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# Mycelial compatibility groups, pathogenic diversity and biological control of *Sclerotium rolfsii* on turfgrass

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## Abstract

*Sclerotium rolfsii* Sacc. (the sclerotial state of *Athelia rolfsii* (Cruzi) Tu and Kimbrough), the soil-borne pathogen on several plants all over the world, has been previously reported from Turkey on certain plants. In this study, turfgrass areas in 9 provinces of Turkey were firstly surveyed for *S. rolfsii*, and samples showing chlorotic, reddish-brown, and frog-eye shaped circular patches were collected. Totally, 32 *Sclerotium rolfsii* isolates were obtained from these areas. One mycelial compatibility group (MCG) was identified among *S. rolfsii* isolates. Disease severity in pathogenicity tests carried out in the greenhouse ranged from 83.74 to 92.87%. Identification of fungal and bacterial isolates used in the study was performed by DNA sequencing analysis. Five antagonistic bacterial strains, previously found as effective on controlling some fungal pathogens, were tested to determine their antifungal effects against the southern blight by using seed coating method in greenhouse conditions. In consequence of the biological control studies, *Bacillus cereus* 44bac and *Stenotrophomonas rhizophila* 88bfp were found more effective than the other strains with the ratio of 91.00 and 90.11%, respectively.

**Keywords:** Turfgrass, Biological control, Mycelial compatibility group, *Sclerotium rolfsii*, Southern blight

## Background

Southern blight, caused by the soil-borne fungus *Sclerotium rolfsii* Sacc. (Atheliaceae: *Athelia rolfsii* (Cruzi) Tu and Kimbrough), is a serious disease for a wide range of plants, including vegetables, fruits, ornamental plants, and field crops (Mullen 2001). The fungus, also, attacks primarily bentgrass, bluegrass, fescues, and ryegrass (Smiley 1992).

The first symptoms of outstanding in turfgrass areas are round crescent-shaped yellow areas about 20 cm in diameter. Grasses grow yellowish over time and become sparse. As long as the disease continues to progress, diseased areas in the form of rings or patches die. But the grass in the center remains green. The color of the dead areas turns reddish brown over time. These rings, formed in dead turfgrass in summer and humid weather, expand quickly (about 20 cm per week). Sometimes, symptoms observed in these areas similar to the “frog eye.” In

*Agrostis* and *Poa* species, the diseased areas caused by this disease are usually seen in the autumn. Under moist conditions, white mycelial growth which develops on the dead grass and later on sclerotia ranging from white or light to dark brown on the mycelium are observed (Smiley 1992).

*S. rolfsii* isolates can be diverged into different mycelial compatibility groups (MCGs) based on mycelial interactions among isolates. The role of MCGs is important in defining field populations of fungi and facilitating genetic variation in fungal species, where the sexual reproductive stage (teleomorph stage) of the life cycle has a minimal impact on the disease cycle (Kohn et al. 1991).

Nowadays, the plantation and conservation of turfgrass areas has become a huge industrial sector in the world. Cultural practices are not efficient for controlling the disease, that is why fungicide usage is very common and widely used in turfgrass areas all around the world. Chemical fungicides are extensively used in turfgrass areas in Turkey, and excessive use of chemical fungicides has led to deteriorating human health, environmental pollution, and development of pathogen resistance to fungicides (Balci and Gedikli, 2012). Due to the harmful

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effects in controlling fungal diseases, new studies are needed to use alternative methods for plant protection, which are less dependent on chemicals and are more environmentally friendly. In this regard, biological control can be an alternative or supplement to current management practices for *S. rolf sii* (Sai et al. 2010).

The most commonly beneficial microorganisms used in the control of plant pathogens are the following bacterial strains: *Bacillus*, *Pseudomonas*, and *Trichoderma* spp. (Raaijmakers et al. 2010). For the biocontrol of *S. rolf sii*, some bacterial genera have been tested for their ability to control. *Pseudomonas* spp. and *Bacillus* spp. have been commonly studied to control *S. rolf sii* on various plants. It was detected that *Pseudomonas* and *Bacillus* strains restricted in vitro hyphal growth or reduced germination of sclerotia of *S. rolf sii* (Rakh et al. 2011 and Tonelli et al. 2011). Several commercial preparations, containing these bacterial and fungal agents, are also recommended on turfgrass diseases in the world. Bio-Trek 22G (*Trichoderma harzianum*) is the first registered biopesticide for dollar spot, brown patch, and Pythium root rot on turfgrass (Harman and Lo 1996). Eco Guard TM (*Bacillus licheniformis*), Rhapsody (*B. subtilis*), Actinovate SP (*Streptomyces lydicus* WYEC 108), and Botrycid (*Pseudomonas aureofaciens*) are the other microbial biocides used against turfgrass diseases (Corwin et al. 2007). Among these, only Rhapsody is recommended against the southern blight disease caused by *S. rolf sii* in turfgrass areas. But there is no registered microbial biocide against turfgrass diseases in Turkey so far.

The objective of this study was to molecularly identify *S. rolf sii* isolates in Turkey, and to detect their virulence and mycelial compatibility groups, using some domestic bacterial and fungal isolates under greenhouse conditions.

## Materials and methods

### Survey and isolation of the pathogens

The survey was performed and samples were collected from the turfgrass areas in İstanbul, Antalya, Ankara, İzmir, Kayseri, Bursa, Aydın, and Muğla Provinces in 2015. Segments of leaves and roots were sterilized for 1 min, in 1% sodium hypochlorite (NaOCl) solution, then washed with sterile water and air dried in a laminar flow cabinet before culturing on potato dextrose agar (PDA, Difco, USA) containing 50 mg/l streptomycin sulfate. Isolates were incubated under the light and dark regimes, respectively on  $28 \pm 1$  °C for 7 days.

### Bacterial isolates

Five antagonistic domestic bacterial strains (215b, 44bac, 88cfp, 166fp, and 88bfp) used in this study were isolated from the tomato and cucumber rhizospheres in a previous study, where isolates 166fp and 88cfp managed with *Pythium deliense*, *Sclerotinia*

*minor*, and *Alternaria solani* on tomato were detected (Aşkın 2008). Also, isolate 44bac managed downy mildew on cucumber (Aşkın and Ozan 2013) under field conditions. Molecular identification of the 5 antagonistic bacterial isolates, used in this study, was first determined in this study.

### Molecular identifications of fungal and bacterial isolates

Isolation of fungal DNA was carried out by Blood and Tissue Kit (QIAGEN Inc. Valencia, CA), as specified by the manufacturer. The PCR reaction mixture and conditions were made by modifying according to Mahadevakumar et al. (2016). DNA amplification was performed, using the optimized cycles optimized with Techne TC-5000 thermal cycler. Primers ITS-1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS-4 (5' TCC TCC GCT TAT TGA TATGC 3') were used for amplification of ITS regions (White et al. 1990). The polymerase chain reaction (PCR) was performed in a 50- $\mu$ l reaction mixture containing 1  $\mu$ l template DNA, 1  $\mu$ l forward primer (10 mM), 1  $\mu$ l reverse primer (10 mM), 5  $\mu$ l reaction buffer (10 $\times$ ), 4  $\mu$ l dNTP (each 2.5 mM), 0.5  $\mu$ l Taq DNA Polymerase (5 U/ $\mu$ l), and 37.5  $\mu$ l sterile double-distilled water. The PCR cycling protocol consisted of initial denaturation at 94 °C for 4 min, followed by 30 cycles of 94 °C for 45 s, 55 °C for 45 s, 72 °C for 2 min, and a final elongation step of 72 °C for 10 min. As a negative control, the template DNA was replaced by sterile double-distilled water.

Molecular definition of bacteria was made according to the protocol of DNA isolation from Blood and Tissue Kit (QIAGEN Inc. Valencia, CA). The 16S rDNA gene fragments were amplified by PCR using the universal primers 27F 5'AGAGTTTGATCMTGGCTCAG3' and 1492R 5'TACGGYTACCTTGTTACGACTT3' (Lane, 1991). The PCR reaction mixture and conditions were modified to carry out the PCR reaction. DNA replications were performed in the ABI Veriti (Applied Biosystem) thermal cycler using the following cycles:

1. The initial denaturation consist of 5 min at 94 °C, 35 cycles of amplification step consisting of denaturation of 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 120 s, and final extension of 10 min at 72 °C (Lane 1991).
2. The PCR product was directly subjected to Sanger sequence treatment in a special Arge Laboratory (BM Gene Research and Biotechnology Company, Ankara, Turkey).
3. Bipartite raw sequence electropherograms were compared to the isolate sequences in Gen Bank after BLAST screening in NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### Determination of mycelial compatibility groups (MCGs) of *S. rolfsii* isolates

In order to determine the mycelial compatibility among 32 isolates, obtained from different areas grown with turfgrass areas, mating each isolate with themselves and with all other isolates was carried out (Punja and Grogan 1983). Mating studies were performed on PDA medium with 0.25% food coloring (Ponceau 4R, Turkey). Mycelial discs of two isolates were reciprocally plated on PDA medium with a distance of 3–4 cm. Cultures were incubated at  $25 \pm 1$  °C and colony growth was observed after 7–14 days (Kohn et al. 1991). Hyphal interaction among the isolates mate was observed at the end of 7 days after culturing. Compatibility between each of the two groups was evaluated according to a red line with separation in the region, where the hyphae collided. When the red line was seen, it was accepted as incompatible, otherwise not (Punja and Grogan 1983).

### Fungal inoculums

*S. rolfsii* was grown on wheat bran medium in bottles of 500 ml, sterilized in an autoclave for 20 min. at 121 °C, for 15 days at  $28 \pm 1$  °C (Aşkın 2008).

### Pathogenicity tests

Pathogenicity tests of *S. rolfsii* isolates were conducted under greenhouse conditions. The fungus inoculums grown on wheat bran (4 g inoculums/kg soil) was added to the sterilized garden soil, fine sand, and burnt fertilizer mixed (2:1:1) and then distributed in the pots (10 cm in diameter). Control pots contained the sterilized garden soil, fine sand, and burnt fertilizer mixed (2:1:1) free from the inoculum. Three pots were replicated for each treatment. All pots were covered by a sanitized polyethylene nylon and incubated for 3 days. At the end of the duration, 30 seeds of turfgrass (cv. *Festuca arundinacea*) were placed on the soil surface, covered with 1 cm of sterile natural soil, and watered with 9–10 ml of water. The infected plants were counted 3 weeks later (Zhang et al. 2014) and recorded. Evaluation was made according to a scale of 0 to 5: 0 = no disease symptoms, 1 = 1–10% hypocotyl infecting and/or shortening, 2 = 11–30% hypocotyl infecting and/or shortening, 3 = 31–50% hypocotyl infecting and/or shortening, 4 = 51–80% hypocotyl infecting and/or shortening, and 5 = entire hypocotyl infecting and/or shortening (Ichielevich Auster et al. 1985). Disease severity was calculated according to the Townsend–Heuberger formula (Townsend and Heuberger 1943):

$$\text{Disease severity}\% = \frac{\sum (N \times V)}{Z \times N} \times 100$$

( $N$  is the number of samples in the scale with different disease grades,  $V$  is the scale value,  $Z$  is the highest scale value, and  $N$  is the total number of samples observed)

### Bacterial inoculums

Bacterial isolates were cultured in potato dextrose broth. After 24 h, the bacterial concentration was verified through spectrophotometry at a wavelength ( $\lambda$ ) of 600 nm seeking for an absorbance between 0.9 and 1 equivalent to a concentration of  $1 \times 10^8$  c/ml and by counting the colony forming units (cfu) per milliliter through the total viable count. Surface-disinfected seeds of turfgrass were inoculated with bacterial solutions by soaking with agitation for 12 h. Rhizobacterial stock cultures were maintained in nutrient agar medium amended with 15% glycerol and stored at  $-80$  °C. Before being used in the bioassays, stock cultures were streaked onto nutrient agar plates and incubated at  $28 \pm 1$  °C for 48 h.

### Biocontrol assays

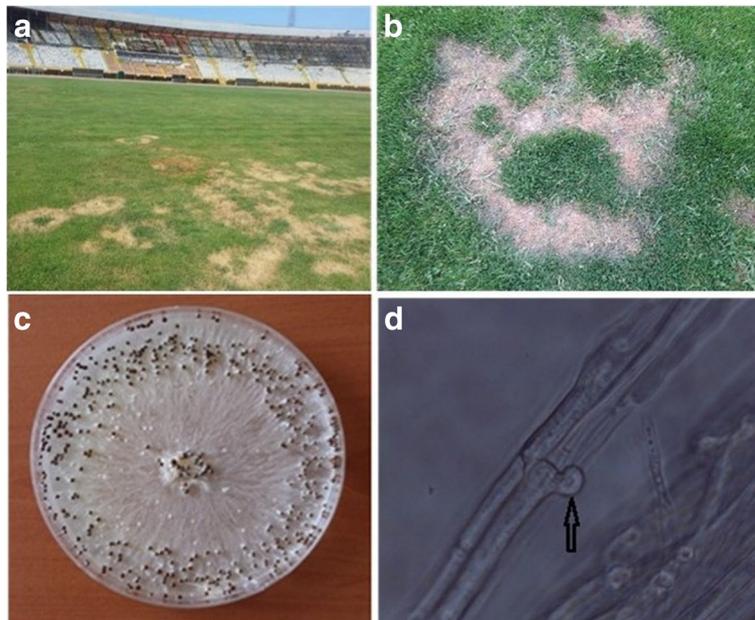
This study was carried out using turfgrass seeds mixture containing 4 cvs: *Festuca rubra*, *Lolium perenne*, *Poa pratensis*, *Festuca arundinacea*, and the most virulent *S. rolfsii* isolate (Sr34-10). The soil used in the experiment was prepared in the form of a mixture of 2:1:1 garden soil to stream sand to burnt fertilizer. Soil mix was sterilized in an autoclave at 121 °C for 45 min. The inoculum of *S. rolfsii* was developed on wheat bran. Antagonistic bacteria were applied by coating to the seeds. Experiments were carried out in both sterilized and non-sterilized soils, where three treatments were performed: (1) negative control by sowing uncoated turfgrass seeds in non-infested soils, (2) positive control by sowing uncoated turfgrass seeds in infested soils to evaluate the varietal sensitivity, and (3) sowing coated turfgrass seeds in infested soils to evaluate the biocontrol efficacy of each antagonistic isolate against *S. rolfsii*. The mixture of inoculum and soil (5 g to 1 kg of soil) were filled in sterile plastic pots (10 cm in diameter). After 4–5 days, 30 coated and uncoated turfgrass seeds were sown at a depth of 1 cm per pot. The plants were grown in a plant growth medium containing 12 h of light, 12 h of darkness, and  $25 \pm 1$  °C temperature. The experiments were carried out in 3 replicates, according to randomized plot design. After the inoculation, observations were made at intervals of 10 days and a 0–5 scale was used 30 days after sowing, and disease severity was calculated as mentioned before.

### Statistical analysis

Variance analyses were carried out, using the SPSS GLM statistical program, to determine the differences among virulence levels of isolates and disease rates in biocontrol assay.

### Disease assessment

Disease ratios were estimated according to the devised scale by Townsend-Heuberger formula (Townsend and



**Fig. 1** General view of disease symptoms on turfgrass areas (a from afar, b close view). Colonial view on PDA (c). Clamp connection on hypha (d) of *Sclerotium rolfsii*

Heuberger 1943). The calculated disease severity and the activity of bacterial isolates using the Abbott formula was determined from the disease severity values. Disease severity was compared by Tukey's multiple comparison test on these ratios.

## Results and discussion

### Survey of the disease and identification of *Sclerotium rolfsii*

The survey was carried out in wide parks, golf courses, stadiums, and recreation areas of 8 provinces among the



**Fig. 2** Mycelial compatibility of some *Sclerotium rolfsii* isolates

**Table 1** Origin, number, MCGs, and disease severity values of *Sclerotium rolfisii* isolates isolated from turfgrass areas

Origin	Isolate numbers	MCGs	Turfgrass composition	Disease severity (%)
İstanbul	Sr34-1	MCG1	<i>Festuca arundinacea</i>	90.68 ± 1.09a
	Sr34-2	MCG1	<i>Lolium perenne</i>	87.58 ± 2.02ab
	Sr34-3	MCG1	<i>Festuca rubra-rubra</i>	87.54 ± 3.18ab
	Sr34-4	MCG1	<i>Festuca rubra-commutata</i>	92.83 ± 1.31a
	Sr34-5	MCG1	<i>Poa pratensis</i>	88.59 ± 2.08ab
	Sr34-6	MCG1		87.21 ± 1.41ab
	Sr34-7	MCG1		83.74 ± 3.45b
	Sr34-8	MCG1		89.17 ± 2.32ab
	Sr34-9	MCG1		91.74 ± 2.69a
	Sr34-10	MCG1		92.87 ± 1.69a
İzmir	Sr35-1	MCG1	<i>Lolium perenne</i>	90.78 ± 2.15a
	Sr35-2	MCG1	<i>Poa pratensis</i>	90.87 ± 1.59a
	Sr35-3	MCG1	<i>Festuca arundinacea</i>	92.70 ± 2.75a
	Sr35-4	MCG1		91.04 ± 1.04a
	Sr35-5	MCG1		90.62 ± 2.58a
	Sr35-6	MCG1		92.29 ± 1.92a
	Sr35-7	MCG1		92.75 ± 2.18a
Antalya	Sr07-1	MCG1	<i>Cynodon dactylon</i>	89.46 ± 1.83ab
	Sr07-2	MCG1	<i>Lolium perenne</i>	90.66 ± 0.04a
	Sr07-3	MCG1	<i>Poa trivialis</i>	90.75 ± 0.63a
	Sr07-4	MCG1		91.75 ± 1.94a
	Sr07-5	MCG1		92.83 ± 1.23a
	Sr07-6	MCG1		89.08 ± 2.35ab
Muğla	Sr48-1	MCG1	<i>Cynodon dactylon</i>	88.47 ± 1.84ab
	Sr48-2	MCG1	<i>Lolium perenne</i>	90.16 ± 2.02a
	Sr48-3	MCG1	<i>Poa trivialis</i>	89.29 ± 4.17ab
	Sr48-4	MCG1		87.54 ± 1.71ab
Ankara	Sr06-1	MCG1	<i>Festuca rubra rubra</i>	88.66 ± 1.65ab
	Sr06-2	MCG1	<i>Lolium perenne</i> <i>Poa pratensis</i> <i>Festuca rubra commutata</i>	88.12 ± 2.38ab
Bursa	Sr16-1	MCG1	<i>Festuca rubra rubra</i>	88.60 ± 4.27ab
	Sr16-2	MCG1	<i>Lolium perenne</i>	90.20 ± 1.10a
	Sr16-3	MCG1	<i>Poa pratensis</i> <i>Festuca arundinacea</i>	91.75 ± 1.25a

There is no difference between the values expressed in the same letter,  $P < 0.0001$

largest turfgrass areas in different regions in Turkey, and totally 1400 samples were collected. In consequence of isolations, distinguishing symptoms, which were chlorotic or reddish-brown frog-eye or crescent-shaped circular patches (Figs. 1 a and b), of 32 *S. rolfisii* isolates were obtained, based on both colony morphology and rDNA internal transcribed spacer (ITS) region sequences. The isolates showed a rapid and a radial mycelial development. The colony color was white on the PDA medium, with a large amount of round, 1–3-mm-diameter brown sclerotia formed (Fig. 1c). “Clamp connection” event in fungus hyphae was observed (Fig. 1d).

On the other hand, molecular studies were also performed on isolates according to ITS-1 and 4 regions, and amplicons displayed by gel transilluminator were found to be between 650–680 bp, which is specific for *S. rolfisii*. The obtained results are parallel to that of Poor-nima et al. (2018) who studied genetic variations among *S. rolfisii* isolates of groundnut by using ITS rDNA sequence data and obtained an amplification fragment of about 650–700 bp. The sequences of the isolates showed

a 99–100% similarity rate compared to those of *S. rolfisii* deposited in the NCBI database. It was concluded that *S. rolfisii* caused more damage in warmer climates than in semi-arid areas in Turkey. *S. rolfisii* was previously found in soybean, peanut, sugar beet, tomatoes, pepper, eggplant, bean, and artichoke in Turkey, causing it to have different names according to crops (Yaşar and Türk 2016 and Aydoğdu et al. 2016). This is the first confirmed study on southern blight on turfgrass in Turkey.

#### Identification of the bacterial bioagents

Unidentified bacterial bioagents, which used in this study to see possible effects for controlling southern blight on turfgrass, were previously isolated from different crops (Aşkın 2008 and Aşkın and Ozan 2013). The sequence data of bacterial isolates showed 99–100% similarity with isolates in GenBank. The results of BLAST analysis showed that *Stenotrophomonas rhizophila* (100% similarity), *Pseudomonas putida* 166fp (100% similarity), *P. putida* 88cfp (99.64% similarity), *Paenibacillus* sp. (99.79% similarity), and *Bacillus cereus* (100% similarity) were

**Table 2** Effect of some bacterial strains against the infection by *Sclerotium rolfsii* on turfgrass

Treatments	Disease severity (%)	Efficacy (%)
<i>Pseudomonas putida</i> 166fp	34.67 ± 3.138 <sup>b</sup>	61.00
<i>Paenibacillus</i> sp.215b	31.47 ± 3.138 <sup>b</sup>	64.60
<i>Bacillus cereus</i> 44bac	8.00 ± 3.138 <sup>a</sup>	91.00
<i>Stenotrophomonas rhizophila</i> 88bpf	8.80 ± 3.138 <sup>a</sup>	90.11
<i>Pseudomonas putida</i> 88cpf	40.27 ± 3.138 <sup>b</sup>	54.75
(+) Control	89.00 ± 3.138 <sup>c</sup>	–
(–) Control	0.00	–

Levels not connected by same letter are significantly different,  $P < 0.0001$

identified. As a result of the study, amplicons displayed by gel transilluminator were found to be between 1500 and 1550 bp. It was detected that these *Pseudomonas* spp. and *Bacillus* spp. protected the infection by *Pythium deliense*, *Sclerotinia minor*, and *Alternaria solani* on tomato (Aşkın 2008) and downy mildew on cucumber (Aşkın and Ozan 2013) under field conditions.

#### Mycelial compatibility groups

In all mates, a red line was not observed in the contact area of the 2 colonies, and complete fusion was observed; all isolates were compatible with each other. Sclerotia were formed at the junction (Fig. 2). As a result of mating type analysis of 32 isolates, all of them were compatible with each other. Thus, it can be concluded that only one mycelial compatibility group (MCG) was seen (Table 1) in *S. rolfsii* in turfgrass, in this study.

To our knowledge, there is no MCG study on *S. rolfsii* on turfgrass in the world. However, in some studies performed in different crops, MCG of *S. rolfsii* isolates obtained from different hosts and geographical regions and even from the same regions and hosts were tested, and it has been reported that there is no relationship between geographic region and host specification of the formation of MCG of *S. rolfsii*. But genetic variations among different MCGs have been reported in different crops (Adandonon et al. 2005).

#### Pathogenicity

Disease severity of 32 *S. rolfsii* isolates ranged between 83.74 and 92.87% in different turfgrass composition (Table 1). The fungus is a destructive pathogen that can infect more than 500 plant species commonly seen in tropical and subtropical regions (Smiley et al. 1992). *S. rolfsii* has been found to cause damage to tomatoes, peppers, lettuce, bean, peanut, and sugarbeet in Turkey (Yaşar and Mert-Türk 2016). But, it has not been previously identified in turfgrass areas in Turkey.

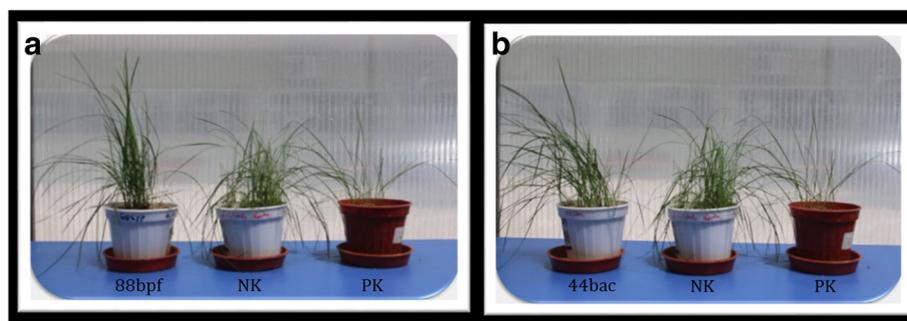
#### Biocontrol assays

In greenhouse experiments, all identified bacterial isolates were found to be effective in controlling the disease comparing to the severity of the disease in control treatment. The lowest disease severities were found at the treatments of 44bac (*B. cereus*) and 88bpf (*S. rhizophila*), being 8.00 ± 3.138 and 8.80 ± 3.138 %, respectively ( $P < 0.0001$ ) (Table 2 and Fig. 2). The highest disease severity was obtained in the treatment of 88cpf (*P. putida*), being 40.27 ± 3.138 % compared to that in the control treatment (Table 2). The highest protection effect was obtained by the isolate 44bac (*B. cereus*) being 91.00%, followed by the isolates 88bpf (*Stenotrophomonas rhizophila*), being 90.11%, and 215b (*Paenibacillus* sp.), being 64.60% (Table 2 and Fig. 3).

For the biocontrol of *S. rolfsii*, several bacterial strains have been studied in the world, and effective results have been obtained in these studies, most of them belonging to the biocontrol of *S. rolfsii* on vegetables with the genera *Pseudomonas* and *Bacillus* (Rakh et al. 2011 and Tonelli et al. 2011). But, there are no adequate studies related to *S. rolfsii* on turfgrass.

#### Conclusion

The presence of *S. rolfsii* as a causal of southern blight disease on the turfgrass areas in Turkey has firstly revealed with this study. Both *Bacillus cereus* 44bac and *Stenotrophomonas rhizophila* 88bpf were found to be effective in controlling *S. rolfsii* under greenhouse conditions. These



**Fig. 3** Effect of some bacterial isolates against southern blight caused by *Sclerotium rolfsii* in greenhouse experiments. **a** *Stenotrophomonas rhizophila* 88bpf, **b** *Bacillus cereus* 44bac (NK uninoculated plants, PK pathogen-inoculated plants)

bacterial strains could be recommended in the management programs of the southern blight disease in turfgrass areas. However, further studies are needed to be experimented under field conditions.

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#### Authors' contributions

FU contributed to the survey, isolation, identification, pathogenicity, mycelial compatibility groups, and biological control studies of *S. rolfii* isolates. All authors designed the study and wrote the manuscript, AA contributed to the provision of bacterial isolates and biological control studies. EK contributed to the identification of bacterial isolates. MY analyzed the data (statistical analyses), mycelial compatibility groups studies, and read and approved the final manuscript. MÜB identified of turfgrass species. All authors read and approved the final manuscript.

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#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Ethics approval and consent to participate

Not applicable

#### Consent for publication

Not applicable

#### Competing interests

The authors declare that they have no competing interests

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