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Peony Stem Rots by *Neopestalotiopsis clavispora* and *Sclerotinia sclerotiorum*, and Antifungal Propineb and *Bacillus siamensis* H30-3 against the Two Fungal Species

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In July 2022, stem rot symptom was found in a peony plant grown in a pot under a greenhouse at Jinju, Gyeongnam Province, South Korea. Two fungal species were isolated from the infected peony stems and cultured on 1/2-strength potato dextrose agar for identification. The morphological characteristics of the fungal isolates were examined, and nucleotide sequences of the internal transcribed spacer region, β-tubulin and translation elongation factor 1-α were analysed. The pathogenicity of the two isolates was confirmed in detached peony leaves, according to Koch's postulates. To our knowledge, this is the report of *Neopestalotiopsis clavispora* and *Sclerotinia sclerotiorum* as the causal agents of peony stem rots. Antifungal activity of chemical fungicide propineb and rhizobacterium *Bacillus siamensis* H30-3 was shown against the two plant pathogenic fungi *N. clavispora* and *S. sclerotiorum*. Unidentified diffusible and volatile compounds from *B. siamensis* H30-3 could suppress *in vitro* mycelial growths of *N. clavispora* JJ 8-2-1 and *S. sclerotiorum* JJ 8-2-2.

Keywords: Antifungal, *Bacillus siamensis*, Herbaceous peony, Propineb, Stem rot

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Introduction

Peony plants (*Paeonia lactiflora*), a herbaceous perennial, have been cultivated in temperate regions worldwide as ornamental flowers in gardens and for cut flowers, and a variety of cultivars were bred with commercial values (Ahn et al., 2018; Kamenetsky-Goldstein and Yu, 2022). The peony plant roots have been used as a raw material for oriental medicines, and the medicinal effects of the root extracts and major constituents have been investigated (Du et al., 2020; He and Dai, 2011; Shi et al., 2016). During plant growing seasons, the peony plant underwent environmental stresses and pathogen invasion. High temperatures in summer led to severe physiological stresses in heat-sensitive peony cultivars (Wang et al., 2022; Wu et al., 2016). Trehalose solution sprayed onto the peony plants was suggested to efficiently alleviate the high temperature-induced tissue

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damage (Zhao et al., 2019). In contrast, shading peony plants decreased stem diameters and strength due to reduced lignin and cellulose contents in stem tissues, which may lead to stem bending (Tang et al., 2022; Zhao et al., 2012). Foliar spraying silicon solution promoted lignin accumulation in peony stems and enhanced the stem strength to prevent stem bending (Zhao et al., 2021).

Herbaceous peony plants also suffer from various pathogen infections (Garfinkel and Chastagner, 2018). In South Korea, 13 fungal, one oomycete and 24 nematode infections have been described as causal agents of peony plant diseases (The Korean Society of Plant Pathology, 2022). Various fungal diseases were found in peony plants in Uiseong County and Youngchun City, Gyeongbuk Province, South Korea. Different fungal species such as *Alternaria* sp., *Erysiphe aquilegiae*, *Pestalotia paeonicola*, *Rhizoctonia* sp., *Cladosporium paeoniae*, *Cylindrocarpon desctructans*, *Cronartium flaccidum* and *Botrytis cinerea*, were isolated from the infected peony plant tissues (Park et al., 1996). In particular, the occurrence of powdery mildew was seasonally investigated in 1992, 1993, 1995, and 1996. The powdery mildew on peony leaves was first found in early June 1992 and 1993, and gradually increased to late July. The first detection of powdery mildew on peony leaves in 1995 and 1996 was in mid-May, earlier than in 1992 and 1993. Peony plants were severely damaged by black root rot caused by soil-borne *Cylindrocarpon destructans* in continuous cropping fields in Uiseong County, Andong City and Yeongcheon City, Gyeongbuk Province, South Korea (Choi et al., 2004). Recently, two fungal diseases such as anthracnose and grey mould have occurred in peony plants (Kim et al., 2020; Ma et al., 2023; Park et al., 2020). The anthracnose was found in peony stems in Gangjin County, Jeonnam Province, South Korea, in mid-April 2019, and the causal agent was identified as *Colletotrichum fioriniae* (Park et al., 2020). In June 2019, leaf blight was found in peony plants grown under a greenhouse in the same county, and *B. cinerea* was identified (Kim et al., 2020). Recently, extensive survey was reported on broad range of fungal and oomycete pathogens in the 12 states of Unite States, and many pathogens were newly identified in the region (Garfinkel and Chastagner, 2019).

Different management has been suggested for peony diseases. Rain sheltering during the peony plant cultivation decreased rust, anthracnose, powdery mildew and root rot compared to open field conditions (Kim et al., 2001). Dazomet fumigation in soils before planting efficiently reduced the black root rot of peony plants (Choi et al., 2004). Four fungicides, pyraclostrobin, phenamacril, flutolanil and boscalid, had differential *in vitro* antifungal activities on different developmental stages of *Cladosporium paeoniae*-causing peony leaf mould (Chai et al., 2022). The fungicides have been applied to control fungal diseases in peony plants, showing more than 60% control efficacies in the peony plant fields (Chai et al., 2022). However, biological control of peony plants using an antagonistic microorganism is rarely described so far.

Stem rot symptoms were found in the peony plants growing in a pot under a greenhouse in Jinju, Gyeongnam Province, South Korea, in July 2022. In this study, two fungal species were isolated from the diseased peony plants, and were identified based on etiological, molecular and phytopathological analyses. In addition, *in vitro* antifungal activities of chemical fungicide propineb and antagonistic rhizobacterium *Bacillus siamensis* H30-3 were also evaluated against two fungal species causing peony stem rot.

Materials and Methods

Isolation and morphological examination of fungal species causing peony stem rot. Peony stems with rot symptoms were cut, and fungal species were isolated from the infected stem pieces according to the slightly modified method described previously (Aktaruzzaman et al., 2022; Nam et al., 2022). The stem pieces were surface-sterilised in 2% sodium hypochlorite for 20 sec, followed by 95% ethanol for 20 sec. The plant tissues were rinsed with sterilised distilled water twice, briefly dried on the sterilised paper, and then transferred on 1/2-strength potato dextrose agar (PDA) medium (Difco, Detroit, MI, USA). After incubation at 25°C for 3 days, a hyphal tip was cut out from a single hypha and placed on the PDA medium for subculture and maintenance.

Two isolates JJ 8-2-1 and JJ 8-2-2 were cultured on the PDA and mycelial plugs (5 mm in diameter) were inoculated on the centre of the PDA media. The fungal colonies were examined at 15 days after culture at 25°C. Photographs of upper and lower sides of colonies were taken. Conidial morphology of isolate JJ 8-2-1 was observed under a light microscope (ECLIPSE Ni-E; Nikon Instruments Inc., Tokyo, Japan).

Molecular identification of two fungal isolates causing peony stem rot. Aerial mycelia of isolates JJ 8-2-1 and JJ 8-2-2 were prepared in 100 µl of distilled water to provide DNA template for polymerase chain reaction. Internal transcribed spacer (ITS) regions of ribosomal DNA were amplified using primer pair ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′- TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Fungal β-tubulin were amplified using primer pair Bt2a ('5-GGTAACCAAATCGGTGCTGCTTTC-3') and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) (Pavlic et al., 2009). Translation elongation factor 1 (TEF1α)-α sequence was amplified with primer pair EF1-728F ('5- CATCGAGAAGTTCGAGAAGG-3') and EF1-986R ('5- TACTTGAAGGAACCCTTACC-3') (Carbone and Kohn, 1999). The polymerase chain reaction (PCR) cycling conditions for the ITS, β-tubulin and translation elongation factor (TEF) regions were 95℃ for 2 min and 40 cycles of 95℃ (30 sec), 55℃ (30 sec), 72℃ (30 sec), and 72℃ for 10 min. The PCR products were subjected to electrophoresis in 1.5% agarose gel with a DNA size marker (Biofact Co., ltd, Daejeon, Korea). The nucleotide sequencing was performed by SolGent Inc. (Seoul, Korea), and nucleotide sequences from isolates JJ 8-2-1 and JJ 8-2-2 were deposited in the National Center for Biotechnology Information (NCBI) GenBank database. The nucleotide sequences from isolates JJ 8-2-1 and JJ 8-2-2 were compared with related nucleotide sequences originated from other fungal isolates by BLAST search (Altschul et al., 1990). The phylogenetic trees were constructed using neighbouring-joining method by MEGA11 in the bootstrap of 1,000 by the maximum likelihood method (Tamura et al., 2021).

Pathogenicity test. The pathogenicity of two fungal isolates for peony stem rot was investigated in detached peony leaves. Peony leaves (cv. Taebaek) were separated and placed on the two-layered cheesecloth saturated with distilled water in plastic boxes. Four mycelial discs (5 mm in diameter per disc) from the growing edge of the fungal colonies were inoculated on the detached leaves, and the plastic boxes were tightly covered to maintain moisture. The inoculated leaves were incubated at 25℃ under dark conditions for symptom development.

***In vitro* antifungal activities of chemical fungicide against two fungal isolates causing peony stem rot.** *In vitro* antifungal activity of the chemical fungicide against the two peony stem rot isolates was evaluated according to our previous studies (Do et al., 2019; Lee et al., 2012). Contact fungicide Antracol (a. i. propineb 70%, wettable powder) (Bayer CropScience Korea Ltd., Seoul, Korea) was used to investigate fungal mycelial growth inhibition on 1/2-strength PDA media amended with different propineb concentrations (0, 6.25, 12.5, 25, 50, and 100 µg/ml). Fungal mycelial discs (5 mm in diameter) were prepared from the growing edge of fungal colonies and inoculated upside down at the centre of media containing propineb. Colony diameters were measured after culture at 25℃ under darkness. Isolate JJ 8-2-1 and isolate JJ 8-2-2 were cultured for 7 days and 2 days, respectively. Five was subtracted from the colony diameter to express mycelial growth (mm).

***In vitro* antifungal activities of *Bacillus siamensis* H30-3 against two fungal isolates causing peony stem rot.** Antifungal activities of *Bacillus siamensis* H30-3 and its volatiles were evaluated against the two fungal isolates originated from peony stem tissues according to the experimental methods performed in our previous studies (Lee et al., 2018; Park et al., 2021).

B. siamensis H30-3 and the fungal isolates (JJ 8-2-1 and JJ 8-2-2) were co-cultured on 1/2-strength PDA media to investigate diffusible antifungal activity from *B. siamensis* H30-3. *B. siamensis* H30-3 was grown in trypticase soy broth (BD, Bergen County, NJ, USA) at 30℃ overnight, and the bacterial culture was centrifuged. The bacterial suspension was prepared in distilled water and adjusted to 10^8 cfu/ml using a spectrophotometer. The bacterial suspension was inoculated thrice on one side on PDA, and the mycelial disc (5 mm in diameter) from the edge of the fungal isolate was placed on the opposite side. Sterile distilled water was inoculated as a mock. The plates were incubated at 25℃ for 7 days and 3 days for isolates JJ 8-2-1 and JJ 8-2-2, respectively, and the radii of the fungal isolates were measured.

Results

Occurrence of stem rots on peony plants in greenhouse. Brown-coloured and water-soaked lesions were found in the stems of peony plants grown in a pot under a greenhouse (Fig. 1A). The infected tissues were observed at the basal (Fig. 1B) and middle (Fig. 1C) parts of the stems, showing similar sunken and collapsed lesions.

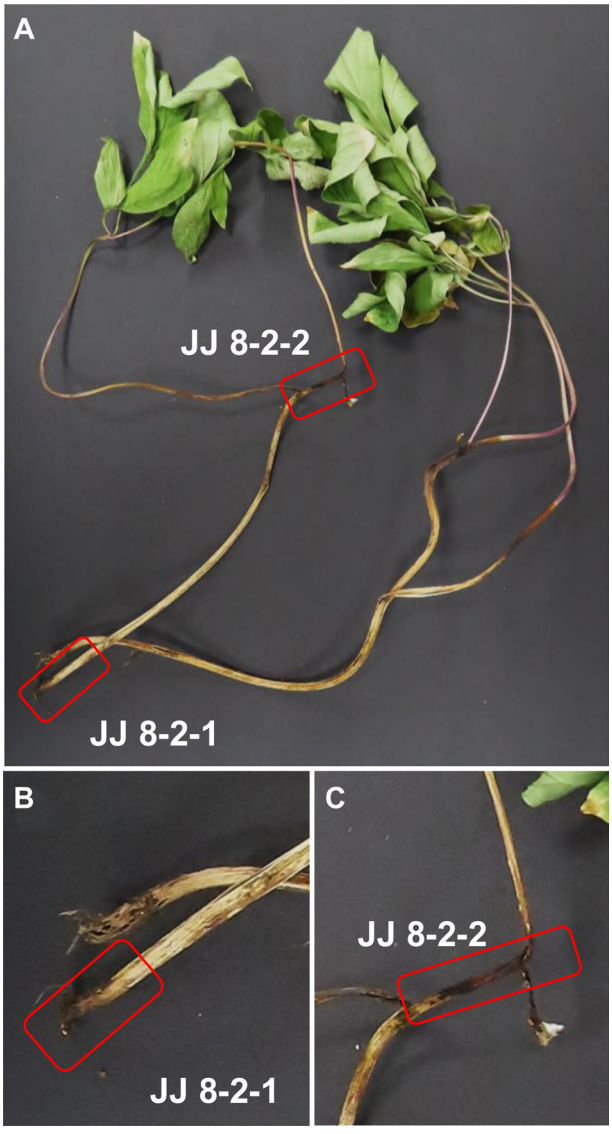

Fig. 1. Stem rot symptoms in peony plants grown under a greenhouse. (A) Brownish discoloured stems indicated by red rectangles. Two fungal species were isolated from the peony stem tissues and designated as isolates JJ 8-2-1 and JJ 8-2-2. (B, C) Close-up views of two areas of infected stem. Isolates JJ 8-2-1 and JJ 8-2-2 were isolated from bottom and middle parts of the infected stem, respectively.

Morphological and molecular identification. To identify the two fungal isolates JJ 8-2-1 and JJ 8-2-2, characteristic features of the fungal cultures on the media were observed (Fig. 2). Isolate JJ 8-2-1 grew with an undulate edge (Fig. 2A). The colony had several concentric layers and produced whitish aerial mycelia as the upper side was examined. The lower side of the colony displayed a pale yellow colour. The isolate JJ 8-2-1 produced black fruiting bodies distinctly found as the lower side was examined compared to the upper side at 15 days. The whitish aerial mycelia were not distinguished and many black averuli were formed on the media (Fig. 2B, C). Fusiform conidia with five cells were observed under a light microscope, and most had three apical appendages and one basal appendage (Fig. 2D). Conidial lengths were 22.4±1.05 µm, and conidial widths of the median cells were 5.8±0.09 µm. The three median cells with horizontal septa were dark brown-coloured, and apical and basal cells were hyaline. The apical and basal appendages were also not pigmented. The isolate JJ 8-2-2 produced a white to light grey fluffy colony on the media, and a number of black sclerotia were formed at the peripheral edge of the media in contact with the Petri dish (Fig. 2E). Arrows indicates 16 sclerotia were found in a Petri dish at 15 days (Fig. 2E) and 16.7±0.54 sclerotia/medium was observed at 28 days (data not shown). The sclerotia from 28-day-old culrues were globosely, cylindrically or irregularly in shape and 2.64±0.14 mm in size (Fig. 2F). The fungal isolates JJ 8-2-1 and JJ 8-2-2 from the diseased peony stem was morphologically identified as *Neopestalotiopsis clavispora* and *Sclerotinia sclerotiorum,* respectively (Bolton et al., 2006; Maharachchikumbura et al., 2014; Willetts and Wong, 1980).

Phylogenetic analysis of isolates JJ 8-2-1 and JJ 8-2-2 were conducted based on nucleotide sequence alignment for molecular identification (Fig. 3). Nucleotide sequences 532 bp and 540 bp were amplified from ITS-5.8S rDNA regions of isolates JJ 8-2-1 and JJ 8-2-2, and deposited in the NCBI database GenBank accession nos. OQ678131 and OQ683862, respectively. Partial nucleotide sequences of TEF1α (279 bp) and β-tubulin (444 bp) were amplified from isolates JJ 8-2-1 and JJ 8-2-2, respectively, and deposited in the NCBI database GenBank accession nos. PP341416 and PP341417, respectively. Phylogenetic trees were generated based on the combined ITS-5.8S rDNA and TEF1α nucleotide sequences for isolate JJ 8-2-1 and the combined ITS-5.8S rDNA and β-tubulin for isolate JJ 8-2-2 to verify the morphological identification. The nucleotide sequence alignment demonstrated that isolate JJ 8-2-1 nucleotide sequences were highly similar to those from other *Neopestalotiopsis clavispora* isolates deposited in the NCBI database (Fig. 3A). Isolate JJ 8-2-2 nucleotide sequences were close in distance to those from other *Sclerotinia sclerotiorum* isolates in the NCBI database (Fig. 3B). The isolate JJ 8-2-2 was clustered with other *S. sclerotiorum* isolates, but distinctly sepa-

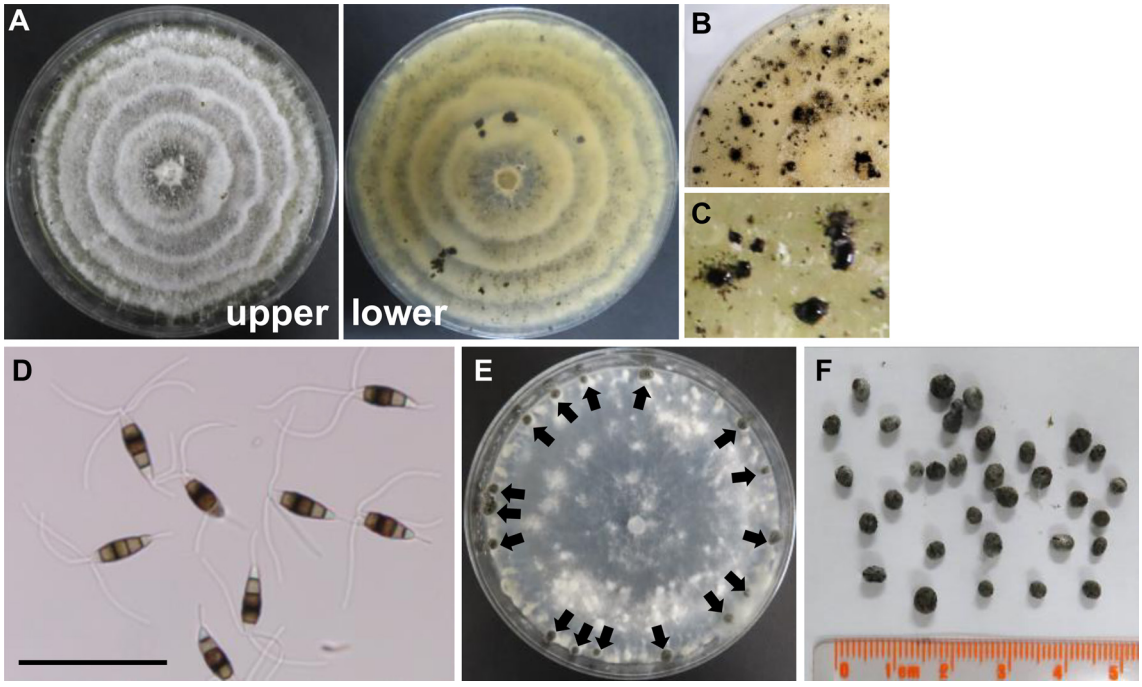

Fig. 2. Morphological characteristics of fungal isolates JJ 8-2-1 and JJ 8-2-2. (A) Fungal colony of isolate JJ 8-2-1 on 1/2-strength potato dextrose agar (PDA) media grown for 15 days at 25°C in the dark. (B) Fungal colony of isolate JJ 8-2-1 and acervuli formation on the media grown for 32 days at 25°C in the dark. (C) Enlarged photo of acervuli of isolate JJ 8-2-1. (D) Conidia of isolate JJ 8-2-1. Three apical appendages and a single basal appendage were shown. Note transparent apical and basal cells, and versicoloured median cells of isolate JJ 8-2-1 conidia. Size bar=50 µm. (E) Fungal colony of isolate JJ 8-2-2 on 1/2-strength PDA media grown for 28 days at 25°C in the dark. Mature black sclerotia of isolate JJ 8-2-2 formed on the media were indicated by black arrows. (F) Sclerotia of isolate JJ 8-2-2 with regular, round and ellipsoidal shape.

rated from other *Sclerotinia* groups, *S. nivalis*, *S. ginseng* and *S. minor*. The isolate JJ 8-2-2 nucleotide sequences have low homology with those from *Clarireedia homoeocarpa*, formerly classified as *S. homoeocarpa*. The phylogenetic properties were consistent with the cultural and morphological fungal classification of isolates JJ 8-2-1 and JJ 8-2-2 shown in Fig. 2.

Pathogenicity on the detached peony leaves. The isolates JJ 8-2-1 and JJ 8-2-2 induced similar soft rot symptoms on the detached peony leaves (Fig. 4). Brown water-soaked lesions were found in the detached peony leaves (two plant lines 9 and 10) inoculated by mycelial plugs from the isolates JJ 8-2-1 and JJ 8-2-2. The fungi were re-isolated from the diseased leaves, but not from uninoculated leaves (data not shown), indicating that two fungal isolates JJ 8-2-1 and JJ 8-2-2 were pathogenic to the peony leaves.

Antifungal activity of propineb. *In vitro* antifungal activity of chemical fungicide propineb was evaluated against isolates JJ 8-2-1 and JJ 8-2-2 (Fig. 5). Suppressed colony formation of isolates JJ 8-2-1 and JJ 8-2-2 was found on the media supplemented with different concentrations of propineb at 7 days and 2 days after culture, respectively (Fig. 5A). Dose-dependent inhibition of isolates JJ 8-2-1 and JJ 8-2-2 mycelial growth was demonstrated on the fungicide media (Fig. 5B). Increasing propineb concentrations to 6.25, 12.5, 25, 50, and 100 µg/ml resulted in gradual decreases in JJ 8-2-1 colony diameters to ca. 64.5, 56.6, 47.9, 42.7, 22.0, and 9.3 mm, respectively. The same propineb concentrations led to the reduced colony diameters of isolate JJ 8-2-2 to ca. 48.8, 37.8, 34.6, 31.5, 24.6, and 13.2 mm, respectively.

Antifungal activity of *B. siamensis* H30-3. Rhizobacteria *B. siamensis* H30-3 showed antifungal activities via two types of dual cultures with isolates JJ 8-2-1 and JJ 8-2-2 (Fig. 6). Mycelial growths of isolates JJ 8-2-1 and JJ 8-2-2 were inhibited on the media by *B. siamensis* H30-3 co-inoculated

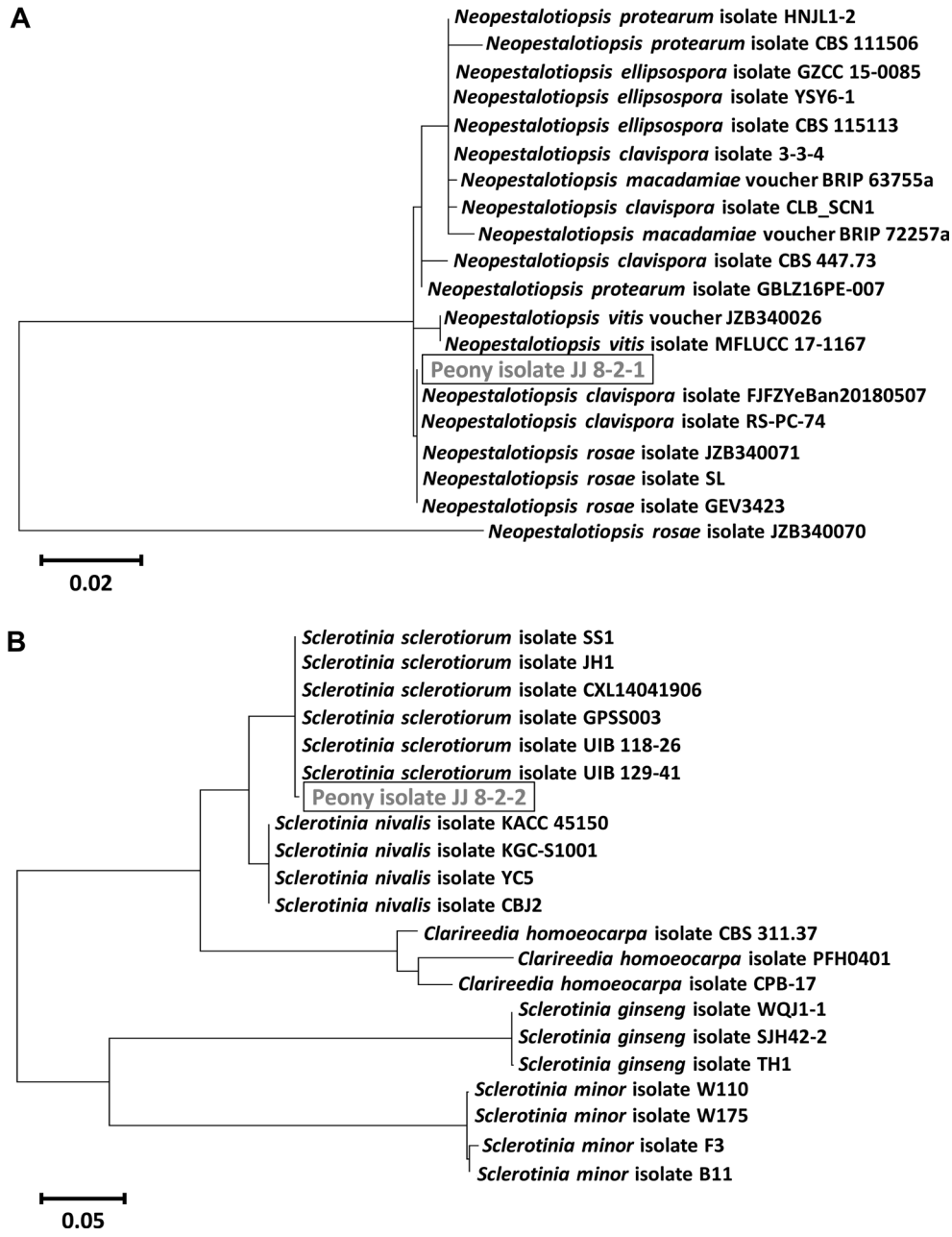

Fig. 3. Multilocus phylogenetic tree based on maximum likelihood analysis through the alignment of (A) the combined sequences of the internal transcribed spacer, β-tubulin and translation elongation factor 1-α genes of isolate JJ 8-2-1 and (B) the combined sequences of the internal transcribed spacer and β-tubulin gene of isolate JJ 8-2-2. The scale bars indicate the number of nucleotide substitutions.

in the same media compared to those in the absence of *B. siamensis* H30-3 (Fig. 6A). The colony radii of isolates JJ 8-2-1 and JJ 8-2-2 were evaluated with or without *B. siamensis* H30-3 co-inoculation (Fig. 6B). The colony radius of isolate JJ 8-2-1 was ca. 37.6 mm at 7 days without the co-inoculation, whereas it was reduced to 29.8 mm by the co-culture with *B. siamensis* H30-3. The colony radius of isolate JJ 8-2-2 reached ca. 48.6 mm at 3 days without the co-inoculation, whereas it was reduced to 40.0 mm by the co-culture with *B. siamensis* H30-3.

The volatile compounds originating from *B. siamensis* H30-3 also exhibited antifungal activities against the mycelial growth of isolate JJ 8-2-1, but not to isolate JJ 8-2-2 (Fig. 6C). The colony diameter of isolate JJ 8-2-1 was ca. 66.1 mm at 7 days without the *B. siamensis* H30-3 volatile compounds, but it was reduced to ca. 54.7 mm by the vola-

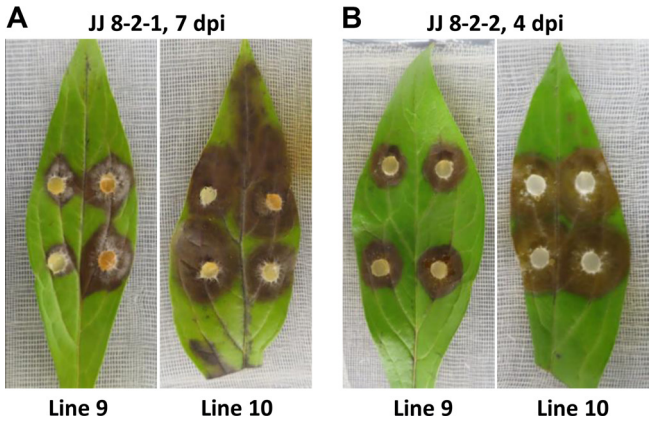

Fig. 4. Symptoms in the peony leaves artificially inoculated by the fungal isolates (A) JJ 8-2-1 and (B) JJ 8-2-2. Peony leaf lesions were taken at 7- and 4-days post-inoculation (dpi) for JJ 8-2-1 and JJ 8-2-2, respectively.

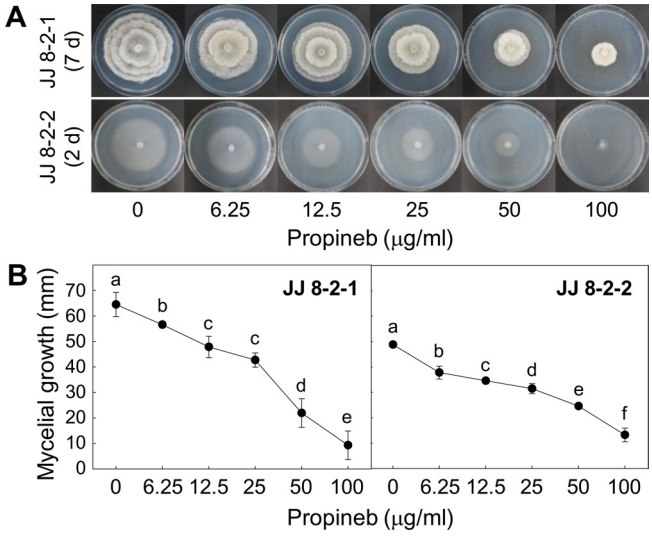

Fig. 5. *In vitro* antifungal efficacies of tow chemical fungicides thiophanate-methyl and propineb on mycelial growths of isolates JJ 8-2-1 and JJ 8-2-2. (A) Colony formation of isolates JJ 8-2-1 and JJ 8-2-2 on 1/2-potato dextrose agar media containing different concentrations of thiophanate-methyl and propineb. The fungal isolates were cultured at 25°C for 7 and 3 days for isolate JJ 8-2-1 and JJ 8-2-2, respectively. (B) Mycelial growth (%) of isolates JJ 8-2-1 and JJ 8-2-2 treated with different concentrations of thiophanate-methyl and propineb. Bars represent the standard errors of the means of the four independent experimental replications. Each experiment has four replications. Means followed by the same letters are not significantly different at 5% level by least significant difference test.

tile compounds. No significant difference was found in the mock- and volatile compounds-treated colony diameters of isolate JJ 8-2-2.

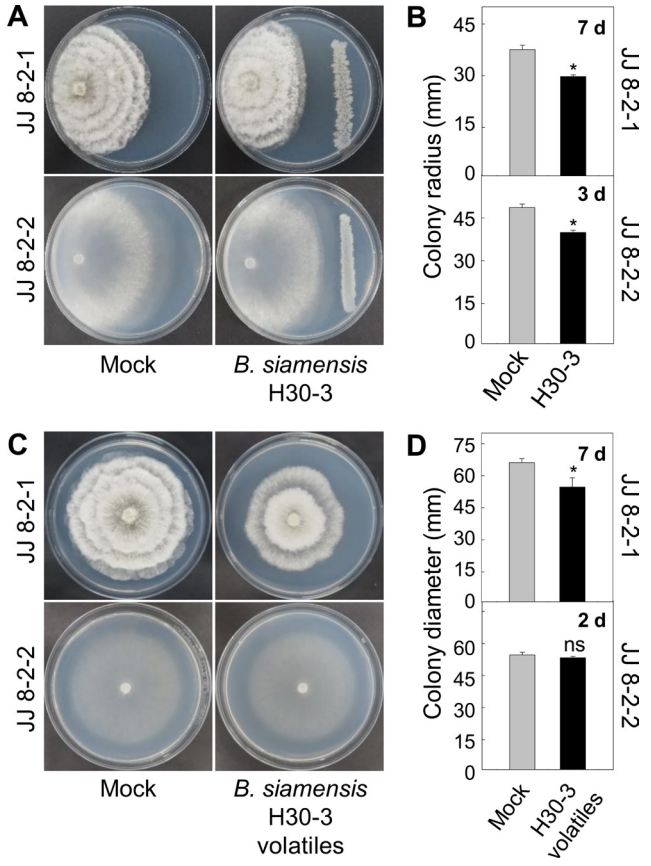

Fig. 6. *In vitro* antifungal activities of *Bacillus siamensis* H30-3 and its volatiles against peony stem rot fungal isolates JJ 8-2-1 and JJ 8-2-2. (A) Arrested mycelial growths of peony stem rot fungal isolates JJ 8-2-1 and JJ 8-2-2 observed in the dual cultures with *B. siamensis* H30-3 on 1/2-potato dextrose agar media. (B) Colony radius of the fungal isolates JJ 8-2-1 and JJ 8-2-2 after co-culture with *B. siamensis* H30-3. (C) Arrested mycelial growths of peony stem rot fungal isolates JJ 8-2-1 and JJ 8-2-2 on 1/2-potato dextrose agar media treated with volatiles from *B. siamensis* H30-3. (D) Colony diameter of the fungal isolates JJ 8-2-1 and JJ 8-2-2 treated with or without volatiles from *B. siamensis* H30-3. Bars represent the standard errors of the means of the four independent experimental replications. Each experiment has four replications. Asterisks indicate significant differences as determined by Student's *t*-test ($P<0.05$).

Discussion

Many fungal pathogens, such as *B. cinerea, C. fioriniae, E. aquilegiae*, have bothered peony plant production and caused economic loss (Kim et al., 2020; Park et al., 1996, 2020). The two fungal species, *Neopestalotiopsis clavispora* and *Sclerotinia sclerotiorum*, causing peony stem rots, are newly reported in South Korea in our current study, based on cultural, morphological, phylogenetical identification and pathogenicity assay. *N. clavispora* and *S. sclerotiorum* identified in peony

stems can be the respectively first report worldwide in our knowledge. *N. clavispora* is taxonomically close to different species of *Pestalotia* and *Pseudopestalotiopsis* (Akinsanmi et al., 2017; Darapanit et al., 2021). *Pestalotia paeoniicola* (synonym of *Pestalotiopsis paeoniicola*) causes brown leaf spots in peony (*P. lactiflora*) and twig blight in tree peony (*P. suffruticosa*) (Marra and Li, 2009; Park et al., 1996; The Korean Society of Plant Pathology, 2022). *S. sclerotiorum* was reported as a causative agent of peony white stem rot in Canada, but only identified in tree peony (*P. suffruticosa*) but not peony plants in South Korea so far (Ginns, 1986; The Korean Society of Plant Pathology, 2022).

The chemical fungicide propineb has been suggested for different fungal diseases in various crops worldwide and is also registered in South Korea for controlling leaf spots caused by *Alternaria* spp. in peony plants. However, the antifungal activity of propineb was not prevalently described against *N. clavispora* and *S. sclerotiorum* so far, and so did plant disease control efficacy as well. We evaluated the *in vitro* antifungal activity of propineb against *N. clavispora* JJ 8-2-1 and *S. sclerotiorum* JJ 8-2-2 to see whether the peony stem rots can be managed by the propineb. The antifungal activities of propineb against the two stem rot fungi *N. clavispora* JJ 8-2-1 and *S. sclerotiorum* JJ 8-2-2 were shown in this study, indicating decreased mycelial growths in dose-dependent manners. The completely suppressed *in vitro* mycelial growth of *N. clavispora* was demonstrated by a propineb dose (3 mg/ml) compared to the concentrations applied in our study (Amrutha and Vijayaraghavan, 2018). Leaf blights of strawberry plants inoculated by *N. clavispora* was efficiently reduced by foliar spraying the propineb twice at 10 days interval (Amrutha and Vijayaraghavan, 2018). The dose-dependent antifungal activity of propineb was also found during the mycelial growth of *S. sclerotiorum* (Krishnamoorthy et al., 2017). Cauliflower Sclerotinia rot was decreased by propineb (2 mg/ml), but the disease control efficacy was relatively lower than those mediated by the same doses of azoxystrobin and thiophanate-methyl (Jajoriya et al., 2022). It needs to investigate the control efficacy of propineb on stem rots in peony plants in the fields. In addition, it is worth testing the antifungal activity and disease protection efficacies of other chemical fungicides on *N. clavispora* and *S. sclerotiorum* causing stem rots in peony plants.

Biological control of fungal diseases in herbaceous and tree peony plants has hardly been demonstrated. Recently, endophytic *B. amyloliquefaciens* Mdgb15 was suggested as a potential biological control agent for grey mould in tree peony plants (Yang et al., 2024). *B. siamensis* H30-3 isolated from the rhizosphere soil of cucumber plants has revealed its *in vitro* antifungal activities against diverse ranges of phytopathogens and plant disease control efficacies (Lee et al., 2018; Park et al., 2021; Shin et al., 2019). Diffusible and/or volatile compounds from *B. siamensis* H30-3 were likely to be involved in the antifungal activity against *Alternaria brassicicola*, *Colletotrichum fructicola*, *C. higginsianum*, *Lasiodiplodia theobromae* and *Phytophthora cactorum*. *B. siamensis* H30-3-suppressed mycelial growths of *N. clavispora* JJ 8-2-1 and *S. sclerotiorum* JJ 8-2-2 suggest that *B. siamensis* H30-3 can be applied to the biological control of peony stem rots. It will be necessary to evaluate that *B. siamensis* H30-3 is playing effectively in integrated disease management in peony plant fields with chemical fungicides including propineb.

Taken together, stem rots were found in herbaceous peony plants and *N. clavispora* JJ 8-2-1 and *S. sclerotiorum* JJ 8-2-2 were identified based on the standard procedures of plant disease diagnosis. *In vitro* antifungal activities of fungicide propineb and rhizosphere bacteria *B. siamensis* H30-3 were evaluated to suggest chemical and biological control agents for peony stem rot diseases. Further investigation into the control efficacies of the chemical and biological control agents in the peony plant fields will provide eco-friendly disease management.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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