Full Length Research Paper

Effect of calcium chloride on anthracnose disease and postharvest quality of red-flesh dragon fruit (*Hylocereus polyrhizus*)

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The study was conducted to examine the effects of $CaCl_2$ postharvest treatment on development of anthracnose, measured as lesion size and quality of red-flesh dragon fruit (*Hylocereus polyrhizus*). Fully matured fruits were treated with varying concentrations of Ca by soaking the fruits for 30 min in solutions containing 0, 1.0, 2.0, 3.0 and 4.0 $CaCl_2$ gL^{-1}. After drying, the fruits were inoculated with spore suspensions of *Colletotrichum gloeosporioides* (10^6 spores L^{-1}). Calcium chloride applied at varying concentrations did not produce significant effect on anthracnose incidence, but the size of lesion was linearly reduced with increasing Ca concentration. Calcium chloride application as postharvest treatment markedly elevated fruit Ca content especially in the fruit peel, but without influencing the N, P, K and Mg contents. Fruit firmness increased with Ca application while pH, soluble solids concentration and titratable acidity were not affected by the treatment. The effect of anthracnose on firmness, pH, SSC and TA of the fruits were reduced with $CaCl_2$ treatments.

Key words: Pitaya, postharvest quality, postharvest disease, fruit firmness, mineral nutrients.

INTRODUCTION

Red flesh dragon fruit [(*Hylocereus polyrhizus* (F.A.C. Weber) Britton and Rose] is a climbing cactus. In the humid tropics it is very susceptible to many diseases, including anthracnose caused by *Colletotrichum gloesoporioides* both at farm and at postharvest stage (Masyahit et al., 2009; Awang et al., 2010) causing serious yield losses. Traditionally growers normally use chemical fungicides to reduce the problem. Our earlier experiences revealed that application of $CaCl_2$ at

preharvest stage could be an effective technique to manage sweet cherry (Ippolito et al., 2005), peach (Elmer et al., 2007), pear (Mouni et al., 2007) and papaya (Eryani- anthracnose infection in red flesh dragon fruit (Muhd et al., 2011). The effectiveness of Ca in reducing fungal diseases were reported for many fruit species such as Raqeeb et al., 2009). Calcium plays important roles in the physiological and biochemical processes in plants, and essential macronutrient for plant to complete its growth cycle including fruit development (Hepler, 2005). Calcium reacts as co-factor of some enzymes in hydrolysis of adenosine triphosphate (ATP) and as second messenger in metabolic and genetic regulation (Jeter and Roux, 2006). Calcium contributed to fruit firmness (Ferguson and Boyd, 2001; Sudha et al., 2007), fruit respiration (Bhattarai and Gautam, 2006), acidity of fruit and soluble solids content (Wojcik, 2001; Mahmud et al., 2008). The texture of fruit is influenced with Ca presence in the middle lamella of the cell wall (Quiles et al., 2004), and

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fruit with low Ca are generally poor in its quality Serrano et al. (2002). Deficiency in Ca can accelerate fruit ripening and reduce suitability for storage (Agusti et al., 2004), and this might affect the fruit quality para-meters such as sugar content, pH and acidity. Fruit is an organ with high metabolic rate and its growth is dependent on continuous supply of Ca. However, Ca concentration in fruit decreases as the fruit grows (Narain et al., 2001; Saure, 2005), which may link to dilution of Ca in fruit tissues following cell growth (Saure, 2005), competition for Ca with other organs (Malone et al., 2002), effectiveness of its transportation (White, 2001) including antagonism with other ions such as K^+ and NH_4^+ at the site of absorption and translocation (Mengel et al., 2001), and lack of available Ca in the soil (Kadir, 2005).

Realising the importance of Ca in fruits and the possibility of it deficiency, Ca application during postharvest has been intensively studied. Postharvest Ca application effectively increases fruit Ca content in both pericarp and mesocarp of the fruit (Ippolito et al., 2005). Postharvest application to increase Ca fruit content is more effective than the soil application (Singh et al., 2007). Calcium application enhance fruit quality by slowing down the fruits ripening processes (Kadir, 2004), maintains firmness and reduces respiration, and increases the fruit marketability (Ishaq et al., 2009). Fruits treated with Ca may have a lower cumulative physiological loss in weight (CPLW), soluble solids content (SSC) and titratable acidity but high in carotenoids and calcium contents (Luna-Guzman and Barret, 2000; Alcaraz et al., 2003; Mahmud et al., 2008). The beneficial effects of Ca treatment on red-flesh dragon fruit was reported earlier as Ca produced a positive impact on the firmness of fresh cut fruit during storage (Chuni et al., 2010).

Fruit lacking in Ca would be more susceptible to disease infection (Fallahi et al., 1997). Application of Ca at postharvest stage was also successful in controlling fungal infection. Calcium dip was effective in inhibiting spore germination, thus provided a good control of *C. gloeosporiodes* infection on papaya (Eryani-Raqeeb et al., 2009). Similar effect of Ca was reported to inhibit the growth of *Botryosphaeria dothidea* on apple and *Monilinia fructicola* on peach (Biggs et al., 1997; Biggs, 2004). As the pathogenic fungi use carbon from sugars and acids of the host for their growth and development (Kamilova et al., 2006), and at the same time secreting cell wall degrading enzymes (Prusky et al., 2001), the quality of fungal infected fruit is expected to be reduced. With the possible role of Ca in inhibiting the growth of the fungus, fruit containing high concentration of Ca may be less susceptible to the fungal infection.

This study was conducted to determine the effects of postharvest application of different concentration of calcium chloride on the occurrence of anthracnose and quality of red-flesh dragon fruits.

MATERIALS AND METHODS

Post-harvest Ca application

The fruits used in the study were obtained from a two-year old commercial dragon fruit farm at Pajam, Negeri Sembilan, Malaysia. To ensure uniformity in fruit maturity, well developed flowers of one day after anthesis (DAA) were tagged, and the selected fruits were harvested at fully ripened stage (33 to 34 DAA). After harvesting, the fruits were cooled in the ambient temperature to release farm heat.

Fruits of similar size and free from diseases, microcracks and wound were used for the study. The fruits were washed using tap water and dried in air-conditioned temperature (22°C) in the laboratory. The fruits were then soaked accordingly in five concen-trations of $CaCl_2$ solutions (0, 1.0, 2.0, 3.0 and 4.0 g·L^{-1}) for 30 min, and left overnight on open shelf at the laboratory at room temperature of 22°C. The fruits were then washed again with distilled water and rinsed.

Fungal inoculation

Four fruits for each plot were artificially wounded with a cork borer (0.5 cm diameter) and inoculated with 10^6 spores mL^{-1} of *C. gloeosporioides*. Although the pathogen can infect the tissue without wounding, but the process is necessary in order to have a more uniform infection among treatments. The control fruits were 'inoculated' with distilled water. The fruits were placed in moisturized plastic trays, covered with cling-films, and incubated for three days after which the disease incidence (% of fruits infected) and size of lesions of the infected fruits were then evaluated.

Determination of fruit firmness, soluble solids content (SSC), titratable acidity (TA) and pH

The measurement techniques employed in this study to measure fruit firmness, SSC, TA and pH were similar as those reported by Muhd Azlan et al. (2011). The firmness of the whole fruit was determined using a texture analyzer (Instron Universal Testing Machine, Model 5543, Instron Corp, USA) by measuring the maximum penetration force (N) required during peel tissue breakage using a 5 mm diameter flat probe.

This was done at two locations for each fruit at 2.0 cm away from the point of inoculation. Digital refractometer meter (Model N-α, Atago, Japan) was used to determine SSC of the fruits by squeezing a few drops of juice of fruits on the prism and the reading was taken immediately. The TA was measured on diluted fruit juice (1 juice: 4 distilled water) prepared by using the same fruits as for the SSC measurement. Ten ml of the diluted juice was titrated with 0.1 N NaOH to pH 8.1 (Model Crison GLP 21, Barcelona, Spain). The TA was calculated and expressed as percentage of citric acid. The pH of the filtered juice prepared using 10 g fruit flesh and made up to 100 ml was also determined using pH meter.

Determination of Fruit N, P, K, Ca and Mg contents

The fruits were divided into flesh and peel portions, and dried at 60°C in an air-circulating oven. 0.25 g of each sample was ground finely and digested in 5 ml sulfuric acid (H_2SO_4) on hot plate at 450°C in a fume chamber for 7 min. 10 ml of hydrogen peroxide (H_2O_2) was then added into the mixtures, and the heating continued for another 4 min. The solution was then made up to 100 ml with

Table 1. Effects of post-harvest $CaCl_2$ treatment on disease incidence of red dragon fruit after inoculation with 10^6 spores.ml^{-1} of *C. gloeosporioides.*

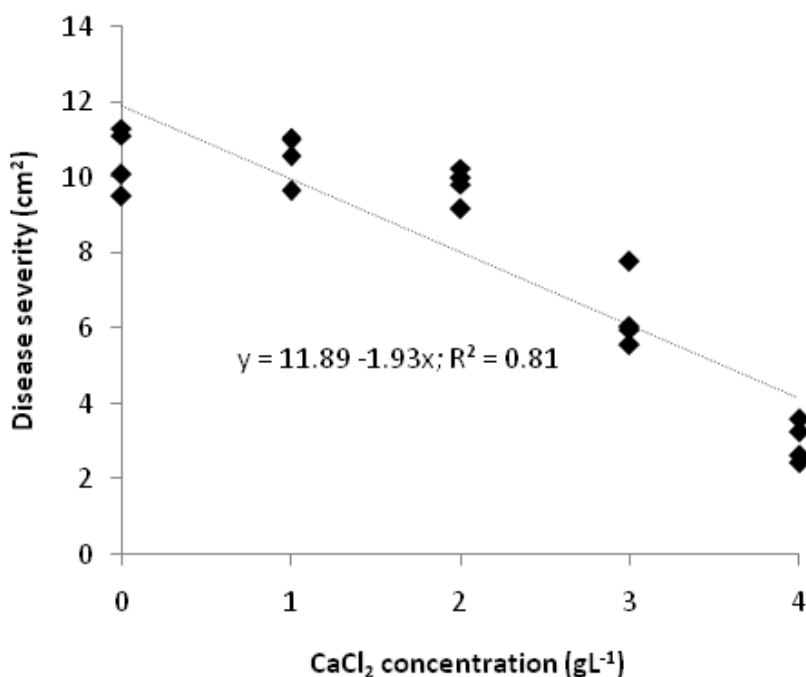| $CaCl_2$ (gL^{-1})	Disease incidence (%)
0 (control)	100
1	100
2	100
3	100
4	100

Figure 1. Relationship between $CaCl_2$ concentration and disease severity of $CaCl_2$ treated fruit at 3 days after inoculated with *C. gloeosporioides* spores (10^6 spores·ml^{-1}).

distilled water and filtered for determination of N and P contents using an auto-analyzer (LACHART Instruments, Model Quikchem IC+FIA 8000 Series, Milwaukee, USA). K, Ca and Mg were measured from the same solution using an atomic absorption spectrophotometer (Perkin Elmer, Model AAS 3110, Palo, Alto, USA) (Muhd Azlan et al., 2011).

Statistical analysis

The study was conducted in a completely randomized design (CRD) with four replications. Data were subjected to analysis of variance (ANOVA) and comparison of mean was performed using Tukey HSD. Regression and correlation analyses were carried out where appropriate.

RESULTS

Effects on disease occurrence

Calcium chloride treatment did not protect the fruits from anthracnose infection. Fruit inoculated with 10^6 spores·ml^{-1} of *C. gloesporioides* exhibited 100% infections after 3 days of incubation (Table 1). However, the size of lesion (infected symptom) on the inoculated fruits was reduced up to 70% as the $CaCl_2$ concentration increased from 0 to 4 gL^{-1} (Figure 1). This result indicated that increased of $CaCl_2$ concentration in the treatment solution could inhibit disease development from the point
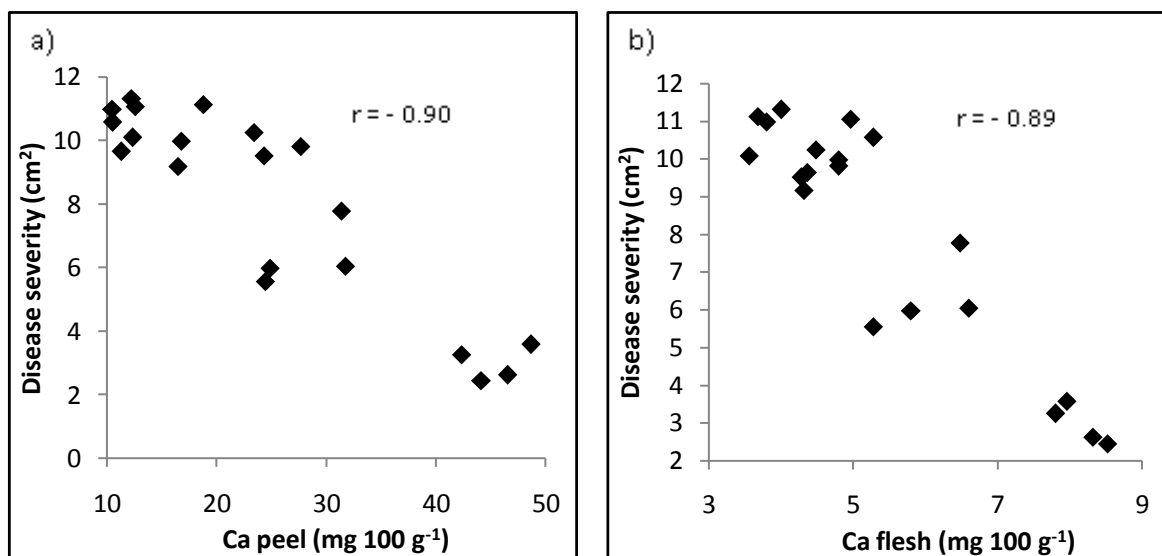
Figure 2. Correlation between disease severity and a) Ca concentration in peel and, b) Ca concentration in flesh of CaCl$_2$ treated fruit at 3 days after inoculated with *C. gloeosporioides* spores (10^6 spores·ml^{-1}).
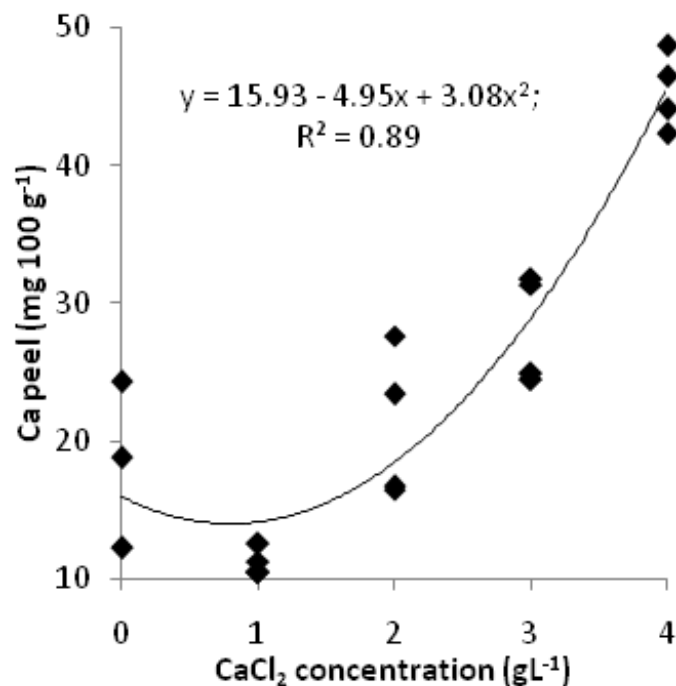

Figure 3. Relationship between CaCl$_2$ concentrations and Ca content of peel of red flesh dragon fruit.

of inoculation, thus may reduce the overall severity of the disease. Significant effects of post-harvest CaCl$_2$ treatment were shown at higher concentrations, but not at concentration of less than 2 gL^{-1}. The size of lesion

caused by *C. gloeosporioides* was negatively related with the content of Ca in the peel and flesh of the fruits, respectively (Figure 2).

Effects on fruit Ca content

Increased CaCl$_2$ concentration in the treatment solutions resulted in higher Ca content both in the peel and flesh of the fruits (Figures 3 and 4). On the average, the Ca content in the peel was higher compared with those in the flesh. Results in Figure 4 show the peel with CaCl$_2$ of 4 gL^{-1} contained the highest Ca content (45.41 mg 100 g^{-1}) followed by treatments at 3 gL^{-1} (28.11 mg 100 g^{-1}), 2 gL^{-1} (21.08 mg 100 g^{-1}), 1 gL^{-1} (11.21 mg 100g^{-1}) and 0 gL^{-1} (16.92 mg 100 g^{-1}). Calcium content in the flesh also showed an increasing trend with the increased CaCl$_2$ concentration in the treatment solutions. Ca content in the flesh ranged from 3.88 to 7.65 mg 100 g^{-1}.

Effects on N, P, K and Mg contents

The contents of N, P, K and Mg in peel and flesh of fruits were not affected by CaCl$_2$ treatments (Table 2). The N and P contents, however, were higher in flesh of fruit compared to the peel. The N content in the flesh range between 151.0 to 173.0 mg 100 g^{-1}, while the P content ranged between 14.3 to 17.5 mg 100 g^{-1}. Meanwhile, the effects of increased Ca treatments on K and Mg content were more apparent in the peel compared to flesh.
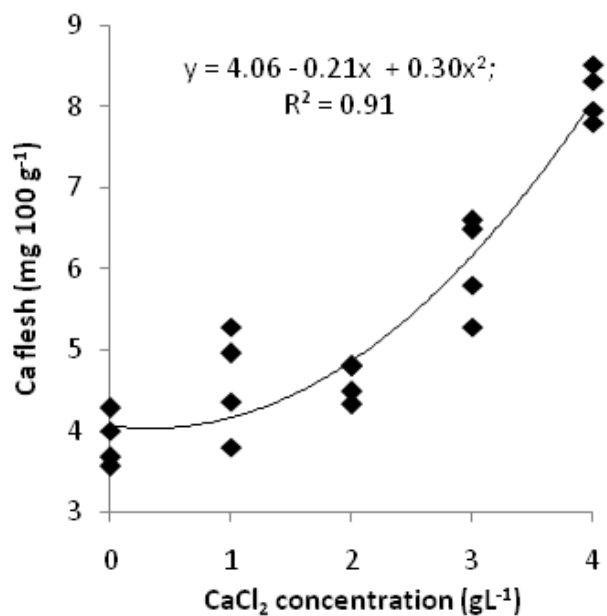
$$y = 4.06 - 0.21x + 0.30x^2;$$
$$R^2 = 0.91$$

Figure 4. Relationship between $CaCl_2$ concentrations and Ca content of flesh of red flesh dragon fruit

Effects on fruits firmness, soluble solids content, titratable acidity and pH

Increasing $CaCl_2$ concentration increased fruit firmness, regardless of fungal infection (Figure 5). The firmness of fruit inoculated with *C. gloeosporioides* ranged from 19.20 to 22.96 N, compared with those inoculated with distilled water which ranged from 22.20 to 23.60 N. The firmness of the infected fruits increased as the $CaCl_2$ concentration in the applied solution increased from 0 to 4 gL^{-1}. Fruit firmness was positively and significantly correlated to Ca content in the peel and flesh as shown positive correlations existed between them as shown in Figure 6).

For the control fruit, the flesh pH was not significantly affected by Ca treatment (Table 3). In contrast, the pH was significantly lowered ($p \leq 0.05$) at higher Ca concentration for the fruit inoculated with *C. gloeosporioides*. Correlation analysis showed that pH was positively correlated with lesion size ($r = 0.86$) and negatively correlated to Ca concentration in fruit tissues (Table 4 and Figure 7).

The SSC of fruit remains constant with Ca treatment, regardless of $CaCl_2$ concentrations in the treatment solution. However, the SSC of the fruit inoculated with *C. gloeosporioides* was significantly lower ($p \leq 0.05$) compared with the untreated fruits (Table 3). Results of correlation analysis showed that SSC was not significantly correlated with the severity of infection, but positively correlated with both Ca in the peel ($r = 0.58$)

and flesh ($r = 0.61$) (Table 4). Titratable acidity was not affected with the treatment. The TA was higher among the inoculated fruits treated with Ca compared with those of the control fruits (Table 3). Results in Table 4 showed that TA and lesion size was negatively correlated ($r = -0.54$), but the TA has a positive correlation with Ca concentration in the peel of fruit ($r = 0.55$). Similar correlation was also shown between TA and Ca in the flesh ($r = 0.62$). Significant correlations were detected between firmness, pH, SSC and TA of fruit (Table 4).

DISCUSSION

Dragon fruit was found to be susceptible to anthracnose caused by *C. gloeosporiodes*. Regardless of $CaCl_2$ concentration, all fruits inoculated with *C. gloeosporiodes* were infected, although $CaCl_2$ treated fruit was reported to reduce fungal disease incidences (Mahmud et al.; 2008; Eryani-Raqeeb et al., 2009). However, $CaCl_2$ treatments were effective in reducing the size of lesion, which may indicate reduction in severity of infection. The result recorded here is in agreement with studies involving other type of fruits; *Botrytis cinerea* on strawberries (Hernandez-Munoz et al., 2006) sweet cherries (Ippolito et al., 2005), and *Alternaria alternate* on pears (Mouni et al., 2007).

The effect of Ca in reducing the disease severity could begin at the initial stage of spore germination. Tian et al. (2002) reported that Ca reduced the germination of *Rhizopus stolonifer* spores on Ca-treated peach, thus reducing the severity of infection. The Ca treatment was also effective in reducing spore germination of *Alternaria alternata* and *Penicillium expansum* on peach (Mouni et al., 2007). Physiologically, the maintenance of low basal concentrations of internal Ca^{2+} is essential for normal cell functions of organisms, and the inability to regulate Ca^{2+} may affect the organisms' normal growth (Biggs, 1999; Eryani-Raqeeb et al., 2009). In this study, increasing $CaCl_2$ concentrations could have increased the free Ca^{2+} in fruit tissues, which may inhibits the growth of *C. gloeosporioides*. Ca^{2+} was reported to inhibit the activity of cell wall degrading enzymes secreted by fungus (Droby et al., 1997).

Reduction in lesion size in Ca-treated fruit could also relate to the role of Ca on fruit cell wall integrity. Ca has a role in fruit texture (Quiles et al., 2004) but it content in the developing fruit declines during maturing process, resulting in the lack of Ca at the end of fruit maturity period (Narain et al., 2001). Deficiency of Ca increases cell membrane permeability which permits ions to escape and lead to the breakdown of intercellular compartmentalization. The escapes of cell wall enzymes such as polygalacturonase and pectin methylesterase would accelerate fruit ripening and softening processes

Table 2. The content of this table is different with the one submitted after reviewer comment sent on 27 May 2011.

Fruit part	CaCl$_2$ (gL^{-1})	Nutrient concentration (mg 100 g^{-1})			
		N	P	K	Mg
Flesh	0 (control)	20.89	1.57	18.03	1.97
	1	23.44	1.59	17.36	2.13
	2	23.84	1.44	18.17	2.04
	3	17.35	1.75	18.58	2.17
	4	20.89	1.65	17.69	2.11
Peel	0 (control)	13.31	1.04	25.83	2.68
	1	11.27	8.39	26.09	2.59
	2	11.40	9.88	25.74	2.66
	3	11.39	1.00	27.37	2.66
	4	12.09	1.13	34.13	2.68
Tukey HSD$_{0.05}$		2.28	0.55	0.95	0.13
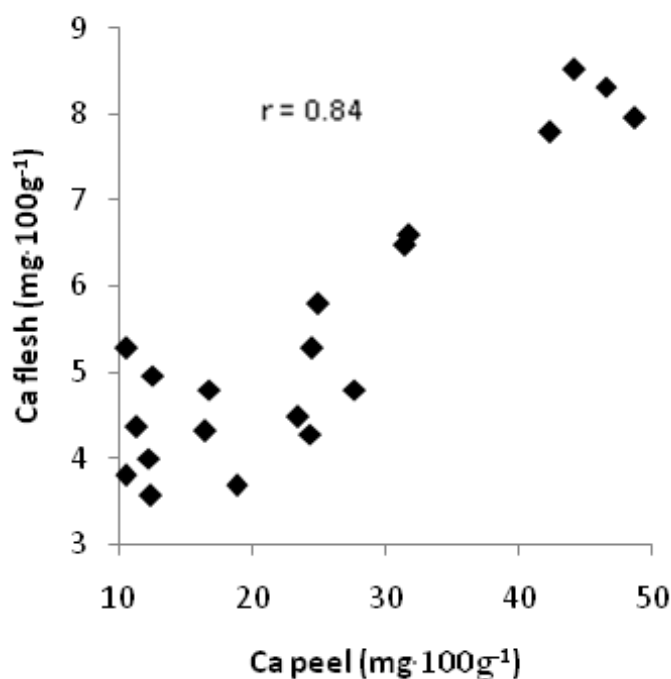
Figure 5. Correlation between Ca content of flesh and peel of Ca treated fruit

(Deytieux-Belleau et al., 2008). This predisposes fruit to fungal attack. Reduction in fungal infection in Ca-treated fruits as observed in this study could be attributed to the increase of cell wall-bound Ca which stabilize cell wall structure (Chardonnet et al., 1999), thus protects it from cell wall degrading enzymes produced by the fungi (Miedes and Lorences, 2006). Calcium increases the mechanical strength of the cell by binding to the carboxyl groups of the pectic homogalacturonan backbone to form cross linkages created by pectin chains. This leads to the
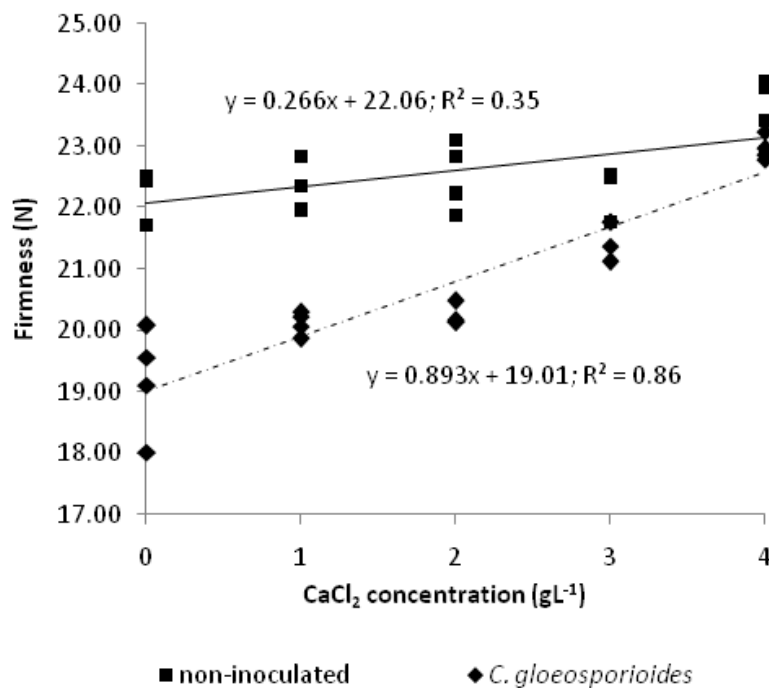
Figure 6. Relationship between fruit firmness and CaCl$_2$ concentration after 3 days of inoculation with *C. gloeosporioides*

formation of insoluble complex that make the cell wall hard for cell wall degrading enzymes to hydrolyse the pectins (Grant et al., 1973), thus protect the pectic backbone from being depolymerised by the enzymes produced by the fungus. In other perspective, plant and fruit are known to produce phytoalexin and phenolics compound as self-defense mechanisms (Andreu et al., 2001). Calcium application increases the synthesis of phytoalexin and phenolics compound thus inhibit the activity of enzymes secreted by the fungus (Rabea et al., 2003).

Calcium chloride treatment increases Ca content in fruit. The Ca content in fruit peel was higher than in the flesh. The peel is the outer most layer of the fruit, thus being first exposed to CaCl$_2$ and therefore it would con-tains higher concentrations of Ca. The result is consistent with the previous study reported for peach (Manganaris et al., 2007) and papaya (Eryani-Raqeeb et al., 2009). Exposing fruit to Ca containing solution created a concentration gradient across the fruit; Ca con-centration in applied solution could be hypertonic to Ca content in fruit. This make Ca transported passively into the fruit (Moraga et al., 2009), thus increasing its Ca content. CaCl$_2$ treatments did not change the concen-trations of N, P, K and Mg in flesh and peel of the fruit; a result which is paralleled to those reported by Tobias et al. (1993). N, P, K and Mg are absorbed by fruits during fruit development, and addition of Ca during post harvest does not affect the level of these nutrients as they are already stored in the fruit. Lack of interaction between Ca and other elements could relate to direct uptake of Ca through the fruit surface (Saure, 2005; Paul and Srivastava, 2006) without competing for the site of absorption and translocation.

A firming effect of Ca treatments on fruit has been reported by Hernandez-Munoz et al. (2006) and Mahmud et al. (2008). Increase in fruit firmness is highly related to the integrity of the tissue cell wall (Ratule et al., 2007). The secretion and activity of cell wall degrading enzymes in the fruit normally increase following fungal infection, and this could lead to loss of firmness. High Ca content in the Ca-treated fruit protects it from severe infection (Saftner et al., 2003; Hernandez-Munoz et al., 2006), and alleviates the effect of disease on fruit firmness. Change of SSC, TA and pH following *C. gloeosporioides* inocula-tion could be attributable to the fungal infection. Fungus secretes cell wall degrading enzymes to break- down the fruit cell wall during infection (Fernando et al., 2001).

One of the most dominant enzymes is pectate lyase, which is responsible for the maceration of fruit tissue leading to decay, and consequently produce symptoms of infection and lesion (Prusky et al., 2001). The secretion and activity of this enzyme are influenced by pH of the host. In order to cope with this problem, NH$_3$ will be

Table 3. Effects of post-harvest $CaCl_2$ on fruit pH, soluble solids content (SSC) and titratable acidity (TA) of red flesh dragon fruits infected by *C. gloeosporioides* after 3 days of incubation.

Inoculum (10^6 spores·mL^{-1})	$CaCl_2$ (gL^{-1})	Quality parameters		
		pH	SSC (%)	TA (%)
Non-inoculated	0	5.34	10.48	0.07
	1	5.30	10.54	0.08
	2	5.32	10.42	0.07
	3	5.27	10.23	0.08
	4	5.28	10.18	0.09
C. gloeosporioides	0	5.66	10.03	0.05
	1	5.64	10.28	0.06
	2	5.61	10.37	0.06
	3	5.57	10.39	0.08
	4	5.47	10.50	0.08
F-test				
Inoculum		**	**	**
$CaCl_2$		**	**	ns
Interaction		ns	**	*
Tukey HSD $_{pooled (0.05)}$		0.20	0.54	0.02

ns, *, ** indicate non-significant and significant at $P < 0.05$ and $P < 0.01$.

Table 4. Correlation coefficients between disease severity, Ca contents and fruit quality attributes following inoculation of *C. gloeosporioides* of red flesh dragon fruit.

Variables	Firmness	pH	SSC	TA
Severity	-0.87**	0.86**	-0.25^{ns}	-0.54**
Ca (peel)	0.83**	-0.84**	0.58**	0.55*
Ca (flesh)	0.87**	-0.80**	0.61**	0.62**
Firmness	1	-0.70**	0.23^{ns}	-0.63**
pH	-	1	-0.18^{ns}	-0.51**
SSC	-	-	1	0.17
TA	-	-	-	1

secreted by the fungus which would act as a buffer, resulting in host tissue alkalinisation which elevates the pH of the fungal inoculated fruit as observed in this study. Calcium source is important for growth and development of fungus (Sati and Bishti, 2006).

The reduction of SSC and TA are associated with the utilization of sugar and acid contents as carbon skeleton sources for the fungus. Kamilova et al. (2006) reported the amount of sugar was reduced approximately by half of concentration of the uninfected tissue of tomato. Therefore, infected fruits would eventually have lower sugar and acid contents. Wang and Galletta (2002) reported that anthracnose infected fruits contained lower SSC and TA than those in the healthy fruit.

Conclusion

Successful uptake and accumulation of Ca in fruit treated with $CaCl_2$ indicated that $CaCl_2$ is a good source to increase fruit Ca content. Increased Ca content in fruit tissue following post-harvest treatment with $CaCl_2$, coupled with the increase in fruit firmness without affecting most of the quality-related parameters, suggest that $CaCl_2$ applied as dipping solution can be utilized in elevating Ca content in red flesh dragon fruit. Furthermore, increasing Ca content has been found to reduce the size of lesion caused by anthracnose on fruit at postharvest stage. Increase fruit firmness following post-harvest Ca application may have an impact on the
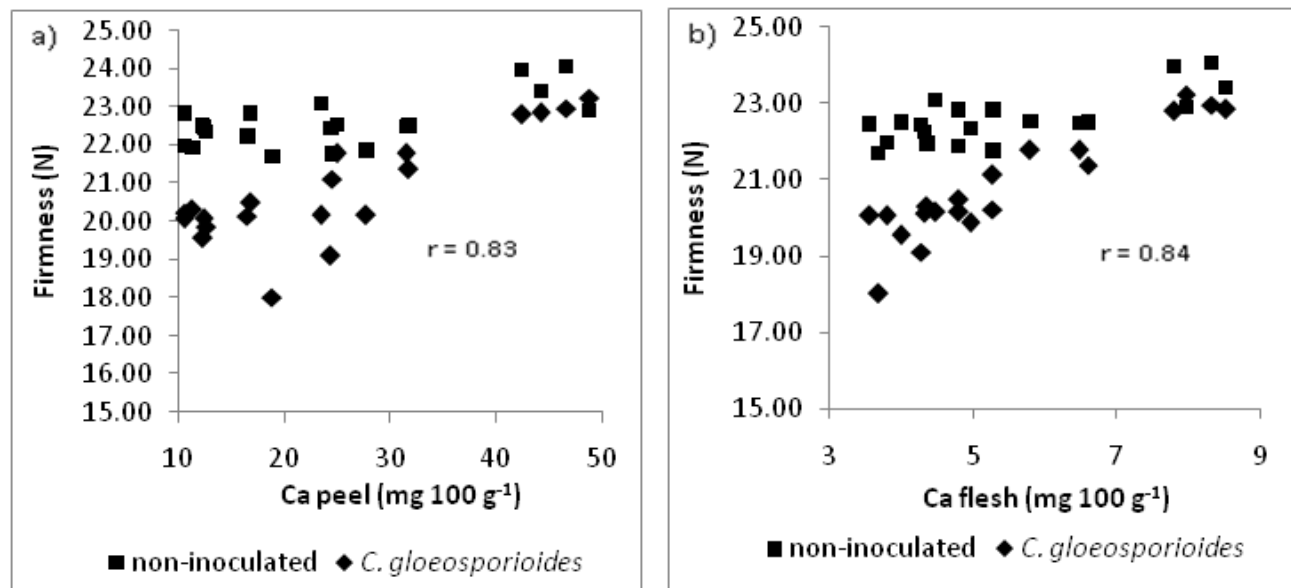
Figure 7. Correlation between (a) Ca concentration in peel and fruit firmness and, (b) Ca concentration in flesh and fruit firmness of CaCl$_2$ treated fruit after 3 days of incubation.

shelf-life and storability of fruits.

REFERENCES

Andreu A, Olivia C, Distel S, Daleo G (2001). Production of phytoalexins, glycoalkaloids and phenolics in leaves and tubers of potato cultivars with different degrees of field resistance after infection with *Phytophthora infestans*. Potato Res., 44: 1-9.
Agusti M, Juan M, Martinez-Fuentes A, Mesejo C, Almela V (2004). Calcium nitrate delays climacteric of persimmon fruit. Ann. Appl. Biol., 144: 65-69.
Alcaraz CF, Alcaraz-Lopez C, Botia M, Riquelme F (2003). Effects of foliar sprays containing calcium, magnesium, and titanium on plum (*Prunus domestica* L.) fruit quality. J. Plant Physiol., 160: 1442-1446.
Awang Y, Ghani MAA, Sijam K (2010). Effects of Collectotrichum gloesporioides and Monilinia fructicola on quality of red flesh dragon fruit (Hylocereus polyrhizus). Acta Hortic. 880:431-438.
Bhattarai DR, Gautam DM (2006). Effect of harvesting method and calcium on post harvest physiology of tomato. Nepal Agric. Res. J., 7: 37-41.
Biggs AR (1999). Effects of calcium salts on apple bitter rot caused by two *Colletotrichum* spp. Plant Dis., 83: 1001-1005.
Biggs AR (2004). Effect of inoculum concentration and calcium salts on infection of apple fruit by *Botryosphaeria dothidea*. Plant Dis., 88: 147-151.
Biggs AR, El-Kholi MM, El-Neshawy S, Nickerson R (1997). Effects of

calcium salts on on growth, polygalacturonase activity and infection of peach fruit by *Monilinia fructicola*. Plant Dis., 81: 399-403.
Chardonnet CO, Sams CE, Conway WS (1999). Calcium effect on the mycelia cell walls of *Botrytis cinerea*. Phytochem., 52: 967-973.
Droby S, Wisniewski ME, Cohen L, Weiss B, Touitou D, Eilam Y, Chalutz E (1997). Influence of CaCl$_2$ on *Penicillium digitatum*, grapefruit peel tissue, and biocontrol activity of *Pichis guilliermondii*. Phytopathol., 87: 310-315.
Deytieux-Belleau, C, Vallet A, Doneche B, Geny L (2008). Pectin methylesterase and polygalacturonase in the developing grape skin.Plant Physiol. and Biochem., 46: 638-646.
Elmer PAG, Spiers TM, Wood P N (2007). Effects of pre-harvest foliar calcium sprays on fruit calcium levels and brown rot of peaches. Crop Protect., 26: 11-18.
Eryani-Raqeeb AA, Mahmud TMM, Syed Omar SR (2009). Effects of calcium and chitosan treatments on controlling anthracnose and postharvest quality of papaya (*Carica papaya* L.). Int. J. Agric. Res., 4: 53-68.
Fallahi E, Conway WS, Hickey KD, Sams CE (1997). The role of calcium and nitrogen in post harvest quality and disease resistance of apples. HortSci., 32: 831-835.
Ferguson IB, Boyd LM (2001). Inorganic nutrients and fruit quality. In: Knee, M. (ed.), Fruit quality and its biological basis. CRC Press, Sheffield, UK. pp. 17-45
Fernando THPS, Jayasinghe CK, Wije Sundera, RLC (2001). Cell wall degrading enzyme secretion by *Colletotrichum acutatum*, the causative fungus of secondary leaf fall of *Hevea brasiliensis*. Mycol. Res., 105: 195-201.
Hepler PK (2005). Calcium: A central regulator of plant growth and development. Plant Cell, 17: 2142-2155.
Hernandez-Munoz P, Almenar E, Ocio MJ, Gavara R (2006). Effects of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria* x *ananassa*). Postharvest Biol. Tech., 39: 247-253.
Grant GT, Morris ER, Rees DA, Smith PJC, Thom (1973). Biological interactions between polysaccharides and divalent cations: The egg-box models. FEBS Lett., 32: 195-198.
Ippolito A, Schana L, Pentimone I, Nigro F (2005). Control of post har-

vest applications of sweet cherries by pre- and postharvest applications of *Aureobasidium pullulans* in combination with calcium chloride or sodium bicarbonate. Postharvest Biol. Tech., 36: 245-252.

Ishaq S, Ahmed Rathore H, Masud T, Ali S (2009). Influence of post harvest calcium chloride application, ethylene absorbent and modified atmosphere on quality characteristics and shelf life of apricot (*Prunus armeniaca* L.) fruit during storage. Pak. J. Nutr., 8: 861-865.

Jeter CR, Roux SJ (2006). Plant responses to extracellular nucleotides: Cellular processes and biological effects. Purinergic Signal., 2: 443-449.

Kadir SA (2004). Fruit quality at harvest of 'Jonathan' apple treated with foliarly-applied calcium chloride. J. Plant Nutr., 27: 1991-2006

Kadir SA (2005). Influence of preharvest calcium application on storage quality of 'Jonathan' apples in Kansas. Trans. Kansas Acad. Sci., 108: 129-137.

Kamilova F, Kravchenko LV, Shaposhnikov AI, Makarova N, Lugtenberg B (2006). Effects of the tomato pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* and of the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the composition of organic acids and sugar in tomato root exudate. Amer. Phytopathol. Soc., 19: 1121-1126.

Luna-Guzman I, Barrett DM (2000). Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. Postharvest Biol. Tech., 19: 61-72.

Mahmud TMM, Eryani-Raqeeb AA, Syed Omar SR, Mohamed Zaki AR, Abdul Rahman AE (2008). Effects of different concentrations and applications of calcium on storage life and physicochemical characteristic of papaya (*Carica papaya* L.). Am. J. Agric. Biol. Sci., 3: 526-533.

Malone M, White P, Angela Morales M (2002). Mobilization of Ca in glasshouse tomato plants by localized scorching. J. Exp. Bot., 53: 83-88.

Manganaris GA, Vasilakakis M, Diamantidis G, Mignani I (2007). The effect of postharvest calcium application on tissue calcium concentration, quality attributes incidence of flesh browning and cell wall physicochemical aspects of peach fruits. Food Chem., 100: 1385-1392.

Masyahit M, Kamaruzaman S, Yahya A, Ghazali M (2009). First report of the occurrence of the anthracnose disease caused by *Colletotrichum gloeosporiodes* (Penz.) Penz. & Sacc. on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia. Am. J. Appl. Sci., 6: 902-912.

Mengel K, Kirkby EA, Kosegarten H, Appel T (2001). Principles of Plant Nutrition (5th ed.). Dordrecht, Netherlands, Kluwer Academic Publishers

Miedes E, Lorences EP (2006). Changes in cell wall pectin and pectinase activity in apple and tomato fruits during *Penicillium expansum* infection. J. Sci. Food Agric., 86: 1359–1364.

Moraga MJ, Moraga G, Fito PJ, Martinez-Navarrele N (2009). Effect of vacuum impregnation with calcium lactate on the osmotic dehydration kinetics and quality of osmodehydrated grapefruit. J. Food Eng., 90: 372-379.

Mouni A, Lamarti A, Aidoun A, Khaddor M, Badoc A (2007). Effect of benzimidazole fungicides and calcium chloride on *Alternaria alternata* and *Penicillium expansum* rot during storage of pears. Afr. J. Biotech., 6: 1289-1292.

Muhd AAG, Yahya A, Kamaruzaman S (2011). Disease occurrence and fruit quality of pre-harvest calcium treated red flesh dragon fruit (*Hylocereus polyrhizus*). Afr. J. Biotech., 10:1550-1558.

Narain N, Bora PS, Holschuh HJ, Vasconcelos MA, Da S (2001). Physical and chemical composition of carambola fruit (*Averrhoa carambola* L.) at three stage of maturity. Ciencia Tech. Aliment., 3: 144-148.

Paul V, Srivastava GC (2006). Role of surface morphology in determining the ripening behaviour of tomato (*Lycopersicon esculentum* Mill.) fruits. Scientia Hortic., 110: 84-92.

Prusky D, McEvoy JL, Levrentz B, Conway WS (2001). Local modulation of host pH by *Colletotrichum* species as a mechanism to increase virulence. Mol. Plant-Microbe Inter., 14: 1105-1113.

Quiles A, Hernando I, Perez-Munuera I, Llorca E, Larrea V, Lluch MA (2004). The effect of calcium and cellular permeabilization on the structure of the parenchyma of the osmotic dehydrated 'Granny Smith' apple. J. Sci. Food Agric., 84: 1765-1770.

Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut W (2003). Chitosan as antimicrobial agent: applications and mode of action. Am. Chem. Soc., 4: 1457-1465.

Ratule MT, Osman A, Saari N, Ahmad SH (2007). Microstructure of peel cell wall and selected physico-chemical characteristics of 'Berangan' banana (*Musa* cv. Berangan [AAA]) ripened at high temperature. Asia Pacific J. Mol. Biol. Biotech., 15: 8-13.

Saftner RA, Bai J, Abbott JA, Lee YS (2003). Sanitary dips with calcium propionate, calcium chloride or a calcium amino acid chelate maintain quality and shelf stability of fresh-cut honeydew chunks. Postharvest Biol. Tech., 29: 257-269.

Sati SC, Bishti S (2006). Utilization of various carbon sources for the growth of waterborne conidial fungi. Mycol. Soc. Am., 98: 678-681.

Saure MC (2005). Calcium translocation to fleshy fruit: its mechanism and endogenous control. Scientia Hortic., 105: 65-89.

Serrano M, Amoro A, Pretel MT, Martinez-Madrid MC, Madrid R, Romojaro F (2002). Effect of calcium deficiency on melon (*Cucumis melo* L.) texture and glassiness incidence during ripening. Food Sci. Tech. Int., 8: 147-154.

Singh R, Sharma RR, Tyagi SK (2007). Pre harvest foliar application of calcium and boron influences physiological disorders, fruit yield and quality of strawberry (*Fragaria* x *ananassa* Duch.). Scientia Hortic., 112: 215-220.

Sudha R, Amutha M, Baby RW, Indira K, Mareeswari P (2007). Influence of pre- and post-harvest chemical treatments on physical characteristics of sapota (*Achras sapota* L.) Var. PKM 1. Res. J. Agric. Biol. Sci., 3: 450-452.

Chuni SH, Yahya A, Mahmud TMM (2010). Cell wall enzymes activities and quality of calcium treated fresh-cut red flesh dragon fruit (Hylocereus polyrhizus). Int. J. Agric. Biol., 12: 713-718.

Tian SP, Fan Q, Jiang AL (2002). Effects of calcium on biocontrol activity of yeast antagonists against the postharvest fungal pathogen *Rhizopus stolonifer*. Plant Pathol., 51: 352-358.

Tobias RB, Conway WS, Sams CE, Gross KC, Whitaker BD (1993). Cell wall composition of calcium-treated apples inoculated with *Botrytis cinerea*. Phytochem., 32: 35-39.

Wang SY, Galletta GJ (2002). Compositional change in *Colletotrichum* (anthracnose) infected strawberry fruit. Acta. Hortic., *567*: 815-819.

White PJ (2001). The pathways of Ca movement to the xylem. J. Exp. Bot., 52: 891-899.

Wojcik P (2001). "Dabrowicka prune" fruit quality as influenced by calcium spraying. J. Plant Nutr., 24: 1229-1241.