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A Pathogenicity of Anthracnose Fungus, *Colletotrichum capsici* on Various Thai Chilli Varieties

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ABSTRACT

Fourteen isolates of *C. capsici* which isolates from various places were characterized into four groups based on morphological characters and growth rate. The fungi and cotyledon of five chilli varieties namely Man Dum, Chee Fah, Mae Ping, Khee Nhu and Luang were tested for their disease interactions. The results revealed that these isolates could be differentiated in pathogenicity. "Mae Ping" variety expressed a largest area of necrotic symptom compared to other four chilli varieties when inoculated with most of *Colletotrichum capsici* isolates. The result suggested that chilli variety "Mae Ping" was the most susceptible variety to all isolates of *C. capsici*. The other four varieties, Man Dum, Chee Fah, Khee Nhu, and Luang showed tiny (or small) area of necrotic symptom.

Key words: chilli anthracnose, *Colletotrichum capsici*,

INTRODUCTION

Anthracnose caused by *Colletotrichum* spp. is an important disease of Chilli (*Capsicum annuum* L.) and many kinds of plants in tropical areas worldwide. It occurs as a pre-harvest or post - harvest fruit rot, causing extensive losses in chilli grown during the warm, wet season in tropical and subtropical climates (AVRDC, 1998).

Anthracnose attacks on a wide range of plants including cereals, legumes, grasses, vegetable, and fruit trees. *C. capsici* (Syd.) Butler & Bisby and *C. gloeosporioides* (Penz) Penz. & Sacc. in Penz, were reported as causal pathogen of chilli anthracnose in Thailand (Sangchote *et al*., 1998; Sangchote, 1999) and also in the tropical Asia (Manandhar *et al*., 1995ab; Kim *et al*., 1989). Apart from these species, *C. graminicola*, and *C. atramentarium* had been reported in India (Verma, 1973), *C. cocodes* in Florida (Roberts *et al*., 2001).

Colletotrichum spp. is capable of causing disease on many the chilli plant parts. However, fruit lesions are the most economically important aspect of this disease. *C. capsici* generally caused disease on ripe red fruit, while *C. gloeosporioides* produced disease both green and ripe fruits (Kim *et al*., 1989; Sangchote *et al*., 1998). Concentric rings of the acervuli within the lesions are commonly found. In some cases, the lesions are brown, not orange, and then turn black from the formation of setae and sclerotia (Roberts *et al*., 2001).

Chilli is one of the popular spicy herbs in Thailand. It is supplied as fresh, dry or food ingredient depended on consumer's requirements for self-sufficient and exported to other countries.

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Most popular varieties with high yield production are susceptible to anthracnose so the growers had to spray fungicides many times. So it is necessary to investigate on interaction between pathogen isolates and various popular and economical varieties to obtain a basic information on their susceptibility and resistance for further studies.

MATERIALS AND METHODS

1. Isolation, identification and maintenance of the fungus from infected chilli fruit

The experiment was conducted at the Environment Laboratory of Central Laboratory and Greenhouse Complex, Kamphaeng Saen campus during February – June, 2003. The anthracnose infected chilli fruits with typical symptoms were obtained from production field of the Asian Vegetable Research and Development Center (AVRDC) at Kasetsart University, Kamphaeng Saen, Nakhon Pathom and Kampheang Sean market. Chilli fruits were washed in running tap water, and dried with tissue paper. The infected fruit tissue was cut out in a size of approximately 3 mm^2 with scalpel. A surface disinfection was made in 1% sodium hypochlorite (Clorox 10%) for 5 minutes then dipped into sterilized water for 2 minutes and once again dried on sterile filter paper. The tissues were placed on Potato Dextrose Agar (PDA), 4 pieces per plate. After 5-10 days incubation at 25°C in alternative 12 hours of darkness and light using near ultraviolet (NUV) light, the fungi were subcultured and sporulated. The isolate was identified to the species under light microscopy.

2. Grouping of the *Colletotrichum capsici* base on morphological characters

The fungal morphology was observed in two categories: 1) growth rate and 2) morphological character of the colony. Fourteen isolates of the pathogenic fungi were maintained at room temperature (25°C in alternative 12 hours of darkness and NUV light). From second to ninth day after inoculation (DAI), the mycelium growth and mycelium characters such as colours of mycelium, acervuli character and spore shape were observed and recorded. The shape, size, colour of spore, and setae were investigated under light microscopy, thereafter fourteen isolates were grouped base on morphological characteristics.

3. Chilli varieties

A various types of chilli were selected as 1) big size, green chilli; 2) big size, red chilli; 3) small size, red chilli; 4) big size, yellow chilli. Name of the chilli varieties used in this study was stated in Table 1.

4. Inoculation

The experiment was arranged in a completely randomised design (CRD) with 15 treatments, ten seedlings per treatment were considered as replication. Seedlings of five chilli varieties were planted in plastic tray for fifteen-day until both cotyledons were fully expanded.

Table 1 Source of five chilli varieties used in the experiment.

No	Thai name	Common name	Type of fruit	Origin of varieties.
1	Man Dum	Hot pepper	Big size, green	Chia Tai seed company
2	Chee Fah	Red chilli	Big size, red	Chia Tai seed company
3	Mae Ping	Hot pepper	Big size, red	Known – You Seed company
4	Khee Nhu	Bird pepper	Small size, red	Chia Tai seed company
5	Luang	Yellow chilli	Big size, yellow	Chia Tai seed company

Mycelium of *C. capsici* was ground in mortar and mixed with sterilized water to make up a liquid mixture. A hole of an inoculation site was made by needle puncture in the middle of cotyledons. A 15-?l droplet of liquid mycelium was dropped on each hole with a micropipette. After 24 hours incubation period in moist chamber, seedlings were placed under open-air environmental conditions in the net-house. The symptom development on cotyledon was observed and the disease severity was recorded during 3 to 7 DAI. Disease severity and plant-pathogen interaction was calculated based on area of necrotic symptom (AONS). The AONS data were transformed to a numerical rank (NR) as shown in Table 2.

Plant and pathogens interaction showed numerical rank (NR) value of 1 was considered as incompatible interaction (I). The others showed NR values from 1.0 to less than 4.0 were considered as an intermediate of incompatible and compatible interaction (IC), and those with NR value of 4.0 were considered as compatible interaction (C).

RESULTS AND DISCUSSIONS

1. Fungal morphological characteristics

Fourteen isolates of *Colletotrichum capsici* were selected from production field of the AVRDC, and Kamphaeng Saen fresh market at Nakhon Pathom (Table 3). A differentiation of fungal colony was seen as morphological character and growth rate. The fungal isolates were classified into 4 groups. Description of each fungal group was shown in Table 4. The first group, *C. capsici*, including isolate No 1, 2, 3, 4 and 15 were characterized with slow growth rate (5.64 mm per day), grey-white type of mycelium, black acervuli, scattered, falcate conidia and non-uniform shape of mass mycelium. Isolate No 10, 13, and 14 with in the second group but slightly difference from the first on slow growth rate (5.86 mm per day), the shape of mass mycelium was irregular, non-uniform, and conidia were falcate. The colour was mixed of grey, white and salmon. Concentric rings of acervuli on mycelium were clear on group 3, *C. capsici* (isolate No 6,7,8 & 11), grey-white in colour, fast growing (7.28 mm per day). The growth rate of group 4 was medium (6.4 mm per day); pink-white colour, and mycelium was non-uniform. All four groups of *C. capsici* are similar to conidial shape but different from growth rate, and mycelium colour.

2. Pathogenicity and reactions of five chilli varieties against anthracnose fungal caused by *Colletotrichum capsici*

Interaction between fourteen isolates of *Colletotrichum capsici* and five chilli varieties was investigated by cotyledon inoculation. The 15 day-old seedlings from five chilli varieties were inoculated with a suspension of macerated mycelium of 8-10 day-old culture of the pathogen. Three to seven days after inoculation, necrotic spot appeared on cotyledon.

The degree of disease severity as a size of necrotic symptom was different on each tested

Table 2 Disease severity of chilli cotyledon infected by *Colletotrichum capsici* expressed by area of necrotic symptom (AONS) transformed to a numerical rank (NR) and interaction.

AONS (mm^2)	Numerical rank (NR)	Interaction
0.00 – 1.50	1	Incompatible (I)
1.51 – 3.00	2	I/C
3.01 – 5.00	3	I/C
> 5.00	4	Compatible (C)

Table 3 Source of fourteen isolates of *Colletotrichum capsici* obtained from infected fruit.

Isolate number	Location	Chilli variety	Species	Fruit symptoms
1	Central Lab	Chilli	*C.c*[1/]	- Dry chilli fruit, symptom is not definite. Mycelium
2	Central Lab	Chilli	*C.c*	covered all over the fruit.
3	Central Lab	Chilli	*C.c*	
4	Central Lab	Chilli	*C.c*	
5	KPS 2/	Chilli	*C.c*	- Necrotic spot concentric rings is white & black
6	KPS 2/	Chilli	*C.c*	- Black dots, the concentric rings are not clear
7	KPS 2/	Chilli	*C.c*	- Concentric rings, black colour & slimy on surface
8	KPS 2/	Chilli	*C.c*	- Sunken, yellowish Mycelium look like spider web
9	KPS 2/	Chilli	*C.c*	- Dry lesion, black, the shape is not definite
10	KPS 2/	Chilli	*C.c*	- Lesion is white-brown & black on rings, near peduncle
11	KPS 2/	Chilli	*C.c*	- Yellowish Mycelium on lesion. fruit was dry.
12	AVRDC	Bell Pepper	*C.c*	- Round, Sunken, brown-black concentric rings. the surface lesion is dry
13	AVRDC	Bell Pepper	*C.c*	- Round, sunken. the colours on concentric rings are differed, white - brown colour
14	AVRDC	Bell Pepper	*C.c*	- Grey - white, curve, lesion located in the middle of fruit.

1/ *Colletotrichum capsici* ; 2/ Kamphaeng Saen market.

variety, varied form 0.00 mm^2 - 19.45 mm^2. Mae Ping variety showed highly susceptible to most *Colletotrichum capsici* isolates. The other varieties, Man Dum, Chee Fah, Khee Nhu, Luang showed smaller area of necrotic spot.

The areas of necrotic tissue (Table 5) was transformed to a numerical data (refer to Table 2), which prepared for clarify a group of severity instead of the area of necrotic symptom. The result indicated that chilli variety "Mae Ping" was susceptible to all isolate of *Colletotrichum capsici*. Since "Mae Ping" variety is one of an export variety and used for Thai curry preparation, then the production procedure should be paid more attention to disease management.

Among the fungal isolates, group 3 (isolate number 6, 7, 8 and 11) showed more virulence than other groups. This group of pathogen was taken from KPS market. The pathogen of group 1 and 2, which were taken from Kamphaeng Saen, Nakhon Pathom, were expressed as moderate virulence on varieties tested. This result showed that there are many strains of pathogen distributed in the same planting area. From disease severity could be transformed in terms of disease reaction or compatible and incompatible reaction as shown in Table 6.

In Table 6, the result showed that chilli variety "Mae Ping" expressed a compatible reaction to pathogen group 3 and group 4. This may be discussed that "Mae Ping" variety contains genes relevant to susceptible response. "Mae Ping" is one of the economical varieties, so it was selected as the model for further investigation, especially for monitoring of plant enzyme changes after applied by various resistance inducers.

Table 4 Morphological characters of 14 isolates of *C. capsici* causing anthracnose of chilli.

Isolate group	Pathogen	Isolate number	Characters of mycelium and conidia	Growth rate / day (mm)	Frequency (%)
		1	- Acervuli scattered, black	5.5	
		2	mycelium are grey to white	6.1	
I	*C. capsici*	3	- Falcate	5.0	33.4
		4		5.5	
		15		6.1	
II	*C. capsici*	10	- Grey black and white salmon	5.5	
		13	colour	5.5	20.0
		14	- Falcate	6.6	
		6	- Grey, pink, white, concentric	5.3	
III	*C. capsici*	7	rings	8.5	26.7
		8	- Falcate	8.1	
		11		7.2	
IV	*C. capsici*	5	- White to pink	6.8	13.3
		9	- Falcate	6.0	

(Record was carried out 9 days after inoculation on PDA)

Table 5 Areas of necrotic symptom (mm^2) produced on chilli cotyledon after 7 days inoculation with a macerated fungal mycelium of *C. capsici.*

Chilli variety	Group of isolates													
	I				II				III				IV	
	1	2	3	4	15	10	13	14	6	7	8	11	5	9
Man Dum	5.35	1.72	1.71	7.53	0.24	0.00	1.63	0.16	7.51	0.69	0.39	7.49	8.26	4.26
She Pha	0.16	0.32	0.43	0.51	0.21	2.94	1.17	2.27	0.98	3.78	7.72	16.58	0.07	5.93
Mae Ping	8.59	11.82	14.97	6.48	0.00	17.42	3.49	14.66	14.13	16.26	15.45	15.20	8.89	19.45
Khee Nhu	0.00	1.27	0.00	1.28	0.00	4.64	0.83	0.00	5.26	0.13	3.17	4.42	0.49	0.53
Luang	0.76	2.05	0.31	1.14	0.31	0.00	0.26	0.31	6.26	0.00	0.66	1.24	3.03	3.19

The result of this study revealed that isolates of *Colletotrichum capsici* were differed from physiological character. Most isolates of *C. capsici* were expressed the compatible interaction to Mae Ping variety. It can be suggested that this variety were susceptible to all isolate of *C. capsici.* Other four chilli varieties reacted to most isolates were not distinct. The plant which shows disease resistant or susceptible depends on genotypes of variety. The study on resistance to disease should be based on knowledge of infection, and pathogenicity of the fungus. Even though the result of this study could not be concluded which variety was susceptible or resistant to anthracnose, but it is the basis for further investigation on resistance induction by various plant inducers among these chilli varieties.

Table 6 Disease severity and pathogenicity of four groups of *C. capsici* on five Thai chilli varieties.

Chilli variety	Group of isolates							
	I		II		III		IV	
	S	P	S	P	S	P	S	P
Man Dum	2.6	I/C	1.33	I/C	2.5	I/C	3.5	I/C
Chee Fah	1.0	I	1.67	I/C	3.0	I/C	2.5	I/C
Mae Ping	3.4	I/C	3.67	I/C	4.0	C	4.0	C
Khee Nhu	1.0	I	1.67	I/C	2.75	I/C	1.0	I
Luang	1.2	I/C	1.0	I	1.75	I/C	3.0	I/C

Note: S = Severity; P = Pathogenicity (disease reaction); MP = Mean point
MP = 1.0 : Incompatible (I)
MP = 1.0 - <4.0 : Intermediate between Incompatible and Compatible (I/C)
MP = 4.0 : Compatible (C)

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