IDENTIFICATION OF *GAEUMANNOMYCES* SPECIES IN TURFGRASS AREAS AND CONTROLLING THE DISEASES BY SOME ENDOPHYTES AS BIOLOGICAL AGENTS

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Abstract. Take-all disease caused by *Gaeumannomyces* species bring about economic loses in turfgrass cereals. Surveys were conducted in turfgrass areas in Türkiye and isolations were done from the 318 samples which were taken from irregularly shaped chlorotic patches, and the roots of which are close to dark brown or black. As a consequence of the isolations, fourteen *Gaeumannomyces* spp. were isolated, and then identifications were performed with rDNA sequence analysis using primers ITS1 and ITS4. Comparison of the ITS sequences with the isolates in the GenBank database, and phylogenetic analysis showed that the isolates belonged to *Gaeumannomyces graminis* var. *graminis*, *Gaeumannomyces californicus* and *Gaeumannomyces cylindrosporus*. As a result of the pathogenicity tests, virulence of *G. graminis* var. *graminis* and *G. californicus* isolates were higher than that of *G. cylindrosporus*. Disease severity rates caused by the isolates, as a result of *in vitro* trials were between 11.83-97.61%, and those in *in vivo* ranged from 3.70-89.64%. As a result of *in vivo* tests, it was detected that the most virulent isolate was *G. graminis* var. *graminis* numbered 732 followed by 966, 1345, 1369, 1385 and *G. californicus* numbered 1573, 1925. In this study, the effects of *Chaetomium globosum, C. bostrychodes, Sordaria fimicola, Clonostachys rosea, Trichoderma harzianum* and *T. hamatum* fungi, which were previously determined as endophyte species in turfgrass areas, against the most virulent species *G. graminis* var *graminis*, were also investigated. As a result of biological control studies, efficacy of *C. globosum* CG07-1, *Chaetomium bostrychodes* CB07-2, *Trichoderma hamatum* Tha34-2 and *Trichoderma harzianum* TH06-5 strains were found as 86.31%, 83.25%, 70.07, and 64.01% respectively. These strains were found promising in the biological control of 'take-all disease' caused by *G. graminis* var *graminis* in turfgrass areas.
Keywords: *biological control, Gaeumannomyces, genetic diversity, take-all, turfgrass, virulence*

Introduction

Green areas with turfgrass, such as parks, golf courses, stadiums, and refuges have been increased in recent years. Especially golf courses are important areas in view of golf tourism. Most of the diseases are caused by plant pathogenic fungi, which are mainly divided as airborne and soilborne fungi in turfgrass. Many root pathogens belonging to different fungal species cause diseases on turfgrasses (Smiley et al., 2005). Among them, *Gaeumannomyces* species result in root rot and patch in turf areas. The disease often is confined to *Agrostis* spp. growing on poorly drained, wet soils or on

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soils that have recently been heavily limed. Although the pathogen may also infect *Cynodon*, *Festuca* and *Poa* spp. (Fouly and Wilkinson, 2000; Smiley et al., 2005).

Four different varieties of *G. graminis* have been reported to infect graminaceous plants. *G. graminis* var. *tritici* Walker is one of the most important root diseases of wheat and barley and causes significant yield losses. *G. graminis* var. *avenae* (Turner) Dennis causes disease especially on oats as well as wheat and barley, this variety also causes 'take-all' patch disease on grasses. *G. graminis* var. *graminis* (Sacc.) is poorly pathogenic or non-pathogenic on grains, but it causes serious infections on St. Augustine grass, Bermuda grass, rice and other warm season grasses (Datnoff et al., 1997; Smiley et al., 2005). *G. graminis* var. *maydis* has been identified on maize and is responsible for the 'take-all' disease of maize. It has been determined to cause mild infection on sorghum and other cereals (Freeman and Ward, 2004). Another species belonging to the *Gaeumannomyces* genus is *G. cylindrosporus* that has been recorded as a non-virulent species, and can colonize grain and grass roots (Ban et al., 2017). All these *Gaeumannomyces* species colonize the roots of graminaceous plants together with *Phialophora* anamorphs as the *Gaeumannomyces-Phialophora* (G-P) complex on their host roots (Bateman et al., 1992; Henson, 1992). Asexual forms of *Gaeumannomyces* species and varieties have characteristic hypopodia structures (Hernández-Restrepo et al., 2016). It is possible to distinguish *Gaeumannomyces* species and varieties by combining these different hypopodia structures and molecular analyzes supported by phylogenetic studies. New *Gaeumannomyces* species and varieties have emerged in recent phylogenetic studies. Recent morphological and molecular studies have identified 19 different species of the agent (Hernández-Restrepo et al., 2016).

Endophytic fungi are microorganisms that live in the underground and aboveground parts of plants in nature without harming them, prevent them from being damaged by enabling them to gain resistance against biotic and abiotic stresses, and encourage their growth (Gond et al., 2010). For this reason, fungal endophytes are significant biological control agents against biotic stress factors like diseases and pests in agriculture. *Trichoderma* and *Clonostachys* (*Gliocladium*) species are the most popular biocontrol agents among used in agriculture. However, the biological control capacities of some endophytic fungi such as *Chaetomium, Sordaria, Myrothecium* have attracted attention and become the focus of attention by researchers (Manoch et al., 2008; Madbouly and Abdel-Wareth, 2020).

Chaetomium, *Sordaria* and *Trichoderma* species are fungi living as saprops in soil, plant residues or cellulose-containing materials. Fungi belonging to these genera have gained importance in the control of pathogenic microorganisms in terms of antagonistic and mycoparasitic relations. *Chaetomium* spp. are also known to produce various metabolites such as BHT, chaetoglobosin and chaetomin besides hydrolytic enzymes (Biswas et al., 2012), which have antifungal properties against phytopathogenic fungi and nematodes (Nitao et al., 2002). The fungi has also been proven to be a mycoparasite on some important pathogens (Gao et al., 2005). They have been found to be effective against pathogenic fungi such as *Alternaria brassicicola, A. raphani, Fusarium* spp. and *Pythium ultimum* (Thiep and Soytong, 2015; Fayyadh and Yousif, 2019). *Chaetomium globosum* showed effective results against rice blast diseases (Soytong and Quimio, 1989), wheat blotch caused by *Cochliobolus sativus* (Aggarwal et al., 2004) and Ascochyta blight of chickpea (Rajakumar et al., 2005). In studies conducted in various parts of the world, it was detected that *Sordaria* spp. have antagonistic effects against

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important phytopathogenic species such as *Rosellinia necatrix, Curvularia lunata, Pestalotiopsis guepinii, Alternaria alternata, Fusarium oxysporum, Colletotrichum capsici, Lasiodiplodia theobromae, Armillaria mellea* and *Pythium aphanidermatum*. *Trichoderma* genus is the most commonly used species in biological control and live in many soils and plants around the world. This genus contains species that are plant growth promoters that act through a variety of mechanisms. They produce various lytic enzymes and antibiotics against plant pathogens, and various products made from these fungi are commercially marketed as biopesticides, biofertilizers and soil conditioners (Kumar et al., 2020).

In this study, the effects of *Chaetomium globosum, C. bostrychodes, Sordaria fimicola, Clonostachys rosea, Trichoderma harzianum* and *T. hamatum* fungi isolated from turfgrass against *Gaeumannomyces graminis* var. *graminis* which causes 'take-all' disease on turfgrass were investigated.

Materials and methods

Collection of samples and isolation of fungi

Surveys were performed in turfgrass areas in Ankara, Antalya, Aydın, Bursa, İstanbul, İzmir, Kayseri and Muğla Cities, and diseased samples were collected from parks, recreation areas, golf courses and stadiums. Collected samples and details of isolates are given in *Table 1*. Isolations were made by samples which were taken from small and big irregularly shaped chlorotic patches (*Fig. 1*) and the roots of which were almost brown or black. Brown root surfaces were sterilized for 30 s in silver nitrate solution (1%), rinsed for 30 s in sterile water, dried on filter paper, and placed on PDA (Difco, USA) amended with streptomycin sulfate (100 µg/ml).

Table 1. *The origin, species and maximum similarity ratios with the GenBank isolates of the isolates in this study*

Isolate number	Isolated city/turfgrass area	The highest similarity rate (%) with GenBank isolates	The name of Genbank isolates and its acc. number	Identified isolate name in this study
732	Muğla/Golf course	99.60	KX306504-*G. graminis*	*G. graminis* var. *graminis*
900	Antalya/Football area	98.43	KX306504-*G. graminis*	*G. graminis* var. *graminis*
920	Antalya/Football area	99.21	KX306502-*G. graminis*	*G. graminis* var. *graminis*
966	Antalya/Football area	99.22	KX306502-*G. graminis*	*G. graminis* var. *graminis*
1345	Antalya/Golf course	99.49	KX306502-*G. graminis*	*G. graminis* var. *graminis*
1369	Antalya/Golf course	99.60	KX306502-*G. graminis*	*G. graminis* var. *graminis*
1385	Antalya/Golf course	99.40	KX306502-*G. graminis*	*G. graminis* var. *graminis*
1686	Antalya/Golf course	98.75	KX306502-*G. graminis*	*G. graminis* var. *graminis*
1573	Bursa/Park	98.99	NR155135-*G. californicus*	*G. californicus*
1590	Ankara/Football area	98.98	NR155135-*G. californicus*	*G. californicus*
1925	Antalya/Park	98.96	NR155135-*G. californicus*	*G. californicus*
1926	Ankara/Football area	99.15	NR155135-*G. californicus*	*G. californicus*
884	Bursa/Park	100	MT242286-*G. cylindosporus*	*G. cylindosporus*
1132	İstanbul/Football area	100	MT242286-*G. cylindosporus*	*G. cylindosporus*

Pathogenicity assays

In vitro (agar plate assays), and *in vivo* (greenhouse assays) pathogenicity tests were performed with all *Gaeumannomyces* isolates.

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In vitro assays

Fungi were incubated on Potato Dextrose Agar (PDA) (Difco, USA) for 5 days at 24 ± 1 °C, 3-4 mm mycelial discs were taken from the tip of the developing fungus colony and transferred to the center of the petri dish containing 1.5% water agar medium and incubated for 3 days. Seeds of the susceptible grass species (*Festuca arundinaceae*) were surfacely disinfected in 0.5% Sodium hypochloride (NaOCl) for 1 min, then washed by sterile distilled water for 1 min. Ten grasses were then placed in a circle at the tip of the growing edge of the fungus colony in each Petri dish (Ichielevich-Auster et al.1985). Three Petri dishes were used for each isolate. Petri dishes without fungi were used as controls. Seeds were incubated for 9-10 days at 24 ± 1 °C under a 12-h photoperiod. After 10 days, disease evaluations were made by using the 0-5 scale (Ichielevich-Auster et al., 1985) on *Table 2*. Disease severity rates (%) were determined with the help of Townsend and Heuberger (1943) formula.

Figure 1. *(a) Small (Turf composition: C. dactylon, L. perenne and P. trivialis) and (b) large (Turf composition: C. dactylon and A. stolonifera) irregular patch symptoms caused by Gaeumannomyces graminis var. graminis in turfgrass areas*

Table 2. *0-5 scale used on disease evaluation*

Scale value	Disease definition
0	No disease
1	1-10% of the hypocotyl with necrosis
2	11-30% with necrosis
3	31-50% with necrosis
4	51-80% with necrosis
5	All hypocotyls with necrosis

In vivo assays

For inoculum production, 125 ml of sterile distilled water was added to bottles containing 500 g of rye seeds and autoclaved twice at 90 °C for 1.5 h, one day apart. Then, 10 agar discs of 7-8 mm in diameter with mycelia of each isolate were added to these bottles and incubated at 25 °C for one month. After incubation, the inoculum was air-dried and smashed using blender. Then, infested rye seeds (5%) were applied to the

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sterilized (two consecutive days at 121 °C for 45 min) sand, soil and fertilizer mixture (1:2:1). There were three replicates with square pots (each one 1 dm^3 in volume) for each treatment. Control pots were not inoculated with fungi. The pots were covered with polyethylene bags and incubated for 72 h. After 72 h, thirty *F. arundinaceae* seeds were planted in pots and covered with about 1 cm of sterile soil. After planting, 15 ml of distilled water was added to each pot. After one month, the grass plants were examined (Datnoff et al., 1997). Disease assessments were evaluated on a scale of 0 to 4 (*Table 3*) (Anonymous, 2008). These scale values were converted to disease severity values using the Townsend and Heuberger formula.

Table 3. *0-4 scale used on disease assessments on biological control studies*

Scale value	Disease definition
0	No disease
1	1-10% of the hypocotyl infected
2	11-30% of the hypocotyl infected
3	31-60% of the hypocotyl infected
4	61-100% of the hypocotyl infected

The data obtained in both studies were analyzed according to a completely random experimental design. The means obtained were analyzed with the LSD (Least Square Difference) multiple comparison test.

DNA isolations and PCR analysis of Gaeumannomyces isolates

DNA isolations were made by using QIAGEN Blood and Tissue Kit, according to the company's instructions for use. In the polymerase chain reactions (PCR) study, general Internal Transcribed Spacer (ITS) primer pairs ITS1 and ITS 4 were used. (White et al., 1990). For PCR analysis, the reaction mixture was prepared as 50 µl; 2 µl of primers (10 mM), 4 µl of BSA, 13 µl of double-distilled water, and 25 µl of GoTaq® Hot Start Green Master mix (2x) (Promega, USA). After this mixture was distributed to the PCR tubes of each isolate, 4 µl of the DNA template of each isolate was added. The PCR cycling protocol consisted of an initial denaturation step at 94 ºC for 4 min, 30 cycles of 45 s at 94 °C, 45 s at 55 °C and 2 min at 72 °C, and finally 10 min extension at 72 °C. PCR products were subjected to direct sanger sequencing in a private R&D Laboratory (BMLabosis, Ankara). Sequence results were compared with isolate sequences in GenBank after BLAST analysis at NCBI.

Evolutionary analysis by maximum likelihood method

Phylogenetic evaluations were made with the Maximum Likelihood Method using the MEGA 7 program (Kumar et al., 2016). As a result of the analysis of finding the most suitable model in the Mega 7 program, it was determined that the most convenient parameter for the tree was Kimura 2-parameter (Kimura, 1980). The phylogenetic tree was constructed with fourteen nucleotide sequences from this study, eight reference isolates (Acc. Numbers: KX306502.1, AJ010031.1, NR155135.1, MT242286.1, AY428780.1, JF414849.1, AY428778.1, U17210.1) and one external isolate (Acc. Number: EU514697.1) from GenBank (*Fig. 2*).

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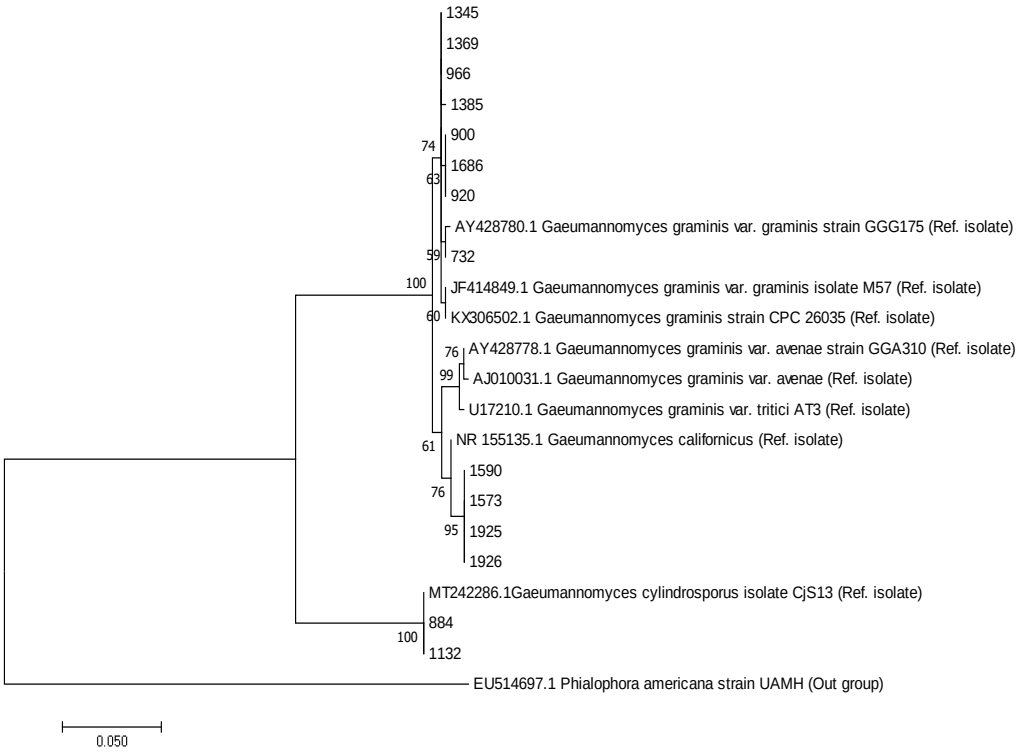

Figure 2. *Dendrogram showing relationships among the 14 isolates of Gaeumannomyces. Genetic distances were obtained by rDNA sequence analysis with ITS 1 and ITS 4 primers*

Biological control studies

Fungal isolates

Chaetomium globosum, C. bostrychodes, Sordaria fimicola, Clonostachys rosea, Trichoderma harzianum and *T. hamatum* isolates used in the study were isolated from the turfgrass areas of Antalya, İzmir, İstanbul and Ankara Cities in Türkiye, in our previous study (Unal, 2020). Isolate 1385 (*Gaeumannomyces graminis* var. *graminis*) was used as pathogen isolate in this study.

Seed treatments

For *C. globosum, C. bostrychodes and S. fimicola;* isolates were developed on filter paper saturated with Malt Extract Broth (Merck, Germany), growing mycelium, and ascospores were passed through a double layer cheesecloth using sterile distilled water. The final solution was then settled by centrifugation for 20 min at 15,000 × g and the supernatant was discarded. The pellets were resuspended using a small amount water and 10×10^7 spores/ml concentration was obtained using a Thoma slide (Hubbard et al., 1982). For methyl cellulose coatings, suspensions were added to methyl cellulose (Sigma, USA) 2%. Turfgrass seeds (*F. arundinaceae*) were treated with the spore suspension as 3 ml of spore suspension per 10 g of seeds in a glass container. The seeds were then dried in a laminar cabinet for 12 h at 25 °C for 2 weeks (Hubbard et al., 1982). *C. rosea*, *T. harzianum* and *T. hamatum* isolates were developed on filter paper saturated with Potato Dextrose Broth (Difco, USA). Growing mycelium and spores were passed through a double layer cheesecloth using sterile distilled water. Spore

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suspensions were centrifuged for 5 min at 2000 × g and the supernatant was discarded. The pellet was resuspended in distilled water and centrifuged again for 5 min at 1000 × g. The supernatant was discarded and the pellet was suspended in 12 ml of distilled water. The spore concentrations were adjusted to 1×10^8 spores/ml (Cliquet and Scheffer, 1997). For methyl cellulose coatings, 2% methyl cellulose was added to the suspensions and 3 ml of this solution was mixed with 10 g seeds. The treated seeds were then left to dry in a laminar flow sterile cabinet for 12 h (Cliquet and Scheffer, 1997).

Data evaluations

Experimental conditions, preparation and contamination of the soil with the pathogen inoculum, and evaluations were the same as in '*in vivo* pathogenicity assays'. Three treatments were performed in the experiment: (1): positive control (with planting uncoated turfgrass seeds in infected soils to detect the varietal sensitivity), (2): planting coated turfgrass seeds in infected soils to detect the biocontrol capacity of the endophyte isolates against pathogen *G. graminis* var. *graminis*, and (3): negative control (with planting uncoated turfgrass seeds in non- infected soils). Each application was designed as three replications. The results were evaluated by calculating the disease severity (%) values by using the "disease severity" formula (Townsend and Heuberger, 1943) by using 0-4 scale values for each replicate. Efficacy (%) of the applications was detected using the Abbott's formula (Abbott, 1925): % Efficacy = (X – Y)X × 100, where X = disease severity in the control and Y = disease severity in the treatments.

Disease severity and efficacy of strains were performed by ANOVA with mean comparisons at $p < 0.05$ level based on LSD range test in the statistical program of JMP Pro 14.3.0. Before that, percent values of means were transformed using arc sin transformation and the assumption of the variance analysis were tested and met.

Results

Surveys were performed in the stadiums, big parks, refuges and golf courses of 8 cities in Türkiye, and a total of 318 samples were collected. During the surveys, in the areas with short grass such as golf courses where the fungus was detected, large patches were observed in those containing *C. dactylon* and *A. stolonifera*, while smaller patches were observed in areas without *A. stolonifera*. In consequence of the isolations from the samples which were obtained from irregularly shaped chlorotic patches and the roots of which are almost brown or black, 14 different *Gaeumannomyces* isolates were obtained. Identifications of the fungi isolates were performed using ITS 1/4 general primers. The bands visualized with the gel transilluminator were found to be between 550-580 bp. Compared with the isolates in Genbank, 8 isolates showed a maximum similarity with *G. graminis* isolates at a rate of 98.43-99.60%, 4 isolates found out 98.96-99.15% similarity rate in *G. californicus* isolates and 2 isolates in *G. cylindrosporus* at a rate of 100% similarity rate. In the phylogenetic analysis performed together with the reference isolates in the Genbank, it was determined that the *G. graminis* isolate was *G. graminis* var. *graminis*. *G. graminis* and *G. californicus* isolates showed rapid mycelial development than *G. cylindrosporus*. The colony colors of all the isolates were gray on PDA.

Virulence analyses of all isolates were performed both *in vitro* and *in vivo* conditions. The isolates showed different pathogenic characteristics in both studies. As a result of *in vitro* studies, disease severity of *G. graminis* var. *graminis* isolates were generally found higher than the other species, and disease severity values of them were

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more than 82.57%. The highest disease severity was obtained by the isolate number 1385 as 97.61% in *in vitro* study and it was in the same group with 732, 920 and 966 isolates. *G. cylindrosporus* showed lower disease severity than the other species with two statistical groups (*Table 4*). In consequence with the *in vivo* pathogenicity study, virulence of the two *Gaeumannomyces* species isolates (*G. graminis* var. *graminis* and *G. californicus*) were higher than *G. cylindrosporus*. Generally, disease severity was higher than 77.49% (in isolate number 900). The highest disease severity was caused by isolate number 732 with the rate of 89.64% in *in vivo* and it was in the same group with 966, 1345, 1369, 1385, 1573, and 1925 isolates. On the other hand, virulence of the two *G. cylindrosporus* isolates were relatively lower than the others, with disease severity rates of 17.78% and 3.70% respectively (*Table 4*).

Table 4. *Disease severity rates (%) of the isolates obtained by in vitro and in vivo pathogenicity trials*

Isolate code numbers	Isolate names	Disease severity (%) in vitro*		Disease severity (%) in vivo*	
732	*G. graminis* var. *graminis*	88.77 ± 5.17	abc	89.64 ± 2.79	a
900	*G. graminis* var. *graminis*	83.66 ± 2.54	c	77.49 ± 4.40	f
920	*G. graminis* var. *graminis*	90.22 ± 4.39	abc	82.94 ± 1.75	c-f
966	*G. graminis* var. *graminis*	92.89 ± 2.72	ab	85.72 ± 2.23	a-d
1345	*G. graminis* var. *graminis*	86.33 ± 1.24	bc	86.33 ± 0.88	a-d
1369	*G. graminis* var. *graminis*	88.43 ± 2.23	bc	86.69 ± 1.67	a-d
1385	*G. graminis* var. *graminis*	97.61 ± 1.07	a	89.47 ± 0.53	ab
1686	*G. graminis* var. *graminis*	82.57 ± 1.53	c	81.24 ± 1.73	def
1573	*G. californicus*	85.66 ± 1.35	bc	84.58 ± 1.19	a-d
1590	*G. californicus*	85.06 ± 2.31	bc	83.75 ± 1.60	b-e
1925	*G. californicus*	84.20 ± 1.17	bc	88.13 ± 1.35	abc
1926	*G. californicus*	82.94 ± 1.84	c	81.25 ± 1.61	def
884	*G. cylindosporus*	32.94 ± 7.36	d	17.78 ± 2.36	g
1132	*G. cylindosporus*	11.83 ± 1.82	e	3.70 ± 1.85	h

*Means in the same column followed by the same letter are not statistically significant from the others according to LSD test at $p < 0.05$

Data obtained with ITS 1 and ITS 4 primers on the 14 isolates of *Gaeumannomyces* were used to produce the dendrogram shown in *Figure 2*. In the phylogenetic evaluation, the isolates belonging the three *Gaeumannomyces* species were seperated into two main clusters. While the first main group included *G. graminis* var. *graminis*, and *G. californicus*, second main group included only *G. cylindrosporus* species. Isolates belonging to *G. graminis* var. *graminis*, and *G. californicus* separated into two subclusters created first main group. It was detected that there were minor differences among *G. graminis* var. *graminis* isolates that took part in the same cluster but *G. californicus* isolates were grouped together and showed no molecular differentiation. On the other hand, within low pathogenic level in this study, *G. cylindrosporus* totally differed from the others. Based on similarity indices, *G. graminis* var. *graminis* and *G. californicus* isolates were similar but *G. cylindrosporus* isolates were different from them. Sequence data of all isolates showed 98.43-100% similarity with isolates in the GenBank and it was given in *Table 1*.

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In the greenhouse trials in which five endophyte fungi were tested against G. *graminis* var. *graminis* in turfgrass. The disease severity values of the tested strains in turfgrass varied between 12.76% and 55.98%, respectively. The lowest disease severity was *C. globosum* CG07-1, while the highest disease severity was 55.98% in *S. fimicola* SF35-1 strain. The efficacy values of the tested strains against disease showed values between 40.19% and 86.31%. (*Table 5*) (*Fig. 3*). The most effective species was *C. globosum* CG07-1 with 86.31% effect value, followed by *C. bostrychodes* CB07-2 with 83.25% effect. *T. hamatum* Tha34-2 was also another the effective isolates with an effect value of 70.07%. *S. fimicola* SF35-1 and *C. rosea* CR34-3 isolates were found as low effective strains with efficacy values of 40.19%, and 48.30% respectively. However, *T. harzianum* TH06-5 was evaluated as a promising strain with an effect value of 64.01% (*Table 5*).

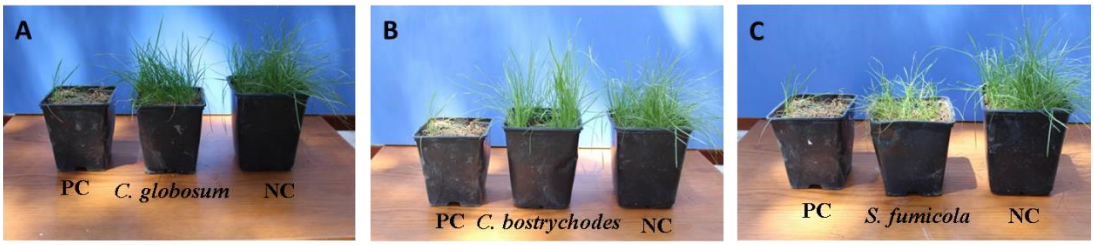

Figure 3. *Effects of endophyte fungal strains against G. graminis var. graminis in greenhouse experiments. A.: Chaetomium globosum CG07-1, B: Chaetomium bostrychodes CB07-2, C: Sordaria fimicola SF35-11: [PC: Positive Control (planting uncoated turfgrass seeds in infested soils), NC: Negative Control (planting uncoated turfgrass seeds in non-infested soils)]*

Table 5. *Disease severity and efficacy values obtained by using six endophytic fungal strains against G. graminis var. graminis on turfgrass*

Treatments	Strain numbers	Disease severity (%)		Efficacy (%)	
Chaetomium globosum	CG07-1	12.76 ± 4.21	d*	86.31 ± 4.67	a
Chaetomium bostrychodes	CB07-2	15.64 ± 6.34	d	83.25 ± 6.94	a
Sordaria fimicola	SF35-1	55.98 ± 6.67	b	40.19 ± 7.13	c
Clonostachys rosea	CR34-3	48.32 ± 12.28	bc	48.3 ± 13.44	bc
Trichoderma harzianum	TH06-5	33.6 ± 9.20	bc	64.01 ± 10.30	ab
Trichoderma hamatum	Tha34-2	27.94 ± 6.66	cd	70.07 ± 7.48	ab
(+) Control		93.6 ± 1.28	a		

*Means in the same column followed by the same letter are not statistically significant from the others according to LSD test at $p < 0.05$

Discussion

By viewing *Gaeumannomyces* isolates obtained from 8 different locations in Türkiye, most of causing agents to ‘take-all’ disease (8 of 14 isolates) in turfgrass were *G. graminis* var. *graminis* species. However, *G. californicus* (4 of 14 isolates) agents were also responsible for ‘take-all’ disease in turfgrass in Türkiye. These *Gaeumannomyces* species were reported in turfgrass in the most part of the world (Wetzel et al., 1996; Fouly and Wilkinson, 2000; Smiley et al., 2005). *Gaeumannomyces* disease on turfgrass is firstly dealt with by this study and species of

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G. graminis var. *graminis, G. californicus* and *G. cylindrosporus* which were detected in this study is the first finding for Türkiye. But *G. g.* var *tritici* damage was known to on wheat in Türkiye (Büyük et al., 2018).

Gaeumannomyces species in turfgrasses areas in Türkiye were differed by two symtoms as large patches and relatively smaller patches according to composition of turfgrass. Especially, it was observed that *G. graminis* var. *graminis* formed large patch symptoms in turfgrass compositions containing *Cynodon dactylon* and *Agrostis stolonifera*. Similarly, it was reported that *G. graminis* var. *graminis* causes decline in bermuda grass species, root rot of *Stenotaphrum secundatum* grass or other warm-season turfgrasses by many researchers (Walker, 1981; Ward and Bateman, 1999).

As a result of PCR studies applied in the diagnosis of isolates, the bands displayed in the gel were found to be between 550-580 bp. Fouly (2004) who studied genetic variation among *G. graminis* var. *graminis* isolates by using ITS rDNA sequence data and obtained an amplification fragment of about 520-582 bp. The molecular studies conducted in recent years have shown that the differences between species are genetically determined by comparing the ITS regions of different species within the genus *Gaeumannomyces* and species close to them (Bryan et al., 1995; Ulrich et al., 2000; Hernández-Restrepo et al., 2016). Similarly, in our study, based on similar rDNA sequence profiles, isolates were categorized two main group, in which two different subgroups were formed in the first group as *G. graminis* var. *graminis* and *G. californicus* (*Fig. 2*). In this study, small genetic differences among *G. graminis* var. *graminis* isolates were determined. Within this clade, *G. cylindrosporus* formed a distict subgroup. This finding is in line with previous results of other researchers that *G. cylindrosporus* and its anamorph *Phialophora graminicola* form a distinct group from *G. graminis* and *G. incrustans* (Bryan et al., 1995; Fouly, 2004). It was concluded that NR155135.1 (Genbank isolate) (Hernández-Restrepo et al., 2016) showed the highest similarity with our *G. californicus* isolates (*Fig. 2*). Conducting pathogenic characterization of 14 *Gaumannomyces* isolates, except *G. cylindrosporus* isolates, most of them were highly pathogenic. *G. cylindrosporus* is shown either weak or none-pathogenic characterization in different studies in the world (Wetzel et al., 1996; Ulrich et al., 2000). Although *G. cylindrosporium* has been reported to cause root symptoms when applied to grass and wheat in some studies, it is seen that the fungus is not considered as a pathogen in some field studies (Hu et al., 1993; Landschoot and Jackson, 1989). With this study, fungi that cause “take all” disease and their virulences in large turfgrass areas in Türkiye were investigated for the first time.

In the biological control studies, the effects of some endophytic fungal species collected from turfgrass areas in Türkiye were investigated against *G. graminis* var. *graminis* on turfgrass. A lot of endophytic fungi have been identified as useful microorganisms for the biocontrol of root pathogens and insects and they are popular in terms of being an alternative to chemical-containing preparations in the control of microorganisms that cause disease in plants (Kumar et al., 2020). An important feature of effective biocontrol agents is their ability to remain in the soil and aggressively colonize the rhizosphere. Since the 1990s, several biological preparations in the form of pellets and powder formulations have been developed from 22 strains of *Chaetomium cupreum* and *Chaetomium globosum.* These formulations have been applied to field soils that have been cultivated for a long time and successful results have been obtained. They provide protection against *Phytophthora cactorum* on strawberry, basal rot of corn caused by *Sclerotium rolfsii, P. parasitica* on tangerine, *Fusarium oxysporum* f. sp.

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lycopersici on tomato and *P. palmivora* on durian and black pepper (Soytong et al., 2001). In this study, the efficacies of *C. globosum* CG07-1 and *C. bostrychodes* CB07-2 strains on pathogen *G. graminis* var. *graminis* were investigated and high efficacy obtained as 86.31% and 83.25%, respectively (*Table 5*).

Trichoderma is the fungal genus most widely and successfully used in the biological control of pathogenic fungi, which is the most important method of agricultural control and have also been in the focus of attention of researchers for years (Avis et al., 2001). Modes of action of *Trichoderma* spp. against phytopathogenic soil-borne fungi include mycoparasitism, antibiosis, competition and induced systemic resistance (ISR) (Ali et al., 2021). Mycoparasitism activities of *T. virens* against *Pythium ultimum* and *Rhizoctonia solani* were demonstrated using the dual culture and RT-PCR techniques (López-Mondéjar et al., 2011). In addition, it was proved that SM1 elicitor produced by *T. virens* induces SR (systemic resistance) in cotton plants against *Colletotrichum graminicola* (Djonović et al., 2007). Also, it was reported that *T. harzianum* had competition ability against *Fusarium oxysporum* for rhizosphere colonization and nutrients uptake (Tjamos et al., 2006). *Trichoderma harzianum* is the most widely used commercial biological preparation in the world in the form of wettable powder and granule formulations (Zin and Badaluddin, 2020). In this study, *Trichoderma harzianum* Th06-5 and *Trichoderma hamatum* Tha34-2 strains were found promising with effect values of 64.01% and 70.07% respectively (*Table 5*).

Conclusion

The future of agricultural production and environmental safety are in danger due to climate change and wrong agricultural practices. Especially in the fighting against diseases in plants, excessive and unconscious use of pesticides causes serious damage to human and the environment. One of the most important solutions to these problems is the use of endophytic microorganisms, which are the elements of natural balance, instead of chemicals in the control against diseases and the dissemination of biological control studies.

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