Efficacy of CaCl₂ against some important postharvest fungi on orange, chilli and Cavendish banana fruits

Hiệu quả của CaCl₂ đối với một số loại nấm quan trọng gây hại sau thu hoạch trên trái cam, ớt và chuối già

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Fruit rot caused by Aspergillus niger or Colletotrichum musae is an important post-harvest disease on orange, chilli and Cavendish banana fruits. The use of synthetic fungicides has been a traditional strategy for the management of the fruit rot disease, but these chemicals adversely affect human health and environment. Therefore, the objective of this study was to evaluate the effects of CaCl₂ on in vitro hyphal growth and in vivo lesion inhibition. First, aqueous solutions of CaCl₂ at three concentrations of 20, 40 and 60 mM were assessed for their inhibitory effect against hyphal growth in vitro. Next, mature fruits were immersed into a solution of 20 mM CaCl₂ for 20 - 30 s, then inoculated by a pathogen suspension at the density of 10⁶ conidia mL⁻¹ and observed for 12 days. The results showed that 20 mM CaCl₂ was the most effective concentration in antifungal assay to Aspergillus isolated from orange rot. The treatment of CaCl₂ continued to gain efficacy on limiting lesions’ development on orange fruits until 12 days after inoculation (DAI). On chilli, CaCl₂ at concentrations of 20 and 40 mM inhibited well on the growth of Aspergillus hypheae isolated from chilli rot. However, calcium treatment was not effective on chilli fruits. On Cavendish banana, solutions of CaCl₂ at concentrations of 20, 40 and 60 mM highly limited fungal growth of Colletotrichum in vitro conditions. The application of CaCl₂ solution could inhibit anthracnose lesion length of Cavendish banana variety, but its efficacy did not prolong until 6 DAI. In general, the good results were obtained from the 20 mM CaCl₂ in almost all the studied assays. Management of rot diseases on fruits by employing 20 mM CaCl₂ could be suitable to replace the current hazardous agro-chemicals.

1. Introduction

The world fruit and vegetable production has quickly increased several folds over the last decades, applies to many kinds of the major crops including orange, chilli, banana, cucurbits, tomato, and cabbage, according to FAO reports (FAO, 2008). The rapid production of fruit and vegetable has occurred for several reasons. Nutritional and medicinal researches elucidate the value and role of these foods in protecting human health. Fruits and vegetables provide essential nutrients as: carbohydrates, vitamins, minerals and fibres. Moreover, the current active global market and internationally intense import/export businesses make it possible for fresh agricultural products which cultivate in one part of the world to be quickly shipped and available to consumers anywhere else in the world. Increased production and business agreements always place a high pressure on farmers that they cannot

Keywords: Aspergillus niger, Colletotrichum musae, hyphal development, lesion inhibition

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allow diseases to affect their harvest products. Fruit rot caused by *Aspergillus niger* Tiegh. or *Colletotrichum musae* (Berk. & Curtis) Arx. is the most important post-harvest disease on fruits (Sarkar, 2016; Dashora and Sharma, 2018; Lema et al., 2018). Average post-harvest losses to these fungi have been estimated at approximately 20% at developed countries, and up to 50% at developing countries (Janisiewicz and Korsten, 2002; Florkowski et al., 2009).

In Vietnam, despite several advances of the production and disease management on fruits and vegetables, farmers often face many challenges (Hue and Nghiem, 2014). Fruit rot caused by *A. niger* plays a major role on losses of orange and chilli production from fields to storage houses (Long, 2012; Xuyen, 2012). *A niger* also causes rot diseases on other fruits and vegetables such as mango, lemon, grapefruit, onion and garlic (Hocking, 2006; Liaquat et al., 2016). This fungi species not only cracks and causes damage on fruit epidermis but also secretes aflatoxins, a group of harmful carcinogenic mycotoxins. These toxic compounds are heat-stable and are non-degradable by a variety of food processing procedures. Especially, low-level exposure to aflatoxins may lead to a suppression of the immune system and increase susceptibility to diseases in humans (Pestka and Bondy, 1994). In banana, anthracnose caused by *C. musae*, usually produces black and brown spots on banana peels, leading to low price and severe economic losses (Lassois et al., 2010). Furthermore, *C. musae* was reported as the popular post-harvest pathogenic fungus in Cavendish bananas in Vietnam (Hang, 2012).

Many strategies are available for managing pathogens in fresh agricultural products. On the first strategy, application of waxes to fruit surfaces was researched. Waxing could improve the fruit appearance, reduces spoilage due to chilling injury, decreases the respiration and transpiration rates and protects fruit epidermis against infection by fungal pathogens. However, waxes could not improve the quality of fruits. Another major disadvantage of using wax for coating the fruits is the development of off-flavor (Sharma and Singh, 2000). In another strategy, UV-C light was applied for managing diseases on fruits and vegetables. The optimum dose and the time required to achieve maximum protection after UV-C treatment against plant pathogens vary depending on the nature of products (D'hallewin et al., 1999). Besides, fruit diseases could be managed using beneficial pathogens. Thanh et al. (2016) indicated that beneficial *Streptomyces fradiae* and *Bacillus polyfermenticus* were capable of inhibiting fungal growth of *Neoscytalidium dimidiatum* causing brown spot disease on dragon fruits in Vietnam. Efficacy of heat treatments and other disease management strategies has also been assessed (Palou et al., 2001; Awang et al., 2011; Mahmud et al., 2008; Ayon-Reyna et al., 2017; Netravati and Jagadeesh, 2018). The efficacy of different heat procedures (aqueous immersion of oranges in water at up to 75 °C for 150 s; 2-4% sodium carbonate aqueous solution at 45 °C for 60 - 150 s, or 1-4% sodium bicarbonate aqueous solution at room temperature for 150 s followed by storage at 20 °C for 7 days) was determined in order to control blue mold disease of oranges caused by *Penicillium italicum*. The incidence of green mold caused by *P. digitatum* was reduced to approximately 1-12%, whereas the untreated fruits were entirely (100%) infected by green mold disease (Palou et al., 2001). Last but not least, calcium treatments have been reported to retain fruit firmness in various agricultural products, including apple (Conway et al., 1994), custard apple (Netravati and Jagadeesh, 2018), peach (Manginaris et al., 2007; Sohail et al., 2015; Gayed et al., 2017), persimmon (Bagheri et al., 2015), pomegranate (Mirdehghan and Ghotbi, 2014), blackberry (Turmanidze et al., 2016), cape gooseberry (Reyes-Medina et al., 2017), tomato (Arthur et al., 2015) and lemon (Valero et al., 1998). Soaking the dragon fruits for 40 min in solutions of CaCl₂ significantly increased the fruit Cu content in the fruit peels, leading to reduce the size of anthracnose lesions (Awang et al., 2011). In mango, solutions containing various concentrations of CaCl₂ could delay the fruit ripening (Mahmud et al., 2008). However, the values of flavors and taste of the mango fruits at ripening were 4.0, 3.0, 2.5 and 2.25 at 2.5, 5.0, 7.5 and 10% CaCl₂, respectively. Treatment of CaCl₂ or combination of hot water - CaCl₂ could reduce mycelial growth and germination of *C. gloeosporioides* in vitro as well as delay anthracnose symptom on papaya fruits (Madani et al., 2016; Ayon-Reyna et al., 2017). In addition, CaCl₂ at a concentration of 4% could reduce the severity of infection from different fungi including *Alternaria alternata*, *Alternaria solani*, *A. niger*, *Botrytis cinerea*, *Fusarium solani* and *Rhizopus stolonifer* on guava fruits (Hassanein et al., 2018). On calcium treated-fruits, the association between firmness retention and reduced rot incidence suggests that calcium might affect both these processes simultaneously through its cellular role in strengthening fruit cell walls (Fallahi et al., 1997; Conway et al., 1999). The major advantage of calcium treatment on fruits is the safety to human health and avoidance of environmental pollutions.

Current researches on preventing *Aspergillus* fruit rot on orange and chilli fruits, as well as anthracnose on Cavendish banana fruits have not been carried out with CaCl₂ yet. Therefore, the objective of this study was to assess the efficacy of CaCl₂ treatment on growth of hyphae and on the diameter of rot lesions on fruits.

2. Materials and methods

2.1 Materials

2.1.1. Fungal strains and culturing

Two samples of virulent strains of *A. niger* isolated from orange and chilli fruits, and one virulent strain of *C. musae* isolated from banana anthracnose lesions were cultivated at Department of Plant Protection, College of Agriculture and Applied Biology, Can Tho University. The fungi *A. niger* and *C. musae* were isolated from infected fruits and identified by molecular analysis (Hang, 2012; Long, 2012; Xuyen, 2012). The fungi were prepared on Potato Dextrose Agar.
inoculation with 2.3.

Orange of A. niger was (Dhinggra and Sinclair, 1995).

Diameter at 55 °C with relative humidity approximately 90%.

2.1.2. Chemicals

CaCl₂·2H₂O (Catalogue No. 1023820500, purity ≥95%, Merck, Germany) was provided from Department of Plant Protection, Can Tho University.

Prior to this research, different concentrations of CaCl₂·2H₂O had been quickly screened to evaluate their effects on spore germination of A. niger and C. musae. Subsequently, CaCl₂·2H₂O at 20, 40 and 60 mM were chosen for this research because it gave the high efficacy in inhibiting spore germination.

2.2. Assessment of CaCl₂-efficacy on hyphal development of post-harvest fungi in vitro conditions

The experiments were carried out in completely randomized design (CRD), with four treatments including CaCl₂ at three different concentrations (20, 40, and 60 mM), and a water control treatment with six replications, one petri dish for each replication. Separated experiment was done with A. niger isolated from orange and chilli fruits, with C. musae of banana anthracnose. Experimental steps conducted were followed the procedure of Dhinggra and Sinclair (1995), Mahmud et al. (2008), and Hajano et al. (2012).

The aqueous solutions of CaCl₂ was prepared according to treatment above description. To assure complete solubility, each sample of aqueous CaCl₂ was magnetically stirred for 30 min. The CaCl₂ solutions were filtered through Whatman papers with pore size of approximately 0.2 µm. The filtered solutions were then poured into the medium of PDA at 55-60 °C for 2 min and gently shaken. Approximately 10 mL of each obtained mixture medium was immediately poured into Petri dishes. After media solidification, a hyphal round slice of fungi at approximately 5 mm diameter was placed at in the center of each petri dish (Dhinggra and Sinclair, 1995). Diameter of fungal colony was measured at 48, 72 and 96 hours for experiments with A. niger, and at 24, 48 and 72 hours for C. musae.

Each experiment was performed 3 times. Based on results of in vitro experiments, an effective concentration of 20mM of CaCl₂ was chosen to conduct following experiments on orange, chilli and Cavendish banana fruits.

2.3. Assessment of treating CaCl₂ at an effective concentration on fruits before inoculation with fungal suspension

The experiment was done in CRD with two treatments including CaCl₂-treated and control treatments, with 12 replications, one fruit per one replication, four inoculated points per one orange fruit, one inoculated point per one chilli or banana fruit.

Experimental fruits including orange, chilli and Cavendish banana were chosen, and surface-disinfected with 95% ethanol (v/v) for one min, and washed two times with sterile distilled water to remove the alcohol residue. Next, the fruits were immersed in a solution of CaCl₂ for 20-30s, then, air-dried for 1-2 h at room temperature. On the untreated control, the fruits were handled identically, but sterile distilled water was used instead of CaCl₂ solution. After that, a bunch of sterile needles was used to create tiny holes with a depth of 2 mm on fruit epidermis, with four positions on orange fruits, one position on chilli or Cavendish banana fruits. One ml of fungal spore suspension at a density of 10⁶ conidia mL⁻¹ was dropped on these tiny holes.

Inoculated fruits were stored in an incubation chamber at 25 °C with relative humidity approximately 98% for 24 h. Finally, inoculated fruits were transferred into transparent nylong bags with wet cotton inside, at room temperature (Sivakumar et al., 2002; Cao et al., 2008; Talibi et al., 2011; Yu et al., 2012) to observe the disease symptoms.

Length of Aspergillus rot lesions was recorded at 8, 10 and 12 days after inoculation (DAI) (on orange fruits), 4, 6 and 8 DAI (on chilli fruits). Separated experiment on orange or chilli fruits was repeated three times.

Anthracnose lesions length on banana fruits were recorded at 5, 6 and 7 DAI (De Costa and Erabadupitiya, 2005). The experiment was done twice.

2.4. Statistical analysis

Data were subjected to an analysis of variance using SPSS 16.0 software package (IBM, USA). Individual comparisons between mean values were performed using Duncan’s Multiple Range Test (DMRT) or t-test with a magnitude of p value at 0.05.

3. Results and discussion

3.1 Effect of CaCl₂ solutions against Aspergillus niger on orange fruits

3.1.1 Efficacy of CaCl₂ on Aspergillus hyphal development in vitro

Each tested concentration of CaCl₂ showed different fungal activity in vitro conditions (Table 1 and Figure 1). Results for the individual concentration varied during three observation time points. The optimal efficacy was observed using
the treatment with as solution of 20 mM CaCl\(_2\). Colonial diameter of the treatment of 20 mM CaCl\(_2\) was 78.2 mm, significantly lower than that of the control sample (85.3 mm) at 96 hours adding putting fungal slices.

Table 1. Efficacy of CaCl\(_2\) on colonial diameter (mm) of Aspergillus niger in vitro

| Treatment          | Time after adding fungal slices (hours) | 48 h | 72 h | 96 h |
|--------------------|----------------------------------------|------|------|------|
| 20 mM CaCl\(_2\)   |                                        | 40.7±5.8\(^c\) | 61.4±7.2\(^c\) | 78.2±6.9\(^b\) |
| 40 mM CaCl\(_2\)   |                                        | 48.0±6.1\(^b\) | 70.8±7.1\(^b\) | 88.0±6.4\(^a\) |
| 60 mM CaCl\(_2\)   |                                        | 56.1±6.3\(^a\) | 77.8±7.4\(^a\) | 84.5±7.2\(^a\) |
| Water control      |                                        | 42.8±5.6\(^c\) | 70.3±6.5\(^b\) | 85.3±7.3\(^a\) |
| Significance       |                                        | *     | *    | *    |
| Coefficient of variance (%) |                                  | 7.26  | 5.38 | 5.41 |

\(^a\) Mean ± SE (standard error) followed by the same letter do not differ significantly according to DMRT at \(p \leq 0.05\).
\(^*\): significant at \(p \leq 0.05\)

Figure 1. Efficacy of 20 mM CaCl\(_2\) on the growth of Aspergillus niger at 96 hours after putting fungal slices.

The round slice of Aspergillus was put at the center of a Petri dish containing PDA medium and solution of CaCl\(_2\) or distilled water. A: The control treatment with distilled water, B: the solution of CaCl\(_2\) at a concentration of 20 mM.

3.1.2 Efficacy of treating CaCl\(_2\) on oranges before an inoculation of Aspergillus suspension

Disease incidence on orange fruits was 100% (data not shown). The effect of CaCl\(_2\) was assessed on the length of rot lesion on orange fruits at three observation time points. The lesion length of calcium treatment was significantly lower than that of the control at 8 DAI, and prolonged until 12 DAI (Table 2 and Figure 2).

The orange fruits were immersed on a solution of 20 mM CaCl\(_2\) first, made tiny holes on orange epidermis at four positions by sterile needles, then inoculated with Aspergillus spore suspension at a density of \(10^6\) conidia mL\(^{-1}\). Control: the orange fruit was treated with sterile distilled water, CaCl\(_2\) 20 mM: the orange fruit was treated with CaCl\(_2\)-solution at a concentration of 20 mM.

Table 2. Efficacy of CaCl\(_2\) treated before Aspergillus inoculation on orange fruits

| Treatment | Days after inoculation | 8 DAI | 10 DAI | 12 DAI |
|-----------|------------------------|-------|--------|--------|
| 20 mM CaCl\(_2\) |                        | 81.92±19.6\(^b\) | 96.17±17.3\(^b\) | 127.83±18.2\(^b\) |
| Non-treated control |                    | 105.5±19.8\(^a\) | 131.17±15.3\(^a\) | 161.00±15.3\(^a\) |

Significance

\(^*\): significant at \(p \leq 0.05\)

Figure 2. Efficacy of CaCl\(_2\) at a concentration of 20 mM on rot lesions caused by Aspergillus niger at 8 DAI (A), 10 DAI (B), 12 DAI (C).

3.2 Effect of CaCl\(_2\) solutions against Aspergillus niger on chilli fruits

3.2.1 Efficacy of CaCl\(_2\) on Aspergillus hyphal development in vitro
The results showed that calcium treatment at a concentration of 20 mM CaCl₂ inhibited hyphal development of *A. niger* during all observation time points. The treatment of 20 mM CaCl₂ had ability from 48 to 96 hours after putting fungal slices, while CaCl₂ at a concentration of 60mM was effective on 72 and 96 hours after putting fungal slice. Therefore, the concentration at 20 mM of CaCl₂ showed a high and stable efficacy than other treatments, and was chosen to do on the following experiment (Table 3).

### Table 3. Efficacy of CaCl₂ on colonial diameter (mm) of *Aspergillus niger in vitro*

| Treatment                  | Time after adding fungal slices (hours) | 48ising % | 72ising % | 96ising % |
|----------------------------|----------------------------------------|-----------|-----------|-----------|
| 20 mM CaCl₂                | 36.80±4.5b                             | 52.50±6.1b| 58.20±6.2b|
| CaCl₂ 2H₂O                | 40.50±5.1a                             | 54.00±5.8b| 62.80±7.2b|
| 60 mM CaCl₂                | 42.00±5.3a                             | 59.80±5.2a| 74.00±7.1a|
| Water control              | 41.20±4.6a                             | 57.50±5.4a| 75.50±8.2a|
| Coefficient of variance (%)| 7.82%                                  | 5.85%     | 10.32%    |

*Mean ± SE (standard error) followed by the same letter do not differ significantly according to DMRT at p ≤ 0.05.

*: significant at p ≤ 0.05

#### 3.2.2 Efficacy of treating CaCl₂ on chilli before an inoculation of *Aspergillus* suspension

Treatment of CaCl₂ at a concentration of 20 mM did not inhibit *Aspergillus* rot lesion on chilli fruits (Table 4).

### Table 4. Efficacy of CaCl₂ treated before *Aspergillus* inoculation on chilli fruits

| Treatment                  | Days after inoculation |
|----------------------------|------------------------|
|                            | 4ising % | 6ising % | 8ising % |
| 20 mM CaCl₂                | 13.51±0.23 | 17.33±0.32 | 20.58±0.24 |
| CaCl₂ 2H₂O                | 10.34±0.19 | 16.76±0.33 | 19.02±0.27 |
| Non-treated control        | ns         | ns       | ns       |
| Coefficient of variance (%)| 15.47%      | 21.78%   | 17.59%   |

*Mean ± SE (standard error) followed by the same letter do not differ significantly according to DMRT at P = 0.05.

*: significant at p ≤ 0.05; ns: non-significant at p ≤ 0.05

#### 3.3 Effect of CaCl₂ solutions against *Colletotrichum musae* on Cavendish banana fruits

3.3.1 Efficacy of CaCl₂ on *Colletotrichum* hyphal development *in vitro*

Efficacy of CaCl₂ was determined by colonial diameters of *Colletotrichum musae* during three observation time points at 24, 48 and 72 hours after *in vitro* culture. At the time point of 24 h, all treatments of three concentrations of CaCl₂ had small colonies ranged from 8.66 mm to 10.00 mm, significantly lower than that of the control treatment of approximately 12.50 mm. At two following observation time points of 48 and 72 h, only CaCl₂ at a concentration of 20 mM was effective on inhibiting development of *Colletotrichum* colony, compared to those of control one (Table 5).

### Table 5. Colonial diameter (mm) of *Colletotrichum musae in vitro* condition

| Treatment                  | Time after adding fungal slices (hours) | 24ising % | 48ising % | 72ising % |
|----------------------------|----------------------------------------|-----------|-----------|-----------|
| 20 mM CaCl₂                | 8.66±2.3bc                            | 29.67±4.5b| 58.50±12.1c|
| CaCl₂ 2H₂O                | 10.00±2.7abc                          | 33.83±4.8a| 63.67±11.3a|
| 60 mM CaCl₂                | 9.17±1.9bc                            | 34.33±5.2a| 63.17±9.3bc|
| Water control              | 12.50±1.8bc                           | 34.00±5.7a| 60.00±11.2bc|
| Coefficient of variance (%)| 12.41                                  | 5.63      | 5.79      |

*Mean ± SE (standard error) followed by the same letter do not differ significantly according to DMRT at p ≤ 0.05.

*: significant at p ≤ 0.05

The effective concentration of CaCl₂ at 20 mM was chosen to carry out assays on Cavendish banana fruits.

3.3.2 Efficacy of treating CaCl₂ on Cavendish bananas before an inoculation of *Colletotrichum*

Lesion lengths of CaCl₂-treatment were short at approximately 11.92 mm, and statistically significant to those of control at 13.00 mm at 5 DAI. However, efficacy of CaCl₂ did not prolong to 6 and 7 DAI (Table 6 and Figure 3).

### Table 6. Efficacy of CaCl₂ treated before *Colletotrichum* inoculation on Cavendish banana fruits

| Treatment                  | Days after inoculation |
|----------------------------|------------------------|
|                            | 5ising % | 6ising % | 7ising % |
| 20 mM CaCl₂                | 11.92±3.4b | 17.42±3.5 | 23.67±4.2 |
| CaCl₂ 2H₂O                | 13.00±3.6b | 18.25±3.2 | 25.08±3.8 |
| Non-treated control        | ns         | ns       | ns       |
| Coefficient of variance (%)| 11.12      | 12.22    | 11.53    |

*Mean ± SE (standard error) followed by the same letter do not differ significantly according to DMRT at P = 0.05.

*: significant at p ≤ 0.05; ns: non-significant at p ≤ 0.05
The banana fruits of Cavendish were immersed on a solution of 20 mM CaCl₂ at approximately 20 s, air-dried for 2 h. Tiny holes were created by sterile needles with a depth of 2 mm on banana epidermis. One mL of Colletotrichum spore suspension at a density of 10⁶ conidia mL⁻¹ was dropped on these tiny holes. CaCl₂: the banana fruit was treated with CaCl₂ at a concentration of 20 mM, Control: the banana fruit was treated with distilled water.

4. Discussion

At in vitro conditions, the results showed that 20 mM CaCl₂ significantly inhibited the growth of the pathogenic fungi A. niger and C. musae, better than CaCl₂ solutions at 40 and 60 mM. Calcium solution at an optimal concentration was required for the inhibition of hyphae, whereas low or high concentrations could non-effect on hyphal growth. Fungal cells exposed to the optimal concentration increased their calcium level in the cytosol, leading to alter the osmotic balance and be toxic to the fungal cells (Ali-Eryani-Raqueeb et al., 2009). The cell walls of fungal pathogens were rich in neutral sugars. When a low concentration of CaCl₂ was applied in vitro conditions, exogenous Ca²⁺ stimulated a production of glucosamine, a kind of amino sugars. The glucosamine is beneficial to fungal growth, resulted in failure on fungal inhibition. On the contrary, high calcium concentration reduced neutral sugars, but lead to an increase in uronic acid, Ca, P and Na in fungal cytosol. Uronic acid is a part of fungal cell walls. Besides, elements of Ca, P and Na play important roles in metabolism and energy transfer as well as an integral component of DNA, RNA, coenzymes and membrane phospholipids of fungal cells. Therefore, fungal pathogens could grow in the PDA medium containing a high calcium concentration (Chardonnet et al., 1999; Stosic et al., 2014).

At in vivo conditions, the results showed that treatment of 20 mM CaCl₂ on orange, chili and Cavendish banana reduced the lesions length. The reduction of rot symptom in CaCl₂-treated fruits could be due to calcium content of fruit peels and host-pathogen interaction. On fruits, calcium is mainly associated with the pectic materials. The Ca²⁺ ions could interact with the anionic pectic polysaccharides, coordinating with the oxygen functions of two adjacent pectin chains, and cross-linking the chains (Rose et al., 2003). The calcium binding could reduce the accessibility of cell wall degrading enzymes from Aspergillus fungus to the substrates of fruits. In the research of Marcelle (1995), the authors indicated that calcium treatment could conserve fruit epidermis, inhibit a post-harvest respiratory peak and delay the ripen process of apple fruits. Moreover, CaCl₂ could be treated at 2-3 weeks before harvesting, leading to prolong shelf life of fruit (Sen et al., 2001). On mango and strawberry fruits, soaking or dipping fruits into the solution of CaCl₂ lead to decrease fruit rot (Uthaibutra et al., 1998; Lara et al., 2004). On red-flesh dragon fruits, CaCl₂ applied at various concentrations at 1, 2, 3 and 4 g·L⁻¹ reduced the anthracnose severity. The concentration of CaCl₂ at 4 g·L⁻¹ (approximately 27 mM) gave the best inhibition on anthracnose lesions (Awang et al., 2011). Our results of 20 mM CaCl₂ on this study are in line to results of Awang et al. (2011). Similarly, CaCl₂ was reported to inhibit the in vitro development of C. gloeosporioides in papaya fruits (Ayonen-Reyna et al., 2017). In a recent study, Hassanin et al. (2018) indicated that 4% (w/v) CaCl₂ could use with gamma irradiation to inhibit fungal growth of A. alternata, A. solani, A. niger, B. cinerea, F. solani and R. stolonifer on guava fruits.

Moreover, host-pathogen interaction on CaCl₂-treated fruits could be an important mechanism. The fungal pathogen must first attack the fruit skin, where fungus secretes cell wall degrading enzymes, favouring the fungal infection (Chardonnet et al., 1999). However, the strengthening of the fruit cell walls by Ca²⁺ binding the polygalacturonase, reduce the susceptibility to fungal infection (Rose et al., 2003), leading to slow the development of rot lesions on CaCl₂-treated fruits. In general, CaCl₂ application in adequate amounts supplied helps to maintain orange, chili and banana fruits’ firmness, decrease on an incidence of fungal attack, leading to limit the diameter of rot lesions.

5. Conclusion

The concentration at 20 mM of CaCl₂ well inhibited the hyphal development of both A. niger and C. musae in vitro. However, this concentration of CaCl₂ was effective on inhibiting Aspergillus rot lesion only on orange fruits in vivo conditions. However, a treatment of 20 mM CaCl₂ on chilli or Cavendish banana fruits had a lower efficacy than on orange fruits. The immersion of orange fruits in a solution...
of 20 mM might be a useful strategy to control fruit rot. Future studies are needed to formulate a commercial solution of CaCl₂.

6. References

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