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Determination of antagonistic effect of bio-agents against cereal damping-off caused by *Fusarium graminearum* in wheat

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Abstract

The antagonistic effect of the isolates of *Gliocladium roseum*, *Saccharomyces cerevisiae*, *Sordaria fimicola*, and their mixtures, at different concentrations against the cereal damping-off pathogen, *Fusarium graminearum*, was examined in vitro and in vivo (foliar, seed, soil, seed + soil) treatments on the susceptible wheat cultivars "Gun 91 and Sultan 95." The 3 isolates inhibited growth of *F. graminearum* at a concentration of 1×10^9 spores/ml with inhibition rates of 84, 88, and 91%, respectively under in vitro conditions. For in vivo assays, the mixture of *S. cerevisiae* + *S. fimicola* exhibited a considerable antagonistic activity even at a concentration of 1×10^5 spores/ml. Particularly, at the seed + soil treatment of the mixture, the pathogen was almost completely suppressed with an inhibition rate above 96% at concentrations of 1×10^8 and 1×10^9 spores/ml for both wheat cultivars, and the percentage of emerged seedlings reached nearly 100%. The results verified that the mixture of *S. cerevisiae* + *S. fimicola* had a high potential, as a promising biocontrol agents and an eco-friendly alternative, to be used against the cereal damping-off caused by *F. graminearum*, to reduce the use of systemic fungicides.

Keywords: Antagonistic effect, Bio-agent, Wheat, Cereal damping-off pathogen, *Fusarium graminearum*

Background

The diseases caused by plant pathogenic bacteria and fungi cause significant reductions in crop yield and huge product losses worldwide (Avelino et al. 2015). Wheat is one of the most important cereal crops as staple food for more than 2 billion people. Many phytopathogens, including *Fusarium graminearum*, reduce the yield quantity and quality in cereal crops and cause severe economic losses throughout the world (Kelly and Ward 2018). This pathogen infects wheat seeds and causes damping-off (Rasiukeviciute and Kelpsiene 2018), which decreases seed germination and seedling emergence in regions with mild and semitropical climates. *F. graminearum* produces sexual spores (ascospores) and asexual spores (macroconidia). The primary infection by ascospores appears in the spring (Leplat et al. 2013). Also, the pathogen can produce a variety of mycotoxins, threatening human health seriously (Leplat et al. 2013 and Omotayo et al. 2019).

The efforts to develop management methods against the disease are not satisfactory and promising. Indeed, non-chemical methods, such as crop residue removal, crop rotation, pathogen free-seed, and cultivation are not the solution for cereal-intensive areas (Peltonen-Sainio et al. 2009 and Vinale et al. 2009). Furthermore, the efficacy of these practices does not contribute to control the disease due to environmental conditions stimulating pathogen growth and disease incidence (Perez et al. 2008 and Landschoot et al. 2013).

Recently, chemical control against plant diseases is proposed as the most effective method. The unconscious and overuse of pesticides causes environmental pollution and degradation of the ecosystem. Besides, the resistance of target organisms as a result of long-term use of pesticides is another disadvantage. Moreover, systemic

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fungicides can be easily absorbed by soil, causing pollution of foods and environment (Schaafsma et al. 2005 and Satapute et al. 2019). Biological control of plant pathogens such as by *F. graminearum* may provide feasible alternatives instead of synthetic fungicides (Jogaiah et al. 2018 and Joshi et al. 2019).

A few documented studies about the possible antagonistic activity of *Gliocladium roseum*, *Saccharomyces cerevisiae*, and *Sordaria fimicola* have been reported against *Fusarium* spp. For instance, *G. roseum* was revealed as a soil-borne endophyte against *Fusarium* spp. (Zhang et al. 2008 and Hue et al. 2009), stimulating plant growth against *F. culmorum* in winter wheat (Knudsen et al. 1995) and reducing the colonization of *Fusarium* species in wheat and maize by suppressing sporulation production (Luongo et al. 2005 and Woo et al. 2006). *S. cerevisiae* was reported as a good producer of antifungal antibiotics against growth of *Fusarium* spp. (Suzzi et al. 1995), promoting seed germination and plant growth due to indole-3-acetic acid (IAA) producing ability on tomato and eggplant seedlings (Attyia and Youssry 2001). The antagonistic activity of *S. fimicola* was only reported in maize against *F. graminearum* under greenhouse conditions (Abdallah et al. 2018).

The present study aimed to evaluate the antagonistic activity of 3 isolates; *Gliocladium roseum*, *Saccharomyces cerevisiae*, and *Sordaria fimicola* and to determine the most effective antagonistic agents against *F. graminearum* in susceptible wheat cultivars.

Materials and methods

Materials

The pathogen was isolated from wheat seed coats of the infected samples. Potato dextrose agar (PDA) medium was used for the isolation and cultivation of the pathogen. Rose Bengal agar (RBA) and PDA were used for the isolation and cultivation of the antagonists obtained from soil samples. The soil samples were taken at 15-cm depth from rhizosphere to obtain a high diversity of antagonists and from 15 various areas in the province of Tekirdag (Turkey) throughout September 2018. The seeds of the susceptible wheat cultivars "Gun 91 and Sultan 95" were purchased and used to determine the antagonistic activity of the bio-agent treatments against *F. graminearum*. The study was conducted in vitro and in vivo assays (foliar, seed, soil and seed + soil treatments) at 5 concentrations of 1×10^5 to 1×10^9 spores/ml. In vivo assays were carried out using 30 samples of the wheat seeds per pot (22 × 15-cm diameter) containing 100 g sterile peat.

Isolation and identification of the causal pathogen and the bio-agents

The wheat seed samples were disinfected by 1% sodium hypochlorite solution (SHS) for 5 min and rinsed 3 times with sterile distilled water (SDW). After drying process on sterile filter paper, the seeds were placed onto PDA plates and incubated at 28 °C for 7 days. After incubation period, the fungal isolates were purified by a single spore technique and kept at − 10 °C throughout the study. The purified isolates were identified according to cultural, morphological (Ellis 1971 1976; Booth 1971 1977; Karaca 1974; Domsch et al. 1980; Nelson et al. 1981; Burgess et al. 1994), microscopic (Nelson et al. 1981; Toussoun and Nelson 1995), and pathological (Nelson et al. 1981; Singh et al. 1991; Toussoun and Nelson 1995) properties. The pathogenicity of 20 isolates of *F. graminearum* was pre-assessed on the basis of the number of dead seedlings of both wheat cultivars after 15 days under plant growth room conditions (at 25 °C, 16 h of photoperiod and 100% RH) to detect the most pathogenic fungal isolate for the next experiments. The experiment was carried out according to the same procedure as in vivo assays.

The soil samples were sieved and dried for a week at 18 °C and then kept at 10 °C. The antagonistic microbial flora was incubated for 7–9 days and isolated with serial dilution technique (Rai and Kovics 2010) on RBA (dextrose, 10 g; soy peptone, 5 g; KH_3PO_4, 1 g; $MgSO_4$, 0.5 g; streptomycin sulfate salt, 1.0 g; Rose Bengal, 0.05 g; agar, 15 g; chloramphenicol, 0.1 g; distilled water, 1000 ml) for

Table 1 Effect of the bio-agents on in vitro mycelial growth of *F. graminearum*

	G. roseum		*S. cerevisiae*		*S. fimicola*	
Concentrations (spores/ml)	Mycelial growth (cm)	Inhibition rate (%)	Mycelial growth (cm)	Inhibition rate (%)	Mycelial growth (cm)	Inhibition rate (%)
1×10^5	4.7 ± 0.56c*	53.0	4.0 ± 0.87d*	60.0	3.7 ± 0.68de*	67.0
1×10^6	3.9 ± 0.45cd	61.0	3.3 ± 0.74de	67.0	3.0 ± 0.21e	70.0
1×10^7	3.2 ± 0.85d	68.0	2.6 ± 0.25e	74.0	2.1 ± 0.74ef	79.0
1×10^8	2.5 ± 0.21de	75.0	2.0 ± 0.22ef	80.0	1.5 ± 0.26efg	85.0
1×10^9	1.6 ± 0.18e	84.0	1.2 ± 0.10f	88.0	0.9 ± 0.12g	91.0
Control	10.0 ± 0.91a	-	10.0 ± 0.83a	-	10.0 ± 0.74a	-

*Values expressed are mean (± standard error) of five replicates
Values given separately for in vitro treatments within each column followed by different letters are significantly different at $p < 0.05$

growth of antagonistic yeasts, and on PDA (agar, 15.0 g; dextrose, 20.0 g; potato extract, 4.0 g; streptomycin sulfate salt, 1.0 g; distilled water, 1000 ml) for growth of fungal antagonists. Consequently, the single spore or hyphal tip was taken from the developing fungal colonies and transferred onto PDA medium containing streptomycin sulfate salt (1.0 g/l) and incubated at room temperature (25 ± 2 °C) for a week. The selected 30 antagonistic isolates were identified according to mycological keys (Lundquist 1972; Vaughan Martini and Martini 1993 and Gams and Bissett 1998). In this study, a preliminary experiment was conducted to assess the identified isolates exhibiting the highest antagonistic activity against *F. graminearum* on PDA medium. The experiment was carried out according to the same procedure as in vitro assays. As a result of the assessment, the most antagonistic isolates were selected and used for the next experiments. In order to prepare the spore suspensions, the pathogen cultures were passed through 2 layers of cheesecloth, diluted with sterile distilled water to a concentration of 1×10^5 conidia/ml and centrifuged at 4.000 rpm for 5 min at 22 °C. The cultures of the bio-agents were blended in an electric blender for 2 min and prepared as a liquid suspension with sterile distilled water. All bio-agents were adjusted at 5 different concentrations from 1×10^5 to 1×10^9 spores/ml and centrifuged at 5500 rpm for 5 min for *S. cerevisiae* (Janson and Elshadei 2012) and at 2500 rpm for 10 min for *G. roseum* and *S. fimicola*. The obtained supernatants were filtered through grade no. 1 Whatman filter paper and used for in vitro and in vivo assays.

Determination of the antagonistic activity of the bio-agents (in vitro)

One disc (0.5-cm diameter) of 7-day-old culture of the pathogen isolate was placed to the center of the plate. The adjusted spore suspensions of *G. roseum*, *S. cerevisiae*, and *S. fimicola* were streaked parallel on either side of the fungal disc at a distance of 2 cm (Jalaluldeen et al. 2014). In addition, a disc (0.5 cm) of *G. roseum-S. cerevisiae, G. roseum-S. fimicola* and, *S. cerevisiae-S. fimicola* was placed at a distance of 2 cm from PDA plate edge to evaluate the antagonistic interaction between each other. The plates were incubated at 25 °C for 7–9 days. After the incubation period, the diameter of each colony was measured to evaluate the antagonistic activity as a result of the average of 5 independent replicates. The antagonist-free PDA medium, containing only SDW and a culture disk of the pathogen, was used as a control. The effect of the bio-agent treatments on mycelial growth of the pathogenic fungus was determined after 7 days and calculated using the following formula (Topps and Wain 1957).

$$I\% = [(C-T)/C] \times 100$$

where I % = inhibition rate, C = average diameter of mycelial growth of pathogenic fungus in control

Fig. 1 The mycelial growth of *F. graminearum* after in vitro treatments with the bio-agents at 1×10^9 spores/ml and the control treatment

treatment, and T = average diameter of mycelial growth of pathogenic fungus after bio-agent treatments.

Determination of in vivo antagonistic activity of the bio-agents on leaf infection

The wheat seed samples were disinfected by 1% SHS for 5 min and rinsed 3 times with SDW before bio-agent treatments. The seeds of wheat cultivars "Gun 91and Sultan 95" were planted in experimental pots (22 × 15-cm diameter) containing a sterile peat and were grown in the plant growth room conditions. When plants reached the two-leaf stage in 2 weeks, *G. roseum*, *S. cerevisiae*, *S. fimicola*, and a mixture of the bio-agents (*G. roseum* + *S. cerevisiae*, *G. roseum* + *S. fimicola*, *S. cerevisiae* + *S. fimicola*) were sprayed as a foliar treatment separately at concentrations of 1×10^5 to 1×10^9 spores/ml to upper and lower surfaces of wheat leaves with a dose-adjusted spray at the rate of 0.2 ml per leaf. After the inocula were allowed to dry on the leaves (approximately 1–2 h), the pathogen inoculum at a concentration of 1×10^5 conidia/ml was applied to wheat leaves in the same way. Following the inoculation process, the plants were

Table 2 Effect of different concentrations of the foliar treatments on lesion length caused by *F. graminearum* in wheat cultivars

Foliar treatments	Concentrations (spores/ml)	Wheat cultivars			
		Gun 91		Sultan 95	
		Lesion length (mm)	Inhibition rate (%)	Lesion length (mm)	Inhibition rate (%)
G. roseum	1×10^5	9.7 ± 1.56c*	34.4	8.4 ± 0.90cd*	36.3
	1×10^6	7.7 ± 0.90d	47.9	6.7 ± 0.81d	49.2
	1×10^7	6.7 ± 1.25d–h	54.7	5.8 ± 0.31e	56.0
	1×10^8	5.6 ± 0.67e–g	62.1	4.3 ± 0.81f–h	67.4
	1×10^9	3.5 ± 0.12g	76.3	2.9 ± 0.21h	78.0
S. cerevisiae	1×10^5	8.9 ± 0.57cd	39.8	7.8 ± 0.55c–e	40.9
	1×10^6	7.0 ± 0.42d–g	52.7	6.1 ± 0.90def	53.7
	1×10^7	5.7 ± 0.71e–g	61.4	5.0 ± 0.45ef	62.1
	1×10^8	4.5 ± 0.21fg	69.5	3.9 ± 0.05g	70.4
	1×10^9	2.7 ± 0.73hi	81.7	2.1 ± 0.01h–j	84.0
S. fimicola	1×10^5	8.5 ± 0.43c–e	42.5	7.3 ± 0.87c–f	44.6
	1×10^6	6.8 ± 0.97d–h	54.0	5.7 ± 0.56e	56.8
	1×10^7	5.5 ± 0.35e–g	62.8	4.6 ± 0.41fg	65.1
	1×10^8	4.0 ± 1.08f–i	72.9	3.2 ± 0.16g–i	75.7
	1×10^9	2.4 ± 0.65h–j	83.7	1.8 ± 0.06i	86.3
G. roseum + *S. cerevisiae*	1×10^5	9.1 ± 0.78cd	38.5	8.0 ± 0.59c–e	39.3
	1×10^6	7.4 ± 0.43d–f	50.0	6.6 ± 0.32d	50.0
	1×10^7	6.0 ± 0.79ef	59.4	5.1 ± 0.68ef	61.3
	1×10^8	4.9 ± 0.41f	66.8	3.5 ± 0.52gh	73.4
	1×10^9	3.1 ± 0.70g–i	79.0	2.4 ± 0.21hi	81.8
G. roseum + *S. fimicola*	1×10^5	9.1 ± 0.83cd	38.5	7.9 ± 0.45c–e	40.1
	1×10^6	7.2 ± 0.50d–g	51.3	6.2 ± 0.94def	53.0
	1×10^7	5.9 ± 0.35ef	60.1	5.0 ± 0.41ef	62.1
	1×10^8	4.7 ± 0.79fg	68.2	3.6 ± 0.91gh	72.7
	1×10^9	2.9 ± 0.72h	80.4	2.3 ± 0.15hi	82.5
S. cerevisiae + *S. fimicola*	1×10^5	6.0 ± 0.70ef	59.4	5.1 ± 0.95ef	61.3
	1×10^6	4.9 ± 0.68f	66.8	4.0 ± 0.41f–i	69.6
	1×10^7	3.2 ± 0.92gh	78.3	2.3 ± 0.91hi	82.5
	1×10^8	1.9 ± 0.06ij	87.1	1.2 ± 0.05ij	90.9
	1×10^9	1.1 ± 0.04j	92.5	0.6 ± 0.01j	94.4
Control	-	14.8 ± 1.34a	-	13.2 ± 1.40a	-

*Values expressed are mean (± standard error) of five replicates

Values given separately for the foliar treatments within each column followed by different letters are significantly different at $p < 0.05$

kept in a plant growth room to provide optimum conditions for disease development for 72 h. Afterwards, the seedlings were kept at room temperature for 24 h (Kawasaki 2008). Consequently, the leaves were detached separately from the plants, and the disease incidence was determined according to visually necrotic lesion and/or sporulation area of the disease. The lesion area was measured as the average of 5 independent replicates and compared with the control pots sprayed with a spore suspension of the fungal pathogen only. The effect of the bio-agent treatments on leaf infection was calculated using the formula of Topps and Wain (1957).

$$I\% = [(C-T)/C] \times 100$$

where I % = inhibition rate, C = average lesion length in control treatment, and T = average lesion length after bio-agent treatments.

Table 3 Effect of different concentrations of the seed treatments on damping-off caused by *F. graminearum* in wheat cultivars

Seed treatments	Concentrations (spores/ml)	Wheat cultivars			
		Gun 91		Sultan 95	
		Incidence of damping-off (%)	Inhibition rate (%)	Incidence of damping-off (%)	Inhibition rate (%)
G. roseum	1×10^5	45.7 ± 0.24c–e*	54.3	40.0 ± 0.25cd*	60.0
	1×10^6	40.3 ± 0.83d	59.7	33.0 ± 0.44de	67.0
	1×10^7	33.7 ± 0.44e–g	66.3	28.3 ± 0.84e–h	71.7
	1×10^8	26.1 ± 0.21f–h	73.9	22.4 ± 0.31f–i	77.6
	1×10^9	18.5 ± 0.45g–i	81.5	14.2 ± 0.79hi	85.8
S. cerevisiae	1×10^5	49.0 ± 0.92b–f	51.0	41.2 ± 0.45c	58.8
	1×10^6	32.7 ± 0.12e–h	67.3	31.8 ± 0.90d–g	68.2
	1×10^7	27.2 ± 0.44fg	72.8	23.6 ± 0.87f–h	76.4
	1×10^8	20.7 ± 0.84g	79.3	16.5 ± 0.61g–j	83.5
	1×10^9	14.1 ± 0.10h–j	85.9	9.5 ± 0.28i–k	90.5
S. fimicola	1×10^5	43.5 ± 0.89c–g	56.5	35.304.91c–h	64.7
	1×10^6	37.0 ± 0.44d–h	63.0	29.5 ± 0.21e–g	70.5
	1×10^7	31.6 ± 0.23e–i	68.4	23.6 ± 0.13f–h	76.4
	1×10^8	21.8 ± 0.90f–l	78.2	16.5 ± 0.91g–j	83.5
	1×10^9	15.3 ± 0.36hi	84.7	11.8 ± 0.21i	88.2
G. roseum + *S. cerevisiae*	1×10^5	46.8 ± 0.71cd	53.2	38.9 ± 0.60c–e	61.1
	1×10^6	35.9 ± 0.67d–k	64.1	33.0 ± 0.13de	67.0
	1×10^7	30.0 ± 0.61e–j	70.6	25.9 ± 0.61f	74.1
	1×10^8	20.7 ± 0.41g	79.3	18.9 ± 0.69gh	81.1
	1×10^9	16.4 ± 0.21g–k	83.6	10.6 ± 0.11ij	89.4
G. roseum + *S. fimicola*	1×10^5	44.6 ± 0.78c–f	55.4	37.7 ± 0.91c–f	62.3
	1×10^6	38.1 ± 0.81d–g	61.9	30.6 ± 0.50ef	69.4
	1×10^7	32.7 ± 0.11e–h	67.3	24.8 ± 0.31fg	75.2
	1×10^8	24.0 ± 0.21f–i	76.0	18.9 ± 0.26gh	81.1
	1×10^9	17.4 ± 0.10g–j	82.6	13.0 ± 0.20h–j	87.0
S. cerevisiae + *S. fimicola*	1×10^5	29.4 ± 0.47e–j	70.6	23.6 ± 0.82f–h	76.4
	1×10^6	22.9 ± 0.89fk	77.1	15.3 ± 0.41g–k	84.7
	1×10^7	13.1 ± 0.67h–k	86.9	8.3 ± 0.17i–l	91.7
	1×10^8	7.7 ± 0.31j	92.3	3.7 ± 0.11j–l	96.3
	1×10^9	2.2 ± 0.11lm	97.8	1.2 ± 0.03kl	98.8
Control	-	61.3 ± 1.11a	-	56.6 ± 1.03a	-

*Values expressed are mean (± standard error) of five replicates
Values given separately for the seed treatments within each column followed by different letters are significantly different at $p < 0.05$

Determination of in vivo antagonistic activity of the bio-agents on damping-off

To evaluate the antagonistic activity of the bio-agents against *F. graminearum*, different concentrations of each of *G. roseum*, *S. cerevisiae*, *S. fimicola*, and their mixtures (*G. roseum* + *S. cerevisiae*, *G. roseum* + *S. fimicola*, *S. cerevisiae* + *S. fimicola*) were examined as the seed, soil, and seed + soil treatments.

To determine the antagonistic activity of the seed treatments against damping-off in wheat cultivars, the pathogen inoculum at the rate of 5% w/v (Hussein 1973) was transferred to pots containing an autoclaved sterile peat, mixed thoroughly, and kept by moisturizing for 7 days. After the incubation period, the bio-agent suspensions were applied at different concentrations to the disinfected wheat seeds by spraying with a dose-adjusted spray to cover the seed surface homogenously at the rate of 20 ml/kg of seeds before sowing in pots.

To determine the antagonistic activity of the soil treatments, the pathogen inoculum at the rate of 5% w/v (Hussein 1973) and the spore suspensions of

Table 4 Effect of different concentrations of the soil treatments on damping-off caused by *F. graminearum* in wheat cultivars

Soil treatments	Concentrations (spores/ml)	Wheat cultivars			
		Gun 91		Sultan 95	
		Incidence of damping-off (%)	Inhibition rate (%)	Incidence of damping-off (%)	Inhibition rate (%)
G. roseum	1×10^5	51.1 ± 0.25b–d*	48.9	45.9 ± 0.41b–d*	54.1
	1×10^6	43.5 ± 0.31c–g	56.5	38.9 ± 0.87c–e	61.1
	1×10^7	38.1 ± 0.96d–g	61.9	30.6 ± 0.22ef	69.4
	1×10^8	31.6 ± 0.33e–i	68.4	25.9 ± 0.29f	74.1
	1×10^9	24.0 ± 0.19f–i	76.0	20.0 ± 0.11g	80.0
S. cerevisiae	1×10^5	54.4 ± 0.78b	45.6	47.1 ± 0.80bc	52.9
	1×10^6	47.9 ± 0.44c	52.1	37.7 ± 0.17c–f	62.3
	1×10^7	38.1 ± 0.81d–g	61.9	29.5 ± 0.47e–g	70.5
	1×10^8	26.1 ± 0.79f–h	73.9	22.4 ± 0.18f–i	77.6
	1×10^9	19.6 ± 0.43gh	80.4	15.3 ± 0.12g–k	84.7
S. fimicola	1×10^5	49.0 ± 0.89b–f	51.0	41.2 ± 0.61c	58.8
	1×10^6	42.4 ± 0.57c–h	57.6	34.2 ± 0.72d	65.8
	1×10^7	38.1 ± 0.31d–g	61.9	27.1 ± 0.43e–i	72.9
	1×10^8	27.2 ± 0.88fg	72.8	23.6 ± 0.21f–h	76.4
	1×10^9	21.8 ± 0.67f–l	78.2	17.7 ± 0.12g–i	82.3
G. roseum + *S. cerevisiae*	1×10^5	53.3 ± 0.42bc	46.7	44.8 ± 0.49b–e	55.2
	1×10^6	44.6 ± 0.92c–f	55.4	36.5 ± 0.41c–g	63.5
	1×10^7	35.9 ± 0.21d–k	64.1	27.1 ± 0.80e–i	72.9
	1×10^8	27.2 ± 0.73fg	72.8	24.8 ± 0.87fg	75.2
	1×10^9	21.8 ± 0.30f–l	78.2	17.7 ± 0.36g–i	82.3
G. roseum + *S. fimicola*	1×10^5	50.0 ± 0.61b–e	50.0	43.6 ± 0.10b–f	56.4
	1×10^6	40.3 ± 0.33d	59.7	36.5 ± 0.82c–g	63.5
	1×10^7	34.8 ± 0.31ef	65.2	27.1 ± 0.98e–i	72.9
	1×10^8	28.3 ± 0.81f	71.7	22.4 ± 0.74f–i	77.6
	1×10^9	20.7 ± 0.61g	79.3	16.5 ± 0.31g–j	83.5
S. cerevisiae + *S. fimicola*	1×10^5	34.8 ± 0.43ef	65.2	28.3 ± 0.25e–h	71.7
	1×10^6	25.0 ± 0.56f–j	75.0	21.2 ± 0.22f–j	78.8
	1×10^7	18.5 ± 0.39g–i	81.5	13.0 ± 0.14h–j	87.0
	1×10^8	10.9 ± 0.38i	89.1	8.3 ± 0.10i–l	91.7
	1×10^9	4.4 ± 0.10k	95.6	3.6 ± 0.07j–l	96.4
Control	-	61.3 ± 1.18a	-	56.6 ± 1.09a	-

*Values expressed are mean (± standard error) of five replicates
Values given separately for the soil treatments within each column followed by different letters are significantly different at $p < 0.05$

tested bio-agents (1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 spores/ml) were added as a soil drench at the rate of 30 ml per each pot, mixed thoroughly, and kept by moisturizing for 7 days. After a week, the wheat seeds were sown in pots following disinfection with SHS.

For the seed + soil treatments, the pathogen inoculum and the spore suspensions of tested bio-agents were added to pots as a soil drench, mixed thoroughly, and kept by moisturizing for 7 days. At the end of a week, disinfected seeds were sprayed by spore suspensions of the tested bio-agents and were sown in pots. The experiment was attempted with an average of 5 independent replicates, compared with the control pots inoculated with a spore suspension of the fungal pathogen only. The study was conducted in a plant growth room under a 16 h photoperiod cycle at 25 °C. The number of dead seedlings was determined after 15 days, and the effect of the bio-agent treatments on damping-off was calculated using the following formula (Topps and Wain 1957).

Table 5 Effect of different concentrations of the seed + soil treatments on damping-off caused by *F. graminearum* in wheat cultivars

Seed + soil treatments	Concentrations (spores/ml)	Wheat Cultivars			
		Gun 91		Sultan 95	
		Incidence of damping-off (%)	Inhibition rate (%)	Incidence of damping-off (%)	Inhibition rate (%)
G. roseum	1×10^5	35.3 ± 0.67d–k*	64.7	29.5 ± 0.73e–g*	70.5
	1×10^6	28.3 ± 0.52f	71.7	22.4 ± 0.56f–i	77.6
	1×10^7	19.6 ± 0.43gh	79.3	16.5 ± 0.33g–j	83.5
	1×10^8	13.1 ± 0.30h–k	86.9	10.6 ± 0.21ij	88.2
	1×10^9	7.7 ± 0.18j	92.3	7.1 ± 0.13i–m	92.9
S. cerevisiae	1×10^5	32.7 ± 0.77e–h	67.3	30.6 ± 0.49ef	69.4
	1×10^6	26.1 ± 0.43f–h	73.9	23.6 ± 0.63f–h	76.4
	1×10^7	19.6 ± 0.65gh	80.4	13.0 ± 0.41h–j	87.0
	1×10^8	10.9 ± 0.32i	89.1	10.6 ± 0.37ij	89.4
	1×10^9	5.5 ± 0.11j–m	94.5	4.8 ± 0.15jk	95.2
S. fimicola	1×10^5	30.5 ± 0.60e–j	69.5	25.9 ± 0.50f	74.1
	1×10^6	21.8 ± 0.23f–l	78.2	17.7 ± 0.39g–i	82.3
	1×10^7	14.2 ± 0.35h–j	85.8	11.8 ± 0.26i	88.2
	1×10^8	9.8 ± 0.21i–k	90.2	8.3 ± 0.21i–l	91.7
	1×10^9	6.6 ± 0.15jk	93.4	5.9 ± 0.11j	94.1
G. roseum + *S. cerevisiae*	1×10^5	26.1 ± 0.34f–h	73.9	24.8 ± 0.67f g	75.2
	1×10^6	15.3 ± 0.27hi	84.7	16.5 ± 0.32g–j	83.5
	1×10^7	10.9 ± 0.21i	89.1	9.5 ± 0.24i–k	90.5
	1×10^8	7.7 ± 0.18j	92.3	5.9 ± 0.18j	94.1
	1×10^9	3.3 ± 0.05k–m	96.7	2.4 ± 0.12k	97.6
G. roseum + *S. fimicola*	1×10^5	29.4 ± 0.89e–j	70.6	27.1 ± 0.31e–i	72.9
	1×10^6	21.8 ± 0.54f–l	78.2	20.0 ± 0.22g	80.0
	1×10^7	15.3 ± 0.41ij	84.7	14.2 ± 0.27hi	85.8
	1×10^8	10.9 ± 0.43i	89.1	9.5 ± 0.15i–k	90.5
	1×10^9	5.5 ± 0.21j–m	94.5	4.8 ± 0.11jk	95.2
S. cerevisiae + *S. fimicola*	1×10^5	9.8 ± 0.12i–k	90.2	7.1 ± 0.09i–m	92.9
	1×10^6	7.7 ± 0.09j	92.3	4.8 ± 0.07jk	95.2
	1×10^7	6.6 ± 0.05jk	93.4	4.8 ± 0.05jk	95.2
	1×10^8	3.3 ± 0.05k–m	96.7	2.4 ± 0.02k	97.6
	1×10^9	1.1 ± 0.03m	98.9	1.2 ± 0.02kl	98.8
Control	-	61.3 ± 1.23a	-	56.6 ± 1.12a	-

*Values expressed are mean (± standard error) of five replicates

Values given separately for the seed + soil treatments within each row followed by different letters are significantly different at $p < 0.05$

$$I\% = [(C-T/C)] \times 100$$

where I % = inhibition rate, C = average of non-emerged seedlings in control treatment, and T = average of non-emerged seedlings after bio-agent treatments.

Statistical analysis

Statistical analysis of data was subjected to ANOVA (one-way analysis of variance). Significant differences ($p < 0.05$) were tested by the general linear model (GLM) procedure, using the Duncan's multiple range test (DMRT) for disease incidence, mycelial growth, and lesion length of the pathogenic fungus after bio-agent treatments.

Results and discussion

The present study is the first report indicating that *G. roseum*, *S. cerevisiae*, and *S. fimicola*, and in particular, the mixture of *S. cerevisiae* + *S. fimicola* was promising and effective against the cereal damping-off caused by *F. graminearum* in wheat cultivars.

In vitro antagonistic activity of the bio-agents

The selected isolates of *G. roseum*, *S. cerevisiae*, and *S. fimicola* showed the highest antagonistic activity inhibiting the growth of *F. graminearum* at a concentration of 1×10^9 spores/ml with an inhibition rate of 84, 88, and 91%, respectively. Accordingly, the decrease of mycelial growth of the pathogen was observed by increasing concentrations, particularly at 1×10^9 spores/ml, in comparison with the lower concentrations (Table 1, Fig. 1). The antagonistic activity of the bio-agents was similar to the in vitro studies as *G. roseum* inhibited growth of *F. graminearum* (Dubey et al. 2014), *F. culmorum*, *F. graminearum*, and *F. nivale* and caused degradation of hyphal walls (Pisi et al. 2001), *S. cerevisiae* and *S. fimicola* inhibited mycelial growth of *Fusarium oxysporum* (Manoch et al. 2008 and Shalaby and El-Nady 2008).

In vivo antagonistic activity of the bio-agents

Antagonistic activity of G. roseum against F. graminearum

In the present study, *G. roseum* had a high antagonistic activity against the pathogen growth in both wheat cultivars at a concentration of 1×10^9 spores/ml of the foliar,

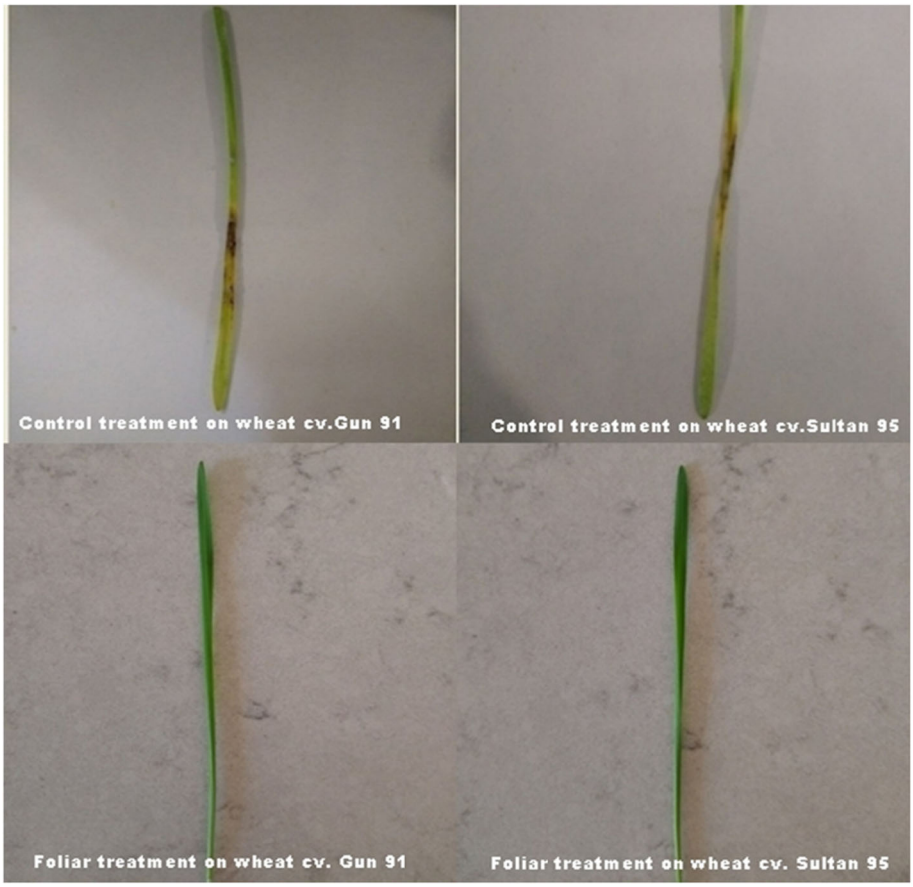

Fig. 2 The leaf infection caused by *F. graminearum* in wheat cultivars after the foliar treatments with *S. cerevisiae* + *S. fimicola* at 1×10^9 spores/ml and the control treatments

soil, and seed treatments with an inhibition rate above 76% (Tables 2, 3, and 4); and the seed + soil treatment in particular exhibited an inhibition rate above 92% (Table 5). These are in agreement with data obtained by other researches, which indicated that *G. roseum* stimulated plant growth and decreased growth of *F. culmorum* in winter wheat (Knudsen et al. 1995), and *Gliocladium* spp. reduced the colonization of *Fusarium* species in wheat and maize plants (Luongo et al. 2005 and Woo et al. 2006). When taking into account that *G. roseum* had biosynthetic gene clusters to encode a large number of enzyme complexes and secondary metabolites, reported by Karlsson et al. (2015) and Sun et al. (2015), it was suggested that the main reason of antagonistic activity was related to the production of antifungal substances such as enzymes and secondary metabolites. In this manner, as a result of the interaction among them, *G. roseum* might damage to the cell structure along with hyphal penetration and promote disruption of pathogen cell.

Antagonistic activity of *S. cerevisiae* against *F. graminearum*

The increment of *S. cerevisiae* concentration resulted in a decrease of the disease incidence, and inhibition rates of the yeast at a concentration of 1×10^9 spores/ml of the foliar, soil, and seed treatments were determined to be above 80% for both wheat cultivars (Tables 2, 3, and 4). However, at the seed + soil treatment, at a 1×10^9 spores/ml concentration of the yeast, the inhibition rate reached to approximately 95% for both wheat cultivars (Table 5). Obtained findings were in agreement with Shalaby and El-Nady (2008), who reported that *F. oxysporum* was adequately controlled by *S. cerevisiae* at $5\ g\ l^{-1}$ (2.78×10^8 cell/ml) and that incidence of damping-off was 6.67%. Based on a study conducted by Attyia and Youssry (2001) related to indole-3-acetic acid (IAA) producing ability of *S. cerevisiae* against *Fusarium* spp. in tomato and eggplant seedlings; it was supposed that the yeast treatments could exhibit plant growth-promoting activity by releasing IAA, which improved plant resistance to *F. graminearum*, and suppressed therefore the pathogen growth. So it was concluded that *S. cerevisiae* increased the division and expansion of plant cells, stimulated plant growth parameters, and consequently decreased the disease incidence.

Antagonistic activity of S. fimicola against F. graminearum

The antagonistic activity of *S. fimicola* at a concentration of 1×10^9 spores/ml of the soil treatment showed

Fig. 3 Damping-off caused by *F. graminearum* in wheat cultivars after the seed + soil treatments with *S. cerevisiae* + *S. fimicola* at 1×10^9 spores/ml and the control treatments

relatively high rates of inhibition (above 78%), following the foliar and seed treatments with an inhibition rate above 83% for both wheat cultivars (Tables 2, 3, and 4). Moreover, the seed + soil treatment at the same concentration showed an inhibition rate above 90% against growth of *F. graminearum* (Table 5). So far, the antagonistic activity of *S. fimicola* was only reported in maize against *F. graminearum* under greenhouse conditions by Abdallah et al. (2018), who found that it inhibited growth of *F. graminearum* and reduced production of zearalenone. Based on a previous study by Dewan et al. (1994), the antagonistic activity was attributed to release compounds (triacontanol and indole-3-carboxaldehyde) from *S. fimicola*, acting as natural growth stimulators to contribute the plant growth and the reduction of disease incidence of *Gaeumannomyces graminis var. tritici*. In the present study, the antagonistic activity of *S. fimicola* and its supportive effect on plant development against the pathogenic fungus were probably related to production of these bioactive compounds.

Antagonistic activity of the mixture of the bio-agents against F. graminearum

When evaluating mixture of the bio-agents, the foliar (Fig. 2), seed and soil treatments of *S. cerevisiae* + *S. fimicola* at a concentration of 1×10^9 spores/ml exhibited an antagonistic activity with an inhibition rate above 87% for both wheat cultivars (Tables 2, 3, and 4). In addition, it was determined that the seed + soil treatment of *S. cerevisiae* + *S. fimicola* had an antagonistic activity by over 90% even at a concentration of 1×10^5 spores/ml; and the pathogen growth was inhibited almost completely at concentrations of 1×10^8 and 1×10^9 spores/ml with an inhibition rate above 96% (Table 5, Fig. 3). It was concluded that there was a synergistic interaction and almost no antagonistic activity between *S. cerevisiae* and *S. fimicola*. Indeed, in vitro results confirmed that they had approximately similar diameters of the mycelial growth (5.5 cm for *S. cerevisiae* and 5.8 cm for *S. fimicola*, respectively) on PDA medium. However, the seed + soil treatment of *G. roseum* + *S. fimicola* and *G. roseum* + *S. cerevisiae* showed similar results with all single treatments of *G. roseum*, *S. cerevisiae*, and *S. fimicola*. It was attributed that the released compounds from *S. fimicola* and *S. cerevisiae* probably suppressed the growth of *G. roseum* in the same way as in vitro results. Therefore, there was no synergistic interaction between *G. roseum* and other bio-agents against *F. graminearum*, emphasizing that *G. roseum* showed a poor mycelial growth with diameters of 3.5 and 3.0 cm against *S. cerevisiae* and *S. fimicola*, respectively. It was considered that a combination of the seed and soil treatments containing *S. cerevisiae* + *S. fimicola* at a concentration of 1×10^9 spores/ml had a synergistic effect on antagonistic activity against *F. graminearum*.

Conclusion

The isolates of *G. roseum*, *S. cerevisiae*, and *S. fimicola* and, in particular, the mixture of *S. cerevisiae* + *S. fimicola* had a high capacity in terms of antagonistic activity at increasing concentrations against *F. graminearum* in both wheat cultivars. It is supposed that application of biological preparations containing these bio-agents will be significantly promising and applicable as an alternative tool to synthetic chemicals and other low-efficiency or harmful methods for the control of damping-off caused by *F. graminearum*.

Abbreviations

IAA: Indole-3-acetic acid; PDA: Potato dextrose agar; RBA: Rose Bengal Agar; SDW: Sterile distilled water; SHS: Sodium hypochlorite solution

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Author's contributions

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