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Major Postharvest Fungal Diseases of Papaya cv. 'Sekaki' in Selangor, Malaysia

Rahman, M.A.[1,*], Mahmud, T.M.M.[1] , Kadir, J.[2], Abdul Rahman, R.[3] and Begum, M.M.[2]
[1]Department of Crop Science, [2]Department of Plant Protection, Faculty of Agriculture,
[3]Department of Food Technology, Faculty of Food Science & Technology,
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
**E-mail: atiqur_2004@yahoo.com*

ABSTRACT

A total of seven fungi were identified from the surface of fully matured papaya fruits cv. 'Sekaki' collected form two different fields namely University Agriculture Park, UPM and MARDI, Selangor and a fruit exporter [Seng Chew Hup Kee (M) Sdn Bhd, Kajang, Selangor, Malaysia]. They were identified as *Botryodiplodia theobromae, Colletotrichum capsici, C. gloeosporioides, Fusarium* sp., *Phomopsis* sp., *Rhizopus stolonifer* and *Stemphylium* sp.. Among the diseases, the highest incidence ranged from 90 to 98% and severity of 25 to 38% were recorded for anthracnose caused by *C. gloeosporioides* for all three sources, followed by stem-end-rot caused by *Botryodiplodia theobromae.* Pathogenicity test showed that both wounded and unwounded fruits inoculated with conidial suspension of *C. gloeosporioides* developed distinct symptoms of anthracnose after three and five days of inoculation, respectively.

Keywords: Disease incidence and severity, fungal pathogens, postharvest, papaya

INTRODUCTION

Papaya (*Carica papaya* L.), a native of tropical America, is grown throughout the tropics and subtropics for its melonlike fruit (Alvarez and Nishijima, 1987). This fruit is rapidly becoming an important fruit internationally, both as fresh and processed products (Sankat and Maharaj, 1997). In Malaysia, it is a smallholders' crop and planting is widespread throughout the country. At present, sekaki is considered as a leading cultivar for export as well as domestic consumption. Papaya has an excellent potential as an export crop in Malaysia. In 2003, the export value of fresh papaya was estimated at RM 100.8 million, up from 23.6 million in 1992 (Anonymous, 2006). Greater commercial production of papaya in Malaysia has increased due to higher returns compared to other fruits. As a result, the production has jumped from 4,938 tonnes in 1980 to 6500 tonnes in 2003 (FAO, 2005). Hong Kong continued to be the major export destination of Malaysian papaya followed by Singapore, United Arab Emirates and Brunei.

Papaya fruits are very susceptible to diseases caused by many microorganisms especially fungi, as papaya fruit is high in moisture and nutrients (Sankat and Maharaj, 1997). Orchard and postharvest diseases are very important in terms of reducing yield and quality of papaya, which are primarily responsible for the losses that occur during shipment of the fruits (Couey *et al.*, 1984; Chau and Alvarez, 1983a; Alvarez, 1980). Papaya postharvest losses of 10-40% in sea shipments and of 5-30% in air shipments are not unusual and losses due to diseases ranged from 1 to 93%, depending on postharvest handling and packing procedure (Alvarez and Nishijima, 1987). Many postharvest diseases are initiated through injuries created during and following harvest. The infection process, particularly during postharvest, is greatly aided by mechanical injuries to the skin of the produce such as fingernail scratches and abrasions, insect punctures and cut (Wills *et al.*, 1989). In some cases, as for latent infections, inoculation occurs prior to harvest but the disease dose not develop until the postharvest period (Kays, 1991). Fruits

* Corresponding Author

may be infected by direct penetration of certain fungi through intact cuticle or through wounds and/or natural opening in their surfaces. For some pathogens, the synthesis of enzymes is essential for initial invasion. Furthermore, the development of fungal infection during the postharvest phase can depend upon the physiological age of the fruit, mechanical injuries, temperature and storage environment (Ilag *et al.*, 1994).

The diseases caused by fungi, virus and bacteria, as well as the damages caused by insects threaten world-wide agriculture and export policy (Albornett and Sanabria de Albarracin, 1994). For this reason, these problems must be solved to guarantee the continuous supply of healthy and fresh fruits of acceptable standards to national and international markets.

Therefore, this study was conducted to determine the status of major postharvest diseases of papaya caused by various fungi that largely affect the fruits of export, thus acquiring knowledge for the implementation of appropriate and effective controls measures for good storage and transport.

MATERIALS AND METHODS

Fruit Materials

Papaya fruits of 'Sekaki' cultivar at color stage two (green with trace yellow) were used in this experiment. Forty five healthy fruits with uniform size, shape, and maturity were collected from each of the two fields namely University Agriculture Park, UPM and MARDI, Selangor and a fruits exporter [Seng Chew Hup Kee (M) Sdn Bhd, Kajang, Selangor, Malaysia].

Isolation and Identification of Pathogenic Fungi Associated with Postharvest Decay of Papaya

Fungi were isolated from naturally infected fruits of papaya. Pieces of tissue were cut from the advancing margin of the lesion, surface sterilized in 5% sodium hypochlorite solution and washed in three changes of sterile distilled water. The tissues were then dried on sterilized filter paper and then plated on potato dextrose agar (PDA). The plates were incubated for seven days at 28±2ºC and observed regularly. After the emergence of mycelial growth, each of the fungal colonies were transferred to fresh PDA plates and incubated at room temperature for 2-4 days to obtain pure cultures. Fungal mycelium from pure cultures were examined under dissecting and compound microscopes and identified by comparing their morphological and cultural characteristics with descriptions published in the literature (Sutton, 1992; Nelson *et al.*, 1983; Sutton, 1980; Booth, 1977; Barnett and Hunter, 1972; Ellis, 1971). Isolates of different fungi were then randomly selected and cultured from a single conidium for further purification. Cultures of fungal isolates were maintained on PDA slants for further use.

Incidence and Severity of Postharvest Diseases of Papaya

Forty five full mature papaya fruits at color stage two were collected from each of the three locations (University Agriculture Park, UPM; MARDI and an exporter, Kajang), Selangor, Malaysia. On arrival at the laboratory, the fruits were surface sterilized with 70% ethanol and air-dried. The fruits were then placed in a commercial packaging, held at room temperature (28±2ºC) for 10 days and observed regularly for the development of disease symptoms. Data on incidence and severity of different postharvest diseases were recorded when disease symptoms developed on the surface of ripened fruits. Disease incidence was calculated by the following formula:

$$\% \text{ Disease incidence} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits assessed}} \times 100$$

Data on disease severity was indexed on a 0-4 scales, where, 0 = no disease symptom on the fruit surface area, 1 = 1–10% diseased area, 2 = 11-20% diseased area, 3 = 21-30% diseased area and 4 = 31% and over diseased area (Illeperuma and Jayasuriya, 2002). Percent disease index (PDI) was calculated according to Singh (1984) as follows:

$$\text{PDI} = \frac{\sum (\text{rating number x number of fruits in the rating})}{\text{Total number of fruits x highest rating}} \times 100$$

Pathogenicity of Colletotrichum gloeosporioides *on Papaya Fruit*

Healthy papaya fruits were surface-disinfested with 75% ethanol and air dried. Inoculations were done on both wounded and unwounded fruits and disease incidence and severity were

compared between them. Each fruit was wounded (3 mm deep and 5 mm diameter) at two different positions with a sterilized cork borer. For inoculum preparation, isolate of *C. gloeosporioides* was grown on PDA at 28±2°C for 7 days. Spores were subsequently harvested by flooding the surface of the media with sterilized distilled water and gently agitating the plate with a bent glass rod to dislodge the spores. The resulting suspension was filtered through two layers of sterile muslin cloth. The concentration of conidia in the filtered suspension was adjusted to $5x10^5$ conidia ml^{-1} with sterile distilled water using a haemacytometer. 100µl of inoculum was placed on each wounded or marked places of unwounded papaya fruit surface. All fruits were incubated for 24 h in moist chambers at room temperature (28±2°C), then packed in a commercial packaging and held at room temperature for 7 days. The fruits were examined daily and disease incidence was recorded after lesion development caused by the test fungus. The experiment was conducted with 10 fruits per treatment. Sterilized distilled water was used on control fruits in place of inoculum.

Statistical Analysis

The experiment was arranged in a complete randomized design with three replicates. All of the percentage data were arcsine transformed before subjecting to analysis of variance (ANOVA) and the means separation was done using the Tukey's Studentized Range (HSD) Test using SAS version 8.2.

RESULTS

Isolation and Identification of Fungi

After eight days of storage at ambient temperature (28±2ºC), more than 90% of the fruits were fully ripened and disease symptoms began to develop on the surface of the fruits. In most cases a diseased fruit had more than one lesion. The diseases appeared in variable degrees of development. Fungi were isolated from rotted papayas and identified based on the morphological and cultural characteristics on PDA plates (Table 1). A total of seven fungi were isolated and identified, namely *Botryodiplodia theobromae, Colletotrichum capsici, C. gloeosporioides, Fusarium* sp., *Phomopsis* sp., *Rhizopus stolonifer and Stemphylium* sp.

Colletotrichum gloeosporioides was mainly isolated from lesions associated with the orange pustules. Hyphae were brown, smooth and septate. Conidia were cylindrical with obtuse ends, hyline, aseptate, uninucleated, and 10-15 µm x 3-5 µm in size. Conidia were formed on the conidiophores in the acervuli, which were round to irregular in shape. Setae were present, sparse to many, dark brown, straight to slightly curved, 2-3 septate, swollen at the base and tapering towards the apex. On the PDA, colony appeared white and gradually turned grayish salmon in color as the culture grew older. *C. capsici* was separated from *C. gloeosporioides* on the basis of lesion color and conidial shape. This fungus was isolated from lesions producing black acervuli, which bear sickle-shaped conidia. Setae were brown in color, 1-5 septate, rigid, hardly swollen at the base and slightly tapered to the apex. Conidia were hyaline, falcate with acute apex, aseptate and uninucleated.

Botryodiplodia theobromae Pat. produced a wide margin of water soaked tissues at the base of the fruit. In advanced stages, the lesion margin remains translucent as the rest of the infected tissues became wrinkled, black and dry. Numerous pycnidia appeared on the affected zone. On PDA plates, the fungal colonies were grayish with abundant mycelium. Initially the conidia were hyaline, unicellular, oblong in

TABLE 1
List of fungal pathogens associated with major postharvest diseases of papaya

Name of the Diseases	Causal Organisms
Anthracnose	*Colletotrichum gloeosporioides, C. capsici*
Stem-end rot	*Botryodiplodia theobromae, Phomopsis* sp.
Fusarium fruit rot	*Fusarium* sp.
Stemphylium rot	*Stemphylium lycopersici*
Rhizopus rot	*Rhizopus stolonifer*
Phomopsis rot	*Phomopsis* sp.

shape, thick-walled with granular content. Mature conidia were two-celled, light brown in color with longitudinal bands resembling striations.

The initial disease symptom of wet rot caused by *Phomopsis* sp. was discoloration of the infected area. Thus, the tissues of the infected area became softer, which was covered with a white mycelial carpet. Fungal colonies on PDA media showed white aerial mycelium with pycnidia scattered on the agar. Conidiophores were simple, septate, sometimes branched. A-conidia were hyaline, fusiform, unicellular, with a guttulate at each end and B-conidia were hyaline, elongated, filiform and curved at the apex.

Fusarium rot caused by *Fusarium* sp. appeared initially as a circular water-soaked lesion, which later became depressed. At the advanced stage of disease development, the soft rotted area was covered with a white mycelial mat of the fungus. Conidia were hyaline, three to four-celled and crescent shaped with sharply pointed ends, which were produced from phialides.

Rhizopus stolonifer, produced a generalized soft and watery lesion on the fruit surfaces. Black masses of fungal sporangia were observed on the surface of infected area. In the advanced stage of disease development, fluids leak out from the rotted portion of the fruit. The fungus first appeared as white cottony colonies on PDA and became heavily speckled by the presence of sporangia and then brownish black with age. Sporangiophores were smooth walled, non-septate and light brown in color. The sporangia were globose to sub-globose with somewhat flattened base, white at first, then black with numerous spores. The sporangiospores were irregular round to oval in shape.

Early symptoms of Stemphylium fruit rot caused by S*temphylium lycopersici*, was small, round and dark brown lesions. The lesions became sunken and developed reddish-brown to purple margins as they enlarged. A velvety, dark green spore mass formed in the center of the lesion. White to gray mycelia were grown over the lesions in the advanced stage of the disease. Conidia were light brown in color, minutely warted with two or three transverse septa, where the medial septum was most prominent.

Disease Incidence and Severity

Among the fungi that caused different postharvest diseases of papaya, significantly higher incidence (90 to 98%) was recorded for anthracnose disease caused by *C. gloeosporioides*, in all three locations, which was followed by stem-end rot disease caused by *Botryodiplodia theobromae,* with disease incidence ranging from 25 to 38% (*Fig. 1*). However, the lowest disease incidence (11.7 to 16.7%) recorded was for Rhizopus soft rot caused by *Rhizopus stolonifer.*

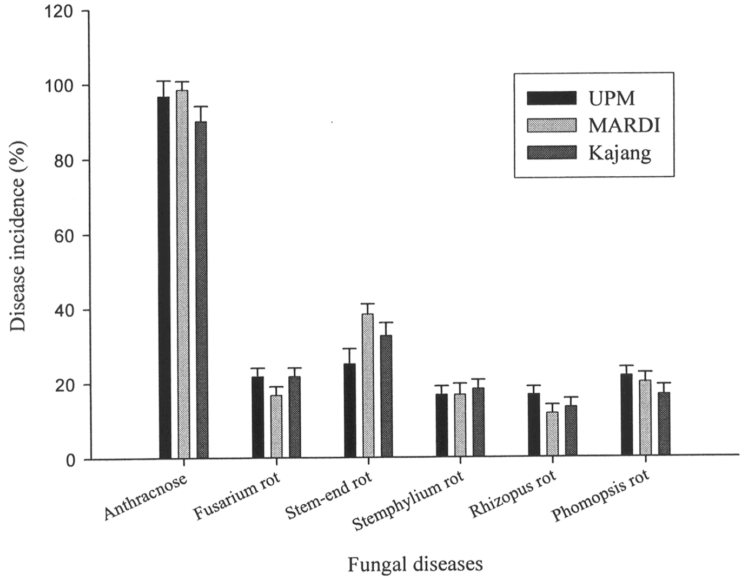


Fig. 1: Incidence of major postharvest diseases of papaya. For each location, values are the mean of three replicates with 15 fruits each. Means were separated by Tukey's Studentized Range (HSD) Test at P≤0.05. Vertical bars represent standard errors of the means

Regarding disease severity, a similar trend was also observed, for all locations, with the highest disease severity ranging from 26 to 34 % for anthracnose followed by stem-end rot, with a severity range of 11 to 16 % (*Fig. 2*).

Pathogenicity Test

Small, round water-soaked lesions were observed on wound inoculated papaya after three days of inoculation. As the infection advanced, lesions became circular and slightly sunken, and covered with dense whitish mycelial growth (*Fig. 3A*). In unwounded fruits, small water-soaked areas were observed on each inoculation site after five days of inoculation (*Fig. 3B*). At the advanced stage of disease development, a round sunken lesion with translucent, light brown margin was formed. After seven days of inoculation the fungus produced light orange spore masses in the central portion of the lesion.

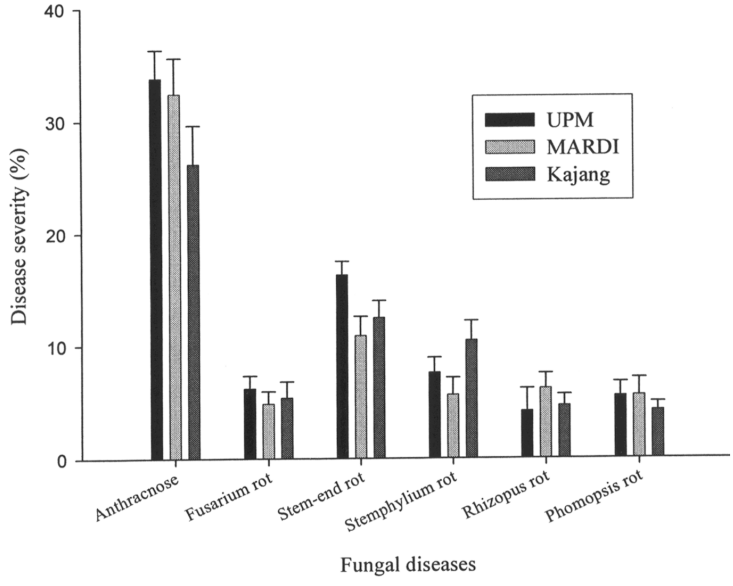
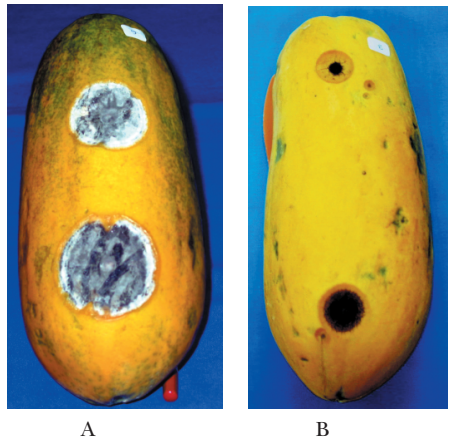


Fig. 2: Severity of major postharvest diseases of papaya. For each location, values are the mean of three replicates with 15 fruits each. Means were separated by Tukey's Studentized Range (HSD) Test at P≤0.05. Vertical bars represent standard errors of the means



A B

Fig. 3: Pathogenicity of C. gloeosporioides *on (A) wounded and (B) unwounded papaya fruits inoculated by the spore suspension (5x 10^5 spore ml^{-1}) of the test fungus. Inoculated fruits were incubated at room temperature (28±2°C) for seven days*

DISCUSSION

Various species of pathogenic fungi such as *Colletotrichum, Phomopsis, Rhizopus, Botryodiplodia*, and *Stemphylium* are responsible for enormous losses of papaya after harvest. Most of these fungi cause rotting that spreads rapidly in the ripe fruit, thus rendering them unfit for consumption (Ilag *et al.*, 1994). There are three general types of postharvest diseases of papaya such as fruit-surface rots, stem-end rots and internal fruit infections (Alvarez and Nishijima, 1987). However, papaya fruits are subjected to several types of postharvest diseases including anthracnose and chocolate spot caused by *Colletotrichum gloeosporioides,* dry rot caused by *Mycosphaerella sp.,* wet rot caused by *Phomopsis sp.,* alternaria fruit spot caused by *Alternaria alternate,* stemphylium fruit rot caused by *Stemphylium lycopersici,* fusarium rot caused by *Fusarium solani,* guignardia spot caused by *Guignardia sp.,* stem-end rot caused by *Ascochyta* sp., *Botryodiplodia, Phomopsis* and *Fusarium,* rhizopus rot caused by *Rhizopus stolonifer* (Albornett and Sanabria de Albarracin, 1994; Ilag *et al.*, 1994; Alvarez and Nishijima, 1987). Among these diseases, anthracnose and stem-end rots continue to cause major postharvest losses of papayas during storage and shipment (de Oliveira *et al.*, 2004; Paull *et al.*, 1997; Alvarez, 1980). Anthracnose of papaya caused by *C. gloeosporioides* is considered to be the most important postharvest disease in the state of Hawaii, and it is important in many other tropical regions where papaya is grown (Snowdon, 1990; Bolkan *et al.*, 1976), including Philippines (Quimio and Quimio, 1974), Malaysia (Lim, 1980) and Sri Lanka (Gamagae *et al.*, 2004; Sivakumar *et al.*, 2002). Similarly in our study, the highest incidence and severity were recorded for anthracnose disease caused by *Colletotrichum* spp. with some degree of variations from location to location. These variations of disease reaction in three selected locations may be due to the variation in climatic conditions, cultural practices and prevalence of the pathogens during the study period. It is reported that the disease severity of sigatoka disease of banana varied with weather conditions (Mishra and Bhattacharyya, 2001).

However, Sepiah *et al.* (1991) and Sepiah (1992) reported that the most important pathogen causing anthracnose of Eksotika papaya, the current important variety in Malaysia, is *C. capsici*. Anthracnose caused by *C. gloeosporioides* was also present on this variety. Lim and Tang (1984) reported that *C. dematium* was the cause for 5% of anthracnose of papaya in Singapore. A single isolate of *C. gloeosporioides* can produce both anthracnose and chocolate spot, but little is known about why some lesions remain superficial while others advance into the fruit parenchyma (Alvarez and Nishijima, 1987).

Anthracnose becomes a problem when fruits have 25% or more skin yellowing (Alvarez and Nishijima, 1987). Infections caused by *C. gloeosporioides* are usually initiated in the field at early stage of fruit development, but the pathogen remains quiescent until the fruit reaches the climacteric phase (Dickman and Alvarez, 1983). The fungus may penetrate the fruit surface directly with an infection peg (Chau and Alvarez, 1983b). An extracellular cutinolytic enzyme is produced, enabling the pathogen to enter green, unwounded fruit. When infected fruits begin to ripen, beads of latex are exuded at the fruit surface, and small water-soaked spots appear. As the infection advances, a circular, sunken lesion with translucent, light brown margins forms. The fungus produces light orange or pink spore masses in the central portion of the lesion. Internal tissue in the infected area is firm with a grayish white discoloration that later turn brown. A layer of callus forms in the parenchyma cells, permitting the infected area to be lifted free of the fruit surface as a plug (Stanghellini and Aragaki, 1966).

Several fungi invade the cut portion of the peduncle after harvest or may enter the fruit through minute injuries and cause stem-end rot. These fungi, individually or in various combinations, cause rotting, shriveling and discoloration of the stem end. Initially the disease was attributed only to *Ascochyta* sp. Later, other genera, including *Botryodiplodia, Phomopsis*, and occasionally *Fusarium* (Hunter and Buddenhagen, 1972) were identified in diseased tissues. Several other fungi including *Alternaria alternate, Stemphylium lycopersici*, *C. gloeosporioides,* and *Mycosphaerella* sp. also may cause stem-end rots when inoculated alone or in various combinations (Chau and Alvarez, 1983c; Chau and Alvarez, 1979; Alvarez *et al.*, 1977). In the Philippines, the pathogen associated with the disease is *Botryodiplodia theobromae* Pat. while in Malaysia, although this fungus is also a causal pathogen, *Phomopsis carica-papayae* is more

common (Ilag *et al.*, 1994). *C. capsici* often causes stem-end rot of papaya when the fruits are kept for long period in cold storage. This disease may also be called stem end anthracnose (Ilag *et al.*, 1994).

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