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Full Length Research Paper

Screening bacterial species for antagonistic activities against the *Sclerotinia sclerotiorum* (Lib.) De Bary causal agent of cucumber white mold disease

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In this study, 23 bacteria strains that belong to 19 bacterial species were tested against *Sclerotinia sclerotiorum* (Lib.) De Bary. *In vivo* and in *vitro* testing of bacterial strains showed that *Serratia plymuthica* strains IK-150 and IK-139, *Burkholderia cepacia* strain IK-16, *Pseudomonas flourocens* strain IK-3, *Pseudomonas putida* strain IK-1, *Paenibacillus macerans* strain IK-36, *Pantoea agglomerans* strain IK-147 and *Burkholderia pyronicia* strain IK-145 totally inhibited mycelial growth and caused loss of viability of sclerotia. The other tested bacterial strains also reduced mycelial growth of the fungus development. These results indicate that bacterial species used in this study could be used in the control of *S. sclerotiorum*.

Key words: *Sclerotinia sclerotiorum*, white mold, biological control, bacteria.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is the causal agent of white mold, stem and fruit rot diseases on more than 400 plant species, including cucumber, on which it causes fruit and stem rots (Boland and Hall, 1994). It is one of the most important diseases of greenhouse grown cucumbers in Western Mediterranean Region of Turkey (Onaran and Yanar, 2009), causing significant yield loss of up to 80% (Tuncer and Damdere, 1997; Onaran and Yanar, 2009). The disease is clearly distinguished by the development of white cottiny mycelium on stem and fruit and resting sclerotia on or in infected tissues of host plant (Agrios, 1997). *S. sclerotiorum* spends 90% of life cycle in soil as a sclerotia, survives up to 5 years in soil (Adams and Ayers, 1979). When sclerotia find suitable conditions, they germinate and produce apothecia that release infectious ascospores (Abawi and Grogan, 1979). Most of the ascospores are produced by sclerotia situated 5 cm below soil (Steadman, 1974). Ascospores require external food source to infect healthy plant tissue.

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Abbreviations: PDA, Potato dextrose agar; **NA,** nutrient agar.

Therefore, senescence flower petals serve as an external energy source to support ascospore infection of healthy plants (Abawi et al., 1975). Once established, infections can spread to leaves, petioles, stems and fruits.

At present, the main management practices are the use of resistant cultivars or repeated fungicide applications. However, the limited availability of commercially acceptable resistant cultivars, lack of resistance in commercial cucumber cultivars and increasing public concerns about potential impact of pesticides on the environment, and also indeterminate flowering characteristic of cucumber which provides many opportunities for infection to occur, even with several foliar fungicide applications, have necessitated alternative or complementary methods that are effective, reliable and environmentally safe. Biological control is one of the effective control methods. Biological control agents have received most of the attention because of their versatile modes of action to protect plants and their potential to be included in integrated management programmes (Köhl and Fokkema, 1998). Therefore, biological control should be implemented as much as possible.

Microorganisms used in biological control of plant pathogens produce extracellular enzymes or metabolites that affect plant pathogens and compete with plant patho-

gens for resources or show antagonism (Cook and Baker, 1983). There are 80 commercially available bio-control agents against plant pathogens (Paulitz and Belanger, 2001). *Trichoderma harzianum* (Inbar et al., 1996) and *Coniothyrium minitans* (Willets and Wong, 1980; Haung et al., 2000) are examples of fungal antagonists used against *S. sclerotiorum*. There are also some bacterial species that affect *S. sclerotiorum* mycelial growth and sclerotia viability. *Pseudomonas putida* and *Pseudomonas flourescens* can be used against *S. sclerotiorum* causing sunflower stem rot disease (Expert and Digat, 1995). Similarly, *Burkholderia cepecia* and *Bacillus subtilis* strains are applied to control rapeseed white mold disease in Taiwan (Gu, 1996). Yuen et al. (1994) reported that *Erwinia herbicola* and *Bacillus polymixa* inhibited the fungal growth of *S. sclerotiorum*.

In the present study, 23 bacterial strains belonging to 19 bacterial species were tested against *S. sclerotiorum in vitro* and *in vivo* to find out new promising bacterial strains in the control of the fungus.

MATERIALS AND METHODS

Isolation of *S. sclerotiorum*

S. sclerotiorum isolates were isolated from cucumber plants grown in greenhouses in Demre, Finike and Kumluca (Antalya) between 2007 and 2009. For isolation, a single sclerotia or infected host tissue, surface-sterilized by dipping in 2% sodium hypochlorite solution for 2 min, and then rinsed three times with sterile distilled water, using dry out blotter for 3 min, was aseptically transferred into potato dextrose agar (PDA) plates. The plates were incubated at 25 ± 2°C. Mycellial discs (diameter of 5 mm) taken from the edge of the actively growing colonies were transferred to the Petri dishes containing PDA to obtain pure cultures of the *S. sclerotiorum*.

Isolation of bacteria

Bacteria were isolated from aerial parts of healthy and diseased cucumber plants grown in greenhouse that are known to be infected by *S. sclerotiorum*. Plant samples were collected and then subjected to surface sterilization as described earlier. Then, they were placed on nutrient agar (NA) and incubated at 28 ± 2°C for 4 days. Pure bacterial cultures were obtained from them. Six bacterial strains were isolated and 3 of them showed antagonistic activity against *S. sclerotiorum*. These antagonistic strains were identified and classified by Ömür Baysal from Western Mediterranean Agricultural Research Institute (Antalya, Turkey). These antagonistic bacterial strains were *B. subtilis* strains AO-2, AO-3 and AO-5 (Table 1).

Additionally, 20 bacterial strains (Table 1), isolated from different insect species and plants were obtained from Isa Karaman (Department of Biology, Gaziosmanpaşa University). These strains were also used in the present study.

Effect of bacteria on growth of *S. sclerotiorum in vitro*

Five-millimeter discs were cut from 4 to 5 days old *S. sclerotiorum* cultures on PDA medium and placed on the edge of a 90 mm Petri dishes containing NA agar. Bacterial cultures were dispersed with a bacterial loop between the fungus discs. The Petri dishes were incubated at 28 ± 2°C for 7 days. Inhibition zones produced by bacterial strains were measured with the aid of calipers. NA inoculated with the pathogen alone was used as control. Each treatment was replicated 4 times. The whole experiment was repeated on 2 different occasions.

Effect of bacteria on viability of sclerotia

Bacterial suspensions were prepared from 48 h old bacteria cultures grown on NA. Sterile distilled water was added to cultures and scratched with aid of bacterial loop. Bacterial suspension concentration was adjusted to 10^8 cfu/ml, adding distilled water. These bacterial suspensions were applied to the fungus sclerotia with a hand spray. Treated sclerotia were placed onto Petri dishes containing PDA. The sclerotia were incubated at 28 ± 2°C for 7 days. Viability of sclerotia and mycelial growth were recorded after 7 days. Each treatment was repeated twice. Whole experiment was replicated on 2 different occasions.

Effect of bacteria on *S. sclerotiorum*, using whole cucumber plants

In the present study, all bacterial strains were tested on *Cucumis sativus* (var. Halley F_1) in greenhouse conditions. Bacterial suspensions were prepared as described earlier and spray inoculation was performed when the plants were at the beginning of flowering (R_2 phase) stage (Nelson et al., 1988). Cucumber plant stems were mechanically injured over 4 cm from the soil surface and 0.5 ml of bacterial suspensions (10^8 cfu/ml) was sprayed onto these wounds. A 5 mm diameter *S. sclerotiorum* mycelium disc was placed onto wounds after 24 h prior to bacterial application. To stimulate fungal development, wet cotton was covered onto wounds. In control, 5 mm mycelium of the fungus was placed after spraying mechanical wounds with sterile distilled water. The plants were incubated three days in these conditions. After 3 days, cotton was removed and plants were incubated for a further 4 days. After 7 days, lesions sizes were measured with calipers. Percentage inhibition rates (I) was calculated by using the formula,

$$I = 100 \times (C.-T)/C$$

Where, C is the lesion length in control and T is the lesion length in treatment.

Testing of bacteria to find out whether bacteria cause any infection onto cucumber plants were also conducted. Mechanically wounded plants were sprayed with 0.5 ml bacterial suspension containing 10^8 cfu/ml and plants were monitored for 7 days for any infection signs.

Statistical analysis

The data were analyzed using analysis of variance (PROC ANOVA; SAS Institute, Inc., Cary, NC), and means were compared by least significant differences (LSD) at P = 0.05.

RESULTS

Effect of bacteria on growth of *S. sclerotiorum in vitro*

Results of inhibition zones caused by bacterial strains are presented in Table 2. All bacterial strains tested inhibited mycelial growth of *S. sclerotiorum in vitro*. However, there

Table 1. Bacterial strains used in the present study.

Strain code	Bacterial strains	Source
IK-1	*Pseudomonas putida-biotype A*	Aphid
IK-3	*Pseudomonas flourocens-biotype G*	Aphid
IK–16	*Burkholderia cepacia*	Aphid
IK-34	*Bacillus cereus –GC subgroup A*	Ant
IK-36	*Paenibacillus macerans-GC subgroup A*	Aphid
IK–55	*Paenibacillus apiarius*	Aphid
IK-81	*Micrococcus luteus-GC subgroup C*	Ant
IK-91	*Bacillus pumilis –GC subgroup B*	Grasshopper
IK-104	*Bacillus amyloliquefaciens*	Aphid
IK-139	*Serratia plymuthica*	Pear-branch
IK-145	*Burkholderia pyrocinia*	Wild pear
IK–147	*Pantoea agglomerans*	Apple-branch
IK-150	*Serratia plymuthica*	Apple-branch
IK-83	*Bacillus subtilis*	Ant
IK–132	*Bacillus lentimorbus*	Pear-branch
IK–178	*Brevibacillus agri*	Pear-branch
IK–22	*Bacillus coagulans*	Mosquito larvae
IK–174	*Serratia marcescens-GC subgroup A*	Cockroach
IK–57	*Brevibacillus laterosporus*	Aphid
IK-146	*Brevibacillus brevis*	Pear-branch
AO-5	*Bacillus subtilis**	Sclerotia
AO-3	*Bacillus subtilis**	Sclerotia
AO-2	*Bacillus subtilis**	Cucumber

*Bacterial strains isolated from plant tissues and sclerotium which were collected from survey areas.

were significant differences among the bacterial strains ($P < 0.05$). Growth inhibition of *S. sclerotiorum* by *Serratia plymutica* strain IK150 was significantly greater than that of the other strains except, *Burkholderia cepacia* strain IK16 (Table 2), but *B. subtilis* strain AO2 had less effect on the growth of the pathogen. The other tested species produced varying sizes of inhibition zones.

Effect of bacterial strains on viability of sclerotia

The effect of bacterial strains on sclerotia viability is presented in Table 3. Application of *P. putida* strain IK-1, *P. flourocens* strain IK-3, *B. cepacia* strain IK-16, *Bacillus cereus* strain IK-34, *Paenibacillus macerans* strain IK-36, *Paenibacillus apiarius* strain IK-55, *Bacillus pumilis* strain IK-91, *Bacillus amyloliquefaciens* strain IK-104, *Burkholderia pyrocinia* strain IK-145, *Pantoea agglomerans* strain IK-147, *S. plymuthica* strain IK-150 and *Serratia marcescens* strain IK-174 on the sclerotia caused loss of viability of the sclerotia and no mycelial growth from these sclerotia with treated bacterial strains were observed. When the sclerotia were treated with *Brevibacillus laterosporus* strain IK-57, *Micrococcus luteus* strain IK-81, *B. subtilis* strains IK-83, AO-2, A0-3 and AO-5, *S. plymuthica* strain IK-139, *Brevibacillus agri* strain IK-178, *Bacillus lentimorbus* strain IK-132, *Bacillus coagulans* strain IK-22 and *Brevibacillus brevis* strain IK-146, the sclerotia remained viable and mycelial germination of the sclerotia were observed when they were transferred to the PDA medium free of bacteria. Interestingly, treatment of sclerotia with *B. coagulans* strain IK-22 and *B. brevis* strain IK-146 stimulated the mycelial germination of scleriotia treated with these bacterial strains and resulted in more mycelial growth than those in controls.

Effect of bacteria on *S. sclerotiorum*, using whole cucumber plants

Bacterial strains that showed activity in *in vitro* test were further tested in *in vivo* conditions on *C. sativus* (var. Halley F_1). Lesion length and percentage prevention of the disease are presented in Table 4. All tested bacterial strains inhibited the fungal growth. *B. cepacia* strain IK-16, *S. plymuthica* strain IK-139, *P. macerans* strain IK-136, *P. agglomerans* strain IK-147, *P. putida* strain IK-1, *B. pyrocinia* strain IK-145, *P. flourocens* strain IK-3, *S. plymuthica* strain IK-150, *S. marcescens* strain IK-174

Table 2. Antagonistic effects of bacterial strains against *S. sclerotiorum* in *in vitro* conditions.

Strains code	Bacterial strains	Inhibition zone (mm)
IK-150	*Serratia plymuthica*	36.70a*
IK–16	*Burkholderia cepacia*	34.50ab
IK-139	*Serratia plymuthica*	32.00cb
IK-3	*Pseudomonas flourocens-biotype G*	30.00cd
IK–147	*Pantoea agglomerans*	26.80ed
IK-145	*Burkholderia pyrocinia*	25.20ef
IK-81	*Micrococcus luteus-GC subgroup C*	22.40gf
IK-1	*Pseudomonas putida-biotype A*	20.60g
IK-36	*Paenibacillus macerans-GC subgroup A*	20.00gh
IK-34	*Bacillus cereus –GC subgroup A*	16.00ih
IK–178	*Brevibacillus agri*	16.00ih
IK-83	*Bacillus subtilis*	14.80ij
IK-104	*Bacillus amyloliquefaciens*	14.00kij
IK-146	*Brevibacillus brevis*	10.90klj
IK–22	*Bacillus coagulans*	10.40klm
IK-91	*Bacillus pumilis –GC subgroup B*	9.40nlm
IK–174	*Serratia marcescens-GC subgroup A*	9.40nlm
IK–55	*Paenibacillus apiarius*	7.00nlom
AO-3	*Bacillus subtilis*	6.40nom
AO-5	*Bacillus subtilis*	6.20no
IK–132	*Bacillus lentimorbus*	5.70no
IK–57	*Brevibacillus laterosporus*	3.60po
AO-2	*Bacillus subtilis*	1.40p

*Means with different letters differ significantly (LSD:4.13).

and *B. brevis* strain IK-146 totally inhibited the fungal growth and no fungal lesion was observed on plants treated with the bacterial strains. The other tested bacterial strains also reduced the fungal development on plants but there were some fungal lesions on leaves and stems of plants. The pathogenicity of the tested bacterial strains was also tested on cucumber plants. No bacterial lesions was observed, confirming that the bacterial strains were non pathogenic to cucumber plant.

DISCUSSION

In this study, effects of antagonistic bacterial strains on *S. sclerotiorum* were tested in *in vivo* and *in vitro* conditions. Some of the tested bacteria appear to be promising as a biocontrol agent against the fungus. *In vitro* results suggest that there is antagonism between the bacterial strains and the fungus. Additionally, most of the bacterial strains caused loss of viability of fungus sclerotia or reduced its mycelial growth. However, increasing mycelial growth of the fungus that were treated with *B. brevis* strain IK-146 and *B. coagulans* strain IK-22 suggests that enzymes produced by bacterial strains may create a suitable environment for the fungus.

This study indicated that bacterial strains showed varying effects depending on the life cycle stage of fungus, e.g. sclerotia or mycelium. Bacterial strains also inhibited the fungal growth on cucumber plants in *in vivo* testing. Results of *in vivo* and *in vitro* tests suggest that bacterial strains affected the fungus in varying degrees. Increased effects of bacterial strains in greenhouse in comparison with *in vitro* test indicate that some of the tested organisms performed much better in real environmental conditions. Similar results with ours were previously reported by other scientists. Kamensky et al. (2003) reported that *S. plymuthica* IC14 strain showed high activity aganists *S. sclerotiorum* in both laboratuary and greenhouse conditions. *Pseudomonas (Burkholderia) cepecia* J82 and J51 strains produced inhibition zones and inhibited mycelial growth of the fungus *in vitro* (Mcloughlin et al., 1992). *B. subtilis* and *B. cereus* strains reduced the mycelial growth of *S. sclerotiorum* and suppress the fungus in sunflower (Zazzerini et al., 1987). Application of *B. cereus* suspension on pea plants reduced prior to sowing, *S. sclerotiorum* ascospor infections (Huang et al., 1993). Treatment of pea seeds with *P. putida* and *P. fluorescens* reduced early *S. sclerotiorum* infections (Expert and Digat, 1995).

It is the first time that *B. laterosporus* strain IK-57, *B.*

Table 3. Effect of bacterial strains on sclerotia viability of *S. sclerotiorum* under *in vitro* conditions.

Strain code	Bacterial strains	Sclerotia viability	Mycelial growth (mm)
Control	*Sclerotina sclerotiorum*	Viable	35.90
IK–57	*Brevibacillus laterosporus*	Viable	10.11
IK-81	*Micrococcus luteus-GC subgroup C*	Viable	16.20
AO-5	*Bacillus subtilis*	Viable	21.05
AO-3	*Bacillus subtilis*	Viable	27.35
IK-139	*Serratia plymuthica*	Viable	27.25
IK–178	*Brevibacillus agri*	Viable	28.00
IK–132	*Bacillus lentimorbus*	Viable	29.80
IK-83	*Bacillus subtilis*	Viable	30.03
AO-2	*Bacillus subtilis*	Viable	32.40
IK–22	*Bacillus coagulans*	Viable	54.22
IK-146	*Brevibacillus brevis*	Viable	54.30
IK-1	*Pseudomonas putida-biotype A*	Dead	0.00
IK-3	*Pseudomonas flourocens-biotype G*	Dead	0.00
IK–16	*Burkholderia cepacia*	Dead	0.00
IK-34	*Bacillus cereus –GC subgroup A*	Dead	0.00
IK-36	*Paenibacillus macerans-GC subgroup A*	Dead	0.00
IK–55	*Paenibacillus apiarius*	Dead	0.00
IK-91	*Bacillus pumilis –GC subgroup B*	Dead	0.00
IK-104	*Bacillus amyloliquefaciens*	Dead	0.00
IK-145	*Burkholderia pyrocinia*	Dead	0.00
IK–147	*Pantoea agglomerans*	Dead	0.00
IK-150	*Serratia plymuthica*	Dead	0.00
IK–174	*Serratia marcescens-GC subgroup A*	Dead	0.00

Table 4. Effects of bacterial strains on disease development of white mold on *C. sativus* (var. Halley F_1) under *in vivo* conditions.

Strain code	Bacterial strains	The lesion length (mm)	Percentage inhibition rates (%)
Control	*Sclerotinia sclerotiorum*	84.60^{a*}	-
AO-2	*Bacillus subtilis*	48.05^{b}	43.20
IK-22	*Bacillus coagulans*	39.85^{c}	52.90
IK-132	*Bacillus lentimorbus*	39.06^{c}	53.83
IK-55	*Paenibacillus apiarius*	38.95^{c}	53.96
IK-57	*Brevibacillus laterosporus*	37.30^{cd}	55.92
AO-3	*Bacillus subtilis*	32.81^{cde}	61.22
IK-34	*Bacillus cereus –GC subgroup A*	31.03^{de}	63.22
IK-104	*Bacillus amyloliquefaciens*	29.51^{e}	65.12
IK-178	*Brevibacillus agri*	29.27^{e}	65.40
AO-5	*Bacillus subtilis*	27.04^{ef}	68.40
IK-91	*Bacillus pumilis –GC subgroup B*	21.06^{fg}	75.11
IK-83	*Bacillus subtilis*	20.26^{fgh}	76.05
IK-81	*Micrococcus luteus-GC subgroup C*	20.21^{fgh}	76.12
IK-146	*Brevibacillus brevis*	15.80^{ghi}	81.32
IK–174	*Serratia marcescens-GC subgroup A*	15.16^{ghi}	82.08
IK-150	*Serratia plymuthica*	14.22^{ghi}	83.19
IK-3	*Pseudomonas flourocens-biotype G*	14.19^{ghi}	83.23
IK-145	*Burkholderia pyrocinia*	13.88^{ghi}	83.59
IK-1	*Pseudomonas putida-biotype A*	13.84^{ghi}	83.64
IK-147	*Pantoea agglomerans*	13.35^{hi}	84.22
IK-36	*Paenibacillus macerans-GC subgroup A*	10.87^{i}	87.15
IK-139	*Serratia plymuthica*	9.93^{i}	88.27
IK-16	*Burkholderia cepacia*	9.12^{i}	89.22

*Means with different letters differ significantly (LSD:7,61).

agri strain IK-178, *M. luteus* strain IK-81, *S. marcescens* strain IK-174 and *B. pyrocinia* strain IK-145 were tested against *S. sclerotium*. All these bacterial strains showed high activity and encourage additional studies with these species.

Previous studies on biocontrol of *S. sclerotiorum* showed that many fungal and bacterial species totally or partially inhibited fungal development of the fungus *in vivo* and i*n vitro* conditions. Some of them are *Coniothyrium minitans Epicoccum purpurascens, Trichoderma virens, Trichothecium roseum* (Huang et al., 2000), *Epicoccum nigrum, Trichoderma viride* (Hannusch and Boland, 1995), *Fusarium* spp., *Penicillium* spp., *Glioclodium roseum* (Ferreira and Boley, 2002), *Glioclodium catenulatum, Trichoderma hamatum* (Krutova, 1987), *Sporidesmium sclerotiorum* (Mischke et al., 1995), *Streptomyces* spp. (Aksay et al., 1991), *Talaromyces flavus* (McLaren et al., 1996), *Trichoderma harzianum* (Inbar et al., 1996), *P. putida, P. fluorescens* (Expert and Digat, 1995), *Bacillus licheniformis* (Sun et al., 2007), *B. cepecia* (Odejijono and Dragar, 1993), *Bacillus amyloliquefaciens* (Fernando et al., 2007), *Ulocladium atrum*, *Ulocladium atrum* (Huang and Erickson, 2007) *Bacillus lenthimorbus, Enterobacter pyrinus, Stenotrophomonas maltophila, Staphylococcus cohnii-cohnii* (Tozlu and Demirci, 2003).

Further studies on promising biocontrol organisms should be conducted to develop a commercial biocontrol agent against *S. sclerotiorum.* Detailed studies on promising bicontrol agents will result in development of a more environmental friendly control strategy against the fungus.

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REFERENCES

Abawi GS, Polach FJ, Molin WT (1975). Infection of bean by ascospores of *Whetzelinia sclerotiorum*. Phytopathology, 65: 673-678.

Abawi GS, Grogan RG (1979). Epidemiology of diseases caused by Sclerotinia species. Phytopathology, 69: 899-904.

Adams PB, Ayers WA (1979). Ecology of *Sclerotinia* species. Phytopathology, 69: 896-899.

Agrios GN (1997). Plant Pathology. Academic Pres, California, p. 635.

Aksay A, Biçici M, Cinar O (1991). Determination of antagonist against white mold pathogen *Sclerotinia sclerotiorum* (Lib) De Bary. J. Çukurova Üniversit Agric. Faculty, 6(2): 55-62.

Boland GJ, Hall R (1994). Index of plant hosts of *Sclerotinia sclerotiorum*. Can. J. Plant Patholol. 16: 93-108.

Cook RJ, Baker KF (1983). The nature and practice of biological control of plant pathogens. American Phytopathological Society, Press. St. Paul, MN. p. 230.

Expert JM, Digat B (1995). Biocontrol of *Sclerotinia* wilt of sunflower by *Pseudomonas fluorescens* and *Pseudomonas putida* strains. Can. J. Microbiol. 41: 685-691.

Fernando WGD, Nakkeeran S, Zhang Y, Savchuk S (2007). Biological control of *Sclerotinia sclerotiorum* (Lib.) De Bary by *Pseudomonas* and *Bacillus* on canola petals. Crop Prot. 26(2): 100-107.

Ferreira SA, Boley RA (2002). *Sclerotinia sclerotiorum.* (online) Available via DIALOG. http://www.extento.hawaii.edu/kbase/Crop/Type/s_scler.htm. Cited 15 May 2010.

Gu BG (1996). Production and uses of biopesticides in China. In: Tang WH, Cook RJ, Rovira A (Eds.). Advances in Biological Control of Plant Diseases, China Agricultural University Press, Beijing, pp. 332-334.

Hannusch DT, Boland GJ (1995). Influence of air temperature and relative humidity on biological control of white mold of bean (*Sclerotinia sclerotiorum*). Phytopathology, 86: 156-162.

Huang HC, Kokko EGI, Yanke J, Phillippe RC (1993). Bacterial suppression of basal pod rot and end rot of dry peas caused by *Sclerotinia sclerotiorum*. Can. J. Microbiol. 39: 227-233.

Huang HC, Bremer E, Hynes RK, Erickson RS (2000). Foliar application of fungal biocontrol agents for the control of white mold of Dry Bean caused by *Sclerotinia sclerotiorum.* Biol. Control, 18(3): 270-276.

Huang H, Erickson RS (2007). Biological control of Sclerotinia stem rot of canola using *Ulocladium atrum*. Plant Pathol. Bull. 16: 55-59.

Inbar J, Menendez A, Chet I (1996). Hyphal interaction between *Trichoderma harzianum* and *Sclerotinia sclerotiorum* and its role in biological control. Soil Biol. Biochem. 28: 757-763.

Kamensky M, Ovadis M, Chet I, Chernin L (2003). Soil-borne strain IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. Soil Biol. Biochem. 35: 323-331.

Köhl J, Fokkema NJ (1998). Strategies for biological control of necrotrophic fungal foliar pathogens. In: Boland GJ, Kuykendall LD (Ed). Plant-Microbe Interactions and Biological Control, Marcel Dekker, New York, pp. 49-88.

Krutova NP (1987). Mycoparasites of Sclerotia of causal agent of Sunflower white rot. Mikologia Fitopatologia, 21: 168-171.

Mclaren DL, Huang HC, Rimmer SR (1996). Control of apothecial production of *Sclerotinia sclerotiorum* by *Coniothyrum minitans* and *Talaromyces flavus*. Plant Dis. 80(12): 1373-1378.

Mcloughlin TJ, Quinn JP, Bettermann A, Bookland R (1992). *Pseudomonas cepacia* suppression of Sunflower wilt fungus and role of antifungal compounds in controlling the disease. Am. Soc. Microbiol., Appl. Environ. Microbiol. 58: 1760-1763.

Mischke S, Mischke CF, Adams PB (1995). A rind-associatiated factor from sclerotia of *Sclerotinia minor* stimulates germination of a mycoparasite. Mycol. Res. 99(9): 1063-1070.

Nelson B, Duval D, Wu H (1988). An *in vitro* technique for large-scale production of Sclerotia of *Sclerotinia sclerotiorum.* Phytopathology, 78: 1470-1472.

Odejijono MAL, Dragar C (1993). Isolation of bacteria antagonistic to a range of plant pathogenic fungi, Soil Biol. Biochem. 25: 247-250.

Onaran A, Yanar Y (2009). Study on the Identification, Distribution, Mycelial Compatibility Groups Pathogenicity and Biological Control of *Sclerotinia sclerotiorum* (Lib.) De Bary (White Mold Disease of Greenhouse Grown Cucumber) in Antalya Province. Ph.D. Theases. Gaziosmanpasa University, Graduate School, Tokat-Turkey. p. 250.

Paulitz TC, Belanger RR (2001). Biological control in greenhouse systems. Annu. Rev. Phytopathol. 39: 103-133.

Steadman JR (1974). Survival of sclerotia of *Whetzelinia* (*Sclerotinia*) *sclerotiorum* in Western Nebraska. Annu. Rep. Bean Improvement Cooperative, 17: 83-84.

Sun QI, Chen XJ, Tong YH (2007). Inhibition of antifungal protein produced by *Bacillus licheniformis* W10 to *Sclerotinia sclerotiorum* and control of rape stem rot by the protein. Editorial Department J. Yangzhou Univ. 28(3): 82-86.

Tozlu E, Demirci E (2003). Identification, distribution, pathogenicity and biological control of sunflower stem rot disease caused by *Sclerotinia*

sclerotiorum (Lib.) De Bary and *Sclerotinia minor* Jagger in Pasinler valli Ph.D. theases, Ataturk University. Graduate School, Erzurum, p. 96.

Tuncer FE, Damdere H (1997). Study on the biological control of white mold (*Sclerotinia sclerotiorum* (Lib.) De Bary) disease of greenhouse vegetable in Antalya. (http://www.tagem.gov.tr/projeler/97/bsag/bsagl8.html.)

Yuen GY, Craig ML, Kerr ED, Steadman JR (1994). Influences of antagonist population levels, blossom development stage, and canopy temperature on the inhibition of *Sclerotinia sclerotiorum* on dry edible bean by *Erwinia herbicola*. Phytopathology, 84: 495-501.

Zazzerini A, Tosi L, Rossi S (1987). Antagonistic effect of *Bacillus spp*. on *Sclerotinia sclerotiorum* sclerotia. Phytopatholol. Mediterranean, 26: 185-187.