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THE USE OF RHIZOBACTERIA ON WHITE ROT DISEASE AND GROWTH OF LETTUCE

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Abstract: White rot caused by *Sclerotinia sclerotiorum* [(Lib.) de Bary] is one of the most important diseases negatively affecting lettuce production. In this study, the effects of rhizobacteria containing different species on *S. sclerotiorum* were investigated. Also effect of rhizobacteria were determined on the growth of lettuce. Eight rhizobacteria strains (*Enterobacter cloacae*, *E. aerogenes*, *Bacillus cereus*, *Microbacterium testaceum*, *Pseudomonas putida*, *P. chlororaphis, Acinetobacter calcoaceticus,* and *Burkholderia cepacia*) were used in the study. Firstly, the *in vitro* effects of rhizobacteria strains were investigated on the mycelial growth and sclerotia viability of *S. sclerotiorum*. Then, pot experiments were carried out under controlled greenhouse conditions to determine the effect of selected strains on white rot disease and the growth of lettuce. The effect of tested bacteria on the mycelial growth of *S. sclerotiorum* ranged between 38.09-79.84%, and the *P. putida* strain had the highest impact. The bacterial strains were also effective on the sclerotia viability of *S. sclerotiorum*. The efficiency in the pot experiment was between 50-90% on white rot, and the highest effect was recorded in *A. calcoaceticus* strain. In the pot experiment rhizobacteria also increased plant growth. In particular, *E. aerogenes* was the most successful strain in plant growth. The results revealed that bacterial strains have different inhibitory effects in *in vitro* and *in vivo* experiments, while having the potential in the biological control of white rot disease and positive results on lettuce growth.

Keywords: Biological control, Lettuce, Plant growth promoting bacteria, *Sclerotinia sclerotiorum*

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1. Introduction

Lettuce (*Lactuca sativa* L.) is a temperate climate vegetable and contains a wide variety of minerals such as vitamins A and C, potassium, calcium and iron. In addition to its rich nutrient content, lettuce also has a large commercial market share among vegetables. Annual lettuce production is 27011747 tons globally, and China produces 10.8 million tons, followed by the USA, India and Spain (FAO, 2021). Turkey ranks 8th in the world with 561990 tons production of 3 prominent commercial lettuce varieties (Iceberg, Kıvırcık and Göbekli) (TUIK, 2022).

Diseases and pest control are highly important in lettuce cultivation as well as practices such as planting, irrigation and fertilization. Many diseases and pests hamper lettuce growth and cause great losses. White Rot Disease, caused by the soil-borne fungal pathogen, *Sclerotinia sclerotiorum* [(Lib.) de Bary], affects both field crops and vegetables and is prevalent all the over world, especially in temperate regions (Ferreira and Boley, 2002; Albert et al., 2022). The *S. sclerotiorum* can form sclerotia by the combination of mycelium and is easily recognized with the identification of sclerots. The white rot disease causes great economic losses every year in lettuce production (Bardin and Huang, 2001; Chitrampalam et al., 2008; Clarkson et al., 2014). The pathogen attacks the root and root collar of the lettuce plant and grows in the plant body causing softening and rotting. A mold layer similar to white cotton forms appears on the root as lettuce withers and dies (Anonymous, 2008). On diseased plants, *S. sclerotiorum* forms white mold and the sclerotia (Smolinska and Kowalska, 2018; Ficker, 2019). *S. sclerotiorum* overwinters on plant residues in soil as sclerotium or mycelium, germinates in the spring, and produces ascospores from the apothecium. Ascospores initiate infection following the penetration and germination in host plants (Agrios, 1997; Bolton et al., 2006).

Cultural measures such as the use of disease-free seedlings/seeds, resistant varieties, seed dressing, removal of plant residues, and chemical control are commonly used to control white rot disease; however, expected efficient results cannot be obtained after the development of resistant fungal strains. Therefore, different methods have been tested as the alternative to cultural and chemical control of the disease. The use of plant growth promoting rhizobacteria (PGPR) is one of such alternatives. The rhizobacteria are densely located in the rhizosphere and are in close contact with the plant roots. The population of these bacteria, composed of different bacterial genera, increases rapidly and spreads in the rhizosphere. The rhizobacteria increase root

growth and plant height and protect plants from diseases caused by soil-borne pathogens (El-Kafrawy, 2008; Soylu, 2011; Imriz et al., 2014; Abdeljalil et al., 2016; Sen et al., 2016).

Many studies have been conducted on the biological control of *S. sclerotiorum* or *S. minor*, and the causative agent of white rot on lettuce (Budge and Whipps, 2001; Fiume and Fiume, 2005; Rabeendran et al., 2006; Chitrampalam et al., 2008; Villalta et al., 2012; Baniasadipour and Shahidi Bonjar, 2014; Elias et al., 2016; Yıldız and Cenberci-Coskun, 2017; Soylu et al., 2021; Ramona et al., 2022). The studies reported that *Coniothyrium minitans*, *Trichoderma harzianum*, and *Gliocladium* spp. species are particularly effective on *S. sclerotiorum*. The *Bacillus* and *Pseudomonas*, which are bacterial species, have been reported as highly effective against soil-borne pathogens such as *Sclerotinia* (Hwang et al., 2006; Soylu et al., 2006; Monteiro et al., 2013; Kara et al., 2020).

Accordingly, this study aimed to determine the effects of rhizobacteria on the mycelial growth and germination of *S. sclerotiorum in vitro*, and biological control of white rot disease, and on growth of lettuce.

2. Materials and Methods

2.1. Materials

Fungal pathogen, *Sclerotinia sclerotiorum*, was isolated from the infected parts of lettuce plants and grown on Potato Dextrose Agar (PDA; Merck) medium. *Enterobacter cloacae* (ZE-2), *E. aerogenes* (ZE-5), *Bacillus cereus* (ZE-7), *Microbacterium testaceum* (ZE-8), *Pseudomonas putida* (ZE-12), and *Acinetobacter calcoaceticus* (ZE-13) bacterial strains, isolated from pepper production areas (Kayaaslan, 2021), and *Burkholderia cepacia* (7-a-2) and *Pseudomonas chlororaphis* (11b) strains, obtained from clove plants and identified using hypersensitivity reaction tests in tobacco (HR) and pectolytic activity test on potato and Matriks assisted laser desorption ionization-time of flight mass spectrometry-MALDI-TOF MS technique; bacteria developed for 24 hours were placed in the device after being treated with ethanol/formic acid and their diagnosis was made by comparing them with the microorganisms in the device's library with BiotyperTM 1.1 software (Bruker Daltonics), were used as the test organisms.

2.2. Methods

2.2.1. Effects of rhizobacteria on mycelial growth and sclerotial germination of *Sclerotinia sclerotiorum*

The bacterial strains were grown in Nutrient Agar (NA; Merck) medium from stock cultures, and *S. sclerotiorum* was grown in PDA medium. Tryptic Soy Agar (TSA; Merck) medium was used in the *in vitro* studies to determine the effect of bacterial strains on mycelial growth of *S. sclerotiorum*. Bacterial strains grown in NA medium for 24 hours were streaked in rings on the end of TSA medium in 90 mm diameter Petri dishes. Following the inoculation of the bacteria at 25±2 °C for 24 hours, a 5 mm diameter mycelium disc taken from the edges of a *S. sclerotiorum* culture was placed in the center of the medium. In the control, no bacteria was added and only a *S. sclerotiorum* mycelial disc was placed on the Petri dishes. All treated petri dishes were sealed with parafilm, and incubated at 25±2 ºC. The diameters of mycelial growth were measured when the fungus completely covered the medium (Tozlu et al., 2016), and the inhibition rate was calculated using the diameters measured (Mari et al., 1996). Five petri dishes were used for each application, and the experiment was repeated twice.

The surface of sclerotia grown in PDA for 10 days was sterilized three times with 2% sodium hypochlorite, and rinsed with sterile distilled water to determine the effect of the bacteria on sclerotial germination of *S. sclerotiorum*. The surface sterilized sclerotia were placed in glass tubes containing 10 ml of Luria Bertani Broth (LB Miller; Merck) and bacterial suspensions at a density of $1x10^8$ cell/ml. Five sclerotia were placed in each glass tube, and 3 glass tubes were used for each bacterial isolate. The tubes were shaken at 175 rpm for 24 hours. In the control treatment, sclerotia was placed in the LB medium without bacterial isolates. Following the incubation period, the bacteria-treated sclerotia were cut in the middle with a sterile scalpel and transferred to the PDA medium, and the petri dishes were incubated at 25±2 °C. The effect of bacterial isolates on the viability of sclerotia was determined according to mycelium growth in the media (Abdeljalil et al., 2016). Each treatment was repeated 2 times.

2.2.2. Biological control of white rot disease using rhizobacteria on lettuce

After the *in vitro* tests, a pot experiment was carried out to determine the effect of bacteria on white rot disease in lettuce under controlled conditions. The pot experiment was carried out in the greenhouse at Tokat Gaziosmanpasa University, Research and Application Center between March and June 2021. A bacterial suspension was prepared by culturing bacteria in NA medium at 25±2 °C for 24 hours, and then adjusting the suspensions to $1x10^8$ cells/ml in saline suspension (0.85% NaCl; Merck). Lettuce seedlings, (Maritima variety) planted in pots filled with sterile soil, peat and perlite (1:1:0.5), were treated with prepared bacterial suspensions for 1 hour. Another 100 ml bacterial suspension was applied to each plant after 10 days. After 10 days, the suspension of bacteria was sprayed on the plant leaves.

Following the bacterial spray, the scar tissue was opened with a scalpel at the root neck of the plant body and a bacterial suspension was applied to the opened part. Immediately after the application, a 5 mm diameter mycelial disc of S. *sclerotiorum* was placed on the opened wound. This inoculated part was wrapped with moist cotton and the plants were placed in a humidity chamber for 1 day. For positive control, plants were inoculated only with *S. sclerotiorum* (Tozlu et al., 2016), while distilled water was applied for negative control. The

experimental layout was randomized plots with 10 replicates, and the treatments were repeated 3 times. After the pathogen application, dead and viable plant counts were carried out according to the growths in the positive control plants (Bayram and Belguzar, 2021).

2.2.3. Effects of rhizobacteria on the growth of lettuce

Under controlled conditions, a pot experiment was carried out to determine the effect of rhizobacteria on the growth of lettuce. The pot experiment was carried out in the greenhouse between March and June 2021. Pathogen wasn't applied to the plants in this part of the study. As in the biological control experiment, bacterial suspensions were prepared, then lettuce seedlings were treated with prepared bacterial suspensions for 1 hour. Another 100 ml bacterial suspension was applied to each plant after 10 days. Then, 10 days after this application, the bacterial suspensions were sprayed onto the plant leaves. Distilled water was applied for negative control. The experimental layout was randomized plots with 10 replicates, and the treatments were repeated 3 times. At the end of approximately 45 days, head diameter, head height, head fresh and head dry weight, stem diameter, root length, root fresh and root dry weight were measured in plants that were daily maintained and irrigated (Bayram and Belguzar, 2021).

2.3. Statistical Analysis

The data obtained were subjected to variance analysis (ANOVA) using SPSS, version 25 statistics software (SPSS Inc. USA), and the differences in the means between the treatments were determined with Duncan multiple comparison test ($p \leq 0.05$).

3. Results

3.1. Effects of rhizobacteria strains on mycelial growth and sclerotial germination of *Sclerotinia sclerotiorum*

The effects of eight rhizobacteria strains on the mycelial growth of *S. sclerotiorum* in the Petri study ranged between 38.09-79.84%. *Pseudomonas putida* (ZE-12) (79.84%) was the most effective isolate. Bacterial strains ZE-5 (78.77%) (Figure 1), ZE-2 (77.43%), ZE-7 (76.37%), ZE-13 (72.24%) were also highly effective on pathogenic fungi. The differences on the mycelial growth of *S. sclerotiorum* among the isolates and control were significantly different (Table 1). The effect of bacterial strains on sclerotial germination of *S. sclerotiorum* was also statistically significant ($p<0.05$). Germination was recorded in sclerotia immersed in bacterial suspensions of ZE-2, ZE-5, ZE-7, ZE-8 and ZE-12, while sclerotia treated ZE-13, 7-a-2 and 11-b strains died. The ZE-5, ZE-8 and ZE-12 strains had a significant effect on the mycelium growth of the pathogen; on the contrary, sclerotia were still viable after exposure. The mycelium growth of sclerotia treated with ZE-2 and ZE-7 bacteria also was significantly suppressed (Table 1). The ZE-13 strain was particularly effective on both mycelium growth and sclerotia germination in *in vitro* study, and thus it was determined as the most successful strain.

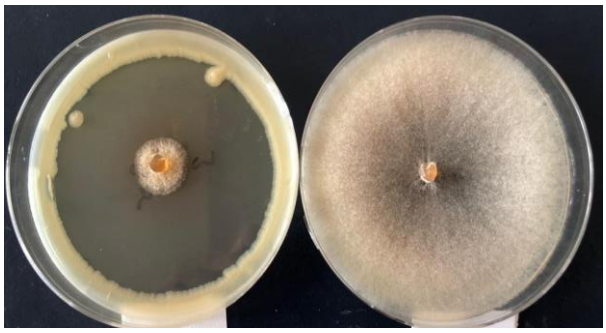

Figure 1. Effect of *Enterobacter aerogenes* (ZE-5) on the mycelial development of *S. sclerotiorum.*

Table 1. The effects of bacterial strains on the mycelial growth and sclerotia viability of *Sclerotinia sclerotiorum*

Codes	Treatments	Diameter of mycelium (mm)	Mean effect (%)	Sclerotia viability	Diameter of mycelium (mm)
C	Control	77.27±0.60 a*	0	Viable	81.32±0.70 a
11b	*Pseudomonas chlororaphis*	48.06±5.10 b	38.09	Dead	0.00±0.00 d
7-a-2	*Burkholderia cepacia*	44.85±7.69 b	42.22	Dead	0.00±0.00 d
ZE-8	*Microbacterium testaceum*	38.49±7.75 c	50.41	Viable	54.64±4.72 b
ZE-13	*Acinetobacter calcoaceticus*	21.55±5.74 d	72.24	Dead	0.00±0.00 d
ZE-7	*Bacillus cereus*	18.34±7.52 de	76.37	Viable	2.59±4.99 d
ZE-2	*Enterobacter cloacae*	17.52±8.89 de	77.43	Viable	1.73±3.97 d
ZE-5	*Enterobacter aerogenes*	16.48±2.99 de	78.77	Viable	42.47±11.26 c
ZE-12	*Pseudomonas putida*	15.66±5.67 e	79.84	Viable	44.64±8.24 c

*The same letters in the same column indicate that differences between applications are not significant ($p \leq 0.05$).

3.2. Biological control of white rot disease using rhizobacteria on lettuce

The plants started to die 6 days after the pathogen application in the pot experiment. Dead/viable plants were counted in the treated groups according to the developments in the positive control plants. All plants in positive control in which only *S. sclerotiorum* was applied, died (100% mortality). No signs of white rot disease were observed in plants of the negative control group. The inhibitory effect of bacteria in bacterial treated plants varied between 50-90%. The highest effect was recorded in strain ZE-13 (90% effect) (Figure 2), followed by strains ZE-5 and ZE-12 (80% effect). The ratio of effect in strains ZE-2 and ZE-7 applications was 70%, and followed by strain 7-a-2 (60% effect). The lowest effect of rhizobacteria was observed in strain 11-b application (Table 2).

Figure 2. Disease development in plants with PC, NC and ZE-13.

Table 2. The effect of rhizobacteria on white rot disease in lettuce

Codes	Treatments	Rate of effect (%)
PC	Treatment with *Sclerotinia sclerotiorum*	0
NC	Treatment with pure water	100
ZE-13	*Acinetobacter calcoaceticus*	90
ZE-5	*Enterobacter aerogenes*	80
ZE-12	*Pseudomonas putida*	80
ZE-7	*Bacillus cereus*	70
ZE-2	*Enterobacter cloacae*	70
7-a-2	*Burkholderia cepacia*	60
11b	*Pseudomonas chlororaphis*	50

3.3. Effects of rhizobacteria on the growth of lettuce

In this experiment, only rhizobacteria were applied to lettuce that were not infected with *S. sclerotiorum* and plant vegetative growth was examined. It is seen that rhizobacteria significantly increase the development of lettuce plants (Table 3). In NC plants, lettuce head diameter was measured as 27.65 cm, and in rhizobacteria-applied plants head diameter was measured in the range of 29.02-36.0 cm. Compared with NC, the highest effect was seen in plants treated *E. cloacae* (ZE-2). Head fresh (93.4-149.8 g) and dry weight (3.27-23.5 g) were also higher in rhizobacteria treated plants compared to the control. Besides, rhizobacteria were also effective on stem and root structure. *E. aerogenes* (ZE-5) significantly increased the stem diameter (6.75 cm) (Figure 3). Compared to the control (17.9 cm), significant increases were observed in the plants to which the rhizobacteria were applied (15.65-28.32 cm). Strain ZE-5 had a high impact on the root length parameter. Also, root wet weight was measured in the range of 8.48-16.08 g in bacteria applied plants. It was observed that strain ZE-2 (16.08 g) and strain 7-a-2 (15.39 g) showed a remarkable effect when compared to the control (8.03 g).

Table 3. Growth parameters in plants treated with rhizobacteria

	Head diameter (cm)	Head height (cm)	Head fresh weight (g)	Head dry weight (g)	Stem diameter (cm)	Root lenght (g)	Root fresh weight (g)	Root dry weight (g)
NC	27.65±2.8c*	19.5±1.21a	91.3±14.03b	3.24±0.94a	5.52±0.65bc	17.9±6.24b	8.03±3.94d	1.14±0.56c
ZE-2	36.0±2.46a	20.7±2.56a	140.3±23.75a	10.02±1.93a	4.5±0.79d	19.8±4.42b	16.08±3.13a	2.06±0.76a
ZE-5	29.02±2.74c	19.75±1.64a	103.1±14.57b	3.5±1.07a	6.75±0.67a	28.32±7.0a	11.89±3.97bc	1.87±0.88abc
ZE-7	30.67±1.67bc	21.17±1.2a	103.4±10.84b	3.9±1.06a	6.05±0.35ab	25.6±5.83a	8.48±2.13cd	1.26±0.58bc
ZE-12	29.57±3.15c	19.85±1.82a	93.4±11.08b	3.27±0.87a	6.02±0.54ab	24.8±4.71a	8.68±2.53cd	1.09±0.32c
ZE-13	33.67±8.24ab	19.75±5.16a	143.1±38.0a	9.8±2.97a	4.6±1.25d	15.65±4.5b	14.6±4.89ab	2.18±1.24a
7-a-2	35.32±2.81a	20.02±2.2a	148.3±30.2a	9.86±2.31a	5.1±0.94cd	17.75±4.0b	15.39±4.73ab	2.03±0.98ab
11b	35.67±2.71a	21.0±3.34a	149.8±32.85a	9.69±3.09a	5.05±0.91cd	18.02±3.2b	14.58±4.08ab	1.83±0.76abc

*The same letters in the same column indicate that differences between applications are not significant (p≤0.05).

Figure3. Development in NC and ZE-5 applied plants.

4. Discussion

In this study, the effects of rhizobacteria on *S. sclerotiorum* were investigated *in vitro* and *in vivo* conditions. The effects of antagonistic bacterial isolates on *S. sclerotiorum* were significantly different in *in vitro* tests. Similar to our study, Fatouros et al. (2018) reported that *Paenibacillus alvei* K165 inhibited the growth of *S. sclerotiorum* in lettuce. The inhibitory effect of *Pseudomonas* spp. AFP104 isolate on mycelial growth of *S. sclerotiorum* was reported as 83.3% by Soylu et al. (2005), while the inhibition ratio of pathogenic fungus by *Streptomyces* sp., *Pseudomonas fluorescens* and *Bacillus subtilis* (Bs1) bacteria was 72.2, 68.0, 62.22%, respectively (Helmy, 2016). El-Tarabily et al. (2000) reported the inhibitory effect of *Serratia marcescens, Streptomyces viridodiasticus* and *Micromonospora carbonacea*, on the growth of *S. minor in vitro.* The inhibitory effect of rhizobacteria can be attributed to the various antibiotics, antimicrobial compounds, enzymes, and organic volatile compounds secreted by the bacteria, that suppress the growth of pathogens and affect systemic resistance in the host plant. For example, *Pseudomonas* species produce numerous antimicrobial compounds such as pyoluteorin, pyrrolnitrin, phenazines, siderophores, cyanide, 2,4-diacetylphloroglucinol (Compant et al., 2005) and enzymes such as cellulose, chitinase, proteases and beta-glucanase (Hernandez-Leon et al., 2015). Antibiotics such as phenazine-1-carboxylic acid, 2-hydroxyphenazine and pyrrolnitrin produced by bacteria are effective on sclerotium and spore germination (Savchuck, 2002; Fernando et al., 2007; Zhang et al., 2004; Selin et al., 2010).

Also in our study, the bacterial strains tested exhibited promising results for sclerotia viability. A significant suppressive effect of *Pseudomonas fluorescens* isolates on sclerotia formation has been indicated by El-Kafrawy (2008). In another study, Onaran and Yanar (2011) stated that 12 of the 23 isolates tested had an inhibitory impact on sclerotia, and the bacterial strains of *Pseudomonas putida, P. fluorescens, Paenibacillus macerans* and *Bacillus pumilis* killed the sclerotia. The mycelial growth of S. *sclerotiorum* by *Bacillus megaterium, B. cereus, B. subtilis, Arthrobacter nicotianae, A. ramosus, Pseudomonas filiscindens, Stenotrophomonas maltophilia, Brevibacterium frigoritolerans* and *Sphingobacterium faecium* was inhibited up to 80% and the sclerotial germination was between 0-100%. Similarly, *Pseudomonas brassicacearum, P. thivervalensis,* and *P. chlororaphis* species were reported to have a significant inhibitor effect on sclerotia viability (Bayram and Belgüzar, 2021).

The potential of bacterial strains, belonging to different genera, in the biological control of white rot disease in lettuce has been investigated in this study. Similarly, Soylu (2011) investigated the effect of antagonistic root bacterial strains (*Lysobacter enzymogenes* C3R5 and N4-7) and PGPR strains (*Bacillus pumilus* T4, *B. amyloliquefaciens* IN937a, *Pseudomonas fluorescens* WCS417r and *P. putida* 89B-61) on *S. sclerotiorum*. In *in vitro* studies, *L. enzymogenes* strain C3R5 and N4-7 significantly inhibited the pathogen whereas in *in vivo*, all strains showed a suppressive effect on disease development compared to the positive control. Chon et al. (2013) reported that the efficiency of *B. megaterium* (DK6) and *B. cereus* (C210) on sclerotia viability of *S. sclerotiorum* in the greenhouse was 20 and 35%, respectively. The disease incidence of 9 out of 26 strains was 20% in a pot experiment carried out in winter. Therefore, 9 strains were considered as potentially antagonistic bacteria for biological control of Sclerotinia rot of lettuce caused by *S. sclerotiorum.* Chen et al. (2016) indicated that two *Streptomyces* species (*S. exfoliatus* FT05W and *S. cyaneus* ZEA17I) inhibited fungal mycelium growth of *S. sclerotiorum* more than 75%. *S. exfoliatus* FT05W inhibited the disease by 40% in lettuce plants under field conditions. In another study, *Bacillus subtilis* GG95 was reported to suppress white rot disease in lettuce by 88% (Lee et al. 2015). *Arthrobacter* FP15 and *Blastobotrys* FP12 isolates also significantly reduced the severity of white rot disease in lettuce (Aggeli et al., 2020). A pot experiment carried out under greenhouse conditions for the biological control of white rot disease in cucumber plants showed that *Pseudomonas chlororaphis* alone and the co-application of *P. brassicacearum* and *P. chlororaphis* had the highest impact on white rot disease. In both applications, 8 out of 10 plants remained viable (80% effect), and had no signs of white rot (Bayram and Belguzar, 2021).

Acinetobacter calcoaceticus exhibited the highest effect on *S. sclerotiorum* in both *in vitro* and *in vivo* experiments. In this study, studies on the action mechanism of antagonistic bacteria have not been conducted. But, in the literature review, *A. calcoaceticus was* also highly effective against *Fusarium* disease caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. The bacteria uses siderophore, biofilm, proteases, endoglucanases and

indole acetic acid, and phosphate solubilization mechanisms, thus promotes plant growth and biocontrol pathogen (Khalil et al. 2021). The *A. calcoaceticus* also increases plant growth by producing malic, succinic, citric and other organic acids (Kang et al., 2012). In our study, besides *A. calcoaceticus, Pseudomonas putida* was successful against the pathogenic fungus. Similarly, the results of a study using cucumber plants under greenhouse conditions, demonstrated that *P. putida* inhibited white rot disease by 83.64% (Onaran and Yanar, 2011). Likewise, *Enterobacter cloacae* strain had a significant effect on the mycelial growth and sclerotia viability of *S. sclerotiorum*. The findings are in agreement with the findings of Mohamed et al. (2020) who also tested the same bacteria against a different pathogen (*Ralstonia solanacearum*). The researchers showed that siderophore, indole acetic acid, hydrogen cyanide, salicylic acid production and *E. clocae* strain PS14 had the highest effect on controlling the agent both *in vitro* and *in vivo*. Similarly, as in this study, *Burkholderia cepecia* had biocontrol activity against various lettuce pathogens including *S. sclerotiorum* and *Burkholderia* strains harbor for the biosynthesis of pyrrolnitrin, burkholdins and cepacin (Biessy et al., 2022).

In this study, rhizobacteria also increased plant growth. There are also studies conducted by Celik (2022) and Karagöz and Kotan (2010) similar to this study. Celik (2022) stated that *Bacillus cereus* provided a yield increase of up to 57.4% compared to the lettuce plants in the control. Rhizobacteria increase plant growth due to the plant hormones and various vitamins, they have produced from the moment they are in the plant, and in this way the root length of the plants is elongated, thus increasing yield. In addition, rhizobacteria provide the transformation of minerals into suitable, degradable forms in the translocation process and incorporation. A large of microorganisms produce Indol-3-acetic acid, giberalic acid and phytohormone, which provide a significant increase in shoot, root, and yield in plants (Çakmakçı, 2005; Chakraborthy et al., 2006).

5. Conclusion

Intense pesticide use in agricultural production areas causes serious environmental as well as health problems. The use of antagonistic bacteria, rhizobacteria that promote plant growth, and beneficial microorganisms are important to reduce pesticide use to sustain agricultural production and increase the yield. The results of this study revealed that microorganisms such as antagonistic bacteria can be widely used as biopreparations or biofertilizers in agricultural areas. White rot disease has a wide host range; therefore, can be a problem in agricultural areas and is very difficult to control. Cultural measures are insufficient to control the disease. Resistance problems and sclerotia formation of the pathogen cause serious difficulties in chemical control. In this study, the effects of 8 antagonistic bacteria against *S. sclerotiorum* were tested both *in vitro* and *in vivo* conditions. The results demonstrated that bacterial isolates with different inhibitory effects have the potential to be used in the biological control of white rot disease. Further studies with these bacteria should be conducted under field conditions, and the effect of antagonistic bacteria tested in this study should also be investigated for other soil-borne pathogens.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	A.C.A.	S.B.
C	50	50
D	50	50
S		100
DCP	50	50
DAI		100
L	50	50
W		100
CR	50	50
SR		100
PM		100
FA		100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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