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Biological Control on Rhizoctonia Blight of Turfgrasses in Golf Courses

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골프장 잔디의 Rhizoctonia 마름병에 대한 생물학적 방제

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ABSTRACT : This study was undertaken to find a new formulation of soil amendment, and selection of antogonists and to effectively control brown and large patch of turfgrasses caused by *Rhizoctonia solani* AG1-1 and AG 2-2. Fourteen inorganic chemicals (1%, w/w) were added individually *in vitro*, and some chemicals showed suppressiveness to *R. solani*. Alum suppressed effectively mycelial growth of *R. solani* in the range of 17 to 77% as compared with control. The four chemicals such as $Al_2(SO_4)_3$, alum, CaO, and NH_4NO_3 were finally selected. Out of three organic compounds, composted pine bark (CPB) showed prominent suppressive effect as compared with milled alfalfa and pine leaves. After inoculation of *R. solani* isolates AG1-1 and AG2-2 on the turf seedlings, water soaked lesions and blight symptoms were developed on the whole seedlings. According to inhibition zone method, mycelial growth of the fungus were greatly suppressed by culture filterates of the antagonists, *Gliocladium virens* (Gl-1) and *Pseudomonas* sp. (P713). CPB soil amendment mixed with antagonists (1% w/w) controlled not only brown and large patch of turfgrasses, but also promote the good growth of the seedlings. In addition, the controlling effect was maintained more than 30 days. Especially, the controlling effect of two antagonists was similar to CPB soil amendment with the antagonists and also stimulated a favorable growth of the seedings. Therefore, it is expected that continuous control of Rhizoctonia blight of turfgrasses can be obtained in field by subsequent applications of the antagonists.

Key words: **soil amendment, microbial product, brown patch, large patch, turfgrass, biological control, *Rhizoctonia solani*.**

Since turfgrasses were grown on golf courses, park and garden, they could be harmed directly by various pesticides, mechanical and physical stresses such as uneven grass cut and golfers. Thus these stresses predisposed them to plant diseases. To maintain healthy turfs, chemical applications and proper agronomical practices have been intensively carried out. After all, the contamination of agroecosystem has been resulted in a serious problem in recent years (1, 2, 3, 5, 19).

Turf is considered as a perennial crop, and remains on a site for generations. Among several fungal soilborne diseases, brown patch caused by *Rhizoctonia solani* (AG 1-1, AG 2-2) were reported in Korea (5). Furthermore, the root disease is known to as one of many factors unsuitable for maintaining healthy turfs (4, 7, 9). Therefore, it is necessary to find a environmentally compatible control method. One of the methods, namely biological control is an alternative method to reduce environmental contamination and to control brown patch effectively. This approach is also an essential method for maintaining sustainable agriculture without any serious yield losses.

In 1926, Sanford (22) attempted to control potato scab by applying green manure in some soils. Since 1960, studies on biological control including soil amendment have been intensively carried out throughout the world (1, 7, 21). Most of the microbial applications were made by treating either on seeds or roots. Although many biocontrol researches on root diseases of horticultural plants including Phytophthora blight of red pepper (2, 3, 5) were successfully carried out in this country, there are still a few difficulties for applying microbial agents in field levels to control soilborne diseases mainly due to inconsistent results.

Thus, to find effectively formulated soil amendment for applying in field conditions is urgently needed to overcome inconsistency of biocontrol. S-H mixture as an organic amendment reduced the incidence of wilt

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and yellows caused by *Fusarium* spp. (24). Avocardo disease incited by *Phytophthora cinamomi* was fairly suppressed by treatment of composted pine bark mixture (9, 10, 11). In addition, Nelson *et al.* (17) reported the active role of antagonists in the suppression of *Rhizoctonia solani* with composted hardwood bark.

Since 1990, biocontrol studies on soil-borne diseases of horticultural plants have been intensively carried out in plant pathology laboratory of Chungbuk National University. Therefore, this study was focused on screening inorganic and organic materials, and effective antagonistic microorganisms, and soil amendment mixed with the antagonists to control brown and large patch of turfgrasses caused by *Rhizoctonia solani* AG 1-1 and AG 2-2.

MATERIALS AND METHODS

Preparation of inoculum. *Rhizoctonia solani* AG 1-1 and AG 2-2, the causal pathogens of brown and large patch of turfgrasses, were obtained from the Korea Turfgrass Research Institute and maintained in potato dextrose agar (PDA) for further use. After the causal fungi were cultured in wheat bran solid medium [wheat bran 15 g, cornmeal 3 g, sand 82 g and moisture 12% (v/w)] for two weeks at 28°C, the inoculum was used for soil inoculation. Seedlings of bent grass (*Agrostis palustris* Huds.) and zoysia grass (*Zoysia japonica* Steud.) were obtained from the nursery plot of Cheongju country club, and then transplanted to plastic pots (20 cm diameter) contained with soil. After the fungal inoculua were evenly inoculated in pot soil which were already planted with the seedlings, pots were kept in inoculation chamber (28°C, 100% RH) for 48 hrs. Readings for disease symptoms were made 10 days after inoculation.

Isolation, screening and identification of antagonistic microorganisms

• *Isolation of antagonist*: 200 rhizosphere soil samples were collected from upland areas across the Chungbuk province, and then soil microorganisms were isolated by soil dilution plate method. Antagonistic microorganisms were screened by triple layers agar and dual culture methods. Finally, *Gliocladium* sp. (Gl-1) and *Pseudomonas* sp. (P 713) were isolated as antagonistic microorganisms.

Studies on mycological characteristics and microscopic observation of the *Gliocladium* sp. (Gl-1) were made 7 days after inoculation of the fungus on PDA (8).

• *Antagonistic activity from microorganisms*: Antagonistic activity was measured in artificial medium. After incubating *Gliocladium* sp. (Gl-1) and *Pseudomonas* sp. (P 713) in nutrient broth, culture filtrates were centrifuged with 3000 rpm, and supernatent was obtained. Suppression of sclerotial germination and lysis in the supernatent of culture filtrate were observed in water agar. Growth of the *R. solani* in the supernatent of culture filtrate was compared with the control.

Evaluation of inorganic and organic amendments. The 14 chemicals used for the experiment were $Al_2(SO_4)_3$, Alum ($Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_20$), $CaCl_2$, $CaCO_3$, $Ca(NO_3)_2$, CaO, glycerine (10%), K_2HPO_4, KCl, K_2SO_4, NH_4NO_3, urea, TSP (Triple super phosphate), $MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$. The test for effectiveness of 14 inorganic chemicals to control Rhizoctonia blight of turf grasses were followed by the the method developed by Huang and Kuhlman (13, 14), The control was not supplemented with an amendment. Composted pine bark (CPB), milled pine leaves and milled alfalfa leaves were used as organic amendment.

Effect of the amendments on biocontrol of *R. solani* AG 1-1 and AG 2-2 was tested in the chemical solutions. Solutions were prepared by dissolving each chemical (1%, w/w) in deionized water. Deionized water was used as a control. Sclerotia of the causal fungus (AG1-1) were obtained after incubation of 10 days on PDA, and placed in the drop of chemical solution for 30 minutes. Sclerotial germination was estimated by direct observation after incubating sclerotia on water agar for 24 hrs at 28°C. Suppression rate of sclerotial germination was measured by counting randomly 100 sclerotia in each chemical solution.

Soil extract media were prepared by placing 1 m*l* of soil extract in 10 m*l* of water agar after shaking 10 g of sterilized soil in 30 m*l* of each chemical 1% (w/v) for 30 min (180 g/min). Then the mycelial growth was estimated by measuring mycelial growth 5 days after inoculation with 9 replicates. the control was not added with soil extract.

To compare the suppressive effect of $Al_2(SO_4)_3$, CaO and Alum in the agar with the rate of 0.5, 1.0, 1.5 and 2.0% (w/w), the each chemical was added separately to water agar, and then inoculated with *R. solani* AG1-1 and AG2-2.

To compare the suppressiveness of chemicals (1%, w/w) to the pathogens in the soil, each chemical was added separately to sterilized soil, and then inoculated with *R. solani* AG1-1 and AG2-2. After one week of

inoculation, survived inoculum was evaluated by the stem colonization method. Colonization of turfgrass root by the pathogen was observed. The inoculum (1%, w/w) and each of the chemical (1%, w/w) were mixed in sterilized pot soil (10 cm diameter) and then kept for 7 days. Twenty stems (2 cm length) were buried in the pot soil with 12% moisture content, and the pots were kept in inoculation chamber for 14 days at 27°C. Then, turfgrass stems were placed on water agar amended with 1 m*l* of streptomycin (100 unit) for the observation of stem colonization.

Effect of organic compounds. The organic materials used in the study were CPB, milled pine leaves and milled alfalfa leaves. Pine bark was composted under natural outdoor conditions for one year and alfalfa leaves which imported from the United States of America as a forage.

To find a suppressiveness of the organic materials to the fungi, each organic material was boiled in 30 m*l* of deionized water for 30 minutes, and then 1 m*l* of 1% (w/v) extract solution was separately mixed in water agar with 5 replicates. The pathogens were placed in the center of each medium and incubated for 5 days, and then suppression of mycelial growth was examined.

Controlling effect of soil amendment mixed with the antagonists. The seedlings of bent and zoysia grasses were obtained from Cheongju country club. For the inoculation of the pots, the causal agents, *R. solani* AG 1-1 and AG 2-2 were cultured on wheat bran sand medium for 10 days. The antagonist, *Gliocladium virens* (Gl-1) was also cultured on wheat bran sand medium for 10 days. *Pseudomonas* sp. (P 713) was cultured on nutrient broth for 2 days and then diluted to 10×10^5 cfu/m*l* with sterilized distilled water. After the bacterial suspension was coated on vermiculite, the vermiculite was dried under indoor conditions.

In a pot test (15 cm diameter × 20 (H), clay loam soil plus sand (1:1 (v/v)) was sterilized. The causal fungi were inoculated on crown part of seedling in a pot soil (1%, w/w). 750 g of CPB were supplemented with the four inorganic chemicals such as $Al_2(SO_4)_3$ (150 g), CaO (40 g), NH_4NO_3 (35 g), Alum (25 g) followed by the formulation SF 21 (3). One percent (w/w) of the CPB was mixed separately with sterilized soil.

The antagonist, *Gliocladium virens* (Gl-1) was cultured on wheat bran medium for 2 weeks, and the *Pseudomonas* sp. (P 713) was cultured on nutrient broth for 4 days and the *Pseudomonas* suspension (10×10^5 cfu/m*l*) was inoculated on vermiculite and air dried. The inoculum of the antagonists was mixed with surface soils, and rate of concentration was 1% (w/w). The inoculated seedlings were kept in inoculation chamber for 48 hrs. Seedlinges obtained from Cheongju country club were transplanted in pot (15 cm diam.× 10 (H)) and each treatment was carried out with 10 replicates.

Details of treatments were as follows. CPB plus the two antagonists, CPB plus *Gliocladium virens* (Gl-1), CPB plus *Pseudomonas* sp. (P713), only two antagonists without amendment, and the pathogen without amendment as control. Seedlings of turfgrasses were grown in standard cultural practices, and after inoculation of the pathogens to the pot soil, blight symptom development was examined everyday up to 5 weeks. Controlling effect was confirmed by disease index. Disease index was based on the following formula;

$$\text{Disease index} = \frac{2\times B+3\times C+5\times D+8\times E+10\times F}{\text{Total culums}}$$

A (0) : Healthy plants; B (2) : Common growing conditions and a little partial blight; C (3) : Partial leaf blight with less than 10%; D (5) : Intermediate blight with 20 to 50%. E (8) : Heavy blight with 50 to 80%; F (10) : Very severe blight with 90 to 100%.

RESULTS

Pathogenicity test. The isolates obtained from the Korea Turfgrass Research Institute were used to test their pathogenicity on seedlings of bent and zoysia grasses. *R. solani* AG 1-1 infected the bent grass, resulting grayish brown blight symptom, and later leaves were killed in the blighted patches with profuse mycelium. On zoysiagrass, *R. solani* AG 2-2 showed typical yellowish brown discoloration and sunken lesions on leaves because of the affected leaves on the soil or the thatch surface. Symptom development by *R. solani* AG 2-2 on bent grass was relatively slower than *R. solani* AG 1-1 about two weeks (Table 1).

Table 1. Pathogenicity of *Rhizoctonia solani* AG 1-1 and AG 2-2 against bent and zoysia grasses

Anastomosis group	Pathogenicity	
	Bent grass	Zoysia grass
Rhizoctonia solani AG 1-1	+++[a]	–[b]
Rhizoctonia solani AG 2-2	–	+++
Control	–	–

[a] +++ : Servere blight symptom
[b] – : No blight symptom was developed

Identification of *Gliocladium* sp. (Gl-1). Mycological characteristics of the antagonist were examined after culturing the fungus on PDA for 7 days. The mycelial growth of the isolate (Gl-1) was almostly coincided with the description made by Domsch in 1980 (8) as listed in Table 3. Thus, *Gliocladium* sp. (Gl-1) was identified as *G. virens*.

Antagonistic ability of the *Gliocladium virens* (Gl-1) to the pathogens. According to triple layers agar method and dual culture method, both *Gliocladium virens* (Gl-1) and *Pseudomonas* sp. (P 713) were finally selected. In dual culture, *Gliocladium virens* (Gl-1) was not only strongly antagonistic to the casual fungi, but also showed hyperparasitic ability by coiling into the mycelium of the pathogen (Photo is not presented). The antagonistic bacterium *Pseudomonas* sp. (P 713) formed inhibition zones of 19 and 31 mm on dual culture medium and 43 and 45 mm of inhibition zones were formed with *Gliocladium virens* (Fig. 1). Dry weight of the fungi treated in extract solution of the antagonists was nearly negligible as compared to the control (Table 2).

Effect of inorganic chemicals on the sclerotial germination and fungal growth. Among the 14 chemicals tested, $Al_2(SO_4)_3$, Alum, $CaCO_3$, CaO, glycerine, K_2HPO_4, KCl, and NH_4NO_3 greatly inhibited sclerotial germination of *R. solani* AG 1-1 (Table 4), although inhibition of mycelial growth was not the same trend. The remaining chemicals did not suppressed sclerotial germination and mycelial growth. Glycerine, K_2HPO_4, KCl and TSP could suppress the sclerotial germination only.

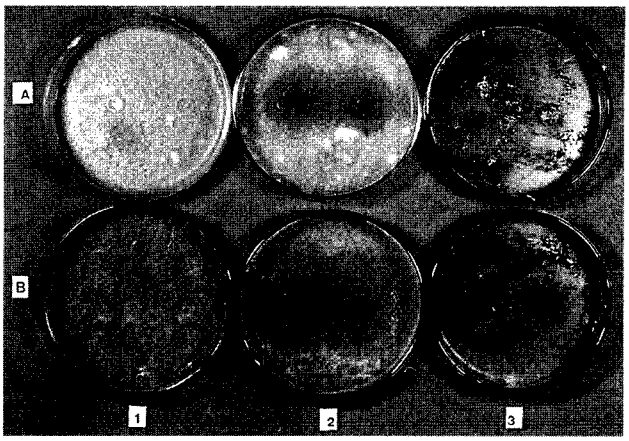

Fig. 1. Inhibition zones produced by the antagonists, *Pseudomonas* sp. (P 713) and *Gliocladium virens* (Gl-1) against plant pathogenic fungus *R. solani* AG 1-1 (A) and AG 2-2 (B). 1; Control, 2; *Pseudomonas* sp.(P 713), 3; *Gliocladium virens* (Gl-1).

The suppression rate of $Al_2(SO_4)_3$, Alum and CaO was higher than 60% at different concentrations of chemicals (0.5, 1.0, 1.5 and 2.0%). In the case of $Al_2(SO_4)_3$, suppression of mycelial growth were in the range of 61.2~86.1% regardless of strains of the fungi, whereas Alum and CaO were in the range of 44.6~92.8 % for mycelial suppression except treating with 0.5% (Table 5).

Effect of inorganic chemicals on the stem segment colonization. After the 14 chemicals (1%, w/w) were individually added to the pot soil infested with the fungi, colonization of stem segment was examined. Stem-segment colonization of the 6 chemicals including $Al_2(SO_4)_3$ was reduced more than 35.0% in comparison with the control. Especially, colonization by *R. solani* treated with $CaCl_2$ resulted in 10 to 30% suppression (Table 6). The other chemicals such as urea showed a little inhibition with 5%.

Table 2. Antagonistic activity of the *Gliocladium* sp. (Gl-1) and *Pseudomonas* sp. (P713) to *Rhizoctonia solani* AG 1-1 and AG 2-2 causing brown and large patch of turfgrass

Antagonist	Mycelial inhibition[a]		Mycelial growth (g dw/50 ml)[b]	
	AG 1-1	AG 2-2	AG 1-1	AG 2-2
Gliocladium virens (Gl-1)	43	45	0.21	0.24
Pseudomonas sp. (P 713)	19	31	0.08	0.05
Untreated	0	0	0.50	0.83

[a]: Inhibition diameter was obtained from dual culture method with 5 replications. Observations were made 3 day after inoculation on PDA at 28°C.

[b]: Dry weight measurement was made 7 days after inoculation of the fungus on extract media of the antagonists *Pseudomonas* sp. (P 713) grown on PDB. and NB. at 28°C.

Table 3. Mycological characteristics of the antagonistic fungus, *Gliocladium* sp. (Gl-1)

Mycological characteristics	Etiological comparison	
	Gliocladium sp. (Gl-1)	*Gliocladium virens* (Domsch, K. H. 1984)
Mycelial growth and color	Colonies very fast growing, 7 cm in 7 days; dark green	Very fast growth, dark green
Conidia	5.0-5.5×4 μm, Ellipsoidal smooth walled conidia	4.5-6×3.5 μm, Ellipsoidal smooth walled conidia
Phialides	Phialides appressed bearing one large drop of green conidia on a whorl	One large drop of green conidia on each whorl

Table 4. Effect of various inorganic chemicals on mycelial growth and sclerotial germination of *Rhizoctonia solani*

Chemicals[w]	Suppression percent (%)			
	Mycelial growth		Sclerotial germination	
	AG 1-1[x]	AG 2-2[y]	AG 1-1	AG 2-2
$Al_2(SO_4)_3$	77.0(1.5) j[z]	69.1(1.4) g	70(30) e	–
Alum	67.7(2.1) g	56.6(2.0) f	30(70) c	–
$CaCl_2$	0.0(7.4) de	6.6(4.3) c	0(100) a	–
$CaCO_3$	0.0(8.2) b	21.8(3.6) d	30(70) c	–
$Ca(NO_3)_2$	0.0(8.7) a	0.0(4.6) ab	0(100) a	–
CaO	17.0(5.4) h	34.8(3.0) e	80(20) f	–
Glycerine	23.1(5.0) i	0.0(4.6) ab	80(20) f	–
K_2HPO_4	0.0(7.5) cd	10.9(4.1) c	70(30) e	–
KCl	0.0(6.9) f	0.0(4.7) ab	70(30) e	–
K_2SO_4	0.0(7.2) e	0.0(4.6) ab	0(100) a	–
NH_4NO_3	17.0(5.4) h	24.0(3.5) d	40(60) d	–
Urea	0.0(8.8) a	6.6(4.3) c	10(90) b	–
TSP	0.0(8.7) a	0.0(4.8) a	30(70) c	–
$MgCO_3 \cdot Mg(OH)_{25}H_2O$	0.0(7.7) c	6.6(4.3) c	10(90) b	–
Control	0.0(6.5) g	0.0(4.6) ab	0(100) a	–

Alum : $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$

TSP : Triple superphosphate

w : One %(w/v) of the chemicals was added to 10 g of sterilized soil and mixed with 30 m*l* of deionized water. Extract media were prepared by pouring 1 m*l* of extract to 10 m*l* of melted water agar.

x : Parenthesis number means mycelial diameter (mm), and measurement were made 3 days after treatment.

y : AG 1-1; Anastomosis group of brown patch, and AG 2-2; Anastomosis groups of *R. solani* on large patch of turfgrass.

z : Parenthesis number means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.

Table 5. Effect of different concentrations of $Al_2(SO_4)_3$, Alum, and CaO on mycelial growth of *Rhizoctonia solani* AG 1-1 and AG 2-2

Chemicals	Conc. (%)	Mycelial growth (mm)		Suppression rate (%)	
		AG 1-1	AG 2-2	AG 1-1	AG 2-2
$Al_2(SO_4)_3$,	0.5	35.5±8.39[a]	21.6±1.66[a]	61.2	82.1
	1.0	21.0±1.08	10.8±0.68	74.7	86.1
	1.5	14.6±0.22	17.7±3.09	82.5	77.1
	2.0	15.1±0.78	11.8±9.70	81.9	84.8
Alum	0.5	76.5±7.23	53.3±0.48	7.9	31.0
	1.0	28.7±2.89	37.6±1.19	65.5	51.3
	1.5	37.6±1.18	23.5±1.33	54.7	69.6
	2.0	24.9±0.69	20.0±0.78	70.4	74.1
CaO	0.5	76.5±7.23	46.6±1.28	7.9	39.7
	1.0	46.0±0.90	13.8±5.10	44.6	82.2
	1.5	17.4±5.35	7.9±1.13	79.1	89.8
	2.0	6.0	6.0	92.8	92.2
Control		83.0±3.49	77.2±0.56	0	0

a : Values are average of 5 replicates and standard error.

Alum : $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$

Table 6. Effect of various chemicals on turfgrass stem-segment colonization by *Rhizoctonia solani* AG 1-1 and AG 2-2

Chemicals[x]	Stem-segment colonization (%)[y]	
	AG 1-1	AG 2-2
$Al_2(SO_4)_3$	20(4) a[z]	15(3) bc
Alum	40(8) c	65(13) d
$CaCl_2$	30(6) b	10(2) a
$CaCO_3$	85(17) f	82.4(14) de
$Ca(NO_3)_2$	75(14) e	90(18) g
CaO	90(18) fg	75(15) ef
Glycerine	55(11) d	80(16) f
K_2HPO_4	75(15) e	90(18) g
KCl	75(14) e	50(10) c
K_2SO_4	85(17) f	90(18) g
NH_4NO_3	45(9) c	20(4) b
Urea	95(19) gh	100(20) h
TSP	40(8) c	45(9) c
$MgCO_3 \cdot Mg(OH)_{25}H_2O$	30(6) b	58.8(10)c
Control	100(20) h	100(20) h

x : One percent of chemicals (w/w) was added to the soil infested with 1% (w/w) of AG 1-1 and AG 2-2 of *Rhizoctonia solani.*

y : Twenty stems were treated with each chemical, and examinations were made one week after treatment. Parenthesis number means number of infected stems.

z : Parenthesis number means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.

Effect of organic compounds on the mycelial growth. 100 g of composted pine bark (CPB), milled pine leaves (MPL) and alfalfa leaves (MAL) were boiled for 30 minutes in 10,000 (w/v, 1%) in deionized water, and extract of each compound was prepared. Suppression of the mycelial growth in the extract medium was examined (Table 7). Out of three organic compounds, CPB suppressed the mycelial growth of *R. solani* AG 1-1 and AG 2-2 from 28.9 to 46.3%. These compounds also showed inhibitory effect against AG2-2 with the range of 14.9~46.3%.

Controlling effect of the antagonists and soil amendment against fungal pathogens. Formulated CPB amendment in sterilized soil infested with *R. solani* AG1-1 and AG 2-2 significantly reduced brown and large patch of turf grasses (Table 8). The CPB (1%, w/w) plus two antagonists was more effective than other treatments, although disease index of each treatment was slightly different. In addition, growing conditions of seedlings were as good as healthy seedlings except pathogen inoculation (Table 8).

Furthermore, CPB (1%, w/w) plus the antagonists either one or both, controlled almost completely Rhizoc-

Table 7. Effect of various organic compound extract media on mycelial growth of *Rhizoctonia solani* AG 1-1 and AG 2-2

Organic compound	Mycelial growth (mm)		Suppression rate (%)	
	R. solani AG 1-1	*R. solani* AG 2-2	*R. solani* AG 1-1	*R. solani* AG 2-2
Milled pine leaves	47.0 c[y]	43.5 b	0 a	28.1 c
Milled alfalfa leaves	60.0 d	51.5 c	0 a	14.9 b
Composted pine bark	32.0 a	32.5 a	28.9 b	46.3 d
Control	45.0 b	60.5 d	0 a	0 a

x : One percent concentration (w/v) of extract media was prepared from each of the organic compound after decoction for 30 minutes in distilled water. Observations were made 5 days after the causal fungi were plated out on the extract medium and values are average of 5 replications.

y : Means within a column followed by the same letter are not significantly differant (p=0.05) according to Duncan's multiple range test.

Table 8. Controlling effect of the antagonists and soil amendment against *Rhizoctonia solani* AG 1-1 and AG 2-2

Treatment	Growing conditions of grass[a]	Disease index (%)	
		Bentgrass (AG 1-1)	Zoysiagrass (AG 2-2)
Two antagonists[b]+ CPB (1%, w/w)	+++	0.0	0.2
Gliocladium virens (Gl-1)+CPB (1%, w/w)	+++	0.4	0.6
Pseudomonas sp.+ CPB (1%, w/w)	++	0.2	1.4
Two antagonists	++	0.2	0.4
	−	8.0	7.0

[a] +++; Grasses with good growth. ++; Grasses with common growth. − ; Grasses with poor growth and blight symptom.

[b] Two antagonists; *Gliocladium virens* (Gl-1) and *Pseudomonas* sp. (P 713) was used. Readings were made 30 days after treatment.

tonia patches of turfgrasses compared with control 8.0 in bent grass and 7.0 in zoysia grass. With regard to blight development, readings on disease were carried out 30 days after inoculation. Although seedlings in the CPB plus *Pseudomonas* sp. (P 713) and treatments of two antagonists were slightly less vigorous than the other pots, seedlings of turfgrasses were all healthy.

With regard to population densities of the antagonists associated with the CPB soil amendment, population densities of the bacterium (P 713) and *Gliocladium virens* (Gl-1) were not significantly affected regardless of soil conditions (data not shown). In addition, wheat bran and vermiculite were known as good additives because of controlling effect and long viability of the antagonists. Such formulation of the antagonists can be applied to field as subsequent application.

DISCUSSION

Out of 14 chemicals, four inorganic chemicals including aluminum sulfate were significantly suppressed the disease in the range of 17~77% (Table 4). These results were similar to the report on SF-21 fomulated by Huang *et al.* (1991). SF-21 includes two major ingredients, namely pine bark and aluminum sulfate. Hoitink reported in 1985 that composted pine bark suppressed Phytophthora root rot successfully. The other successful case on *F. oxysporum* f. sp. *vasinfectum* using a soil amendment was also reported by Chung *et al.* (2). According to stem segment colonization method, effect of each inorganic chemical can be easily examined at indoor conditions rather than the field. However, further modification on the method is needed. In the case of *R. solani* strains, one percent (w/w) of each inorganic chemical appeared to be not enough to suppress colonization of the fungus because of rapidly growing mycelia (Table 6).

Since controlling effect of soil amendment against soil-borne pathogens is known to be caused by mutual factors such as biological and chemical factors. Hoitink (11) pointed out that composted pine bark suppressed soil-borne plant pathogenic fungi and to promote activity of indigenous thermophilic bacterial and fungal antagonists such as *Trichoderma* and *Gliocladium* spp.. Additionally, tannin, resin, saponin, starch, other carbohydrates and ethylester were contained in pine bark were toxic and fungistatic to the pathogens (13).

The inhibition of sclerotial germination were also caused by abiotic factors in the extract solution of composted pine bark (Table 4). According to the report by Davey *et al.* (7, 20), the suppression of *R. solani* in soil by composted organic amendment and supplementary nitrogen was related to the general microbial activity in the soil.

Aluminum sulfate, an acidifying agent, was used for controlling damping off of pine seedlings caused by soil-borne fungi due to low soil pH (13, 14). In addition, exchangeable Al^{+3} ion retarded the development of *P. capsici* in soil as described in the report by Muchovej *et al.* (16). Naturally occurring soil aluminum reduced pathogenesis of sunflower pathogens such as *Verticillium albe-atrum Whethzelinia sclerotiorrum* (18). In this study, both mycelial growth and sclerotial germination of *R. solani* were singificantly reduced by sup-

plementation of $Al_2(SO_4)_3$, Alum and CaO (Table 4).

Since Howell (12) demonstrated that *Gliocladium virens* which producing viridin and gliotoxin could promote the biocontrol of damping off of cotton seedlings caused by *Rhizoctonia* sp.. Even though we did not try to find the antibiotic prduction from antagonistic *Gliocladium virens* (G1-1), this fungus inhibited mycelial growth of the *R. solani* greatly. Therefore, further study on antibiotc production by *Gliocladium virens* (G1-1) should be needed in the near future.

The indigenous *Pseudomonas* sp. (P713) also suppressed brown and large patch of turfs. The results were also similar to the report by Jeong *et al.* published in 1993 (15). Their results showed that compatibility and synergistic effects of *G. virens* and *Pseudomonas putida* were higher to compete with Fusarium wilt fungus of cucumber.

Probably, different mechanisms of suppressiveness explain the CPB amendment for controlling brown and large patch of turfgrasses. However, our results could be interpreted as a combined factor effects. We have shown that a formulated CPB amendment plus the antagonists completely controlled patch diseases of turfs (Table 8). In addition, our results could be applied in the field conditions because seedlings were directly transplanted in pot after being transferred from the nursery of Cheongju country club.

In the case of two antogonists, controlling effect was very similar to CPB plus the antagonists except seedling vigor. This result strongly suggested that application of antagonist could control the fungal disease effectively in field for an appropriate interval but could not promote the plant vigor effectively.

The application of biocontrol agents with the formulation, will require further research efforts to find out how widely this treatment is applicable in different soil-borne plant pathogens and how economically this control method is feasible.

요 약

잔디의 Rhizoctonia 마름병에 효과적인 새로운 토양 첨가제의 조제, 길항균 선발 및 방제효과를 구명코저 본 연구를 1995년부터 1996년까지 지난 2년간 수행하였다. 황산 알미늄등 14종의 무기성분(1%, w/w)을 성분별로 검정하여 $Al_2(SO_4)_3$ 포함하여 최종 4종의 무기물이 선발되었고 무처리에 비하여 17~77%의 균사신장 억제 효과가 확인되었다. 부숙 소나무수피 등 3종의 유기물중 부숙 소나무 수피는 알팔파 잎가루나 소나무 잎가루에 비하여 균사신장억제 효과가 우수 하였다. *Rhizoctonia solani* AG1-1와 AG2-2의 서양잔디와 한국잔디에서의 병원성은 수침상 병반이 확대되면서 잔디전주가 갈변고사 하였다. 분리한 길항균 *Gliocladium* sp.(Gl-1)과 *Pseudomonas* sp.(P 713)을 저지원법과 배양여액에서의 균사저지 상황 등 대조구와 비교하였을때 그 길항력의 우수함이 확인되었고 *Gliocladium* sp.(Gl-1)는 배양기상에서의 균사 생장, 균총색, 포자 및 소병의 형태를 문헌과 비교 검토한 후 *G. virens*로 동정 하였다. 잔디에 토양 첨가제(1%, w/w)와 두종의 길항균의 처리에 의한 방제효과는 묘생육도 양호할 뿐만 아니라 무처리에 비교하여 거의 완전 방제 경향을 나타내었고 그 효과는 30일 이상 지속되었다. 특히 2가지 길항균만의 접종구도 토양 첨가제 및 길항균 처리구와 그 효과가 유사할뿐 만아니라 잔디 생육도 양호하였으므로 포장에서의 잔디 후기산포제로서 활용할 경우 지속적인 방제효과의 유지가 가능할 것으로 크게 기대된다.

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