CHAPTER 1

Introduction

1.1 Rationale

Mango, known as the 'king of fruits' or 'apple of the tropics', is ranked as the world's fifth most important fruit, which Thailand is being one of its major producers (Nelson, 2008; Prabakar *et al.*, 2008). In 2005, the global production of this fruit was 28.2 million tons. Major export markets for Thai mangoes include Japan, Malaysia, China, Hong Kong, Singapore, the Netherlands, Canada, and the United States of America (USA). In 2006, Thailand had a total planted area for mangoes of 286,697 hectares, with a total export of 29,600 tons valued at 1,147 million baht (Chomchalow and Na Songkhla, 2008). The fruit is known for its nutritional value, delicious taste, sensory appeal and health promoting qualities (Mukherjee, 1997; Prakash, 2004; Singh *et al.*, 2008).

In Thailand, high temperatures and humidity favour disease development in both the pre- and postharvest phase (Yahia, 1999). Many mango diseases can reduce fruit quality and cause severe losses. Disease incidence is therefore one of the key factors constraining mango production. Anthracnose, which is caused by *Colletotrichum gloeosporioides*, is the most devastating disease in mangoes and causes major constraints to mango production and export (Sangchote, 1987; Rawal, 1997; Arauz, 2000; Ploetz, 2003; Akem, 2006; Nelson, 2008). Benzimidazole fungicides such as carbendazim, benomyl and thiabendazole are widespread and have

been used for over 20 years because they are believed to control plant diseases better than any other method (Pongsuwan, 1993). Although these fungicides effectively suppress and control a wide variety of plant diseases, particularly in their long-term use, their effectiveness as systemic fungicides could be reduced in controlling disease pathogens. Pathogens often become resistant to fungicides, due to mutation in a gene encoding in the target site of the fungicide. An increase in fungicide resistance causes problems for mango growers leading to limitations of efficacy and usefulness.

In addition to good quality mango, consumers demand food safety and environmental friendly production practices. Since the promulgation of the World Trade Organization (WTO) in 2004, the FAO/WHO Codex Alimentarus Commission standards for pesticide residues have been used as a reference point for sanitary and phytosanitary measures (Arauz, 2000). Because of toxicity, harm to the environment and high expenses, farmers are looking for alternatives to fungicides. Chitosan is one such alternative for controlling plant diseases, as it is a nontoxic agent and biodegradable polymer. Chitosan is therefore promising for use as an antifungal agent and a substance to promote the plant's self-defence system against pathogens (Rabea *et al*., 2003; Bautista-Baños *et al.*, 2006). In addition, fungicide-resistant strains of microorganisms and regulations for using fungicides have reduced the attractiveness of chemical-based control strategies (Johnson and Sangchote, 1994).

1.2 Mango anthracnose disease

1.2.1 The host

Mango (*Mangifera indica* L.) is a perennial tree in the Anacardiaceae family. It is branched and evergreen, and grows up to 30-40 feet tall. The plant originated in the Indo-Myanmar region and has been cultivated for over 4,000 years (Toohill, 1985). It was domesticated and adapted throughout the tropics and subtropics, and its widespread distribution is associated with human settlement, because the mango plays an important role in the diet of many people. The mango has more than 1,000 local names throughout the world, which indicates how greatly valued it is by humankind. Mango is also a common garden tree throughout the tropics. Ripe mango is rich in vitamin A and forms a delicious dessert (Bally, 2006), but the fruit can be eaten also unripe, processed into pickles, pulps, jams, and chutneys, frozen or dried.

Mango is one of the most popular fruits in Thailand as well as in many other countries. There are many mango cultivars in Thailand that can be eaten as green mango and riped mango. The major Thai cultivars include Namdokmai, Chokanan, Mahachanok and Khiaosawoei (Eiadthong *et al*., 1999).

1.2.2 The anthracnose diseases

Mango anthracnose was first reported in Puerto Rico in 1903 (Prakash, 2004), and now it is one of the most serious disease in many parts of the world, especially in areas with high humidity, frequent rains and temperatures ranging of 24 ^{O}C to 32^{O}C (Philippine Mango Seeding Farm Crop, 2010). The disease appears in both the pre- and post-harvest stages, and its incidence in the post-harvest phase causes most

economic damage worldwide. The disease directly affects the market value of the fruit, thus causing severe economic losses.

Disease cycle

The disease cycle consists of dissemination, inoculation, infection and pathogen development, symptoms and disease development, pathogen reproduction and pathogen survival (Figure 1.1).

Dissemination: In the field, fungus produces fruiting bodies on the symptom of leaves, twigs, panicles, and mummified fruit. The conidia are spread throughout the orchard by means of water droplets, i.e. heavy dew, irrigation water, and raindrops, etc. Wet weather contributes to conidia production, and its dispersal and infection (Arauz, 2000; Akem, 2006).

Inoculation: Conidia formed on the anthracnose lesions in the mango canopy are considered as a primary source of inoculum, sporulate and infection sites, and those attached to the plant cuticle become uniseptate during the germination stage, which takes about 12-48 hr after attachment. Conidium attachment is mediated by the presence of a mucilaginous polymer on the surface of the conidium. Once the adhesion has occurred, the conidium germinates and the germ tube grows a short distance before showing a formation of terminal appressorium. Young appressoria are lightly pigmented or hyaline, and thicken and melanise as the appressorium ages. Infection pegs are produced where the structure depends on the stage of fruit development at the time of infection. Variation in length of infection pegs may be

attributed to differences in concentration of antifungal compounds in the peel of the fruit before and after harvesting (Arauz, 2000; Akem, 2006).

Infection and pathogen development: In immature fruit and young tissue, spores germinate and penetrate through the cuticle and epidermis to ramify through the tissue. In mature fruit, the infection penetrates the cuticle and remains quiescent infection until the climacteric of the fruit begins to ripen. The fruit infection is associated with mechanical damage from rainfall at all stages of fruit development (Arauz, 2000; Akem, 2006).

Symptoms and disease development: Mango fruits can be infected by *C. gloeosporioides* in all stages of the development and caused fruit loss in the pre-harvest period. Anthracnose on fruits in the post-harvest stage due to its infection in the field and remains quiescent until ripening begins. Sometimes the disease appears after harvest, and then the fruit becomes black and sunken, with rapidly expanding lesions on mango fruits. Once the climacteric period starts, the lesions begin to develop. There are no reports of fruit-to-fruit infection (Arauz, 2000). Symptomology of *C. gloeosporioides* is characterized by dark and depressed lesions on ripe fruit. The symptoms are often accompanied by pink and slimy spore masses which developed as the acervuli structures. Infections on stems, leaves and young inflorescences manifest as sub circular or angular black lesions which are enlarged and coalesced. The infection frequently destroys leaf edges or entire inflorescences (Arauz, 2000; Akem, 2006).

Leaf anthracnose appears in irregular shaped black necrotic spots on both sides of the mango leaf. The lesions often coalesce and form large necrotic areas

along the leaf margins. Severely affected leaves are usually curled. The lesions develop primarily on young tissue. The conidia are formed later and can be observed in the lesions at all stages. In older leaves, the anthracnose lesions do not develop, but latent infections form and the fungus remains dormant until the tissue senesces. Under favourable conditions, the fungus can invade twigs and cause dieback in some cases (Arauz, 2000; Akem, 2006).

Panicle anthracnose or blossom blight can affect both the inflorescence stalk and individual flower. Dark grey to black elongated lesions appear in the stalk. The colour of blighted flowers varies from brown to black as they dry. Larger fruit are aborted because of normal self-thinning or other physiological causes that are usually mummified (Arauz, 2000; Akem, 2006).

Pathogen reproduction: The sticky masses of conidia are produced in a fruit body or acervuli on symptomatic tissue, especially during moist (rainy, humid) conditions. Many cycles of the disease can occur as the fungus continues to multiply during the season (Arauz, 2000; Akem, 2006).

Pathogen survival: The pathogen survives in the season through infected and defoliated terminal branches and mature leaves. In addition, the inoculum production has also been reported in dry leaves on the ground (Ann, 1995). Nevertheless, the role of the sexual stage in the disease cycle is unclear. The conidia can be disseminated by rainfall onto other leaves, flowers and young fruits (Arauz, 2000; Akem, 2006).

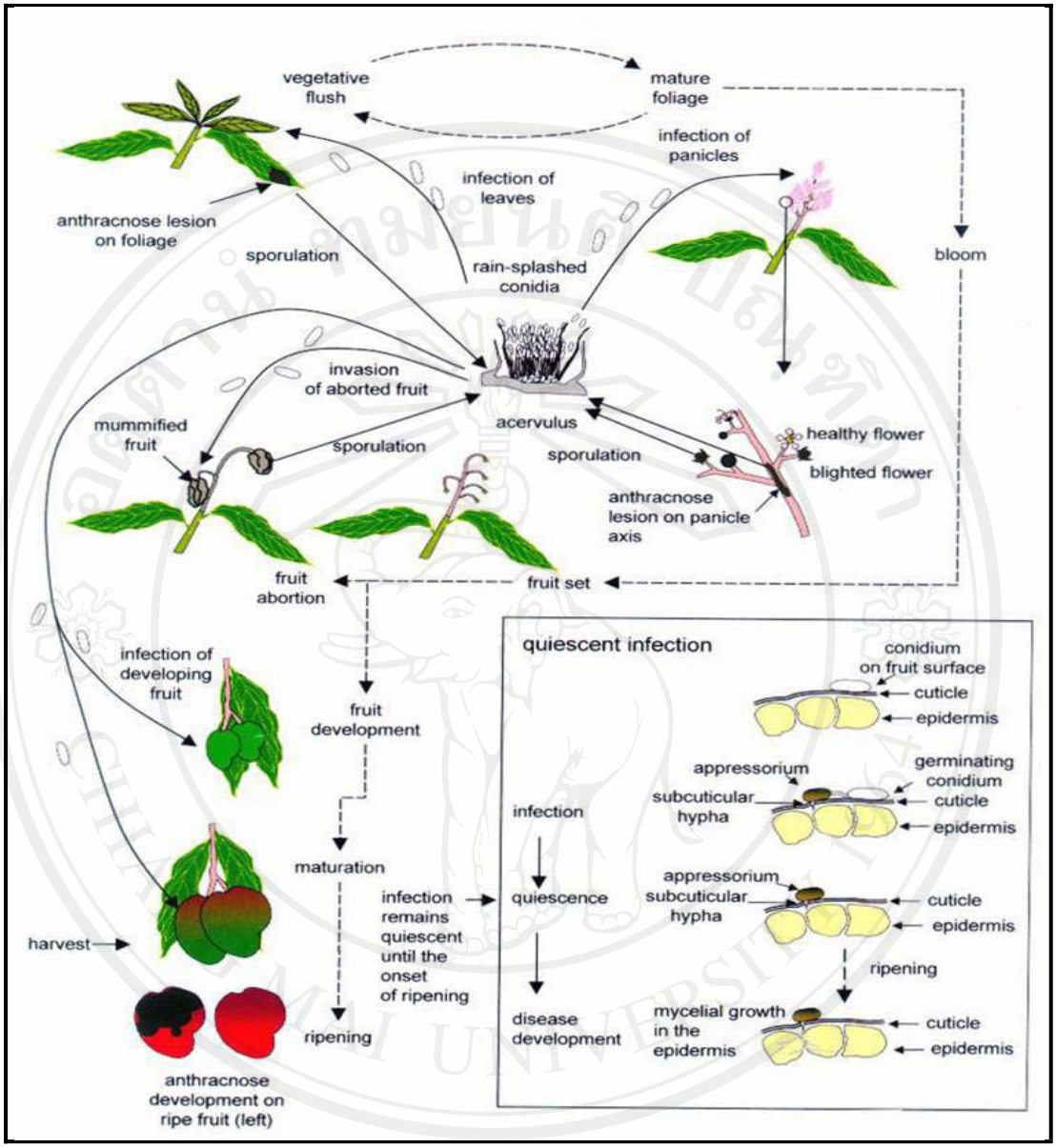

Figure 1.1 Disease cycle of mango anthracnose. Solid lines indicate the disease cycle; dotted lines indicate mango phenology.

Source: Arauz (2000)

1.2.3 The pathogen: *Colletotrichum* spp.

The genus, *Colletotrichum* spp. causes anthracnose disease in mango. Three closely related taxa of fungi cause anthracnose in most mango producing areas as follows:- *C. gloeosporioides*, *C. acutatum* and *C. boninense*. Pre- and post-harvest anthracnoses on mango have been reported extensively in the USA, West Indian Islands, South Africa, India, Malaysia, Thailand and Australia. *C. gloeosporioides* (teleomorph: *Glomerella cingulata*) is the major cause of the disease and a large number of host species has been reported (Dodd *et al*., 1997). *Colletotrichum acutatum* plays a minor role in mango plantation areas of Australia, India, Japan, Taiwan and Florida in the USA. Moreover, *Colletotrichum boninense* has been reported to infect mango anthracnose in Columbia.

The genus *Colletotrichum* (teleomorph *Glomerella*) is classified on the basis of molecular phylogeny classification reported by Hibbett *et al.* (2007) as follows:-

Kingdom: Fungi

Phylum: Ascomycota

Subphylum: Pezizomycotina

Class: Sordariomycetes

Form Order: Phyllachorales

Form Family: Phyllachoraceae

Form Genus: *Colletotrichum*

The morphological and cultural characteristics are characterized as *C. gloeosporioides* (von Arx, 1957; Mordue, 1971; Sutton, 1992). Mycelium is mostly greyish white to deep grey on potato dextrose agar (PDA). The colour of the underside in colonies varies from white to dark green or brown. The colour becomes darker with aging. The conidia are produced usually as pale salmon masses in acervuli, often as setae, and sometimes as glabrous with a 500 μm diameter. The conidia are straight, hyaline, and normally uninucleate, with much variation in length (9-24 μm). They usually have a width of 3-4.5 μm but according to von Arx (1957), up to 6 μm, and they have varying proportions of obtuse apices. The appressoria are 6–20 μm in length and 4–12 μm in width, ovoid to clavate, and sometimes lobed. The conidia germinate and form appressoria at one extremity or both points, ending with very short or long hyphae. In culture media, appressoria are formed often from mycelium and become complex (Sutton 1980; 1992).

1.2.4 Control of mango anthracnose

Control of post-harvest anthracnose can be achieved by field management, post-harvest treatment, or a combination of both. The management must be efficient and cost-effective, as well as safe for consumers, agricultural workers and the environment.

Chemical control

Much attention and effort in anthracnose control have focused on the application of fungicides. The aim of fungicide use is to reduce damage to inflorescences and fruits. In extreme situations, where fruit has developed under

disease-favourable conditions, the use of protectant sprays and systemic fungicides has been reported (Dodd *et al.*, 1997; Nelson, 2008). Despite the long recognition of fungicide applications, few of them are currently approved by importing countries. The European Union, for instance, follows the guidelines of the FAO/WHO Codex Alimentarius whereas the U.S. Environment Protection Agency uses different maximum residue limits as shown in Table1.1. The choice of fungicides therefore depends on the requirements of importing countries.

Table 1.1 Fungicides labelled for controlling anthracnose of mangoes

Fungicide	Maximum residue limits	
	Codex (FAO/WHO)	**EPA (U.S.A.)**
Carbendazim	2.0[1/]	3.0
Prochloraz[2/]	2.0	Not labelled
Captan	Not labelled	50.0
Ferbam	2.0[3/]	7.0
Thiabendazole	-	10.0
Copper fungicides	No data	Exempt

[1/], [3/] As benomyl.

[2/] Allowed in postharvest use.

Sources: Food and Agriculture Organization (FAO) (1997) and United States Environmental Protection Agency (EPA) (1999)

Non-chemical control

Cultural practices: Sanitation has been done by pruning trees annually and removed fallen plant debris from the ground. Plant spacing is performed by widen the

space between plant stands to inhibit severe epidemics. Intercropping is introduced by planting non-host plants that are not hosts to inhibit epidemics (Nelson, 2008).

Disease forecasting system: The predictive models for *C. gloeosporioides* infection on mangoes based on temperature and moisture requirements (Arauz, 2000; Akem, 2006).

Prospects for biological control: The biological fungicide for controlling mango anthracnose caused by *C. gloeosporioides* have been reported in commercial products of Thailand, Vietnam and China, that are *Chaetomium globosum*, *Chaetomium cupreum*, *Trichoderma harzianum* and *Trichoderma viride* (Soytong, 2004; Soytong *et al*., 2005; Kaewchai *et al*., 2009). Soytong (2004) reported that Ketomium® is a broad spectrum biological fungicide in the form of pellets or powder that is formulated from mixing 22-strains of *Ch. globosum* and *Ch. cupreum*. It applied to control mango anthracnose which spraying at the rate of 10-20 g/20 l of water mixed with sticker and spreader at every 15-20 days until harvest to protect the disease of upper plant parts above soil. Moreover, Noiaium and Soytong (2000) reported that Chaetomium's pellet at the rate of 20 g/plant every 4 months amended with 5 kg of organic compost could reduce the pathogen inoculums and disease incidence of 79.88 and 55.93 percent, respectively in the field. The plants treated with Trichoderma's pellets could also reduce the pathogen inoculums and incidence of anthracnose in mango var. Choke Anan of 81.26 and 55.53 percent, respectively. It was observed that the biological treatments gave better yield than the chemical fungicide treatment.

Post-harvest treatment: The following treatment has been reported to retard growth and symptom development e.g. keeping in refrigeration at 50°F (10°C), but do not chill unripe fruit to avoid chilling injury, hot water dip for 15 minutes at about 120-130°F (49-55°C), vapour heat and forced-air dry heat for 3-6 hr at various temperatures (Nelson, 2008).

Integrated disease control The integrated disease control has been reported by Dirou and Stovold (2005), Nelson (2008) and Singh *et al*. (2008) as follows:- site selection**,** cultivar selection, cultural practices in the field such as sanitation, plant spacing, intercropping etc., fungicide sprays in the field, and postharvest treatments.

1.3 Carbendazim

Carbendazim is a broad spectrum benzimidazole fungicide group. Its systemic activity plays a very important role in plant disease control. The fungicide was first reported in 1973 and developed by three companies, i.e., BASF, Hoeschst and Dupont (Pfeil and Dellarco, 2005). Carbendazim is used to control a broad range of diseases in fruit, vegetables and ornamental plants. It is also used in post-harvest handling, food storage and seed treatment. Furthermore, it is recommended for controlling anthracnose. Carbendazim is registered in Thailand for foliar application on mangoes, and growers are recommended to spray several times in cropping season (Banasiak, 2003). The molecular formula of carbendazim is characterized as $C_9H_9N_3O_2$, and its structure is shown in Figure 1.2.

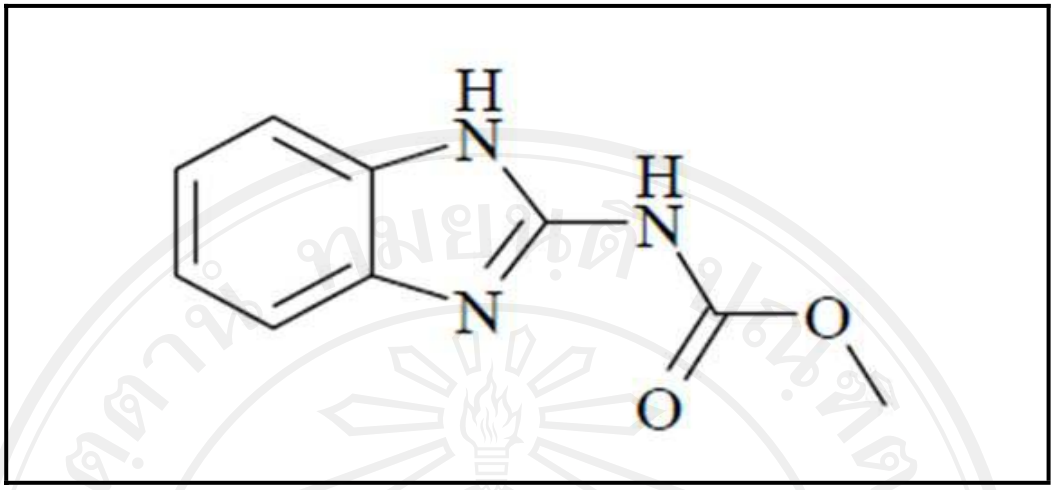

Figure 1.2 Structure formula of carbendazim.

Source: Haute (2007)

Mode of action:

Carbendazim inhibits fungal mitotic microtubule formation. The mode of carbendazim action is considered widely as binding to free tubulin, particularly beta-tubulin at the colchicine binding site, and disrupting microtubule formation, thereby inhibiting mitosis (Figure 1.3). The primary action of benzimidazoles as anthelmintics is considered to be through binding by the free beta-tubulin and inhibiting its polymerization. These results affect the microtubule-dependent glucose uptake. A number of benzimidazoles also have been shown to inhibit mammalian tubulin polymerization and be aneugenic *in vivo* (Davidse, 1986).

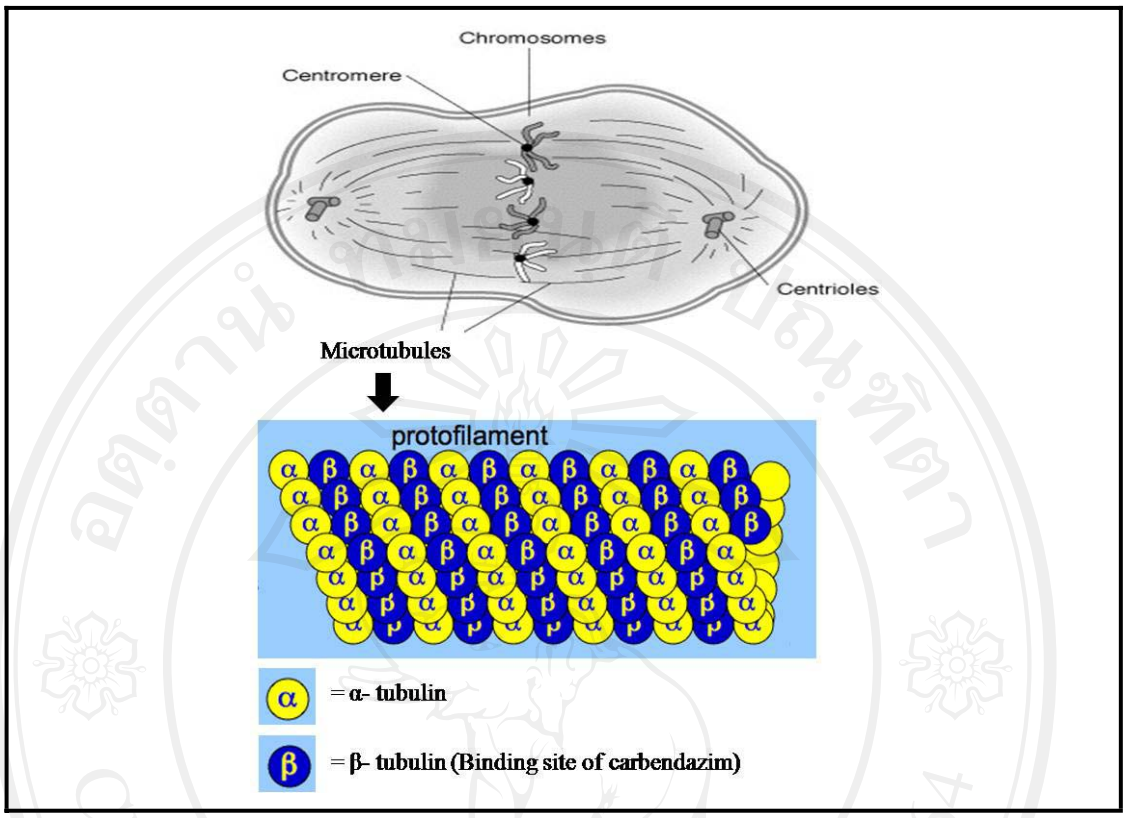

Figure 1.3 Binding site of carbendazim.

Sources: Metaphase.png (2005) and Koning (2010)

1.4 Fungicide resistance

Fungicides are important tools for maintaining healthy, reliable, and high-quality agricultural products. Failure of fungicides to control a disease in a crop may due to several reasons, including poor sprayer calibration, operator error, excessive wind, wash off by rain, poor quality fungicide product and pathogen become resistant to the fungicide (Beresford, 1994; Ma and Michailides, 2005).

Fungicide resistance is a very serious problem. It is defined as the decreased sensitivity of an isolate from a particular pathogen species against a particular inhibitor. Field resistance is observed when the frequency of resistant individuals in a pathogen population resulting in poor disease control (Ma and Michailides, 2005).

1.4.1 Development of fungicide resistance

Fungicides at risk from pathogen resistance are mainly synthetic ones developed in the early 1970s, and are very selective in the way they affect their target fungi (Beresford, 1994; Ma and Michailides, 2005). The fungicide groups are important to horticulture at risk, including benzimidazoles, dicarboximides and phenylamides. Many fungicides, such as captan, copper, mancozeb, metiram, sulphur, and thiram, are non-selective, broad spectrum and active against diseases (Konstantinidis *et al*., 2003).

Brent and Hollomon (1998) and Damicone and Smith (2009) estimated the risk of benzimidazole resistance development. The incidence of acquired fungicide resistance in the field has become an important factor limiting the efficacy of disease control strategies. The growers have been usually increased dosage and application frequency. The development of resistance might become an important aid in understanding the mechanism of action in fungicides at the molecular level.

Deising *et al*. (2008) reported that development of fungicide resistance is a selection process, with the fungicide as the selecting agent. In qualitative resistance, mutation-based insensitive mutants are selected, and strains are either sensitive or resistant to the chemical fungicide. In quantitative resistance, individuals that express genes leading to reduce fungicide sensitivity. Sub-lethal fungicide stress leads to induce genes that resist subsequent chemical fungicide treatment. As a consequence the population is shifted to increase resistance and numbers of individuals with higher degrees of resistance (Figure 1.4).

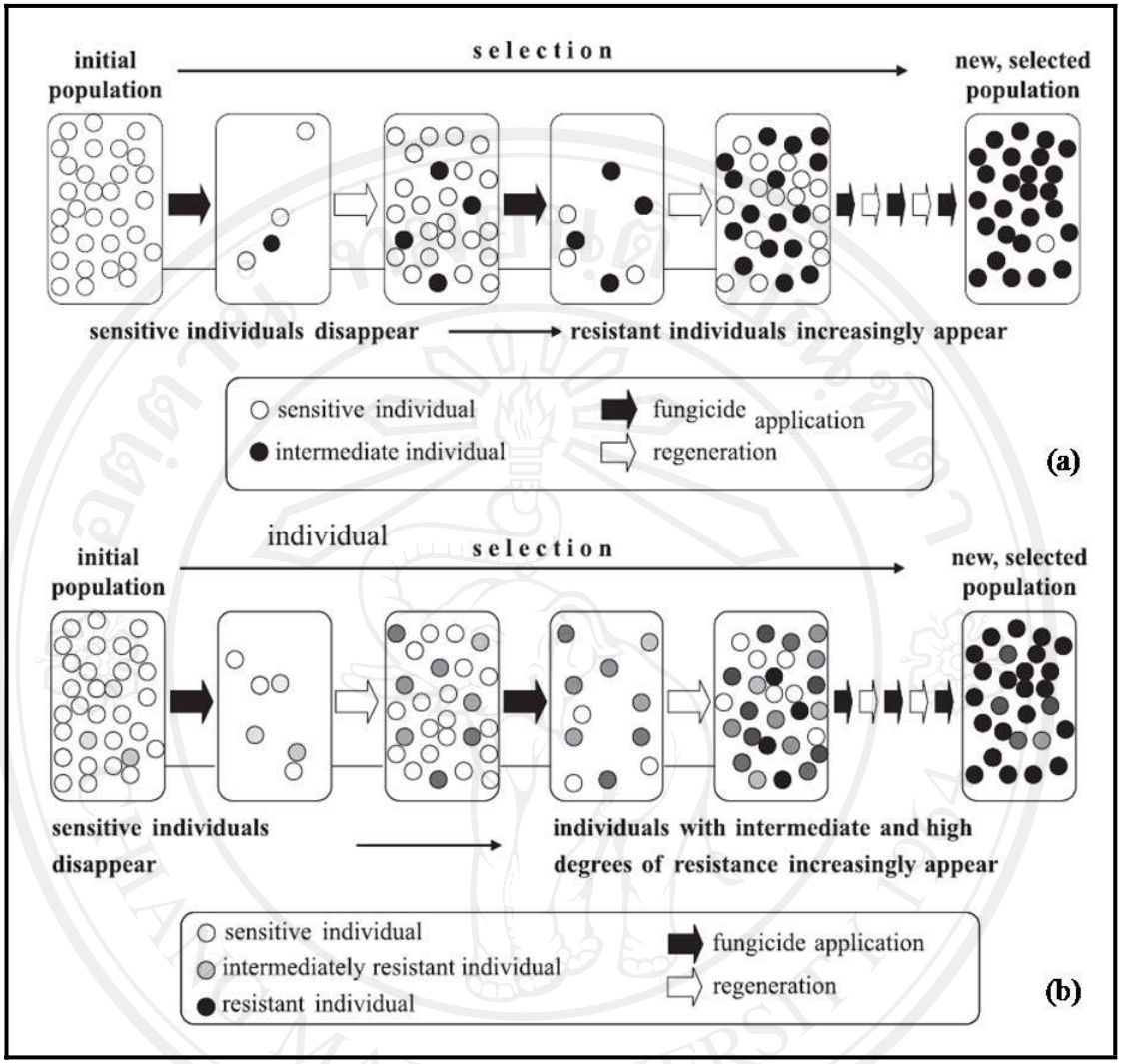

Figure 1.4 Development of fungicide resistance; In qualitative resistance (a), In quantitative resistance (b).

Source: Deising *et al*. (2008)

1.4.2 Benzimidazole fungicide resistance

Most plant-pathogenic fungi develop high or very high resistance to benzimidazole fungicides, such as carbendazim, benomyl, and thiophanate-methy after being exposed to them for 2-3 years (Brent and Hollomon 1998; Deising *et al*., 2008; Damicone and Smith, 2009). Fungicide resistance to benzimidazoles has been reported in many fungal species. In most cases, resistance was correlated with point mutations in the beta-tubulin gene, which resulted to alter amino acid sequences at the benzimidazole-binding site (Davidson *et al*., 2006). There are many reports on the most resistant isolates of plant pathogenic fungi showed amino acid substitution at codon 6, 50, 167, 198, 200, and 240 in the beta-tubulin gene suggesting a possible cause of benzimidazole resistance in field isolates of pathogenic fungi as seen in Table 1.2.

Various mechanisms can confer fungicide resistance, but the most common resistance mechanism of phytopathogenic fungi is an alteration of the biochemical target site of the fungicide. Molecular techniques can be developed and based on this mechanism, to detect resistant isolates rapidly, and thus improved the ability to speed up resistant genotype detection and understanding the evolution of fungicide resistance at the population level. The timely detection of resistance levels in populations of phytopathogenic fungi in the field could help growers for making correct decision on resistance management programs that aim to control plant diseases (Staub, 1991; Brent and Hollomon, 1998; Deising *et al*., 2008; Damicone and Smith, 2009).

Table 1.2 Point mutations of some phytopathogenic fungi at the beta-tubulin gene, causing resistance to benzimidazole fungicides

Amino acid		Phytopathoginic fungi	Fungicide	Phenotype definition	References
Codon	Substitution				
6	His to Tyr (**C**AT)-(**T**AT)	*Monilinia fructicola*	benomyl or thiophanate-methyl	low resistance	Ma *et al.* (2003)
50	Tyr to Cys (T**A**C)-(T**G**C)	*Cladobotryum dendroides*	carbendazim	resistant	McKay *et al.* (1998)
	Tyr to Asn (A**T**C)-(A**A**C)	*Fusarium moniliforme*	benomyl	resistant	Yan and Dickman (1996)
167	Phe to Tyr (T**T**C)-(T**A**C)	*Cochliobolus heterostrophus*	benomyl	resistant	Gafur *et al.* (1998)
		Penicillium expansum	thiabendazole	resistant	Baraldi *et al.* (2003)
		Neurospora crassa	benomyl	resistant	Orbach *et al.* (1986)
198	Glu to Ala (G**A**G)-(G**C**G)	*Botrytis cinerea*	carbendazim	highly resistant	Yarden and Katan (1993)
			carbendazim	highly resistant	Ziogas *et al.* (2009)
		Cercospora beticola	methy-benzimidazolecarbamate	tolerant	Davidson *et al*. (2006)
		Colletotrichum gloeosporioides	benomyl	resistant	Peres *et al*. (2004)
			thiophanate-methyl	highly resistant	Chung *et al*. (2006)
			benomyl	resistant	Maymon *et al.* (2006)
			Bennomyl or Carbendazim or thiophanate-methyl	resistant	Kim *et al*. (2007)
			carbendazim	high resistant	Ru-Lin and Jun-Sheng (2007)
		C. gloeosporioides f. sp. *aeschynomene*	benomyl	resistant	Buhr and Dickman (1994)
		Helminthosporium solani	benomyl or thiophanate-methyl	resistance	Cunha and Rizzo (2003)
		M. fructicola	benomyl or thiophanate-methyl	high resistance	Ma *et al.* (2003)

Table 1.2 Continued

Amino acid		Phytopathoginic fungi	Fungicide	Phenotype definition	References
Codon	Substitution				
		Mycosphaerella fijiensis	benomyl	medium or high resistance	Cañas-Gutiérrez *et al*. (2006)
		Penicillium aurantiogriseum	benomyl	very high resistance	Koenraadt *et al.* (1992)
		Penicillium expansum	benomyl	very high resistance	
			benomyl or thiabendazole	highly resistant	Ru-Lin and Jun-Sheng (2007)
			benomyl or thiabendazole	highly resistant	Sholberg *et al*. (2005)
		Tapesia acuformis *Tapesia yallundae*	carbendazim or thiabendazole	very high resistance	Albertini *et al.* (1999)
		Penicillium puberrulum	benomyl	very high resistance	Koenraadt *et al.* (1992)
		Venturia inaequalis	benomyl	very high resistance	
		Venturia pirina	benomyl	very high resistance	
198	Glu to Lys (**G**AG)-(**A**AG)	*Botrytis cinerea*	carbendazim	highly resistant	Yarden and Katan (1993)
		Colletotrichum cereale	thiophanate-methyl	resistance	Wong *et al*. (2008)
		P. aurantiogriseum	benomyl	highly resistant	Koenraadt *et al.* (1992)
		Penicillium digitatum	benomyl	highly resistant	
		Tapesia yallundae	Carbendazim or thiabendazole	resistance	Albertini *et al*. (1999)
198	Glu to Lys (**G**AA)-(**A**AA)	*Monilinia fructicola*	benomyl	highly resistant	Koenraadt *et al.* (1992)
		V. inaequalis	benomyl	highly resistant	
198	Glu to Gly (G**A**G)-(G**G**G)	*B. cinerea*	carbendazim	highly resistant	Ziogas *et al*. (2009)
		T. acuformis *T. yallundae*	carbendazim or thiabendazole	highly resistant	Albertini *et al.* (1999)
		V. inaequalis	benomyl	medium resistance	Koenraadt *et al.* (1992)

Table 1.2 Continued

Amino acid		Phytopathoginic fungi	Fungicide	Phenotype definition	References
Codon	Substitution				
198	Glu to Val (G**A**G)-(G**T**G)	*Botrytis cinerea*	carbendazim or thiophanate-methyl	resistant	Zhang *et al.* (2009)
		Penicillium expansum	benomyl or thiabendazole	highly resistant	Sholberg *et al*. (2005)
		Penicillium digitatum	benomyl	highly resistant	Koenraadt *et al.* (1992)
200	Phe to Tyr (T**T**C)-(T**A**C)	*B. cinerea*	carbendazim	moderately resistant	Yarden and Katan (1993)
		C. gloeosporioides	thiophanate-methyl	intermediately resistant	Chung *et al*. (2006)
		P. aurantiogriseum	benomyl	medium resistance	Koenraadt *et al.* (1992)
		Penicillium italicum	benomyl	medium resistance	
		V. inaequalis	benomyl	medium resistance	
		Venturia pirina	benomyl	medium resistance	
		T. acuformis *Tapesia yallundae*	carbendazim or thiabendazole	highly resistant	Albertini *et al.* (1999)
240	Leu to Phe (**C**TC)-(**T**TC)	*T. yallundae*	carbendazim or thiabendazole	moderate to low resistance	Albertini *et al.* (1999)

1.5 Chitosan

Chitosan is produced commercially by deacetylation of chitin, a structural element in the exoskeleton of crustacean shells (from crabs and shrimps for example) whose main attributes correspond to its polycationic nature (Figure 1.5) (Sandford, 1989). It is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (Figure 1.6).

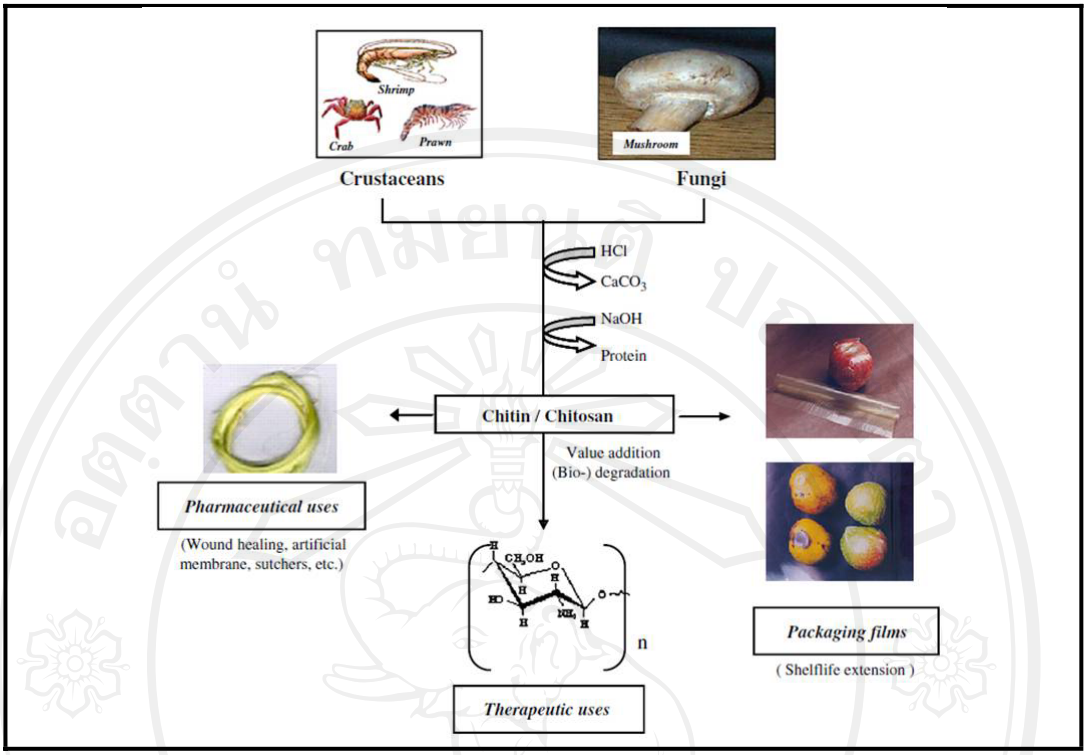

Figure 1.5 Production and advantages of chitosan.

Source: Harish Prashanth and Tharanathan (2007)

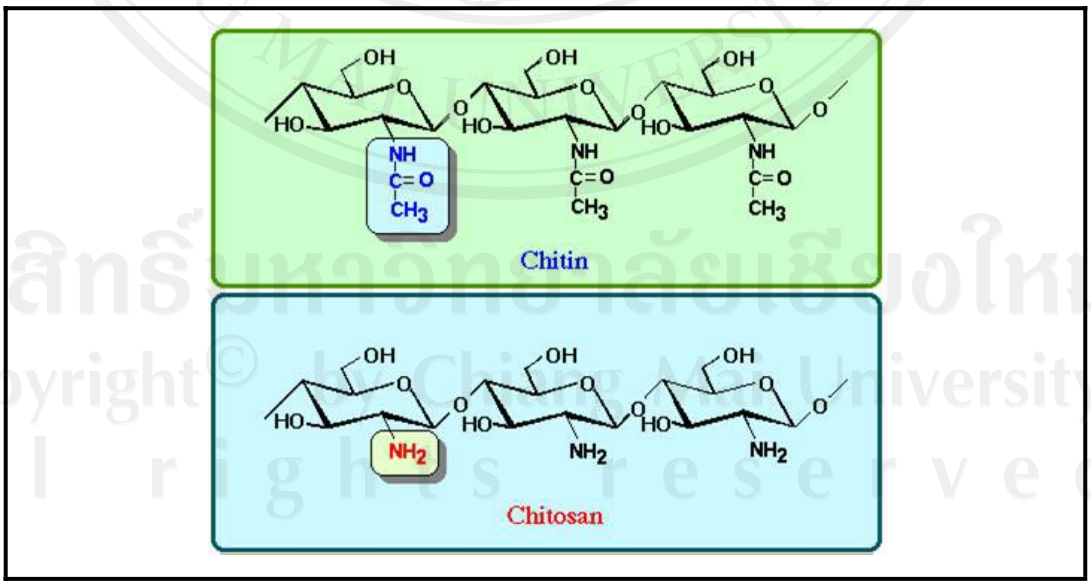

Figure 1.6 Primary structures of chitin (a) and chitosan (b).

Source: Dalwoo (1999)

Chitosan has been interested over decades because of its wide range of potential applications. Studies have shown great potential of the chemical in terms of biocompatibility, biodegradability, non-toxicity and absorption properties. Therefore, chitosan has been applied in various fields such as biotechnology, the food industry, material science, medical science, pharmaceuticals, and agriculture (Kumar, 2000; Tsigos *et al.*, 2000; Rabea *et al.*, 2003; Bautista-Baños *et al*., 2006; Nge *et al.*, 2006).

1.5.1 Applications and mechanisms of chitosan for plant production

In agriculture, chitosan has been used primarily as coating seeds, leaves, fruits, vegetables, and fertilizers to control agrochemical release that increase crop yield, to stimulate the plant immunity system, and protect plants against microorganism and stimulate their growth (Kumar, 2000; Tsigos *et al.*, 2000; Rabea *et al.*, 2003; Bautista-Baños *et al*., 2006; Nge *et al.*, 2006; Harish Prashanth and Tharanathan, 2007).

- **Nutrient sources**

Chitosan has a high carbon and nitrogen content and is a selective binder for transitional metal ions, for example, iron, copper and zinc. This unique selective chelating property means chitosan can bind in the soil to iron, copper and zinc, then release these micronutrient ions making them available to plant roots when they and microbes release hydrogen ions to the soil water in the vicinity of the root system. This makes chitosan a useful chelating agent. This characteristic is especially important in sandy soils or deficient in transitional metal ions (Bautista-Baños *et al*., 2006; Nge *et al.*, 2006; Harish Prashanth and Tharanathan, 2007).

- **Antimicrobial agents**

The antimicrobial activity of chitosan was observed and compared with a wide range of microorganisms including fungi (*Fusarium oxysporum*, *Botrytis cinerea*, *Piricularia oryzae*, *Rhizoctonia solani*, *Colletotrichum lindemuthianum*, etc), algae, and some bacteria (*Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas fluorescens*, *Xanthomonas campestris*, etc). Binding of the cationic amino group of chitosan to anionic groups of these microorganisms is resulted in growth inhibition of the fungi. Khieokachee (2008) reported that chitosan completely inhibited the growth of *C. gloeosporioides* on PDA. This is probably associated with its ability to bind DNA and therefore inhibited mRNA transcription in microorganisms (Vasyukova *et al*., 2001). Chitosan has been applied as a soil amendment, seed treatment, and foliar treatment to control the diseases.

- **Chitosan as an elicitor of mechanism response in plants**

Elicitors are substances that can induce defence responses when applied to tissues or cultured cells in plants. Many studies evaluated the ability of chitosan to elicit natural plant defence responses, and reported the physiological and biological changes occurring within plants due to the elicitation of chitosan. The primary physiological change has been observed the reduction of stomatal aperture after treating plants with chitosan, and reduced fungal access to the inner leaf tissue. Guard cells in plant leaves can produce H_2O_2 that mediates the elicitor induction to decrease stomatal apertures in response to chitosan treatment (Figure 1.7). In addition, chitosan elicits the accumulation of antibiotic phytoalexins, thus inducing pathogen-related (PR) proteins, proteinase inhibitors, and stimulating lignifications (Benhamou, 1996;

Terry and Joyce, 2003). Synthesis of phenolic acids is stimulated in the primary leaves following chitosan treatment. For example, Kleekron (2005) studied the ability of chitosan to stimulate the plant's self-defence system against rice blast disease. The results showed that chitosan decreased the severity of rice blast under field conditions. It was nevertheless unclear whether chitosan also led to plant resistance to various pathogens other than rice blast. In addition, the mechanism of how chitosan induces the resistance of plants to pathogens remains vague.

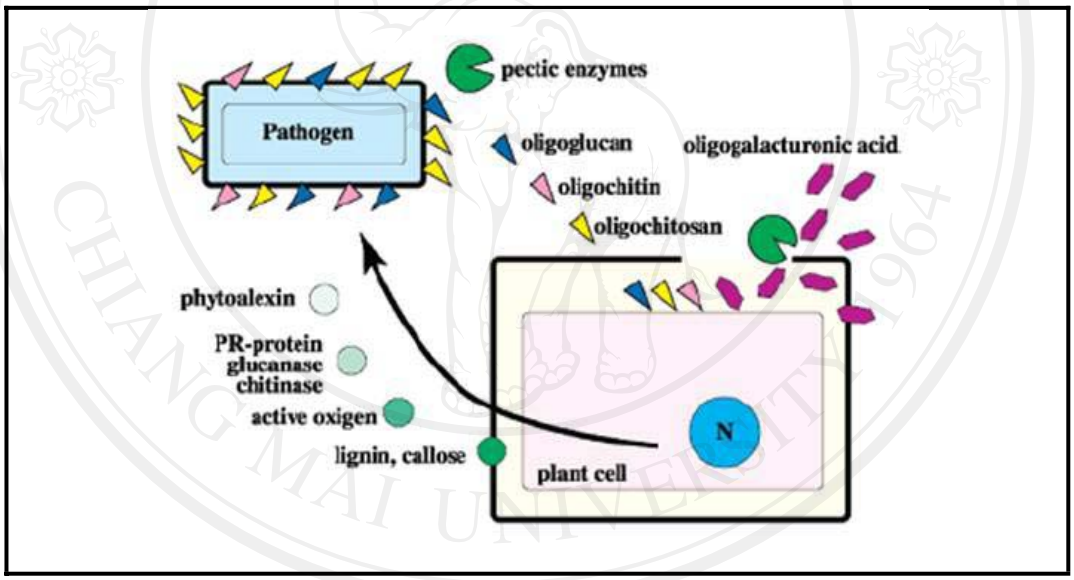

Figure 1.7 Production mechanism of elicitor in a plant cell.

Source: Rabea *et al*. (2003)

1.6 The objectives of this study

The main objectives were as follows:-

1. To detect by phenotype, the carbendazim-resistant *Colletotrichum* spp. that causes mango anthracnose.
2. To detect the mutants of carbendazim-resistant *Colletotrichum* spp. by molecular techniques.
3. To evaluate the antifungal activity of chitosan against carbendazim-resistant *Colletotrichum* spp.

1.7 Scope of this thesis

This thesis is divided into three parts. Part 1 (Chapter 2) describes isolation and morphological characterisation of *Colletotrichum* spp. causing mango anthracnose. Part 2 (Chapter 3 and 4) describes detection by phenotypic and genotypic assays of the carbendazim-resistant *Colletotrichum* spp. causing mango anthracnose, and Part 3 (Chapter 5) evaluates the antifungal activity of chitosan against carbendazim-resistant *Colletotrichum* spp. causing mango anthracnose.

Part 1: Isolation and morphological characterisation of *Colletotrichum* spp. causing mango anthracnose

This part presents the collection, isolation and morphological characterizations of *Colletotrichum* spp.; the causal agents of mango anthracnose disease. The naturally infected mango fruit and leaves, which showed anthracnose symptoms, were collected from markets and orchards. The cultures were purified for further study, and the

preliminary morphological characterisations of the pathogens were observed on potato dextrose agar (PDA).

Part 2: Carbendazim-resistance detection

This part describes the phenotypic and genotypic detections of *Colletotrichum* spp., the causal agents of mango anthracnose disease. Chapter 3 presents the detection of carbendazim-resistant *Colletotrichum* by phenotypic response on carbendazim supplemented with PDA, the pathogenicity test and identification of *Colletotrichum* species by analyzing the sequence of the ITS region. Chapter 4 provides the detection of carbendazim-resistant *Colletotrichum* spp. by analyzing a partial sequence of the beta-tubulin gene related to the carbendazim-resistant phenotype.

Part 3: Control of mango anthracnose

Chapter 5 focuses on evaluation of the antifungal activity of chitosan against the carbendazim-resistant *Colletotrichum* spp. for mango anthracnose. The conclusion and future perspectives are presented in Chapter 6. Finally, some part of this thesis was contributed by published in the esteemed journal.