Identification and Characterization of *Colletotrichum* spp. from Anthracnose of Guava (*Psidium guajava*) and Banana (*Musa* spp.), and *Gliocephalotrichum* spp. from Fruit Rot of Rambutan (*Nephelium lappaceum*)

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by

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LIST OF ABBREVIATIONS

µl	Microliter
µm	Micrometer
AFLP	Amplified Fragment Length Polymorphism
bp	Base pair
BA	Banana Awak
BB	Banana berangan
BE	Banana emas
BN	Banana nangka
BR	Banana rastali
CLA	Carnation leaf agar
cm	Centimeter
ddH_2O	Double-distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EtBr	Ethidium bromide
f. sp.	Formae specials
G	Guava
g	Gram
h	Hour
Ha	Hectares
ITS	Internal transcribed spacer
kb	Kilobase
kg	Kilogram
L	Liter
mA	Miliampere
mg	Miligram

min	Minutes
ml	Mililiter
ML	Maximum likelihood
mm	Milimeter
mM	Milimolar
Mt	Metric tan
NJ	Neighbour-joining
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
p.s.i	Per. Square inc
R	Rambutan
RAPD	Random amplified polymorphic DNA
rDNA	Ribosomal deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
rpm	Revolutions per minute
s	Second
spp.	Species
TBE	Tris-Boric acid-EDTA
U	Unit
UV	Ultraviolet light
V	Volt
WA	Water agar

LIST OF SYMBOLS

%	Percentage
®	Registered
°C	Degree of Celsius
±	Plus minus
™	Trade mark

Pengecaman dan Pencirian *Colletotrichum* spp. daripada Antraknos Jambu Batu (*Psidium guajava*) dan Pisang (*Musa* spp.), dan *Gliocephalotrichum* spp. daripada reput buah Rambutan (*Nephelium lappaceum*)

ABSTRAK

Tanaman buah-buahan terdedah kepada penyakit lepas tuai yang menyebabkan kerugian yang teruk dan antara penyakit lepas tuai yang paling lazim adalah antraknos dan reput buah. Kajian mengenai penyakit lepas tuai pada tiga tanaman buah-buahan iaitu jambu batu (*Psidium guajava*), pisang (*Musa* spp.) dan rambutan (*Nephelium lappaceum)* telah dijalankan. Daripada jambu batu dan pisang, penyakit antraknos telah diperhatikan, dan dua spesies *Colletotrichum* telah dikenalpasti. Berdasarkan ciri-ciri morfologi warna koloni, konidia, apresoria, kehadiran dan ketiadaan seta, dua spesies *Colletotrichum* telah dikenalpasti iaitu *C. gloeosporioides* (52 pencilan) dan *C. musae* (enam pencilan). Untuk pengesahan spesies dan analisis filogenetik, penjujukan kawasan penjarak transkripsi dalaman (ITS) dan gen ß-tubulin telah digunakan. Walau bagaimanapun, berdasarkan keputusan BLAST, 55 pencilan telah dikenalpasti sebagai *C. gloeosporioides* dan hanya tiga pencilan sebagai *C. musae*. Analisis filogenetik menggunakan jujukan kawasan ITS dan gen ß-tubulin, berdasarkan set data individu dan set data gabungan menggunakan kaedah penyambungan- jiran (NJ) dan kebolehjadian maksimum

(ML) menunjukkan *C. gloeosporioides* dan *C. musae* dikelompokkan dalam klad yang berbeza. Pencilan-pencilan *C. gloeosporioides* dikelompokkan kepada beberapa sub-klad menunjukkan variasi intraspesies dan dari segi genetik pencilan-pencilan tersebut berbeza daripada strain epitip *C. gloeosporioides*, dan oleh itu pencilan-pencilan *C. gloeosporioides* daripada jambu batu dan pisang dianggap sebagai kompleks spesies *C. gloeosporioides*. Pencilan *C. musae* dikelompokkan bersama dan tidak menunjukkan variasi intraspesifik dan juga secara genetiknya adalah serupa dengan strain epitip *C. musae*. Daripada ujian kepatogenan, pencilan *C. gloeosporioides* daripada jambu dan pisang serta pencilan *C. musae* daripada pisang adalah patogen terhadap perumah masing-masing. Patogen penyebab penyakit telah berjaya dipencilkan dan Postulat Koch telah ditepati. Untuk menguji kespesifikan perumah spesies *Colletotrichum*, jangkitan persilangan antara jambu batu dan pisang telah dijalankan. Pencilan *C. gloeosporioides* daripada jambu batu boleh menjangkiti pisang dan pencilan daripada pisang boleh menjangkiti jambu batu. Walau bagaimanapun, pencilan *C. musae* daripada pisang merupakan perumah spesifik dimana jangkitan hanya berlaku pada pisang. Daripada reput buah rambutan, 19 pencilan *G. bacillisporum* telah dikenalpasti berdasarkan pencirian secara morfologi dan jujukan DNA kawasan ITS dan gen ß-tubulin. Berdasarkan analisis filogenetik set data individu dan set data gabungan menggunakan kaedah NJ dan ML, variasi intraspesies telah diperhatikan di kalangan pencilan *G. bacillisporum*. Ujian kepatogenan menunjukkan *G. bacillisporum* adalah patogen terhadap buah rambutan dan Postulat Koch telah ditepati dimana pencilan *G. bacillisporum* yang sama telah dipencilkan semula daripada gejala reput buah rambutan. Kajian ini menunjukkan penyakit lepas tuai, antraknos pisang dan jambu batu disebabkan oleh dua spesies *Colletotrichum*, *C. gloeosporioides* dan *C. musae*, dan reput buah rambutan disebabkan oleh *G.*

bacillisporum. Kajian ini merupakan laporan pertama tentang kejadian *C. gloeosporioides* yang berasosiasi dengan pisang dan *G. bacillisporum* daripada reput buah rambutan.

Identification and Characterization of *Colletotrichum* spp. from Anthracnose of Guava (*Psidium guajava*) and Banana (*Musa* spp.), and *Gliocephalotrichum* spp. from Fruit Rot of Rambutan (*Nephelium lappaceum*)

ABSTRACT

Fruit crops are vulnerable to postharvest diseases causing severe losses and among the most common postharvest diseases are anthracnose and fruit rot. Studies on postharvest disease on three fruit crops, namely guava (*Psidium guajava*), banana (*Musa* spp.) and rambutan (*Nephelium lappaceum*) were conducted. From guava and banana, anthracnose disease was observed, and two species of *Colletotrichum* were identified. Based on morphological characteristics of colony colours, conidia, appressoria and presence or absence of setae, the two species of *Colletotrichum* were identified as *C. gloeosporioides* (52 isolates) and *C. musae* (six isolates). For confirmation of species and phylogenetic analysis, sequencing of Internal Transcribed Spacer (ITS) regions and ß-tubulin gene were applied. However, based on BLAST results, 55 isolates were identified as *C. gloeosporioides* and only three isolates were identified as *C. musae*. Phylogenetic analysis using ITS regions and ß-tubulin gene sequences based on individual and combine datasets using Neighbour-joining (NJ) and Maximum likelihood (ML) methods showed that *C. gloeosporioides* and *C. musae* were clearly separated into different clades. The groupings of *C.*

gloeosporioides isolates into several sub-clades showed intraspecific variation and the isolates were genetically different from *C. gloeosporioides* epitype strain and thus the isolates of *C. gloeopsorioides* from guava and banana are regarded as species complex. Isolates of *C. musae* were grouped together and did not show any intraspecific variations as well as genetically the same with *C. musae* epitype strain. From pathogenicity test, *C. gloeosporioides* isolates from guava and banana as well as *C. musae* isolates from banana were pathogenic to their respective hosts. The pathogen was successfully isolated and thus, Koch's postulate was fulfilled. To test the host specificity of *Colletotrichum* species, cross infection between guava and banana were conducted. *Colletotrichum gloeosporioides* isolates from guava was able to infect banana and isolates from banana was able to infect guava. However, *C. musae* from banana was host specific as the infection only occurs on banana. From fruit rot of rambutan, 19 isolates of *G. bacillisporum* were identified based on morphological and DNA sequencing of ITS regions and ß-tubulin gene. Based on phylogenetic analysis of individual and combined dataset using NJ and ML methods, intraspecific variations were observed among *G. bacillisporum* isolates. Pathogenicity tests showed that *G. bacillisporum* was pathogenic to rambutan and Koch's postulate was fulfilled as the same *G. bacillisporum* isolates were reisolated from the fruit rot symptoms of rambutan. This study showed that postharvest disease of anthracnose on guava and banana was caused primarily by two species of *Colletotrichum, C. gloeosporioides* and *C. musae,* and fruit rot of rambutan by *G. bacillisporum*. These are the first report on the occurrence of *C. gloeosporioides* associated with anthracnose of banana and and *G. bacillisporum* from fruit rot of rambutan.

CHAPTER 1

INTRODUCTION

Guava, banana and rambutan are among the most economically important fruits crop in Malaysia. Banana remains as the major fruits crops while guava and rambutan are considered as minor fruits in the fruit industry in Malaysia (International Tropical Fruits Network, www.itfnet.org/v1/tropical-fruit-info). These fruits have high nutritional value and can be processed into various types of food products (Abeyrathne and Jaenicke, 2006). Like any other crops, these fruits were reported to be seriously infected by postharvest diseases which may cause losses in terms of quality and quantity as well as economic losses to farmers, processors, marketers and also to the consumers (Michailides *et al*., 2010).

Postharvest diseases on fruit crops are caused primarily by fungi and infections can occur before, during or after harvest. It can also be latent infections that occur in the field and infections can continue to develop on the fruits by mechanical and insect injury during harvest, handling and storage. Fungal infection of postharvest diseases depends on the physiological age of the fruits, wounds such as puncture and bruises, temperature and storage environment (Agrios, 2005). Common postharvest diseases on fruit crops are anthracnose and fruit rot.

Anthracnose is one of the most common postharvest diseases of guava and banana, caused primarily by *Colletotrichum* spp.. Symptoms of anthracnose are characterized as black and round to irregular necrotic lesions on the fruits with orange conidial masses and acervuli in the middle of the lesions. *Colletotrichum* spp. causing anthracnose disease has a wide host range, infect a variety of host plant and different plant parts (Agrios, 2005). Several species such as *C. gloeosporioides*, *C.*

acutatum and *C. musae* have been reported to be associated with anthracnose of different types of fruit crops including guava (*Psidium guajava*) and banana (*Musa* spp.) (Soares *et al*., 2008; Abd-Elsalam *et al*., 2010; Phoulivong *et al*., 2010).

In addition to anthracnose, fruit rot or fruit decay is also a common postharvest disease. Typical symptom of fruit rot appear as light brown with water-soaked areas develop in the pericarp and pulp then later enlarged and turn dark brown in colour (Sivakumar *et al*., 1996). *Gliocephalotrichum* spp. are often associated with fruit rot disease and is one of the most common postharvest disease of rambutan (*Nephelium lappaceum*). Two *Gliocephalotrichum* spp., *G. bulbilium* and *G. simplex* have been reported to be associated with fruit rot of rambutan (Nishijima *et al*., 2002; Serrato-Diaz, 2012).

The identification of anthracnose and fruit rot pathogens is important especially to formulate effective control method and for quarantine purposes (Phoulivong, 2011). The first step for identification of plant pathogenic fungi is using morphological and cultural characteristics. Commonly used morphological characteristics are microscopic characters such as the shapes and sizes of conidia and formation of conidiophores. Cultural or macroscopic characters include structures of mycelia and colony characteristics (Anaissie *et al*., 2009). Morphological characteristics are not always reliable as the characteristics can be easily influenced by environmental conditions as well as cultural media and incubation conditions (Cooke *et al*., 2006; Madden *et al*., 2007). Therefore, DNA sequencing is used for identification and characterization of plant pathogenic fungi.

DNA sequencing is not only used for identification and characterization but also applied to determine genetic variations and phylogenetic relationships. For

molecular identification of plant pathogenic fungi including *Colletotrichum* and *Gliocephalotrichum*, ITS regions are commonly used. The region has also been chosen as DNA barcode for identification of plant pathogenic fungi (Schoch *et al*., 2012) as the ITS1 and ITS2 regions consist of highly variable sites that have potential targets for species specific identification of plant pathogenic fungi (Liu, 2012).

For phylogenetic analysis, ITS regions are not always sufficient especially to determine the relationships among closely related species. Therefore, a protein coding gene such as ß-tubulin gene is also included to infer robust phylogenetic relationships at various taxonomic levels (Begerow *et al*., 2004). The ß-tubulin sequences have been applied to determine phylogenetic relationship of *Colletotrichum* from anthracnose of guava and banana (Peres *et al*., 2002; Phoulivong *et al*., 2010) and *Gliocephalotrichum* from fruit rot of rambutan (Nijishima *et al*., 2002; Serrato-Diaz, 2012).

Pathogenicity test by fulfilling Koch's postulates is essential to determine the causal pathogens of anthracnose and fruit rot of fruit crops. Moreover, many species of *Colletotrichum* and *Gliocephalotrichum* have a wide host range, and the same species are often found to infect more than one host plant. Pathogenicity test is also used to determine the degree of virulence of plant pathogenic fungi on the hosts (Schafer, 1994).

Although there are reports of *Colletotrichum* causing anthracnose on fruit crops in Malaysia, detailed studies on the identification, characterization and pathogenic ability of the species have not been conducted. Moreover, most studies only rely on morphological characteristics which can lead to misidentification. The

same scenario is also applied to *Gliocephalotrichum* in which reports on this genus in Malaysia are very limited.

Therefore, the specific objectives of the present study were:

(i) To isolate and identify *Colletotrichum* spp. from anthracnose of guava and banana, and *Gliocephalotrichum* spp. from fruit rot of rambutan based on morphological characteristics and DNA sequencing of ITS regions and ß-tubulin gene.

(ii) To determine phylogenetic relationship of *Colletotrichum* and *Gliocephalotrichum* isolates by using ITS regions and ß-tubulin gene sequences.

(iii) To confirm pathogenic isolates by conducting pathogenicity test and cross infection of *Colletotrichum* spp. on guava and banana, and pathogenicity test of *Gliocephalotrichum* isolates on rambutan.

CHAPTER TWO

LITERATURE REVIEW

2.1 Tropical Fruits

Malaysia is the second world's largest exporter of tropical fruits after Thailand with 71 191 Mt, valued at US$ 21 682 million (Chomchalow *et al*., 2008) and is one of the country involve in import and export of fruit crops for decades (Suntharalingam *et al*., 2011). Fruit crops has been recognized as one of the contributors in the agricultural sector to the economic growth in Malaysia besides oil palm and rubber (Fatimah *et al*., 2008).

Tropical fruits can be divided into two categories, seasonal such as mango, rambutan, durian, dokong, lansium, mangosteen and non-seasonal such as carambola, pineapple, melons, guava and banana (Arora and Ramanatha, 1995). There are five major tropical fruits grown in Malaysia, namely banana, mango, pineapple, papaya, and avocado whereas citrus, durian, mangosteen, rambutan, jack fruit, lychee, passion fruits and guava are minor tropical fruits (Ooi *et al*., 2002).

Malaysia produced tropical fruits with total cultivated area of 210 171 Ha with production of 1 213 084 Mt in 2012. Various tropical fruits are currently cultivated in Malaysia (**Table 2.1**) and based on the data by Department of Agriculture, cultivated area and production of banana decreased from 2011 to 2012 but production is still the largest which was about 334 302 Mt in 2011 and 318 976 Mt in 2012. The cultivated area and production of guava was also decreased from 2011 to 2012 with 2 557 Ha and 24 923 Mt to 1 582 Ha and 22 060 Mt. However, rambutan cultivated area and production increased from 19 882 Ha and 70 569 Mt (2011) to 26 442 Ha and 89 572 Mt (2012) (**Table 2.1**).

Table 2.1: Typical tropical fruits planted in Malaysia

Fruit name	2011		2012	
	Cultivated area (Ha)	Production (Mt)	Cultivated area (Ha)	Production (Mt)
Star fruit	1 318	12 934	1 353	13 162
Papaya	2 681	44 928	3 641	55 511
Cempedak	8 726	35 236	11 352	58 394
Ciku	797	5 707	1 177	7 196
Durian	82 832	299 184	100 267	303 291
Guava	2 557	24 923	1 582	22 060
Langsat	5 724	25 785	6 450	25 750
Mango	7 688	22 709	9 813	27 650
Jackfruit	3 534	19 614	4 597	32 504
Banana	31 300	334 302	29 916	318 976
Rambutan	19 882	70 569	26 442	89 572
Watermelon	14 488	237 072	13 581	259 018

(Source: Department of Agriculture, 2012)

2.2 Guava (*Psidium guajava*)

Apple guava or common guava (*Psidium guajava*) belongs to the family Myrtaceae and is a native of tropical America and probably originate from Peru, north to Mexico and the Caribbean (Kwee and Chong, 1990; Verheij and Coronel, 1991). Guava fruit was cultivated by the Carribean Indians and was also common in West Indies. Today, this crop is widely distributed in all subtropical and tropical parts of the world which is cultivated in more than 60 countries. Guava is widely cultivated in Brazil (Rocha and Bernelmans, 2005), India (Prasad *et al*., 1952; Morton, 1987; Radha and Mathew, 2007), California (Webber, 1944), Malaysia (Augustin and Azizah, 1988; Ali and Lazan, 1997), USA primarily in Florida (Murray and Campbell, 1989; Dehgan, 1998; Langeland and Hall, 2000), Hawaii (Hamilton and Seagrave-Smith, 1959) and Puerto Rico (Rodriguez and Iguina, 1971).

Guava fruit is one of the fruit crops given priority for cultivation in Malaysia with two major areas planted with guava, Johor and Perak (Abd Rahman *et al*., 2008). The land area cultivated with guava increased from 1 375 Ha (2008) to 2 557 Ha (2011). The production of guava also increased steadily since 2008 - 2011 from 18 143 to 24 923 060 Mt (Department of Agriculture, 2012). In 2012, export of guava was estimated to be about RM 790 000 (Department of Agriculture, 2012).

There are about 31 local guava cultivars and the most common are Hong Kong Pink, GU4, GU5 and GU7. There are also two main types of guava cultivar namely, seedless cultivar known as clone GU15 and seed guava cultivar known as clones GU8, GU9 and GU10. Local guava cultivar can be classified into three groups based on the length, weight and also the diameter of the fruit (Ali and Lazan, 1997). The cultivar exhibit variations in shape, color, smoothness of skin, size and presence or absence of seed in the fruits (Radha and Mathews, 2007).

Guava fruit contains about 74% - 87% moisture, 13% - 26% dry matter, 0.5 - 1% ash, 0.4% - 0.7% crude fat and 0.8% - 1.5% crude protein but has a low energy value (275 kJ). The fruit also contains calcium (14 - 30 mg), phosphorus (23 - 37 mg) and iron (0.6 - 1.4 mg) and rich in vitamin C (ascorbic acid) which is 3 to 6 times more than oranges, 10 to 30 times more than banana and 10 times more than papaya. It also contains vitamin A (b-carotene) and pink-fleshed cultivar has higher amount of vitamin A compared to white-fleshed cultivar (Kwee and Chang, 1990).

2.2.1 Diseases and Pests of Guava

All parts of guava tree such as seedling, root, leaf, shoot and fruits can be infected by diseases. Most reported diseases on guava are caused by fungi. Although,

damping-off of seedling is caused by several fungal genera such as *Pythium, Rhizoctonia, Fusarium* and *Phytophthora* but only *Rhizoctonia solani* has been isolated from infected seedlings. Pre-emergence and post-emergence damping-off on guava seedlings would result in seed decay and wilting (Ali and Lazan, 1997; Prakash, 2012).

On guava tree, white root disease is more common compared to brown or red root disease. The causal agent of white root disease is a basidiomycetes fungus, *Rigidoporus lignosus* and this disease has been reported in Perak, Selangor and Pahang. Usually, white or yellowish-white rhizomorph of the fungus attached to the infected roots. The white root disease also show symptom of leaves wilting which become yellow and then turn to brown (Ali and Lazan, 1997).

Pink disease is a disease that infect leaf and shoot of guava and is caused by a basidiomycete fungus, *Corticium salomonicolor*. The symptoms occur when all the infected leaves and shoot are dead, forming crust caused by penetration of the fungus. The disease is easily recognised by the pink color mycelial on the twigs (Ali and Lazan, 1997).

Common diseases infect guava fruit are anthracnose caused by *C. gloeosporioides,* Stylar end ring rot by *Phomopsis psidii,* brown fruit rot by *Botryosphaeria* and fruit rot by several fungal species such as *Rhizopus stolonifer, Lasiodiplodia* and *Aspergillus* (Ali and Lazan, 1997). The infection affect the nutrient compositions of guava which in turn affect the market value of the fruit (Amusa *et al.,* 2005).

Besides fungal diseases, guava is also infected by algae and lichen. *Cephaleuros virescens* is the most common algal found on the leaves which appeared in the form of orange, rust colored, velutinous spots on the upper and lower leaf

surfaces. Lichen can be found on the bark of the trunk, branches, twigs and also the leaf surfaces of guava. It can be seen in the form of whitish, pinkish patches of different shapes on the main trunk and branches of the tree. Cructose lichens are the most common lichens found on guava. The effect of algae and lichen on guava are regarded as minor infection (Ali and Lazan, 1997; Nelson, 2008).

Mosaic virus of guava has been found on leaves of the shoot. The symptoms appear as deformed, puckered, rugose with dark and light green mosaic. The leaves of the shoot reduced in size compared to normal healthy leaves (Ali and Lazan, 1997).

Pests also reduce the yield and quality of guava fruit. Fruit flies (*Dacus dorsalis*) is one of the important guava pests and most commonly found in the tropics as well as other part of the world (Lim and Khoo, 1990). Other species of fruit flies, *D. zonatus* has been reported in India (Rana *et al*., 1992); the oriental fruit fly, *Bactrocera dorsalis* in Hawaii (Stark *et al*., 1994) and the Caribbean fruit fly *Anastrepa* spp. reported in Florida (Coledonio - Hurtado *et al*., 1995). The female fruit fly infect the guava fruit by puncturing the skin to lay eggs. The tissue damage caused by the presence of larvae and then soon infected by fungi or bacteria which cause further deterioration of the fruits. Other common pests of guava are mealybugs which are sap-sucking insects such as *Ferrisia virgata, Ferrisia psidii, Planococcos citri, P. pacifificus* and *P. lilacinus* (Mukhopadhyay and Ghose, 1994; Mania, 1994, 1995).

2.3 Banana (*Musa* spp.)

In Malaysia, banana is the second largest fruit crop cultivated which contributes about 16% to the total fruit production areas. Land area cultivated with

banana has increased in the past 5 years from 26 855 Ha (2006) to 29 790 Ha (2010). Three major states that produce banana are Johor (7 161 Ha), Pahang (3 927 Ha) and Sarawak (3 729 Ha). Export of banana was estimated to be about RM12 190 000 in 2012 (Department of Agriculture, 2012). The fruit is mainly exported to Singapore, Brunei, Hong Kong and the Middle East. However, Malaysia still imported banana from other countries such as from the Phillippines and Thailand (Mokhtarud-din, 2011).

Banana plant is a herbaceous flowering plant of the genus *Musa*, and a member of Musaceae which include banana and plaintain. *Musa* spp. are native throughout the Indo-Malaysian region, in the tropical and subtropical areas of Sri Lanka and eastern India, across south China and Southeast Asia to the southwest Pacific and northern Australia, and domesticated widely in all tropical regions of the world (Kennedy, 2008). Cultivated banana or edible banana originates from two wild banana, *Musa acuminata* and *M. balbisiana* or hybrids of *M. acuminata* and *M. balbisiana*, depending on the genomic constitutions (Price, 1995).

The most popular and widely grown banana cultivar is the Cavendish subgroup (AAA) (Chang, 2011; Mokhtarud-din and Robert, 2010; Tengku Ab. Malik, 2011; Liew and Lau, 2012) which is extremely important for consumption and in the banana trade (Stover and Simmonds, 1987). The largest commercial crops of Cavendish are generally found in South Africa, Somalia and Ethiopia (Karamura *et al*., 1998). Furthermore, cavendish cultivar shows higher yield compared to other cultivars (Molina and Escalant, 2002). In Malaysia, about 50% of the banana cultivated areas are cavendish and berangan type (Mokhtarud-din and Robert, 2010). The most common cultivars of edible banana are mas, berangan, cavendish and rastali while for cooking are nangka, lang, relong, tanduk, nipah and awak.

Banana contains about 70% water, 27% carbohydrate, 0.3% fat and 1.2% protein. Eleven vitamins have been recorded in banana and the fruit is considered a good source of vitamins A, B1, B2 and C (Sharrock and Charlotte, 2000). Additionally, banana fruit is rich in essential minerals which mainly contain high concentrations of potassium (K) and low in sodium (Na) (Sharrock and Charlotte, 2000; Oliveira *et al*., 2007; Haslinda *et al*., 2009). Banana is recommended for obese and geratric patients due to low fat and high energy value. Banana is also used in nutrition for infants, for an individual suffering from various intestinal disorders and for treatment of peptic ulcers, infant diarrhoea, coeliac disease and colitis (Sharrock and Charlotte, 2000).

2.3.1 Diseases and Pests of Banana

Banana plant is susceptible to bacterial, fungal and viral diseases. The most well-known bacterial disease is bacterial wilt or Moko disease caused by *Ralstonia solanacearum*. Moko disease affects all stages of banana plant development including the fruit. The first sign of Moko disease can be seen in yellowing and wilting of the oldest leaves which then become necrotic and collapse. Symptoms are spread to the younger leaves which develop to pale green or yellow before becoming necrotic and collapse after a week (Jeger *et al*., 1995). Banana fruit becomes brown and dry rot and some of the fruit may ripen prematurely or split. Internal symptoms are seen in vascular bundles which are initially cream or yellow, then become brown or black (Jeger *et al*., 1995; Eyres, 2001).

Other banana disease caused by bacteria is blood disease and *Pseudomonas celebensis* was identified as the causal agent of the disease. Plantains are the primary hosts of blood disease which affects all banana plant parts and no banana cultivars

are resistant to the disease (Mackie, 2007). Symptoms observed in blood disease are similar to Moko disease in which yellowing of the oldest leaf margins are observed and it become necrotic and collapse. The young leaves also become bright yellow, then necrotic and become dry. Internal symptoms is discoloration of vascular bundles which is also similar with Moko disease. However, the yellowing of leaves and discolored vascular bundles are more conspicuous in blood disease compared to Moko disease. Banana fruit internal symptoms are shown by the appearance of reddish-brown discoloration and the fruit become rotten (Jeger *et al.*, 1995; Mackie, 2007).

Banana plant is also susceptible to Xanthomonas wilt caused by *Xanthomonas vasicola pv. Musacearum* (Xvm). Banana Xanthomonas wilt is a vascular disease that shows yellowing and wilting of leaves, premature ripening of the bunch, a yellowish bacterial ooze, rotting of fruit and internal yellow discolaration of the vascular bundles (Biruma *et al*., 2007). Initially symptoms are blackening of the male bud, extending into an immature fruit bunch followed by premature ripening of the fruits. Even though, banana bunch may appear green but the internal parts of the fruits exhibit reddish brown discoloration. A cream or yellow-colored ooze may exudes within a few minutes after cutting the tissues which is the characteristic to distinguish Xanthomonas wilt from other bacterial wilts of banana (Biruma *et al*., 2007).

Banana plant is also infected by several pathogenic fungi. One of the most destructive fungal disease of banana is Panama disease or Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Stover, 1962). The disease infects banana plants through the roots and later blocks the vascular tissues which cut off the supply of water and nutrients to the whole plant parts. Initial symptoms appear by yellowing of

the older leaves and spread to the younger leaves, which become wilted and die. The obvious symptoms can be seen in the xylem vessels of the roots and the rhizome which turns from reddish-brown to maroon color when the fungus invades the tissues (Daly and Walduck, 2006; Newley, 2010).

Other banana disease caused by fungi is Sigatoka leaf spots which is caused by *Mycosphaerella musicola* (yellow sigatoka) and *M. fijiensis* (black sigatoka). The spots appear on the upper leaf surface in pale yellow streaks for yellow sigatoka and dark brown on the lower leaf surface for black sigatoka (Mourichon *et al*., 1997). The spot become enlarged to form necrotic lesions with yellow haloes and light grey centres. Numerous spots can coalesce and destroy large areas of leaf tissues. Black sigatoka is reported to be more serious than yellow sigatoka because symptoms appear on younger leaves (Mourichon *et al*., 1997).

Anthracnose of banana fruits is caused by *Colletotrichum* spp. and *C. musae* is the most common species that infect green banana fruits and ripening fruit following wounding. *Colletotrichum musae* is reported to be host specific to *Musa* spp. and reduces banana yield during preharvest and postharvest (Costa and Kalpage, 2006). The disease symptoms are characterized by sunken brown to black lesion and covered with salmon-coloured acervuli of *C. musae*. The lesions become enlarged during fruits ripening (Stover and Simmonds, 1987).

Several viruses also cause serious problem to banana plant. Banana bunchy top virus (BBTV) is one of the most serious viral diseases of banana and is caused by genus *Babuvirus* in the family Nanoviridae (Vetten *et al*., 2005). The Cavendish cultivar is reported to be easily infected with the virus compared with the other cultivars (Laughlin, 1997). The symptoms on infected leaves are characterized by stunted growth, chlorotic at the margins, the leaves growing upright and bunching at

the apex of the plant to form a rosette. Banana bunchy top virus is transmitted by banana aphid (*Pentalonia nigroervosa*) which occurs by movement of infected vegetative planting such as suckers, corms and tissue–culture plantlets (Thomas *et al*., 1994).

Another viral disease is banana streak disease which commonly occurs in Dwarf Cavendish cultivar (Lockhart, 1986). Banana streak disease is caused by genus *Badnavirus*, from family Caulimoviridae and is transmitted only within family Musaceae. The primary symptoms of banana streak disease include chlorotic and necrotic streaks to the veins of the leaf lamina, then the leaf become darker and turn dark brown-black. Another symptoms are stunting, constriction of the bunch on emergence, pseudostem splitting and fruit distortion (Lockhart and Jones, 2000). The disease is reported to be transmitted by several species of mealybug such as *Planococcus citri* (Risso), *Saccharicoccus sacchari* (Cockerell) and *Pseudococcus comstiki* (Kuwana) (Lackhart and Olszewski, 1993; Su, 1998; Dahal *et al*., 2000).

Bract mosaic is also one of the viral diseases that infect banana and the viral causal agent is the family Potyviridae (Magnaye and Espino, 1990; Jeger *et al*., 1995). The mosaic disease symptoms appear as dark reddish-brown mosaic pattern on subtending male flowers inflorescence. The symptoms can also be seen on the leaf which became spindle-shaped lesions and streaks running parallel to the veins and also mosaic pattern stripes and spindle-shaped on the pseudostem (Anonymous, 1995). Bract mosaic is transmitted by three species of aphids, namely *Aphis gossypii, Rhopalosiphum maidis* and *Pentalonia nigronervosa* (Diekmann and Putter, 1996).

A few pests have been reported on banana plant. Insect pests can attack banana rhizome, pseudostem, fruit and leaves. The most serious insect pest of banana is *Cosmopolite sordidus* or banana weevil which cause internal damage to the

rhizome. The adult borers of the weeevil deposit eggs in the holes of the rhizome and the larvae burrow into it. *Odoiporus longicollis* is pseudostem weevil in which the larvae burrow in the pseudostem. The insects tend to infect rotting tissues and stems of harvesting tissues (Gowen, 1995; Tinzaara and Gold, 2008).

The most serious insect pests attacking banana fruit is banana moth, *Nacoleia octasema*, causing brown scabs on the developing fruits. Thrips such as *Chaetanaphothrips* spp. attack a large number of fruits when the flowering axis emerges by develop red blemishes on the fruits (Gowen, 1995). Another insect pest, banana aphid (*Pentalonia nigronervosa*) also cause damage to the banana fruits and able to transmit Banana Bunchy Top Virus which is the only known vector transmitting in circulative and nonpropagative manner (Hafner *et al.*, 1995; Hu *et al.*, 1996).

Caterpillars from several species of *Lepidoptera* can cause defoliation of banana leaves. For example, *Spodoptera litura* is the most common banana leaf eating caterpillar and the damages is often more apparent on the leaves (Gowen, 1995; Hill, 2008)

Minor pests of banana also includes banana mealybug (*Pseudococcus comstocki*), banana lace bug (*Stephanitis typica*), banana fruit fly (*Dacus curvipennis*) and banana flower thrips (*Thrips florum*) (Gowen, 1995; Hill, 2008).

2.4 Rambutan

Rambutan (*Nephelium lappaceum*) belongs to the family Sapindaceae which is native to Malaysia and Indonesia. It is closely related to lychee, longan and pulasan. Rambutan is widely spread from southern China through the Indo-Chinese region, Malaysia, Indonesia to the Philippines (Stone, 1992). Rambutan remains as

minor fruit and highly prized fruit crop in its region of origin (Ooi *et al.*, 2002; Capinera, 2008).

Rambutan has large genetic variation due to cross-pollination and more than 50 cultivars have been reported in Southeast Asia. Most of these cultivars are vegetatively propagated by bud grafting or inarching. Names of each rambutan cultivar may refer to the fruit characteristics, area of production or to a specific cultivar. In Malaysia, there are 31 rambutan cultivars reported but only seven are recommended for cultivation which are R3 (Peng Thing Cheng), R134, R156 (Muar Gading), R160 (Khaw Tow Bak), R161 (Lee Long), R162 (Ong Heok or Daun Hijau) and R170 (Deli Cheng) (Salma, 1986). Many rambutan cultivar can be found in other tropical regions include three most popular cultivars grown in Thailand (Rongrien, Seechompoo or Srichompoo and Bangyeekhan), Indonesia (Lebakbulus, Binjai, Rapiah and Simacan) and the Philippines (Maharlika, Seematjan and Seejonja). The characteristics of a good commercial rambutan cultivars are fruit weight must be over 40g, red or yellow color must be resistant to insect, tolerance of temperature below 15°C and early flowering to reduce fruit loss from bird attack (Lim and Diczbalis, 1998).

Rambutan is the fourth largest fruit crop cultivated in Malaysia after banana, durian and watermelon. The area cultivated with rambutan has decreased from 24 929 Ha (2006) to 19 783 (2010) but the total production steadily increased in 2006-2008 from 67 091 Mt to 76 474 Mt. However, the production decrease in 2009-2010 from 71 232 Mt to 70 569 Mt. (Department of Agriculture, 2012). The total value of export was about RM 750 000 in 2012 (Department of Agriculture, 2012). Rambutan is cultivated in a large scale in Perak, Pahang, Kedah, Kelantan, Johor and Terengganu (Alfredo, 2004).

Rambutan fruit is rich with minerals and nutritional values. Composition of 100g sample of rambutan include 82.1% water, 0.9% protein, 0.3% fat, 2.8g glucose, 3.0g fructose, 9.9g sucrose, 2.8g dietary fibre, 0.05g malic acid, 0.31g citric acid, 0.5g niacin, 15g calcium, 70g vitamin C, 0.01g thiamine, 0.07g riboflavin, 140g potassium, 2g sodium and 10g magnesium (Chang, 2011). Rambutan has also been reported as a supplement to reduce hypocholesterolemic effects (Mongkolsiririkie *et al*., 1989) .

In Malaysia, rambutan plant parts are used for medicinal uses. The roots are boiled and used for treating fever (Chang, 2011). The fruits may used as antihelmintic, the bark to treat disease of the tongue and the leaves can be used in poultices for treatment of headache. The skin of rambutan can also be use in medicines as it contains saponin to treat dysentery rind and fever (Tindall, 1994).

2.4.1 Diseases and Pests of Rambutan

Like many other crops, rambutan tree is also tend to be infected by serious preharvest and postharvest diseases. During preharvest, several studies showed *Oidium nephelli* caused powdery mildew which is one of the most widespread disease of rambutan. The fungus infects young leaves, inflorescence and young fruits and the symptoms appear as white-yellow on the leaves, inflorescences and also fruits (Tindall, 1994).

Stem-end rot is one of postharvest disease of rambutan fruit caused by *Botryodiplodia theobromae*. The pathogen infect rambutan through the cut stem end by rapid penetration and the symptom appears as rot at the stem end of the fruit. Fruit rot of rambutan is caused by *G. bulbilium* and is reported to be the major postharvest rot in the Philippines (Pordesimo and Lun-Ilag, 1982). Anthracnose caused by *C.*

gloeosporioides can also infect rambutan fruit in which aerial hyphae develop on the fruits (Alahakoon and Brown, 1994; Sivakumar *et al*., 1997; Wijeratnam *et al*., 2008).

Seven major insect pests of rambutan in SouthEast Asia are leaf miner (*Acrocercops cramella*), armoured scale (*Phenacaspis* sp.), citrus mealy bug (*Planococcus citri*), yellow peach moth (*Conogethes punctiferalis*), Oriental fruit fly (*Bactrocera dorsalis*) and dried fruit beetles (*Carpophilus dimidiatus* and *C. marginelius*). All these insect pests cause external damage except *A. cramerella* in which the larvae normally burrow inside the fruit. Dried-fruit beetles can be a secondary pest as it usually enters the holes of fruits made by other insects. Oriental fruit flies (*B. dorsalis*) attack ripe rambutan but are not a problem unless overripe fruit are left on the tree (Watson, 1984; Osman and Chettanachitara, 1987).

2.5 Postharvest Disease of Fruit Crops

Postharvest diseases can be classified based on how infection is initiated. It can be the result of latent infection that occur in the field and infection through wounding that is created by mechanical or insect injury during harvest and handling operations (Coates and Johnson, 1997; Michailides and Manganaris, 2009). Infections of fruit crops by postharvest disease may occur during harvesting, grading, packing, transportation to the market and also after purchasing by the consumer. During transportation, postharvest disease may develop and continue either during storage at room temperature or under refrigeration until the moment of actual consumption or use (Dennis, 1983; Agrios, 2005).

Infection by postharvest disease may be influenced by environmental conditions such as temperature, relative humidity and atmosphere during storage

which favor the attack by microorganisms or by mechanical injury (Coates and Johnson, 1997; Freeman *et al*., 1998; Agrios, 2005).

Many of the postharvest pathogens are unable to directly penetrate the surface of fruits. Thus, the pathogens infect through injury or natural openings such as stomata and lenticles (Coates and Johnson, 1997). Mechanical injury caused wound which provide entry of pathogens (Arthey and Ashurst, 1996).

Fungi are the most prevalent pathogens causing postharvest disease of tropical and subtropical fruits (Agrios, 2005; Cacciola and Lio, 2008). Most of the fungi which can cause postharvest disease belongs to the phylum Ascomycota such as *Colletotrichum* causing anthracnose disease, *Fusarium* spp. causing crown rot, *Gliocephalotrichum* causing fruit rot, *Aspergillus niger* causing black mould and *Penicillium expansum* causing blue mould (Coates and Johnson, 1997; Narayasamy, 2006). Fleshy fruits such as banana, papaya, mango, rambutan, kiwifruit and citrus contain higher amount of water and sugar content which are more susceptible to infection (Cipollini and Stiles., 1992; Agrios, 2005).

2.5.1 Anthracnose Disease

Anthracnose is one of the most serious postharvest disease of fruit crops which also infect stem, foliage, root, leaves, flowers and twigs (Agrios, 2005). Sharma and Rana (1999) reported that the first symptom of anthracnose on green fruits are dark brown to black spot with a pale margin and lenticular shape and then, the spot areas increase in size and become sunken and coalesce to form large spots on ripening fruits. There are two types of anthracnose disease, those that occur during development in the field and during postharvest which damage mature fruit during storage (Freeman *et al*., 1998).

Anthracnose disease is caused primarily by *Colletotrichum* species (Agrios, 2005; Than *et al*., 2008a; Crouch *et al*., 2009) that infect a wide range of crops in both temperate and tropical regions. *Colletotrichum* spp. not only infect the fruits but the leaves, roots, stems and flowers (Bailey *et al*., 1992). Some *Colletotrichum* spp. have a wide host range and some species only infect a single host (Freeman *et al*., 1998).

Infection and disease development caused by *Colletotrichum* species requires high humidity which usually depends on rain water, optimal temperature between 20 - 25^{o}C for conidia to germinate and infection to occur (Waller, 1992; Agrios, 2005). Infection begins with germination of conidia to produce appresoria which firmly attached to the host surface. The infection peg develops from the appresoria, penetrate the host cuticle and cell wall (Agrios, 2005).

There are two ways of infection by *Colletotrichum* after the conidia lands on the surface of the host. The conidia can germinate immediately after landing, or it needs some time before germination occurs. The period between the landing and germination is referred to as latent infection (Verhoeff, 1974). The latent phase may occur for a short or long time. Activation of latent infection can occur when tissues of the host are damaged by physiological or mechanical processes (Waller, 1992; Agrios, 2005).

Besides *Colletotrichum*, there are other genera of ascomycetes fungi that caused anthracnose disease such as *Diplocarpon, Elsinoe* and *Gnomonia. Diplocarpon rosae* cause black spot on the leaves and stems of roses. The infected leaf tissue turn yellow, followed by dropping and weakening of the plant (Agrios, 2005; Nelson, 2012). *Elsinoe ampelina* causes anthracnose of grapes which produces bird's eye lesion and sunken black lesion on leaves, shoots and berries (Ellis and

Erincik, 2002; Agrios, 2005). The first report of anthracnose causes by *Elsinoe ampelina* on grapes was observed in Michigan (Schilder *et al.,* 2005). Anthracnose disease can decrease the quality of grapes which infect *Vitis* and *Rubus* spp. (Ellis and Erincik, 2002). *Gnomonia* causes anthracnose diseases on shade tree such as oak, sycamore and walnut. The typical symptoms of anthracnose varys such as dieback of branch, extensive blighting of leaves, circular lesions and also premature defoliation. Walnut anthracnose is the most important disease caused by *Gnomonia leptostyla* which attacks the leaves and nut. The symptoms on leaves appears as dark circular area, surrounded by a yellow margin while on nuts, brown to black sunken spots develop on the husk (Kennelly and O'Mara, 2010).

2.5.2 Fruit Rot

Fruit rot is also known as fruit decay which involves decomposition of fruit. The process of decomposition involves the changes of physical properties of the fruit surface and also the internal volume of the fruit (Kider *et al*., 2011).

Fruit rot mainly occurs due to the activity of fungi and bacteria (Agrios, 2005). The infection starts when the fungus and bacteria starts to grow on the outer skin of the fruit in which the growth are influenced by the moisture, temperature and nutrient concentration of the fruit. Fruit has high percentage of water content in proportion to their weight. During infection, water content in the fruit is reduced and then decay occurs (Kider *et al*., 2011). Environmental factors can also influence the occurence of fruit rot such as temperature and humidity that contribute to fungal and bacterial growth. Warmer temperature and high humidity can lead to more rapid growth of fungi and bacteria while colder temperature can retard or slow the growth of both microorganisms (Kider *et al*., 2011).

Typical symptoms of fruit rot are not visible in the field but infections become active after the fruit is picked and starts to soften which begins as small lesions, irregular brown to reddish discoloration on the peel. The infection usually initiates by a wound or an injury to facilitate attacks by fungi and bacteria. When the fruits mature, the lesions enlarge and rotting spread throughout the fruit and has an offensive odor (Barkai-Golan, 2001). The decay affects both the surface and internal tissues of the fruit (Kider *et al*., 2011).

A few genera of Ascomycete fungi such as *Penicillium, Aspergillus, Rhizopus*, *Alternaria* and *Botrytis* are common causal pathogen of fruit rot. *Penicillium*, *Aspergillus* and *Rhizopus* commonly cause postharvest rot on wounded or senescent fruits. Penicillium rot cause postharvest rot on citrus, pears, apples, grapes, melons and many other fruit crops commonly known as blue mold and green mold rots which occur during picking and handling especially in humid conditions. Penicillium rot can also occur during transit, storage and in the market (Agrios, 2005). Aspergillus rot also occurs on grapes, apples and citrus especially fruits that have been exposed to the sun for several days. Rhizopus rot occurs on peach, pears and strawberry (Agrios, 2005; Snowdon, 2010).

Alternaria causes fruit rot before and after harvest that may appear as brown or black lesion, with flat or sunken spots or also the decay areas appear shallow or extend deep into the flesh fruits. *Alternaria* rot infect lemons, grapes, strawberries, cherries, and black rot of oranges. *Botrytis* cause gray mold rots of fruits in the field and during storage. Decay of the fruits may start at the blossom or stem end of wounded areas, and cut of tissues or crack. *Botryris* can infect many fleshy fruits such as pears, strawberries, citrus and apples (Agrios, 2005).

Bacteria can also cause fruit rot in which *Erwinia* and *Pseudomonas* often cause soft rot of fleshy fruits that occur in the field or after harvest, during transit, storage and marketing. Both *Erwinia* and *Pseudomonas* cause infection on fleshy fruits such as strawberries, blueberries, raspberries, peaches, pears and cantaloupes (Capinera, 2008).

2.6 *Colletotrichum* species

2.6.1 *Colletotrichum* Systematics

Description of *Colletotrichum* started with the genus *Vermicularia* by Tode in 1790 (Sutton, 1992). Later, Corda (1831) introduced the generic name of *Colletotrichum* for *C. lineola* and *Glomerella* which was first introduced as the teleomorph or sexual stage of *Colletotrichum* by Von Schrenk and Spaulding (1903) with five original species, including *G. cingulata* (Sutton, 1992). About 20 species from 80 species of the genus *Glomerella* have been reported to be associated as *Colletotrichum* teleomorph (Sutton, 1992).

The first monograph of *Colletotrichum* was written by Von Arx (1957) with 750 names but drastically reduced to 11 species based on morphological characters. From the reduced number of species, a new taxonomic concept was developed as variant forms which was considered to be host-specific. For example, *C. gloeosporioides* which was reported to have around 600 synonyms were included in a series of variant forms which could not reliably distinguished based on morphological characteristics. Then, the taxonomic concept, focused on species groups associated with a particular crop plants was applied by Simmonds (1965) on *Colletotrichum* spp. causing ripe fruit rots and later by Sutton (1966, 1968) on *C. graminicola* complex and appressorial morphology was considered an important

character for identification. After that, classification of *Colletotrichum* species by Sutton (1980) accepted 22 species and Baxter *et al*. (1983) contributed to the classification of *Colletotrichum* in South Africa by describing 11 species. Based on morphological and molecular methods, there are more than 40 accepted *Colletotrichum* species and several new species have been identified (Sutton, 1980, 1992; Cai *et al*., 2009; Hyde *et al*., 2009). According to Cannon *et al*. (2012) more than 100 species have been accepted.

Identification of *Colleotrichum* species have always relied on morphological characteristics such as colony color, size and shape of conidia, presence or absence of setae and teleomorph and cultural criteria (Sutton, 1980; Gunnel and Gubler, 1992; Sutton, 1992; Agrios, 2005). These features have been used by Smith and Black, (1990) to differentiate between species of C. *fragariae, C. acutatum* and *C. gloeosporioides* associated with anthracnose disease on strawberry. Besides that, conidial morphology and growth rate can also be applied to distinguish between *C. acutatum* and *C. gloeosporioides* (Vinnere *et al*., 2002; Talhinhas *et al*., 2005).

Host specificity has also been suggested for identification of *Colletotrichum* species as there are some species of *Colletotrichum* which have been reported to be restricted to only one host. For example, *C. tabacum* is only found on tobacco, *C. musae* on banana, *C. falcatum* on sugarcane and *C. piperis* on pepper (Waller, 1992). However, host specificity may not be reliable to differentiate *Colletotrichum* species that infect a variety of hosts such as *C. gloesporioides, C. acutatum* and *C. graminicola* (Freeman *et al*., 1998).

Identification of *Colletotrichum* species solely based on morphological characteristics is still insufficient due to large variation among and within morphological features especially species within a species complex such as *C.*