CHAPTER 2

Isolation, morphological characterization and pathogenicity test of *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) causing *Fusarium* wilt in tomato

2.1 Introduction

Fusarium wilt disease of tomato is one of the major diseases contributing to the loss in the production of this important crop, which is caused by pathogenic soil-inhabiting fungus *Fusarium oxysporum*. Such pathogens are specific for certain plant hosts and known as 'forma speciales'; individual isolates within this fungus normally have a narrow host range, and the species is classified into forma specialis based on specific pathogenicity on a host plant. Therefore, *Fusarium* wilt disease of tomato (*Lycopersicon esculentum* Mill) are named as *F. oxysporum* f. sp. *lycopersici* W. C. Snyder & H. N. Hans (*Fol*) (Marasas *et al*., 1984; Joffe, 1986; Cartia *et al*., 1988; Rivelli, 1989; Fletcher, 1994; Mushtaq and Hashmi, 1997; Jovicich *et al.*, 1999). The fungus produces three types of spores: microconidia, macroconidia and chlamydospores, but no sexual stage (telemorph) has been reported (Booth, 1971; Nelson *et al*., 1981; Windels, 1992). *Fusarium* species are commonly found in soils, and persist as chlamydospores or as hyphae in plant residues and organic matter (Booth, 1971; Burgess, 1981).

The devastating disease is now occurs worldwide, which having been reported in more than 40 countries and particularly severe in countries with warm climate, most prevalent on acid and sandy soils (Cai *et al*., 2003). The loss in yield varies between 10% to 90% depending on the stage of the plant growth at which section occurs and the environmental conditions (Kumar and Sood, 2002; Singh, 2005). In severe cases it may cause up to 80% loss in tomato production (Malhotra *et al*., 1993). *Fusarium* wilt syptoms have been characterized as causing vascular wilt (the most important), yellows,

corm rot, root rot, and damping-off (Agrios, 1988; Smith *et al*., 1988). Plants afflicted with *Fusarium* wilt first develop yellowing of the lowest leaves that is often restricted to one side of the plant or a single shoot. The affected leaves wilt and die. Wilting progresses up the stem until the foliage is killed and the stem decays (Agrios, 1997).

The objectives of this chapter were as follows:-

1. To isolate *F. oxysporum* f. sp. *lycopersici* (*Fol*), the causal agent of *Fusarium* wilt disease in tomato
2. To describe morphological characterization of *F. oxysporum* f. sp. *lycopersici* that causes *Fusarium* wilt disease in tomato
3. To evaluate the pathogenicity and select a highly virulent strain as the representative experimental strain
4. To identify race of the selected virulent *F. oxysporum* f. sp. *lycopersici* strain

2.2 Materials and methods

2.2.1 Collection and isolation of the pathogen

Naturally infected tomato plants that showed typical *Fusarium* wilt symptoms were collected from eleven commercial fields at Doi Inthanon National Park, Chiang Mai, Thailand. The vascular tissues of infected plants were collected to isolate the pathogen by tissue transplanting technique. The leaves and secondary roots were trimmed for leaving only the main stem, and then surface sterilized by soaking in 10% Clorox (1% sodium hypochlorite, NaOCl) solution for 1 - 2 min. Subsequently, the pieces of tissue were rinsed twice in sterile distilled water for 2 - 3 times, and blotted dry on sterile paper towels in a laminar flow cabinet. The base of the stem of a diseased plant lengthwise was cut to reveal the xylem just below the epidermis, and then placed on a Petri dish containing Potato Dextrose Agar (PDA), and the cultures were incubated at a room temperature (RT) of approximate 28 - 30ºC. The cultures were daily observed for the colony growth daily. The hyphal tips were transferred to a new PDA Petri dish after 1 - 3 days of culture, and incubated at RT to get pure culture.

Single spore isolation

The single spore isolation was conducted to avoid the inclusion of other fungi. All isolated cultures were separately grown on PDA Petri dish for 10 days before separately streak on a new PDA Petri dish using a sterile loop, and incubated for 12 h at RT. A light compound microscope was used to observe single germinated conidia. A piece of agar containing single conidia was then removed and transferred to a new PDA Petri dish, and incubated for 7 days. The pure culture dishes of each isolate was transferred into tube containing mineral oil and preserved at 4^{o}C for further use.

Isolate designation

The isolates were coded by using the acronym of the genus (Figure 2.1) as follows:- "*Fusarium oxysporum* f. sp. *lycopersici*" (Fol), followed by the host variety as follows:- "Cherry" (C) or "Thomas" (T), followed by fields;- "Sawat's field" (A), "Puttinun's field" (B), "Somkid's field" (C), "Yhing's field" (D), "Arnont's filed" (E), "Boonserm's field" (F), "Manit's field" (G), "Surasit's field" (H), "Sordee's field" (I), "Boonthurm's field" (J), or "Suveera's field" (K), and the isolate number after underscore character (001, 002, 003, up to 126). The diagrams are shown as follows;

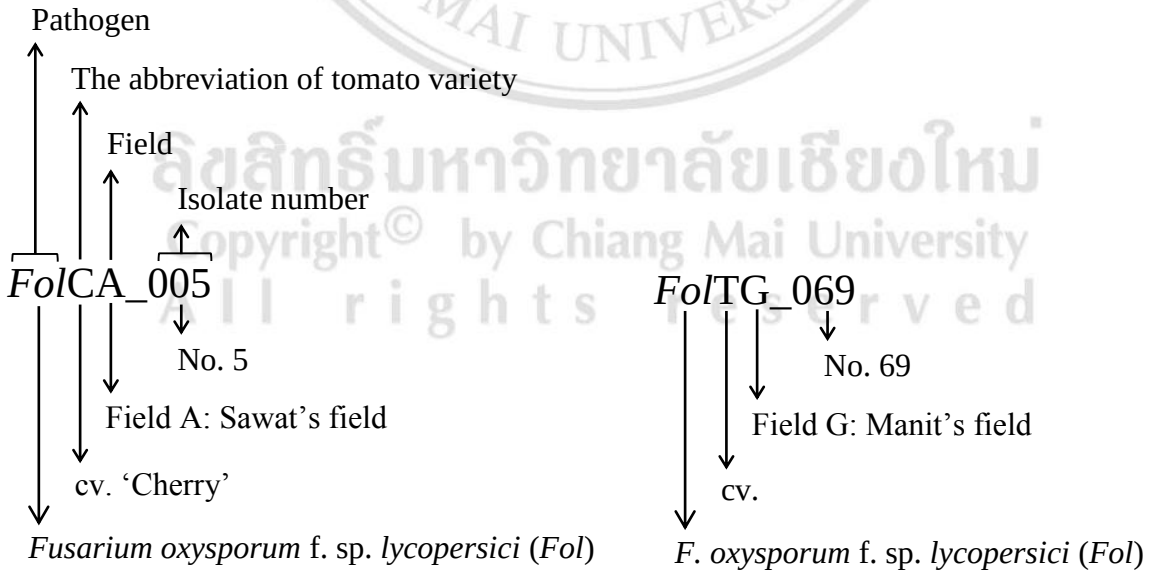

Figure 2.1 Diagram for coding the *Fusarium oxysporum* f. sp. *lycopersici* isolates

2.2.2 Morphological characterization of *Fusarium oxysporum* f. sp. *lycopersici*

Each isolate of *Fol* was cultured by transferring the mycelial discs (5 mm diameter) from stock onto PDA at RT for 7 days. The morphological characteristics of colony types and reproductive structures were daily observed. Colony diameter was measured daily until fully developed. Its growth rate was calculated from an average mean of daily growth (mm/day). The colony color of all isolates was recorded after 10 days. The conidia were measured after 14 days using micrometer at 400x magnification (10x ocular, 40x objectives). After measuring the colony growth, thirty conidia per replicate were measured to average size.

The identification of the *Fusarium* species was based on the published description; distinctive characters of the shapes and sizes of macro- and microconidia, presence and absence of chlamydospores as well as colony appearances, pigmentations and growth rates on agar media (Nelson *et al.*, 1994; Leslie and Summerell, 2006).

A basic key for *Fol*, mycelia are initially produces colorless to pale yellow mycelium that turns pink or purple with age or delicate white to pink, often with purple tinge, and are sparse to abundant. The fungus produces three types of spores; microconidia, macroconidia and chlamydospores. Microconidia are borne on simple phialides arising laterally and are abundant, oval-ellipsoid, straight to curved, 5-12 × 2.2-3.5 µm, and nonseptate. Macroconidia, sparse to abundant, are borne branched conidiophores or on the surface of sporocochia and are thin walled, three- to five-septate, fusoid-subulate and pointed at both ends, have pedicellate base. Three-septate conidia measured 27-46 × 3-5 µm while five-septate conidia measured 35-60 × 3-5 µm. Three-septate spores are more common. Chlamydospores, both smooth and rough walled, are abundant and form terminally or on an intercalary basis. They are generally solitary, but occasionally form in pairs or chains. No perfect stage is known (Wong, 2003).

2.2.3 Pathogenicity tests

Fol inoculum

Fol isolates were grown on PDA at RT for 10 days. The *Fol* isolates were then prepared as conidial suspension by flooded *Fol* colony with 10 ml of sterile distilled water (Singleton *et al*., 1992). Mycelia were dislodged by scraping the surface of *Fol* colony with a sterile microscope glass slide. The mycelia suspension was then filtered through a sterile cheese cloth. The concentration of conidia in suspension was determined on a heamacytometer and adjusted to 1×10^7 conidia/ml.

Plant materials

Seeds of tomato (*Lycopersicon esculentum* Mill.) were surface-sterilized with 1% sodium hypochlorite (10% Clorox) for 1 minute, then rinsed three times in sterile distilled water and dried under a sterile air stream. Sterilized seeds were separately soaked for 12 h, then air dried overnight before sowed in seedling tray (28 × 54 cm, 72 wells) containing Peat Moss growing media (KLASMANN-DEILMANN®, Germany) mixed with coconut coir dust (1:1 ratio) and maintained in greenhouse at 30 ± 2ºC with 12 h photoperiod. The experiments were performed with the uniform 30-day-old tomato plants with four expanded leaves. The tomato cv. 'Bonny Best' (susceptible to *Fusarium* wilt) and cv. 'EWS-37434' (resistant to *Fusarium* wilt) were used for comparison. The cultivars were kindly provided by Hortigenetics Research (S.E. Asia) Limited.

Pathogenicity test

All successfully isolated *Fol* were tested for pathogenicity testing following Kochs' postulates. The root dip method (Applied from Rowe, 1980; Windels, 1992; Marlatt *et al*., 1996) were performed to record for pathogenic and non-pathogenic isolates. Tomato seedling cv. 'Bonny Best' (susceptible to *Fusarium* wilt) was used in this experiment. Roots were trimmed to a length of approximately 2.5 cm, and submerged in 50 ml of each inoculum suspension for approximately 15 min. Inoculated seedlings were planted into each pot at a rate single plant per pot. Seedlings dipped in sterilized water were served as controls. The plants were maintained in the greenhouse.

Three replications were arranged in a Randomized Completely Block Design (RCB), with three plants per replicate. Disease severity was assessed daily starting 10 days after inoculation. Disease was rated on 1 to 5 scales (Applied from Marlatt *et al.*, 1996), as follow: 1 = no symptom; 2 = slight chlorosis, wilting, or stunting of plant; 3 = moderate chlorosis, wilting, or stunting of plant; 4 = severe chlorosis, wilting, or stunting of plant and 5 = dead plant. Final assessments were made 21 days after inoculation. Then, pathogenicity group was categorized (Sibounnavong, 2012) based on DSI as avirulent (DSI = 1); low (DSI ≤ 3.50); moderate (DSI > 3.50 to 4.50) and high virulent (DSI > 4.50). The most virulent *Fol* was reisolated from inoculated plant and used as the representative an experimental strain.

Fol isolates of different pathogenic groups were randomly selected and repeated for pathogenicity testing following the previously described method. Greenhouse-grown tomato seedlings cv. 'Bonny Best' and cv. 'EWS-37434' (resistant to *Fusarium* wilt) (East-West Seed Co., Ltd.) were used for comparisons. The cultivars were kindly provided by Hortigenetics Research (S.E. Asia) Limited.

2.2.4 Race identification of *F. oxysporum* f. sp. *lycopersici* isolate *Fol*CK_117

Tested host plants were the set of standard differential tomato varieties consisted of cv. 'EWS-S' as the susceptible control (susceptible to race1, 2 and 3), while cv. 'EWS-R1' as resistance to race 1, cv. 'EWS-R12.1' and 'EWS-R12.2' as resisted to race 1 and 2, and cv. 'EWS-R123' as resistance to race1, 2 and 3 (Table 2.1). The cultivars were kindly provided by Hortigenetics Research (S.E. Asia) Limited.

The most virulrent isolate that selected for further experiment was examined for pathogenicity on the set of standard differential tomato varieties using previously described method.

Table 2.1 Resistance and susceptibility of 5 differential tomato varieties used in this study to different races of *Fusarium oxysporum* f. sp. *lycopersici*

Race of *Fol*	Standard differential varieties*				
	EWS-S	EWS-R1	EWS-R12.1	EWS-R12.2	EWS-R123
race 1	S	R	R	R	R
race 2	S	S	R	R	R
race 3	S	S	S	S	R

*S = susceptible, R = resistant

Statistical analysis

Data were computed analysis of variance (ANOVA). Treatments mean were compared using Fisher's Least Significant Difference (LSD) at $P = 0.05$.

2.3 Results

2.3.1 Collection and isolation of the pathogen

Tomato stem samples showing typical *Fusarium* wilt symptoms were collected from eleven commercial fields at Doi Inthanon National Park, Chiang Mai, Thailand from September 2013. In infested tomato fields, a high incidence of symptomatic plants, including yellowing, wilting, and stunting of plants, was observed throughout a field (Figure 2.2A). Vascular red- to dark-brown discoloration was observed when cutting longitudinal sections into the xylem at the stem base (Figure 2.2B). Yellow discoloration was clearly observed in infected leaflet, comparable to green color in healthy leaflet. Afterwards, the browning discoloration vascular was conducted to isolate the causal agent using the tissue transplanting technique (Figure 2.2C). The results showed that one hundred and twenty-six isolates were isolated successfully from 2 tomato varieties; cv. 'Cherry' (5 fields, 71 isolates) and Thomas (6 fields, 55 isolates) (Table 2.2). Each isolate was purified by single spore isolation and the culture was preserved at 4°C for further study.

(A)

(B)

(C)

Figure 2.2 *Fusarium* wilt symptoms on naturally-infected tomato plants from commercial fields at Doi Inthanon National Park, Chiang Mai, Thailand; (A) *Fusarium* wilt infested tomato fields under naturally infection, (B) vascular browning discoloration in fields and (C) leaflet discoloration, the cut tomato vascular tissue in infected with *Fusarium* wilt pathogen

Table 2.2 Number of *Fusarium oxysporum* f. sp. *lycopersici* isolates causing *Fusarium* wilt in tomato from commercial fields at Doi Inthanon National Park, Chiang Mai, Thailand

Commercial fields		Tomato varieties	Isolates	Percentage
A	Sawat's field	Cherry	8	6.35
B	Puttinun's field	Thomas	13	10.32
C	Somkid's field	Cherry	12	9.52
D	Yhing's field	Cherry	10	7.94
E	Arnont's filed	Cherry	10	7.94
F	Boonserm's field	Thomas	10	7.94
G	Manit's field	Thomas	12	9.52
H	Surasit's field	Thomas	9	7.14
I	Sordee's field	Thomas	11	8.73
J	Boonthurm's field	Cherry	18	14.28
K	Suveera's field	Cherry	13	10.32
	Total		126	100.00

2.3.2 Morphological characterization of *Fusarium oxysporum* f. sp. *lycopersici*

All isolates were observed for morphological characterization by growing on PDA for 10 days. Colonies are usually fast growing and have a cottony aerial mycelium. The initial color of colonies on PDA was white, and then the color turned to deepen with aging. Then, the colony characteristics were divided into 3 categories according to a visual color as follows:- 1) purplish-white cottony colony (P) (67 isolates, 53.17%), 2) white cottony colony (W) (43 isolates, 34.13%) and 3) orangish-white cottony colony (O) (16 isolates, 12.70%) (Table 2.3; Figure 2.3A).

Growth rate of the colonies ranged between 6.93 - 10.46 mm/day with the average 8.51 ± 0.13 mm/day. Mycelia were delicated, septated, branched, hyaline and smooth margins. Conidiophores typically scattered as solitary phialides on the aerial mycelium (Figure 2.3B). *Fol* produces three types of asexual spore as follow:- 1) microconidia are borne on simple phialides arising laterally and abundant, one-celled hyaline oval-ellipsoid, straight to curved with an average 6.63-8.50 × 2.65-2.82 µm 2) macroconidia are sparse to abundant, borne on branched conidiophores, thin walled with 3 to 5 septate

(3-septate conidia are more common), pointed at both ends, pedicellate base and fusoid-subulate with an average 35.37-39.20 × 3.43-5.50 µm and 3) chlamydospores are both smooth and rough walled, abundant and form terminally or on an intercalary basis, generally solitary, but occasionally form in pairs or chains (Table 2.3; Figure 2.3C).

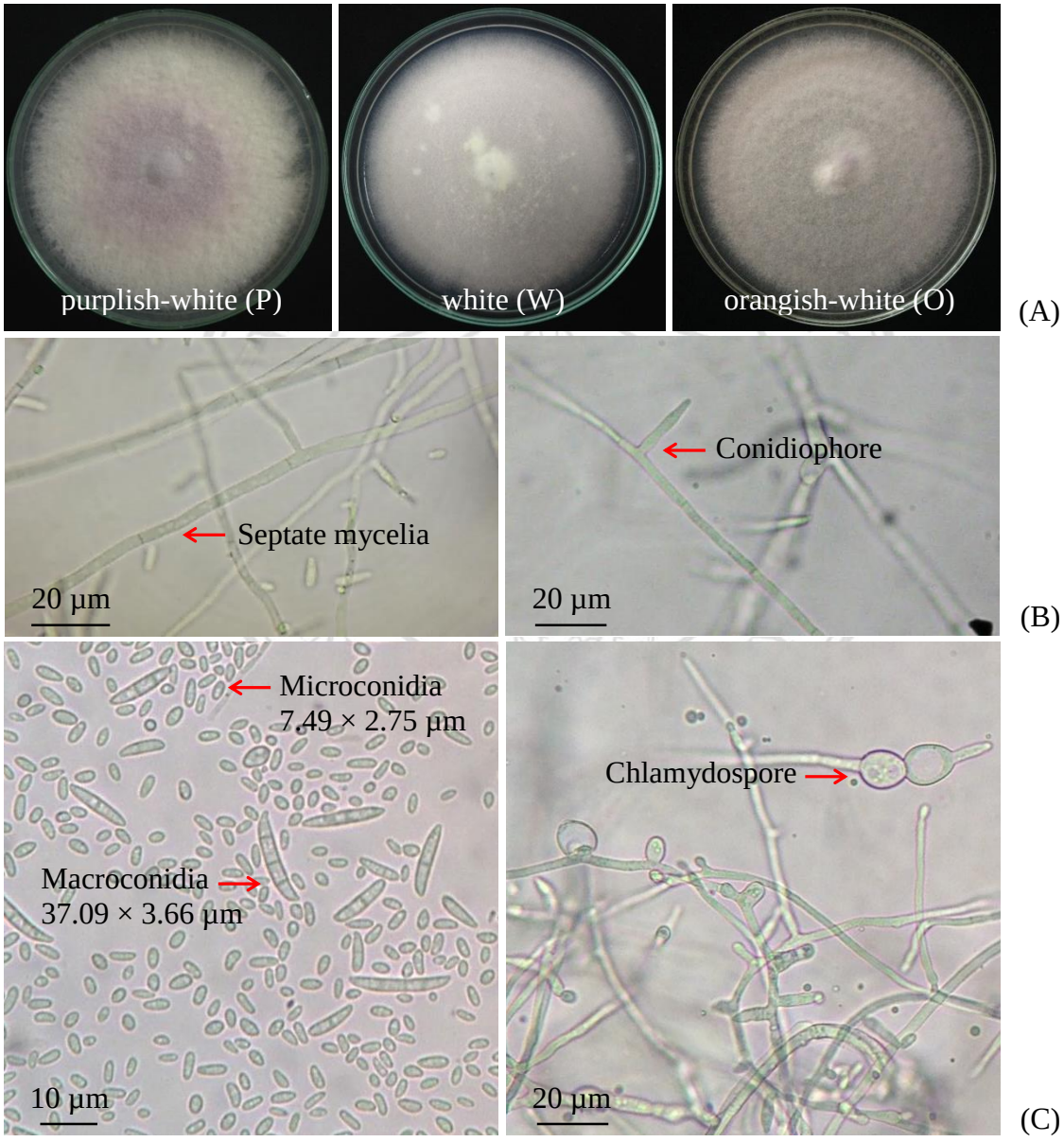

Figure 2.3 Morphological characterization of *Fusarium oxysporum* f. sp. *lycopersici* isolates grown on PDA for 10 days causing *Fusarium* wilt in tomato; (A) colony color, (B) mycelium and conidiophore and (C) micro-, macroconidia and chlamydospore

Table 2.3 List of *Fusarium oxysporum* f. sp. *lycopersici* isolates causing *Fusarium* wilt in tomato cv. 'Cherry' and 'Thomas' from commercial fields at Doi Inthanon National Park, Chiang Mai, Thailand

No.	Isolate code	Colony[1/]		Mean size of conidia (µm)[2/]				Appearance of chlamydospore
		Color[3/]	Growth rate (mm/day)	Macroconidia		Microconidia		
				Length	Width	Length	Width	
A. Mr. Sawat's farm = 8 isolates								
1.	*Fol*CA_001	P	10.40 ± 0.31	36.80 ± 3.48	3.73 ± 0.73	7.27 ± 1.50	2.74 ± 0.16	✓
2.	*Fol*CA_002	P	7.64 ± 0.18	37.30 ± 3.83	3.67 ± 0.70	7.17 ± 1.85	2.80 ± 0.15	✓
3.	*Fol*CA_003	P	7.59 ± 0.47	37.17 ± 4.04	3.57 ± 0.72	7.63 ± 1.94	2.79 ± 0.16	-
4.	*Fol*CA_004	P	9.28 ± 0.26	36.33 ± 3.61	3.73 ± 0.68	7.03 ± 1.62	2.73 ± 0.19	-
5.	*Fol*CA_005	P	7.64 ± 0.34	37.17 ± 3.62	3.67 ± 0.70	7.67 ± 1.87	2.72 ± 0.17	✓
6.	*Fol*CA_006	P	7.56 ± 0.19	37.20 ± 3.73	3.67 ± 0.70	6.70 ± 1.64	2.72 ± 0.16	✓
7.	*Fol*CA_007	P	7.59 ± 0.18	37.07 ± 4.04	3.60 ± 0.71	7.43 ± 1.82	2.75 ± 0.17	-
8.	*Fol*CA_008	P	9.33 ±0.26	35.80 ± 3.86	3.60 ± 0.71	7.27 ± 1.73	2.78 ± 0.18	✓
B. Mr. Puttinun's farm = 13 isolates								
9.	*Fol*TB_009	W	8.40 ± 0.25	36.35 ± 4.31	3.77 ± 0.62	7.43 ± 1.89	2.79 ± 0.16	-
10.	*Fol*TB_010	W	7.65 ± 0.21	36.57 ± 3.98	3.47 ± 0.62	7.37 ± 1.68	2.76 ± 0.16	✓
11.	*Fol*TB_011	P	8.40 ± 0.26	37.83 ± 4.16	3.73 ± 0.68	7.30 ± 1.72	2.69 ± 0.15	✓
12.	*Fol*TB_012	P	8.40 ± 0.10	39.20 ± 3.94	3.67 ± 0.65	7.70 ± 1.55	2.79 ± 0.17	-
13.	*Fol*TB_013	P	8.40 ± 0.17	37.10 ± 3.48	3.73 ± 0.68	7.00 ± 1.63	2.74 ± 0.17	-
14.	*Fol*TB_014	W	8.40 ±0.20	36.93 ± 3.83	3.77 ± 0.62	7.60 ± 1.70	2.73 ± 0.18	✓
15.	*Fol*TB_015	P	7.00 ± 0.19	38.33 ± 3.94	3.63 ± 0.66	7.60 ± 1.60	2.77 ± 0.16	✓
16.	*Fol*TB_016	P	9.28 ± 0.30	36.53 ± 3.64	3.57 ± 0.67	7.53 ± 1.61	2.72 ± 0.17	✓
17.	*Fol*TB_017	P	7.64 ± 0.25	38.13 ± 3.59	3.77 ± 0.72	7.37 ± 1.70	2.69 ± 0.19	✓
18.	*Fol*TB_018	P	7.61 ± 0.17	36.10 ± 3.82	3.60 ± 0.66	7.53 ± 1.45	2.81 ± 0.15	-
19.	*Fol*TB_019	P	9.28 ± 0.14	36.87 ± 4.30	3.53 ± 0.67	7.47 ± 1.77	2.73 ± 0.18	✓

[1/], [2/] Mean of three replications (5 plates/rep) with the corresponding standard error of the mean.
[3/] Colony color: P = purplish-white, Y = yellowish-white, O = orangish-white
[4/] Appearance of chlamydospore production on PDA: ✓= present, - = absent

Table 2.3 (Continued)

No.	Isolate code	Colony[1/]		Mean size of conidia (µm)[2/]				Appearance of chlamydospore
		Color[3/]	Growth rate (mm/day)	Macroconidia		Microconidia		
				Length	Width	Length	Width	
20.	*Fol*TB_020	P	7.56 ± 0.19	36.73 ± 3.55	3.63 ± 0.71	7.50 ± 1.77	2.78 ± 0.16	-
21.	*Fol*TB_021	W	7.56 ± 0.41	37.57 ± 3.26	3.73 ± 0.63	8.23 ± 1.63	2.70 ± 0.17	✓
C. Mr. Somkid's farm = 12 isolates								
22.	*Fol*CC_022	W	8.32 ± 0.09	35.97 ± 3.56	3.60 ± 0.66	7.53 ± 1.56	2.72 ± 0.18	✓
23.	*Fol*CC_023	P	8.40 ± 0.17	35.77 ± 3.55	3.63 ± 0.66	7.77 ± 1.71	2.77 ± 0.19	✓
24.	*Fol*CC_024	P	7.64 ± 0.18	38.87 ± 3.39	3.73 ± 0.63	7.10 ± 1.66	2.77 ± 0.17	-
25.	*Fol*CC_025	P	8.40 ± 0.25	37.00 ± 3.56	3.60 ± 0.66	7.07 ± 1.50	2.74 ± 0.17	✓
26.	*Fol*CC_026	W	9.28 ± 0.25	38.10 ± 3.55	3.57 ± 0.62	7.30 ± 1.73	2.72 ± 0.16	✓
27.	*Fol*CC_027	P	9.28 ± 0.16	38.33 ± 3.46	3.57 ± 0.67	7.77 v 1.52	2.75 ± 0.15	✓
28.	*Fol*CC_028	W	9.33 ± 0.17	37.50 ± 3.98	3.67 ± 0.65	7.37 ± 1.72	2.74 ± 0.18	-
29.	*Fol*CC_029	P	8.40 ± 0.17	36.90 ± 2.97	3.73 ± 0.63	7.17 ± 1.65	2.77 ± 0.17	✓
30.	*Fol*CC_030	P	8.40 ± 0.14	37.93 ± 4.04	3.70 ± 0.69	7.03 ± 1.80	2.79 ± 0.17	-
31.	*Fol*CC_031	P	6.93 ± 0.29	36.97 ± 4.05	3.67 ± 0.70	7.40 ± 1.76	2.68 ± 0.18	✓
32.	*Fol*CC_032	W	7.64 ±0.18	38.03 ± 3.60	3.63 ± 0.66	7.80 ± 1.66	2.76 ± 0.19	✓
33.	*Fol*CC_033	W	7.64 ± 0.11	36.53 ± 3.56	3.70 ± 0.64	7.77 ± 1.69	2.72 ± 0.17	✓
D. Mr. Ying's farm = 10 isolates								
34.	*Fol*CD_034	W	9.33 ± 0.28	37.43 ± 3.76	3.47 ± 0.67	7.67 ± 1.90	2.73 ± 0.15	✓
35.	*Fol*CD_035	W	9.33 ± 0.27	36.73 ± 3.49	3.83 ± 0.64	7.87 ± 1.63	2.77 ± 0.17	-
36.	*Fol*CD_036	W	8.37 ± 0.16	37.10 ± 3.08	3.63 ± 0.66	7.43 ± 1.80	2.73 ± 0.17	✓
37.	*Fol*CD_037	O	8.40 ± 0.14	37.33 ± 4.20	3.67 ± 0.65	7.93 ± 1.61	2.76 ± 0.16	✓
38.	*Fol*CD_038	O	7.64 ± 0.13	35.80 ± 3.61	3.80 ± 0.65	7.67 ± 1.81	2.78 ± 0.16	✓
39.	*Fol*CD_039	W	8.40 ± 0.11	37.27 ± 3.96	3.57 ± 0.62	7.47 ± 1.63	2.77 ± 0.20	-
40.	*Fol*CD_040	W	7.65 ± 0.27	36.77 ± 3.09	3.60 ± 0.55	7.47 ± 1.67	2.75 ± 0.16	-

[1/], [2/] Mean of three replications (5 plates/rep) with the corresponding standard error of the mean.
[3/] Colony color: P = purplish-white, Y = yellowish-white, O = orangish-white
[4/] Appearance of chlamydospore production on PDA: ✓= present, - = absent

Table 2.3 (Continued)

No.	Isolate code	Colony[1/]		Mean size of conidia (µm)[2/]				Appearance of chlamydospore
		Color[3/]	Growth rate (mm/day)	Macroconidia		Microconidia		
				Length	Width	Length	Width	
41.	*Fol*CD_041	O	8.40 ± 0.11	36.83 ± 3.43	3.73 ± 0.63	7.93 ± 1.53	2.80 ± 0.15	-
42.	*Fol*CD_042	W	9.33 ± 0.21	36.20 ± 3.30	3.77 ± 0.67	7.87 ± 1.52	2.74 ± 0.19	✓
43.	*Fol*CD_043	P	9.24 ± 0.16	36.37 ± 3.28	3.47 ± 0.62	7.30 ± 1.55	2.81 ± 0.14	-
E. Mr. Arnonth's farm = 10 isolates								
44.	*Fol*CE_044	P	7.64 ± 0.15	36.33 ± 3.62	3.60 ± 0.61	6.73 ± 1.63	2.79 ± 0.15	✓
45.	*Fol*CE_045	P	8.40 ± 0.16	37.07 ± 3.97	3.67 ± 0.60	7.87 ± 1.50	2.75 ± 0.15	-
46.	*Fol*CE_046	W	7.64 ± 0.28	37.07 ± 4.19	3.63 ± 0.66	7.57 ± 1.73	2.71 ± 0.16	✓
47.	*Fol*CE_047	W	9.33 ± 0.21	36.77 ± 3.56	3.57 ± 0.67	7.73 ± 1.57	2.75 ± 0.17	-
48.	*Fol*CE_048	W	9.33 ± 0.19	36.83 ± 4.15	3.57 ± 0.67	7.50 ± 1.88	2.75 ± 0.17	-
49.	*Fol*CE_049	W	9.33 ± 0.19	37.73 ± 4.05	3.67 ± 0.65	7.33 ± 2.02	2.75 ± 0.13	✓
50.	*Fol*CE_050	W	9.33 ± 0.31	37.03 ± 3.82	3.80 ± 0.60	7.57 ± 1.71	2.73 ± 0.16	✓
51.	*Fol*CE_051	W	9.33 ± 0.25	36.70 ± 3.53	3.60 ± 0.66	7.77 ± 1.61	2.78 ± 0.15	-
52.	*Fol*CE_052	W	8.40 ± 0.25	37.77 ± 3.31	3.63 ± 0.66	7.00 ± 1.51	2.76 ± 0.14	✓
53.	*Fol*CE_053	W	9.28 ± 0.29	37.47 ± 4.08	3.77 ± 0.62	7.27 ± 1.71	2.72 ± 0.18	-
F. Mr. Boonserm's farm = 10 isolates								
54.	*Fol*TF_054	P	8.42 ± 0.32	37.00 ± 3.87	3.67 ± 0.65	7.13 ± 1.69	2.76 ± 0.18	-
55.	*Fol*TF_055	P	9.33 ± 0.21	37.70 ± 4.13	3.50 ± 0.56	6.63 ± 1.38	2.73 ± 0.16	✓
56.	*Fol*TF_056	P	8.37 ± 0.19	36.70 ± 3.36	3.57 ± 0.62	8.13 ± 1.59	2.79 ± 0.21	✓
57.	*Fol*TF_057	P	9.33 ± 0.21	37.01 ± 4.04	3.63 ± 0.60	7.83 ± 1.69	2.70 ± 0.17	-
58.	*Fol*TF_058	O	8.35 ± 0.27	36.63 ± 3.83	3.47 ± 0.56	7.87 ± 1.65	2.80 ± 0.15	✓
59.	*Fol*TF_059	O	8.40 ± 0.18	37.60 ± 3.78	3.57 ± 0.67	7.53 ± 1.73	2.71 ± 0.18	✓
60.	*Fol*TF_060	P	10.46 ± 0.57	38.03 ± 3.52	3.77 ± 0.62	7.37 ± 1.80	2.77 ± 0.17	✓
61.	*Fol*TF_061	W	9.33 ± 0.17	37.87 ± 3.76	3.67 ± 0.65	7.23 ± 1.76	2.82 ± 0.15	✓

[1/], [2/] Mean of three replications (5 plates/rep) with the corresponding standard error of the mean.
[3/] Colony color: P = purplish-white, W = white, O = orangish-white
[4/] Appearance of chlamydospore production on PDA: ✓= present, - = absent

Table 2.3 (Continued)

No.	Isolate code	Colony[1/]		Mean size of conidia (µm)[2/]				Appearance of chlamydospore
		Color[3/]	Growth rate (mm/day)	Macroconidia		Microconidia		
				Length	Width	Length	Width	
62.	*Fol*TF_062	P	9.30 ± 0.15	37.23 ± 3.62	3.77 ± 0.62	6.90 ± 1.68	2.73 ± 0.18	-
63.	*Fol*TF_063	W	9.33 ± 0.17	36.40 ± 3.62	3.77 ± 0.62	7.50 ± 1.80	2.80 ± 0.18	-
G. Mr. Manit's farm = 12 isolates								
64.	*Fol*TG_064	W	9.36 ± 0.24	36.23 ± 2.96	3.60 ± 0.55	7.63 ± 1.72	2.73 ± 0.18	✓
65.	*Fol*TG_065	O	9.33 ± 0.15	37.50 ± 3.62	3.70 ± 0.64	6.63 ± 1.72	2.76 ± 0.17	✓
66.	*Fol*TG_066	O	7.65 ± 0.38	39.07 ± 3.10	3.67 ± 0.65	7.43 ± 1.86	2.69 ± 0.15	-
67.	*Fol*TG_067	O	7.64 ± 0.39	38.33 ± 3.68	3.70 ± 0.64	7.60 ± 1.94	2.73 ± 0.18	-
68.	*Fol*TG_068	P	8.40 ± 0.20	36.40 ± 3.01	3.67 ± 0.65	7.80 ± 1.80	2.71 ± 0.15	✓
69.	*Fol*TG_069	P	7.70 ± 0.26	37.60 ± 3.55	3.47 ± 0.67	7.67 ± 1.58	2.68 ± 0.16	-
70.	*Fol*TG_070	P	7.65 ± 0.31	35.63 ± 3.48	3.47 ± 0.62	7.87 ± 1.48	2.74 ± 0.18	✓
71.	*Fol*TG_071	P	8.40 ± 0.19	37.07 ± 3.87	3.50 ± 0.62	7.33 ± 1.94	2.77 ± 0.18	-
72.	*Fol*TG_072	W	6.93 ± 0.30	36.57 ± 3.96	3.57 ± 0.62	6.97 ± 1.49	2.71 ± 0.15	-
73.	*Fol*TG_073	P	7.64 ± 0.22	37.13 ± 3.48	3.57 v 0.62	7.07 ± 1.69	2.76 ± 0.16	✓
74.	*Fol*TG_074	P	7.64 ± 0.19	37.17 ± 4.02	3.50 ± 0.63	7.93 ± 1.71	2.76 ± 0.16	✓
75.	*Fol*TG_075	W	8.32 ± 0.32	37.00 ± 3.95	3.73 ± 0.63	7.70 ± 1.85	2.77 ± 0.17	-
H. Mr. Ying's farm = 10 isolates								
76.	*Fol*TH_076	W	8.40 ± 0.22	36.77 ± 3.53	3.80 ± 0.60	7.47 ± 1.67	2.71 ± 0.19	✓
77.	*Fol*TH_077	W	9.33 ± 0.27	37.93 ± 4.00	3.47 ± 0.62	7.57 ± 1.84	2.78 ± 0.17	-
78.	*Fol*TH_078	W	8.45 ± 0.21	37.63 ± 3.48	3.63 ± 0.60	7.17 ± 1.69	2.71 ± 0.18	✓
79.	*Fol*TH_079	O	9.33 ± 0.22	37.60 ± 3.48	3.53 ± 0.67	7.33 ± 1.40	2.76 ± 0.17	✓
80.	*Fol*TH_080	W	9.36 ± 0.17	37.33 ± 3.52	3.60 ± 0.76	7.90 ± 1.66	2.75 ± 0.17	✓
81.	*Fol*TH_081	P	9.33 ± 0.19	37.23 ± 3.26	3.70 ± 0.64	7.57 ± 1.63	2.75 ± 0.16	-
82.	*Fol*TH_082	P	8.40 ± 0.18	35.83 ± 3.92	3.60 ± 0.55	8.50 ± 1.36	2.72 ± 0.17	-

[1/], [2/] Mean of three replications (5 plates/rep) with the corresponding standard error of the mean.
[3/] Colony color: P = purplish-white, Y = yellowish-white, O = orangish-white
[4/] Appearance of chlamydospore production on PDA: ✓= present, - = absent

Table 2.3 (Continued)

No.	Isolate code	Colony[1/]		Mean size of conidia (µm)[2/]				Appearance of chlamydospore
		Color[3/]	Growth rate (mm/day)	Macroconidia		Microconidia		
				Length	Width	Length	Width	
83.	*Fol*TH_083	P	8.40 ± 0.22	36.97 ± 3.87	3.50 ± 0.56	7.40 ± 1.74	2.74 ± 0.15	✓
84.	*Fol*TH_084	W	7.64 ± 0.22	36.73 ± 3.39	3.53 ± 0.62	7.50 ± 1.73	2.79 ± 0.17	✓
I. Mr. Sordee's farm = 11 isolates								
85.	*Fol*TI_085	P	8.40 ± 0.42	36.97 ± 3.77	3.57 ± 0.56	7.30 ± 1.73	2.76 ± 0.18	-
86.	*Fol*TI_086	P	7.64 ± 0.38	37.23 ± 3.70	3.57 ± 0.56	7.63 ± 1.64	2.80 ± 0.15	✓
87.	*Fol*TI_087	P	7.64 ± 0.36	37.23 ± 4.21	3.43 ± 0.56	6.97 ± 1.52	2.78 ± 0.16	-
88.	*Fol*TI_088	P	7.59 ± 0.30	36.67 ± 3.97	3.63 ± 0.55	7.43 ± 1.80	2.75 ± 0.16	✓
89.	*Fol*TI_089	P	7.64 ± 0.22	35.37 ± 3.20	3.60 ± 0.55	7.53 ± 1.67	2.73 ± 0.18	-
90.	*Fol*TI_090	P	7.64 ± 0.28	36.37 ± 3.55	3.57 ± 0.62	7.93 ± 1.81	2.74 ± 0.18	-
91.	*Fol*TI_091	P	7.64 ± 0.40	37.20 ± 3.77	3.77 ± 0.62	7.77 ± 1.65	2.76 ± 0.19	-
92.	*Fol*TI_092	O	9.33 ± 0.31	37.70 ± 3.87	3.60 ± 0.55	7.83 ± 1.67	2.81 ± 0.15	✓
93.	*Fol*TI_093	P	9.33 ± 0.25	36.00 ± 3.51	3.53 ± 0.62	7.63 ± 1.92	2.72 ± 0.16	✓
94.	*Fol*TI_094	P	9.33 ± 0.29	37.00 ± 3.98	3.53 ± 0.67	7.73 ± 1.63	2.72 ± 0.7	-
95.	*Fol*TI_095	W	7.64 ± 0.18	37.90 ± 3.57	3.70 ± 0.59	7.20 ± 1.74	2.74 ± 0.17	✓
J. Mr. Boonthum's farm = 18 isolates								
96.	*Fol*CJ_096	W	7.64 ± 0.34	36.70 ± 3.72	3.67 ± 0.65	7.10 ± 1.60	2.77 ± 0.17	-
97.	*Fol*CJ_097	O	7.59 ± 0.18	38.50 ± 3.88	3.60 ± 0.65	7.70 ± 1.42	2.75 ± 0.16	✓
98.	*Fol*CJ_098	W	9.33 ± 0.27	36.60 ± 3.04	3.60 ± 0.61	7.87 ± 1.59	2.77 ± 0.18	✓
99.	*Fol*CJ_099	P	8.40 ± 0.17	37.07 ± 3.59	3.50 ± 0.56	7.73 ± 1.63	2.76 ± 0.15	✓
100.	*Fol*CJ_100	P	9.28 ± 0.30	36.90 ± 4.02	3.47 ± 0.56	7.77 ± 1.69	2.75 ± 0.18	-
101.	*Fol*CJ_101	P	7.64 ± 0.25	37.30 ± 3.99	3.57 ± 0.50	7.80 ± 1.62	2.78 ± 0.18	✓
102.	*Fol*CJ_102	P	7.56 ± 0.19	37.60 ± 3.76	3.80 ± 0.60	7.53 ± 1.52	2.74 ± 0.18	-
103.	*Fol*CJ_103	W	8.40 ± 0.17	36.97 ± 3.60	3.70 ± 0.64	7.30 ± 1.64	2.73 ± 0.17	-

[1/], [2/] Mean of three replications (5 plates/rep) with the corresponding standard error of the mean.
[3/] Colony color: P = purplish-white, Y = yellowish-white, O = orangish-white
[4/] Appearance of chlamydospore production on PDA: ✓= present, - = absent

Table 2.3 (Continued)

No.	Isolate code	Colony[1/]		Mean size of conidia (µm)[2/]				Appearance of chlamydospore
		Color[3/]	Growth rate (mm/day)	Macroconidia		Microconidia		
				Length	Width	Length	Width	
104.	*Fol*CJ_104	P	7.64 ± 0.22	37.03 ± 4.05	3.60 ± 0.61	7.07 ± 1.67	2.72 ± 0.16	✓
105.	*Fol*CJ_105	O	7.64 ± 0.28	36.97 ± 3.78	3.63 ± 0.60	7.03 ± 1.64	2.79 ± 0.16	-
106.	*Fol*CJ_106	P	9.28 ± 0.16	38.77 ± 3.41	3.53 ± 0.67	8.03 ± 1.54	2.73 ± 0.18	-
107.	*Fol*CJ_107	W	7.64 ± 0.38	36.90 ± 3.80	3.67 ± 0.06	7.40 ± 1.84	2.69 ± 0.15	-
108.	*Fol*CJ_108	W	9.33 ± 0.25	36.10 ± 3.70	3.57 ± 0.56	7.37 ± 1.54	2.76 ± 0.18	✓
109.	*Fol*CJ_109	W	7.64 ± 0.11	36.87 ± 3.65	3.70 ± 3.64	7.17 ± 1.61	2.70 ± 0.16	-
110.	*Fol*CJ_110	O	9.33 ± 0.28	36.37 ± 3.75	3.67 ± 0.65	7.80 ± 1.74	2.71 ± 0.15	✓
111.	*Fol*CJ_111	O	7.64 ± 0.13	36.67 ± 3.87	3.47 ± 0.56	7.07 ± 1.73	2.76 ± 0.16	✓
112.	*Fol*CJ_112	P	7.65 ± 0.27	36.57 ± 3.69	3.50 ± 0.62	7.20 ± 1.62	2.75 ± 0.18	✓
113.	*Fol*CJ_113	P	8.40 ± 0.11	37.83 ± 3.47	3.60 ± 0.55	7.73 ± 1.65	2.69 ± 0.17	✓
K. Mr. Suveera's farm = 13 isolates								
114.	*Fol*CK_114	P	7.64 ± 0.15	37.70 ± 3.71	3.63 ± 0.55	7.63 ± 1.82	2.68 ± 0.15	✓
115.	*Fol*CK_115	P	9.33 ± 0.19	35.53 ± 4.09	3.60 ± 0.61	7.17 ± 1.67	2.69 ± 0.17	-
116.	*Fol*CK_116	W	9.33 ± 0.25	36.20 ± 3.15	3.77 ± 0.56	7.63 ± 1.76	2.74 ± 0.16	✓
117.	*Fol*CK_117	P	8.40 ± 0.25	36.83 ± 3.15	3.60 ± 0.61	7.67 ± 1.64	2.72 ± 0.17	✓
118.	*Fol*CK_118	P	8.37 ± 0.19	37.77 ± 3.73	3.77 ± 0.56	7.53 ± 1.67	2.75 ± 0.18	-
119.	*Fol*CK_119	W	8.35 ± 0.27	36.90 ± 3.34	3.60 ± 0.61	7.43 ± 1.67	2.70 ± 0.16	-
120.	*Fol*CK_120	W	9.33 ± 0.17	36.90 ± 4.07	3.63 ± 0.66	7.80 ± 1.85	2.71 ± 0.19	✓
121.	*Fol*CK_121	O	9.36 ± 0.24	37.00 ± 3.81	3.87 ± 0.56	7.53 ± 1.75	2.71 ± 0.13	✓
122.	*Fol*CK_122	O	7.70 ± 0.26	37.30 ± 3.92	3.63 ± 0.66	7.47 ± 1.84	2.74 ± 0.16	-
123.	*Fol*CK_123	P	6.93 ± 0.30	37.57 ± 3.51	3.70 ± 0.59	7.03 ± 1.70	2.65 ± 0.16	✓
124.	*Fol*CK_124	P	8.40 ± 0.22	37.13 ± 3.71	3.53 ± 0.62	7.93 ± 1.88	2.74 ± 0.16	✓
125.	*Fol*CK_125	P	9.36 ± 0.17	36.97 ± 3.87	3.63 ± 0.55	7.53 ± 1.56	2.78 ± 0.18	-
126.	*Fol*CK_126	P	7.64 ± 0.22	37.87 ± 3.82	3.77 ± 0.62	7.37 ± 1.66	2.78 ± 0.17	✓

[1/], [2/] Mean of three replications (5 plates/rep) with the corresponding standard error of the mean.
[3/] Colony color: P = purplish-white, Y = yellowish-white, O = orangish-white
[4/] Appearance of chlamydospore production on PDA: ✓= present, - = absent

2.3.3 Pathogenicity test

The pathogenicity test confirmed that one hundred and twenty-six isolates of *F. oxysporum* f. sp. *lycopersici* (*Fol*) from infected tomato vascular tissue were pathogenic to tomato seedlings cv. 'Bonny Best' (susceptible to *Fusarium* wilt). Disease severities (Figure 2.4) were observed differently depending on isolates of the pathogenic *Fol*. Initial symptoms appeared as slightly yellow at lower leaves on one site of plants within 3 - 4 days after inoculation, and turned to deepen color within 7 - 10 days together with slight chlorosis, wilting, or stunting symptom. Afterwards, these symptoms were clearly observed since 14 days after inoculation, especially wilting symptom. At the end of observation (21 day after inoculation), dead plants found in tomato seedlings inoculated with the virulent isolates, while the susceptible isolates gave difference levels verified from low to high of chlorosis, wilting, or stunting of plant. Vascular browning discoloration was observed in *Fol*-infected seedlings (Figure 2.5). Moreover, adventitious roots formation was developed, which usually first demonstrated on basal stem on one site of basal stem before distributed around the stem (Figure 2.6).

At final assessment, disease severity index (DSI) of 126 *Fol* isolates were rated there was 59 (46.83%), 57 (45.24%), 4 (3.17%) and 5 (3.97%) isolates displayed variable scale 1 – 4 of disease severity index, respectively. Only one isolate (0.79%), *Fol*CK_117, showed high virulence and DSI scale at 5. Afterwards, pathogenicity groups (Sibounnavong, 2012) were categorized according to disease severity index. The results implied that there was 3 (2.38%), 113 (89.69%) and 9 (7.14%) isolates which were categorized as avirulent (DSI = 1), low (DSI ≤ 3.5) and moderate (DSI > 3.5 to 4.5) pathogenicity group, respectively. Similarly, only the isolate *Fol*CK_117 was categorized as high virulent group (DSI > 4.5) (Table 2.4).

The purplish-white colony group of pathogens *Fol* gave the highest range of pathogenicity that found between scales 2.0 – 5.0 with an average 2.56 ± 0.67. Meanwhile, the pathogenicity scales of white colony group were found resemble to orangish-white colony group; the pathogenicity scales of white colony group were found between scales 1.0 – 4.5 with an average 1.26 ± 0.13, while between scales 1.1 – 1.6 with an average 1.25 ± 0.13 were found in orangish-white colony group (Table 2.5).

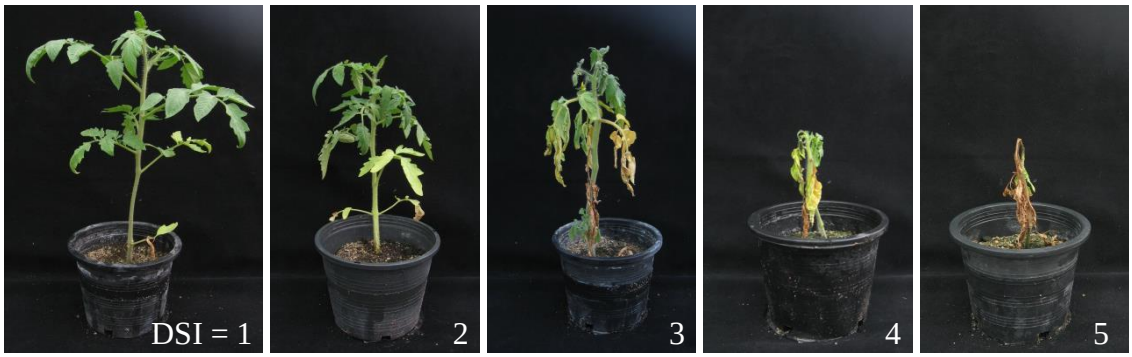

Figure 2.4 Disease severity index (DSI) of *Fusarium* wilt in tomato at 21 days after inoculation with *Fusarium oxysporum* f. sp. *lycopersici* (Marlatt *et al*., 1996); 1 = no symptom, 2 = slight chlorosis, wilting, or stunting of plant, 3 = moderate chlorosis, wilting, or stunting of plant, 4 = severe chlorosis, wilting, or stunting of plant, and 5 = dead plant

Figure 2.5 Vascular browning discoloration of tomato seedlings cv. 'Bonny Best' at 21 days after inoculation with *Fusarium oxysporum* f. sp. *lycopersici*

Figure 2.6 Adventitious root development of tomato seedlings cv. 'Bonny Best' at 21 days after inoculation with *Fusarium oxysporum* f. sp. *lycopersici*

Table 2.4 Disease severity index (DSI) and pathogenicity group of *Fusarium oxysporum* f. sp. *lycopersici* isolates causing *Fusarium* wilt in tomato seedlings cv. 'Bonny Best' (susceptible to *Fusarium* wilt)

No.	Isolate code	Colony color[1/]	DSI[2/]		Pathogenicity group[4/]
A. Mr. Sawat's farm = 8 isolates					
1.	*Fol*CA_001	P	2.45	DEF[3/]	low
2.	*Fol*CA_002	P	2.45	DEF	low
3.	*Fol*CA_003	P	2.33	DEF	low
4.	*Fol*CA_004	P	2.22	EF	low
5.	*Fol*CA_005	P	4.00	BC	moderate
6.	*Fol*CA_006	P	2.56	DEF	low
7.	*Fol*CA_007	P	2.33	DEF	low
8.	*Fol*CA_008	P	2.22	EF	low
B. Mr. Puttinun's farm = 13 isolates					
9.	*Fol*TB_009	W	1.33	I	low
10.	*Fol*TB_010	W	1.11	I	low
11.	*Fol*TB_011	P	2.00	FGH	low
12.	*Fol*TB_012	P	2.45	DEF	low
13.	*Fol*TB_013	P	4.00	BC	moderate
14.	*Fol*TB_014	W	1.00	I	avirulent
15.	*Fol*TB_015	P	2.34	DEF	low
16.	*Fol*TB_016	P	4.22	B	moderate
17.	*Fol*TB_017	P	2.33	DEF	low
18.	*Fol*TB_018	P	3.56	C	moderate
19.	*Fol*TB_019	P	2.00	FGH	low
20.	*Fol*TB_020	P	2.89	D	low
21.	*Fol*TB_021	W	1.33	I	low
C. Mr. Somkid's farm = 12 isolates					
22.	*Fol*CC_022	W	1.22	I	low
23.	*Fol*CC_023	P	2.33	DEF	low
24.	*Fol*CC_024	P	2.11	EFG	low
25.	*Fol*CC_025	P	2.56	DEF	low
26.	*Fol*CC_026	W	1.45	HI	low
27.	*Fol*CC_027	P	2.00	FGH	low
28.	*Fol*CC_028	W	1.22	I	low
29.	*Fol*CC_029	P	2.33	DEF	low
30.	*Fol*CC_030	P	2.11	EFG	low
31.	*Fol*CC_031	P	2.44	DEF	low
32.	*Fol*CC_032	W	1.45	HI	low
33.	*Fol*CC_033	W	1.00	I	avirulent
D. Mr. Sawat's farm = 8 isolates					
34.	*Fol*CD_034	W	1.45	HI[3/]	low
35.	*Fol*CD_035	W	1.33	I	low
36.	*Fol*CD_036	W	1.11	I	low

[1/] Colony color: P = purplish-white, Y = yellowish-white, O = orangish-white

[2/] Disease severity index (DSI); 1 = no symptom; 2 = slight chlorosis, wilting, or stunting of plant; 3 = moderate chlorosis, wilting, or stunting of plant; 4 = severe chlorosis, wilting, or stunting of plant and 5 = dead plant

[3/] Means of three replications (3 plants/rep). Means followed by a common letter in each column are not significantly different by LSD at $P<0.05$.

[4/] Pathogenicity group was categorized according to DSI as avirulent (DSI = 1); low (DSI ≤ 3.50); moderate (DSI > 3.50 to 4.50) and high virulent (DSI > 4.50).

Table 2.4 (Continued)

No.	Isolate code	Colony color[1/]	DSI[2/]		Pathogenicity group[4/]
37.	*Fol*CD_037	O	1.56	GHI[3/]	low
38.	*Fol*CD_038	O	1.11	I	low
39.	*Fol*CD_039	W	1.33	I	low
40.	*Fol*CD_040	W	1.11	I	low
41.	*Fol*CD_041	O	1.11	I	low
42.	*Fol*CD_042	W	1.22	I	low
43.	*Fol*CD_043	P	2.56	DEF	low
E. Mr. Puttinun's farm = 13 isolates					
44.	*Fol*CE_044	P	2.33	DEF	low
45.	*Fol*CE_045	P	2.45	DEF	low
46.	*Fol*CE_046	W	1.33	I	low
47.	*Fol*CE_047	W	1.45	HI	low
48.	*Fol*CE_048	W	1.22	I	low
49.	*Fol*CE_049	W	1.44	HI	low
50.	*Fol*CE_050	W	1.45	HI	low
51.	*Fol*CE_051	W	1.00	I	avirulent
52.	*Fol*CE_052	W	1.22	I	low
53.	*Fol*CE_053	W	1.33	I	low
F. Mr. Somkid's farm = 12 isolates					
54.	*Fol*TF_054	P	2.11	EFG	low
55.	*Fol*TF_055	P	3.89	BC	moderate
56.	*Fol*TF_056	P	3.89	BC	moderate
57.	*Fol*TF_057	P	2.34	DEF	low
58.	*Fol*TF_058	O	1.22	I	low
59.	*Fol*TF_059	O	1.33	I	low
60.	*Fol*TF_060	P	2.22	EF	low
61.	*Fol*TF_061	W	1.11	I	low
62.	*Fol*TF_062	P	2.45	DEF	low
63.	*Fol*TF_063	W	1.11	I	low
G. Mr. Manit's farm = 12 isolates					
64.	*Fol*TG_064	W	1.33	I	low
65.	*Fol*TG_065	O	1.11	I	low
66.	*Fol*TG_066	O	1.44	HI	low
67.	*Fol*TG_067	O	1.22	I	low
68.	*Fol*TG_068	P	2.00	FGH	low
69.	*Fol*TG_069	P	4.11	BC	moderate
70.	*Fol*TG_070	P	2.45	DEF	low
71.	*Fol*TG_071	P	2.22	EF	low
72.	*Fol*TG_072	W	1.22	I	low
73.	*Fol*TG_073	P	2.11	EFG	low
74.	*Fol*TG_074	P	2.22	EF	low
75.	*Fol*TG_075	W	1.33	I	low

[1/] Colony color: P = purplish-white, W = white, O = orangish-white

[2/] Disease severity index (DSI); 1 = no symptom; 2 = slight chlorosis, wilting, or stunting of plant; 3 = moderate chlorosis, wilting, or stunting of plant; 4 = severe chlorosis, wilting, or stunting of plant and 5 = dead plant

[3/] Means of three replications (3 plants/rep). Means followed by a common letter in each column are not significantly different by LSD at $P<0.05$.

[4/] Pathogenicity group was categorized according to DSI as avirulent (DSI = 1); low (DSI ≤ 3.50); moderate (DSI > 3.50 to 4.50) and high virulent (DSI > 4.50).

Table 2.4 (Continued)

No.	Isolate code	Colony color[1/]	DSI[2/]		Pathogenicity group[4/]
H. Mr. Surasith's farm = 9 isolates					
76.	*Fol*TH_076	W	1.33	I[3/]	low
77.	*Fol*TH_077	W	1.22	I	low
78.	*Fol*TH_078	W	1.33	I	low
79.	*Fol*TH_079	O	1.33	I	low
80.	*Fol*TH_080	W	1.33	I	low
81.	*Fol*TH_081	P	2.33	DEF	low
82.	*Fol*TH_082	P	2.45	DEF	low
83.	*Fol*TH_083	P	2.22	EF	low
84.	*Fol*TH_084	W	1.45	HI	low
I. Mr. Sordee's farm = 11 isolates					
85.	*Fol*TI_085	P	2.67	DE	low
86.	*Fol*TI_086	P	2.56	DEF	low
87.	*Fol*TI_087	P	4.00	BC	moderate
88.	*Fol*TI_088	P	2.45	DEF	low
89.	*Fol*TI_089	P	3.89	BC	moderate
90.	*Fol*TI_090	P	2.11	EFG	low
91.	*Fol*TI_091	P	2.33	DEF	low
92.	*Fol*TI_092	O	1.11	I	low
93.	*Fol*TI_093	P	2.11	EFG	low
94.	*Fol*TI_094	P	2.56	DEF	low
95.	*Fol*TI_095	W	1.22	I	low
J. Mr. Boonthum's farm = 18 isolates					
96.	*Fol*CJ_096	W	1.11	I	low
97.	*Fol*CJ_097	O	1.22	I	low
98.	*Fol*CJ_098	W	1.11	I	low
99.	*Fol*CJ_099	P	2.56	DEF	low
100.	*Fol*CJ_100	P	2.00	FGH	low
101.	*Fol*CJ_101	P	2.11	EFG	low
102.	*Fol*CJ_102	P	2.00	FGH	low
103.	*Fol*CJ_103	W	1.44	HI	low
104.	*Fol*CJ_104	P	2.11	EFG	low
105.	*Fol*CJ_105	O	1.22	I	low
106.	*Fol*CJ_106	P	2.22	EF	low
107.	*Fol*CJ_107	W	1.22	I	low
108.	*Fol*CJ_108	W	1.33	I	low
109.	*Fol*CJ_109	W	1.11	I	low
110.	*Fol*CJ_110	O	1.22	I	low
111.	*Fol*CJ_111	O	1.11	I	low
112.	*Fol*CJ_112	P	2.45	DEF	low
113.	*Fol*CJ_113	P	2.67	DE	low

[1/] Colony color: P = purplish-white, W = white, O = orangish-white

[2/] Disease severity index (DSI); 1 = no symptom; 2 = slight chlorosis, wilting, or stunting of plant; 3 = moderate chlorosis, wilting, or stunting of plant; 4 = severe chlorosis, wilting, or stunting of plant and 5 = dead plant

[3/] Means of three replications (3 plants/rep). Means followed by a common letter in each column are not significantly different by LSD at $P<0.05$.

[4/] Pathogenicity group was categorized according to DSI as avirulent (DSI = 1); low (DSI ≤ 3.50); moderate (DSI > 3.50 to 4.50) and high virulent (DSI > 4.50).

Table 2.4 (Continued)

No.	Isolate code	Colony color[1/]	DSI[2/]		Pathogenicity group[4/]
K. Mr. Suveera's farm = 13 isolates					
114.	*Fol*CK_114	P	2.00	FGH[3/]	low
115.	*Fol*CK_115	P	2.11	EFG	low
116.	*Fol*CK_116	W	1.22	I	low
117.	*Fol*CK_117	P	5.00	A	high
118.	*Fol*CK_118	P	2.22	EF	low
119.	*Fol*CK_119	W	1.22	I	low
120.	*Fol*CK_120	W	1.22	I	low
121.	*Fol*CK_121	O	1.22	I	low
122.	*Fol*CK_122	O	1.45	HI	low
123.	*Fol*CK_123	P	2.11	EFG	low
124.	*Fol*CK_124	P	2.34	DEF	low
125.	*Fol*CK_125	P	2.45	DEF	low
126.	*Fol*CK_126	P	2.22	EF	low
F-test			***		
$LSD_{0.05}$			0.64		
CV (%)			20.24		

[1/] Colony color: P = purplish-white, W = white, O = orangish-white
[2/] Disease severity index (DSI); 1 = no symptom; 2 = slight chlorosis, wilting, or stunting of plant; 3 = moderate chlorosis, wilting, or stunting of plant; 4 = severe chlorosis, wilting, or stunting of plant and 5 = dead plant
[3/] Means of three replications (3 plants/rep). Means followed by a common letter in each column are not significantly different by LSD at $P<0.05$.
[4/] Pathogenicity group was categorized according to DSI as avirulent (DSI = 1); low (DSI ≤ 3.50); moderate (DSI > 3.50 to 4.50) and high virulent (DSI > 4.50).

Table 2.5 Correlation between colony color of *Fusarium oxysporum* f. sp. *lycopersici* isolates causing *Fusarium* wilt and disease severity index (DSI) in tomato seedlings cv. 'Bonny Best' (susceptible to *Fusarium* wilt)

Colony color[1/]	Number		DSI[2/]	
	Isolates	Percentage	Range	Averange
P	67	53.17	2.0 – 5.0	2.56 ± 0.67
W	43	34.13	1.0 – 4.5	1.26 ± 0.13
O	16	12.70	1.1 – 1.6	1.25 ± 0.13

[1/] Colony color: P = purplish-white, W = white, O = orangish-white
[2/] Disease severity index (DSI); 1 = no symptom; 2 = slight chlorosis, wilting, or stunting of plant; 3 = moderate chlorosis, wilting, or stunting of plant; 4 = severe chlorosis, wilting, or stunting of plant and 5 = dead plant

Afterward, twenty-three of *Fol* isolates of each pathogenicity group were randomly selected to re-test for pathogenicity in cv. 'Bonny Best' (susceptible to *Fusarium* wilt) and compare to cv. 'EWS-37434' (resistant to *Fusarium* wilt) (Table 2.5). The selected *Fol* were pathogenic to seedlings cv. 'Bonny Best' and gave disease severity resemble the first test, whereas unable to infect the resistant cv. 'EWS-37434' plants (Table 2.6). The result of the pathogenicity test showed a significant difference ($P \leq 0.05$) in pathogenicity test between isolates when compared to the control.

Table 2.6 Randomly selected isolates from the pathogenic group of *Fusarium oxysporum* f. sp. *lycopersici* in tomato seedlings cv. 'Bonny Best' (susceptible to *Fusarium* wilt)

No.	Isolate code	Tomato variety	Colony color[1/]	DSI[2/]
A. High[3/] = 1 isolates				
1.	*Fol*CK_117	Cherry	P	5.00
B. Moderate = 9 isolates				
2.	*Fol*CA_005	Cherry	P	4.00
3.	*Fol*TB_013	Thomas	P	4.00
4.	*Fol*TB_016	Thomas	P	4.22
5.	*Fol*TB_018	Thomas	P	3.56
6.	*Fol*TF_055	Thomas	P	3.89
7.	*Fol*TF_056	Thomas	P	3.89
8.	*Fol*TG_069	Thomas	P	4.11
9.	*Fol*TI_087	Thomas	P	4.00
10.	*Fol*TI_089	Thomas	P	3.89
C. Low = 10 isolates				
11.	*Fol*CA_003	Cherry	P	2.33
12.	*Fol*TB_012	Thomas	P	2.45
13.	*Fol*CC_025	Cherry	P	2.56
14.	*Fol*CD_039	Cherry	W	1.33
15.	*Fol*TF_061	Thomas	W	1.11
16.	*Fol*TG_071	Thomas	P	2.22
17.	*Fol*TH_079	Thomas	O	1.33
18.	*Fol*TI_094	Thomas	P	2.56
19.	*Fol*CJ_099	Cherry	P	2.56
20.	*Fol*CJ_112	Thomas	P	2.45
D. Non-pathogenic = 3 isolates				
21.	*Fol*TB_014	Thomas	W	1.00
22.	*Fol*CC_033	Cherry	W	1.00
23.	*Fol*CE_051	Cherry	W	1.00

[1/] Colony color: P = purplish-white, W = white, O = orangish-white

[2/] Disease severity index (DSI); 1 = no symptom; 2 = slight chlorosis, wilting, or stunting of plant; 3 = moderate chlorosis, wilting, or stunting of plant; 4 = severe chlorosis, wilting, or stunting of plant and 5 = dead plant

[3/] Pathogenicity group was categorized according to DSI as non-pathogenic (DSI = 1); low (DSI ≤ 3.50); moderate (DSI > 3.50 to 4.50) and high (DSI > 4.50).

Table 2.7 Disease severity index (DSI) of selected *Fusarium oxysporum* f. sp. *lycopersici* to isolates in tomato seedlings cv. 'Bonny Best' (susceptible to *Fusarium* wilt) and cv. 'EWS-37434 (resistant to *Fusarium* wilt)

No.	Isolate code	Colony color[1/]	DSI[2/]			
			cv. 'Bonny Best'		cv. 'EWS-37434'	
A. High = 1 isolates						
1.	*Fol*CK_117	P	5.00	A[4/]	1.00	H
B. Moderate = 9 isolates						
2.	*Fol*CA_005	P	4.00	BC	1.00	H
3.	*Fol*TB_013	P	4.22	BC	1.00	H
4.	*Fol*TB_016	P	4.33	B	1.00	H
5.	*Fol*TB_018	P	3.89	CD	1.00	H
6.	*Fol*TF_055	P	4.00	BC	1.00	H
7.	*Fol*TF_056	P	3.56	D	1.00	H
8.	*Fol*TG_069	P	4.22	BC	1.00	H
9.	*Fol*TI_087	P	3.89	CD	1.00	H
10.	*Fol*TI_089	P	4.00	BC	1.00	H
C. Low = 10 isolates						
11.	*Fol*CA_003	P	2.22	F	1.00	H
12.	*Fol*TB_012	P	2.67	E	1.00	H
13.	*Fol*CC_025	P	2.56	EF	1.00	H
14.	*Fol*CD_039	W	1.78	G	1.00	H
15.	*Fol*TF_061	W	1.56	GH	1.00	H
16.	*Fol*TG_071	P	2.22	F	1.00	H
17.	*Fol*TH_079	O	2.44	EF	1.00	H
18.	*Fol*TI_094	P	2.67	E	1.00	H
19.	*Fol*CJ_099	P	2.44	EF	1.00	H
20.	*Fol*CJ_112	P	2.44	EF	1.00	H
D. Non-pathogenic = 3 isolates						
21.	*Fol*TB_014	W	1.00	H	1.00	H
22.	*Fol*CC_033	W	1.00	H	1.00	H
23.	*Fol*CE_051	W	1.00	H	1.00	H
	A (cv. of tomato plant)		***		$LSD_{0.05}$ = 0.08	
	B (isolate of *Fol*)		***		$LSD_{0.05}$ = 0.26	
	A*B		***		$LSD_{0.05}$ = 0.37	
	CV (%)		12.54			

[1/] Colony color: P = purplish-white, W = white, O = orangish-white

[2/] Disease severity index (DSI); 1 = no symptom; 2 = slight chlorosis, wilting, or stunting of plant; 3 = moderate chlorosis, wilting, or stunting of plant; 4 = severe chlorosis, wilting, or stunting of plant and 5 = dead plant

[3/] Pathogenicity group

[4/] Means of three replications (3 plants/rep). Means followed by a common letter in each column are not significantly different by LSD at $P<0.05$.

2.2.4 Race identification of *F. oxysporum* f. sp. *lycopersici* isolate *Fol*CK_117

The isolate *Fol*CK_117 was identified for different races by pathogenicity test on the set of reference differential tomato varieties. Tomato seedlings cv. 'EWS-20996' and 'EWS-20987' showed susceptibility to *Fol*CK_117, whereas other three varieties showed resistance (Table 2.7). The results indicated that the *Fol*CK_117 was identified as race 2 virulent phenotype.

Table 2.8 Race identification of *Fusarium oxysporum* f. sp. *lycopersici* isolate *Fol*CK_117 using 5 tomato varieties with different races

race of *Fol*	standard differential varieties*				
	EWS-S	EWS-R1	EWS-R12.1	EWS-R12.2	EWS-R123
race 1	S	R	R	R	R
race 2	S	S	R	R	R
race 3	S	S	S	S	R
*Fol*CK_117	S	S	R	R	R

*S = susceptible, R = resistant

2.4 Discussion

Fusarium wilt disease affects the quality and quantity of tomatoes in almost all tomato producing areas of Thailand. The causal agent was isolated based on the symptoms on vascular of 2 tomato cultivars; cv. 'Cherry' and cv. 'Thomas' which collected from eleven commercial fields at Doi Inthanon National Park, Chiang Mai, Thailand. The samples collection was done in September 2013 (rainy season). Although both tomato cv. 'Cherry' and cv. 'Thomas' are claimed as resistance cultivars, the *Fusarium* wilt disease is still occurred because of favourable conditions. *Fusarium* wilt, a serious disease of tomato, occurs during periods of cool, rainy weather that may come at the end of a growing season. Local dissemination is by transplants, tomato stakes, windborne, farm machinery and in particularly the waterborne infested soil. They cause a 30 – 40% yield loss and may go up to 80% under favourable conditions (Kapoor, 1988; Wong, 2003; Kirankumar *et al*., 2008).

Traditionally, characterization of *Fusarium* species was based on the published description; distinctive characters of the shapes and sizes of macro- and microconidia, presence and absence of chlamydospores as well as colony appearances, pigmentations and growth rates on agar media (Nelson *et al.*, 1994; Leslie and Summerell, 2006). In this study, morphological characteristics of isolated fungi were observed PDA and under microscopic, which all identified as *F. oxysporum* which agreement with previous reports from Nelson *et al*. (1983) and Synder and Hans (2003). Such pathogens are specific for certain plant hosts and known as 'forma speciales' (Marasas *et al*., 1984; Joffe, 1986; Cartia *et al*., 1988; Rivelli, 1989; Fletcher, 1994; Mushtaq and Hashmi, 1997; Jovicich *et al*., 1999), the more detailed tests comfirmed that all isolates were *F. oxysporum* f. sp. *lycopersici* (*Fol*). However, *Fol* causing *Fusarium* wilt has been reported in many major growing areas in Thailand, such as Nong Khai, Udon Thani, Chiang Mai, Lampang, Phetchabun, Khon Kaen and Nakhon Pathom provinces (Wuttiwanit, 2002). It has been reported that it was found in in Northern Thailand, where rotating grown tomatoes between plains and highlands region throughout the year, including San Sai, Fang, Chom Thong, Hot and Omkoi district, Chiang Mai province, Mae Sariang district and Mae Hong Son province (Lumyong and Inwang, 1984).

In present study, pathogenicity tests were conducted using the susceptible cultivar to avoid genetic factor of resistant gene. Based on this principle, tomato cv. 'Bonny Best' was selected to use as standard susceptible host because they do not contain *Fusarium* resistant gene (Grattidge, 1982; Marlatt *et al*., 1996). In this study, the pathogenicity test confirmed that a total 126 isolates of *Fol* were pathogenic to tomato seedling cv. 'Bonny Best', which the isolate *Fol*CK_117 was considered as the most virulent strain. Seedlings inoculated with each isolate of *Fol* showed yellowing, chlorosis, wilting, stunting of plant and dead plant, which differentiate virulence level verified from low to very high virulent isolates. The colony colors (pigmentation) were correlated with disease severity index (DSI); the purplish-white colony group gave the highest of an average DSI, followed by the white and orangish-white colony group, respectively. Moreover, the stem was cut lengthwise, browning vascular system was observed. This browning vascular tissue is characteristic of the disease and generally can be used for its tentative identification and conformation of the fungal isolates as *F. oxysporum* f. sp. *lycopersici* (Armstrong and Armstrong, 1968; Jones, 1991; Reis *et al*.,

2005). The symptoms were the same as those described by Massee (1895) who was first explained the *Fusarium* wilt disease of tomato in England, and other reports (Walker, 1971; Jones *et al*., 1982; Agrios, 1988; Smith *et al*., 1988; Jones, 1991; Frank, 1998; Messenger and Braun, 2000).

Furthermore, some of representative *Fol* was randomly selected to recheck. Their pathogenicity was compared tests using a resistant cultivar, cv. EWS-37434. Two pathogenicity tests were evaluated as a method to identify the suitable experimental strain. The results showed resemble the first test seedlings cv. 'Bonny Best', whereas no any symptom was found in the resistant cv. 'EWS-37434' seedlings due to resistance gene. This results clearly indicated the suitable tentative susceptible and resistance cultivars for further experiments.

The forma specialis of *F. oxysporum* is classified on the basis of virulence on a particular host (Correll, 1991). Variation in virulence within a forma specialis has been categorized by signing phenotypes the pathogenic races. Races are defined by their differential interaction with host genotypes, which in some cases are varieties known to carry one or more major genes for resistance (Gordon and Mattyn, 1997). Four specific dominant resistant genes of tomato varieties of *Fol* are known and designed *I*, *I*-1, *I*-2 and *I*-3 and only three races of *Fol* have been found (race1, 2 and 3). The three known races of *Fol* are distinguished by their pathogenicity to varieties with specific dominant resistance genes (Elias and Schneider, 1992). The initiation and development of plant disease is caused by interaction of specific genes for virulence in the pathogen and of specific genes for susceptibility in the host. These resistant genes are monogenic specific dominant resistant genes. They were introduced into commercial tomato varieties. These varieties carrying resistant genes were provided as a set of the race differential varieties for determining the race of *Fol*. A set of tomato varieties consisting of differential tomato varieties has 5 trails; cv. 'EWS-S' as the susceptible control (susceptible to race1, 2 and 3), cv. 'EWS-R1' as resisted to race 1, cv. 'EWS-R12.1' and 'EWS-R12.2' as resisted to race 1 and 2 and cv. 'EWS-R123' as resisted to race1, 2 and 3. These host set is related to standard varieties that represented in most studies. The cv. 'EWS-S' is not contain any resistant gene, which resemble to cv. 'Bonny Best' and cv. 'Ponderosa'. The cv. 'EWS-R1' is owing to the present the locus *I* which resemble to cv. 'UC82-L' and cv. 'IPA-5'. The cv. 'EWS-R12.1' and 'EWS-

R12.2' are due to the presence of the loci *I* and *I*-2 which resemble to cv. 'Walter', cv. 'Peto94', cv. 'MH-1' and cv. 'Floradade'. The cv. 'EWS-R123' is probably due to the presence of the locus *I*-3 which resemble to cv. I3R-1' and cv. 'BHRS-2,3'. Nevertheless, the interaction between these resistant genes and race is not completely clear (Grattidge, 1982; Marlatt *et al*., 1996; Reis *et al*., 2005; Reis *et al*., 2005; Bunyatratchata1 *et al*., 2005; Sheu and Wang, 2006; Elena and Pappas, 2006). In this study, race identification of the isolate *Fol*CK_117 was examined for pathogenicity on a set of standard differential tomato varieties, which provided from Hortigenetics Research (S.E. Asia) Limited. The results of this present study showed that the isolate *Fol*CK_117 are designated as race 2 because this isolate can infect cv. 'EWS-S' (susceptible to race1, 2 and 3) and cv. 'EWS-R1' (resisted to race 1). Huang and Lindhout (1997) reported that tomato lacking *I* genes are susceptible to *Fol*. The *Fol* race 1 attacks only this cultivar, race 2 overcomes resistant varieties which contain the *I* genes (single dominant resistant gene which resists race 1 and the race 3 overcomes the *I*-2 gene that is resistant to *Fol* race 2. The gene *I*-3 has been proposed for resistance to *Fol* race 3.

2.5 Conclusions

The 126 isolates of *F. oxysporum* f. sp. *lycopersici* were obtained from *Fusarium* wilt infected vascular of 2 tomato cultivars; cv. 'Cherry' and cv. 'Thomas'. Generally, all isolates showed the initial white color of colonies, and then the color turned to deepen with aging, which divided according to a visual representation of colors into 3 categories as follows:- purplish-white, white and orangish-white cottony colony on PDA after 10 days. The growth rate of the colonies ranged between 6.93 - 10.46 mm/day in diameter, at an average of 8.51 ± 0.13 mm/day. They formed three types of asexual spore, including microconidia, macroconidia and chlamydospores.

A pathogenicity test confirmed that 126 of *Fol* were pathogenic to tomato seedlings cv. 'Bonny Best' (susceptible to *Fusarium* wilt), but not to cv. 'EWS-37434 (resistant to *Fusarium* wilt). Moreover, the isolate *Fol*CK_117 was selected to represent as the most virulent strain for further experiments. In addition, the *Fol*CK_117 was identified as race 2 virulent phenotype.