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Alternative Postharvest Treatments to Control Anthracnose Disease in Papaya during Storage

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ABSTRACT

Papaya (*Carica papaya* L.) is an important fruit crop consumed both as a fresh fruit and as a processed product. Besides, being in domestic use in providing food to the people, the economic value of the crop has potential to be exploited as an income generator by exporting and strategic marketing especially in countries where the exotic fruits are in high demand. Anthracnose disease caused by a fungus *Colletotrichum gloeosporioides* is a major cause for the postharvest loss of papaya particularly when attempting to extend the storage life. Postharvest fungicides applied as spray or dips with or without a food grade wax have been shown to be effective in reducing anthracnose. A hot water dip treatment, double dip hot water treatment that was developed to eradicate fruit fly, and hot water dip treatment in combination with fungicides can be used to control anthracnose. However, hot water treatment may affect the ripening process of the fruit and use of fungicides for extended periods may lead to the emergence of fungicide-resistant strains of the fungus. Furthermore, residuals of fungicides present on the fruit may be harmful to consumers. The growing concerns of the consumer have generated an interest in the development of alternative approaches to postharvest disease control using non-chemical methods while retaining the overall quality. Therefore, this review summarizes the alternative postharvest treatments for the control of this pathogen without the use of chemical fungicides.

Keywords: 1-MCP, alternative postharvest treatments, bio-control agents, CA and MA storage, chitosan, GRAS compounds, irradiation

Abbreviations: 1-MCP, 1-methyl cyclopropene; **CA**, Controlled atmospheric storage; **GRAS**, Generally Regarded as Safe; **MA**, modified atmosphere storage; **USFDA**, United States Food and Drug Administration

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INTRODUCTION

The papaya (*Carica papaya* L.) is a popular tropical fruit belonging to the family *Caricaceae*. It is native to tropical America and seeds of papaya were taken from the Caribbean to Malacca (or Philippines) and then to India (Storey 1941). Papaya is a melon like fruit, which varies greatly in shape and size. The skin of unripe fruit is smooth, green, thin and changes to deep orange or yellow when ripe.

The papaya is rapidly becoming an important fruit crop as a fresh fruit or in the processed form. In Asia, green fruit are served in salads, as a vegetable or made into preserves. Papaya is processed into various forms such as slices for fruit salads and cocktails, dehydrated slices, chunks and as fruit leathers or bars. Papaya can also be processed into puree for juices, nectar, mixed drinks and jams. Papaya provides a cheap source of vitamins and minerals in the daily diet of the people. It is an excellent source of provitamin-A, ascorbic acid and also a good source of calcium. The fruit is also popular with dieters since it is low in fat, carbohydrate and calories. The major nutrients in papaya are listed in **Table 1**.

In Asia papaya cultivation is now being transformed from back yard home gardening to organized production systems due to steady domestic market with potential for increased production to serve export markets. Main papaya

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Table 1 Nutritional composition of papaya (per 100 g edible portion).

Nutrient	Amount per 100 g edible portions
Water	90.8 g
Energy	32.8 kCal
Protein	0.6 g
Fat	0.1 g
Carbohydrates	7.2 g
Calcium	17.0 mg
Phosphorus	13.0 mg
Iron	0.5 mg
Vitamin C	57.0 mg
Carotene	660.0 µg
Thiamine	40.0 µg
Riboflavin	250.0 µg

Source: Tables of composition for use in Sri Lanka – 1989, Medical Research Institute, Sri Lanka

Table 2 Common diseases of papaya and their causal organisms.

Disease	Causal organism
Stem-end-rot	*Botrydiplodia theobromae*
Internal yellowing disease	*Enterobacter cloacae*
Purple stain	*Erwinia herbicola*
Fruit surface rots (infect intact fruits)	
Anthracnose (mostly by)	*Colletotrichum gloeosporioides*
Cercospora black spots	*Cercospora* sp.
Phytophthora fruit rot	*Phytophthora* sp.
Cladosporium spots	*Cladosporium oxysporum*
Fruit surface rots (infect through wounds)	
	Mycospharella sp.
	Phomopsis sp.
	Alternaria sp.
	Stemphylum sp.
	Fusarium sp.

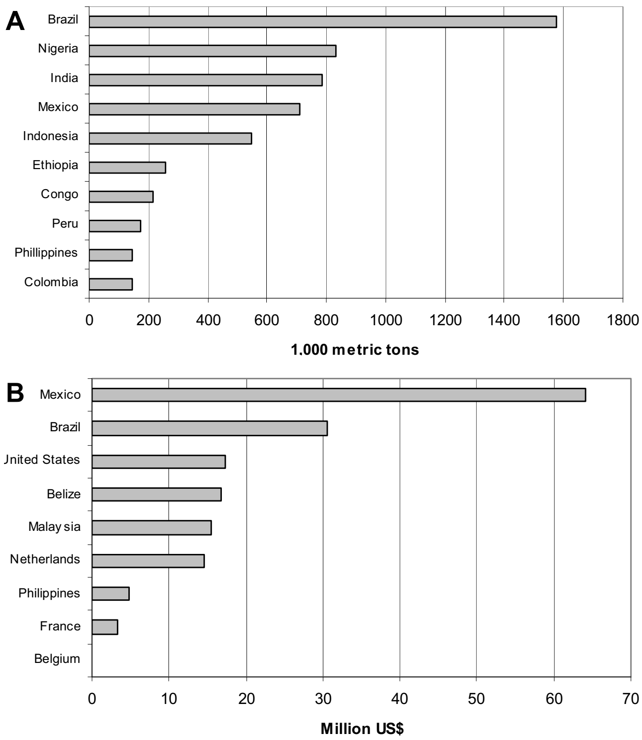

Fig. 1 Top world producers (**A**) and Exporters (**B**) of papaya in 2005. Source: www.usda.gov/Data/

cultivars reported in the world are 'Solo' (Hawaii), Hortus Gold (South Africa), Improved Petersen (Australia), Betty (Florida), Coorg Honey Dew & Honey Dew (India) Maradol (Cuba) and Red Lady (Taiwan) cultivars (Sankat and Maharaj 1997). World production of papaya was 8.5 million tons in year 2004 and the top papaya producing countries (**Fig. 1**) in the world are Brazil (25% of world production), Nigeria (13%), India (12%), Mexico (12%), Indonesia (9%), Congo (4%), Peru (3%), China (3%) and Colombia (2%). Papaya exports increased significantly by 47% in volumes in 2004. Mexico was the largest exporter followed by Malaysia and Brazil.

To increase the exports to European markets it is necessary to improve postharvest management to meet the stringent quality standards that exist in these countries. At present a considerable quantity of this commodity is lost in the postharvest phase.

CONSTRAINS IN PAPAYA EXPORT

Common postharvest diseases of papaya

Papayas are very susceptible to invasion by certain pathogenic fungi and bacteria, as they are high in moisture and nutrients (**Table 2**). The development of fungal infection during the postharvest phase can depend upon the physiological age of the host, mechanical injuries and storage conditions (Salunkhe *et al.* 1991).

Anthracnose is a major cause for the postharvest loss of papaya particularly when attempting to extend the storage life. The causal organism *Colletotrichum gloeosporioides* (Bolkan *et al.* 1976) is of common occurrence in home garden environments. Symptoms of the disease are known to be particularly destructive once ripening has been initiated. Incidence of this disease limits the storage life of the commodity in both local and export markets during the ripening process, storage and transportation (Alvarez and Nishijima 1987).

Symptoms of anthracnose disease in papaya

Anthracnose in papaya is caused by *Colletotrichum* species. Five different species have been reported as pathogens of the fruit. They are *C. capsici*, *C. circinans*, *C. dematium*, *C. gloeosporioides* and *C. papayae. Colletotrichum gloeosporioides* is the most frequently reported pathogen (Sankat and Maharaj 1997). *C. gloeosporioides* Penz. is a facultative parasite. Its teleomorph is *Glomerella cingulata* and belongs to order Phyllachoraceae. The fungus produces hyaline, one celled, ovoid to oblong, sometimes curved or dumbbell shaped conidia, 10-15 µm in length and 5-7 µm in width. Masses of conidia appear pink or salmon coloured. The fungus is favoured by high temperatures and humid or moist weather. The pathogen initially infects non-wounded, immature green fruit in the field. Spores germinate from appressoria on the fruit surface. The fungus, using its appressorium enzymatically penetrates the cuticle and then remains dormant as sub cuticular hypae. The fungus resumes growth during ripening and causes the characteristic symptoms. Thus, papaya anthracnose has a latent stage in its development that is similar to many other anthracnose diseases of tropical fruits (Adikaram 1991).

Disease symptoms are normally not apparent at the time of harvest, but appear when the fruit are ripening or has ripened. When fruit are stored for long periods in cold storage, disease symptoms may appear on the green unripe fruit (Rohani 1994). The first symptoms of papaya anthracnose are small round, water soaked and sunken spots on the body of the ripening fruit. Lesions may become as large as 5 cm in the ripening fruit. The fungus forms pinkish-orange conidial masses that cover the lesion center (**Fig. 2**). Conidia are frequently produced in a concentric ring pattern. Infection involves the fruit tissue, which becomes softer with the diseased portion eventually falling out or readily separating from the unaffected parts of the fruit (Rohani 1994).

METHODS OF CONTROL OF POSTHARVEST PATHOGENS

Disease control involves the prevention of infection, eradication of infection or delaying of symptoms until the fruit would normally have been consumed. It should be the con-

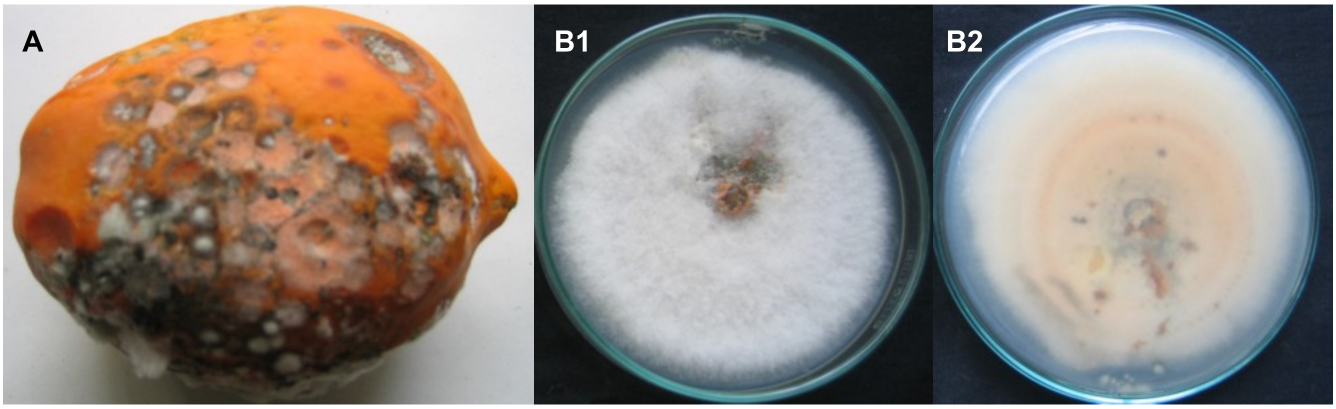

Fig. 2 Symptoms of anthracnose disease on papaya (**A**) and colony characteristics of anthracnose-causing fungi (**B1**: top view of the plate, **B2**: bottom view of the plate) isolated from papaya, incubated at 28°C for 10 days.

cern not only of growers but also pickers, packers and people engaged in transporting storing and marketing the fruit.

Chemical method

Available literature suggests that benomyl or thiobendazole are amongst the more important fungicides, used to reduce diseases of papaya. These fungicides are used with or without hot water treatment after harvest (Broadrick *et al.* 1972; Nishijima *et al*. 1972; Quimio *et al*. 1975; Bolkan *et al.* 1976; Couey *et al.* 1984; Sulsusi *et al*. 1993). However, Sepiah *et al*. (1991) and Sepiah (1993) reported that prochloraz or propiconazole was more effective than these two fungicides in controlling disease caused by *Colletotrichum* species on papaya in Malaysia. Postharvest loss due to anthracnose has been significantly reduced with the introduction of fungicides e.g., thiobendazole-incorporated wax coating of fruit (Alvarez and Nishijima 1987). This is now a common practice especially for fruit shipped to overseas markets. However, chemical control measures can have detrimental effects on humans and also the environment. Extended periods of fungicide use may lead to the emergence of resistant strains of the fungus (Delp 1980; Spotts and Cervants 1986; de Waaurd *et al*. 1993; Bruton 1994) and residues of fungicide present on the fruit may be harmful to consumers (Johnson and Sangchote 1993).

Hot water treatment

Hot water treatment can also be used to reduce postharvest infection (Coates *et al.* 1993). A short dip, 5-10 min at 50-52°C is used to control postharvest rots of papaya (Aragaki *et al.* 1981; Couey *et al*. 1984). A double dip hot water treatment consisting of an initial 30 min immersion at 42°C followed by a 20 min immersion at 49°C, which is used effectively against fruit flies, was effective against anthracnose, stem-end rots and other fungal fruit rots of papaya in Hawaii (Alvarez and Nishijima 1987). However, hot water dip treatments are reported as affecting the ripening process of papaya (Paull 1990) by failing to soften the fruit and having skin scaled after ripening. Though the hot water treatment reduce the incidence of anthracnose by reducing the available inoculum on the fruit surface, this treatment affects the polygalacturonase activity, ascorbic acid content and titrable acidity levels (Ferguson *et al.* 2000).

Polymeric films and wax coating

Modification of the storage environment can be achieved by holding the fruit in an atmosphere consisting of low levels of O_2 or elevated CO_2. Wrapping the fruit in packaging materials such as polyethylene and polyvinyl chloride can create this environment. The application of various dipping solutions (which provide a membrane over the fruit thereby limiting gaseous exchange) has also been found to be a satisfactory way of achieving modified atmospheres. Semi permeable coatings have been used to improve the storability of perishables crops. Waxing of fruit serves a two-fold purpose of reducing water loss and improving the fruit appearance to the consumer (Mitra 1997). Applying a cellulose base film to papaya altered the internal gas concentration, retarded ripening and extended shelf life (Baldwin *et al*. 1992)

Plastic film wraps were more effective than waxing for reducing water loss from papaya. Seal packing (low density polythene) retarded the development of peel colour and reduced the increase in titratable acidity during ripening and also reduced fruit softening. Fruit waxing reduced weight loss by 14-40%, while plastic shrink-wraps reduced it by about 90%. Occasionally, off flavours develops in waxed and wrapped fruit when the fruit cavity CO_2 levels exceeds 7% at the full ripe stage. It is reported that the storage life of ‘Eksotica’ papaya could be extended up to 4 weeks at 10-12°C by wrapping the fruits with low-density polyethylene bags (Rohani and Zaipun 2007).

Controlled atmospheric storage

Use of controlled atmospheres (CA) is considered as a supplement to proper temperature and relative humidity. CA storage can directly or indirectly affect the growth of postharvest pathogens and consequently delay the disease incidence and severity. At 10°C papaya fruit can be stored for 36 days in 8% CO_2 and 3% O_2 followed by another 5 days at 25°C to facilitate the retail market conditions (Cenci *et al*. 1997). CA condition of 2-5% of O_2 and 5-8% of CO_2 is recommended to extend the storage life of papaya (Kader 2002). However, storage of papaya under CA condition has not been practiced commercially.

Use of bio-control agents

In the recent past microbial antagonist have been used to control postharvest diseases of fruits. Most of the reported yeast and bacteria antagonists were naturally occurring organisms on fruit surfaces. Use of bio-control agents shows some important advantages compared to traditional chemical pesticides. The organisms are considered as non-hazardous to human and animals, biodegradable and environmentally friendly. They are target specific and do not affect beneficial organisms. The most important feature is these organisms can be developed in commercial formulations. However, limited shelf-life, limited bio control efficiency in situations where several pathogens are involved in disease development and limited efficiency under high disease pressure (Droby *et al.* 1993) are some of the disadvantages encountered in use of these organisms.

Available literature suggested that bio-control agent *Crytococcus magnus* at a concentration of 10^7-10^9 cells/mL is effective in controlling anthracnose disease in papaya

(Guy *et al*. 2007). Gamagae *et al*. (2003, 2004) reported that use of 2% sodium bicaorbonate incorporated wax coating with *Candida oleophila* is a commercially acceptable alternative to disease control in papaya during storage.

Use of Generally-Regarded-As-Safe compounds

GRAS (Generally Regarded as Safe) compounds, a group of substances already approved by United States Food and Drug Administration (USFDA) for human consumption are used at present to control postharvest diseases. These compounds have been given more emphasis in disease control strategies on account of the growing international concerns regarding the effect of chemical pesticides on the environment and human health. Some of the commonly used GRAS compounds are calcium chloride, magnesium chloride, magnesium sulphate, ascorbic acid, sodium benzoate, benzoic acid, citric acid, sodium chloride, tartaric acid, potassium metabisulphite, sodium metabisulphate, calcium phosphate, sodium phosphate, sorbic acid, calcium carbonate and potassium carbonate.

Treatments with sodium bicarbonate provided satisfactory control of green mold on lemons (Smilanick *et al.* 1995, 1999) and oranges (Similanick *et al*. 1999) and blue mould on oranges (Palou *et al*. 2001). Combined treatment of sodium bicarbonate (2%) and bio control agent *Candida oleophila* strain (1-182) controlled the anthracnose disease in papaya by reducing the incidence of the disease at 13.5°C and 95% RH for 14 days ± 2 days at 25°C, 75% RH (Gamagae *et al*. 2003).

Use of irradiation

Gamma irradiation in low doses has satisfactorily increased the shelf life of papaya by delaying ripening and senescence. It is also effective as a quarantine treatment against fruit flies (Thomas 1986). Combined treatment of hot water dip and irradiation could be used to extend the shelf life of papaya by reducing the fungal rot (Pimentel and Walder 2004).

Use of 1-methylcyclopropene (1-MCP)

The ethylene inhibitor 1-MCP has been recommended to extend the storage life of many fruits including papaya (Hofman *et al*. 2001; Jacomino *et al*. 2002; Ergun and Huber 2004). Mature Solo papaya treated with 1-MCP after harvest is reported to increase the days to reach the ripe stage by 325%, from 5 to 20 days (Hofman *et al*. 2001). However, this increase in days to reach the ripe stage is associated with higher disease severity and external blemishes. Sunrise Solo papaya treated with 1-MCP at the 10-20% skin yellow and 70-80% skin yellow stage had a delay of 4-6 days with the treatment while non-treated control softened in 5 days from 10-20% yellow stage and 2 days from the 70-80% yellow stage (Ergun and Huber 2004). Manenoi *et al*. (2007) reported that papaya must be more than 25% yellow before treatment with 1-MCP and fruit with less than that maturity did not ripen completely and had a delayed ethylene production. The beneficial effect of 1-MCP in reducing decay caused by anthracnose and Rhizopus rot in guava was clearly explained by Singh and Pal (2008). However, report on effect of 1-MCP on disease control of papaya is limited.

Chitosan treatment on postharvest disease control

Chitosan (Poly (1-4) β, D-Glucosamine), the deacetylated form of chitin, which is soluble in acidic solutions, has been shown to be fungicidal against several fungi (Benhamou 1996). The effect of chitosan may be fungistatic or fungicidal against pathogens of various fruits and vegetables (Bautista-Banos *et al*. 2003). Growth of important postharvest fungi such as *Alternaria alternata*, *Fusarium oxysporum*, *Rhizopus scolonifer*, *C. gloeosporioides* and *Penicillium* sp. is inhibited on nutrient media amended with various concentrations of chitosan (Hirano and Nagao 1989; Benhamou 1992; Bhaskara Reddy *et al.* 1997).

A significant reduction of storage rots has also been recorded in chitosan treated fruits such as apples, kiwifruit, pears (Du *et al*. 1997; Bautista-Baños *et al.* 2004). In strawberries and raspberries, chitosan coatings reduced two of the main postharvest diseases, grey mould and Rhizopus rot. Moreover, chitosan's fungicidal performance was equivalent to that of the synthetic fungicides such as ipriodione and thiabendazole (TBZ), commonly used to reduce the disease (El-Ghaouth *et al*. 1991a, 1992a, 1992b; Zhang and Quantick 1998).

Chitosan forms a semipermeable film that regulates the gas exchange, reduces transpiration loss and slows down fruit ripening. Since, chitosan is applied as a coating, respiration rate is slowed down and thereby water loss is reduced. This effect has been reported for numerous horticultural commodities such as tomato, strawberries, longan, apples, mangoes, bananas, bell peppers, papaya, etc. (El-Ghaouth *et al*. 1991b, 1992c; Du *et al.* 1997, 1998; Jiang and Li 2001; Hewajulige *et al.* 2006, 2007). Combined treatment of calcium infiltration (2.5%) and chitosan coating (0.75%) extended the storage life of papaya cv. Eksotica up to 35 days with better retention of firmness and water loss (Al eryani *et al*. 2008). Bautista-Baños *et al.* (2003) and Sivakumar *et al*. (2005) reported that chitosan incorporated with plant extracts and salts of carbonic acids respectively also had a protective effect against *C. gloeosporioides* the causal organism of anthracnose disease of papaya. The biodegradable nature of natural compounds derived from animals and plants have aroused interest among researchers. Chitosan, a high molecular polymer, non-toxic, bioactive agent has become a useful compound due to its fungicidal effects and elicitation of defense mechanisms in plant tissue (Wilson *et al.* 1994; Terry and Joyce 2004).

Several studies have demonstrated that chitosan is an exogenous elicitor of many inherent host defense responses, including accumulation of chitinase, β-1,3-glucanase and phenolic compounds, induction of lignification and synthesis of phytoalexins by the infected host tissue (Tejchgraber *et al*. 1991; Arlorio *et al*. 1992; Bhaskara Reddy *et al*. 1997; Zang and Quantic 1998). Microscopic observations of fungi treated with chitosan revealed that it can affect the morphology of the hyphae and the tip of the hyphae became thin in the presence of chitinase and β-1,3-glucanases (Arlorio *et al*. 1992). Recent reports have shown that chitosan has the capacity to induce resistance to *F. oxysporum* in susceptible tomato plants when applied as a root dressing, foliar spray or seed dressing by restricting pathogen growth to the outer root tissues and eliciting a number of defense reactions, including structural barriers (Benhamou 1998). Induced resistance response was also reported in groundnut treated with chitosan, where a significant increase of endogenous salicylic acid, inter-cellular chitinase and glucanase activities were measured (Sathyabama and Balasubramanian 1998). Studies on fresh strawberries and raspberries with a chitosan coating showed a significant increase of chitinase and β-1,3-glucanase activities, compared with fruit used as uncoated controls (Zhang and Quantick 1998). Peel samples of papaya varieties Rathna and Red Lady grown in Sri Lanka showed enhanced chitinase and β-1,3-glucanase activities when subjected to chitosan dip treatment compared to untreated controls (Hewajulige *et al*. 2008). The inhibitory effect of chitosan on anthracnose in papaya seems to originate from the combination of is antifungal property and its ability to stimulate defense response in the host.

CONCLUSION

This review has illustrated the possible non-chemical methods to control anthracnose disease in papaya in order to extend the storage life for 14-21 days to facilitate sea shipment of the commodity. However, proper pre harvest management of orchard to reduce the inoculum potential, harvest at the correct stage of maturity, method of harvesting, and proper handling of the fruit throughout the supply chain and the selection of suitable packaging material also equally important to reduce the anthracnose severity in papaya. Effect of use of GRAS compounds, biocontrol agents and chitosan coating are promising to extend the storage life of papaya while retaining the overall quality of the fruit and should be investigated further. Use of I-MCP is also observed for promising results on other climacteric fruits like guava. More investigation has to be focused on improving or inducing the defense mechanism of the fruit and thereby reduce the disease incidence and severity of anthracnose. Combined treatment of the above promising technologies could result in better disease control over chemical control while maintaining the postharvest quality of papaya.

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