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# Enhancement of Disease Control Efficacy of Chemical Fungicides Combined with Plant Resistance Inducer 2,3-Butanediol against Turfgrass Fungal Diseases

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**Turfgrass, the most widely grown ornamental crop, is severely affected by fungal pathogens including *Sclerotinia homoeocarpa*, *Rhizoctonia solani*, and *Magnaporthe poae*. At present, turfgrass fungal disease management predominantly relies on synthetic fungicide treatments. However, the extensive application of fungicides to the soil increases residual detection frequency, raising concerns for the environment and human health. The bacterial volatile compound, 2,3-butanediol (BDO), was found to induce plant resistance. In this study, we evaluated the disease control efficacy of a combination of stereoisomers of 2,3-BDO and commercial fungicides against turfgrass fungal diseases in both growth room and fields. In the growth room experiment, the combination of 0.9% 2R,3R-BDO (levo) soluble liquid (SL) formulation and 9% 2R,3S-BDO (meso) SL with half concentration of fungicides significantly increased the disease control efficacy against dollar spot and summer patch disease when compared to the half concentration of fungicide alone. In field experiments, the disease control efficiency of levo 0.9% and meso 9% SL, in combination with a fungicide, was confirmed against dollar spot and large patch disease. Additionally, the induction of defense-related genes involved in the salicylic acid and jasmonic acid/ethylene signaling pathways and reactive oxygen species detoxification-related genes under *Clarireedia* sp. infection was confirmed with levo 0.9% and meso 9% SL treatment in creeping bentgrass. Our findings suggest that 2,3-BDO isomer formulations can be combined with chemical fungicides as a new integrated tool to control *Clarireedia* sp. infection in turfgrass, thereby reducing the use of chemical fungicides.**

***Keywords*** **:** 2,3-butanediol stereoisomers, fungal diseases, fungicides, induced resistance, turfgrass

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Turfgrass is an agriculturally and economically important cultivar of grass that encompasses lawns, parks, roadsides, sports fields, and golf courses. Humans have been using turfgrasses for over 10 centuries, as it offers many recreational health benefits and positive environmental impacts (Beard and Green, 1994; Stackhouse et al., 2020; Zhou et al., 2011). However, turfgrass species are quite susceptible to a wide range of fungal pathogens. Dollar spot, brown patch, summer patch, and large patch are some common fungal diseases caused by *Sclerotinia homoeocarpa*, *Rhizoctonia solani*, and *Magnaporthe poae*, which are responsible for severe damage to turfgrass lawns (Benelli et al., 2018; Kobayashi et al., 1995; Shi et al., 2018). Presently, the control of turfgrass fungal diseases mainly depends on synthetic fungicides. However, continuous use of fungicides for turf disease increases the frequency of residual detection above the standard level (Kim et al., 2014). In agricultural systems, fungicide persistence raises concerns

about soil function, crop production, nutrient cycling, soil biodiversity, and food safety, as well as off-site migration of chemicals (e.g., due to run-off and leaching) (Liu et al., 2018; Ogura et al., 2021; Wightwick et al., 2010). Such off-site migration adversely affects humans and other nontarget organisms (Damalas and Eleftherohorinos, 2011; Ogura et al., 2021). Hence, integrated turfgrass management strategies are essential to reduce the level of fungicide residue in soil.

Several studies have focused on the usage of induced systemic resistance (ISR) and systemic acquired resistance (SAR) plant defense mechanisms as alternative strategies for a broad spectrum of fungal pathogen control (Rahman et al., 2015; Sharma et al., 2019; Tyagi et al., 2020; Urooj et al., 2021). Previous studies reported that the volatiles emitted from plant growth-promoting rhizobacteria (PGPR) were responsible for inducing crop resistance (Chung et al., 2016; Ryu et al., 2003). A well-characterized bacterial volatile 2,3-butanediol (BDO) is produced by many PGPR bacteria such as *Bacillus* spp., *Klebsiella* spp., and *Paenibacillus* spp. (Barrett et al., 1983; Garg and Jain, 1995; Ryu et al., 2004). It consists of three stereoisomers, i.e., two enantiomers (2R,3R- and 2S,3S-BDO) and one meso compound (2R,3S-BDO). It has been reported that 2,3-BDO treatment promotes plant growth and ISR in *Arabidopsis thaliana* and tobacco (Han et al., 2006; Ryu et al., 2003). Treatment of maize with 2,3-BDO and 2-butanol promotes plant growth and induced defense against the insect pest *Spodoptera littoralis* (D'Alessandro et al., 2014). Furthermore, (2R,3R)-BDO application induced plant defense in *Capsicum annuum* under pathogen infection (Kong et al., 2018; Yi et al., 2016). In *Agrostis stolonifera* (creeping bentgrass), application of (2R,3R)-BDO activates ISR against foliar diseases caused by *Microdochium nivale, R. solani,* and *S. homoeocarpa* (Cortes-Barco et al., 2010). Recently, 2,3-BDO application in creeping bentgrass also activated disease-resistance against *R. solani* by inducing phytohormone and antioxidant responses (Shi et al., 2018). However, the efficacy of 2,3-BDO isomers in combination with fungicides to turf disease control remains unknown.

In this study, we investigated the efficacy of 2,3-BDO isomers formulations in combination with the half concentration of fungicides such as azoxystrobin, pencycuron, and tebuconazole, for controlling economically important turfgrass fungal diseases in South Korea under growth room and field conditions. First, we examined the effect of the combinations of soluble liquid (SL) formulation of (R,R)-2,3-BDO (levo 0.9% 2,3-BDO SL) and (R,S)-2,3-BDO (meso 9%-2,3-BDO SL) with half concentrations of tebuconazole and azoxystrobin against dollar spot and brown patch, and summer patch, respectively, under growth room conditions. We then assessed the disease control efficacy of the combinations of 2,3-BDO formulations and half concentration of chemical fungicides such as tebuconazole and pencycuron against dollar spot disease and large patch disease, respectively, under field experiments. Further, the expression of plant defense-related genes in 2,3-BDO-treated creeping bentgrass were analyzed.

## Materials and Methods

**Preparation of levo and meso 2,3-BDO SLs formulations.** (R,R)-2,3-BDO (0.9 g) was mixed with 1.0 g of ethoxylated C12-14 secondary alcohol (Softanol 90, Nippon Shokubai Co., Ltd., Osaka, Japan) as an emulsifier, 30.0 g of propylene glycol monomethyl ether as a solvent, and 68.1 g of water to prepare levo 0.9% 2,3-BDO SL formulation. Bio-based (R,S)-2,3-BDO (9 g) was blended with 5.0 g Softanol 90 (Nippon Shokubai Co., Ltd.) as an emulsifier, 30.0 g of propylene glycol monomethyl ether as a solvent, and 56.0 g of water to make a meso 9% 2,3-BDO SL formulation.

**Fungal isolates.** *Clarireedia* sp. (causing dollar spot on creeping bentgrass was isolated by Prof. J. W. Kim, University of Seoul), a fungal pathogen of turfgrass dollar spot, and *R. solani* AG1-1B KACC40111, a fungal pathogen of turfgrass large patch, were incubated in a wheat-rice bran medium (9 g of wheat bran, 1.5 g of rice bran, and 10 ml of distilled water in a 250-ml Erlenmeyer flask) at 25°C. After 7 days, the culture was mixed with 110 ml of distilled water containing 200 μg/ml streptomycin sulfate and macerated for 10 s to prepare an inoculum suspension.

*M. poae* KACC48138, a fungal pathogen of turfgrass summer patch, was incubated on potato dextrose agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 25°C for 5 days. Then, the mycelia-containing agar plugs were inoculated into the sand–oatmeal mixture (sand:oatmeal:water = 20:1:4, v/v/v), followed by incubation at 25°C for 7 days. The solid culture of *M. poae* was used as an inoculum.

**Plant material.** Seeds of creeping bentgrass (*Agrostis stolonifera* 'Penncross') and Kentucky bluegrass (*Poa pratensis* L) were soaked in sterile distilled water and incubated at 4°C for 48 h. Soaked seeds were sown in sterilized soil (horticultural nursery soil:sand, 3:1, v/v) filled in vinyl pots (diameter 7 cm, height 6 cm). The pots were incubated at 25 ± 5°C (day/night) with a 16-h photoperiod for 4 weeks. The bentgrass plants were used as hosts for dollar spot and

large patch disease bioassays. Meanwhile, Kentucky bluegrass (*Poa pratensis*, midnight) was selected as host grass to evaluate summer patch disease. The seeds were soaked and grown in vinyl pots (diameter 17.4 cm, height 19.7 cm) filled with a sterilized mixture of peat moss (10%), sand (USGA standard), and fertilizer (100 g/m$^2$). The pots were incubated for 3 weeks as mentioned earlier.

**Combination of levo and meso 2,3-BDO SLs with fungicides against turfgrass fungal disease: growth room experiments.** To investigate the disease control efficacy of the combination of levo and meso 2,3-BDO SLs with tebuconazole against dollar spot disease, the 4-week-old creeping bentgrass was treated with 10 ml of 2,3-BDO formulations (levo 0.9% 2,3-BDO SL, 1,000-fold dilution; meso 9% 2,3-BDO SL, 1,000-fold dilution), tebuconazole EC (a.i 25%, Horikuo, 2,000-fold dilution), and the combinations of 2,3-BDO isomer formulations (1,000-fold dilution) with half concentration of tebuconazole (4,000-fold dilution) 4 days prior to pathogen inoculation, followed by soil inoculation with 3.5 ml of the *Clarireedia* sp. inoculum. The experiments were carried out twice in triplicates. The plants were maintained at 25°C with a 16-h light per day at 95-100% relative humidity; disease severity was assessed 7 days post-inoculation (dpi). Disease severity was determined in terms of the yellowing of turfgrass leaves. The control value for each treatment was calculated using the mean values of six estimates.

To investigate the disease control efficacy of the combination of levo and meso 2,3-BDO SLs with tebuconazole against brown patch disease, 4-week-old Kentucky bluegrasses were treated with 10 ml of 2,3-BDO isomer formulations (levo 0.9% 2,3-BDO SL, 1,000-fold dilution; meso 9% 2,3-BDO SL, 1,000-fold dilution), tebuconazole EC (2,000-fold dilution), and the combinations of 2,3-BDO isomer formulations (1,000-fold dilution) with half concentration of tebuconazole (4,000-fold dilution) 4 days prior to pathogen inoculation. An aliquot of 2 ml of *R. solani* suspension was sprayed evenly on leaf blade surfaces, and soil was inoculated with 8 ml of suspension. The inoculated plants were maintained at 25°C with a daily photoperiod cycle of 16 h, and disease severity was assessed at 11 dpi. The experiments were carried out twice in triplicates. The control value of each treatment was calculated using the mean values of six estimates.

To investigate the disease control efficacy of the combination of levo and meso 2,3-BDO SLs with azoxystrobin against summer patch disease, 3-week-old Kentucky bluegrasses were treated three times with 200 ml 2,3-BDO isomer formulations (levo 0.9% 2,3-BDO SL, 1,000-fold dilution; meso 9% 2,3-BDO SL, 1,000-fold dilution), azoxystrobin WG (a.i 50%, Heritage, Syngenta, 1,000-fold dilution), and the combinations of 2,3-BDO isomer formulations (1,000-fold) with half concentration of azoxystrobin (2,000-fold dilution) at 3-day intervals beginning 9 days before pathogen inoculation. To inoculate *M. poae*, 8 g of soil inoculum added to the center of the Kentucky bluegrass. The pots were maintained at 25°C, with a 16-h light per day and 95-100% relative humidity; disease severity was calculated at 19 dpi. The experiments were carried out twice in triplicates. The control value of each treatment was calculated using the mean values of six estimates.

**Field experiment.** The field experiment was conducted in the experimental field of Daejung Turfgrass Research Institute, South Korea. Three replicates were arranged in a randomized complete block design. The disease control efficacy of the combinations of 2,3-BDO isomer formulations and tebuconazole against dollar spot disease was evaluated on the creeping bentgrass. The treatments were arranged in a completely randomized design; each treatment consisted of four replicates with 4 m$^2$ (2 × 2 m$^2$) per plot/replicate. To induce dollar spot disease in the field, 4 holes (8 mm dia. 10 cm deep) were made per plot, and each hole was inoculated with 8 g of *Clarireedia* sp. After the inoculation, creeping bentgrass was treated with 250 ml of 2,3-BDO isomer formulations (levo 0.9% 2,3-BDO SL, 1,000-fold dilution; meso 9% 2,3-BDO SL, 1,000-fold dilution), tebuconazole EC (2,000-fold dilution), and the combinations of 2,3-BDO isomer formulations (1,000-fold dilution) with half concentration of tebuconazole (4,000-fold dilution), twice at 2-week intervals. Disease severity was assessed at 21 dpi, and the control value was calculated.

The disease control efficacy of the combinations of 2,3-BDO isomer formulations and pencycuron was also tested against large patch disease on naturally infected zoysiagrass. The experiment was conducted in Icheon Midas Country Club, Gyeonggi-do, South Korea. The treatments were arranged in a completely randomized design; each treatment consisted of four replicates with 4 m$^2$ (1 × 4 m$^2$) per plot/replicate. An aliquot of 200 ml of 2,3-BDO isomer formulations (levo 0.9% 2,3-BDO SL, 1,000-fold dilution; meso 9% 2,3-BDO SL, 1,000-fold dilution), pencycuron (2,000-fold dilution), and the combinations of 2,3-BDO isomer formulations (1,000-fold dilution) with half concentration of pencycuron (4,000-fold dilution), four times at 2-week intervals. Disease severity was assessed 28 days after treatment, and the control value was calculated.

**Real-time PCR analysis of defense-priming gene ex-**

**Table 1.** Primers used for the expression analysis of induced resistance and ROS-detoxifying enzyme-related genes

| Target | Gene name | Primer pair | Scale |
|---|---|---|---|
| Constitutive | *AsUBI3* | Forward: 5'-CAGGACAAGGAGGGCATC-3' | 0.02 |
| | | Reverse: 5'-TTCCTGAGCCTGGTGACC-3' | |
| Induced resistance-related gene | *AsNPR1* | Forward: TCTGCAAGTGCGATGTCCAG | |
| | | Reverse: CCCGAAGACATGGTCTCTCCTA | |
| | *AsPR3* | Forward: TGTTCATCCAGAACCAGAGCG | |
| | | Reverse: ACTGCGACAACAAGAACACG | |
| | *AsPR4* | Forward: AGAGGTATCGGCAATGGAGG | |
| | | Reverse: TCTCCGGGTTGTACAGGTTG | |
| | *AsAOS1* | Forward: GGCGCTGGAGAAGATGGA | |
| | | Reverse: GGACGTGGCCTTGGTGAC | |
| | *AsOPR4* | Forward: CAACGACCGCACCGACGAG | |
| | | Reverse: GAGGAAGGGGTAGTCGGTGTA | |
| | *AsGns5* | Forward: CCTGCAGGCCCTCAGCGG | |
| | | Reverse: GCCACCCGCTCTCCGACA | |
| | *AsLOX* | Forward: CCGCGAGATCAAGGTCGTCT | |
| | | Reverse: GCATCAACGGCGCCCTAATC | |
| | *AsERF* | Forward: GCAAGGTTGTTGACTGCTGG | |
| | | Reverse: CAACAGTGCCGAAGAAGCTG | |
| ROS-detoxifying enzyme-related gene | *AsDHAR* | Forward: GAAAGGTGCCTGTGTTTAATG | |
| | | Reverse: GTGATGGAGTTGGGTACTTC | |
| | *AsSOD* | Forward: CACTGGACCTCACTTCAAC | |
| | | Reverse: GTAGCAACACCATCCACTC | |
| | *AsMDHAR* | Forward: CCATGAAGCTCTACAACGAG | |
| | | Reverse: GTAGAAGTAGGGCAGGTAGT | |
| | *AsCAT* | Forward: TTGCCAATAAGAGGGAGAATG | |
| | | Reverse: CGAAGCCGAGCATGTAAG | |
| | *AsGR* | Forward: GATGGAGGCTACTTGCTTTG | |
| | | Reverse: GCTAAGACCCACGACAGATA | |
| | *AsPOD* | Forward: CTTCGACAACGCCTACTAC | |
| | | Reverse: TTTGCCCATGTTCACCA | |

ROS, reactive oxygen species.

**pression.** 2,3-BDO isomer-treated creeping bentgrass samples were ground in liquid nitrogen, and the total RNA was extracted using the IQeasy Plant RNA Extraction mini kit (iNtRON, Seongnam, Korea), followed by quantification with a NanoDrop spectrometer (NP80, IMPLEN, Munchen, Germany). cDNA was synthesized using a SuperScript IV First-Strand Synthesis System for RT-PCR kit (Invitrogen, Waltham, MA, USA). The primers for selected genes are listed in Table 1. A total of 10 µl reaction mixtures containing 5 µl of 2 IQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), 4 µl of forward and reverse primers, and 1 µl of cDNA template were used to perform quantitative real-time polymerase chain reaction (qRT-PCR) (BioRad CFX96Real-Time System, Singapore). The PCR cycling parameters were denaturation at 95°C for 3 min, followed by 40 cycles at 95°C for 15 s, and 53°C for 1 min.

## Results

**Enhancement of fungal disease control in turfgrass by the combination of levo and meso 2,3-BDO SLs with chemical fungicides: growth room experiments.** The combinations of 2,3-BDO isomer formulations and chemical fungicides were tested against dollar spot, brown patch, and summer patch diseases caused by *Clarireedia* sp., *R. solani*, and *M. poae*, respectively. The disease control efficacy against creeping bentgrass dollar spot at 7 dpi with *Clarireedia* sp. is shown in Fig. 1. The plants treated with levo 0.9% and meso 9% SL did not control the creeping bentgrass dollar spot disease. However, a significant reduction in disease severity was observed in treatments with

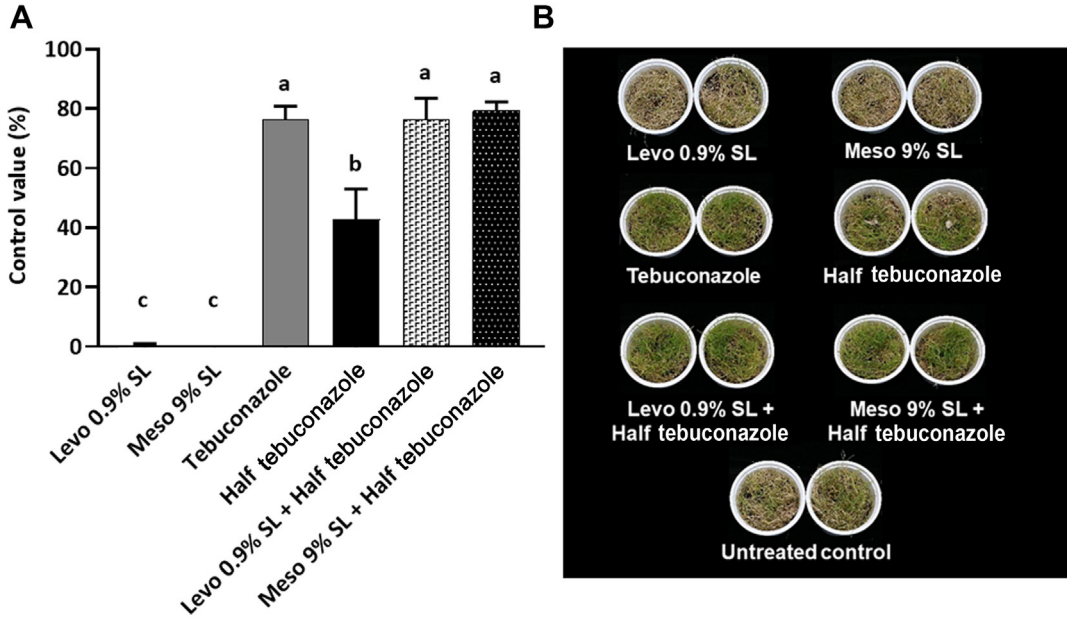


**Fig. 1.** Effects of the combination of levo and meso 2,3-BDO soluble liquid (SL) with tebuconazole against dollar spot disease caused by *Clarireedia* sp. on creeping bentgrass at 7 days post-inoculation (dpi). (A) Control values at 7 dpi. (B) Symptoms of dollar spot disease on creeping bentgrass at 7 dpi. Tebuconazole and half tebuconazole: 2,000-fold and 4,000-fold, respectively. Levo 0.9% and meso 9% SLs: 1,000-fold. The bars with different letters represent significant differences between the treatments ($P < 0.05$). Error bars represent mean ± standard deviation.

the combinations of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL with the half concentration of tebuconazole, showing control values of 76.2% and 79.3%, respectively, which were significantly higher than that of half concentration (4,000-fold dilution) of tebuconazole alone, which had a control value of 42.8%. The development of creeping bentgrass dollar spot was similarly suppressed by the combinations of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL with the half concentration of tebuconazole, compared to the commercial concentration (2,000-fold dilution) of tebuconazole, with a control value of 76.3% (Fig. 1A and B).

The disease control efficacy against Kentucky bluegrass brown patch at 11 dpi with *R. solani* is shown in Fig. 2. The disease control values for the treatments of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL alone were 16.3% and 37.4%, respectively. The combinations of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL with a half concentration of tebuconazole showed significantly similar disease control efficacy, with control values 45.1% and 48.2%, respectively, compared with the half concentration of tebuconazole alone, which had a control value of 50.5%. However, the development of Kentucky bluegrass brown patch disease was not significantly suppressed compared with the commercial concentration (2,000-fold dilution) of tebuconazole, which had a control value of 73.6%. Thus,

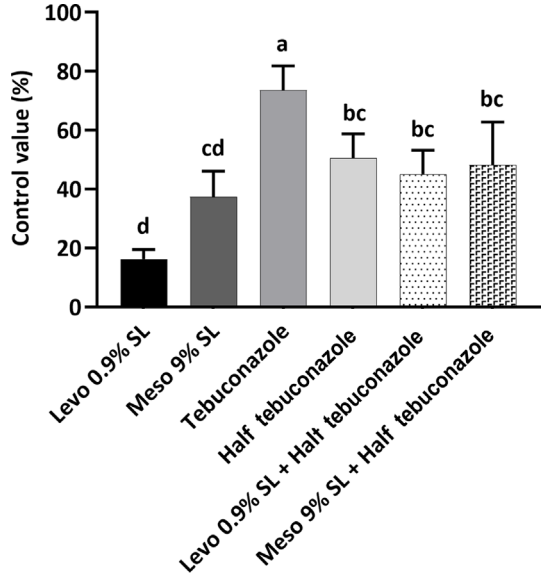


**Fig. 2.** Effects of the combination of levo and meso 2,3-BDO soluble liquid (SL) with tebuconazole against brown patch disease caused by *Rhizoctonia solani* on Kentucky bluegrass at 11 days post-inoculation. Tebuconazole and half tebuconazole: 2,000-fold and 4,000-fold, respectively. Levo 0.9% and meso 9% SLs: 1,000-fold. The bars with different letters represent significant differences among treatments ($P < 0.05$). Error bars represent mean ± standard deviation.

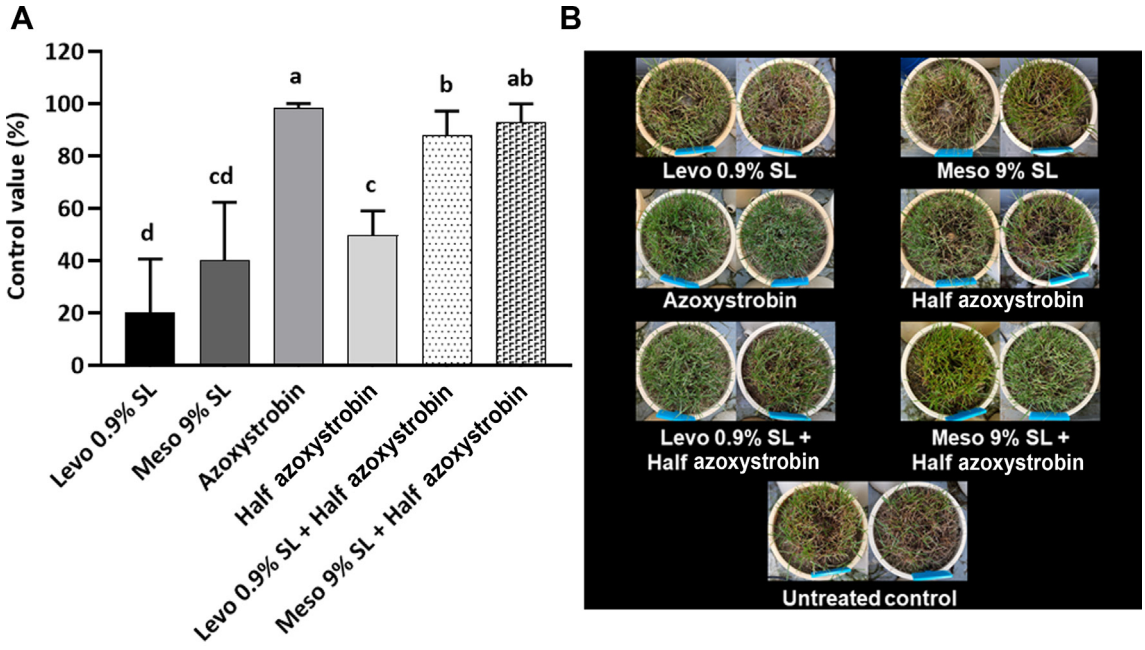


**Fig. 3.** Effects of the combination of levo and meso 2,3-BDO soluble liquid (SL) with azoxystrobin against summer patch disease caused by *Magnaporthe poae* on Kentucky bluegrass at 19 days post-inoculation (dpi). (A) Control values at 7 dpi. (B) Symptoms of summer patch disease on Kentucky bluegrass at 19 dpi. Azoxystrobin and half azoxystrobin: 10,000-fold and 20,000-fold, respectively. Levo 0.9% and meso 9% SLs: 1,000-fold. The bars with different letters represent significant differences between the treatments ($P < 0.05$). Error bars represent mean ± standard deviation.

the combinations of 2,3-BDO isomer formulations with tebuconazole did not show any synergistic effects against Kentucky bluegrass brown patch disease.

We next evaluated the disease control efficacy of the combinations of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL with a half concentration of azoxystrobin against Kentucky bluegrass summer patch disease at 19 dpi with *M. poae*. The disease control values were 20.4% for levo 0.9% 2,3-BDO SL and 40.3% for meso 9% 2,3-BDO SL. Treatment with the combinations of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL with a half concentration of azoxystrobin significantly increased the disease control efficacy in Kentucky bluegrass with control values of 87.7% and 92.9%, respectively, which were comparable with that of commercial concentration of azoxystrobin, which had a control value of 98.4% (Fig. 3A and B). Moreover, these control values were higher than that of half concentration of azoxystrobin, which had a control value of 49.7%.

**Enhancement of fungal disease control in turfgrass by the combination of levo and meso 2,3-BDO SLs with chemical fungicides: field experiments.** Similar to the results of growth room experiments, the combination of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL with a half concentration of tebuconazole was applied in the Kentucky bluegrass field to assess the disease control efficacy against dollar spot disease. The dollar spot disease was most effectively controlled by the combination of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL with a half concentration of tebuconazole with control values of 100% and 98.1%, respectively (Fig. 4A and B). Their control values were higher than those of levo 0.9% 2,3-BDO SL alone, meso 9% 2,3-BDO SL alone, and a half concentration of tebuconazole and comparable with that of the commercial concentration of tebuconazole.

We then evaluated the disease control efficacy against large patch disease on zoysiagrass. The results showed that the control values of 48.7% and 43.6% were observed for levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL treatments, respectively (Fig. 5). The combinations of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL with the half concentration of tebuconazole highly suppressed the development of zoysiagrass large patch disease with the control values of 56.7% and 60.8%, respectively, compared to the half concentration of tebuconazole with a control value of 46.8%. The disease control values of the two combinations were comparable to that of the commercial concentration of tebuconazole with a control value 68.2%.

**Effect of levo and meso 2,3-BDO SLs on induced resis-**

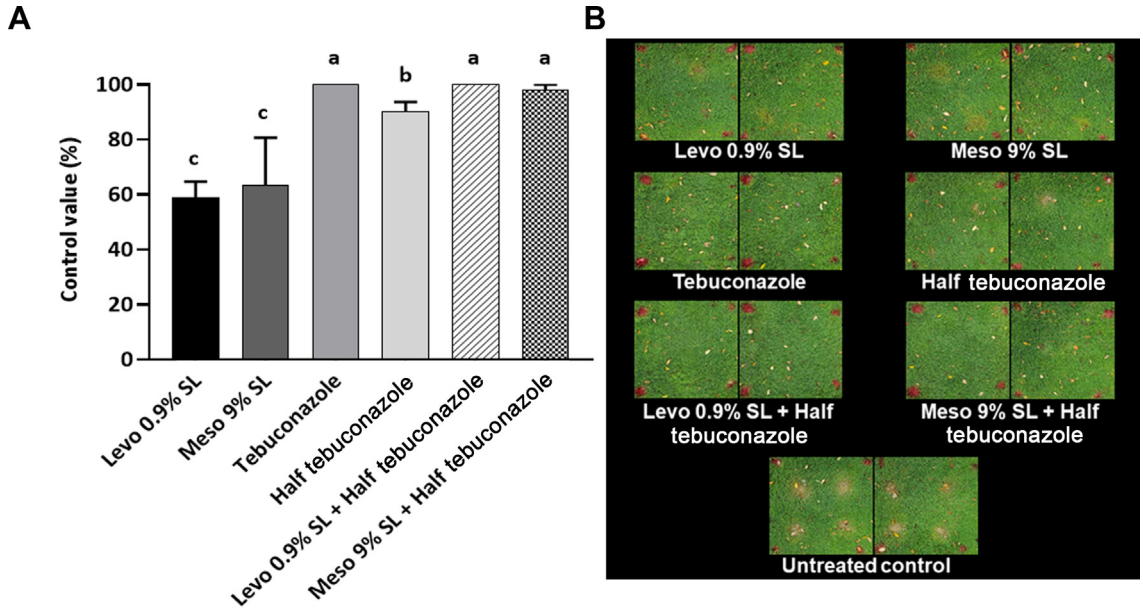


**Fig. 4.** Effects of the combination of levo and meso 2,3-BDO soluble liquid (SL) with tebuconazole on Kentucky bluegrass in a field artificially infected by *Clarireedia* sp. (A) Control values at 21 days post-inoculation . (B) Symptoms of dollar spot disease on Kentucky bluegrass. Tebuconazole and half tebuconazole: 2,000-fold and 4,000-fold, respectively. Levo 0.9% and meso 9% SLs: 1,000-fold. The bars with the different letters represent significant differences between the treatments ($P < 0.05$). Error bars represent mean ± standard deviation.

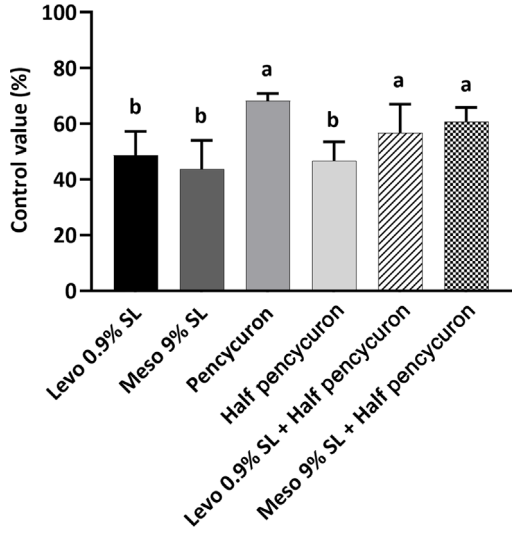


**Fig. 5.** Effects of the combination of levo and meso 2,3-BDO soluble liquid (SL) with pencycuron on zoysiagrass in a field naturally infected by *Rhizoctonia solani*. Pencycuron and half pencycuron: 1,000-fold and 2,000-fold, respectively. Levo 0.9% and meso 9% SLs: 1,000-fold. The bars with different letters represent significant differences between the treatments ($P < 0.05$). Error bars represent mean ± standard deviation.

**tance against dollar spot disease.** To confirm the induced resistance in creeping bentgrass against *Clarireedia* sp., we assessed the induction of defense-related genes *AsNPR1* for salicylic acid (SA), *AsLOX*, *AsAOS1*, *AsGns5*, *AsOPR4*, *AsPR3*, and *AsPR4* for jasmonic acid (JA), and *AsERF* for ethylene (ET) signaling pathways at 1 and 2 dpi. Expression of *AsNPR1* for SA signaling was significantly upregulated by 3.34-fold in creeping bentgrass treated with levo 0.9% 2,3-BDO SL, compared to the control at 2 dpi. At 1 dpi, the increased transcript level 2.11-fold was noticed for meso 9% 2,3-BDO SL compared to the control (Fig. 6). The relative expression level of *AsPR4*, which is a marker gene of SA and JA signaling pathways, was significantly upregulated by both levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL treatments by 42.28-fold and 69.19-fold, respectively, compared to the control at 1 dpi. The expression level of *AsPR4* in levo 0.9% 2,3-BDO SL treatment was significantly induced 9.19-fold higher than that of the control at 2 dpi. The transcript levels of the signature genes of JA signaling pathways are shown in Fig. 6. The 2.84-fold enhanced expression of *AsAOS1* was also observed in the creeping bentgrass treated with levo 0.9% 2,3-BDO SL at 1 dpi. At 2 dpi, *AsLOX* and *AsGns5* expressions were also significantly 2.91- and 2.71-fold higher than the control, respectively. The relative expression levels of *AsLOX* and *AsAOS1* in the meso 2,3-BDO treatment were significantly enhanced, by 1.85-fold and 3.08-fold, respectively, at 1 dpi. Furthermore, the transcription level of *AsERF* for ET signaling was significantly increased by 2.27-fold higher in

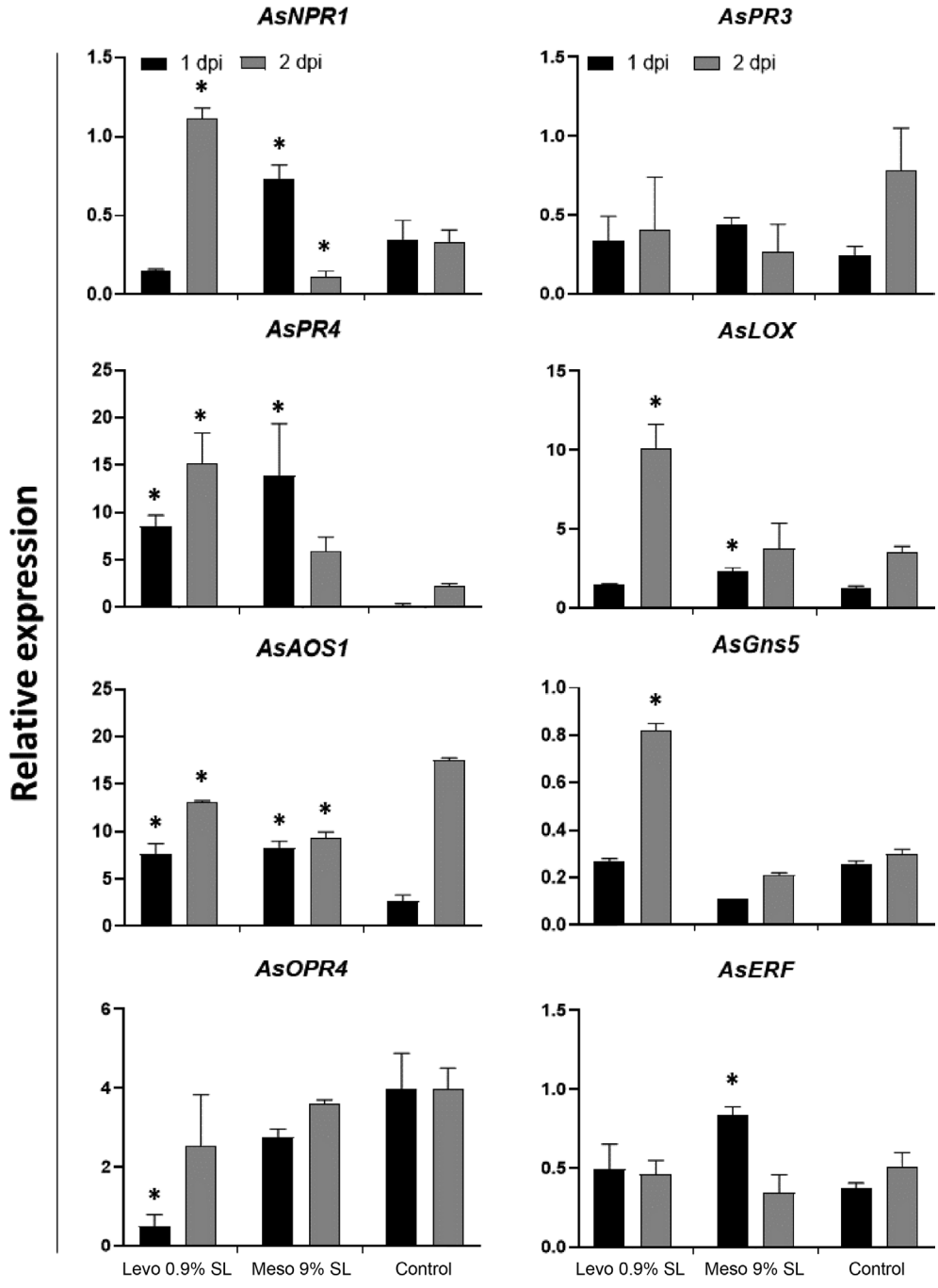


**Fig. 6.** Induction of defense-related marker genes of salicylic acid (*AsNPR1*, *AsPR3*, and *AsPR4*), jasmonic acid (*AsPR3*, *AsPR4*, *AsLOX*, *AsAOS1*, *AsGns5*, and *AsOPR4*), and ethylene (*AsERF*) signaling pathways in creeping bentgrass by levo 0.9% and meso 9% soluble liquids (SLs) treatment at 1,000-fold. dpi, days post-inoculation. Asterisks indicate significant differences at $P < 0.05$ level. Error bars represent mean ± standard deviation.

the treatment of meso 9% 2,3-BDO SL at 1 dpi. However, levo 0.9% 2,3-BDO SL treatment did not cause enhanced expression of *AsERF* at either 1 dpi or 2 dpi.

**Effect of levo and meso 2,3-BDO SLs on reactive oxygen species–detoxifying enzyme gene expression under fungal infection.** The effect of 2,3-BDO stereoisomers on the induction of reactive oxygen species (ROS)–detoxifying enzyme genes *AsDHAR*, *AsCAT*, *AsMDHAR*, *AsSOD*, *AsGR*, and *AsPOD* at 1 and 2 dpi is shown in Fig.

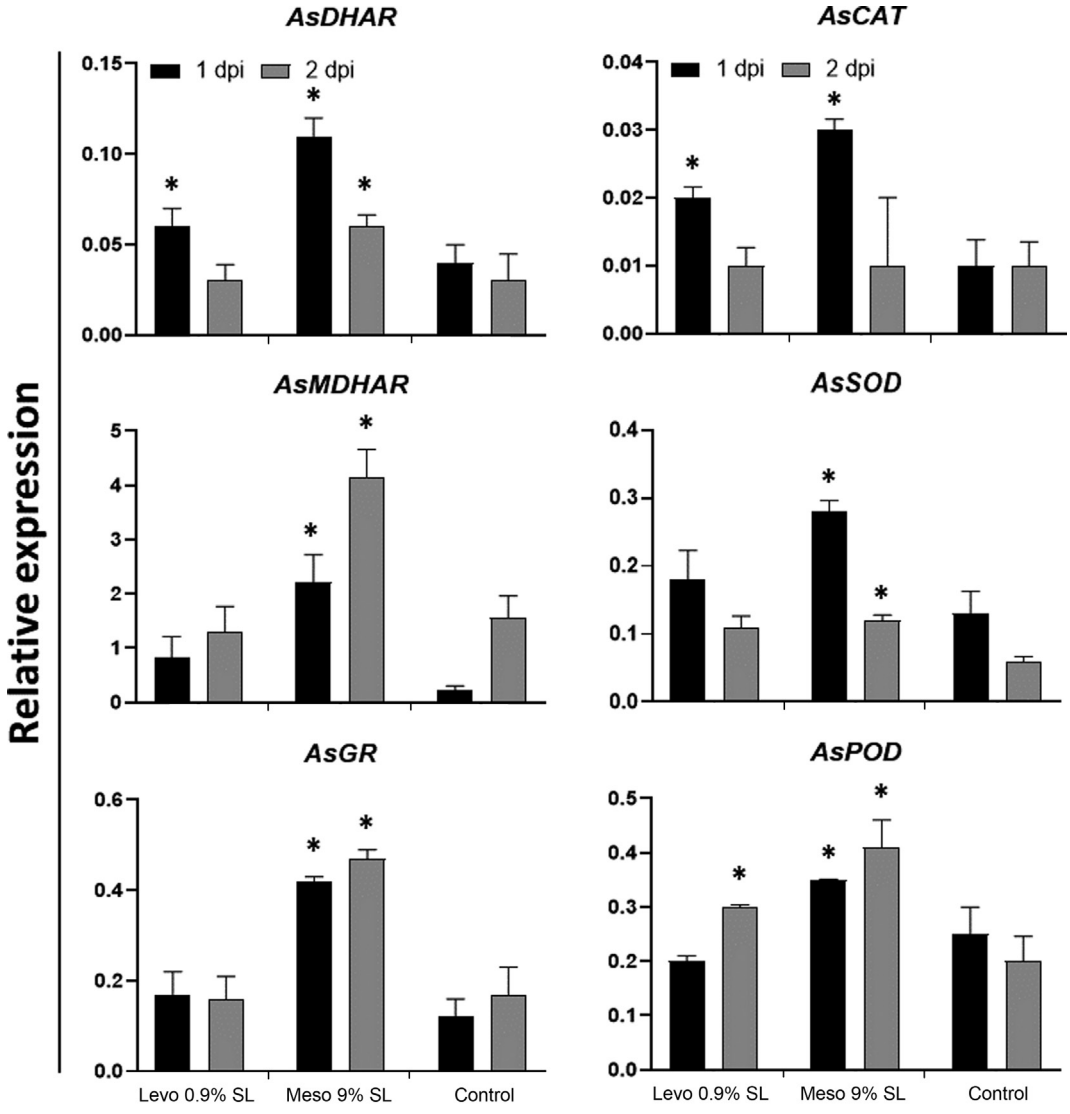


**Fig. 7.** Induction of reactive oxygen species detoxification-related marker gene (*AsDHAR*, *AsGR*, *AsMDHAR*, *AsCAT*, *AsSOD*, and *AsPOD*) expression in creeping bentgrass by levo 0.9% and meso 9% soluble liquids (SLs) treatment at 1,000-fold. dpi, days post-inoculation. Asterisks indicate significant differences at $P < 0.05$ level. Error bars represent mean ± standard deviation.

7. Expressions of *AsDHAR* and *AsCAT* were significantly upregulated by levo 0.9% 2,3-BDO SL treatment, with increases of 1.80-fold and 2.42-fold, respectively, at 1 dpi. Notably, the expression of *AsPOD* at 2 dpi was upregulated by 1.53-fold for the treatment of levo 0.9% 2,3-BDO SL, compared to the control. Expressions of *AsDHAR*, *AsCAT*, *AsMDHAR*, *AsSOD*, *AsGR*, and *AsPOD* were significantly upregulated by meso 9% 2,3-BDO SL treatment with increases of 3.13-, 3.42-, 10.14-, 2.17-, 3.36-, and 1.40-fold, respectively, compared to the control at 1 dpi. At 2 dpi, significantly increased transcript levels of 2.11-, 2.66-, 2.20-, 2.73-, and 2.08-fold in response to meso 9% SL treatment were noticed for *AsDHAR*, *AsMDHAR*, *AsSOD*, *AsGR*, and *AsPOD*, respectively, compared to the control.

## Discussion

Plants have evolved a great variety of defense mechanisms to fight against pathogens. Therefore, priming of defense responses in plants by triggering the signaling pathways is a sustainable strategy in integrated pest management that can reduce fungicide application in agriculture. However, few studies have reported priming of plant defense responses against fungal pathogens in the turfgrass industry. This study aimed to effectively control various turfgrass fungal diseases by combining the plant resistance inducer

2,3-BDO with synthetic fungicide to reduce the amounts of synthetic fungicides used. This study revealed that the application of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL, in combination with the half concentration of chemical fungicides, effectively suppressed the development of dollar spot disease in creeping bentgrass and Kentucky bluegrass, summer patch disease in Kentucky bluegrass, and brown summer patch in zoysiagrass. The results were comparable to the commercial dosages of synthetic fungicides in pot and field experiments. However, the combinations were less effective at suppressing the development of Kentucky bluegrass brown patch than the half concentration of chemical fungicide alone. This may be due to the variable host physiologies or pathosystems.

Several research groups have reported the disease control efficacy of 2,3-BDO against turfgrass fungal diseases. Cortes-Barco et al. (2010) reported that the treatment of 100 μM levo 2,3-BDO significantly suppressed mycelial growth and necrosis caused by *Clarireedia* sp. in *A. stolonifera* (creeping bentgrass) when compared to the control treatment. In our study, both levo and meso 2,3-BDO were virtually ineffective against dollar spot disease in creeping bentgrass. Thus, the difference between the two experiments may have been due to different disease pressure. Additionally, Cortes-Barco et al. (2010) reported that the application of levo 2,3-BDO suppressed the development of brown patch of *A. stolonifera* caused by *R. solani*. Shi et al. (2018) also evaluated the disease control efficacy of 2,3-BDO against brown patch caused by *R. solani* at five different concentrations of 150-550 μM. 2,3-BDO showed the highest disease control efficacy at 250 μM, with control values of 58% at 10 dpi and 42% at 15 dpi. In this study, both levo 0.9% 2,3-BDO SL (100 μM) and meso-2,3-BDO 9% SL (1 mM) were able to moderately suppress the development of brown patch disease in Kentucky bluegrass. Although there are several reports on the disease control efficacy of 2,3-BDO alone, to our knowledge, there are no studies on the enhancement of disease control efficacy against turfgrass fungal diseases by combining chemical fungicides with 2,3-BDO.

The present study revealed that the combination of both levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL, with a half concentration of tebuconazole fungicide, showed higher disease control values against dollar spot disease caused by *Clarireedia* sp. than that of the half concentration of tebuconazole alone. The increased disease control efficacy by 2,3-BDO isomer formulations might be achieved by inducing resistance in turfgrass. To confirm this, we investigated the relative expression of the genes related to SA, JA, and ET signaling pathways by qRT-PCR, because these plant hormones play significant roles in regulating plant defense responses. Several findings have shown that NPR1 protein, a transcription coactivator, functions as a master regulator of SA-mediated responses and SAR in plants (Backer et al., 2019; Pajerowska-Mukhtar et al., 2013). Our present study found that *AsNPR1* was induced by both levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL treatments. The results indicate that both levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL treatments activated the SA signaling pathway in creeping bentgrass after pathogen infection. This result was consistent with that reported by Park et al. (2018), who reported that 2,3-BDO treatment effectively upregulated NPR1 transcription in tobacco plants upon *Phytophthora parasitica* var. *nicotianae* infection.

SA and JA signaling pathways are usually implicated in plant resistance to biotrophic and necrotrophic pathogens, respectively (Pieterse et al., 2009). Activating these signaling pathways during the plant–pathogen interaction leads to the accumulation of PR proteins, which prevents pathogen growth and the spread of disease to other organs (González-Bosch, 2018). We also observed the induction of SA and JA signaling pathways, as evidenced by the transcript levels of their marker gene *AsPR4*. The expression of *AsPR4* was induced by both levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL treatments, implying enhanced pathogen tolerance in creeping bentgrass. These findings were inconsistent with those observed by Kong et al. (2018) that the expression level of *Capsicum annuum* PR4 was increased by levo 2,3-BDO treatment but not by meso 2,3-BDO treatment. Thus, the difference may be due to different responses between plants. Further, both levo 0.9% and meso 9% SL treatment induced the expression of JA marker genes such as *AsLOX* and *AsAOS1*, while *AsGns5* was induced only by levo 0.9% treatment. These results were consistent with those reported by Cortes-Barco et al. (2010) that levo 2,3-BDO application significantly increased the expression of *AsAOS1*, *AsGns5*, and *AsOPR4* in creeping bentgrass after *M. nivale* inoculation. However, *AsOPR4* was not induced by levo 0.9% and meso 9% SL treatment in our study, which was inconsistent with Cortes-Barco et al. (2010). This may be due to the difference in pathogens. Additionally, the expression of *AsERF*, a marker gene of the ET signaling pathway, was induced only by meso 9% 2,3-BDO SL treatment. These findings confirm that the increased disease control efficacy of the combination of levo 0.9% 2,3-BDO SL and meso 9% 2,3-BDO SL with the half concentration of fungicides was due to the induction of plant defense marker genes in the SA, JA, and ET signaling pathways.

Next, the effects of levo 0.9% 2,3-BDO SL and meso

9% 2,3-BDO SL on ROS detoxification were determined for creeping bentgrass under *Clarireedia* sp. infection. The plant's primary response to oxidative stress is producing enzymatic and nonenzymatic ROS scavengers via an endogenous defensive mechanism. Fungal infections cause the over accumulation of free radicals, such as hydroxyl radicals ($OH^{\bullet}$), hydrogen peroxide ($H_2O_2$), and superoxide dismutase (SOD) production in plants (Mofidnakhaei et al., 2016). Among the enzymatic antioxidants, SOD initiates the first line of defense in plants by rapidly converting $OH^{\bullet}$ to $H_2O_2$. Generated $H_2O_2$ can be then converted to water and dioxygen by catalase, ascorbate peroxidase (APX), and glutathione peroxidase enzymes or catalyzed by the ascorbate-reduced glutathione (AsA-GSH) cycle (Apel and Hirt, 2004; Biczak, 2016). In an antioxidant defense system, AsA-GSH is a major pathway of $H_2O_2$ detoxification, consisting of four enzymatic antioxidants, APX, MDHAR, DHAR, and GR, as well as the nonenzymatic antioxidants AsA and GSH (Hasanuzzaman et al., 2019; Mohammadi et al., 2021).

In our present study, levo 0.9% 2,3-BDO SL treatment significantly upregulated the *AsDHAR*, *AsCAT*, and *AsPOD* expressions after *Clarireedia* sp. infection, while meso 9% 2,3-BDO SL treatment significantly upregulated all of the 6 ROS-detoxifying enzyme genes tested in this study. Activation of the ROS-detoxifying enzymes suggests that levo 2,3-BDO and meso 2,3-BDO participated in resistance mechanisms or prohibited further progress of plant cell damage in creeping bentgrass. Moreover, these findings indicate that plant defense response induction depends on which isomers of 2,3-BDO are used.

Through growth room and field experiments, this study has presented an efficient method to control turfgrass fungal diseases based on a combination of 2,3-BDO isomer formulations and chemical fungicides. Our findings offer a new integrated approach for controlling turfgrass disease while minimizing fungicide usage in the environment. Additionally, we confirmed that levo and meso 2,3-BDO could effectively protect turfgrasses via the induction of plant systemic resistance.

## Conflicts of Interest

This work was financially supported by GS Caltex Corporation, Republic of Korea.

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