

Effect of Biological Seed Treatment with *Cylindrocarpon olidum* var. *olidum* on Control of Common Bunt (*Tilletia laevis*) of Wheat

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The goals of the present study were to determine if *Cylindrocarpon olidum* var. *olidum* exerts antibiotic activity on teliospore germination of *Tilletia laevis* *in vitro* and to evaluate the efficacy of a seed treatment with this antagonist against artificial seedborne infection in the field under natural cropping conditions. Germination of bunt teliospores was completely inhibited *in vitro* on water agar medium supplemented with the antagonist's culture filtrate. In field experiments seed application reduced the incidence of common bunt from 82.9% to 40.4%, and from 81.4% to 42.0% in 1995/1996 and 1997/1998, respectively. *C. olidum* var. *olidum* may have a potential against *T. laevis* and is worthy of further research on its use as a biocontrol agent for common bunt.

KEY WORDS: Biological control; common bunt; *Cylindrocarpon olidum*; *Tilletia foetida*; *Triticum aestivum*.

INTRODUCTION

Common bunt in wheat (*Triticum aestivum* L.) caused by *Tilletia caries* (DC.) Tul. and C. Tul. and *T. laevis* J.G. Kühn is one of the most important diseases worldwide wherever wheat is grown. The seedborne pathogens infect the germinating seedling and grow systemically in infected wheat plants. Just when the ears are formed, mycelia invade the developing seeds and convert into masses of teliospores that form 'bunt balls' in place of kernels. These structures break easily during threshing and the liberated teliospores attach to healthy seeds or contaminate the soil. When spore-contaminated seeds are sown, the spores germinate synchronously with the seeds and infect the germinating plants (1,9). The causal agents show differences in geographical distribution in Turkey as in other countries. Özkan and Damgacı (*Bull. Plant Prot.* (1985) in Turkish) stated that *T. laevis* was the dominant species in Turkey, except for southeast Anatolia, with a prevalence rate between 84.1% and 96.7%.

Being a seedborne disease, general crop protection measures including rotation are inadequate to control common bunt (11) and seed treatment with fungicides seems to be necessary. However, particularly in the last decades, partly because of the perceived health and environmental risks associated with chemical pesticides, the trend in agriculture toward alternative disease management strategies has increased (6).

In order to find substitutes for chemicals to control common bunt, studies are being conducted of alternative methods, mainly focusing on different organic seed treatments

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such as uses of plant extracts, cereal and mustard flour, milk powder and other organic compounds (2,4,5,7,20) as well as antagonistic microorganisms (3,8,10,12-15).

There have been several reports dealing with biological control of common bunt by antagonistic microorganisms. Some isolates of *Streptomyces* and *Bacillus* species (3,14), some strains of *Pseudomonas fluorescens* (15), several isolates of *P. fluorescens* and *P. putida* (8), a *Pseudomonas chlororaphis* strain – MA 342 (3,10,12), some *Gliocladium* species and *Trichoderma harzianum* (3) were evaluated for their effectiveness in controlling common bunt pathogens *in vitro* or *in vivo*. Antibiotic activity of *Cylindrocarpon olidum* var. *olidum* (Wolenz.) Wolenz. against 25 important soilborne pathogenic fungi was first reported by Turhan (19). Two organic acids isolated from cultures of *C. olidum* were found to have inhibitory properties towards some fungi and Gram-positive bacteria (16).

The current study was designed to evaluate the efficacy of *C. olidum* var. *olidum* against *T. laevis* *in vitro* and to investigate this antagonist for its potential to reduce the incidence of common bunt in wheat caused by this pathogen.

MATERIALS AND METHODS

Origin of *C. olidum* var. *olidum* and *T. laevis* The *C. olidum* var. *olidum* isolate tested in this study was originally obtained from the rhizosphere of *Liquidambar orientalis* Mill. (oriental sweet gum, syn. levant storax) in Marmaris, Turkey, and identified by Prof. Dr. W. Gams, CBS, the Netherlands (18). The teliospores of *T. laevis* were collected from bunt balls of naturally infected spikes obtained from the Plant Protection Research Institute, in Bornova, Turkey. The bunt-sensitive wheat cultivar (*Triticum aestivum* L. cv. ‘Cumhuriyet 75’), was used as host plant.

Testing the inhibition of teliospore germination *in vitro* *Cylindrocarpon olidum* var. *olidum* was grown in stationary flasks in potato dextrose (PD) broth at 25°C for 12 days in the dark and the culture filtrate was separated with Whatman 42 filter paper. The culture filtrate was added to sterile molten water agar (0.8% and supplemented with 0.3% Ca(NO₃)₂) at a ratio of 1:40, before pouring it into the petri dishes, 20 ml each (17).

Control groups were designed in two ways: (a) petri dishes having water agar with Ca (NO₃)₂ only; and (b) petri dishes having the same medium supplemented with PD, in place of culture filtrate, at a ratio of 1:40 before pouring it. The final pH of all media was measured as 5.39.

Preparing the teliospore suspension of *T. laevis*, two bunt balls were surface-sterilized in a 1% sodium hypochlorite solution for 2 min and washed up with sterile distilled water (15). Then they were crushed in 20 ml distilled water and filtered through two layers of cheesecloth allowing the teliospores to pass through. Then, 0.2 ml of the just prepared suspension was applied as a drop on the surface of the agar layer and smeared over it with the aid of a glass loop. After incubation at 10°C for 18 days in darkness (17), the agar surface is examined directly under the microscope and by taking 100 teliospores into account on each petri plate, the germination rate was evaluated. Teliospores were recorded as germinated if the germ tubes (young promycelia) were visible. *In vitro* experiments were set up in four replicates, each consisting of three petri plates and arranged in a randomized plot design. The data were logit transformed and analyzed using the SPSS software (SPSS for Windows, release 9.0.0; SPSS Inc.). The germination rates in the treatments were compared using Duncan’s multiple range test at $P \leq 0.05$.

Antagonist inoculum for field trials Still surface liquid cultures of *C. olidum* var. *olidum* were grown on 50 ml PD broth in 250 ml Erlenmeyer flasks at 25°C in the dark for 25 days. Culture filtrate was separated through a tea strainer. The filtrate containing conidia was adjusted to 5×10^5 conidia ml⁻¹ and used after being kept overnight in a refrigerator.

Seed treatment Teliospores were obtained by crushing the bunt balls from the previous year. Seeds were inoculated with dry spores of the pathogen (3.5 g teliospores per 1 kg seed) by mixing them in a jar (20). Then wheat seeds were treated using a method modified from Weller and Cook (19). For each treatment, 650 g infested seeds were wetted either with 75 ml water (control #1) or with the culture filtrate of the antagonist (75 ml) supplemented with 375 mg methylcellulose (Methocel A 15) to enhance the stickiness, and the jar was shaken vigorously for approximately 5 min for uniform distribution over the seed surface. A second control (control #2) was created by wetting seeds with water supplemented with methylcellulose to determine whether the latter had any effect on bunt incidence.

The treatments were as follows: (i) sowing the seeds infested with the teliospores and wetted with distilled water only (control #1); (ii) sowing the seeds infested with the teliospores and wetted with distilled water supplemented with methylcellulose (control #2); and (iii) sowing the seeds infested with teliospores and wetted with culture filtrate of *C. olidum* var. *olidum* supplemented with methylcellulose.

Since it was found in a previous biotest (Turhan, unpublished) that the culture filtrate of *C. olidum* var. *olidum* displayed no phytotoxicity in the germination of cress seeds, the effect of the antagonist on wheat germination was not examined in this study.

Experimental design The field trials were performed in a randomized block design with four replications and repeated twice, during the growing periods of 1995/96 and 1997/98. Experimental units (plots) consisted of six rows, each 3 m long. In each row, 27 g of seeds was sown. Two rows consisting of untreated wheat seeds were sown between the neighboring replicates. During the experiments, the plants received no special treatment (including fertilizer and pesticide applications) except weeding in spring.

Field experiments were carried out in a non-sterilized sandy loam soil consisting of 53.5% sand, 30.5% silt, 17.0% clay and 2.8% organic matter, with a pH of 8.2 and a moisture holding capacity of 19.4%.

At maturity, all plants in each plot were harvested separately and the reaped spikes were transported to the laboratory in plastic bags to be evaluated later as healthy or bunted. The number of infected ears in each treatment was recorded and the percent disease control was calculated as follows:

$$100 - 100 \times (\text{no. of diseased plants in the treatment} / \text{no. of diseased plants in the control})$$

Statistical analysis was performed using the SPSS software (SPSS for Windows, release 9.0.0; SPSS Inc.). Differences between means were tested with Duncan's multiple range test, $P \leq 0.05$. All analyses with percent values were done after logarithmic transformation.

RESULTS

Inhibition of teliospore germination *in vitro* The results of the biotests with adding the culture filtrate of *C. olidum* var. *olidum* into the agar medium showed that the antifungal metabolites excreted by the antagonist prevented teliospore germination of *T.*

laevis completely. The germination rates were found to be 88.5% on water agar, 88.3% on water agar with PD broth, and zero on water agar with culture filtrate of the antagonist.

Effect of applying *C. olidum* var. *olidum* on bunt incidence Application of the antagonist culture onto the infested seeds significantly reduced the disease incidence in both plantings, compared with control (Table 1). The rate of protection was 51.3% and 48.4% in the consecutive trials, showing that the effect of the antagonist was consistent over 2 years. The use of methylcellulose as an adhesive resulted in no statistically significant difference in bunt incidence in the first year. Therefore, the control character without methylcellulose application (control #1) was excluded from the experiment and not tested in the second year.

DISCUSSION

Common bunt pathogens infect the germinating wheat plants *via* teliospores attached to the seeds or from inoculum in the soil. Germination of both seeds and teliospores, production of infectious hyphae and attack of the coleoptile occur synchronously at the pre-emergence stage in soil. Thus, biological control of common bunt can be achieved if the initial infection of seedlings can be prevented by the antagonist.

It was previously reported that *C. olidum* var. *olidum* possessed antibiotic activity against 25 soilborne fungi (18). So far there seems to be no information about the effectiveness of this antagonist as a biocontrol agent. The current study was designed to evaluate the efficacy of *C. olidum* var. *olidum* against *T. laevis* *in vitro* and in the field. The findings suggested that this antagonist completely inhibited the germination of bunt teliospores *in vitro*, as germination rates of the diagnosed spores were reduced from 88.5% in water agar and 88.3% in water agar supplemented with PD broth, to zero on agar medium with the antagonist's culture filtrate. In earlier studies, Kollmorgen and Jones (14) investigated the effects of soilborne microorganisms on the germination of chlamydospores of *T. caries* and *T. foetida* and found that some isolates of *Streptomyces* and *Bacillus* possessed inhibitory activity on both pathogens. In another study a strain of *P. fluorescens* (2-79) proved to inhibit teliospore germination of *T. laevis* (15).

The results of the present study (Table 1) support the control effect of *C. olidum* var. *olidum* on common bunt in both years under field conditions. Antagonist application reduced the rate of bunted spikes by 51.3% and 48.4% in the first and second plantings, respectively. Although the suppression was consistent over years, the effectiveness was not very high. This may have been due to the fact that the initial contamination was probably unnaturally high. It is possible that a greater degree of protection would have been attained under natural conditions. In our experiments, seeds were contaminated with teliospores at a rate of 3.5 g spores per kg in order to achieve a consistent infection. In a previous study of similar nature, pathogen inoculation had been accomplished by using 750 mg spores per kg. Seed treatment with *P. fluorescens* (2-79) reduced the infection level of common bunt (*T. laevis*) by up to 65% under field conditions (15). In another study, one kg of seeds was artificially infested by mixing it with 2 g of crushed *T. caries*-infected ears. Seed application with *P. chlororaphis* isolate MA342 controlled the disease and was as effective as the tested fungicide, significantly suppressing *T. caries* in the field (10).

In earlier studies dealing with biological control of common bunt (*T. caries*), bacterial isolates were generally evaluated as biocontrol agents. Elsherif and Grossmann (8) tested the antagonistic activity of several isolates of *P. fluorescens* and *P. putida* against the

TABLE 1. Results of two field trials showing the effect of antagonist application on bunt incidence (data are means of four replicates)

Treatments	Harvested heads (no.)		Bunted heads (no.)		Bunted heads (%)		Rate of protection (%)	
	1995/96	1997/98	1995/96	1997/98	1995/96	1997/98	1995/96	1997/98
<i>Tilletia laevis</i> (control #1)	1449.5 a ^z	nt ^y	1218.8 a	nt	84.2 a	nt	–	–
<i>T. laevis</i> + MC ^x (control #2)	1377.8 a	1566.3 a	1141.3 a	1274.3 a	82.9 a	81.4 a	–	–
<i>T. laevis</i> + MC + <i>Cylindrocarpon olidum</i>	1194.2 a	1481.0 a	492.0 b	622.5 b	40.4 b	42.0 b	51.3	48.4

^zWithin columns, means followed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's multiple range test.

^ynt, Not tested.

^xMethylcellulose.

pathogen in pot trials. The bacteria were applied by seed or root treatment or by placement in the planting hole. One of the isolates tested reduced the disease incidence, with an efficacy comparable to that of the fungicide tested. In field experiments, Johnsson *et al.* (12) controlled seedborne *T. caries* by seed application with isolate MA342 as effectively as guazatine+imazalil. Borgen and Davanlou (3) obtained the most promising results in the field, among the well characterized biologically based products, by a combination of milk powder and *P. chlororaphis* isolate MA342, which gave almost full control. However, a significant reduction in germination vigor was recorded at the dose providing maximum control and all higher doses.

It is concluded that *C. olidum* var. *olidum* may have a potential against *T. laevis* in organic agriculture and is worthy of further research on its use as a biocontrol agent for common bunt.

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REFERENCES

1. Agrios, G.N. (1988) Plant Pathology. Academic Press, London, UK.
2. Becker, J. and Weltzien, H.C. (1993) Bekämpfung des Weizensteinbrandes (*Tilletia caries* (D.C.) Tul. & C.Tul.) mit organischen Nährstoffen. *Z. PflanzenKr. PflanzenSchutz* 100:49-57.
3. Borgen, A. and Davanlou, M. (2000) Biological control of common bunt in organic agriculture. *J. Crop Prod.* 3: 159-174.
4. Borgen, A. and Kristensen, L. (2001) Use of mustard flour and milk powder to control common bunt (*Tilletia tritici*) in wheat and stem smut (*Urocystis occulta*) in rye in organic agriculture. *Proc. BCPC Symposium 2000, Seed Treatment: Challenges and Opportunities* (Birmingham, UK), pp. 141-150.
5. Borgen, A. and Nielsen, B.J. (2001) Effects of acetic acid in control of seed borne diseases. *Proc. BCPC Symposium 2000, Seed Treatment: Challenges and Opportunities* (Birmingham, UK), pp. 135-140.
6. Cook, R.J. and Baker, K.F. (1983) The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN, USA.
7. El-Naimi, M., Toubia-Rahme, H. and Mamluk, O.F. (2000) Organic seed-treatment as a substitute for chemical seed-treatment to control common bunt of wheat. *Eur. J. Plant Pathol.* 106:433-437.
8. Elsherif, M. and Grossmann, F. (1991) Versuche zur biologischen Bekämpfung einiger pathogenen Pilze durch fluoreszierende Pseudomonaden unter Anwendung verschiedenen Applikationsverfahren. *Z. PflanzenKr. PflanzenSchutz* 98:236-249.
9. Hoffmann, J.A. (1982) Bunt of wheat. *Plant Dis.* 66:979-986.
10. Hökeberg, M., Gerhardson, B. and Johnson, L. (1997) Biological control of cereal seedborne diseases by seed bacterization with greenhouse-selected bacteria. *Eur. J. Plant Pathol.* 103:25-33.
11. Johnsson, L. (1990) Survival of common bunt (*Tilletia caries* (DC) Tul.) in soil and manure. *Z. PflanzenKr. PflanzenSchutz* 97:502-507.
12. Johnsson, L., Hökeberg, M. and Gerhardson, B. (1998) Performance of the *Pseudomonas chlororaphis* biocontrol agent MA 342 against cereal seed-borne diseases in field experiments. *Eur. J. Plant Pathol.* 104:701-711.
13. Knudsen, I.M.B., Hockenhull, J., Jensen, D.F., Gerhardson, B., Hökeberg, M., Tahvonen, R. *et al.* (1997) Selection of biological control agents for controlling soil and seedborne diseases in the field. *Eur. J. Plant Pathol.* 103:775-784.
14. Kollmorgen, J.F. and Jones, L.C. (1975) The effects of soil-borne micro-organisms on the germination of chlamydospores of *Tilletia caries* and *T. foetida*. *Soil Biol. Biochem.* 7:407-410.
15. McManus, P.S., Ravenscroft, A.V. and Fulbright, D.W. (1993) Inhibition of *Tilletia laevis* teliospore germination and suppression of common bunt of wheat by *Pseudomonas fluorescens* 2-79. *Plant Dis.* 77:1012-1015.
16. Quaghebeur, K., Coosemans, J., Toppet, S. and Compennolle, F. (1994) Cannabiorci- and 8-chlorocannabiorci chromenic acid as fungal antagonists from *Cylindrocarpon olidum*. *Phytochemistry* 37:159-161.

17. Saydam, C., Copcu, M. and Ögüt, M. (1972) Effects of some chemicals against *Tilletia foetida* (Wallr.) Liro in vitro. *J. Turk. Phytopathol.* 1:108-114.
18. Turhan, G. (1994) *Cylindrocarpon olidum* (Wollenw.) Wollenw. var. *olidum* als starker Antagonist gegen Pilze und ein neuer Kandidat für die biologische Bekämpfung. *Mitt. Biol. Bundesanst. Land- Forstwirtschaft.* 301:356.
19. Weller, D.M. and Cook, R.J. (1983) Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* 73:463-469.
20. Winter, W., Rogger, C., Bänziger, I., Krebs, H., Rügger, A., Frei, P. et al. (1997) Weizenstinkbrand: Bekämpfung mit Magermilchpulver. *Agrarforschung* 4:153-156.