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The Suppressive Effects of Calcium Compounds against *Botrytis cinerea* in Paprika

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Abstract. Plant diseases including gray mold caused by *Botrytis cinerea* are often reduced when calcium compounds are used as alternative materials in paprika. However, much less information is available about the effects of calcium compounds on controlling of *B. cinerea*. Seven calcium compounds such as calcium sulfate dihydrate, calcium chloride, calcium nitrate, calcium oxide, calcium hydroxide, calcium carbonate, and calcium hydride were evaluated for their effectiveness against *B. cinerea* on potato dextrose agar medium. The pH of selected calcium compounds was higher (pH 8.2-10) than that of the control (pH 6.6). Calcium carbonate, calcium oxide, calcium hydride, and calcium hydroxide among seven calcium compounds were more effectively inhibited the growth of *B. cinerea* than other calcium compounds. In the case of spraying the spore suspension on paprika applied with the selected four calcium compounds and supplied with the selected calcium supplements in a hydroponic culture system, the paprika treated with calcium compounds showed less severity of disease than those untreated plants. On the basis of our results, we propose that the suppressive effects of calcium compounds on *B. cinerea* in paprika resulted from the supply of calcium and a certain degree of salt stress.

***Additional key words*:** alternative strategy for disease management, gray mold, plant disease

Introduction

The gray mold caused by the fungus *Botrytis cinerea* Pers.: Fr. develops mainly on fruits, leaves or petals of many crops (Guillem et al., 2007). It is one of the serious diseases of several crops grown in a greenhouse around the world and is usually controlled by the use of fungicides (Raposo et al., 1996). However, the resistance of *B. cinerea* to several fungicides is already widespread in many growing areas around the world (Yoon et al., 2008). Furthermore with increasing popular preference for organic agricultural products, the development of alternative disease management strategies is vital (Mikani et al., 2008). One possible alternative method of disease management is the application of inorganic elements. It was recently reported that inorganic elements had a suppressive effect on the mycelium growth of *B. cinerea*. When plant tissues are infected by a pathogen like *B. cinerea*, the cell walls are degraded, leading to a leakage of electrolytes from both the plant cell wall and cytoplasm (Chardonnet et al., 1999). The cell wall-degrading enzymes produced by this fungus play an important role for tissue maceration (Charlotte and Donèche, 2002). In plants, calcium (Ca) strengthens cell walls by chelating pectin substances and increases the resistance of plant tissue to plant pathogens in plant cells (Chardonnet et al., 1999). Calcium plays an important role in tissue protection against fungal infection (Charlotte and Donèche, 2002). It has been reported in many studies that the application of calcium has resulted in reducing diseases caused by several pathogens (Sugimoto et al., 2005; Volpin and Elad, 1991). In the present study, $Ca(NO_3)_2$ treatment markedly suppressed infection caused by fungi and affected resistance to pathogens (Sugimoto et al., 2005). Stanghellini et al. (1996) demonstrated that the amending nutrient solution resulted in control of *Pythium aphanidermatum* in crops. It was shown that the amending nutrient solutions with calcium supplements also reduced the severity of *B. cinerea*. However, few researches have been done to examine the interactions of nutrition with disease resistance of host plant and improve nutrient efficiency in hydroponic system as an alternative way to control *B. cinerea*. It is necessary to understand the effect of nutrient levels on plant growth and against disease. It should be possible to regulate the growth rate of *B. cinerea* by applying

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calcium compounds or using them in the nutrient solutions.

The first objective was to study the ability of calcium compounds to enhance the control activity against *B. cinerea* on potato dextrose agar (PDA) media containing calcium compounds as well as the infection of paprika plants by *B. cinerea*. The second objective was to examine the responses of the amending nutrient solutions with calcium supplements against *B. cinerea* during the periods of occurrence on paprika grown in nutrient solutions.

Materials and Methods

Isolates of *B. cinerea* from paprika

During gray mold epidemic months between August and November in 2007, isolates of *B. cinerea* were obtained from diseased leaves, stems and fruits of paprika collected from Gangwondo, Korea. *B. cinerea* was isolated by placing fragments of diseased leaves, stems and fruits on potato dextrose agar (PDA) amended with 100 $\mu g \cdot mL^{-1}$ of streptomycin. Tips of mycelia which were grown from infected tissue samples were transferred to fresh PDA medium and then incubated at 20℃. Each isolate was maintained at 20℃ on PDA slants and fresh subcultures were made when necessary.

Mycelial inhibition effects of calcium compounds

Seven calcium compounds (calcium sulfate dihydrate, calcium chloride, calcium nitrate, calcium oxide, calcium hydroxide, calcium carbonate, and calcium hydride) were used to evaluate their efficacy against *B. cinerea* on PDA media containing calcium compounds (concentration: 3.3 $g \cdot L^{-1}$), on which mycelia disks of *B. cinerea* were inoculated. One week after incubation, the diameters of the colonies were measured. The pH of each calcium compound was measured using a pH meter (Multi 340i, Mettler Toledo, Germany). All tests were replicated three times.

Seedling test

Two cultivars of paprika ('Cupra' and 'Boogie') grown in pots with a diameter of 12 cm for 6 weeks were treated with each calcium compound solution (concentration: 3.3 $g \cdot L^{-1}$) and soaked fully as the method of foliage spray one time before inoculation. We used the *B. cinerea* isolate obtained from paprika in Gangwon province and then plated on petri dishes with PDA medium and incubated them for 3 weeks at 20℃. The resulting suspension was filtered through two layers of cotton gauzes. The concentration of *B. cinerea* in spore suspension was adjusted to 12.5×10^6 conidia/mL by counting with a hemocytometer. Only the fully expanded leaves and stems from each plant were inoculated with 50 mL of the suspensions using a low-pressure sprayer. Then all the seedlings of paprika were moved into a limited room and covered with plastic bag to keep a humid condition. We observed the disease symptoms every day after inoculation and then assessed them as the following disease index: 0 = no disease, 1 = disease occurred < 10%, 2 = disease occurred 10-30%, 3 = disease occurred 30-60%, 4 = disease occurred < 60%, 5 = disease occurred < 80%.

Nutrient solutions for hydroponic culture

The nutrient solutions were followed by the Horticultural Research of Areum (Table 1). Post-transplanted seedlings were put into the slab (15 × 90 cm) and were grown under a hydroponic culture system in the greenhouse. Each nutrient solution was irrigated three times a day (each time supplying nutrient solutions with 100 mL) for six weeks. Also, programmable timers were used to schedule irrigation length and frequency (Agro. 3000, Namgyeong, co., Korea). The pH of the nutrient solution was maintained between 5.7-6.3 using HNO_3 solution.

Greenhouse experiments with amending nutrient solutions

The seeds of paprika ('Cupra' and 'Boogie') were sown in 240 cell-trays (Mifko Co., Korea) at the greenhouse, on 6 February, 2008. After sprouting, seedlings were transplanted into the rockwool (10 × 10 cm, Mifko co., Korea) on 16 March, 2008. Thereafter, seedlings were managed in the same manners as described previously and the standard nutrient solution was amended with the selected four calcium compounds, which were diluted 10 times more than the containing of calcium compounds on PDA media, separately and supplied each nutrient system line for one month, especially in the period of occurrence of *B. cinerea* between September and October in 2008, as an alternative method to control of *B. cinerea*. To measure the pH of each amending nutrient solution,

Table 1. The composition of standard nutrient solutions to grow paprika plants.

Nutrient solution	Ions ($meq \cdot L^{-1}$)[z]						
	NH_4^+	K^+	Ca^{++}	Mg^{++}	NO_3^-	SO_4^{--}	$H_2PO_4^-$
Standard	1.2	6.7	4.5	3.0	16.7	2.5	1.2

[z]treatment solution contained equal concentration of micronutrient ($meq \cdot L^{-1}$): Fe-EDTA; 1.08, $MnSO_4 \cdot H_2O$; 0.17, $ZnSO_4 \cdot 7H_2O$; 0.15, H_3BO_3; 0.22, $CuSO_4 \cdot 5H_2O$; 0.02, $Na_2MoO_4 2H_2O$; 0.01.

we used the portable pH meter (Argus, Sentron, Netherlands) and tried to maintain between 5.7-6.3 using HNO_3 solution. The concentration of spore suspension was adjusted as 1.09 $\times 10^6$ conidia/mL by using a hemocytometer. Only the fully expanded leaf and stem from each plant was inoculated with 50 mL of the suspension using a low-pressure sprayer. All of the treatments were applied as post-inoculation foliar sprays into plants on the 25th of October and arranged in a randomized block design with 10 replicate plants per treatment. Three replicates were performed per treatment. The existing disease for each infected site per plant was measured one month later, on the 25th of November in 2008. Data on the occurrence of disease (%) of plants per treatment was analyzed.

Data analysis

All of the data was analyzed by analysis of variance, and mean separation done with Duncan's multiple range test (DMRT) at $P = 0.05$ using Statistical Analysis System (SAS, 2003, Institute Inc., Cary, NC, USA) and figure program (Sigma Plot 2001, Phil Science Co., Korea).

Results

Effects of calcium compounds against *B. cinerea* on PDA

In the results of the experiment which evaluated the effect of calcium compounds on PDA media, the mycelial growth rates of *B. cinerea* on PDA containing different calcium compounds are shown in Fig. 1. Among them, on the PDA media containing calcium carbonate, calcium oxide, calcium hydride and calcium hydroxide, the colony sizes of mycelium of *B. cinerea* were reduced significantly. However, calcium hydride was a little dangerous when it was mixed with water. Therefore, instead of using calcium hydride, it was determined that calcium nitrate was one of the selected calcium compounds to compare other calcium compounds.

Suppressive effects of selected calcium compound solutions on *B. cinerea*

The effect of calcium on disease severity in 'Cupra' and 'Boogie' are summarized in Table 2. According to the results of the disease rates, the seedling plants treated with calcium compound solutions occurred less than those of the control. Among them, the solutions of calcium hydroxide and calcium carbonate were more suppressive than others in 'Cupra' (Table 2).

The analysis of pH

According to the results of the pH of selected calcium compounds, the pH of selected calcium compounds were higher (pH 8.2-10) than that of control (pH 6.6) as shown in Fig. 2. Among them, calcium oxide (pH 12.34) and calcium hydroxide (pH 12.27) were over pH 12.0. The treatment of

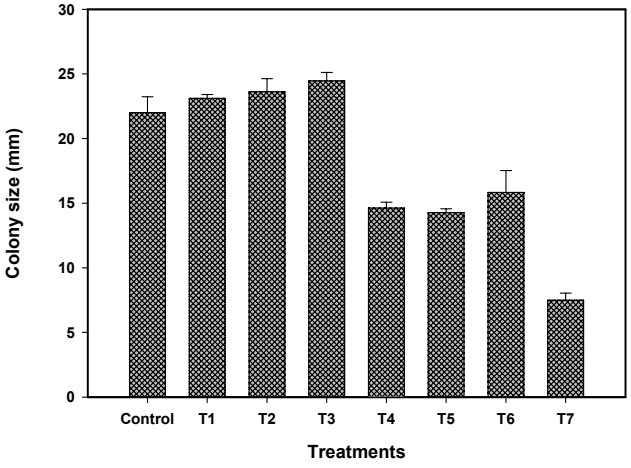

Fig. 1. Comparison of colony size (mm) treated with different calcium compounds to control *B. cinerea* on PDA media at 3 days after inoculation. Control: Potato dextrose agar (PDA) media, T1: control + calcium sulfate dehydrate, T2: control + calcium nitrate, T3: control + calcium chloride, T4: control + calcium carbonate, T5: control + calcium oxide, T6: control + calcium hydride, T7: control + calcium hydroxide.

Table 2. Effects of calcium compound treatments of paprika seedlings in 2 days and 3 days after inoculation of gray mold.

Treatments	Grades of *B. cinerea*[z] disease (0-5)			
	Boogie		Cupra	
	2 days	3 days	2 days	3 days
Calcium nitrate	1.08 b[y]	2.24 b	2.31 a	3.70 a
Calcium oxide	1.62 b	2.90 b	2.06 a	3.06 a
Calcium hydroxide	1.21 b	2.36 b	0.86 b	2.12 b
Calcium carbonate	1.31 b	2.40 b	0.20 b	2.22 b
Control	2.42 a	4.00 a	2.50 a	3.62 a

[z]Disease severity: 0 = no disease, 1 = disease occurred < 10%, 2 = disease occurred 10-30%, 3 = disease occurred 30-60%, 4 = disease occurred < 60%, 5 = disease occurred < 80%.
[y]Mean separation within columns by Duncan's multiple range test $P = 0.05$.

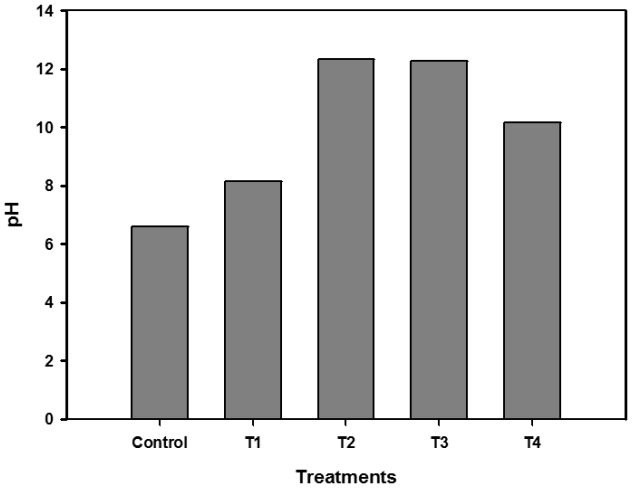

Fig. 2. Comparison of pH of selected calcium compound solutions. Control: Distilled water, T1: calcium nitrate, T2: calcium oxide, T3: calcium hydroxide, T4: calcium carbonate.

calcium hydroxide was significantly higher than others in comparison of colony size (mm) on PDA media containing seven calcium compounds in Fig. 1 and comparison of seedling test in Table 2. The results showed that the increased pH of calcium compounds was more effective to inhibit the growth of *B. cinerea* than the lowered pH of calcium compounds.

Greenhouse experiments with amending nutrient solutions

The results of greenhouse experiments with amending nutrient solutions adding selected calcium supplements; calcium oxide, calcium hydroxide, calcium carbonate and calcium nitrate are shown in Fig. 3. As shown in the calculations of disease occurrence (%) in this experiment, similar results were obtained in experiments that evaluate the effect of calcium application

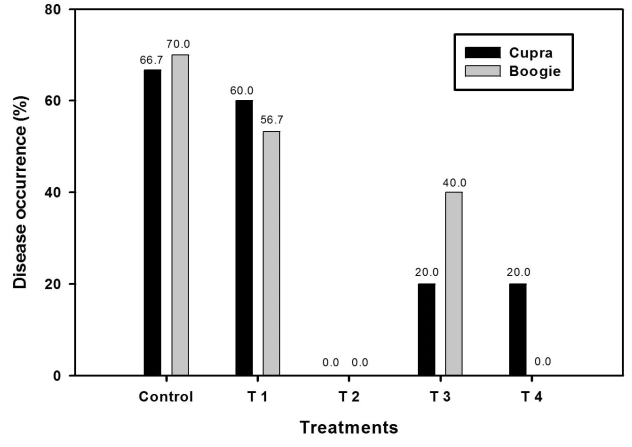

Fig. 3. Disease suppressive effect against gray mold according to the treatments of calcium compound solutions. Control : Standard nutrient solution, T1: control + calcium nitrate, T2: control + calcium oxide, T3: control + calcium hydroxide, T4: control + calcium carbonate.

on disease severity in 'Cupra' and 'Boogie'. The greatest decrease in disease occurrence was recorded in the calcium oxide treatment, which showed 0% disease incidence in 'Cupra' and 'Boogie', respectively, compared with control (standard nutrient solution) as 66.7% and 70.0% in 'Cupra' and 'Boogie', respectively. The disease occurrence of the plants grown in calcium hydroxide showed 20% and 40% in 'Cupra' and 'Boogie'; also, the disease occurrence of the plants grown in calcium carbonate were presented as 20% and 0% in 'Cupra' and 'Boogie', respectively. With the exception of plants grown in calcium nitrate as comparison, the disease occurrence was 60% and 56.7% between 'Cupra' and 'Boogie', respectively.

Discussion

For the purpose of this investigation, we assessed the mycelial growth of *B. cinerea* occurring in paprika plants, depending on different calcium compounds on PDA media, together with the role of calcium for controlling *B. cinerea*. Our data showed that the colony sizes of *B. cinerea* were reduced in comparison with the control (PDA media) without calcium compounds in Fig. 1. Although these results were based on *in vitro* assays, it was likely that similar results could occur in the fruit if free calcium ions were present. As shown in Fig. 2, the solutions of calcium hydroxide and calcium carbonate were more suppressive than any others between 'Cupra' and 'Boogie'. The developments of disease, by passing time, were more processed than expected. Due to the high concentration of spores and the humid conditions in the plastic covered pots, the occurrence of disease of *B. cinerea* was accelerated. The picture was not shown but the spores of *B. cinerea* by the treatment of calcium carbonate, calcium hydroxide and calcium nitrate were changed more than those of the control, similar to results of when we controlled *B. cinerea* using dicarboximide fungicide. However, the mechanism by which calcium compounds inhibit fungal spore germination and germ tube elongation was not known. One mechanism by which Ca^{2+} is known to reduce decay is by stabilizing the host cell wall and thus reducing maceration by cell wall degrading enzymes produced by fungal pathogens. According to the results of the measured pH of each calcium compound as shown in Fig. 2, the pH of the calcium compound solution correlated with colony size and disease severity in Fig. 1 and Table 2.

This study demonstrated that applications of a few calcium compound solutions have the ability to reduce the severity of *B. cinerea* on paprika. The effects of applying calcium before and after inoculation of *B. cinerea* will be studied to determine whether the longer calcium stays in the plants will lessen the disease percentage or not. Improved control

of *B. cinerea* by combinations of calcium salts was reported by the addition of calcium salts to cell suspensions in better control of *B. cinerea* on plants (McLaughlin et al., 1990). These results indicate that mechanisms of osmotic pressure may be involved in improving inhibition of infection by *B. cinerea*. We observed myceliar inhibition of *B. cinerea* on PDA media as well as inhibition of *B. cinerea* on seedling plants, which may be due to direct contact with the fungus or by drying of the moisture on leaf surface, thus inhibiting the disease development.

The results of greenhouse experiments show that by amending nutrient solutions and adding selected calcium supplements in Fig. 3, the disease of *B. cinerea* in paprika grown in nutrient solutions occurred less. These results support the notion that the use of calcium compounds as growth media reduces disease in aerial parts of plants attacked by foliar pathogens (Guillem et al., 2007). According to the study of Wojcik and Lewandowski (2003), foliar treatment with $CaCl_2$ led to foliar Ca increases and was effective against *Botrytis* strawberry leaves and fruits. Moreover, Elad et al. (1993) reported that *Botrytis* incidence in cucumber was reduced to 50% when Ca supplements were applied to the growth media. However, the relatively high pH of the calcium compounds could have interfered with nutrient uptake. Even though the amending nutrient solutions including calcium compounds were diluted to supply paprika for one month, paprika plants and fruits from high concentration of calcium treatments wilted at the beginning of irrigation. So, regulation of the pH of amending nutrient solutions when applied to calcium compounds in hydroponic as an alternative method to control plant disease must be considered.

Whatever the mechanism may be, calcium is inexpensive, safe, and more acceptable than fungicide in organic agriculture. The results suggest that calcium compounds have the potential to control *B. cinerea* under greenhouse conditions. It is necessary to determine effective levels of calcium for disease suppression in the field or in the nutrient solutions. Moreover, further research is needed on the central role of the mechanisms involved in disease reduction to the application of calcium.

Literature Cited

Chardonnet, C.O., C.E. Sams, and W.S. Conway. 1999. Calcium effect on the mycelial cell walls of *Botrytis cinerea*. Phytochem. 52:967-973.

Charlotte, C. and B. Donèche. 2002. Purification and characterization of two isozymes of polygalacturonase from *Botrytis cinerea*. Effect of calcium ions on polygalacturonase activity. Microbiol. Res. 157:183-189.

Elad, Y., H. Yunis, and H. Volpin. 1993. Effect of nutrition on susceptibility of cucumber, eggplant, and pepper crops to *Botrytis cinerea*. Can. J. Botany 71:602-608.

Guillem, S., C. Eva, B. Celia, A. Mannuel, and T. Isabel. 2007. The suppressive effects of composts used as growth media against *Botrytis cinerea* in cucumber plants. J. Eur. Plant Pathol. 117:393-402.

McLaughlin, R.J., M.E. Wisniewski, C.L. Wilson, and E. Chalutz. 1990. Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. J. Phytopathol. 80:456-461.

Mikani, A., H.R. Etebarian, P.L. Sholberg, D.T.O. Gorman, S. Stokes, and A. Alizadeh. 2008. Biological control of apple gray mold caused by *Botrytis mali* with *Pseudomonas fluorescens* strains. Posthar. Biol. and Technol. 48:107-112.

Raposo, R., J. Delcan, V. Gomez, and P. Melgarejo. 1996. Distribution and fitness of isolates of *Botrytis cinerea* with multiple fungicides resistance in Spanish greenhouses. Plant Pathol. 45:497-505.

Stanghellini, M.E., S.L. Rasmussen, D.H. Kim, and P.A. Rorabaugh. 1996. Efficacy of nonionic surfactant in the control of zoospore spread of *Pythium aphanidermatum* in a recirculating hydroponic system. Plant Dis. 80:422-428.

Sugimoto, T., M. Anio, M. Sugimoto, and K. Watanabe. 2005. Reduction of Phytophthora stem rot disease soybeans by the application of $CaCl_2$ and $Ca(NO_3)_2$. J. Phytopathol. 153:536-543.

Volpin, G. and Y. Elad. 1991. Influence of calcium nutrition on susceptibility of rose flowers to *Botrytis* blight. Phytophthol. 81:1390-1394.

Wojcik, P. and M. Lewandowski. 2003. Effect of calcium and boron sprays on yield and quality of "Elsanta" strawberry. J. Plant Nutr. 26:671-682.

Yoon, C.S., E.H. Ju, Y.R. Yeoung, and B.S. Kim. 2008. Survey of fungicide resistance for chemical control of *Botrytis cinerea* on Paprika. J. Plant Pathol. 24:447-452.

파프리카 양액재배에서 발생하는 잿빛곰팡이병 방제에 대한 칼슘제제의 효과

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초 록. 본 실험은 7가지의 칼슘제제; calcium sulfate dihydrate, calcium chloride, calcium nitrate, calcium oxide, calcium hydroxide, calcium carbonate, calcium hydride가 포함된 PDA배지에서 잿빛곰팡이 균의 방제효과를 알고자 실시되었다. 선발된 칼슘제제의 pH는 8.2-10으로 대조구인 pH 6.6보다는 높게 측정되었다. 7가지 칼슘제제가 포함된 PDA배지에서의 잿빛곰팡이병 방제 colony size(mm) 결과는 calcium carbonate, calcium oxide, calcium hydride, calcium hydroxide가 다른 칼슘제제들 보다 잿빛곰팡이병 억제에 효과가 좋았다. 선택된 4개의 칼슘제제를 식물체에 처리 한 후 잿빛곰팡이병의 포자를 살포한 실험과 희석된 4개의 칼슘제제)를 표준 양액재배에 첨부하여 한 달간 양액을 공급한 후 잿빛곰팡이병의 포자를 접종한 실험결과는 칼슘제제를 처리하지 않은 대조구보다는 칼슘제제를 처리한 실험구에서 잿빛곰팡이병의 발생률이 대체적으로 낮았다. 칼슘제제를 처리한 파프리카 식물체에서 잿빛곰팡이병 억제에 대하여서는 칼슘제제 처리에 따른 어느 정도의 염기스트레스의 영향 등으로 볼 수가 있겠다.

추가 주요어 : 병 방제를 위한 대체적인 전략, 잿빛곰팡이, 식물병