

TABLE OF CONTENTS

	Page
Acknowledgements	iii
Abstract (English)	v
Abstract (Thai)	ix
Table of Contents	xiii
List of Tables	xv
List of Figures	xix
List of Proceeding and Publications	xxiii
Chapter 1 Introduction	1
Chapter 2 Isolation and morphological characterisation of <i>Colletotrichum</i> spp. causing mango anthracnose	27
Chapter 3 Phenotypic assay detection of carbendazim-resistant <i>Colletotrichum</i> spp. causing mango anthracnose	50
Chapter 4 Detection of carbendazim-resistant <i>Colletotrichum</i> spp. using the second beta-tubulin gene sequences	84
Chapter 5 Effect of chitosan on carbendazim-resistant <i>Colletotrichum</i> spp. causing mango anthracnose	100

TABLE OF CONTENTS (CONTINUED)

	Page
Chapter 6 Conclusion	135
References	138
Appendices	158
Appendix A	159
Appendix B	167
Curriculum Vitae	174
Proceeding and Publications	175

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LIST OF TABLES

Table	Page
1.1 Fungicides labelled for controlling anthracnose of mangoes	10
1.2 Point mutations of some phytopathogenic fungi at the beta-tubulin gene causing resistance to benzimidazole fungicides	18
2.1 List of <i>Colletotrichum</i> spp. isolates causing anthracnose on fruits and leaves of various mango cultivars from markets and orchards	36
2.2 Morphological characterizations of <i>Colletotrichum</i> spp. isolates	43
3.1 Phenotype-resistant levels of <i>Colletotrichum</i> spp. to carbendazim fungicide	53
3.2 Carbendazim-resistant assay of <i>Colletotrichum gloeosporioides</i> causing mango anthracnose on potato dextrose agar amended with carbendazim	59
3.3 Selected isolates from the phenotype of carbendazim-resistant <i>Colletotrichum gloeosporioides</i> from leaf and fruit of each mango cultivar	68

LIST OF TABLES (CONTINUED)

Table	Page
3.4 Lesion diameter of anthracnose symptoms on fruits and leaves of the mango cv. Namdokmai inoculated with carbendazim-resistant <i>Colletotrichum</i> spp. for 4 days	70
3.5 The blast result of rDNA ITS sequences (430 bp) from <i>Colletotrichum gloeosporioides</i> causing mango anthracnose and their most closely related sequences in GenBank during October 2010	76
3.6 ITS sequences used in phylogenetic analyses from GenBank	78
4.1 The second beta-tubulin sequences used in genetic analyses from GenBank	91
4.2 The nucleotide and amino acid substitution in the second beta-tubulin gene at codon 198 and 200 causing changes to the carbendazim resistibility	95
5.1 Description of chitosan samples used in this study	102
5.2 Disease index (DI) on mango anthracnose	112

LIST OF TABLES (CONTINUED)

Table	Page
5.3 The effect of various chitosan on conidial inhibition of carbendazim-resistant <i>Colletotrichum gloeosporioides</i> causing mango anthracnose for 6 hr	116
5.4 The effect of various chitosan on conidial inhibition of carbendazim-resistant <i>Colletotrichum gloeosporioides</i> causing mango anthracnose for 12 hr	117
5.5 The effect of various chitosan on conidial inhibition of carbendazim-resistant <i>Colletotrichum gloeosporioides</i> causing mango anthracnose for 18 hr	118
5.6 The effect of various chitosan on conidial inhibition of carbendazim-resistant <i>Colletotrichum gloeosporioides</i> causing mango anthracnose for 24 hr	119
5.7 The effect of various chitosan on colony diameter of carbendazim-resistant <i>Colletotrichum gloeosporioides</i> causing mango anthracnose	121

LIST OF TABLES (CONTINUED)

Table	Page
5.8 Lesion diameter and decreased disease incidence of harvested mango fruits before and after inoculation with carbendazim-highly resistant <i>Colletotrichum gloeosporioides</i> for 4 day	124
5.9 Disease index and disease reduction of chitosan treated to control harvested mango anthracnose caused by <i>Colletotrichum gloeosporioides</i> for 7 days	128

LIST OF FIGURES

Figure	Page
1.1 Disease cycle of mango anthracnose	7
1.2 Structure formula of carbendazim	13
1.3 Binding site of carbendazim	14
1.4 Development of fungicide resistance	16
1.5 Production and advantages of chitosan	21
1.6 Primary structures of chitin and chitosan	21
1.7 Production mechanism of elicitor in a plant cell	24
2.1 Anthracnose symptoms on naturally-infected leaves in various mango cultivars	33
2.2 Anthracnose symptoms on naturally-infected fruits in various mango cultivars	34
2.3 Numbers of <i>Colletotrichum</i> spp. isolates causing mango anthracnose	35
2.4 Morphological characterizations of <i>Colletotrichum</i> spp. isolates causing mango anthracnose	42

LIST OF FIGURES (CONTINUED)

Figure	Page
3.1 Diagrammatic representation of <i>Colletotrichum</i> spp. ribosomal DNA. The species-specific primer positions, CgInt and ITS4, in highly conserved 18S and 28s ribosomal gene sequences flanking the spacer regions	57
3.2 The carbendazim resistibility assays of <i>Colletotrichum gloeosporioides</i> causing mango anthracnose on potato dextrose agar amended with carbendazim	64
3.3 Isolate number of carbendazim-resistant <i>Colletotrichum gloeosporioides</i> causing mango anthracnose	65
3.4 The mycelial characterisation of carbendazim-resistant <i>Colletotrichum gloeosporioides</i>	67
3.5 Lesions on fruits of the mango cv. 'Namdokmai' inoculated with carbendazim-resistant <i>Colletotrichum gloeosporioides</i> for 4 days	72
3.6 Lesions on leaves of the mango cv. Namdokmai inoculated with carbendazim-resistant <i>Colletotrichum gloeosporioides</i> for 4 days	73

LIST OF FIGURES (CONTINUED)

Figure	Page
3.7 Amplification of the partial ITS region for species identification of various mango anthracnose pathogen isolates by PCR using species-specific primers of CgIT and ITS4	75
3.8 Phylogenetic tree based on the rDNA ITS sequence data representing relationships between <i>Colletotrichum gloeosporioides</i> isolates within the published sequence	79
4.1 Primers in nested polymerase chain reaction (PCR) in the partial second beta-tubulin gene based on nucleotide sequence of <i>Colletotrichum gloeosporioides</i> f. sp. <i>aeschynomene</i>	88
4.2 PCR amplification of the partial region of the second beta-tubulin gene from various isolates of <i>Colletotrichum gloeosporioides</i> causing mango anthracnose	90
4.3 Comparison of deduced nucleotide and amino acids sequences of the second beta-tubulin from <i>Colletotrichum gloeosporioides</i> f. sp. <i>aeschynomene</i> , benomyl-resistant and –sensitive <i>C. gloeosporioides</i> at the target sites of benzimidazole between carbendazim-resistant phenotype of <i>C. gloeosporioides</i> isolates causing anthracnose disease from various mango cultivars	93

LIST OF FIGURES (CONTINUED)

Figure	Page
4.4 Amino acid substitution in the second beta-tubulin at codon 198 and 200 can change the carbendazim resistibility	96
5.1 The effect of chitosan on the mycelial growth of carbendazim-resistant <i>Colletotrichum gloeosporioides</i> causing mango anthracnose	122
5.2 The effect of chitosan on decreasing anthracnose on mango fruits that treated before and after inoculation of highly carbendazim-resistant <i>Colletotrichum gloeosporioides</i>	125
5.3 The effect of chitosan on reducing anthracnose on mango fruit when sprayed chitosan before and after inoculation of highly carbendazim-resistant <i>Colletotrichum gloeosporioides</i>	129

LIST OF PROCEEDING AND PUBLICATIONS

1. **Kongtragoul, P.**, Chaichana, S. and Nalumpang S. 2008. Studies on fungicide carbendazim resistance of *Colletotrichum gloeosporioides* causing mango anthracnose. p. 53. In: The 3rd Annual Meeting of Thai Mycological Association (TMA) and Mycology Conference in Thailand. October 11, 2008. Khon Kaen University, Khon Kaen, Thailand. (Poster presentation in English)
2. **Kongtragoul, P.** and Nalumpang S. 2009. Preliminary assay of carbendazim-resistant detection of *Colletotrichum* spp. causing anthracnose of mango cv. Nam Dok Mai. p. 77-83. In: The 6th Proceeding of Agriculture Graduate Conference. March 12-13, 2009. Faculty of Agriculture. Chiang Mai University. Chiang Mai, Thailand (Oral presentation in Thai).
3. **Kongtragoul, P.**, Chaichana, S. and Nalumpang S. 2009. Effect of ginger extracts and some fungicide on carbendazim-resistant *Colletotrichum* spp. causing mango anthracnose. p. 159-164. In: Proceeding of the Third Botanical Conference of Thailand. March 25-27, 2552. Faculty of Science, Mahidol University, Bangkok, Thailand. (Poster presentation in Thai).
4. **Kongtragoul, P.**, Miyamoto, Y., Miyake, C., Izumi, Y., Akitmitsu, K. and Nalumpang S. 2009. Detection of carbendazim-resistant *Colletotrichum gloeosporioides* causing mango anthracnose disease. p. 9-15. In: CMU-KU symposium: The 2nd International Meeting for Development of International Network for Reduction of Agrochemical Use; Food Safety Technologies in

Southeast Asia. September 22-23, 2009. The Imperial Mae Ping Hotel, Chiang Mai, Thailand. (Oral presentation in English)

5. **Kongtragoul, P.**, Miyamoto, Y., Miyake, C., Izumi, Y., Akitmitsu, K. and Nalumpang, S. 2009. Determination of beta-tubulin gene point mutation in *Colletotrichum gloeosporioides* causing mango anthracnose. p. 15. In: The 4th Annual Meeting of Thai Mycological Association and Mycology Conference in Thailand. October 24, 2009. Faculty of agricultural production, Maejo University, Chiang Mai, Thailand. (Oral presentation in English)
6. **Kongtragoul, P.**, Miyamoto, Y., Miyake, C., Izumi, Y., Akitmitsu, K. and Nalumpang S. 2010. Point mutation in the β -tubulin gene of carbendazim-resistant *Colletotrichum gloeosporioides* causing mango anthracnose. p. 22. In: The Annual Meeting of Thai Phytopathological Society (TPS) Conference on Plant Pathology in Thailand. May 15, 2553. Convention Center, Kasetsart University. Bangkok, Thailand. (Oral presentation in Thai)
7. Nalumpang S., Miyamoto, Y., Miyake, C., Izumi, Y., Akitmitsu, K. and **Kongtragoul, P.** 2010. Point mutation in the beta-tubulin gene conferred carbendazim-resistant phenotype of *Colletotrichum gloeosporioides* causing 'Nam Dok Mai' mango anthracnose. International Journal of Agricultural Technology. 6 (2): 365-378.
8. **Kongtragoul, P.** and Nalumpang, S. 2010. Characterization of carbendazim-resistant *Colletotrichum gloeosporioides* causing mango anthracnose disease on fruits from fresh markets. p. 189. In: The 8th National Postharvest

Technology Conference 2010. September 1-3, 2553. The Empress Hotel, Chiang Mai , Thailand. (Poster presentation in Thai)

9. **Kongtragoul, P.** and Nalumpang, S. 2010. Single Nucleotide transversion in the *TUB2* gene associated with carbendazim-resistant *Colletotrichum gloeosporioides* from mango. p. 3. In: The 4th AG-BIO/PERDO Graduate Conference on Agricultural Biotechnology and UT-KU Joint Seminar. December 9-10, 2010. Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. (Oral presentation in English).
10. **Kongtragoul, P.** and Nalumpang, S. 2010. Characterization of Carbendazim-resistant *Colletotrichum gloeosporioides*. *Journal of Agriculture*. 26 (3): 203-212.