Australian Journal of Crop Science AJCS

AJCS 6(1):17-22 (2012) ISSN:1835-2707

Sensitivity of *Colletotrichum gloeosporioides* to sodium bicarbonate on the development of anthracnose in papaya (*Carica papaya* L. cv. Frangi)

Hasan M.F[1], Mahmud T.M.M[1,4*], Kadir J[2], Ding P[1], Zaidul I.S.M[3]

[1]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[3]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[4]Institute of Tropical Agriculture (ITA), Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[*]Corresponding author: mtmm59@gmail.com

Abstract

Using of non-chemical control methods to reduce postharvest decay is becoming increasingly important in both economic and environmental view point. Sodium bicarbonate (SBC) is a common food additive recognized as safe substance in food industry. This study had been carried out to look at the ability in both *in vitro* and *in vivo* conditions of sodium bicarbonate (SBC) to suppress postharvest anthracnose disease of papaya. SBC at various concentrations, 0, 1, 1.5, 2, 2.5 and 3% (w/v) was used as treatment against mycelial growth and spore germination of *Colletotrichum gloeosporioides*. After seven days of incubation, mycelial growth was completely inhibited by 3% SBC, which was statistically similar with 2 and 2.5% SBC (99.5% and 96.5% inhibition, respectively). Similar trend was observed with spores germination, where no spores were germinated after 8 h in 3% SBC solution, which was statistically identical with 2 and 2.5% SBC solution. In *in vivo* test, 2% SBC significantly reduced the disease incidence and severity where anthracnose disease was reduced up to 60% compared to the control. Therefore, 2% SBC can be expected to provide more effective control of anthracnose in papaya during storage.

Keywords: Mycelial growth; Spore germination; Disease incidence; Disease severity.
Abbreviations: SBC - Sodium bicarbonate; PDA - potato dextrose agar; PIRG - percent inhibition of radial growth; DI - disease incidence; DS - disease severity; GRAS - generally regarded as safe.

Introduction

Papaya fruits are very susceptible to diseases caused by many microorganisms especially fungi. Various genuses of fungi such as *Colletotrichum, Phomopsis, Phytophthora, Rhizopus, Stemphylium* and *Fusarium* are responsible for enormous losses after harvest (Rahman et al., 2007). Most of the fungi cause rotting that spreads rapidly in the ripe fruits, thus rendering them unfit for consumption. Among the postharvest diseases, anthracnose caused by *Colletotricum gloeosporioides* Penz. Sacc., is the major postharvest disease of papaya in Malaysia (Rahman et al., 2007). Disease symptoms are normally not apparent at the time of harvest, but appear when the fruits are ripening or ripened (Ilag et al., 1994). Generally, the field contaminations are due to the conidia which, originate from the dying infected petioles of the lower leaves of the papaya plant. In a few hours of moist environment, conidia develop appressoria from which infection pegs penetrate the skin of the fruit and remain latent until fruit ripens. Subsequently, during the postharvest period symptoms become evident (Snowdon, 1990). Normally, the initial symptom of the disease appeared five days after harvest and by 9-10 days after storage, most of the fruits were fully ripened and 91% were invaded by the pathogens. It is also reported that fruits which were stored for long periods in cold storage, disease symptoms may appear on the green unripe fruit. The first symptoms of papaya anthracnose are round, water-soaked spots on the surface of the ripening fruit. The spots enlarged and turn light brown before sunken spots develop on the surface. Lesions may become as large as 5 cm in diameter (Dickman, 2005; Pernezny and Litz, 2005). Pinkish-orange areas are formed by the conidial masses that cover the lesion center and are frequently produced in a concentric ring pattern (Dickman, 2005). Infection involves the fruit tissues which become softer and eventually the disease portion falls out or can be readily separated from the uninfected parts of the fruit. It can also cause off flavor of the pulp. Control of anthracnose of papaya as well as increase the shelf life commonly depends on the use of synthetic fungicides. The enormous use of synthetic fungicides cause numerous environmental problems such as, the use of fungicides for extended periods may lead to the emergence of resistant strains of fungus. Moreover, fruits may become harmful to the consumers due to the presence of residues of fungicides. This is why consumer demand is increasing for non-chemically treated fruits at postharvest level. Considering the potential benefits of alternatives, which include both suppression of fungicide resistance and reduction of the risks associated with the use of fungicides to humans, animals and the environment, alternative studies are inevitable. Selected organic and inorganic salts act as active antimicrobial agents

and are widely used as alternatives to chemical control in the food industry. One of these compounds is sodium bicarbonate (SBC), commonly known as baking soda (Kuepper et al., 2001), which is generally regarded as safe (GRAS) by the United States Food and Drug Administration. In addition, it is listed as an approved ingredient in organic products (Mazzini, 2002). Immersing fruit in solutions of SBC to control the postharvest incidence of *Penicillium digitatum* on citrus was first described in 1928 (Barger, 1928) and has since been used in California to control postharvest decay of lemons because it is economical, readily available and can be used with little risk of fruit injury (Palou et al., 2001). The efficacy of bicarbonate salts for the control of postharvest pathogens has been studied in citrus fruit (Usall et al., 2008), apple (Ilhan et al., 2006), potato (Mills et al., 2006), bell pepper (Fallik et al., 1997), melon (Aharoni et al., 1997) and carrots (Punja and Gaye, 1993). However, research on the control of anthracnose of papaya is still scarce. As far we know, only Gamagae et al., (2003) showed the efficacy of SBC in *in vivo* to control anthracnose of papaya. To explain the potentiality of an inorganic compound as alternative agent, both in laboratory and field experiments are necessary. Therefore, our objectives in this study were to examine the influence of SBC treatment in both *in vitro* and *in vivo* on controlling the anthracnose of papaya during storage.

Results

Effect of SBC on growth and spore germination of C. gloeosporioides

Increase in concentrations of SBC significantly ($p \leq 0.05$) inhibited the radial growth of *C. gloeosporioides* after seven days of incubation (Figs 1 & 2). The complete inhibition of mycelial growth was found with 3% treatment, which was not sizeably different from 2 and 2.5%. However, concentrations above 2% were significantly different from 1 and 1.5% treatments. The latter two were substantially different between each other. These results showed that SBC at any concentrations will be able to inhibit the growth significantly whereas for the control no inhibition was observed. Visual observation showed that overall spore germination was lower on PDA plates amended with higher concentrations of SBC compared to control (Fig 3). No spore germination was observed on PDA plates treated with concentrations of more than 2% of SBC after seven hours of incubation. Whereas, few spores were germinated in 1-1.5% SBC amended plates but numerous were found on the control plates after seven hours of incubation. In the figure, the results of 2 and 2.5% SBC overlapped with the results of 3% SBC treatment. This is because of equal in efficacy of these treatments to inhibit the spore germination throughout the incubation period.

SBC markedly reduced spore germination of *C. gloeosporioides* (Fig 4). Spore germinations were completely inhibited with concentrations above 2% even after only 7h of inoculation. Whereas, on PDA plates amended with 1 and 1.5% SBC, germination was inhibited by 60 and 78%, respectively and decreased drastically after that. Zero inhibitions were obtained after 13 and 15h of inoculation respectively, which was similar to the control. Observation on inhibition of spore germination *in vitro* was carried out by light microscope investigations (Figure 5). On SBC free agar, germination of *C. gloeosporioides* was found to be regular and smooth (Fig 5a). In contrast, on SBC treated agar medium, noticeable morphological changes occurred. At

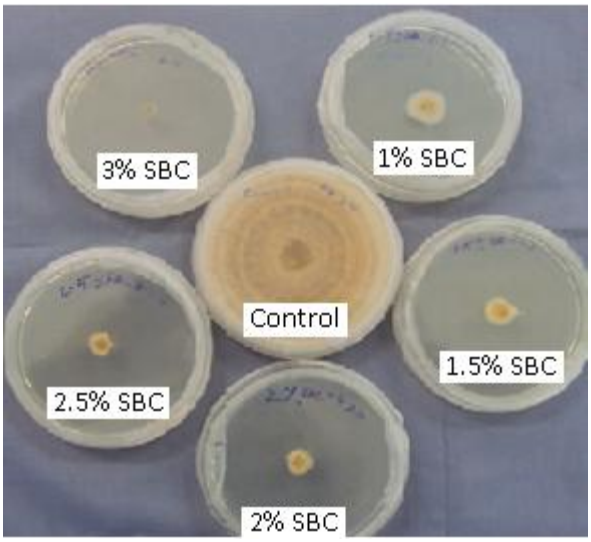

Fig 1. Effect of different concentrations of SBC on the radial growth of C. gloeosporioides after seven days of incubation at 28 ± 2 ºC.

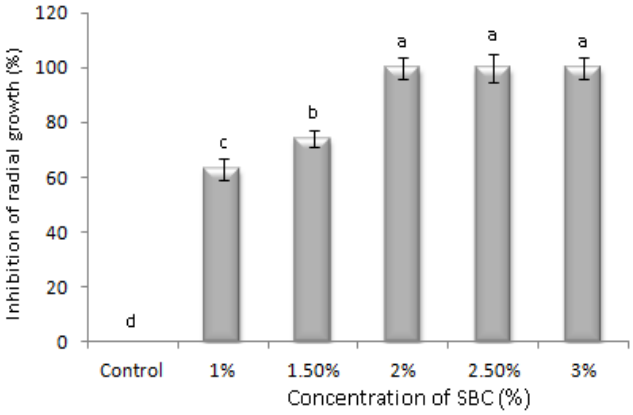

Fig 2. Effect of different concentrations of SBC on the radial growth of *C. gloeosporioides* after seven days of incubation at 28 ± 2ºC. Means with different letters were significantly different at $P \leq 0.05$ on the arcsine transformed values according to Tukey's Studentized Range Test. Vertical bars represent the standard error.

1.5% SBC, germ tube grew with abnormal shapes and after 10 h many swellings, thickenings and vacuoles were observed on germinated spores (Fig 5b).

Disease control efficacy of SBC

Artificially inoculated fruits

Generally, lesion diameter of anthracnose in papaya fruits increased with increasing storage duration. Lesion diameter in water treated negative control fruits increased rapidly in a significant quadratic relationship with storage duration (R^2 = 0.93) compared with SBC treatments (Fig 6). However, benocide (positive control) showed the slower rate of development of lesion diameter during 18 days of storage duration. On the other hand, lesion diameter rate in 2% SBC was intermediate. There were significant positive quadratic relationships between lesion diameter of fruits treated with

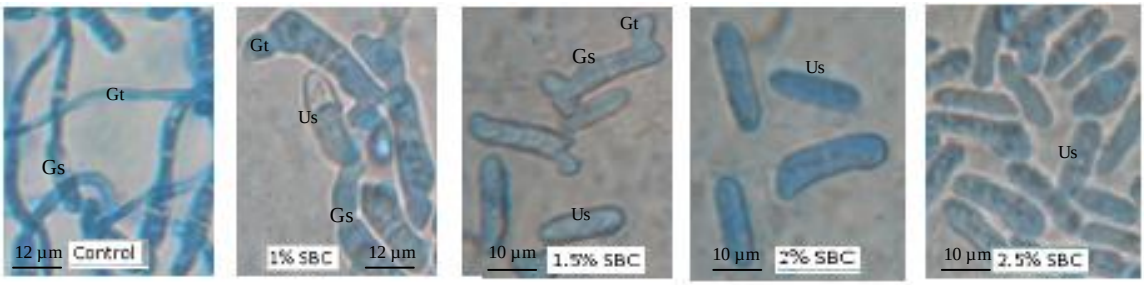

Fig 3. Effect of different concentrations of SBC on spore germination of *C. gloeosporioides* after seven hours of incubation at 28 ± 2 ºC. Gs = Germinated spore; Us = Ungerminated spore; Gt = Germ tube.

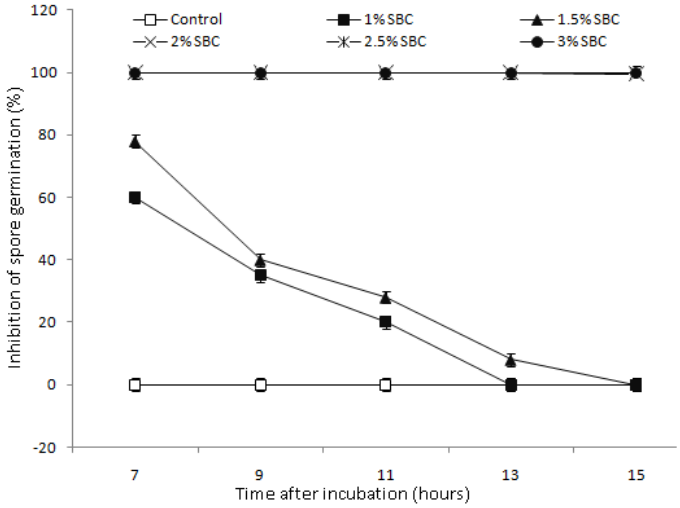

Fig 4. Effect of different concentrations of SBC on spore germination of *C. gloeosporioides* after 7-15h of inoculation at 28 ± 2 ºC. Means separation was done on the arcsine transformed values at $P \leq 0.05$ according to Tukey's Studentized Range Test. Vertical bars represent the standard error.

benocide (R^2 = 0.29), 2% SBC (R^2 = 0.89) and storage duration.

Disease incidence in non-inoculated fruits

Anthracnose incidence on naturally infected papaya fruits subjected to different treatments is presented in Figure 7. Disease incidence was significantly ($P \leq 0.05$) reduced when the fruits were subjected to all treatments compared to water-treated control fruits during 14 days of storage and six days of ripening at ambient temperature (28±2ºC). The 2% SBC showed significantly lower anthracnose incidence on naturally infected fruits than in fruits dipped in water. The incidence of the disease gradually increased with ripening period. Water-treated control fruits showed anthracnose spots even after 14 days of storage and the incidence increased to 60 and 100% by the third and fourth day of ripening period, respectively. By the end of the ripening period lowest disease incidence (58%) was recorded in fruits subjected to benocide® treatment. Disease incidence recorded in fruits treated with SBC was 39% after three days of ripening but, increased gradually to 85% by the 6th day of ripening. The data of present study showed that the SBC not only is effective in reducing the disease incidence but also delayed the onset of anthracnose infection.

Disease Severity in non-inoculated fruits

Both SBC and positive control (benocide®) treatments significantly ($P \leq 0.05$) reduced the disease severity when compared to water treated negative control (Fig 8). The naturally infected fruits subjected to benocide® showed lowest anthracnose severity. The SBC treatment reduced the disease severity with complete absence of symptoms until 14 days of storage at 12ºC. But, since anthracnose infection is latent and occurs especially during ripening, only by the end of ripening period, disease severity was recorded as 7%. In contrast, anthracnose symptoms appeared in water-treated control fruits by the end of the 14 days storage and the severity increased gradually reaching 51% by the end of ripening.

Discussion

Recently, many studies have demonstrated problems arising from the presence of pesticide residues in the environment, foods and feeds. This has led to restrictions and reduction of availability of some chemical fungicides previously used to control disease of plants and spoilage of their produce used for food. Alternative methods to control these pathogens and spoilage organisms are now being investigated. About 34 inorganic salts are used to control different disease (Deliopolus et al., 2010). Among them, sodium bicarbonate is one of vital candidate used to control disease. In this study, we have described the use of sodium bicarbonate against anthracnose of papaya caused by *C. gloeosporioides*. Results showed that *C. gloeosporioides* is very sensitive to SBC since growth was affected even at 1% concentration. Mycelial growth and spore germination were affected by SBC indicating that SBC affected various stages of development. Moreover, no spore was formed by the fungus in higher SBC concentrations. Similar results were found by Ilhan et al. (2006), who reported that mycelial growth of *Venturia inaaequalis* were markedly reduced by SBC with greater effect at higher concentrations. Literatures generally report that the level of inhibition of fungi is highly correlated with SBC concentration, indicating that SBC performance is related to rate of application. Aharoni et al. (1997) reported that SBC inhibited radial growth of *Rhizopus, Alternaria* and *Fusarium in vitro*. Inhibition by 0.5% SBC on conidial germination and germ tube elongation of *Venturia inaaequalis* was 59.0% and 92.3%, respectively and complete inhibition was achieved by 2% SBC (Ilhan et al., 2006). Overall, spore germination of fungi treated with SBC is generally reported to be lower than in untreated. In this study, no spore was germinated in higher concentrations (2 to 3%) of SBC amended plates, whereas, numerous spores were germinated in the control plates. SBC is not only effective in halting the growth of the pathogen, but also induces marked

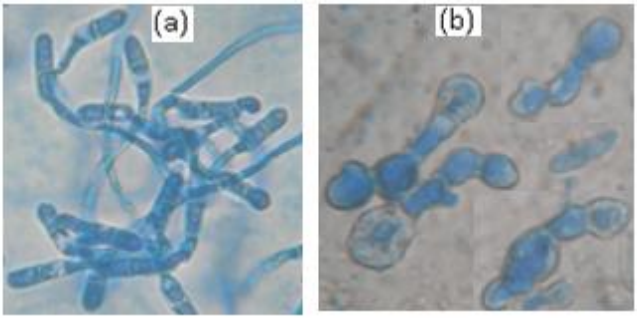

Fig 5. Effect of 1.5% SBC on germinated spores morphology of *C. gloeosporioides*. (a) = germinated spore with normal shape after 7h of incubation; (b) = germinated spore with abnormal shape at 1.5% SBC after 10h of incubation.

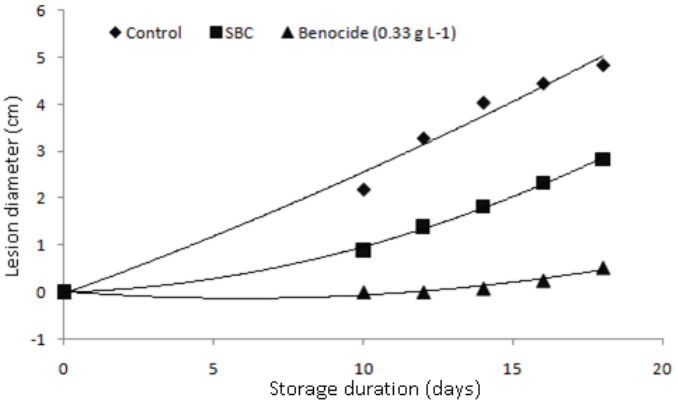

Fig 6. Relationship between lesion diameter of anthracnose in pre-inoculated papaya fruits and inoculation duration for various treatments. Solid lines represent significant quadratic responses. The quadratic regression equations for each treatment are: Lesion diameter (Control ◆) = $1.199x - 0.031x^2 - 6.644$ ($R^2 = 0.93$); lesion diameter (2%SBC ■) = $0.036x^2 - 0.672x + 4.092$ ($R^2 = 0.89$); lesion diameter (Benocide ▲) = $0.011x^2 - 0.251x + 1.382$ ($R^2 = 0.29$).

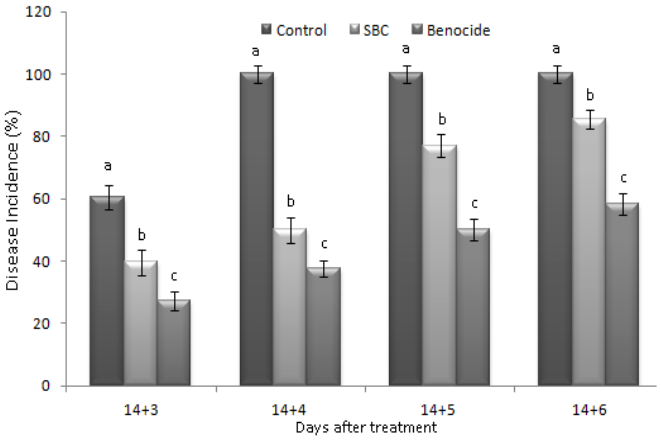

Fig 7. Effects of 2% SBC, benocide and water control treatments on the incidence of anthracnose in naturally infected papaya fruits stored at 12º C for 14 days and six days of ripening under ambient temperature (28 ± 2 ºC). Means with different letters are significantly different at $P \leq 0.05$ on the arcsine transformed values according to Tukey's Studentized Range Test. Vertical bars represent the standard error.

morphological changes, structural alterations and molecular disorganization of the fungal cells. Results of this study showed that morphology of the SBC treated spores of *C. gloeosporioides* was affected. Germinated spore of the fungus became malformed, thickened and swelled compared with germinated spore in the control plate. Result of *in vitro* experiment was reflected in the *in vivo* experiment. In terms of disease severity and disease incidence were significantly lower in 2% SBC treated fruits. These findings were in close agreement with that of Gamagae et al. (2003), who found that the control of anthracnose disease with 2% SBC was significantly higher than control. It was also reported that 1 and 2% SBC significantly reduced the disease incidence and severity of *V. inaequalis* (Ilhan et al., 2006).

The mechanism by which SBC affects the growth of several phytopathogenic fungi has not been fully elucidated, but several hypotheses have been proposed in the explanation of antimicrobial activity. It is believed that, the inhibitory effect of SBC on microorganisms are due to reduction of cell turgor pressure with collapse and shrinkage of hyphae and spores, resulting in fungistasis (Davide et al., 2004; Fallik et al., 1997). SBC has inhibitory action against postharvest pathogens and are more fungistatic than fungicidal and not very persistent (Palou et al., 2002). Likewise, Olivier et al. (1999) claimed that, pathogen inhibition by sodium bicarbonate is primarily due to the alkaline pH, which is detrimental to most microorganisms.

Materials and methods

Plant materials

Papaya fruits of 'Frangi' cultivar at color stage two (green with tinge of yellow) were used in this experiment. Healthy fruits with uniform size, shape, and maturity were purchased from a farm belonged to Malaysian Agricultural Food Corporation (MAFC) located in Lanchang, Pahang, Malaysia.

Pathogen culture

Pure culture of *C. gloeosporioides* was grown on potato dextrose agar (PDA, Oxoid Ltd., Basingstoke, Hampshire, England) at 28 ± 2°C for seven days (Rahman et al., 2007). Spores were subsequently harvested and suspension was prepared with sterilized distilled water. The concentration of conidia in the suspension was adjusted to 5×10^5 conidia mL^{-1} using haemacytometer (Obagwu and Korsten, 2003).

Culture media

Solutions of SBC (Systerm, Classic Chemicals Sdn. Bhd.) at concentrations of 0 (control, sterilized water only), 1, 1.5, 2, 2.5 and 3% (w/v) were used. Different concentrations of SBC were filtered through a 0.45µm Millipore filter before adding them to the autoclaved PDA.

Effect of SBC to C. gloeosporioides

The effect of SBC on mycelia growth of *C. gloeosporioides* was conducted by placing a 5 mm diameter of a seven days old culture in the center from the periphery of a 9 cm petri dish containing PDA with appropriate SBC concentrations. Radial measurements of growth were taken after seven days of incubation until the fungus reached the edge of the control plates (Bautista-Banos et al., 2003). The percent inhibition of

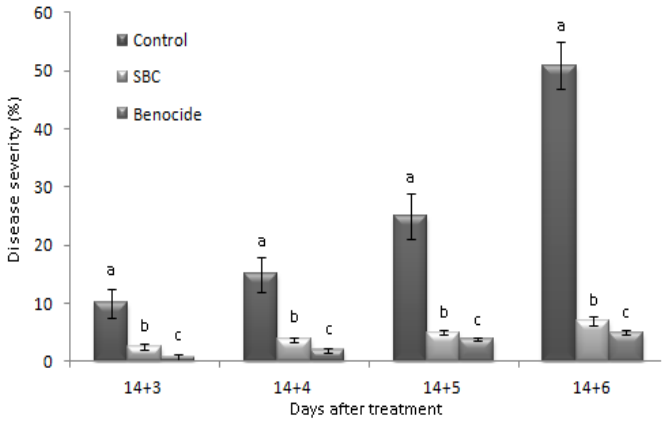

Fig 8. Effect of 2% SBC on the disease severity of anthracnose in naturally infected fruits stored at 12ºC for 14 days and six days of ripening under ambient temperature (28 ± 2 ºC). Means with different letters are significantly different at $P \leq 0.05$ on the arcsine transformed values according to Tukey's Studentized Range Test. Vertical bars represent the standard error.

radial growth (PIRG) was calculated according to the formula described by Sivakumar et al., (2000).

Spore germination assays

For spore germination test, 100 µL aliquots of the spore suspensions (5 x 10^5 spore mL^{-1}) were pipetted onto PDA plate amended with SBC and spread with a sterile bent glass rod. Inoculated plates were incubated at 28 ± 2 ºC for 13h. Data on spore germination were collected every two hours after seven hours of inoculation. Microscopically, germination of 100 spores per plate was determined. A spore was considered to have germinated when the germ tube length exceeded or equaled the length of the spore (El-Ghaouth et al., 1992).

Fruit inoculation and lesion measurement

A total of 36 fruits at color stage two were surface sterilized with 75% ethanol followed by rinsing with distilled water. Each of the fruit was wounded (3 mm deep and 5 mm diameter) with a sterilized cork borer. Two wounds were made at the mid region of fruit with six centimeters apart. Each of the wound was then inoculated with 50 µl conidial suspension of *C. gloeosporioides* (5 x 10^5 spores mL^{-1}) and held at 28 ± 2 ºC for two hours (Gamagae et al., 2003). Each of the 12 inoculated fruits was then dipped for 15 min in 2% (based on the *in vitro* study) SBC solution. The control treatment consisted of a set of 12 inoculated fruits that were immersed either in sterilized distilled water or commercial fungicide Benocide® (Benomyl 50% WP) @ 0.33 g L^{-1} acted as negative and positive controls, respectively. Fruits were allowed to air dry for five minutes after treatment. Each fruit was then wrapped using 70gm offset paper and held at 12 ºC and 95% RH for 18 days (Rahman et al., 2007). Data on lesion diameter were recorded on each alternate day started on day tenth after inoculation.

Naturally infected fruits

Fruits selection and sterilization were done as in artificially inoculated fruits. Fruits inoculation and lesion measurement were done as previously described. Treated fruits were packed and stored for 14 days at the same conditions as with the artificially inoculated fruits. At the completion of storage time, fruits were removed and ripened with ethylene at room temperature (28 ± 2 ºC). Ten mL^{-1} 2% ethylene was injected in a sealed polythene bag of fruits for 24 h. Ethylene was then removed by opening the sealed polythene bag and the fruits were allowed to ripen at room temperature for another six days. Data on anthracnose incidence and severity were recorded daily started on third day of post-storage, when disease symptoms began to appear in ripened fruits. Disease incidence was calculated using the formula.

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

Data on disease severity (DS) 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). Disease severity was recorded according to the following formula described by Singh (1984):

$$\text{Disease Severity (DS)}(\%) = \frac{\Sigma(\text{Severity rating} \times \text{number of fruits in that rating})}{\text{Total number of fruits assessed}} \text{X highest scale X } 100$$

Statistical analysis

All experiments in this study were arranged in a completely randomized design and repeated twice with five replications for *in vitro* and twelve for *in vivo* experiments. Data were statistically analyzed using SAS software version 9.2 (SAS, Institute Cary, NC, 1987), by analysis of variance and significant differences among the treatments were determined using Tukey's Studentized Range Test at $P \leq 0.05$.

Conclusion

The key findings of this study demonstrated that the control activity of SBC against anthracnose of papaya was significantly enhanced by 2% SBC. The level of control conferred by the SBC was superior to control. Therefore, 2% SBC can be expected to provide more effective control of anthracnose in papaya during storage and its efficacy can be enhanced with coating and antagonistic biocontrol agent.

References

Aharoni Y, Fallik E, Copel A, Gil M, Gringberg S, Klein JD (1997) Sodium bicarbonate reduces postharvest decay development on melons. Postharvest Biol Tec. 10: 201-206.

Barger WR (1928) Sodium bicarbonate as a citrus fruit disinfectant. Calif.Citrogr. 13: 164–174.

Bautista-Banos S, Hernandez-Lopez M, Bosquez-Molina E, Wilson CL (2003) Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit. Crop Prot. 22: 1087–1092.

Davide S, Angelo G, Maria LG (2004) Control of Penicillium expansum and Botrytis cinerea on apple combining a biocontrol agent with hot water dipping and acibenzolar-S-methyl, baking soda, or ethanol application. Postharvest Biol Tec. 33: 141–151.

Deliopoulos T, Kettlewell PS, Hare MC (2010) Fungal disease suppression by inorganic salts: A review. Crop Prot. 29: 1059-1075.

Dickman MB (2005) *Colletotrichum gloeosporioides*. In: Crop Knowledge Master. Retrieved 17 June 2005 from www.extento.hawaii.edu/kbase/crop/Type/c_gloeo.htm

El Ghaouth A, Arul J, Grenier J, Asselin A (1992) Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. Phytopathology. 82: 398–402.

Fallik E, Grinberg S, Ziv O (1997) Potassium bicarbonate reduces postharvest decay development on bell pepper fruits. J Hort Sci. 72: 35-41.

Gamagae SU, Sivakumar D, Wilson WRS, Wijesundera RLC (2003) Use of sodium bicarbonate and *Candida oleophila* to control anthracnose in papaya during storage. Crop Prot. 22: 775-779.

Ilag LL, Sepiah M, Tan SC, Sulusi P, Suchat V (1994) Postharvest pathology and control of diseases. In Papaya-Fruit development, postharvest physiology, handling and marketing in ASEAN, ed. M.Y. Rohani, pp. 83-98. ASEAN Food Handling Bureau, Kuala Lumpur, Malaysia.

Ilhan K, Arslan U, Karabulut OA (2006). The effect of sodium bicarbonate alone or in combination with a reduced dose of tebuconazole on the control of apple scab. Crop Prot. 25: 963-967.

Illeperuma CK, Jayasuriya P (2002) Prolonged storage of 'Karuthacolomban' mango by modified atmosphere packaging at low temperature. J Hort Sci. 77(2): 153-157.

Kuepper G, Thomas R, Earles R (2001) Use of Baking Soda as a Fungicide. National Centre for Appropriate Technology, Fayetteville, AR. http://attra.ncat.org/attra-pub/bakingsoda.html.

Mazzini F (2002) Come cambiano le norme sui prodotti fitosanitarie loro coadiuvanti. Inf Tore Fitopatol. 52: 42–46.

Mills AAS, (Bud) Platt HW, Hurta RAR (2006) Sensitivity of *Erwinia* spp. To salt compounds in vitro and their effect on the development of soft rot in the potato tubers in storage. Postharvest Biol Tec. 41: 208-214.

Obagwu J, Korsten L (2003) Integrated control of citrus green and blue molds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. Postharvest Biol Tec. 28: 187-194.

Olivier C, Macniel CR, Loria R (1999) Application of organic and inorganic salts to field-grown potato tubers can suppress silver scurf during potato storage. Plant Dis. 83: 814–818.

Palou L, Smilanick JL, Usall J, Vinas I (2001) Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Dis. 85: 371–376.

Palou L, Usall J, Smilanick JL, Aguilar MJ, Vinas I (2002) Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum*on citrus fruit. Pest Manag Sci. 58: 459–466.

Pernezny K, Litz RE (2005) Some Common Diseases of Papaya in Florida. Retrieved 17 June 2005 from http://edis.ifas.ufl.edu/VH050

Punja Z, Gaye MM (1993) Influence of postharvest handling practices and dip treatments on development of black root on fresh carrots. Plant Dis. 77: 986–995.

Rahman MA, Mahmud TMM, Kadir J, Abdul Rahman R, Begum MM (2007) Major postharvest fungal diseases of papaya cv. sekaki in selangor, Malaysia. Pertanika J Trop Agric Sci. 31 (1): 27-34.

Singh RS (1984) Introduction to Principles of Plant Pathology, 3rd Edition. Oxford and IBH Publishing Co., New Delhi, India.

Sivakumar D, Wilson Wijeratnam RS, Wijesundera RLC, Marikar FMT, Abeyesekere M (2000) Antagonistic effect of *Trichoderma harzianum* on postharvest pathogen of rambutan (*Nephelium lappaceum*). Phytoparasitica. 28: 240-247.

Snowdon AL (1990) A colour atlas of postharvest diseases and disorders of fruits and vegetables. Vol. 1, Wolfe Scientific Ltd., London, UK.

Usall J, Smilanick J, Palou L, Denis-Arrue N, Teixido N, Torres R, Vinas I (2008) Preventive and curative activity of combined treatments of sodium carbonates and Pantoea agglomerans CPA-2 to control postharvest green mold of citrus fruit. Postharvest Biol Tec. 50: 1–7.