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Antagonistic effect of endophytes against several root-rot pathogens of wheat

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SUMMARY – Four different endophyte species were isolated from Rye grasses, *Triticum* spp. and Tall fescue. All *Neotyphodium* and *Acremonium* spp. significantly affected the growth rate of five root-rot pathogens of wheat in PDA plates. Culture filtrates of endophytes have had some effect against these test fungi. However nonfiltrated treatments are more efficient than culture filtrates. Conidia of *A. typhinum* and *D. sorokiniana* were mixed for germination test. The pathogen has shown abnormal elongation of the hypha, lysis of the conidia and abnormal germ tubes.

Key words: Endophytic fungi, wheat, antagonist, root-rot pathogens, *Neotyphodium*, *Acremonium*.

RESUME – *"Effet antagoniste de champignons endophytes contre plusieurs pathogènes de pourriture des racines du blé". Quatre espèces différentes d'endophytes ont été isolées dans des ray-grass,* Triticum *spp. et* Festuca arundinacea. *Toutes les espèces de* Neotyphodium *et* Acremonium *ont affecté de manière significative le taux de croissance de cinq champignons pathogènes de pourriture des racines de blé dans des PDA. Les filtrats de culture des endophytes ont eu un certain effet contre ces champignons de l'essai. Cependant, les traitements sans filtre sont plus efficaces que les cultures avec filtres. Les conidies de* A. typhinum *et de* D. sorokiniana *ont été mélangées pour l'essai de germination. Ces champignons pathogènes ont montré une élongation anormale des hyphes, une lyse des conidies et des tubes anormaux de germe.*

Mots-clés : *Champignons, endophyte, blé, antagoniste, pourriture des racines,* Neotyphodium, Acremonium.

Introduction

Endophytic fungi was recognized as early as 1887 by De Bary, Freeman (1903), McLennan (1920), Sampson (1937) and Neill (1941) observed them many years ago. But endophytes were published in the mid-1970's, Bacon *et al.* (1977) discovered an association between presence of an endophyte in *Festuca arundinacea* (Tall fescue) and poor performance cattle which was known as "summer syndrome". On the other hand, it has been demonstrated that many endophytes protect their host plants against natural enemies. It is known that *Acremonium* infected grasses produce chemicals that have a wide range of biological activity (Webber, 1981; Siegel *et al.,* 1985; Bacon *et al.,* 1986; Carroll, 1986; Bacon and Siegel, 1988). Marshall *et al*. (1999) concluded that fungal endophytes of genera *Neotyphodium* and *Acremonium* inhabit some wild wheat species grown indigenously in Turkey. These endophytes may influence the ecology and distribution of *Triticum* species and may also serve as a source of biological control agents of pests or abiotic stress factors in wheat.

The systemic, seedborne, nonpathogenic, fungal endophytes of most interest as biological control agents belong to the genus *Neotyphodium* Glenn, Bacon, Price, Hanlin (formerly *Acremonium* section Albolanosa Morgan-Jones and Gams) (Glenn *et al*., 1996). These fungi are conidial anamorphs of *Epichloë* spp. (persoon: Fries) Tulasne (Schardl and Philips,1997).

White and Cole (1985, 1986), Schmith (1991), McGee *et al*. (1991) and Chu-Chou *et al*. (1992) have demonstrated that isolates of *A. lolii, A. coenophialum, A. strictum, Phialophora*-like sp. and *E. typhina* from various grass spp. suppressed fungal pathogens in culture.

Materials and methods

Acremonium and *Neotyphodium* spp. were used in this study, which were isolated from *Poaceae*. Four different endophytes were present, *Acremonium typhinum* were isolated from *Triticum dichasians* and *T. cylindricum*, *N. coenophialum* isolated from rye grass and *A. strictum*, *A. starrii* isolated from tall fescue. Root-rot pathogens of wheat were used in this study, *Drechslera sorokiniana*, *Rhizoctonia cerealis*, *Gaeumannomyces graminis*, *Fusarium culmorum* and *F. graminearum*.

First experiment

Four disks of 4 mm diameter of *A. starrii*, *A. strictum* and *A. typhinum* cultures were incubated for 2 weeks except *N. coenophialum,* which was kept in an incubator for three weeks. 1 mm^2 pieces cut off 10 day-colonies of root-rot pathogens were placed in the centre of the potato dextrose agar (PDA) plates. Only the pathogens were placed on the PDA as a control. After 3 days the cultures were measured every day up to 10 days and daily growth rates were calculated. All plates were incubated 21 ± 1ºC in the dark incubator condition.

Second experiment

In this experiment Czabek-dox (N/6) broth medium were used. It contained 0.5% yeast extract with 50 µg/l Streptomycin sulfate, to supress bacteria. 20 ml each N/6 liquid medium were inoculated with three plugs of *Acremonium* spp.'s. and *N. coenophialum*. All of them were left at room temperature for one week. After centrifugation, the liquid was sterilised by filtration through a 0.2 µm filter and infiltrated on to 1 cm disk of sterile filter paper (Whatman No. 1) Mc Gee *et al.* (1991). Each disk placed on PDA plates, 2 cm of edge, then every five plates 1 mm^3 pieces from 10 days cultures root-rot pathogens were incubated. After three days, the cultures were measured every day up to ten days. Endophyte filtrates were used as a control.

Third experiment

D. sorokiniana and *A. typhinum* cultures were used in this experiment. *A. typhinum* isolate which was isolated from *Triticum dichasians* and S96 race of *D. sorokiniana* conidia were mixed together for their germination test. *A. typhinum* and *D. sorokiniana* cultures were grown on PDA which had 50 µg/l Streptomycin sulfate. Cultures were incubated at 21 ± 1ºC for three weeks. Then, 4 ml of sterile water added gradually to each dish, and conidia were removed with a soft brush. In order to remove mycelium, four layers of cheese cloth were used. *A. typhinum* spore concentration was determined with a hemosytometer. It was 5 x 10^6 conidia/ml. To determine *D. sorokiniana* spore concentration each one 1 µl, conidia suspension were poured on slides and counted with stereomicroscope. *D. sorokiniana* conidia were too big for counting with hemosytometer. The spore concentration was adjusted to 10^5 conidia/ml. Spores of two fungi were mixed together 5 x 10^6 conidia/ml, *A. typhinum* and 10^6 conidia/ml *D. sorokiniana*. 100 µl of the suspenson was placed on each of three petri plates with 0.5% water agar. They had three replicates and controls of *A. typhinum* and *D. sorokiniana*. The effect of *A. typhinum* on the germination of *D. sorokiniana* and conidiophores were examined under light microscopy after three days up to five days.

Results and discussion

Two isolates of *A. strictum*, one isolate of *N. coenophialum*, one isolate of *A. starrii* were obtained from *Festuca* two isolates of *A. typhinum* isolated from *Triticum dichasians* and *T. triunciale*, and one isolate of *N. coenophialum* isolated from Rye grass. All test fungi were isolated from wheat in Central Anatolia and Marmara Region of Turkey. All of the test fungi examined for their inhibition by Endophtes on PDA medium. Growth of all test fungi were significantly inhibited by *Acremonium* spp. *A. strictum* and *N. coenophialum* produced melanin against adjacent hypha of *F. culmorum* and *F. graminearum.* However only *N. coenophialum* had no effect on *G. graminis* which was isolated from *F. arundinacea* (Table 1). On the other hand when Endophytes was incubated one more week, Test fungi were placed in the centre of PDA plates. Growing of the test fungi were completely blocked or strongly affected by Endophytes.

Besides, this examination showed that their mycelial growth were sparcer, weak and slow, lost their natural colours, pigment formation was generally reduced and pale.

Table 1. Effect of *Acremonium* and *Neotyphodium endophytes* on growth of several root-rot pathogens,1995

Pathogen	Endophyte species	Growth (mm/day)	Percent growth reduction
Drecshlera sorokiniana	Control	6.50 a	
	N. coenophialum	4.18 b	35.7
	A. typhinum†	3.80 b	41.5
	N. coenophialum††	3.80 b	41.5
	A. strictum††	3.16 c	51.4
	A. starrii††	2.80 c	56.9
	A. typhinum†	2.70 c	58.5
	A. strictum††	0.14 d	97.8
Fusarium graminearum	Control	11.38 a	–
	A. strictum††	7.70 b	32.3
	A. strictum††	7.56 b	33.6
	A. typhinum†	7.34 b	35.5
	N. coenophialum†	6.02 c	47.1
	A. starrii††	5.96 c	47.6
	A. typhinum†	5.30 c	53.4
	N. coenophialum††	4.10 d	64.0
Fusarium culmorum	Control	13.24 a	–
	A. starrii††	8.90 b	32.8
	N. coenophialum††	8.90 b	32.8
	A. strictum††	8.40 bc	36.6
	A. strictum††	8.36 bc	36.9
	N. coenophialum†	8.00 c	39.6
	A. typhinum†	7.36 d	44.4
	A. typhinum†	4.52 e	65.9
Rhizoctonia cerealis	Control	10.00 a	–
	N. coenophialum††	8.78 b	12.2
	N. coenophialum†	7.86 c	21.4
	A. strictum††	7.22 cd	27.8
	A. starrii††	6.88 d	31.2
	A. typhinum†	6.60 d	34.0
	A. strictum††	3.78 e	62.2
	A. typhinum†	3.66 e	63.4
Gaumonamyces graminis	*N. coenophialum*††	6.98 a	–
	Control	6.88 a	–
	N. coenophialum†	6.14 b	10.8
	A. strictum††	4.90 c	28.8
	A. starrii††	4.88 c	29.1
	A. typhinum†	4.24 d	38.4
	A. strictum††	3.68 de	47.3
	A. typhinum†	3.40 e	50.6

†Isolated from Rye grass.
††Isolated from Fescue.

D. sorokiniana was strongly affected by *A. strictum*, *A. typhinum* and *A. starrii* respectively. *F. graminearum* was strongly retarded by *N. coenophialum*, *A. typhinum* and *A. starii* respectively. *F. culmorum*

was inhibited the most by *A. typhinum*, then respectively *N. coenophialum* and *A. strictum*. *R. cerealis* and *G. graminis* were affected the most by *A. typhinum*. Secondarily *A. strictum* inhibited to both of them.

Culture filtrates of endophytes effects were determined on the test fungi (Table 2).

Table 2. Effect of endophytes culture filtrates on growth of several root-rot pathogens,1995

Pathogen	Endophyte species	Growth (mm /day)	Percent growth reduction
Drecshlera sorokiniana	A. strictum††	5.38 a	-2.28
	N. coenophialum†	5.30 a	-0.76
	Control	5.26 a	–
	A. strictum††	5.24 a	0.38
	A. *typhinum*†	5.18 a	1.53
	A. *typhinum*†	5.18 a	1.53
	A. starrii††	5.06 a	3.80
	N. coenophialum††	4.98 a	5.32
Fusarium graminearum	*N. coenophialum*†	8.60 a	-13.16
	A. strictum	7.76 b	-2.10
	N. coenophialum††	7.74 bc	-1.84
	Control	7.60 bc	–
	A. strictum††	7.26 cd	3.48
	A. starrii††	7.10 d	6.58
	A . *typhinum*†	7.04 d	7.37
	A. *typhinum*†	7.04 d	7.37
Fusarium culmorum	*A. strictum*††	10.00 a	-4.94
	Control	8.70 b	–
	N. coenophialum†	8.58 b	1.38
	N. coenophialum††	8.56 b	1.61
	A. starrii††	7.98 c	8.28
	A. strictum††	7.96 c	8.51
	A. typhinum†	7.96 c	8.51
	A. typhinum†	7.96 c	8.51
Rhizoctonia cerealis	Control	10.24 a	–
	N. coenophialum†	7.74 b	24.42
	N. coenophialum††	7.46 bc	27.15
	A. strictum††	7.22 c	29.50
	A. starrii††	7.20 c	29.69
	A. typhinum†	7.08 c	30.86
	A. typhinum†	7.08 c	30.86
	A. strictum††	6.56 d	35.94
Gaumonamyces graminis	*N. coenophialum*†	5.72 a	0
	Control	5.72 a	–
	N. coenophialum††	5.50 a	3.85
	A. *typhinum*†	5.10 b	10.84
	A. *typhinum*†	5.10 b	10.84
	A. strictum††	5.08 b	11.19
	A. starrii††	4.14 b	13.64
	A. strictum††	4.82 b	15.74

†Isolated from Rye grass.
††Isolated from Fescue.

The test fungi were not affected by *N. coenophialum* isolates. Also *D. sorokiniana*'s growth rate was not retarded by any *Neotyphodium* species. Culture filtrates effects were compared with nonflitrated culture media. Mostly nonfiltrated treatments had reduced growth of test fungi more significant than culture filtrates.

In the other experiments, conidia of *D. sorokiniana* and *A. typhinum* were observed after one day. On the first day they began to germinate in 0.5% water agar. After 5 days germination increased average 69.7% of *D. sorokiniana* and average 91.6%. *A. typhinum*. Germination rate of *D. sorokiniana*'s conidia were not decreased significantly by *A. typhinum*. But some septa of conidia had lysis and abnormal growth. Also their germ tubes became abnormally branched and swollen. Hypha of *D. sorokiniana* had abnormal spiral growth. On the other hand, *A. typhinum* did not have any abnormal developing conidia and other frames.

All of the test fungi were affected by endophytes in PDA. Moreover, *Acremonium* spp. were incubated one more week and then pathogens were placed in the centre of petri plates. Growth of the test fungi were completely blocked or strongly affected by *Acremonium* spp., *Alternaria alternata*, *Cladosporium cladosporioides* and *Rhizoctonia cerealis* showed susceptibility to antibiosis by *N. coenophialum* of *Festuca arundinacea*. Besides, *A. coenophialum* and *A. lolii* of *F. versuta* inhibit growth of *R. cerealis* (White and Cole, 1985, 1986). Also Schmith (1991) found that growth of *D. sorokiniana* were reduced by *A. typhinum*, *A. uncinatum* and *A. coenophialum in vitro* respectively.

Melanine pigment were produced by *N. coenophialum* and *A. strictum*. They clearly affected *F. culmorum* and *F. graminearum* to melanise hyphal walls. When this area was examined under microscope, thick hyphal walls in dark pigmentation was observed.

D. sorokiniana and *A*. *typhinum* was mixed for germination test and *A*. *typhinum* was slightly affected on *D. sorokiniana* germination. However, abnormal elongation was caused, lysis on septa of conidia and abnormal germ-tubes, spiral hypha, etc. *A. typhinum* continued developing normally as before.

Conclusion

Culture filtrates of Endophytes. have less effect than their liquid cultures against root-rot pathogens of wheat. During the filtration period, it was not known that antibiosis was produced or which amount of antibiosis passed through 0.2 μm filter. McGee *et al*. (1991) showed that culture filtrates of *A. strictum'*s two isolates failed to inhibit significantly the mean rate of growth of the opposing fungi. However, it was known that culture filtrates of some *Acremonium* spp. and *Neotyphodium* spp. have inhibition characteristics against some pathogens. On the other hand, these are even very complex *in vivo* and further studies should be done on the biochemical relationship of endophytes, their hosts and pathogens.

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