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RESEARCH ARTICLE

Efficacy of $CaCl_2$ against some important postharvest fungi on orange, chilli and Cavendish banana fruits

Hiệu quả của $CaCl_2$ đối với một số loại nấm quan trọng gây hại sau thu hoạch trên trái cam, ớt và chuối già

LE, Thanh Toan*; VO, Trong Ky; NGUYEN, Thi My Linh; TRIEU, Phuong Linh; NGO, Van Toan; NGUYEN, Huy Hoang

College of Agriculture and Applied Biology, Can Tho University, 3/2 Str., Can Tho city, Viet Nam

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 $CaCl_2$ on *in vitro* hyphal growth and *in vivo* lesion inhibition. First, aqueous solutions of $CaCl_2$ 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 $CaCl_2$ for 20 - 30 s, then inoculated by a pathogen suspension at the density of 10^6 conidia mL^{-1} and observed for 12 days. The results showed that 20 mM $CaCl_2$ was the most effective concentration in antifungal assay to *Aspergillus* isolated from orange rot. The treatment of $CaCl_2$ continued to gain efficacy on limiting lesions' development on orange fruits until 12 days after inoculation (DAI). On chilli, $CaCl_2$ at concentrations of 20 and 40 mM inhibited well on the growth of *Aspergillus* hyphae isolated from chilli rot. However, calcium treatment was not effective on chilli fruits. On Cavendish banana, solutions of $CaCl_2$ at concentrations of 20, 40 and 60 mM highly limited fungal growth of *Colletotrichum in vitro* conditions. The application of $CaCl_2$ 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 $CaCl_2$ in almost all the studied assays. Management of rot diseases on fruits by employing 20 mM $CaCl_2$ could be suitable to replace the current hazardous agro-chemicals.

Thối trái do nấm Aspergillus niger hay nấm Colletotrichum musae là bệnh sau thu hoạch thường gặp trên cam, ớt và chuối già. Thuốc trừ nấm tổng hợp là biện pháp truyền thống quản lý bệnh thối trái nhưng lại ảnh hưởng bất lợi đến sức khỏe con người và môi trường. Vì vậy, mục tiêu của nghiên cứu là đáng giá ảnh hưởng của $CaCl_2$ đối với sự sinh trưởng in vitro của nấm và sự ức chế vết bệnh ở điều kiện in vivo. Đầu tiên, dung dịch $CaCl_2$ ở các nồng độ 20, 40 và 60 mM được sử dụng để đánh giá khả năng ức chế sự sinh trưởng in vitro của nấm bệnh. Tiếp theo, trái trưởng thành được nhúng vào dung dịch $CaCl_2$ 20 mM trong 20 - 30 s, rồi lây nhiễm với huyền phù mầm bệnh ở mật số 10^6 bào tử mL^{-1} và quan sát đến 12 ngày. Kết quả cho thấy $CaCl_2$ 20 mM có hiệu quả ức chế tốt đối với nấm Aspergillus phân lập từ bệnh thối trái cam. $CaCl_2$ tiếp tục thể hiện hiệu quả ức chế bệnh trên trái cam đến 12 ngày sau lây bệnh. Trên ớt, $CaCl_2$ 20 và 40 mM cho hiệu quả ức chế sự phát triển nấm Aspergillus phân lập từ bệnh thối trái ớt. Tuy nhiên, xử lý $CaCl_2$ không mang lại hiệu quả mong đợi trên trái ớt. Trên chuối già, dung dịch $CaCl_2$ ở các nồng độ 20, 40 và 60 mM ức chế tốt sợi nấm Colletotrichum trong điều kiện in vitro. Dung dịch canxi có thể ức chế vết bệnh thán thư trên chuối già, nhưng hiệu quả không kéo dài đến 6 ngày sau lây bệnh. Nhìn chung, các kết quả tốt đều đạt được khi xử lý bằng $CaCl_2$ 20 mM ở hầu hết các thí nghiệm. Việc kiểm soát bệnh thối trái bằng $CaCl_2$ 20 mM có thể thay thế cho hóa chất nông nghiệp độc hại hiện nay.

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

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

* Corresponding author
Email: lttan134@gmail.com

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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 Viet Nam, 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 harmfully 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 Viet Nam (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 dimitiatum* 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 ºC for 150 s; 2-4% sodium carbonate aqueous solution at 45 ºC for 60 - 150 s, or 1-4% sodium bicarbonate aqueous solution at room temperature for 150 s followed by storage at 20 ºC 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 (Manganaris 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 $CaCl_2$ significantly increased the fruit Ca content in the fruit peels, leading to reduce the size of anthracnose lesions (Awang et al., 2011). In mango, solutions containing various concentrations of $CaCl_2$ 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% $CaCl_2$, respectively. Treatment of $CaCl_2$ or combination of hot water – $CaCl_2$ 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, $CaCl_2$ 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 $CaCl_2$ yet. Therefore, the objective of this study was to assess the efficacy of $CaCl_2$ 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

(PDA) at pH 6.5 at about 10-12 days before conducting experiments. Medium of PDA was prepared followed the procedure of Atlas (2004). The fungi were incubated at 28±2 ºC with relative humidity approximately 90%.

2.1.2. Chemicals

$CaCl_2{\cdot}2H_20$ (Catalogue No. 1023820500, purity ≥95%, Merck, Germany) was provided from Department of Plant Protection, Can Tho University.

Prior to this research, different concentrations of $CaCl_2{\cdot}2H_20$ had been quickly screened to evaluate their effects on spore germination of *A. niger* and *C. musae*. Subsequently, $CaCl_2{\cdot}2H_20$ 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 $CaCl_2$-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 $CaCl_2$ 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 $CaCl_2$ was prepared according to treatment above description. To assure complete solubility, each sample of aqueous $CaCl_2$ was magnetically stirred for 30 min. The $CaCl_2$ 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 ºC 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 $CaCl_2$ was chosen to conduct following experiments on orange, chilli and Cavendish banana fruits.

2.3. Assessment of treating $CaCl_2$ at an effective concentration on fruits before inoculation with fungal suspension

The experiment was done in CRD with two treatments including $CaCl_2$-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 $CaCl_2$ 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 $CaCl_2$ 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 10^6 conidia mL^{-1} was dropped on these tiny holes.

Inoculated fruits were stored in an incubation chamber at 25 ºC 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 $CaCl_2$ solutions against *Aspergillus niger* on orange fruits

3.1.1 Efficacy of $CaCl_2$ on *Aspergillus* hyphal development *in vitro*

Each tested concentration of $CaCl_2$ 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 [1/]	72 [1/]	96 [1/]
20 mM $CaCl_2 \cdot 2H_2O$	40.7±5.8^c	61.4±7.2^c	78.2±6.9^b
40 mM $CaCl_2 \cdot 2H_2O$	48.0±6.1^b	70.8±7.1^b	88.0±6.4^a
60 mM $CaCl_2 \cdot 2H_2O$	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

[1/] 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*

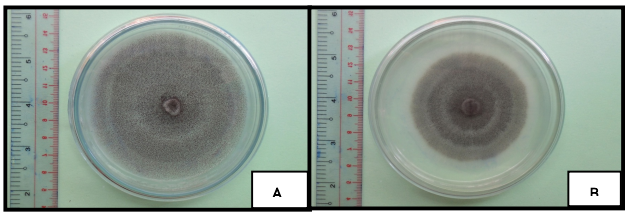

Figure 1. Efficacy of 20 mM $CaCl_2$ on the growth of *Aspergillus niger* at 96 hours after putting fungal slices.

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 [1/]	10 [1/]	12 [1/]
20 mM $CaCl_2 \cdot 2H_2O$	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	*	*	*
Coefficient of variance (%)	27.99	27.17	21.57

[1/] 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*

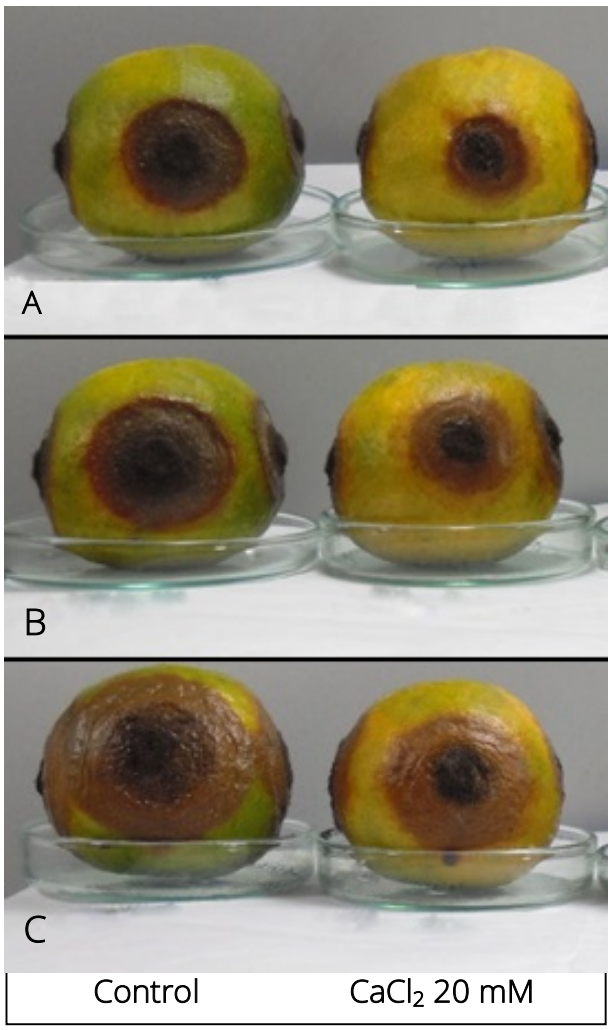

Figure 2. Efficacy of $CaCl_2$ at a concentration of 20 mM on rot lesions caused by *Aspergillus niger* at 8 DAI (A), 10 DAI (B), 12 DAI (C).

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 $CaCl_2$ inhibited hyphal development of *A. niger* during all observation time points. The treatment of 20 mM $CaCl_2$ had ability from 48 to 96 hours after putting fungal slices, while $CaCl_2$ at a concentration of 60mM was effective on 72 and 96 hours after putting fungal slice. Therefore, the concentration at 20 mM of $CaCl_2$ showed a high and stable efficacy than other treatments, and was chosen to do on the following experiment (Table 3).

Table 3. Efficacy of $CaCl_2$ on colonial diameter (mm) of *Aspergillus niger in vitro*

Treatment	Time after adding fungal slices (hours)		
	48 [1/]	72 [1/]	96 [1/]
20 mM $CaCl_2{\cdot}2H_2O$	36.80±4.5[b]	52.50±6.1[b]	58.20±6.2[b]
40 mM $CaCl_2{\cdot}2H_2O$	40.50±5.1[a]	54.00±5.8[b]	62.80±7.2[b]
60 mM $CaCl_2{\cdot}2H_2O$	42.00±5.3[a]	59.80±5.2[a]	74.00±7.1[a]
Water control	41.20±4.6[a]	57.50±5.4[a]	75.50±8.2[a]
Significance	*	*	*
Coefficient of variance (%)	7.82%	5.85%	10.32%

[1/] *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 $CaCl_2$ on chilli before an inoculation of *Aspergillus* suspension

Treatment of $CaCl_2$ at a concentration of 20 mM did not inhibit *Aspergillus* rot lesion on chilli fruits (Table 4).

Table 4. Efficacy of $CaCl_2$ treated before *Aspergillus* inoculation on chilli fruits

Treatment	Days after inoculation		
	4 [1/]	6 [1/]	8 [1/]
20 mM $CaCl_2{\cdot}2H_2O$	13.51±0.23	17.33±0.32	20.58±0.24
Non-treated control	10.34±0.19	16.76±0.33	19.02±0.27
Significance	ns	ns	ns
Coefficient of variance (%)	15.47%	21.78%	17.59%

[1/] *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 $CaCl_2$ solutions against *Colletotrichum musae* on Cavendish banana fruits

3.3.1 Efficacy of $CaCl_2$ on *Colletotrichum* hyphal development *in vitro*

Efficacy of $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ 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)		
	24 [1/]	48 [1/]	72 [1/]
20 mM $CaCl_2{\cdot}2H_2O$	8.66±2.3[c]	29.67±4.5[b]	58.50±12.1[c]
40 mM $CaCl_2{\cdot}2H_2O$	10.00±2.7[b]	33.83±4.8[a]	63.67±11.3[a]
60 mM $CaCl_2{\cdot}2H_2O$	9.17±1.9[bc]	34.33±5.2[a]	63.17±9.3[ab]
Water control	12.50±1.8[a]	34.00±5.7[a]	60.00±11.2[b]
Significance	*	*	*
Coefficient of variance (%)	12.41	5.63	5.79

[1/] *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 $CaCl_2$ at 20 mM was chosen to carry out assays on Cavendish banana fruits.

3.3.2 Efficacy of treating $CaCl_2$ on Cavendish bananas before an inoculation of *Colletotrichum*

Lesion lengths of $CaCl_2$-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 $CaCl_2$ did not prolong to 6 and 7 DAI (Table 6 and Figure 3).

Table 6. Efficacy of $CaCl_2$ treated before *Colletotrichum* inoculation on Cavendish banana fruits

Treatment	Days after inoculation		
	5 [1/]	6 [1/]	7 [1/]
20 mM $CaCl_2{\cdot}2H_2O$	11.92±3.4[b]	17.42±3.5	23.67±4.2
Non-treated control	13.00±3.6[a]	18.25±3.2	25.08±3.8
Significance	*	ns	ns
Coefficient of variance (%)	11.12	12.22	11.53

*1/ 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*

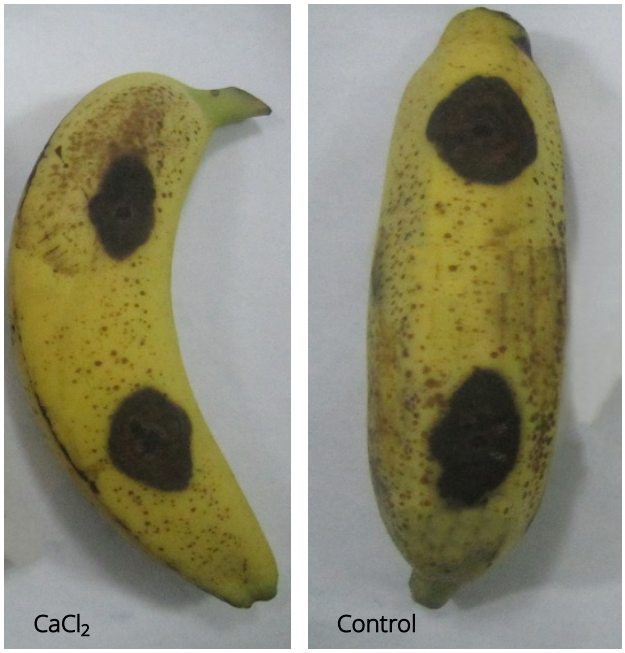

Figure 3. Efficacy of $CaCl_2$ treatment before *Colletotrichum* inoculation on Cavendish bananas at 7 DAI.

The banana fruits of Cavendish were immersed on a solution of 20 mM $CaCl_2$ 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 10^6 conidia mL^{-1} was dropped on these tiny holes. $CaCl_2$: the banana fruit was treated with $CaCl_2$ 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 $CaCl_2$ significantly inhibited the growth of the pathogenic fungi *A. niger* and *C. musae*, better than $CaCl_2$ 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 (Al-Eryani-Raqeeb et al., 2009). The cell walls of fungal pathogens were rich in neutral sugars. When a low concentration of $CaCl_2$ was applied *in vitro* conditions, exogenous Ca^{2+} 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 $CaCl_2$ on orange, chilli and Cavendish banana reduced the lesions length. The reduction of rot symptom in $CaCl_2$-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 Ca^{2+} 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, $CaCl_2$ 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 $CaCl_2$ lead to decrease fruit rot (Uthaibutra et al., 1998; Lara et al., 2004). On red-flesh dragon fruits, $CaCl_2$ applied at various concentrations at 1, 2, 3 and 4 gL^{-1} reduced the anthracnose severity. The concentration of CaCl2 at 4 gL^{-1} (approximately 27 mM) gave the best inhibition on anthracnose lesions (Awang et al., 2011). Our results of 20 mM $CaCl_2$ on this study are in line to results of Awang et al. (2011). Similarly, $CaCl_2$ was reported to inhibit the *in vitro* development of *C. gloeosporioides* in papaya fruits (Ayon-Reyna et al., 2017). In a recent study, Hassanein et al. (2018) indicated that 4% (w/v) $CaCl_2$ could use with gramma irradication to inhibit fungal growth of *A. alternate*, *A. solani*, *A. niger*, *B. cinerea*, *F. solani* and *R. stolonifer* on guava fruits.

Moreover, host-pathogen interaction on $CaCl_2$-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 Ca^{2+} binding the polygalacturonase, reduce the susceptibility to fungal infection (Rose et al., 2003), leading to slow the development of rot lesions on $CaCl_2$-treated fruits. In general, $CaCl_2$ application in adequate amounts supplied helps to maintain orange, chilli 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 $CaCl_2$ well inhibited the hyphal development of both *A. niger* and *C. musae in vitro*. However, this concentration of $CaCl_2$ was effective on inhibiting *Aspergillus* rot lesion only on orange fruits *in vivo* conditions. However, a treatment of 20 mM $CaCl_2$ 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 $CaCl_2$.

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