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Characterization and evaluation of carbendazim-resistance response of *Colletotrichum* species

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Isolates of *Colletotrichum* associated with mild and severe *symptoms* of *anthracnose* on long cayenne peppers (*Capsicum annuum* var. *acuminatum*) *were collected from* Chiang Mai, Nakorn Pathom and Pichit provinces. A total of fifteen *Colletotrichum* spp. isolates were obtained as pure cultures by using single-spored isolation. Based on morphological characteristics, eight isolates were identified as *C. gloeosporioides* and seven isolates were *C. capsici*. Each isolate was tested for resistance to the carbendazim fungicides by measuring radial growth on the series of PDA plates amended with 0, 0.1, 1, 10, 100, 500 and 1000 µg/ml of carbendazim. On the basis of their fungicide resistant response, the fifteen fungal isolates obtained in this study could be divided into 4 groups as follow; nine isolates were highly resistant (Car^{HR}), one isolate was moderately resistant (Car^{MR}), two isolates were weakly resistant (Car^{WR}) and three isolates were sensitive (Car^{S}). The *Colletotrichum* spp. isolates which expressed Car^{HR}, Car^{WR}, Car^{MR} and Car^{S} phenotype were proved and confirmed for pathogenicity on *fruits* of long cayenne pepper. In order to gain further information on pathogenicity test, the *C. gloeosporioides* isolate TPCMCg60 (Car^{HR}) was exhibit very high virulence on pepper fruit.

Key words: *Colletotrichum*, carbendazim-resistance, chili anthracnose

Introduction

Chili peppers (*Capsicum annuum*) is an economically important crop in Thailand and used for flavor dishes and for medicinal purposes. Pre-harvest and post-harvest chilies always affected by many disease and insects. Diseases caused by fungi, bacteria, and viruses, however, are the major limitation to chili production in Thailand, such as anthracnose disease caused by *Colletotrichum*

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species, bacterial wilt caused by *Pseudomonas solanacearum*, mosaic caused by chili veinal mottle virus (CVMV) and cucumber mosaic virus (CMV) which are the most serious destructive diseases of chili. One of the most important diseases in chili is anthracnose caused by *Colletotrichum capsici* and *C. gloeosporioides* (Than *et al.*, 2008, Waller *et al.*, 2002) and is favored by warm temperatures, high moisture and poor circulation among the plants. The infected chili pepper by *Colletotrichum* spp. results in yield loss up to 50% (Pakdeevaraporn *et al.*, 2005) and reduced marketability (Mananadhar *et al.*, 1995). Anthracnose disease can infect on every part of chili and typical symptoms on stem such as leaf tip die-back, stem die-back, foliar blight, leaf spot, leaf lesion and fruit leading to sunken necrotic tissues, with concentric rings of acervuli that are often wet, conidial masses may also occur scattered or in concentric rings on the lesions (Mananadhar *et al.*, 1995; Than *et al.*, 2008). The management and control of anthracnose disease are still under extensive research. In some case, eradicative sanitation methods are not feasible, systemic fungicides are substituted. Due to rely on chemical fungicide, pathogen resistance to systemic fungicides after repeatedly used throughout cropping season and the deleterious to the environment and consumers. The benzimidazole fungicides, particularly carbendazim, have been used to control of many diseases caused by Deuteromycetous pathogens, its systemic properties and their great efficacy in controlling plant diseases. It has also been recommended for a long period to control of ripe rot disease of chili (*Capsicum annum* L.) caused by *C. capsici* (Sariah, 1989). However, continuous using those chemical fungicides showed frequency of resistant isolates in some regions of China which gradually increased (Zhang and Huang, 2007). Thus, the objectives of this study were to characterize and evaluate the sensitivity of *Colletotrichum* species to the benzimidazole fungicide, carbendazim.

Materials and methods

Morphological characterization and evaluation of carbendazim-resistance response of Colletotrichum species

Isolation and identification of Colletotrichum spp.: Naturally-infected chili fruits were randomly collected from local markets in Chiang Mai, Nakorn Pathom and Pichit provinces, then kept in moist chamber and brought to laboratory. The infected chili fruits were diagnosed from sign and symptoms of anthracnose disease. The fruiting structures of *Colletotrichum* spp. were observed and identified referred to Sutton (1980). The isolation of pathogen from diseased tissues was conducted by single spore isolation method (Choi *et al*., 1999), acervuli of *Colletotrichum* spp. was picked up directly from infected

tissue under stereomicroscopic (40X) and placed in sterilized distillated water to make spore suspension before spread over the entire water agar (WA) plate. After incubated at room temperature overnight (8-12 h) the germinated spores as single colony were transferred to new PDA plate by using sterilized needle and to get pure culture. The pure cultures were observed daily and subcultured for stock culture (4^oC) and further experiment.

Carbendazim-resistant phenotype assay.: Carbendazim resistance was studied for detection all isolates of *Colletotrichum* spp. by phenotypic assay. The resistibility of each isolate to carbendazim was observed from the growth of colony. All isolates were tested to screen carbendazim sensitivity to *Colletotrichum* spp. The experiment was done by using Completely Randomized Design (CRD) with three replications. Treatments were different concentrations of carbendazim of 0, 0.1, 1, 10, 100, 500 and 1000 µg/ml amended in PDA before autoclaved at 121 °C, 15 lbs/inch^2 for 20 min. A mycelia plug of *Colletotrichum* spp. was cut from peripheral of colony by sterilized cock borer and transferred into the middle of PDA amended with carbendazim in each concentration and incubated for 14 days at 25-28 °C. Colony growth of each fungal isolate and the level of resistance to carbendazim was evaluated and grouped into four representative phenotype reactions which highly resistant (Car^{HR}) and able to grow on carbendazim at ≥ 500 mg/l; moderately resistant (Car^{MR}) at ≤ 100 mg/l; weakly resistant (Car^{WR}) at ≤ 10 mg/l; and sensitive (Car^{S}) at ≤ 1 mg/l as, Percentage of inhibition was equivalent compared to the level of carbendazim resistant by phenotype reactions were described in Table 1.

Table 1 Phenotype-resistant levels of *Colletotrichum* spp. to carbendazim fungicide (modified from Farungsang and Farungsang, 1992; Farungsang *et al*., 1994; Koenraadt *et al*., 1992; Peres *et al*., 2004)

Categories	Percent colony growth	Phenotype reactions
+ =	<10%	sensitive (Car^{S}) at ≤1 µg/ml
++ =	≥10–35%	weakly resistant (Car^{WR}) at ≤10 µg/ml;
+++ =	>35–65%	moderately resistant (Car^{MR}) at ≤100 µg/ml
++++ =	>65–100%	highly resistant (Car^{HR}) at ≥500 µg/ml

Pathogenicity test

The isolates of *Colletotrichum gloeosporioides* and *C. capsici* which expressed Car^{HR}, Car^{WR}, Car^{MR} and Car^{S} phenotype were randomly selected to be representative of carbendazim-resistant phenotypes for pathogenicity test.

Ten microlitre of *Colletotrichum* spp. spore suspensions (10^6 conidia/mL) were dropped into wounded area and distillated water used as control. Moistened paper towels were placed in the plastic box containing the inoculated fruits and incubated at room temperature. The lesion diameters were measured after three days of incubation. The experiment was done by using completely randomized design (CRD) with three replications. *Colletotrichum* spp. isolates were grouped into five categories based on lesion size as followed: 0 mm = non-pathogenic isolates (N), 0.1-5.0 mm. = low virulent isolates (L), 5.1-10.0 mm = moderately virulent isolates (M), 10.1-15.0 = high virulent isolates (H) and ≥ 15.1 mm = very high virulent isolates (VH). The highest virulent isolate was selected for further experiment.

Results

Morphological characterization and evaluation of carbendazim-resistance response of Colletotrichum species

Isolation and identification of Colletotrichum spp.: Fifteen isolates of *Colletotrichum* spp. were obtained from naturally infected chili fruits which thirteen isolates collected from long cayenne pepper (*Capsicum annuum* L.) in Chiang Mai province, one isolate from Nakorn Pathom province and another from Pichit province. The fungi were observed under microscopic out of eight isolates were *C. capsici* and seven isolates were *C. gloeosporioides*, respectively. These isolates were cultured on PDA for 14 days and observed periodically for morphological characters such as colony character, conidial size, conidial masses, conidial shape and setae (Table 2). From the morphological characters of *Colletotrichum* spp. isolates were identified referred to Sutton (1980).

Carbendazim-resistant phenotype assay: Fifteen isolates of *Colletotrichum* spp. (*C. capsici* and *C. gloeosporioides*) were tested for carbendazim-resistant by growth assays on PDA amended with carbendazim at concentrations of 0.1, 1, 10, 100, 500, and 1000 mg/l, respectively. The isolates were classified into four representative phenotype reactions as follows: highly resistant (Car^{HR}; ≥ 500 mg/l), moderately resistant (Car^{MR}; ≤ 100 mg/l), weakly resistant (Car^{WR}; ≤ 10 mg/l), and sensitive (Car^{S}; ≤ 1 mg/l) (Figure 1).

Three isolates (20%) include MHCMCc11, MHCMCg10 and SSCMCg30 isolates were classified as the Car^{S} phenotype, be able to grown on carbendazim amended PDA at 0.1 mg/l concentration. Two isolates (13%) of MPJCc1 and TPCMCc9 were classified as the Car^{WR} phenotype which able to grown on carbendazim amended PDA at 0.1, 1.0 and 10 mg/l concentrations. The isolate MRCMCg46 was classified as the Car^{MR} phenotype (7%) which

able to grown on carbendazim amended PDA at 0.1, 1.0, 10 and 100 mg/l concentrations. Nine isolates (60.0%) of KNKCg5, SSCMCc86, SSCMCg28, SSCMCg78, TPCMCc53, TPCMCc43, TPCMCc75 and TPCMCg60 were classified as the Car^{HR} phenotype which able to grown on carbendazim amended PDA at 0.1, 1.0, 10, 100 and 500 mg/l concentrations. Among nine isolates of Car^{HR} phenotype, the isolate SSCMCg27 was capable to grow on maximum carbendazim amended PDA at concentration of 500 mg/l.

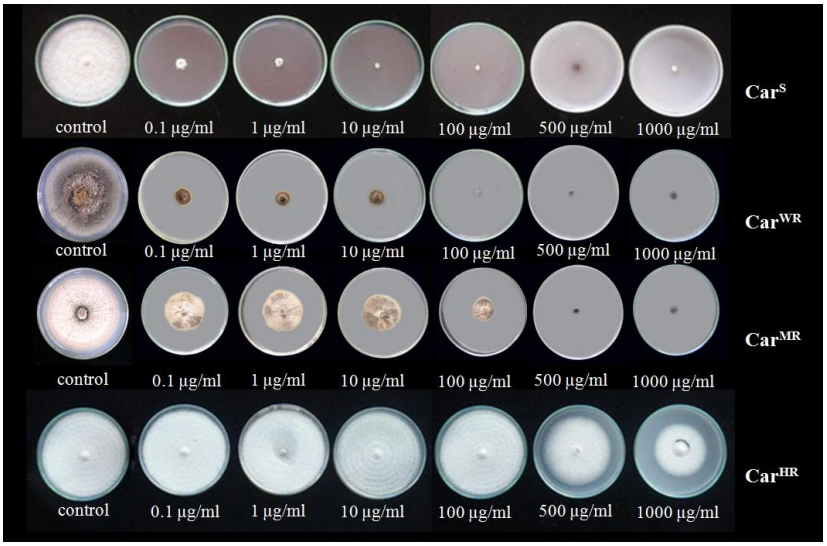

Fig. 1. The carbendazim-resistant assays of *Colletotrichum* spp. causing chili anthracnose on potato dextrose agar amended with various carbendazim concentrations; sensitive (Car^{S}; SSCMCg30), weakly resistant (Car^{WR}; MPJCc1), moderately resistant (Car^{MR}; MRCMCg46) and highly resistant (Car^{HR}; TPCMCg60).

Three isolates were classified as the Car^{S} phenotype which isolate MHCMCc11 identified as *C. capsici* while isolates MHCMCg10 and SSCMCg30 were identified as *C. gloeosporioides*. The isolate MRCMCg46 was classified as the Car^{MR} phenotype and also identified as and *C. gloeosporioides*. Two isolates of the Car^{WR} phenotype, MPJCc1 and TPCMCc9 were identified as *C. capsici*. Additionally nine isolates which classified as the Car^{HR} phenotype, KNKCg5, SSCMCg27, SSCMCg28, SSCMCg78 and TPCMCg60 were identified as *C. gloeosporioides* and isolates SSCMCc86, TPCMCc53, TPCMCc43 and TPCMCc75 were identified as *C. capsici*, respectively (Table 3).

Table 2. Morphological character of *Colletotrichum* spp. isolates

Isolate[1/]	Colony character	Conidial size (μm) Width	Conidial size (μm) Length	Conidial masses	Shape	Setae	Species
KPNPCg5	white to grey colony	2.38-3.94	22.82-24.45	absent	falcate	absent	*Colletotrichum gloeosporioides*
MHCMCc11	white to grey colony	1.98-3.58	20.56-24.89	absent	falcate	present	*Colletotrichum capsici*
MHCMCg10	white to orange colony and cottony mycelia	2.62-3.93	9.17-14-41	absent	cylindrical	absent	*Colletotrichum gloeosporioides*
MRCMCg46	white to orange colony and cottony mycelia	2.63-3.93	7.85-11.12	absent	cylindrical	absent	*Colletotrichum gloeosporioides*
MUPJCc1	white to grey colony	2.34-3.95	18.34-25.58	present	falcate	present	*Colletotrichum capsici*
SSCMCc86	white to grey colony	2.14-3.95	22.96-24.89	absent	falcate	present	*Colletotrichum capsici*
SSCMCg27	white colony and cottony mycelia	2.62-4.33	15.72-18.07	absent	cylindrical	absent	*Colletotrichum gloeosporioides*
SSCMCg28	white colony and cottony mycelia	2.63-3.94	7.86-11-79	absent	cylindrical	absent	*Colletotrichum gloeosporioides*
SSCMCg30	white to orange colony and cottony mycelia	2.62-3.93	9.17-12.79	absent	cylindrical	absent	*Colletotrichum gloeosporioides*
SSCMCg78	white to orange colony and cottony mycelia	3.01-3.53	8.46-12.11	absent	cylindrical	absent	*Colletotrichum gloeosporioides*
TPCMCc53	white to grey colony	2.34-3.95	20.96-24.89	absent	falcate	present	*Colletotrichum capsici*
TPCMCc43	white colony	2.34-3.95	22.27-25.30	absent	falcate	present	*Colletotrichum capsici*
TPCMCc75	white to grey colony	2.14-3.91	18.34-21.58	absent	falcate	present	*Colletotrichum capsici*
TPCMCc9	white to grey colony	2.32-3.93	17.82-24.44	present	falcate	present	*Colletotrichum capsici*
TPCMCg60	white to orange colony and cottony mycelia	2.52–4.37	7.02-11.51	absent	cylindrical	absent	*Colletotrichum gloeosporioides*

[1/]KPNP=Kham Pang Saen, Nakorn Pathom; MHCM=Mae Hea, Chiang Mai; MRCM=Mae Rim, Chiang Mai; MUPJ=Muang, Pichit; SSCM; San Sai, Chiang Mai; TPCM=Ton Payom, Chiang Mai; Cc= *Colletotrichum capsici*; Cg= *C. gloeosporioides*

Table 3. Carbendazim-resistant assay of *Colletotrichum* spp. causing chili anthracnose on potato dextrose agar amended with carbendazim

No.	Isolate	Carbendazim concentrations (mg/l)						Phenotype-resistant level [1/]
		0.1	1	10	100	500	1000	
1	KNKCg5	+++	+++	+++	+++	+++	+++	Car^{HR}
2	MHCMCc11	+	-	-	-	-	-	Car^{S}
3	MHCMCg10	+	-	-	-	-	-	Car^{S}
4	MPJCc1	+	+	+	-	-	-	Car^{WR}
5	MRCMCg46	+++	+++	++	++	-	-	Car^{MR}
6	SSCMCc86	+++	+++	+++	+++	++	++	Car^{HR}
7	SSCMCg27	+	+	+	+	+	-	Car^{HR}
8	SSCMCg28	+++	+++	+++	+++	++	++	Car^{HR}
9	SSCMCg30	+	-	-	-	-	-	Car^{S}
10	SSCMCg78	++++	+++	+++	+++	+++	++	Car^{HR}
11	TPCMCc53	++++	+++	+++	+++	+++	++	Car^{HR}
12	TPCMCc43	++	++	++	++	++	+	Car^{HR}
13	TPCMCc75	+++	+++	+++	+++	++	+	Car^{HR}
14	TPCMCc9	+	+	+	-	-	-	Car^{WR}
15	TPCMCg60	++++	+++	+++	+++	+++	++	Car^{HR}

[1/] Resistant response against carbendazim on PDA: Car^{HR} = highly resistant (≥ 500 mg/l), Car^{MR} = moderately resistant (≤ 100 mg/l), Car^{WR} = weakly resistant (≤10 µg/ml) and Car^{S} = sensitive (≤ 1 mg/l), respectively.

Pathogenicity test

The pathogenicity test confirmed that Car^{HR} (KNKCg5, SSCMCc86, SSCMCg27, SSCMCg28, SSCMCg78, TPCMCc53, TPCMCc43, TPCMCc75 and TPCMCg60), Car^{WR} (MPJCc1 and TPCMCc9), Car^{MR} (MRCMCg46) and Car^{S} (MHCMCc11, MHCMCg10 and SSCMCg30) isolates of *Colletotrichum* spp. caused the initial symptoms appeared as brown circular sunken lesions on chili fruits at 2 days after incubation in a plastic box with moist conditions at room temperature. Four days after inoculation, the lesions were well developed and produced orange masses of fungal spores in concentric rings on the lesions of fruits (Figure 2).

Figure 2 Pathogenicity test of carbendazim-resistant *Colletotrichum gloeosporioides* on chili fruits. Non-inoculated (A) and inoculated fruits (B-D)

The lesion size from pathogenicity tests grouped into five categories as followed: 3 isolates (20.00%) include isolates MPJCc1, TPCMCc53 and TPCMCg60 were very high virulent group, another 3 isolates (20.00%) were high virulent group include isolates MRCMCg46, SSCMCg78 and TPCMCc9. Seven isolates (46.67%) include isolates KNKCg5, MHCMCc11, SSCMCc86, SSCMCg27, SSCMCg30, TPCMCc43 and TPCMCc75 were moderately virulent group while 2 isolates (13.33%) include MHCMCg10 and SSCMCg28 were low virulent isolates and no non-pathogenic group was investigated (Table 4).

The virulence categories were based on lesion diameter on chili fruits and the Car^{HR} phenotype performed moderately virulent (KNKCg5, SSCMCc86, SSCMCg27, SSCMCg78, TPCMCc43 and TPCMCc75), high virulent (SSCMCg78) and very high virulent (TPCMCc53 and TPCMCg60) categories, respectively. The Car^{MR} phenotype (MRCMCg46) presented high virulent categories and for Car^{WR} phenotype presented high virulent (TPCMCc9) and very high virulent (MPJCc1) categories whereas the Car^{S} phenotype presented moderately virulent (MHCMCc11 and SSCMCg30) and low virulent (MHCMCg10) categories, respectively (Table 4). *Colletotrichum gloeosporioides* isolate TPCMCg60 gave the highest virulent disease incidence on chili fruits (25 mm of lesion size) and also showed highly resistant phenotypic response to carbendazim (Car^{HR}) (Figure 3).

Table 4. Lesion diameters of *Colletotrichum* spp.

Isolate		**Lesion diameter**[1/] **(mm.)**		**Virulence categories**[2/]
control		0.0	k	-
KNKCg5	Car^{HR}	9.3	def	M
MHCMCc11	Car^{S}	7.8	efg	M

MHCMCg10	Car^{S}	2.5	j	L
MPJCc1	Car^{WR}	15.3	b	VH
MRCMCg46	Car^{MR}	10.7	cd	H
SSCMCc86	Car^{HR}	6.3	gh	M
SSCMCg27	Car^{HR}	7.5	fg	M
SSCMCg28	Car^{HR}	4.0	ij	L
SSCMCg30	Car^{S}	10.0	de	M
SSCMCg78	Car^{HR}	11.3	cd	H
TPCMCc53	Car^{HR}	17.5	b	VH
TPCMCc43	Car^{HR}	7.6	fg	M
TPCMCc75	Car^{HR}	5.2	hi	M
TPCMCc9	Car^{WR}	12.8	c	H
TPCMCg60	Car^{HR}	25.0	a	VH
CV. (%)		13.95		

[1/] Means of three replication. Means followed by a common letter in each column are not significantly different by LSD at $P < 0.05$

[2/] Virulence categories; N = non-pathogenic isolates, L = low virulent isolates, M = moderately virulent isolates, H = high virulent isolates and VH = very high virulent isolates

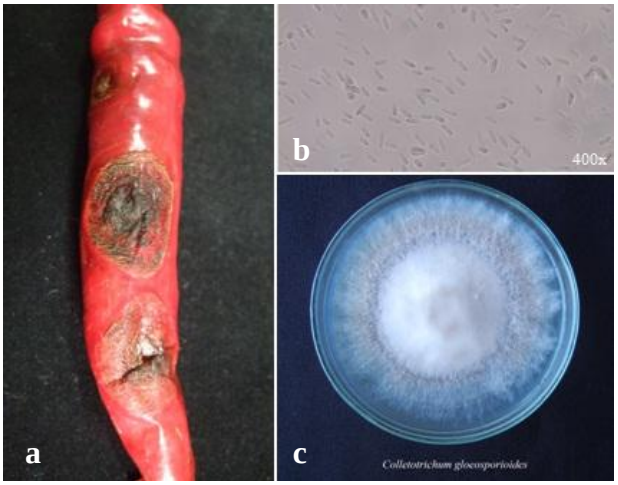

Fig. 3. Naturally infected chili fruit with *Colletotrichum gloeosporioides* (TPCMCg60) (a), conidia (b), colony on PDA medium (c)

Discussions

In this study, fifteen isolates of *Colletotrichum* isolates were isolated from long cayenne peppers (*Capsicum annuum* var. *acuminatum*) which were collected from Chiang Mai, Nakorn Pathom and Pichit provinces, respectively. The fungi were observed under microscopic and of eight isolates were *C. capsici* and seven isolates were *C. gloeosporioides*, respectively. Than *et al*. (2008) demonstrated *C. capsici* and *C. gloeosporioides* were main pathogen of

chili anthracnose in Thailand. Similar to Ratanacherdchai *et al*. (2010) reported that *C. capsici* and *C. gloeosporioides* were causal agent of chili anthracnose in Thailand and also demonstrated that genetic diversity of *C. capsici* and *C. gloeosporioides* were correlated to geographic distribution. Anthracnose has been recognized as one of the most destructive diseases on chili peppers (*Capsicum annuum*) are grown under hot and rainy season in the tropics and subtropics areas (Park, 2007). Among 15 isolates the Car^{HR} phenotype presented in larger number and one of all able to grown on carbendazim at concentration 1000 $mg.L^{-1}$. In agreement, with report of Ru-Lin and Jun-Sheng (2007), *Colletotrichum* species causing chili anthracnose in China which expressed Car^{HR} phenotype and showed a nucleotide mutation at codon 198 and amino acid substitution. This indicated emergence of carbendazim resistant in chili growing field would lead to decrease efficient of fungicide and failure in disease control. Likewise, Nakaune and Nakano (2007) suggested that continuous and widespread use of similar fungicide would lead the rapid development of resistant strains in the target fungi. The resistance mechanisms of pathogen to the fungicide related to the modification of the target binding site, the membrane transport system and the metabolic pathways for fungicide toxification or detoxification (Hewitt, 1998). Moreover, in this study found the Car^{MR} phenotype, moderately resistant isolate as previous reports of *Botytris cinerea* (Yarden and Katan, 1993), *C. gloeosporioides* (Chung *et al*., 2006), *Penicillium italicum*, *P. aurantiogriseum*, *Venturia inaeqalis, V. pirina* (Koenraadt *et al*., 1992), *Tapesia. yallundae* and *T. acuformis*, (Albertini *et al*., 1999). Besides, the Car^{MR} phenotype of *C. gloeosporioides*, causing mango anthracnose, related to a single nucleotide transversion at codon 200 of *TUB2* gene in as the report of Kongtragoul *et al*. (2011). It could be demonstrated that the Car^{MR} phenotype possibly in order to develop resistance to carbendazim in brief time and Car^{HR} phenotype population increased and difficult to control, subsequently. The detection of carbendazim resistance fungi in recent years as a result of frequently and singly used over a long period of time. Accordingly, this data would provide benefit for monitoring fungicide-resistant in field population and prepare for disease management approach guideline since anthracnose is an important disease of chili in Thailand. From the pathogenicity test confirmed that Car^{HR}, Car^{WR}, Car^{MR} and Car^{S} isolates of *Colletotrichum* spp. from chili fruits were showed differentiate virulence level verified from low to very high virulent isolates. The pathogenicity of *Colletotrichum* spp. in this study showed no relationship with carbendazim responses phenotype. In agreement with Ratanacherdchai *et al*. (2010) reported that there was no clear relationship between genetic diversity and pathogenicity variability of *C. capsici* and *C. gloeosporioides*. And also supported by Denoyes-Rothan *et al*.

(2003) that the absence of correlation between genetic polymorphism and geographical origin of *Colletotrichum* spp. but probably occurred through international plant exchanges. Hence, in further investigations of Smith and Black (1990) were preformed that low virulence isolates of *C. gloeosporioides*, could be considered as nonpathogenic in a difference climatic conditions. This hypothesis is supported by the fact that optimal temperatures for growth of *C. gloeosporioides in vitro* is high, that could be related to limitation of epidemics of this species in temperate regions. According to pathogenicity test *Colletotrichum gloeosporioides* isolate TPCMCg60 (Car^{HR}) was showed the highest virulent disease incidence on chili fruits (25 mm of lesion size) and also showed highly resistant phenotypic response to carbendazim.

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References

Albertini, C., Gredt, M. and Leroux, P. (1999). Mutations of the *β*-tubulin gene associated with different phenotypes of benzimidazole resistance in the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis*. Pestic. Biochem. Physiol. 64:17-31.

Choi, Y.W., Hyde, K.D. and Ho, W.H. (1999). Single spore isolation of fungi. Fungal Divers 3:29–38.

Chung, W.C., Huang, J.W. and Huang, H.C. (2005). Formulation of a soil biofungicide for control of damping-off of Chinese cabbage (*Brassica chinensis*) caused by *Rhizoctonia solani.* Biol. Control. 32:287–294.

Denoyes-Rothan, B., Guérin, G., Délye, C., Smith, B., Minz, D., Maymon, M., and Freeman, S. (2003). Genetic diversity and pathogenic variability among isolates of *Colletotrichum* species from strawberry. Phytopathology. 93(2):219-228

Farungsang, U. and Farungsang, N. (1992). Benomyl resistance of *Colletotrichum* spp. associated with rambutan and mango fruit rot in Thailand. Acta Hort. (ISHS) 321: 891-897.

Farungsang, U., Farungsang, N. and Sangchote, S. (1994). Benomyl resistance of *Colletotrichum* species associated with mango and rambutan fruit rots in Thailand. *In*: Proceedings of an International Conference held in Bangkok, Thailand: Development of Postharvest handling technology for tropical tree fruits. G. I. Johnson and E. Highley eds. ACIAR No. 58: 45-50.

Hewitt, H.G. (1998). Fungicide resistance. *In:* Fungicides in Crop Protection. Centre for Agriculture and Biosciences International, Wallingford, UK, pp.155–181.

Koenraadt, H., Somerville, S.C. and Jones, A.L. (1992). Characterization of mutations in the beta-tubulin gene of benomyl-resistant field isolates of *Venturia inaequqlis* and other plant pathogenic fungi. Phytopathology 82(11):1348-1354.

Kongtragoul, P., Nalumpang, S., Miyamoto, Y., Izumi, Y. and Akimitsu, K. (2011). Mutation at Codon 198 of TUB2 gene for carbendazim resistance in *Colletotrichum gloeosporioides* causing mango anthracnose in Thailand. J. Plant Prot. Res. 51(4):377-384.

Mananahar, J.B., Hartman, G.L. and Wang, T.C. (1995). Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. Plant Dis. 79:380–383.

Nakaune, R. and Nakano, M. (2007). Benomyl resistance of *Colletotrichum acutatum* is caused by

enhanced expression of β-tubulin 1 gene regulated by putative leucine zipper protein CaBEN1. Fungal Genetics and Biology. 44:1324–1335.
Pakdeevaraporn, P., Wasee, S, Taylor, P. W. J. and Mongkolporn, O. (2005). Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum*. Plant Breeding. 124:206-208.
Park, H.G. (2007). Problems of anthracnose in pepper and prospects for its management. pp. 19. *In*: Abstracts of the First International Symposium on Chilli Anthracnose. Oh, D.G. and Kim, K.T. (eds.). Seoul: National Horticultural Research Institute, Rural Development of Administration.
Park, H.G. (2007). Problems of anthracnose in pepper and prospects for its management. pp. 19. *In*: Abstracts of the First International Symposium on Chilli Anthracnose. Oh, D.G. and Kim, K.T. (eds.). Seoul: National Horticultural Research Institute, Rural Development of Administration.
Peres, N. A. R., Souza, N.L., Peever, T. L. and Timmer, L.W. (2004). Benomyl sensitivity of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from citrus. Plant Dis. 88(2):125-130.
Ratanacherdchai, K., Wang, H.K., Lin, F.C. and Soytong, K. (2010). ISSR for comparison of cross-inoculation potential of *Colletotrichum capsici* causing chilli anthracnose. Afr. J. Microb. Res. 4(1):76-83.
Ru-Lin, Z. and Jun-Sheng, H. (2007). Cloning of a carbendazim-resistant gene from *Colletotrichum gloeosporioides* of mango in South China. Afr. J. Biotechnol. 6(2):143-147.
Sariah, M. (1989). Detection of Benomyl resistance in the anthracnose pathogen, *Colletotrichum capsici*. Journal of Islamic Academy of Science. 2(3):168-171.
Smith, B.J. and Black, L.L. (1990). Morphological, cultural, and pathogenic variation among *Colletotrichum* species isolated from strawberry. Plant Dis. 74:69-76
Sutton, B. C. 1980. The *Coelomycetes*: fungi imperfect with pycnidia acervuli and stromata. CMI. Kew Surrey, England, pp. 696.
Than, P.P., Jeewom, R., Hyde, K.D., Pongsupasamit, S., Monkholporn, O. and Taylor, P.W.J. (2008). Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chili (*Capsicum* spp.) in Thailand. Plant Pathol. 57:562–572.
Yarden, O. and Katan, T. 1993. Mutations leading to substitutions at amino acids 198 and 200 of *β*-tubulin that correlates with benomyl-resistance phenotypes of field isolates of *Botrytis cinerea*. Phytopathology. 83:1478-1483.
Zhang, R.L. and Huang, J.S. (2007). Cloning of a carbendazim-resistant gene from *Colletotrichum gloeosporioides* of mango in South China. Afr. J. Biotech. 6:143-147.

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