

Determination of Seedborne Fungi in Onion and Their Transmission to Onion Sets

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Samples of onion (*Allium cepa* L.) seeds were obtained from seven regions in Turkey. The seed coat, embryo and endosperm were cultured, the seedborne fungi were determined and their transmission to onion sets was investigated in both sterile and field soils. Among the fungi determined, *Aspergillus alutaceus* Berk. and Curt., *Beauveria bassiana* (Bals.) Vuill., *Cladosporium cladosporioides* (Fres.) de Vries, *Geotrichum* sp., *Hemicola fuscoatra* Traaen, *Trichoderma harzianum* Rifai and *T. pseudokoningii* Rifai in onion seeds, and *Fusarium culmorum* (W.G.Sm.) Sacc., *F. graminearum* Schwabe and *F. sambucinum* Fuckel in onion sets, were recorded for the first time. *Aspergillus niger* v. Tieghem was found at the highest rate in seed samples (especially in the seed coat), and in bulbs and roots of onion sets that developed from these seeds, whether in sterile or field soil. *Fusarium oxysporum* Schlecht was isolated at a higher rate from onion sets grown in sterile or field soil, than from seeds. *F. acuminatum* Ellis and Everhart, *F. sambucinum*, *F. equiseti* (Corda) Sacc. and *F. graminearum* were isolated only from onion sets grown in sterile soil. In dual culture tests, these *Fusarium* isolates were inhibited by *A. niger* and thus, except for *F. oxysporum*, could not develop in agar plate. The *Fusarium* spp. appeared in onion sets grown in sterile soil and were inhibited by other fungi in field soil. It was concluded that all fungi were seedborne and that *A. niger* and *Fusarium* spp., but not the other fungi, were transmitted from the seeds to onion sets. *A. niger* and *F. oxysporum* were also transmitted through the soil.

KEY WORDS: Onion; *Allium cepa* L.; seedborne fungi; transmission.

INTRODUCTION

Various species of fungi have been determined in onion seeds produced in different climatic regions. They include *Botrytis allii* Munn, *B. cinerea* Pers. ex Pers. (2,8,12,13), *Fusarium oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *F. moniliforme* Sheldon, *F. avenaceum* (Corda ex Fr.) Sacc., *F. moniliforme* var. *subglutinans* Wr. and Reink. (1,12,16), *Alternaria alternata* (Fr.) Keissler, *A. porri* (Ell.) Cif. (9,12,15), *Aspergillus niger* v. Tieghem, *A. flavus* Link ex Gray, *A. fumigatus* Fres. (5,6,7), *Stemphylium botryosum* Wallr., *S. vesicarium* (Wallr.) Simmons (12,15), *Penicillium* spp., *P. cyclopium* Wastling, *Trichothecium roseum* (Pers.) Link ex Gray, *Rhizopus nigricans* Ehr., *Cladosporium* sp., *Trichoderma* sp., *Mucor* sp., *Colletotrichum circinans* (Berk.) Vogl. (12), *Drechslera (Pseudocochliobolus) australiensis* (Bugnicourt) Subram. and Jain ex M.B. Ellis, *Rhizoctonia solani* Kühn (1), and *Pythium* spp. (3). Onion seed samples were examined by placing them intact on agar plate or moist filter paper (6,12), which enabled us to investigate transmission of *A. niger* from naturally contaminated seeds to seedlings and sets (6,7).

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In the present study, onion seeds produced in different provinces of Turkey were screened for the presence of fungi in the embryo, endosperm and seed coat. The possible transmission of the isolated fungi from the seeds to onion sets was examined in sterilized and in field soil.

MATERIALS AND METHODS

Occurrence of the fungi on onion seeds

Eighteen samples of seeds of local (not yet registered) varieties from seven different regions of Turkey were collected and screened for the presence of fungi in their different parts. The regions, and the number of samples obtained from each, were: (i) Aegean, 3; (ii) Black Sea, 3; (iii) central Anatolia, 3; (iv) eastern Anatolia, 1; (v) Marmara, 5; (vi) Mediterranean, 1; and (vii) southern Anatolia, 2. First, the seeds were sterilized by dipping them in 1% sodium hypochlorite solution for 3 min, followed by several rinses in sterile distilled water. They were then soaked in sterile distilled water for 2 h, filtered and dried on sterile filter paper. The embryos and endosperms were separated with a sterile scalpel under a stereomicroscope. Ten dissected seed embryos were placed individually in ten petri dishes containing potato dextrose agar (PDA), and ten endosperms in another ten petri dishes. To screen the seed coats, ten intact seeds, soaked for 2 h and not sterilized, were placed in petri dishes containing moist filter paper; ten petri dishes without seeds were prepared as controls. All petri dishes were incubated at 25°C for 10 days and the colonies of fungi which developed around the samples on agar and filter papers were examined and identified under a stereomicroscope. The fungi which were not identified, were transferred to test tubes containing agar.

Seed transmission of fungi in sterile and field soils

Seed transmission of fungi in sterile soil was investigated in naturally contaminated seeds among the samples from seven regions in Turkey. Thirty-five seeds were sown in 30x15 cm plastic pots containing a sterilized mixture of field soil:manure:sand, 1:1:1; and three such pots were allocated for each sample.

Another replicate of the seeds from the same samples was sown in an experimental field of the Tekirdağ Faculty of Agriculture. There were three rows in each bed. Seeds were sown by hand and plants were harvested during the fourth month of growth, after development of the onion sets in the pots and in the field. Onion sets for each sample were removed from the soil, and 1–2 mm pieces of sets and their roots were placed on the agar plates. Identification of the colonies was done after 10 days of incubation at 25°C.

The occurrence of a certain type of fungal development from seed and from sets grown in sterile and field soils, was investigated. For this purpose pairs of the fungi were tested using the method of dual cultures. Antagonistic activity of a fungus isolated at the highest rate from seeds and onion sets grown in sterile soil, was sought. Isolates from questionable fungi – those obtained from onion sets grown in sterile soil although they were not isolated from seeds (or only at a very low rate) – were chosen at random. Assays were conducted in 10-cm-diameter petri dishes filled with PDA: 5-mm-diameter discs, taken from the cultures of each isolate of the fungi, were placed equidistantly on opposite points on the discs. The petri dishes were incubated at 20°C for 10 days, after which the diameter of the fungal colony growth was measured. Antagonistic activity was evaluated by percent inhibition of growth as compared with the growth of the pathogen in the absence of the antagonist.

Soil samples were taken from the center of two plots at one experimental site. One (the main) plot was in an onion-growing area with naturally contaminated seeds; the other plot had never been used for onion production.

Soil was taken from three depths at each station: the soil surface (0 cm), in the vicinity of the onion set roots (0–10 cm), and below the roots (15 cm). From each soil sample, a 10-g portion was added to 100 ml of sterile distilled water and shaken. This soil suspension was diluted 1:10,000 with distilled sterile water and 10 ml of the dilution was incorporated into the PDA (200 ml) medium. This mixture was poured into ten petri dishes (20 ml in each dish). The plates were incubated for 4 days at 25°C. The total number of colonies of every one of the fungi was determined in 0.1-mg soil samples and recorded.

In the sterile soil and field experiments, the daily average maximum and minimum temperatures were 22° and 15°C, respectively, and the daily average relative humidity ranged from 71% to 54%.

RESULTS AND DISCUSSION

Occurrence of the fungi in onion seeds

Eleven species of fungi, belonging to nine genera, were identified in onion seeds and their tissues (Table 1). Among them, *A. niger* was the dominant species in all seed samples from the various regions. It was isolated from all samples and seed parts especially in the Black Sea and Marmara regions. In these two regions average temperatures during seed development, in July, are 20° and 24°C, respectively. In southern Anatolia and central Anatolia, the highest rate of contamination of the seed coat was by *A. niger*. In these regions ambient temperatures generally average 31.5° and 23°C, respectively, during July. It has been reported that the incidence of this species is as high as 88.9% on seeds produced under such warm temperatures (6).

Other species of fungi were isolated at different rates from samples from different regions. Among them, *Aspergillus alutaceus*, *Beauveria bassiana*, *Cladosporium cladosporioides*, *Geotrichum* sp., *Humicola fuscoatra*, *Trichoderma harzianum* and *T. pseudokoningii*, at low rates, were identified for the first time on onion seeds and their parts.

Seed transmission of fungi in sterile and field soils

Some species of fungi which were determined in seed parts – *Alternaria alternata*, *A. alutaceus*, *B. bassiana*, *Botrytis* spp., *C. cladosporioides*, *Geotrichum* sp., *S. botryosum*, *T. harzianum*, *T. pseudokoningii* and *T. roseum* – were not isolated from sets grown in sterile or field soil. In all regions, the highest rate of infection of root and bulbs was by *A. niger* (Tables 2 and 3).

In other studies in field soil, the mean incidence of bulb and root infection by *A. niger* was, respectively, 65% and 35.8% in cv. Saggai Red, and 52.5% and 37.5% in cv. 'Nassi' (7). *Fusarium oxysporum* ranked second in incidence on onion sets in sterile and field soils. It was reported that *F. oxysporum* was present in onion sets (bulbs) and roots (4,10,13). *F. acuminatum*, *F. culmorum*, *F. equiseti*, *F. graminearum* and *F. sambucinum* were isolated at lower rates from sets grown in sterile soil. It was also observed that the frequency of occurrence of the identified species of fungi differed between the sets which developed in the field and in sterile soil. In the present study, we thought that these fungi might also be seedborne but inhibited by other species of fungi, such as *A. niger*, in artificial media.

TABLE 1. The incidence of fungi detected in different parts of onion seeds from different regions

Region ^z	Seed part ^y	Percentage of the parts of onion seeds contaminated with														Total fungi
		A. alter. ^a	A. aluta.	A. niger	B. bass.	Botry. sp.	C. clado.	F. oxy.	Geotr. sp.	H. fusc.	S. botry.	T. harz.	T. pseudo-	T. roseum	Other fungi	
i	Emb.	0	0	0.3	0	0	0	0	0.3	0	0	0.7	0	0	6.3	8.3
	End.	0	0.3	0	0	0	0	0	1.0	0	0	0.3	0	0.3	5.7	11.7
	SC	0	0	0.3	0	0	0	0	0	0	0	0	0	0	27.7	44.3
ii	Emb.	0	0	1.7	0	0	0	0	0	0	0	3.7	0.6	0	2.0	20.6
	End.	0	0	10.3	0	0	0	0	0	0	0	0.3	0.6	0	2.3	12.99
	SC	0	0	41.0	0	0	0	0	0	0	0	0	0	0	13.3	61.7
iii	Emb.	0	0	0	0	0	0	0	0	0	0	0	0.7	0	3.7	4.3
	End.	0	0	0	0	0	0	0	0	0	0	0	0.7	0	5.3	6.0
	SC	0	0	33.7	0	0	0	0	0	0	0	0	0	0	20.3	54.0
iv	Emb.	0	0	0	0	0	0	0	0	0	0	0	0	0	3.0	3.0
	End.	0	0	0	0	0	1.0	0	0	0	0	0	1.0	0	7.0	9.0
	SC	0	0.3	6.0	0	0	0	0	0	2.0	0	0	0	0	25.0	33.3
v	Emb.	0	1.4	10.2	0.2	0	0	0.8	0	0	0	0.2	0	0	5.4	18.2
	End.	0	0.4	9.6	0	0	0	0	0	0	0	0	0	0	3.0	13.0
	SC	1.8	15.6	28.4	0	0	0	0	0	0	0.2	0	0	0	6.0	52.0
vi	Emb.	0	0	0	0	0	0	0	0	0	0	0	0	0	3.0	3.0
	End.	0	0	7.0	0	0	0	0	0	0	0	0	0	0	9.0	16.0
	SC	0	0	20.0	0	0	0	0	0	0	0	0	0	0	0	20.0
vii	Emb.	1.0	0	0	0	0	0	0	0	0	0	0	0	0	5.0	6.0
	End.	0.5	0	1.5	0	0.5	0	0	0	1.0	0	0	0	0.5	5.0	9.0
	SC	0	0	54.5	0	0	0	0	0	0	0	0	0	0	1.5	56.0

^z See Materials and Methods.

^y Emb., embryo; End., endosperm; SC, seed coat.

^a A. alter., *Alternaria alternata*; A. aluta., *Aspergillus alutaceus*; A. niger, *Aspergillus niger*; B. bass., *Beauveria bassiana*; Botry. sp., *Botrytis* sp.; C. clado., *Cladosporium cladosporioides*; F. oxy., *Fusarium oxysporum*; Geotr. sp., *Geotrichum* sp.; H. fusc., *Humicola fuscoatra*; S. botry., *Stemphylium botryosum*; T. harz., *Trichoderma harzianum*; T. pseudo., *Trichoderma pseudokoningii*; T. roseum, *Trichothecium roseum*.

TABLE 2. The incidence of fungi on the root and bulb of onion sets developed from seed samples grown in sterile soil

Region ^z	Plant part	Percentage of onion set parts bearing							Other fungi	Total fungi
		A. niger ^y	F. acum.	F. cul.	F. equi.	F. gram.	F. oxy.	F. samb.		
i	Root	8.0	0.7	0	2.0	0	2.7	0	7.3	18.7
	Bulb	2.0	0	0	0	0	0.7	0	4.7	7.3
ii	Root	24.0	0.7	0	0	0	1.3	0	4.7	28.7
	Bulb	22.7	0	0	0	0	0	0	5.3	28.0
iii	Root	63.3	0.7	0.7	0	0	2.7	0	0.7	67.3
	Bulb	55.3	0	0	0	0	2.7	0.7	1.3	60.0
iv	Root	20.0	2.0	0	4.0	0	6.0	0	0	32.0
	Bulb	18.0	0	0	0	0	4.0	0	2.0	24.0
v	Root	68.4	0	0	0.8	0	7.2	0	2.0	78.4
	Bulb	57.6	0	0	0	0.8	3.2	0	1.6	63.2
vi	Root	30.0	0	0	0	0	22.0	0	0	52.0
	Bulb	8.0	0	0	0	0	10.0	0	2.0	20.0
vii	Root	14.0	0	0	0	0	2.0	1.0	20.0	37.0
	Bulb	13.0	0	0	0	0	0	0	20.0	33.0

^z See Materials and Methods.

^y A. niger, *Aspergillus niger*; F. acum., *Fusarium acuminatum*; F. cul., *Fusarium culmorum*; F. equi., *Fusarium equiseti*; F. gram., *Fusarium graminearum*; F. oxy., *Fusarium oxysporum*; F. samb., *Fusarium sambucinum*.

To examine this assumption, we conducted a dual culture experiment and soil tests. The results of the experiment (Table 4) indicated that the *Fusarium* spp. were inhibited by

TABLE 3. The incidence of fungi on the root and bulb of onion sets developed from seed samples grown in field soil

Region ^z	Plant part	Percentage of onion set parts bearing					Total fungi
		<i>Aspergillus niger</i>	<i>Fusarium culmorum</i>	<i>Fusarium oxysporum</i>	<i>Macrophomina phaseoli</i>	Other fungi	
i	Root	47.3	0	7.3	1.3	0	56.0
	Bulb	34.0	0	0	0.7	0	34.7
ii	Root	50.7	2.0	12.0	0	0	64.7
	Bulb	50.0	0	0.7	0	0	50.7
iii	Root	24.7	0	1.3	0	0	26.0
	Bulb	28.0	1.3	0	0	0	29.3
iv	Root	54.0	0	12.0	4.0	0	70.0
	Bulb	48.0	0	2.0	0	0	50.0
v	Root	47.2	0.4	4.0	0.8	1.2	53.6
	Bulb	43.6	0	0.4	0	1.2	45.2
vi	Root	94.0	0	4.0	0	0	98.0
	Bulb	66.0	0	0	0	0	66.0
vii	Root	47.0	0	4.0	0	0	51.0
	Bulb	45.0	0	2.0	0	0	47.0

TABLE 4. Rate of inhibition of *Fusarium* spp. by two *Aspergillus* isolates

Species ^z	<i>A. niger</i> ^y	<i>A. niger</i> ^x
<i>F. acuminatum</i>	48.2 ^w	29.6
<i>F. culmorum</i>	44.4	34.9
<i>F. equiseti</i>	74.0	62.9
<i>F. graminearum</i>	56.2	53.3
<i>F. oxysporum</i>	66.7	63.1
<i>F. sambucinum</i>	57.1	54.2

^zThere were no significant differences among the species at $P=0.05$.

^ySource: seed.

^xSource: onion set in sterile soil.

^wEach value is the mean of three replicates.

TABLE 5. Recovery of *Aspergillus niger* and *Fusarium oxysporum* from soil samples^z

Location	Sample depth	cfu /0.1 mg soil			Percentage of	
		<i>F. oxysporum</i>	<i>A. niger</i>	Total	<i>A. niger</i>	<i>F. oxysporum</i>
Main plot	Soil surface (0 cm)	0.2	0.2	1.6	12.5	12.5
	Root depth (0-10 cm)	0.6	0.2	6.8	8.8	2.9
	15 cm depth	1.6	0.4	6.0	26.7	6.7
Outside ^y	Soil surface	0	1.2	4.2	0	28.6
	Root depth	1.0	0.4	7.4	13.5	5.4
	15 cm depth	0.2	1.0	5.8	3.5	17.2

^zEach value is the mean of five replicates.

^yAn area of the field not used for onion production.

A. niger, and it was therefore concluded that they could develop in sterile soil. It was found that *A. niger* and *F. oxysporum* were both present in the soil samples (Table 5).

The proportion of colony-forming units (cfu) of *A. niger* and *F. oxysporum* in soil samples taken from the surface (0 cm), the root region (0–10 cm depth), and 15 cm deep in

the main plot, was approximately 16% and 8%, respectively, of the total cfu recovered. The ratios of *A. niger* colonies from soil samples from the same depths, but taken from an area of the field not being used for onion production (outside) were lower, and of *F. oxysporum* colonies higher, than those of the soil samples from the area with naturally contaminated onion sets (differences not significant). Soil samples taken from the main plot and from outside harbored a variety of fungi, but no other *Fusarium* spp.

There appear to be three principal possibilities for infection in epigeal hosts like onion, in which the cotyledons are carried above ground: (a) direct invasion from seed into the seedlings; (b) invasion into the soil for a longer or shorter time, followed by local or systemic invasion of the host such as exemplified by the soil inhabitants and soil invaders; and finally (c) subsistence for a limited period of time in host debris, including decaying seeds (10). If onion seeds are naturally contaminated by *Fusarium* spp., the latter may be transferred from seeds to soil, which is expected as a second possibility (10). However, the occurrence of *A. niger* and *F. oxysporum* in soil samples both within and outside the main onion plot suggests that they are probably a natural component of the soil mycoflora of our experimental area. Thus, their incidence on the parts of onion sets was higher than in the seeds. Hayden and Maude (6,7) noted that *A. niger* is transmitted from naturally contaminated onion seeds to seedlings and bulbs, that it is infrequent in the soil in the UK, but is a major component of the soil in Sudan. Because of the high incidence of the fungus in the field in Sudan (6,7), most of the bulbs grown there are contaminated with *A. niger*.

In conclusion, all of the determined fungi occurred commonly as a natural contaminant of seeds. Thus, *F. acuminatum*, *F. culmorum*, *F. equiseti*, *F. graminearum* and *F. sambucinum* likely could be transmitted from seeds to onion sets in sterile soil, but were apparently inhibited by other organisms in field soil. *A. niger* and *F. oxysporum* were transmitted from both seed and soil to onion sets. Other determined fungi could not be seed-transmitted. *Macrophomina phaseoli* was isolated only from onion sets grown in field soil, which indicates that the sets might have been contaminated in the soil.

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