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RESEARCH NOTE

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Identification of Fusarium Basal Rot Pathogens of Onion and Evaluation of Fungicides against the Pathogens

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ABSTRACT

Onion (*Allium cepa* L.) is an economically important vegetable crop worldwide. However, various fungal diseases, including Fusarium basal rot (FBR), neck rot, and white rot, reduce onion production or bulb storage life. FBR caused by *Fusarium* species is among the most destructive onion diseases. In this study, we identified *Fusarium* species associated with FBR in Jeolla and Gyeongsang Provinces in South Korea and evaluated fungicides against the pathogens. Our morphological and molecular analyses showed that FBR in onions is associated with *Fusarium commune*, *Fusarium oxysporum*, and *Fusarium proliferatum*. We selected seven fungicides (fludioxonil, hexaconazole, mandestrobin, penthiopyrad, prochloraz-manganese, pydiflumetofen, and tebuconazole) and evaluated their inhibitory effects on mycelial growth of the pathogens at three different concentrations (0.01, 0.1, and 1 mg/mL). We found that prochloraz-manganese was highly effective, inhibiting 100% of the mycelial growth of the pathogens at all concentrations, followed by tebuconazole. Fludioxonil showed < 50% inhibition at 1 mg/mL for the tested isolates.

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The genus *Allium* (family Amaryllidaceae) comprises more than 600 species, including cultivated onion (*Allium cepa* L.), garlic (*Allium sativum* L.), leek (*Allium ampeloprasum* var. *porrum* L.), and chive (*Allium schoenoprasum* L.) [1]. Onion is among the most commonly grown and consumed vegetable crops worldwide, accounting for 23.8% of the total global vegetable farming area in 2019 [2]. Onion is consumed in salads, with seasoned vegetables, and in side dishes, and is recognized as a valuable healthy food containing bioactive compounds and phytochemicals such as flavonoids, fructo-oligosaccharides, thiosulfinates, and other sulfur compounds [3–5]. China is the largest onion-producing country worldwide, followed by India, the United States, Egypt, and Turkey, with South Korea ranked 12th in 2019 [6]. Onion production in South Korea was 1,168,227 tons over a cultivated area of 14,673 ha in 2020, increasing to 1,576,756 tons over 18,461 ha in 2021 [7,8]. The largest onion-producing region in South Korea is Jeonnam Province, which recorded 579,042 tons of onion production in 2021, corresponding to 36.7% of the total onion production in South Korea [8].

Onion production can be affected by various diseases caused by bacteria, fungi, nematodes, and viruses, among which fungal pathogens cause severe yield losses [9]. These pathogens include *Fusarium oxysporum* f. sp. *cepae* (Fusarium basal rot), *Botrytis aclada* and *Botrytis allii* (neck rot), *Aspergillus niger* (black mold), *Penicillium* spp. (blue mold), *Sclerotium cepivorum* (white rot), *Peronospora destructor* (downy mildew), and *Stemphylium vesicarium* (Stemphylium leaf blight) [10–19]. Fusarium basal rot (FBR) caused by *F. oxysporum* f. sp. *cepae* is among the most destructive onion diseases worldwide, causing yield losses of >50% [20]. The fungus invades onions through direct penetration or natural wounds in the basal plate and roots and causes disease at any stage of onion development, leading to delayed seedling emergence, seedling death, and pre- and post-harvest bulb rot [21–23]. Although FBR can be caused by a single *Fusarium* species, it is also associated with complexes of different *Fusarium* species, including *F. oxysporum* f. sp. *cepae*, *Fusarium proliferatum*, *Fusarium redolens*, and *Fusarium avenaceum* [24–27]. The compositions of these FBR pathogenic complexes vary regionally

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and among growing seasons [28]. The identification of *Fusarium* complexes associated with FBR in onions is required for better disease management.

In recent years, FBR has become a serious problem in onion production and storage in South Korea. However, information on the *Fusarium* species associated with FBR in onions remains limited, and no fungicides are available to manage the disease. The objectives of this study were to determine which *Fusarium* species are associated with FBR in onions and identify effective fungicides against the pathogens. We collected FBR-infected onion bulbs, roots, and stems in onion cultivation fields in Jeolla and Gyeongsang Provinces, identified the causal agents of FBR, and evaluated different classes of fungicides against the pathogens.

Fusarium species were isolated from bulbs, roots, and stems of onions showing symptoms of FBR from onion fields in Jeolla and Gyeongsang Provinces in South Korea during 2016–2022 (Table S1). Plant samples were placed individually in plastic bags and moved to the laboratory. For fungal isolation, plant samples were cut into 10-mm pieces, surface-sterilized with 1% sodium hypochlorite solution for 1 min, rinsed three times in sterile distilled water (SDW), and grown on potato dextrose agar (PDA). Pure fungal isolates were obtained by transferring emerging hyphal tips or single spores to new PDA plates. Mycelial agar plugs of each isolate were mixed with 25% glycerol and stored in a deep freezer at −75 °C before use. PDA and carnation leaf agar were used to examine the morphological characteristics of *Fusarium* isolates as described by Nelsone et al. [29].

Fusarium isolates were identified based on morphological analysis and sequencing of the translation elongation factor 1 alpha (*TEF1*) gene region. Genomic DNA was extracted from each isolate using the HiGene Genomic DNA Prep Kit (Biofact, Daejeon, Korea), according to the manufacturer's instructions. The *TEF1* region was amplified using H-Star *Taq* PCR Master Mix (Biofact, Daejeon, Korea) using the primers EF1-728F (5′-CATCGAG AAGTTCGAGAAGG-3′) and EF1-986R (5′-TACTT GAAGGAACCCTTACC-3′) or EF1-1251R (5′-CCT CGAACTCACCAGTACCG-3′) [30,31], and sequenced by Biofact (Daejeon, Korea). Oligonucleotide primers were synthesized by Bioneer (Daejeon, Korea). The obtained sequences were analyzed using the National Center for Biotechnology Information BLAST program (http://blast.ncbi.nlm.nih.gov/). Sequence alignment was performed using the ClustalW program in the MEGA 6.0 software, and the phylogenetic relationship was constructed using the maximum-likelihood (ML) method and Tamura-Nei model with 500 bootstrap replicates [32,33].

Onions showing FBR symptoms, including yellowing leaves, wilt, bulb rot, and root rot, were collected from onion fields in Jeolla and Gyeongsang Provinces (Figure 1(A,B)). Severely rotted bulb tissues were dark brown, sunken, and watery, with white mycelia on the bulb surface (Figure 1(C)). These disease symptoms were identical to those previously reported for FBR [25,28]. Although the internal transcribed spacer (ITS) is widely used for the identification of fungi, closely related *Fusarium* species are not effectively discriminated against by ITS [34]. Many protein-encoding genes have been investigated for the identification of *Fusarium*, and the *TEF1* gene was found to be a powerful marker for this genus [34–36]. To identify fungal pathogens associated with FBR in onions, fungi were isolated from the collected samples and identified based on *TEF1* sequences. A total of 41 isolates were identified as *Fusarium* through BLAST searches of GenBank using the *TEF1* sequences of each fungal isolate: 15 isolates of *F. commune*, 12 of *F. oxysporum*, and 14 of *F. proliferatum* (Table S1). This result indicates that *F. commune*, *F. oxysporum*, and *F. proliferatum* are mainly responsible for FBR disease in onions. To analyze the genetic relationships among *Fusarium* isolates, an ML-based phylogenetic tree was constructed using the *TEF1* sequences of *Fusarium* isolates, with those of *Fusarium* spp. from other studies used as reference sequences (Figure 2). *Fusarium commune* and *F. proliferatum* isolates were each clustered within a single clade, and *F. oxysporum* isolates were clustered within four clades, indicating a high degree of genetic diversity (Figure 2).

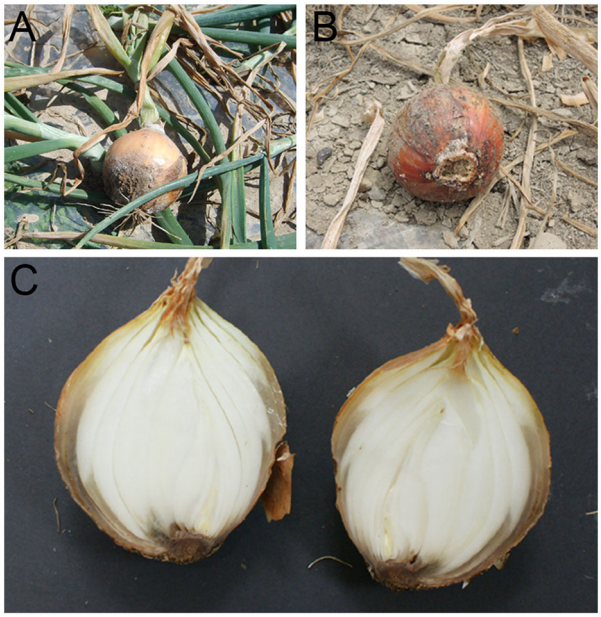

Figure 1. Typical symptoms of Fusarium basal rot (FBR) in onion. (A, B) FBR symptoms include leaf curling, yellowing, and rotting of bulbs, with whitish mycelium on the bulb surface. (C) Bulb tissue appears dark brown when cut open.

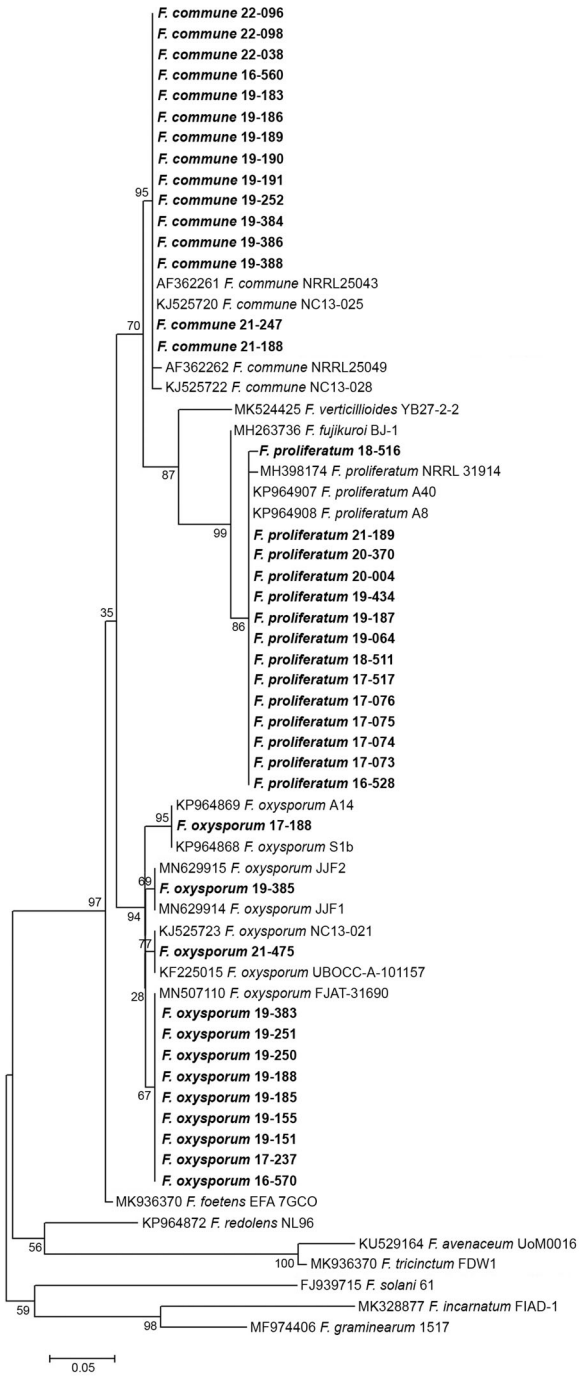

Figure 2. A phylogenetic tree of *Fusarium* isolates generated through maximum-likelihood analysis of *TEF1* sequences. Node numbers indicate bootstrap values from 500 replicates. The scale bar indicates 0.05 nucleotide substitutions per site.

Combining the result of molecular and morphological analyses provides more reliable data for *Fusarium* species identification compared to the analysis of molecular data alone [36,37,46,47]. Therefore, we further identified *F. commune*, *F. oxysporum*, and *F. proliferatum* isolates based on their morphological characteristics. Microconidia of *F. oxysporum* were oval-shaped, with 0–1 septae but usually aseptate, and produced on monophialides (Figure 3(F,G)). Macroconidia were slightly curved, with foot-shaped or notched basal cells and 3–5 septae (Figure 3(G)). Microconidia of *F. proliferatum* were club-shaped with a flattened base, aseptate, and produced in chains or false heads on mono- and polyphialides (Figure 3I–K). Macroconidia were straight or slightly curved with poorly developed basal cells and 3–5 septae (Figure 3K). *Fusarium commune* shared similar morphological features to *F. oxysporum*, except that *F. commune* produced polyphialides as well as monophialides (Figure 3(A–D)). *Fusarium commune* and *F. oxysporum* produced abundant chlamydospores singly or in pairs, whereas chlamydospores were absent in the culture of *F. proliferatum* (Figure 3(E,H,L). *Fusarium commune* was originally misidentified as *F. oxysporum* due to their similar morphological features [37,38]. Both species produce conidia in false heads on monophialides, and chlamydospores are present. However, *F. commune* was identified as a new species in 2003 according to morphological features and DNA sequence data from *TEF1* [37]. These species differ in that only *F. commune* produces conidia on both mono- and polyphialides [37]. *Fusarium proliferatum* is morphologically distinguishable from *F. commune* and *F. oxysporum* by its microconidial chains, which are not observed in the latter two species [29,39]. Our morphological observations of *F. commune*, *F. oxysporum*, and *F. commune* isolates were consistent with these previous reports, supporting the phylogenetic analysis constructed using the *TEF1* sequences of *Fusarium* isolates.

Damping off caused by FBR is a common disease in onion seedlings, found in almost all onion production fields [28,40]. To determine whether *F. commune*, *F. oxysporum*, and *F. proliferatum* were the causal agents of FBR in onions, we investigated the pathogenicity of the isolates in onion seedlings based on Koch's postulates. Conidia were obtained from *F. commune* 16-560, *F. oxysporum* 19-385, and *F. proliferatum* 17-073 in 2-day-old cultures with carnation leaf pieces shaken at 150 rpm in potato dextrose broth (PDB). The conidial suspension was filtered through two layers of cheesecloth, counted using a hemocytometer, and adjusted to 8×10^5 conidia/mL. Onion seeds (*A. cepa* L. cv. Hwangryongball) were surface-sterilized with 1% sodium hypochlorite solution for 3 min, rinsed twice in SDW, and incubated for 2 days in sterile Petri dishes with wet paper towels in the dark. The onion seeds were then soaked in the conidial suspension of *Fusarium* isolates or in non-inoculated PDB as a control for 90 min. For each treatment, 50 seeds were planted in a pot and incubated at 25 °C under a 12-h light/12-h dark light cycle for 12 days. This experiment was conducted in triplicate and repeated three times. All tested isolates significantly reduced seedling survival rate compared with the non-inoculated control (Figure 4(A,B)). *Fusarium proliferatum*

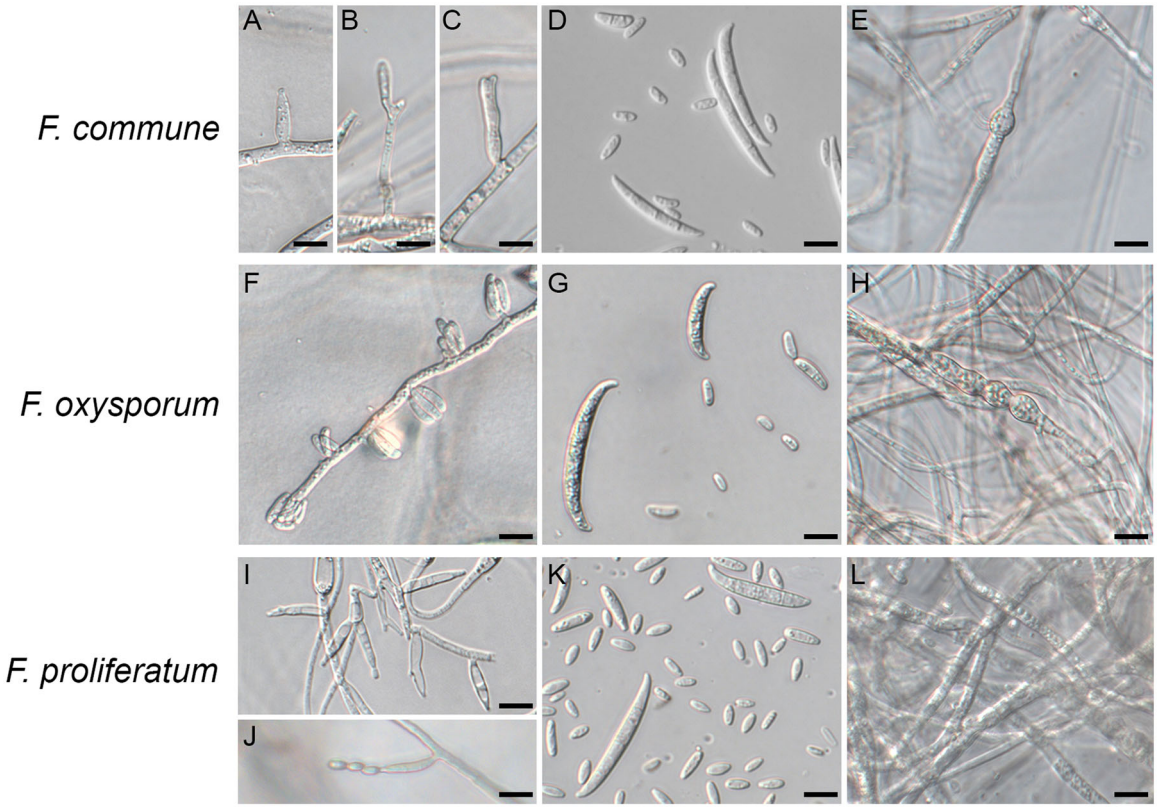

Figure 3. Morphological characteristics of *Fusarium commune*, *Fusarium oxysporum*, and *Fusarium proliferatum*. (A) Monophialides, (B, C) polyphialides, (D) micro- and macroconidia, and (E) chlamydospores in the middle of hyphae of *F. commune*. (F) Microconidia in false heads from monophialides, (G) micro- and macroconidia, and (H) chlamydospores in the middle of hyphae of *F. oxysporum*. (I) Mono- and polyphialides, (J) a chain of microconidia, and (K) micro- and macroconidia of *F. proliferatum*. (L) Chlamydospore formation was absent in the culture of *F. proliferatum*. Scale bars = 10 μm.

17–073 was the most pathogenic isolate, with approximately 12% of seedling survival rate, followed by *F. commune* 16-560 (48%) and *F. oxysporum* 19–385 (53%). Seedling survival rate in the control pots was approximately 98%. We observed the appearance of white mycelium on dead seeds or seedlings by damping off in all fungal treatments (Figure 5), from which these pathogens were isolated, fulfilling Koch's postulates. Next, we performed an onion bulb test. Mature onion bulbs were rinsed with SDW three times and cut vertically. Mycelial agar plugs from 7-day-old PDA cultures of *F. commune* 16–560, *F. oxysporum* 19-385, and *F. proliferatum* 17-073 were inoculated onto the vertically cut basal plates of mature onion bulbs and incubated for 8 days in a moistened box. This experiment was conducted in triplicate and repeated three times. All isolates caused typical FBR symptoms, with the development of white mycelium on the bulb surface, water soaking, and rotting of bulb tissues (Figure 4(C)). These pathogens were re-isolated from infected plants, but not from control plants, fulfilling Koch's postulates. Collectively, these results show that *F. commune*, *F. oxysporum*, and *F. proliferatum* are the causal agents of onion basal rot. Although *F. oxysporum* f. sp. *cepae* is well known to be an FBR pathogen in onion, FBR is also associated with various *Fusarium* complexes [24–27]. In Finland, *F. oxysporum*, *F. proliferatum*, and *F. redolens* were reported as the major *Fusarium* species causing FBR [26]. In East Azerbaijan Province, Iran, *F. oxysporum*, *F. solani*, *F. proliferatum*, and *F. redolens* were reported to cause FBR [25]. Consistent with these reports, we demonstrated that different *Fusarium* species were associated with FBR in South Korea. Previous studies have isolated *F. oxysporum* and *F. proliferatum* from market onion bulbs or diseased onion bulbs in low-temperature storage facilities in South Korea [10,18]. However, in our study, *F. commune* was frequently isolated from onion fields, which has not previously been reported in South Korea. Therefore, to our knowledge, this is the first report of FBR in onion caused by *F. commune* in South Korea.

Seven fungicides that are used to manage other fungal pathogens, including *Sclerotium cepivorum*, which causes white rot in onions, were selected to evaluate their inhibitory effect on mycelial growth in *Fusarium* isolates (Table 1). The chemical groups, active ingredients, and formulations of these fungicides are listed in Table 1. Fungal isolates used for this experiment are shown in Table 2. The fungicides were dissolved in SDW and added to PDA after autoclaving when the media had cooled to

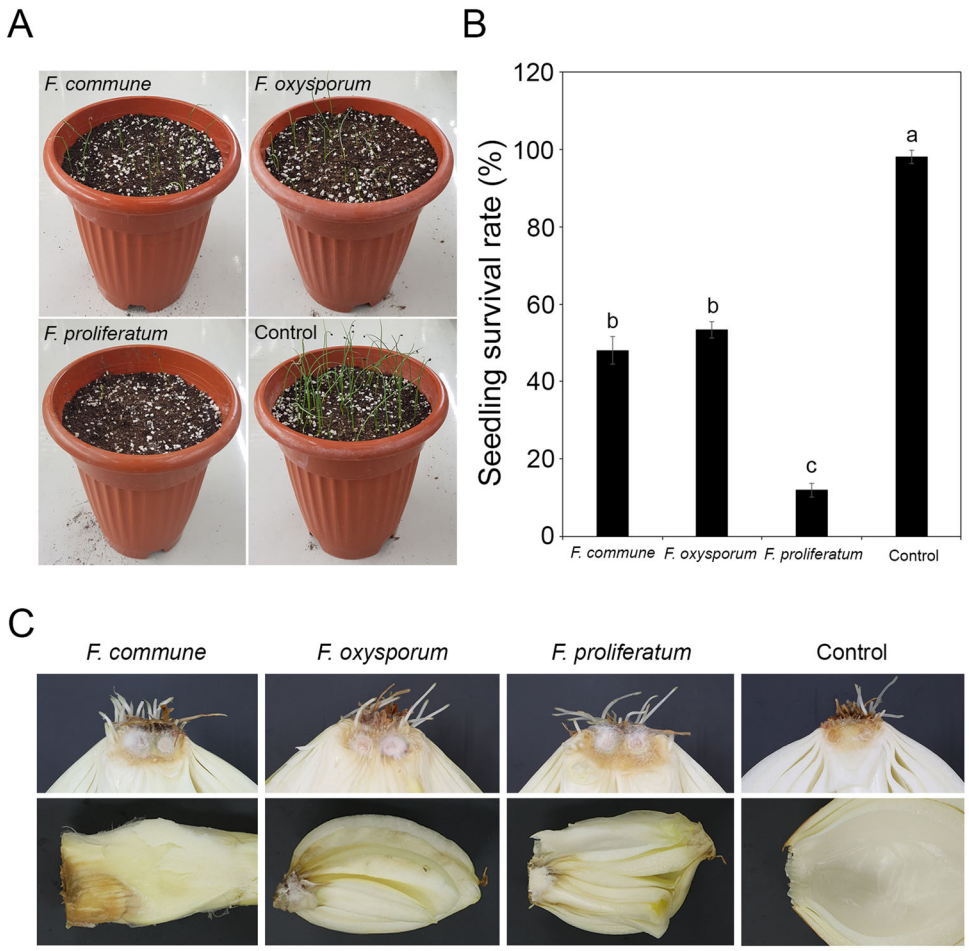

Figure 4. Pathogenicity tests of *Fusarium. commune* 16-560, *Fusarium. oxysporum* 19-385, and *Fusarium. proliferatum* 17-073. Onion seeds (*Allium cepa* L. cv. Hwangryongball) were soaked in the conidial suspension (8×10^5 conidia/mL) of each isolate for 90 min and planted in pots. Pots without the tested pathogens were used as controls. At 12 days after inoculation, photographs were taken (A) and seedling survival rate was measured (B). different letters on bars indicate significant differences according to Tukey's test ($p < 0.05$). (C) Mycelial agar plugs from PDA cultures of each isolate were inoculated on the vertically cut basal plates of onion bulbs. Agar plugs without mycelia were used as controls. Photographs were taken 8 days after inoculation.

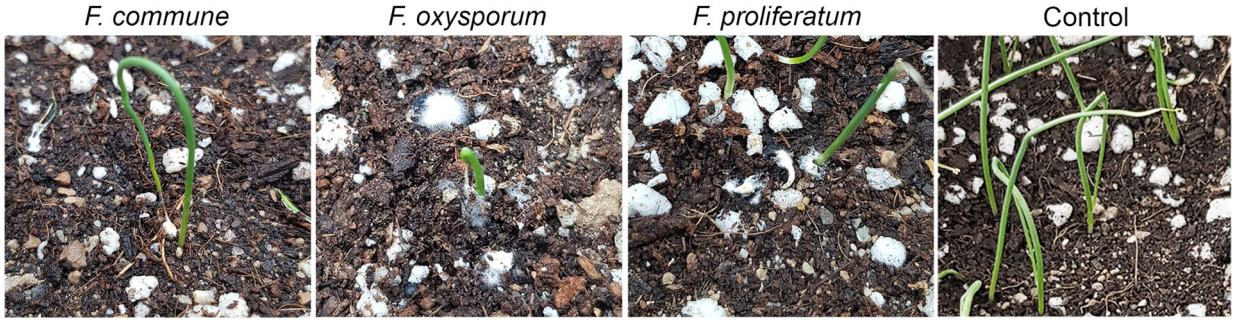

Figure 5. Onion seeds and seedlings infected with Fusarium basal rot pathogens under pathogenicity test.

Table 1. Fungicides used in this study.

Chemical group	Active ingredient	Formulation[a]
Phenylpyrroles	Fludioxonil	20% SC
Triazoles	Hexaconazole	2% SC
Methoxy-acetamide	Mandestrobin	40% SC
Pyrazole-4-carboxamides	Penthiopyrad	20% EC
Imidazoles	Prochloraz-manganese	50% WP
N-methoxy-(phenyl-ethyl)-pyrazole-carboxamides	Pydiflumetofen	18.35% SC
Triazoles	Tebuconazole	20% SC

[a]EC: emulsifiable concentrate; SC: suspension concentrate; WP: wettable powder.

Table 2. Effect of seven fungicides on mycelial growth inhibition of *Fusarium commune*, *Fusarium oxysporum*, and *Fusarium proliferatum* isolates. Colony diameters were measured at 4 days after inoculation, and the inhibition rate was compared to the control.

		Mycelial growth inhibition (%)											
		F. commune isolate				*F. oxysporum* isolate				*F. proliferatum* isolate			
Fungicide	Concentration (mg/mL)	16-560	19–252	19–388	21–247	17–188	19–155	19–385	21–475	17–073	17–076	19–064	21–189
Fludioxonil	0.01	23.0 j	21.6 j	25.6 l	4.4 ij	19.3 i	16.6 k	8.5 n	12.4 i	23.0 k	19.4 jk	26.7 k	28.1 j
(20% SC)	0.1	39.3 i	26.7 i	43.7 ij	12.2 i	26.7 h	25.5 j	13.9 m	18.0 h	27.7 j	22.4 j	34.8 j	30.4 j
	1	46.2 h	46.6 g	47.4 i	30.3 gh	36.2 g	43.0 h	37.1 j	37.7 g	39.9 h	36.3 i	44.9 i	39.9 i
Hexaconazole	0.01	40.3 i	36.5 h	31.1 k	36.8 g	28.9 h	35.5 i	27.7 l	35.1 g	35.6 i	23.2 j	33.7 j	27.1 j
(2% SC)	0.1	71.1 e	76.7 c	73.6 d	65.8 de	68.0 d	68.5 d	59.3 h	71.4 d	75.8 d	62.3 fg	75.9 d	68.3 f
	1	92.1 b	89.2 b	93.7 b	88.9 b	85.6 b	92.8 b	79.6 c	91.0 b	91.3 b	83.5 b	90.9 b	91.1 b
Mandestrobin	0.01	58.2 g	39.7 h	42.7 j	52.2 f	50.7 f	54.7 g	54.2 i	61.1 f	61.0 g	50.8 h	64.5 h	49.8 h
(40% SC)	0.1	65.3 f	48.8 g	52.7 h	58.9 ef	52.7 f	67.0 de	64.5 ef	65.6 ef	67.2 f	52.4 h	67.6 gh	52.0 h
	1	71.1 e	55.3 f	57.1 g	61.7 e	56.8 e	75.3 c	66.5 e	68.9 de	71.9 e	59.7 g	72.3 ef	72.6 ef
Penthiopyrad	0.01	2.4 k	19.6 j	21.0 m	2.1 j	13.8 j	10.4 l	1.8 o	8.9 i	9.8 l	15.7 k	10.2 l	15.7 k
(20% EC)	0.1	36.7 i	49.5 g	52.4 h	22.9 h	36.7 g	45.3 h	33.8 k	39.2 g	40.7 h	37.2 i	43.2 i	39.5 i
	1	80.8 c	78.4 c	80.3 c	74.6 c	77.7 c	88.6 b	77.9 c	77.2 c	78.8 c	77.0 cd	80.5 c	78.8 d
Prochloraz-manganese	0.01	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
(50% WP)	0.1	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
	1	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Pydiflumetofen	0.01	70.4 e	59.2 ef	61.8 f	61.9 e	66.0 d	58.7 fg	59.8 gh	68.2 de	67.3 f	66.1 ef	69.4 fg	61.9 g
(18.35% SC)	0.1	72.9 de	61.1 e	63.5 f	65.5 de	68.0 d	61.0 f	62.0 fg	71.9 d	70.2 e	68.2 e	73.1 de	67.7 f
	1	76.9 cd	65.3 d	67.8 e	71.1 cd	74.8 c	63.4 ef	71.8 d	73.5 cd	74.3 d	75.9 d	77.0 d	72.1 e
Tebuconazole	0.01	100 a	100 a	100 a	100 a	86.3 b	78.0 c	82.8 b	87.4 b	90.8 b	81.1 bc	91.1 b	86.9 c
(20% SC)	0.1	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
	1	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a

Means with different letters in each column indicate significant differences according to Duncan's test ($p < 0.05$).

approximately 65 °C. Mycelial agar plugs from 7-day-old PDA cultures was placed at the center of each fungicide-containing medium. The fungicides were tested at concentrations of 0.01, 0.1, and 1 mg/mL based on the formulated product, consisting of an active ingredient plus inert ingredients. The control medium contained only PDA. Colony diameters were measured at 4 days after inoculation at 25 °C in the dark, and the inhibition rate was compared to those of the control media. Experiments were conducted in triplicate and repeated three times, and the resulting data were analyzed using Duncan's test ($p < 0.05$) with the SPSS software (IBM Corp., Armonk, NY, USA). We assessed the inhibitory effect of the seven fungicides on the mycelial growth of *F. commune*, *F. oxysporum*, and *F. proliferatum* isolates. The most effective fungicide was prochloraz-manganese, showing 100% growth inhibition for the tested fungi at all concentrations, followed by tebuconazole (Table 2). The imidazole fungicide prochloraz and the triazole fungicide tebuconazole are sterol demethylation inhibitors, which inhibit the C-14 α-demethylation of 24-methylene dihydro lanosterol, a precursor of ergosterol in fungi [41]. Several studies conducted in other countries have reported that prochloraz alone or in combination with other fungicides has strong inhibitory effects on the mycelial growth of *F. oxysporum* f. sp. *cepae* and FBR incidence in onion [2,28,42,43]. Tebuconazole was also found to be effective against *F. oxysporum* f. sp. *cepae* [2]. Our findings are consistent with these previous results. Hexaconazole, a triazole fungicide, was less effective than tebuconazole, but still showed a high inhibitory effect at 1 mg/mL, ranging from 79.6% to 93.7% inhibition of the tested fungi (Table 2). Fludioxonil, a phenylpyrrole fungicide, has broad-spectrum activity against various plant pathogenic fungi, including *Botrytis*, *Fusarium*, *Sclerotinia*, *Aspergillus*, and *Penicillium* [44,45]. A recent study reported that fludioxonil has an inhibitory effect against FBR [42]. In this study, fludioxonil was less effective than the other tested fungicides, ranging from 30.3–47.4% inhibition at 1 mg/mL. Penthiopyrad exhibited similar or weaker inhibitory effects to fludioxonil at 0.01 mg/mL, but was more effective than mandestrobin and pydiflumetofen at 1 mg/mL (Table 2). Mandestrobin and pydiflumetofen showed relatively high inhibitory effects at 0.01 mg/mL, with 42.7–64.5% and 58.7–70.4% inhibition, respectively. However, increasing the concentration to 1 mg/mL did not exceed 80% inhibition. In conclusion, we identified the causal agents of FBR in onion in South Korea through molecular and morphological analyses and explored the inhibitory effects of seven fungicides against the pathogens. The results of this study provide much-needed data for the development of effective FBR management strategies.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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