Plant Pathol. J. 28(2) : 156-163 (2012)
http://dx.doi.org/10.5423/PPJ.OA.10.2011.0197
pISSN 1598-2254 eISSN 2093-9280

The Plant Pathology Journal

Chemical and Biological Controls of Balloon Flower Stem Rots Caused by *Rhizoctonia solani* and *Sclerotinia sclerotiorum*

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(Received on October 22, 2011; Revised on February 20, 2012; Accepted on February 21, 2012)

Stem rots caused by *Rhizoctonia solani* and *Sclerotinia sclerotiorum* have been known as devastating diseases in balloon flower plants. Antifungal activities of four fungicides, azoxystrobin, polyoxin B, trifloxystrobin and validamycin A were evaluated *in vitro*, showing effective suppression with mycelial growth of the fungal isolates on PDA media. Efficacies of the four fungicides were also demonstrated in stem tissues of balloon flower plants against *R. solani* and *S. sclerotiorum*. A commercially available *Bacillus subtilis* strain Y1336 was tested in terms of antagonistic biological control of stem rot disease of balloon flower plants. The bacterial strain revealed its antifungal activities against *R. solani* and *S. sclerotiorum* demonstrated by dual culture tests using paper discs and two plant pathogenic fungi on PDA media, as well as by plant inoculation assay, indicating that this antagonistic bacterial strain can be incorporated into disease management program for balloon flower stem rot diseases together with the four chemical fungicides.

Keywords: antagonistic bacteria, fungicide, *Platycodon grandiflorum*, stem rot diseases

Balloon flower (*Platycodon grandiflorum* A. DC.) has been cultivated as an edible rootcrop and traditional medicinal herb to treat diverse disease symptoms such as inflammation (Kim et al., 2006). Continuous cropping of balloon flower more than 3 years provoked drastic increase of soil-borne disease occurrence such as stem rot (personal communication). A variety of fungal species including *Colleotrichum dematium*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Septoria platycodonis* were identified as causal agents for balloon flower diseases in South Korea (Korean Society of Plant Pathology, 2009). Species of *Cladosporium* sp., *Fusarium* sp., *Phyllosticta* sp. and *Stemphylium* sp. were not identified so far requiring to be confirmed. *R. solani* infection led to disease occurrence in a wide range of plant species including monocots and dicots (Jeon et al., 2010). Different *R. solani* isolates originated from balloon flower plants grown in six locations of South Korea, showed differential pathogenicity in relation to their anastomosis and tissue specificity (Kim et al., 1992). However, stem rot disease of balloon flower plants by *S. sclerotiorum* was rarely demonstrated.

Cultural, chemical and biological controls of soil-borne disease such as *R. solani* and *S. sclerotiorum* have been applied for many different crop productions in combination with each other (Csinos and Stephenson, 1999; Howell et al., 2000; Vinale et al., 2009; Van Beneden et al., 2010). Large number of fungicides was evaluated and suggested for disease control of *R. solani* and *S. sclerotiorum* in many crops. Among those, fungicides like flutolanil, iprodione and pencycuron were effective to limit mycelial growth of *R. solani* (Campion et al., 2003; Csinos and Stephenson, 1999). Use of boscalid and vinclozolin were suggested for control of Sclerotinia stem rot in canola and soybean, respectively (Bradley et al., 2006; Mueller et al., 2002). Only azoxystrobin and polyoxin B are allowed to be applied to balloon flower plants to control leaf spot diseases caused by *Phyllosticta* sp. and *Septoria* sp. in South Korea. In addition, no fungicidal activities including these two fungicides have been determined to control balloon flower stem rot diseases yet.

Pseudomonas aeruginosa strain JS2 and endophytic *Bacillus* sp. strain CY22 isolated from plant growing soil and root interior of balloon flower, respectively, showed *in vitro* antibiotic activity against several phytopathogenic fungi, *Fusarium oxysporum*, *Phytophthora capsici*, *Pythium ultimum* and *R. solani* (Cho et al., 2002; Ryu et al., 2000). Treatment of balloon flower seedlings with the strain CY22 protected the seedlings against stem rot disease by *R. solani* (Cho et al., 2003). 2,4-Diacetylphloroglucinol and iturin were proven as the antifungal compounds produced by the *P. aeruginosa* and *Bacillus* sp., respectively (Cho and Yun,

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2005; Ryu et al., 2000). Biological control of Sclerotinia stem rot of balloon flower by antagonistic bacteria was not found.

In this study, we evaluate *in vitro* and *in planta* antifungal effects of commercially available fungicides and antagonistic bacteria against balloon flower stem rot diseases by *R. solani* and *S. sclerotiorum.*

Materials and Methods

Plant and pathogens. Balloon flower roots were purchased from commercial traditional market in Jinju, South Korea, and planted in soil mixture. A voucher specimen has been deposited in Laboratory of Plant Pathology and Protection, Jinju National University, South Korea. Newly coming balloon flower stems from the roots were cut and used for fungal inoculation. *R. solani* AG-2-2 isolate K13, originated from balloon flower stems, was obtained from Korea Agricultural Culture Collection (KACC 40125) and *S. sclerotiorum* isolate SRUi-1 was isolated from water cress greenhouse, Ui-Ryeong, South Korea.

***In vitro* antifungal activities of pesticides.** Fungicides used in the *in vitro* plate assays were as followed: azoxystrobin, trifloxystrobin, polyoxin B and validamycin A, described in Table 1. Effect of each fungicide on radial growth rate of *R. solani* and *S. sclerotiorum* were tested on potato dextrose agar (PDA) media amended with a range of six concentrations determined according to manufacturers' recommendation dosage. Radial mycelial growth was determined by placing 5-mm mycelial disc cut from the growing edge of 2-day-old PDA cultures upside down in the center of the test plate. *R. solani* and *S. sclerotiorum* were cultured at 25 °C. Colony diameter of *R. solani* and *S. sclerotiorum* were measured 2 or 3 days after inoculation, respectively.

Antagonistic bacteria. An antagonistic *Bacillus subtilis* Y1336, which is registered for powdery mildew and Sclerotinia rot disease of several vegetable crops in South Korea, was obtained from commercial market. *In vitro* antifungal activities were evaluated with paper discs containing different numbers of the *B. subtilis* Y1336 against *R. solani* and *S. sclerotiorum*. The sterilized paper discs (8 mm in diameter) were loaded with suspension of potential antagonistic bacterial, air-dried and then placed on the margin of PDA media. Mycelial discs of *R. solani* and *S. sclerotiorum* were also inoculated at the center of the PDA, and formation of clear inhibition zone was examined during dual culture of antagonistic bacteria and plant pathogenic fungi. Experiments were conducted repeatedly 4 times. Four replicates were prepared for each experiment.

Table 1. Fungicides used in this study

Name	Type	Recommend Conc. (μg/ml)
Azoxystrobin	FL	100
Trifloxystrobin	FL	110
Polyoxin B	SP	100
Validamycin A	Lq	100

FL, flowable; Lq, liquid; SP, soluble powder

Pathogen inoculation and disease evaluation. *In vivo* disease control efficacy of the four fungicides and an antagonistic bacterium were tested using balloon flower stems. Balloon flower stem segments (5 cm in length) were sprayed with different concentrations of the fungicides or the bacterial strain, and left to air-dry for 1 day at room temperature. *R. solani* and *S. sclerotiorum* were cultured on PDA media for 2 days at the temperature described previously. Untreated-, fungicide- or bacteria-applied balloon flower stem segments were put vertically at the growing edge of the growing fungal mycelia as shown in Fig. 2A. Inoculated stems were transferred to the moist chamber, and mycelial growth progress and disease development on the stem tissues from the inoculation bottom line was observed. Experiments were repeated 4 times with 4 replicates per experiment. Disease progress was evaluated by measuring stem lengths covered by the fungal mycelia. Efficacy of fungicide or antagonistic bacteria was expressed as relative mycelial progress (%) on the stem segments from the inoculation bottom site compared to that of inoculated stem segments without any pretreatment.

***In vitro* antibacterial activities of pesticides.** Different doses of four different pesticides (azoxystrobin, trifloxystrobin, polyoxin B and validamycin A) were added into nutrient agar (beef extract 3 g, peptone 5 g, and agar 16 g per liter) media, and 0.2 ml of bacterial suspension of *B. subtilis* Y1336 (10^4 cfu/ml) was spread onto the media. Number of the bacterial colonies was counted after incubation at 30°C for 2 days. *In vitro* bacterial growth was expressed as percentage compared to colony number on nutrient agar media without any pesticide.

Statistical analyses. All experiments were conducted in a randomized design with three replications. Data were subjected to analysis of variance using SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA) and means were separated by Duncan's multiple range test (DMRT) at $P < 0.05$.

Results

***In vitro* fungicidal effects against *R. solani* and *S. sclerotiorum*.** Four fungicides, azoxystrobin, trifloxystrobin,

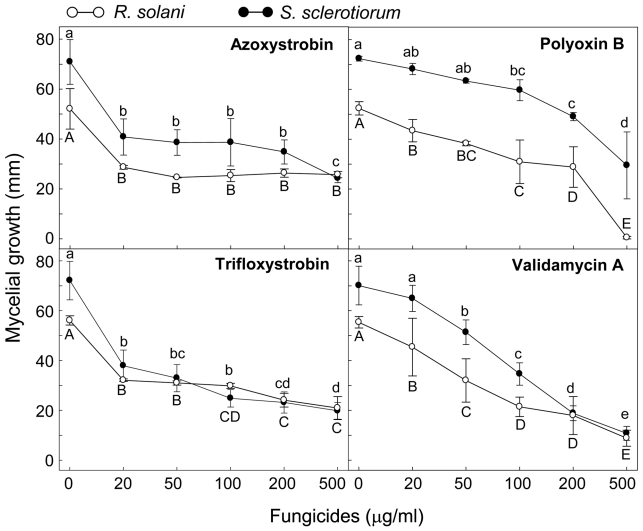

Fig. 1. Inhibition activities of different fungicides (azoxystrobin, trifloxystrobin, polyoxin B and validamycin A) against *in vitro* mycelial growth of *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* Vertical bars represent the standard errors. Means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

polyoxin B and validamycin A, were used to examine *in vitro* antifungal activities against *R. solani* and *S. sclerotiorum.* All fungicides showed significant antifungal activities against both fungal species in a dose-dependent manner, demonstrated by reduced mycelial growth of the plant pathogenic fungi (Fig. 1). Although azoxystrobin was the only registered fungicide to leaf spot disease of balloon flower in South Korea, similar antifungal efficacy to *R. solani*, causing balloon flower stem rot was revealed. Colony diameter of *R. solani* was reduced to ca. 45% at 20 μg/ml of strobilurin fungicide azoxystrobin, but no further mycelial growth arrest was observed at more than 50–200 μg/ml of the azoxystrobin. Treatment with 500 μg/ml of azoxystrobin much limited the *R. solani* mycelial growth. Mycelial growth of *S. sclerotiorum* was also affected by treatment of azoxystrobin about ca. 45% at 20–500 μg/ml. We applied another strobilurin fungicide, trifluoxystrobin, to determine antifungal activity against *R. solani* and *S. sclerotiorum in vitro* (Fig. 1). With 20–100 μg/ml of trifloxystrobin, mycelial growth of *R. solani* was drastically inhibited, and higher concentration of the trifloxystrobin (200–500 μg/ml) led to much reduced fungal growth. *S. sclerotiorum* was sensitive to trifluoxystrobin at the 20–50 μg/ml, showing arrested fungal growth. The fungal growth retardations were much significant to the application of more than 100 μg/ml of the trifluoxystrobin.

Antifungal activities of two antibiotics, polyoxin B and validamycin A was evaluated on the two fungal species. Increasing concentration of polyoxin B resulted in gradual reduction of mycelial growth of *R. solani* and *S. sclerotiorum.* Relatively lower dose 20–50 μg/ml of polyoxin B was not enough to mediate growth inhibition of *R. solani*. More than 200 μg/ml of polyoxin B began to reduce *R. solani* growth. Polyoxin B acted effectively to *S. sclerotiorum* than to *R. solani* in the mycelial growth inhibition with lower concentrations of 20–50 μg/ml. Mycelial growth of *S. sclerotiorum* was much inhibited by 100–500 μg/ml of polyoxin B. Both *R. solani* and *S. sclerotiorum* growth was drastically limited by increasing concentration of validamycin A (Fig. 1). With 50 μg/ml of the validamycin A treatment, inhibition of the *R. solani* mycelial growth was revealed by ca. 24%. Antifungal activity of validamycin A against *R. solani* increased to ca. 86% by 500 μg/ml. *S. sclerotiorum* growth was affected by validamycin A treatment as well even by 20 μg/ml. Increasing dose of the validamycin A from 50 to 500 μg/ml effectively restricted the fungal growth.

Disease symptoms of balloon flower stem rot caused by artificial inoculation of *R. solani* and *S. sclerotiorum.* Balloon flower stem tissues were inoculated by *R. solani* and *S. sclerotiorum* to investigate temporal disease development and symptom of stem rots (Fig. 2A). After inoculation with *R. solani* and *S. sclerotiorum,* balloon flower stem tissues showed susceptibilities to the fungal pathogens (Fig. 2B).

Growing *R. solani* were observed on the surface of the stem tissues at 2 days after inoculation. Half of the stem tissues in length were colonized by white fungal hyphae of the *R. solani,* and turned to pale brown color 4 days after inoculation. At 10 days after inoculation, whole stem tissue segments discolored were covered with the fungal mycelia. Disease development by *S. sclerotiorum* was more or less faster than that of *R. solani* on the balloon flower stem tissues. Fifty percent of the stem from the bottom part was water-soaked by the infection and discolored with the fungal growth of *S. sclerotiorum* 2 days after inoculation. A number of cottony and white mass of the mycelia were formed onto the stem tissues 4 days after inoculation, and distinctly matured to black sclerotinia varied in size 10 days after inoculation. Some of the matured sclerotinia were fallen to the ground 10 days after inoculation.

Disease protection of fungicides on the balloon flower stem rots. *In vivo* control efficacy of the four fungicides against balloon flower stem rots was evaluated under a controlled environment using the stem segments (Fig. 3). Treatment with four fungicides demonstrated differential protection effect in balloon flower stems against two different stem rot causing fungal species.

No disease control was found at the *R. solani*-infected

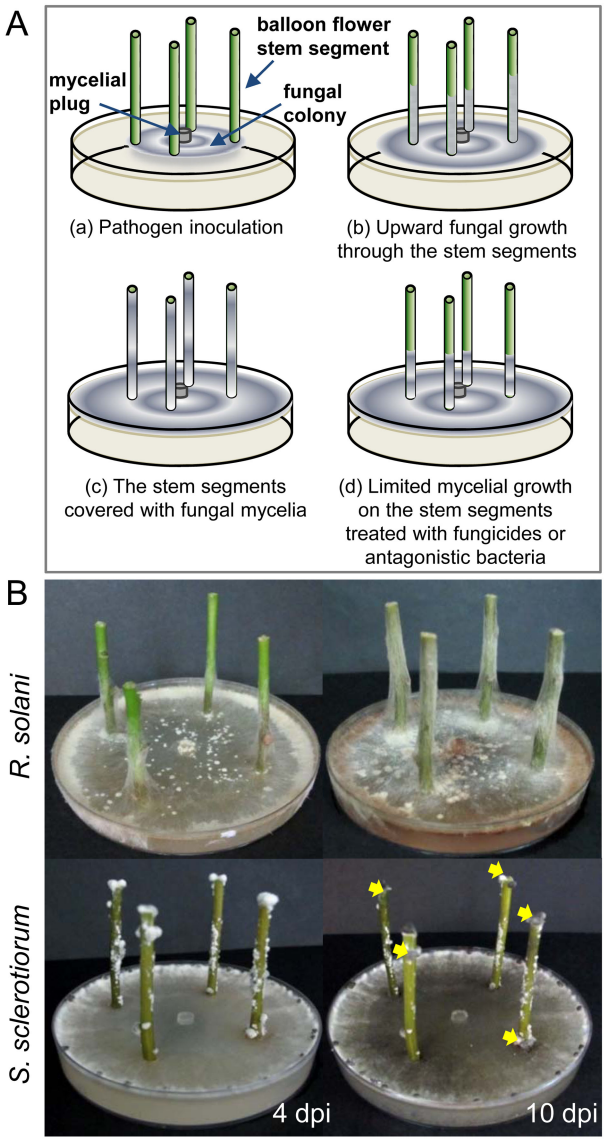

Fig. 2. Fungal inoculation of balloon flower stems. (A) Inoculation schemes and disease evaluation procedures based on upward mycelial growth on the stem segments from the base. (B) Disease symptom development on the balloon flower stem rots caused by *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* Pictures were taken 4 and 10 days after inoculation. Arrows indicate sclerotinia formed on the infected stem tissues by *S. sclerotiorum*.

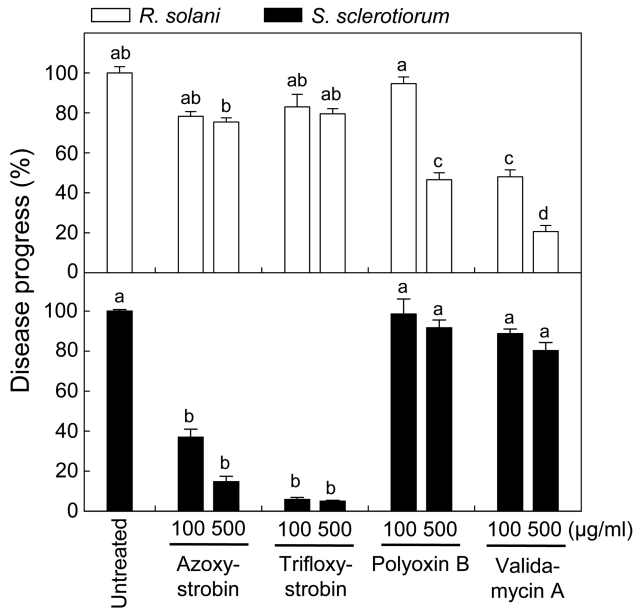

Fig. 3. Protective efficacies of different fungicides (azoxystrobin, trifloxystrobin, polyoxin B and validamycin A) against disease progress in the balloon flower stems by *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* Lesion length on the stems was measured at 5 and 2 days after inoculation by *R. solani* and *S. sclerotiorum*, respectively. Relative lesion lengths caused by the fungal inoculation on the fungicide-pretreated stems were expressed compared to that produced on the stems untreated with fungicides as percentage for disease protection. Vertical bars represent the standard errors. Means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

stem tissues pretreated with two strobilurin fungicides, azoxystrobin and trifloxystrobin. However, antibiotics suchas polyoxin B and validamycin A revealed significant disease suppression to *R. solani* infection. Five hundred μg/ml of polyoxin B significantly reduced balloon flower stem rot disease by *R. solani*. Validamycin A could suppress the stem rot disease at 100 μg/ml relatively lower concentration, and 500 μg/ml of validamycin A showed ca. 80% disease protection against *R. solani* (Fig. 3). Interestingly, disease protection against *S. sclerotiorum*-infected stem rot was achieved by treatment with two strobilurin fungicides, while two antibiotics, polyoxin B and validamycin A did not show any disease protection effect even at higher concentration of 500 μg/ml.

Effect of antagonistic bacteria on *in vitro* growth of *R. solani* and *S. sclerotiorum*. Commercial biological control agent *B. subtilis* Y1336 was subjected to dual cultures with stem rot disease fungi *R. solani* and *S. sclerotiorum* (Fig. 4A). Two plant pathogenic fungi were not affected by paper discs without the bacterial species, showing fungal mycelia growing and spreading across the paper discs. However, clear inhibition zone was observed between the pathogenic fungi and the paper discs containing different numbers of the bacterial strain, indicating that the presence of fungistatic substances released by the bacterial strain. However, a bacterial number-dependent increase in clear zone diameter was not found. Pretreatment with lower concentration (10^4 cfu/ml) of the *B. subtilis* could not provide any disease protection against both stem rot diseases. However, higher concentration (10^8 cfu/ml) of the strain protected effectively

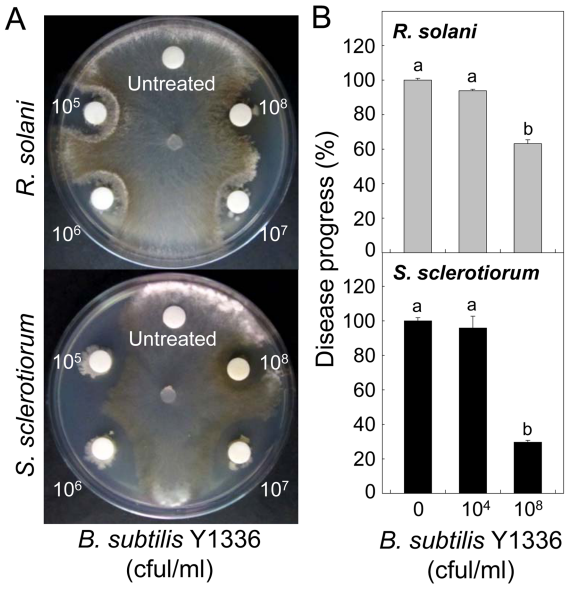

Fig. 4. Biological control of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* by antagonistic bacterial strain *Bacillus subtilis* Y1336. (A) *In vitro* inhibition of mycelial growth of *R. solani* and *S. sclerotiorum* shown by paper discs containing different dose (10^5 to 10^8 cfu/ml) of the antagonistic bacteria. Pictures were taken 4 days after fungal cultures. (B) *In planta* antifungal activity of the *B. subtilis* on balloon flower stems inoculated with *R. solani* and *S. sclerotiorum.* Relative lesion lengths caused by the fungal inoculation on the antagonistic bacteria-pretreated stems were expressed compared to that produced on the untreated stems as percentage for disease protection. Vertical bars represent the standard errors. Means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

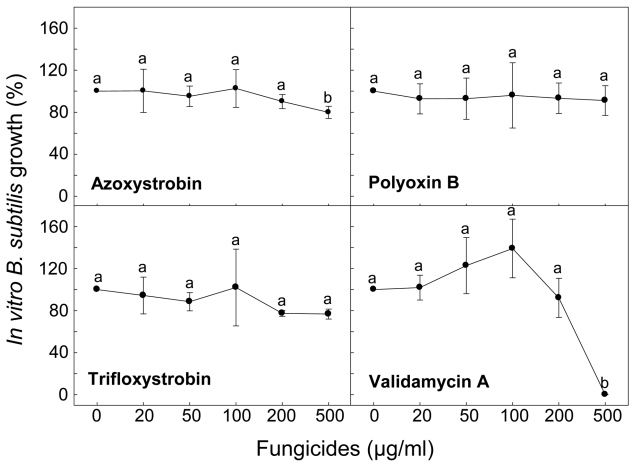

Fig. 5. Effects of different fungicides (azoxystrobin, trifloxystrobin, polyoxin B and validamycin A) on *in vitro* growth of antagonistic bacterial strain *Bacillus subtilis* Y1336. Vertical bars represent the standard errors. Means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

balloon flower stems against *R. solani* and *S. sclerotiorum* (Fig. 4B).

Effect of fungicides on growth of antagonistic bacteria. We investigated viability of the antagonistic *B. subtilis* strain in response to the 4 fungicides showing *in vitro* antifungal activities against *R. solani* and *S. sclerotiorum* to examine possibility of biological control of balloon flower stem rots in a combination with chemical fungicides. Among 4 fungicides, azoxystrobin, trifloxystrobin and polyoxin B did not inhibit the growth of *B. subtilis* strain in the range of 20–500 μg/ml assayed (Fig. 5). However, 500 μg/ml of validamycin A significantly decreased number of the bacterial colonies.

Discussion

Increases in stem rots during balloon flower cultivation and limited information on the disease management prompted us to find various ways to limit the disease spread. In South Korea, two fungicides azoxystrobin and polyoxin B have been allowed to control leaf spot disease of balloon flower plants. In this study, disease protection efficacies of these fungicides were evaluated against stem rot diseases caused by *R. solani* and *S. sclerotiorum*. Additionally, antifungal activities of another strobilurin fungicide trifloxystrobin and another antibiotic validamycin A were also tested.

Azoxystrobin fungicide has been used for controlling a variety of diseases including mango anthracnose (*Colletotrichum gloeosporioides*), wheat leaf scorch (*Didymella exitialis*), and wheat take-all (*Gaeumannomyces graminis*) (Cromey et al., 2004; Jenkyn et al., 2000; Sundravadana et al., 2007). Use of azoxystrobin provided disease control of stem rot (*Sclerotium rolfsii*), Rhizoctonia pod rot (*Rhizoctonia solani*) and early leaf spot (*Cercospora arachidicola*) during peanut production, and followed by increased yields (Grichar et al., 2000). However, certain crops are hypersensitive to azoxystrobin so that treatment with the fungicide was suggested to be reconsidered. Some apple cultivars showed phytotoxicity to the azoxystrobin fungicide treatment (Tobutt et al., 2009). Azoxystrobin also have direct detrimental effects on stigma, showing localized damage and collapse of stigmatic cells of almond flowers (Yi et al., 2003).

It was found that azoxystrobin and its related chemical trifloxystrobin clearly decrease mycelial growth of *R. solani* and *S. sclerotiorum in vitro*. Twenty mg/ml of the fungicides were effectively limited growth of the two fungal species, however, further increase of the chemicals did not drastically restrict mycelial growth. Foliar spray of the azoxystrobin and trifloxystrobin onto balloon flower did not lead any damage in stems used in our experiments. Pre-treat-

ment with these fungicides demonstrated strong disease protection against *S. sclerotiorum.* However, *in planta* disease protection by azoxystrobin and trifloxystrobin against *R. solani* was not significant, although these have antifungal activities against *R. solani in vitro*. Application of azoxystrobin sufficiently can be suggested to control stem rot disease by *S. sclerotiorum*. Recently, concern about fungicide resistance was growing in the crop fields, because frequent application of azoxystrobin may cause decreased sensitivity of *Alternaria solani*, *Podosphaera fusca* and *Pseudoperonospora cubensis* (Ishii et al., 2001; Rosenweig et al., 2008). Strobilurin fungicides currently have not been used in balloon flower seasoning so far. Although the fungicide was revealed to be effective to control stem rot disease of balloon flower plants in this study, occurrence of resistant isolates of *R. solani* and *S. sclerotiorum* regularly monitored. In addition, using mixture of strobilurin fungicides with other chemically unrelated fungicides of different modes of action should be considered to minimize resistance development.

Two antibiotics, polyoxin B and validamycin A originated from *Streptomyces* spp. were tested for balloon flower stem rot control. Polyoxin B, inhibitor of fungal cell wall synthesis, has been used for various plant fungal diseases, including pepper gray mold (Park et al., 1999), tomato gray leaf spot (Kim et al., 1997), cucumber powdery mildew (Kim et al., 2004) and apple moldy-core disease (Reuveni et al., 2002). However, antifungal activity of polyoxin B on *R. solani* and *S. sclerotinia,* causing balloon flower stem rots, were hardly detected so far. Disease controls of Rhizoctonia root and crown rot of sugar beet by polyoxin D was reported (Bolton et al., 2010). Polyoxin B was revealed as one of efficient controlling fungicides to inhibit fungal growth of *R. solani in vitro* and *in planta*, but it only showed *in vitro* antifungal activity against *S. sclerotiorum*. Validamycin A, strong inhibitor of trehalase, was likely to act efficiently to Basidiomycotina, while it could not inhibit most Ascomycotina and Oomycetes (Robson et al., 1988). Increasing dose of validamycin A reduced radial growth of *R. solani* and *S. sclerotiorum in vitro*. Pretreatment with the validamycin in balloon flower stems significantly delayed symptom development by *R. solani*.

Interestingly, 4 fungicides having *in vitro* antifungal activities against *R. solani* and *S. sclerotiorum*, showed differential disease protection efficacies in balloon flower stems. Strobilurin fungicides were effective to control *S. sclerotiorum*-infected stem rot, while the antibiotics were useful for *R. solani*-infected stem rot of balloon flower plants although mode-of-action of the two antibiotics were different. Although it should be further elucidated that fungicides with *in vitro* antifungal activities have no disease protection efficacy *in planta*, mixtures of strobilurin fungicides with antibiotics can be further explored for probable occurrences of stem rots by *R. solani* or *S. sclerotiorum* in the balloon flower fields. For a long time, fungicide application with different mode-of-action fungicides is generally accepted to enhance disease protections synergistically, as well as to escape occurrence of fungicide-resistance isolates in nature (Gisi, 1996). Fungicide synergism was well demonstrated during the control of oomycete pathogen-causing plant diseases, *Pythium aphanidermatum* on perennial ryegrass (Couch and Smith, 1991), *Pseudoperonospora cubensis* on cucumber (Samouch and Cohen, 1988), *Plasmopara viticola* on grapevine (Gisi et al., 1985) and *Phytophthora infestans* on tomato (Gisi et al., 1985). Recently, fungicide synergism was reassessed for control of turfgrass dollar spot caused by *Sclerotinia homeocarpa* (Burpee and Latin, 2008). Synergistic effects of different combinatorial mixtures of 4 fungicides, azoxystrobin, trifloxystrobin, polyoxin B and validamycin A primarily evaluated in this study remain investigated for effective field application in balloon flower production.

Biological controls of *R. solani* and *S. sclerotiorum*-mediated soil borne diseases have been demonstrated in recent decades. Tomato damping-off and root-rot by *R. solani* were suppressed by antagonistic bacteria *B. subtilis* RB14-C and *Pseudomonas fluorescens* A6RI, respectively (Asaka and Shoda, 1996; Berta et al., 2005). Sclerotinia rots by *S. sclerotiorum* in soybean and witloof chicory decreased by antagonistic bacteria *B. subtilis* SB24 and mycoparasite *Coniothyrium minitans*, respectively (Benigni et al., 2010; Zhang and Xue, 2010). Mycoparasitic fungi *Clonostachys rosea* strain ACM941 reduced disease severity of pea root rots caused by a variety of fungal pathogens including *R. solani* and *S. sclerotiorum* (Xue, 2003). Both *R. solani* and *S. sclerotiorum* were sensitive to treatment with bacteria *B. subtilis* Y1336 *in vitro*, and pretreatment with the strain reduced stem rot diseases by the both in the current study, indicating that *B. subtilis* Y1336 can act as antagonistic bacteria for suppression of balloon flower stem rots.

Integration of microbial antagonists with fungicides can achieve disease protection by reduced fungicide application. It is critically important that fungicides with efficient antifungal activities against phytopathogenic fungi should not have any growth delaying effect on antagonistic bacteria or fungi. Polyoxin B was suggested as promising agent for integration of chemical and biological control, because it inhibited *in vitro* growth of *B. cinerea* causing pepper gray mold rot whereas antagonist *Trichoderma harzianum* grew well on the media containing polyoxin B (Park et al., 1999). Bacterial strain *B. subtilis* RB14-C antagonistic to *R. solani* was resistant to fungicide flutolanil, and usage of the bacterial strain reduced amount of flutolanil for effective

control of tomato damping-off (Kondoh et al., 2001). Chemical control of witloof chicory infected by *S. sclerotiorum* was achieved by mixture of fungicides fludioxonil and cyprodinil, as well as by mycoparasite *C. minitans* (Benigni et al., 2010). However, integration of the chemical mixtures and *C. minitans* could not be suggested because mycelial growth of *C. minitans* was completely inhibited at 1 mg/ml of the mixture. We found that *B. subtilis* Y1336 strain were resistant to all 4 fungicides, showing that only high validamycin A of 500 mg/ml have bactericidal effect on the *B. subtilis* Y1336. These findings suggest that biological control by *B. subtilis* Y1336 can be integrated into the disease management with the 4 chemical fungicides.

Taken together, efficient antifungal activities of several fungicides were demonstrated *in vitro* against two fungal species *R. solani* and *S. sclerotiorum* causing stem rot disease of balloon flower, as well as under the controlled environment using cut stem tissues. Antagonistic bacterial strain was also observed for balloon flower stem rot control. Further studies using these chemical fungicides and antagonistic bacteria remained under the greenhouse and field conditions.

Acknowledgements

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ008801)", and "The Agricultural Genome Center (TAGC) in the Next-Generation BioGreen 21 program (Project No. PJ008211)" Rural Development Administration, Republic of Korea, and was partially supported by Gyeongnam National University of Science and Technology Grant 2012 to Jeum Kyu Hong.

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