aug. 24, 05		Treatment	Experiment 4 Oct. 12 – Nov. 2, 05	
	Disease incidence (%)	AUDPC		Disease incidence (%)	AUDPC
Canoron® (WP) 0.5 g a.i./liter	11.52	66.37 a	Canoron® (WP) 0.5 g a.i./liter	29.97	81.18 ab
Phytomycin® 0.1g a.i./liter	8.96	59.76 a	*B. subtilis* (CH_6) 1.6 $OD_{600\ nm}$ 10 ml/L	40.56	94.74 a
Cupravit® 1.27 g a.i./liter	14.61	77.70 a	Cupravit® 1.27 g a.i./liter	9.93	45.41 b
Control (water)	16.72	84.26 a	Control (water)	35.94	90.56 a
%CV		31	%CV		29

[y]The treatments were applied 5 times at 1, 2, 4, 5 and 6 weeks after new flush of leaves by foliar spray at 2 L/min for a total of 7 L/plant . Disease incidence was recorded at weekly from the 3rd to 7th week of the first spray. The number of diseased leaves on each tagged branch was recorded and the results were calculated as percentage of disease incidence, and transformed to AUDPC.

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased leaves per branch}}{\text{Number of leaves per tagged branch}} \times 100$$

[z]Means within column followed by the same letter were not significantly different at the $P = 0.05$ according to Duncan's multiple range test.

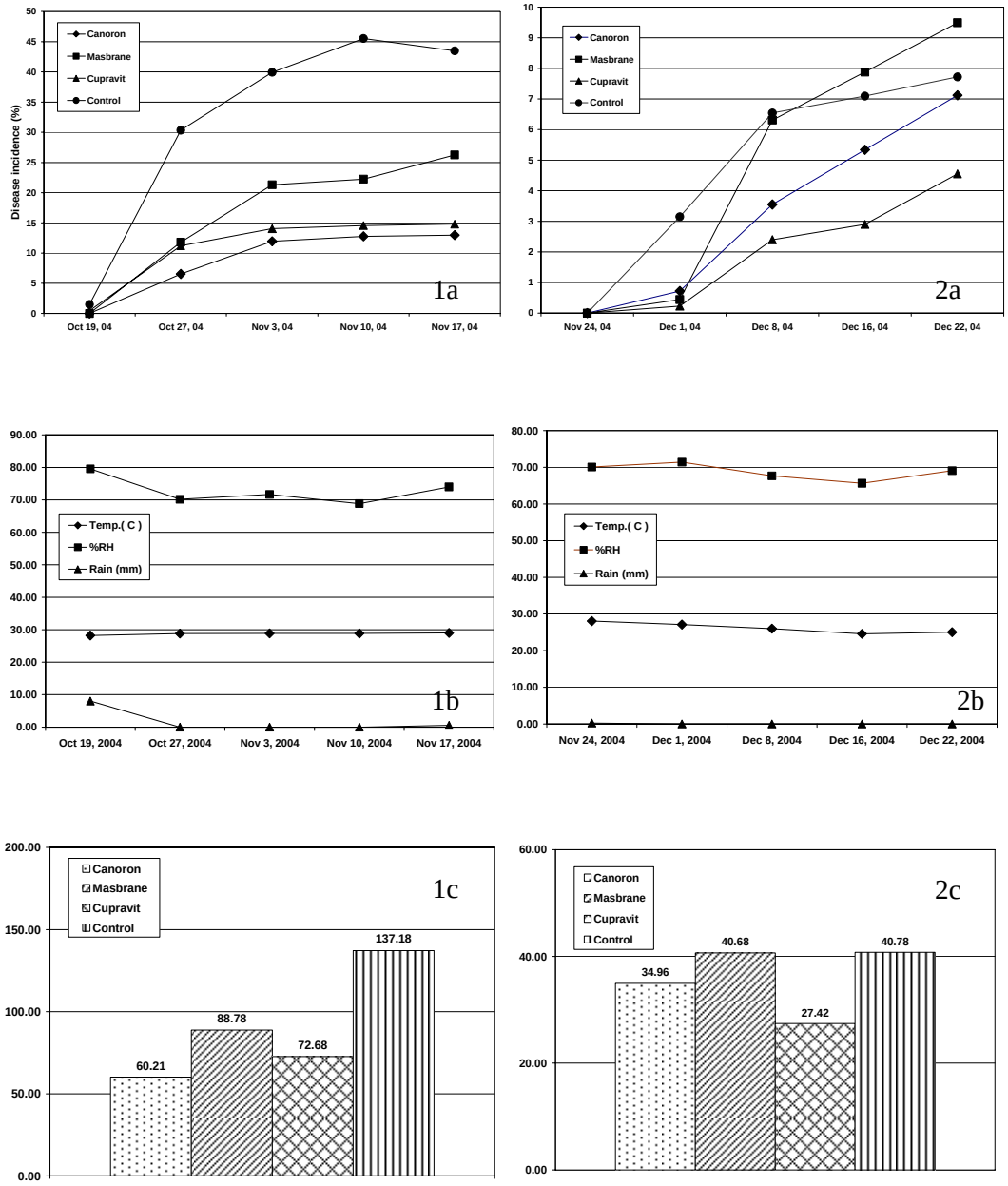

Figure 11 Percentage of disease incidence (a), weather data (b) and area under the disease progress curve of disease incidence (c) after spraying of Canoron® (FP), Masbrane®, Cupravit® for control citrus canker on lime in Phetchaburi Province, Thailand on Oct. 7 – Nov. 17, 2004 (1) and Nov. 10 – Dec. 22, 2004 (2)

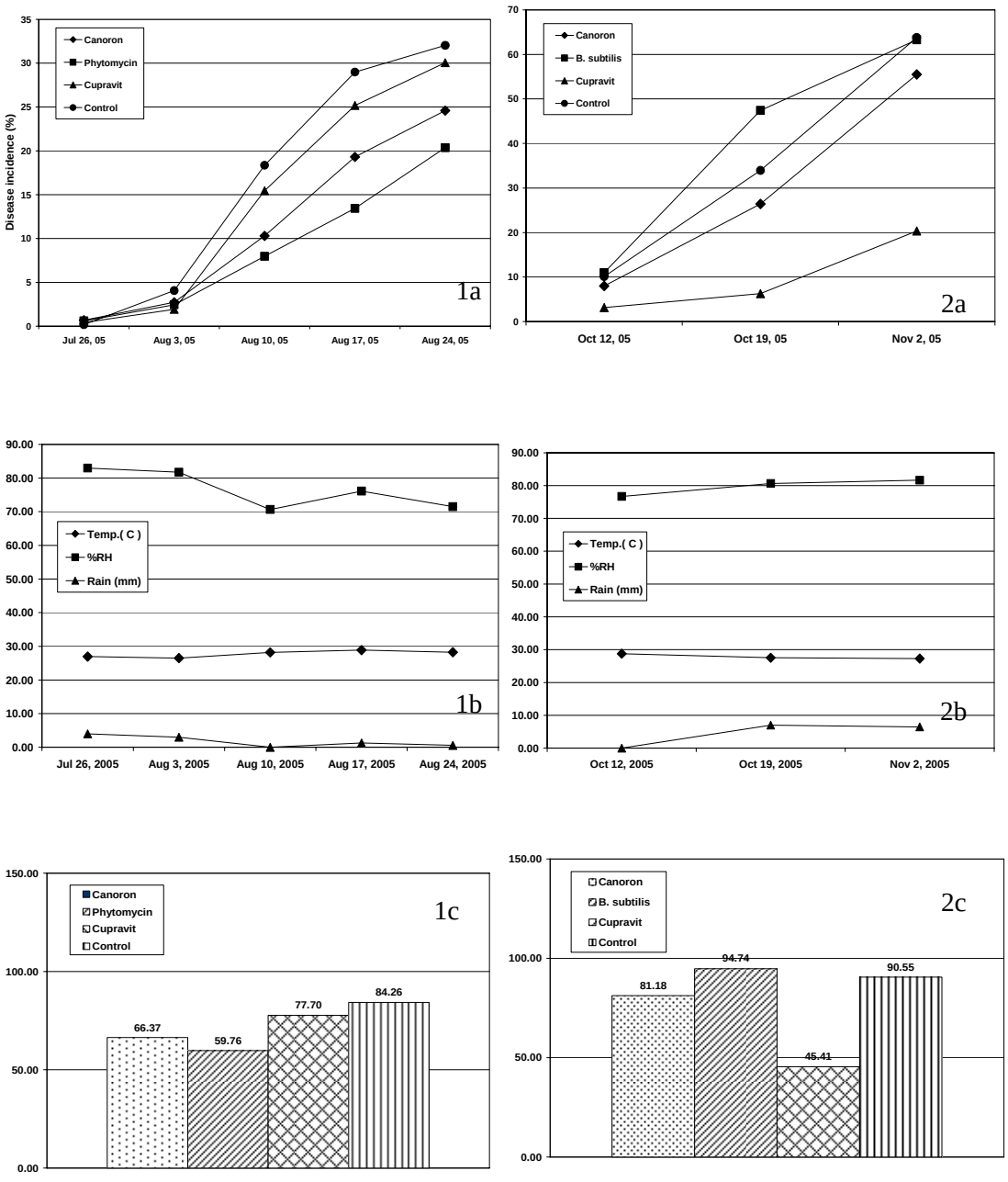

Figure 12 Percentage of disease incidence (a), weather data (b) and area under the disease progress curve of disease incidence (c) after spraying of Canoron® (WP), *Bacillus subtilis* (CH6) and Cupravit® for control citrus canker on lime in Phetchaburi Province, Thailand on Jul. 26– Aug. 24, 2005 (1) and Oct. 12 – Nov. 2, 2005 (2)

Table 14 Data of air temperature (^{o}C), relative humidity (%RH) and rain volume (mm) during experiments at Phetchaburi Province in 2004-2005.

Experiment	Air temp.(^{o}C)	% RH	Total rain vol.(mm)
1) Oct. 7 – Nov. 17, 04	28.68	73.74	108.30
2) Nov. 10 – Dec. 22, 04	26.48	68.94	3.90
3) Jul. 26 – Aug. 24, 05	27.87	75.49	38.00
4) Oct. 12 – Nov. 2, 05	27.42	81.08	133.00

1) Oct. 7 – Nov. 17, 04 and 2) Nov. 10 – Dec. 22, 04 received data from Phetchaburi Province weather station (15km from field trials).

3) Jul. 26 – Aug. 24, 05 and 4) Oct. 12 – Nov. 2, 05 collected from LICOR® data logger LI-1400 (LI-COR Biosciences, NE, USA) (6 m from field trials).