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Biocontrol Effect of *Gliocladium virens* G1 and Soil Amendment on Astragal Stem Rot Caused by *Rhizoctonia solani*

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In order to find an environment-friendly method to suppress astragal stem rot caused by the isolates of *Rhizoctonia solani* AG 1 and AG 4, we tested an antagonistic fungus *Gliocladium virens* G1 was evaluated as a biocontrol agent and estimated inorganic compounds and organic materials were tested for their effect of the disease suppression. *G. virens* G1 effectively inhibited mycelial growth in a dual culture and caused mycelial lysis in the culture filtrate. No adverse effect was observed when examined for seed germination and seedling growth. Promoted seedling growth was observed with the seed treatment. Seeds of astragal plant were germinated higher in the sterile soil than the natural soil. Of 14 inorganics tested, alum, aluminum sulfate and calcium oxide significantly suppressed the mycelial growth and sclerotial germination. Milled pine bark and oak sawdust also suppressed the mycelial growth. Soil amended with 1% of *G. virens* G1 composted with pine bark (w/v) significantly controlled astragal stem rot in the glasshouse experiments.

KEYWORDS: Astragal stem rot, Biological control, *Rhizoctonia solani*, Soil amendment

Astragal plant (*Astragalus membranaceus*) has been used for medicinal purpose to robustness, urination, antibiosis and protection of liver and kidney (You, 1988). This cash crop has been intensively cultivated throughout the whole country. In 1994, there was approximately 443 ha of astragal plant cultivated in the alpine Chungbuk province (Yun, 1994). Two soilborne diseases, Fusarium wilt caused by *Fusarium oxysporum* and Rhizoctonia stem rot caused by *Rhizoctonia solani*, are becoming the major limiting factors in production. In 1996, 16.4% of root diseases were reported from a field survey conducted by Chungbuk provincial office of agricultural technology, Korea (Yun, 1996). *R. solani* infects the plant immediately after seedling emergence, and the typical stem rot develops mainly during the rainy summer season in Korea (Yun, 1994). Although cultural practices and fungicide applications have been mainly practiced to control the disease, they have not been often successful in disease control. Excessive fungicide applications may partially contaminate the agroecosystem.

Biological control of soilborne diseases with introduced microorganisms has been successfully practiced in many crops (Cook and Baker, 1983). Furthermore, soilborne diseases may also be effectively controlled by soil amendment. In 1926, Sanford attempted to control potato scab by applying green manure composted with *Trichoderma* species. Thereafter, intensive studies on soil amendment have been conducted. An avocado Phytophthora root rot caused by *Phytophthora cinamomi* was fairly suppressed by using a composted pine bark mixture (Hoitink, 1986). Development of an effective soil amendment applicable to natural field conditions is urgently needed for environment-friendly control of astragal stem rot.

This study was conducted to find an optimum formulation of soil amendment by 1) evaluating the antagonist *G. virens* as a biocontrol agent against astragal stem rot, 2) screening inorganic compounds, 3) screening organic materials and 4) evaluating *G. virens* composted with inorganic compounds and inorganic materials to control astragal stem rot.

Materials and Methods

Isolation of causal fungus and pathogenicity. Pathogens were directly isolated from diseased plants and used in the study. Diseased astragal plants were collected from Jechon and Danyang, Korea in 1997. Infected roots and stems were surface-disinfected by imbibing for 3 min with 0.5% solution of sodium hypochloride. The samples were then plated on 2% of water agar (WA) and incubated at 27°C for three days. The extending mycelial tips were isolated, and transferred on potato dextrose agar (PDA) slant, incubated at 27°C for 5 days, and stored at 5°C for long term storage. *R. solani* isolates were tentatively grouped into R-1 and R-2 based on cultural characteristics such as growth rate and sclerotial formation on PDA after incubation at 27°C for 5 days. R1 isolates grew faster than R2 isolates. Furthermore, R1 isolates produced profuse sclerotia on PDA, wheras R2 isolates did not. Anastomosis grouping was conducted with the type cultures kindly obtained from National Institute of Agricultural Science and Technology, Suwon, Korea. (Kim *et al.*, 1995). All the isolates were grouped into either of

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AG 1 or AG 4. Two isolates of *R. solani* AG 1 and AG 4 were respectively tested for their pathogenicity by inoculating the fungus grown on potato-dextrose-rice-bran-agar (PDRBA) consisting of potato dextrose broth (100 m*l*), rice bran (30 g) and agar (17 g). Inoculum was prepared by mixing soil with mycelial suspension (mycelial suspension : soil = 2 : 3, v/w) in the pot (700 g soil, 20 cm in diameter, 25 cm in depth). The pots inoculated and transplanted with 30-day-old seedlings were kept in the glasshouse for disease development. Disease severity was assessed on the basis of symptom (percent of infected stems).

Antagonistic activity. *G. virens* G1, originally isolated from turfgrass (Chung and Chung, 1998) was used in the study. Antifungal activity was confirmed by the dual culture between *G. virens* G1 and isolates of *R. solani* based on inhibition of mycelial growth 3 days after incubation on PDA at 26°C. Mycelial contact point by the dual culture was microscopically observed for coiling, penetration and hyperparasitism. Suppression of sclerotial germination and mycelial lysis were determined by observing the growth on WA after soaking sclerotia and mycelia in the supernatant of broth culture of *G. virens* G1. Sclerotial germination and mycelial lysis were observed 5 days after incubation at 27°C. Each treatment was made with three replicates.

Seed germination with *G. virens* G1. Seeds of astragal were soaked in the suspension of *G. virens* G1 (1×10^6 conidia/m*l*) for 60 min, plated on WA and incubated at 27°C for 15 days. Seed germination was determined with germination ratio, height and weight of seedling. Each treatment was made with three replicates consisting of 20 seeds, respectively. These seeds were challenged by reinoculating *R. solani* at (20 mycelial fragments/ml). These seeds were sown in the test tube (1.4 × 17 cm) filled with vermiculite and incubated at 27°C for 15 days under diurnal conditions. Each treatment was made with five replicates consisting of seven seeds, respectively.

Antifungal activity of inorganic compounds and organic materials. Inorganic compounds tested were SF-21 (Huang and Kuhlman, 1991) and 14 chemicals which were aluminum sulfate [$Al_2(SO_4)_3$], alum [$Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$], calcium chloride ($CaCl_2$), calcium carbonate ($CaCO_3$), calcium nitrate [$Ca(NO_3)_2$], calcium oxide (CaO), glycerine, potassium phosphate (K_2HPO_4), potassium chloride (KCl), potassium sulfate (K_2SO_4), ammonium sulfate[$(NH_4)_2SO_4$], urea (H_2NCONH_2), triple superphosphate (T.S.P.), magnesium carbonate [$MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$]. Six organic materials tested were composted pine bark, sawdust of pine tree, oak tree, larch tree, rice bran and wheat bran. A bulk of milled pine bark naturally composted for longer than 6 months was used in this experiment. The organic materials were boiled in a beaker for 30 min and used to prepare the extract medium same as described for inorganic compounds. Extract solution was prepared by dissolving 0.3 g of each compound (1%, w/w) in 30 m*l* of distilled water and mixing with 10 g of sterilized soil. One milliliter of the extract solution was added to 10 m*l* of WA before solidification. Mycelial growth was estimated by measuring colony diameter on the extract medium at 5 days after incubation. Sclerotial germination was determined on the soil extract medium Every treatment was made with three replicates.

Control effect of *G. virens* G1. Autoclaved sterilizing and natural loam soil was used in this study. The seeds were soaked in the spore suspension (1×10^8 cells/m*l*) of *G. virens* G1 for 30 min and placed on Petri dish in which a moist filter paper is placed. *G. virens* was applied twice by drenching 50 m*l* of conidial suspension of *G. virens* G1 (1×10^6 cells/m*l*) onto the pots prepared as described previously in this study. Disease severity was determined 30 days after incubation in the glasshouse. Each treatment was made with three replicates consisting of 20 seeds per pot.

Biocontrol effect of *G. virens* G1 amended with CPM on stem rot. Antagonist with composted pine bark mixture (CPM), (1%, w/v), seedlings soaked in the conidial suspension of *G. virens* (1×10^6 conidia/m*l*) for 30 min, and antagonist drenched on the pot soil at 1.0×10^6 and 1.0×10^4 conidia/m*l*, respectively were examined. CPM consisted of 375 g of composted pine bark, 375 g of saw dust of oak tree, 150 g of aluminum sulfate, 25 g of alum, 20 g of calcium chloride, 30 g of calcium oxide, 30 g of urea, 30 g of triple superphosphate, 20 g of potassium chloride, and 750 m*l* of 10% glycerine. A 40-day-old astragal seedlings were transplanted in the pot. *R. solani* was inoculated at 10% (w/v) rate into the soil. In case of soil drench, 50 m*l* of the antagonist suspension at two concentrations of 10^6 and 10^4 conidia/m*l* was drenched twice on the pots with 3 days interval. After transplanting, disease severity was estimated every 2 days from the fifth day after transplanting based on percentage of seedlings with stem rot. Each treatment was made with 20 seedlings.

Results

Effect of *G. virens*. Inhibitory activity of *G. virens* G1 against *R. solani* was confirmed; the inhibition zone was 35.1 mm for *R. solani* AG 1 and 40.7 mm for AG 4 (Table 1). In the broth culture, supernatant of *G. virens* G1 significantly reduced mycelial weight of *R. solani* AG 1 and AG 4. The antagonist *G. virens* G1 was inhibitory to the mycelial growth of *R. solani* AG 1 and AG 4 (Fig. 1). Mycelial coiling of *G. virens* G1 was observed around the mycelia of *R. solani*. Furthermore, abnormal hyphae or mycelial lysis of *R. solani* was also confirmed at the contact points

Table 1. Inhibition zone formed by the antagonist *Gliocladium virens* G1 to *Rhizoctonia solani* AG 1 and AG 4 and effect on mycelial growth of the fungus in the culture filtrate of the antagonist

Treatment	Inhibition zone (mm)		Dry weight (g/100 ml)	
	AG 1	AG 4	AG 1	AG 4
Gliocladium virens G1	35.1±1.17[1)]	40.7±3.87	0.97±0.004	1.00±0.059
Control	0	0	1.06±0.031	1.94±0.124

[1)]Standard error (P = 0.05).

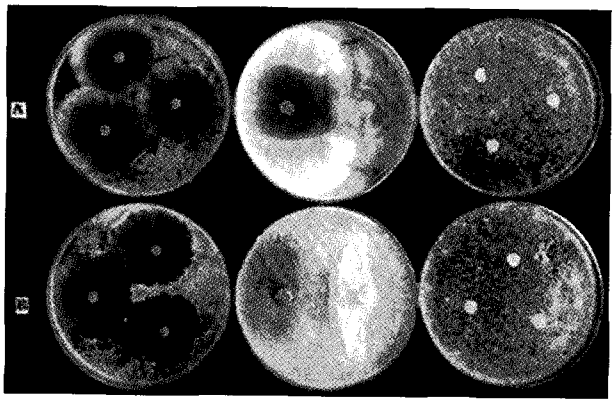

Fig. 1. Inhibitory activity of the antagonist *Gliocladium virens* G1. A : *Rhizoctonia solani* AG 1, B : *Rhizoctonia solani* AG 4, 1. dual culture of *R. solani* and *G. virens* G1, 2. dual culture of *G. virens* G1 and *R. solani*, 3. culture of *R. solani* as controls.

between the antagonist and the pathogens. No adverse effect was observed on seed germination of astragal plant when the seeds were soaked in the conidial suspension of *G. virens* G1 and germinated on WA (Table 2). Growth promotion was observed with germination ratio, seedling height and weight. In terms of germination, 78.3% of astragal seeds were germinated with treatment of *G. virens* G1 whereas 51.7% without the treatment. The seedling height was much longer with the treatment of *G. virens* G1; the height was 3.45 cm with the treatment where 2.26 cm without the treatment. This also resulted in increased seedling with the treatment of *G. virens* G1; the weight was 50 mg per seedling with the treatment whereas 40 mg per seedling without the treatment. Disease severity was much lower with treatment of *G. virens* G1; the diseased seedlings were 22.9% for *R. solani* AG 1 and 28.6% for *R. solani* AG 4 when treated with the antagonist treatment, and 71.4% and 65.7%, respectively, without the treatment.

Table 2. Effect on seed germination and seedling growth of astragal plant by soaking in the suspension of the antagonist *Gliocladium virens* G1

Treatment	Seed germination (%)	Seedling height (cm)	Seedling weight (g)
G. virens	78.3±5.7[1)]	3.4±0.8	0.05±0.01
Control	51.7±2.8	2.2±0.6	0.04±0.01

[1)]Standard error (P = 0.05).

Effect of inorganic compounds and organic materials. Fourteen inorganic chemicals formulated in SF-21 and 6 organic materials included in CPM were confirmed for their effect of disease suppression in the soil extract medium. Seven inorganic compounds, aluminum sulfate, alum, calcium nitrate, calcium oxide, glycerine and ammonium nitrate, showed inhibitory activity against mycelial growth of *R. solani* AG 1 and AG 4 (Table 3). These compounds except calcium oxide were also inhibitory against sclerotial germination. Among the compounds, alum was most effective in inhibition of mycelial growth and sclerotial germination. Only milled oak, milled pine bark, and milled larch sawdusts showed some inhibitory activity against mycelial growth of *R. solani* AG 1, but the inhibitory activity was not significantly dif-

Table 3. Effect of the inorganic compounds on mycelial growth and sclerotial germination of *Rhizoctonia solani* causing stem rot of astragal plant

Chemicals[1)]	Supperssion (%)[2)]			
	Mycelial growth		Sclerotial germination	
	AG 1[3)]	AG 4[4)]	AG 1	AG 4[5)]
$Al_2(SO_4)_3$	2.3 (66.7)x	4.7 (60.7)y	3.3 (29)	-
Alum[6)]	51.2 (33.3)y	29.4 (45.0)z	16.7 (25)	-
$CaCl_2$	0 (70.0)x	5.3 (60.3)y	0 (30)	-
$CaCO_3$	0 (73.7)x	0 (68.0)xy	0 (30)	-
$Ca(NO_3)_2$	3.4 (66.0)x	0 (68.0)xy	6.7 (28)	-
CaO	6.7 (63.7)x	1.6 (62.7)y	0 (30)	-
Glycerine	2.3 (66.7)x	1.1 (63.0)y	6.7 (28)	-
K_2HPO_4	1.5 (67.3)x	0 (64.7)y	0 (30)	-
KCl	0 (70.7)x	0 (77.0)x	0 (30)	-
K_2SO_4	0 (72.0)x	3.8 (61.3)y	6.7 (28)	-
NH_4NO_3	2.9 (66.3)x	0 (71.3)xy	0 (30)	-
Urea	0 (69.0)x	0 (70.7)xy	0 (30)	-
TSP[7)]	0 (70.3)x	0 (67.3)xy	0 (30)	-
$MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$	0 (73.0)x	0 (68.3)xy	0 (30)	-
Control	0 (68.3)x	0 (63.7)y	0 (30)	-

[1)]Each compound (1%, w/v) was added to 10 g of sterilized soil and mixed with 30 ml of deionized water. Extract media were prepared by pouring 1 ml of extract to 10 ml of melted water agar.

[2)]Means within the same column followed by the same letter are not significantly different (P = 0.01) according to Duncan's multiple range test.

[3)]Parenthesis number means mycelial diameter (mm), and records were made 3 days after treatment.

[4)]*R. solani* AG 1 and AG 4 were used after isolated from stem rot of astragal plant.

[5)]*R. solani* AG 4 did not form sclerotia.

[6)]Alum : $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$.

[7)]TSP : triple superphosphate.

Table 4. Effect of extract media of organic compounds for suppression of mycelial growth of *Rhizoctonia solani* causing stem rot of astragal plant

Organic compound[1]	Mycelial growth (mm)		Suppression (%)[2]	
	AG 1	AG 4	AG 1	AG 4
Rice bran	83.7	55.7[3]	0 >x	8.7 xy
Wheat bran	85.0	56.0	0 >x	8.2 xy
Milled pine sawdust	70.0	49.7	0 >y	18.5 yz
Milled oak sawdust	66.0	42.3	3.4y	30.7 z
Milled larch sawdust	66.0	48.7	3.4y	20.2 yz
Milled pine bark	65.3	46.0	4.4y	24.6 z
Control	68.3	61.0	0 y	0 x

[1]1% (w/v) concentration of extract media were prepared from each of the organic sources after decoction for 30 minutes.
[2]Means within a column followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.
[3]Means mycelial diameter (mm), and records were made 3 days after treatment. Values are average of 3 replicates.

ferent (Table 4). *R. solani* AG 4 was more sensitive to the organic materials; all the six organic materials inhibited the mycelial growth. Among the organic materials, milled oak and milled pine sawdust were most effective in the disease suppression.

Effect of *G. virens* G1 on seed germination and growth. *G. virens* G1 resulted in higher seed germination and better growth in the sterilized soil than in the natural soil. Furthermore, application of *G. virens* G1 by soaking of the seeds in the conidial suspension was slightly better than by soil drenching (Table 5).

Effect of *G. virens* G1 with the amended CPM. *G. virens* G1 amended with CPM (1%, w/v) resulted in relatively low disease severity both for *R. solani* AG 1 and AG 4 (Table 6). *G. virens* G1 alone also resulted in low disease severity when the seeds were soaked in the conidial suspension. However, high disease severity was observed when applied by drenching the conidial suspension of *G. virens* G1 onto the top of soils in the pot. Application dose of the conidial suspension affected the disease severity; the severity was lower with the higher dose at 1.0×10^6 conidia/m*l* than with that at 1.0×10^4 conidia/m*l*.

Table 6. Effect of soil drenching and soaking of the antagonist on *Rhizoctonia* stem rot of astragal plants under the greenhouse conditions

Pathogen	Treatment	Infection[1] percent (%)
AG 1	Antagonist amended[2] with CPM (1%, W/v)	10 a[3]
	Antagonist soaked (1×10^6 conidia/m*l*)	10 a
	Antagonist drench (1×10^6/m*l*)	40 b
	Antagonist drench (1×10^4/m*l*)	45 b
	Control	85 c
AG 4	Antagonist amended with CPM (1%, W/v)	10 a
	Antagonist soaked (1×10^6 conidia/m*l*)	15 a
	Antagonist drench (1×10^6/m*l*)	25 b
	Antagonist drench (1×10^4/m*l*)	55 c
	Control	90 d

[1]Infection percent was obtained 18 days after treatment, and 20 seedlings were used as a replication.
[2]CPM is formulated with 7 effective in organic chemicals and composted milled pine bark and composted sawdust of oak tree, respectively.
[3]Means within a column followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

Discussion

Recently biological control has been globally drawing high attention of researchers because it is an alternative strategy of disease management for environment-friendly agriculture. The main mechanisms of biological control are well known to be due to competition, lysis, hyperparasitism and antibiosis by the introduced antagonist. In practice, the introduced antagonists are not persistent in the soil because of difficulties to adapt to the soil microenvironment. Thus it is recognized that soil amendments may have potential to provide consistent biological control. Effect of soil amendment is elucidated as mutual phenomenon between biological and nonbiological factors. Furthermore the application of composts is also an effective methods in disease control and often as effective as the fungicide application (Hoitink *et al.*, 1991). Hoitink pointed out in 1986 that composted pine bark suppresses soilborne fungi and to promote activity of indigenous thermophilic bacteria and *Trichoderma* and *Gliocladium* spp. as antagonists. However, tannin, resin, saponin,

Table 5. Effect of the antagonist on seed germination and seeding growth of astragal plants in the pot experiment

Soil	Antagonist	Inoculation method	Seed germination (%)	Diseased seedling (%)	Healthy seedling (%)
Sterilized soil	*G. virens* Gl	Soaking	73.3	13.6 a[1]	86.4 a[1]
		Drenching	71.7	37.2 b	62.8 b
	Control		68.3	56.1 c	43.9 c
Natural soil	*G. virens* Gl	Soaking	63.3	31.6 a	68.4 a
		Drenching	51.7	35.5 a	64.5 a
	Control		31.7	57.9 b	42.1 b

[1]Means within a column followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

starch, other carbohydrates and ethylates in the pine bark are also known to be toxic and fungistatic to the pathogens (Huang and Kuhlman, 1991). There is a high positive correlation between increase of antagoinst population and activity of biological control (Bin *et al.*, 1991). Nonbiological factors were identified for the growth of fungal antagonists including *Gliocladium* sp. (Papavizas, 1985).

In this research, the extract solution of the composted pine bark demonstrated the inhibitory activity to mycelial growth and sclerotial germination of *R. solani* AG 1 and AG 4. Suppressed damping off at early seedling stage by soaking the seeds in the conidial suspension of *G. virens* Gl suggests that the antagonistic reaction results from the production of antifungal substance and hyperparasitism by mycelial coiling. It is known fact that a diverse microbial population including pathogens are found in nonsterilized natural soil than in the sterilized soil, and the seed germination is higher in the sterilized soil. This observation was confirmed again in this study and may be due to lack of saprophytes including the causal fungus. In this study, CPM (1%, w/v) and *G. virens* G1 synergistically suppressed the stragal stem rot caused by *R. solani* AG 1 and AG 4. There are many reports (Papavizas, 1985) on plant growth promoting fungi (PGPF). This study also confirmed growth promotion in seed germination and seedling height and weight with the treatment of *G. virens* G1 confirmed. Therefore, further intensive study on PGPF is needed to characterize *G. virens* G1. Development of application method of the antagonist is very important; *G. virens* G1 applied by seed coating or soil drenching could be significantly different in disease suppression by biological control. Further researches are needed to extend the application of the inorganic and organic materials amended with *G. virens* G1.

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