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Detection and Location of Seed-borne Fungi of Black Cumin and Their Transmission in Seedlings

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Abstract

Eighty-eight samples of black cumin seed were collected from commercial markets in Egypt and tested for seed-borne fungi. Both blotter and deep freezing methods were used in order to detect the maximum number of internal and external seed-borne fungi. A total of 37 species of fungi belonging to 20 genera were identified. The pathogenicity of *Fusarium oxysporum*, *F. solani*, *F. moniliforme* and *Verticiilium* sp. was studied on black cumin seeds and seedlings, using both seed and soil inoculation. A higher percentage of pre- and post-emergence damping off developed from seed inoculation with each of the four fungi than from soil infestation. The transmission of *Fusarium oxysporum*, *F. solani*, moniliforme and *Verticillium* sp. from seed to mature plants of black cumin was also studied. All of the four tested fungi were restricted to the roots and lower portions of the stem with the exception of *F. moniliforme*, which was found in the vascular system of middle stem sections when sampled at 60 and 120 days after planting.

Introduction

Black cumin (*Nigella sativa* L.) is a herbaceous plant, which belongs to the family Ranunculaceae. Black cumin seeds are used for many purposes; as a flavoring agent in cheese and bakery products and in anti-microbial drug products (Hanafy and Hatem, 1991; Al Gaby, 1992; Mahmoud, 1993; El-Kayati *et al*., 1995).

Like many culinary herbs, black cumin seeds benefit the digestive system, relieve stomach pain and colic, serve as a carminative and, in large quantities, as a diuretic, a promoter for menstruation and, in India, are used to increase breast milk production (Akhtar and Riffat, 1991; Chevallier, 1996).

Thymoquinone and thymohydroquinone, the predominant compounds in the essential oil, exhibit an anti-microbial activity (Al Gaby, 1992). Nigellone (alkaloid) is the carbonyl polymer of thymoquinone (Ansari *et al*., 1988). Nigellone isolated from the essential oil of black cumin seed is used as an antispasmodic for the treatment of bronchitis, asthma and whooping cough (Hussein, 1985; Chakravarty, 1993). Akhtar and Riffat (1991) reported that powdered Nigella sativa seeds are used against cestode infections in children.

Hilal *et al*. (1994) published the first report on root diseases of black cumin in Egypt. They isolated *Fusarium moniliforme*, *F. oxysporum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Alternaria* spp. and *Nigrospora* spp. Pathogenicity tests indicated that *F. oxysporum* and *M. phaseolina* were the most virulent, while *R. solani* was the least on black cumin. Stunting was the predominant symptoms on plants. Seed yield of attacked plants was more or less nil. Because they had either empty fruit capsules or the capsules contained immature and malformed seeds.

Dubey (1995) recorded a new fusarium wilt disease of *Nigella sativa* in India. Plants remained susceptible to infection at all growth stages; infected plants showed vascular discoloration. The author proposed a new forma specialize, nigella, naming the fungus *F. oxysporum* f.sp. *nigella* and showing that it differed from *F. oxysporum* f.sp. *cumini*.

Since little information on the diseases of black cumin is available, the present work was planned to:

1. Detect, identify and survey the seed-borne fungi of local and imported black cumin seed in Egypt
2. Study the fungi isolated from the seed and their effects on black cumin plants
3. Elucidate the mode of transmission of some of the pathogenic fungi from seeds to mature plants

In compiling the list of seed-borne fungi attacking black cumin, no attempt was made to differentiate between seed infection, in which infected seed produced infected plants or was the focus of an epidemic and seed transport, in which an organism was present on the seed but not shown to subsequently cause infection.

Materials and Methods

Sources of seed samples: Eighty-eight samples of black cumin seed, collected from commercial markets in different parts of Egypt (including Gharbia, Alexandria, Cairo, Assiut, Sohag, Damietta and Dakhlia) during 1994 and 1995, were used in these studies. The samples were stored at home temperature for a maximum of six months.

Seed health testing: The detection of seed-borne mycoflora was carried out following the Rules of the International Seed Testing Association (ISTA, 1993). Two hundred seeds of each sample were tested using standard blotter and deep-freezing methods.

Blotter method: Twenty-five seeds were plated in a 9-cm diameter Petri dish containing three layers of moist blotter paper. The plates were incubated at 20±2°C for 7 days under cool white fluorescent lights with alternating cycles of 12 hours light and 12 hours darkness.

Deep freezing method: After plating the seeds as described for the blotter method, the dishes were incubated at 20°C for 24 hours and transferred to a -20°C freezer for 24 hours. This was followed by 5 days incubation at 20±2°C under cool white fluorescent lights with alternating cycles of 12 hours light and 12 hours darkness.

The incubated seeds were examined under a stereoscopic microscope (6-50X magnification) after 7 days for the presence of fungi and their morphological characteristics. Whenever necessary, a compound microscope was used to confirm identification by examining the morphology of conidia and conidiophores. The seed-borne fungi were identified using the Commonwealth Mycological Institute, Kew, Surrey, England (CMI) description sheets, Danish Government Institute of Seed Pathology (DGISP) publications and papers by Booth (1985), Burgess *et al*. (1988), Chidambaram *et al*. (1973), Ellis (1971), Raper and Fennell (1965) and Singh *et al*. (1991).

Using a stereoscopic microscope, hyphal tips from the fungi were transferred to potato dextrose agar (FDA) plates using the tips of heat-stretched capillary tubes. Pure cultures of the fungi were obtained and all isolates were maintained on PDA slants.

Pathogenicity test of important seed-borne fungi: Four fungi, *Fusarium oxysporum*, *F. solani*, *F. moniliforme* and *Verticillium* sp., were tested for their pathogenic effects on black cumin seeds and seedlings.

Preparation of inocula: Seed lots showing a high incidence of infection with *Fusarium oxysporum*, *F. solani*, *F. moniliforme* and *Verticillium* sp. were plated on blotters. A micromanipulation technique was followed to isolate the fungal spores. Capillary tubes were stretched using a Bunsen burner and the very fine tips were used to isolate the spores of the fungus under a stereoscopic microscope. The isolated spores were transferred to PDA media in a Petri dish and incubated for several days in the dark at 24±2°C. The incubation period differed from one fungus to another and from one isolate to another of the same fungus. When hyphal growth reached a diameter of 3 cm, one 0.5 cm disk cut from each of the cultures was transferred to 50 ml of potato dextrose broth media and incubated in the dark for 10 days at 24±2°C until the hyphal mat covered the open surface of the liquid medium. Pure cultures of each fungus were maintained on PDA slants for further studies. The hyphal mats were harvested and washed with sterile distilled water. Fifty grams of fresh mat were blended in 500 ml of sterile distilled water to produce a suspension of each fungus. The prepared suspensions were used for inoculating seeds and soil.

Seed inoculation with the isolated fungi: Black cumin seeds from pathogen-free seed lots were disinfected in 1% sodium hypochlorite solution for one minute, rinsed in tap water three times and placed on sterilized tissue paper at room temperature until dry. The sterile seeds were then soaked in the fungal suspensions for 2 hours and left to dry at room temperature. The inoculated seeds were planted in plastic bags containing sterilized soil.

Conventional growing-on test: Both inoculated and non-inoculated seeds were planted (5 seeds per bag) in plastic bags (8 cm diam.) containing autoclaved sandy soil and allowed to grow in greenhouse under ambient conditions during the winter season. Daily observations for germination and symptoms of pre- and post-emergence damping off were recorded for one month.

Soil infestation with pathogenic fungi: Fifty grams of fresh mat were blended in 500 ml of sterile distilled water to produce a sticky suspension of each fungus. Each prepared suspension was added to 2 kg of peatmoss and mixed well. About 150 grams of the inoculated peatmoss was added to a plastic pot (8 cm diam.) containing 350 grams of steam sterilized sandy soil and mixed thoroughly. Sixteen replicates (pots) were used per treatment. The pots were kept in the greenhouse for seven days at 20-24°C to allow the fungus to spread through the soil before the seeds were planted. Another 16 pots, prepared in the same manner without the fungus suspension, served as checks. The soil was kept moist by watering when needed.

Pathogen-free seeds were planted (5 seeds/pot) in infested and non-infested soil and kept in a greenhouse during the winter season. Daily observations for germination and symptoms of pre-and post-emergence damping off were recorded for one month.

Transmission of pathogenic fungi from seed to mature plants: Black cumin seeds were inoculated with *Fusarium moniliforme*, *F. oxysporum*, *F. solani* and *Verticillium* sp. The methods of inoculum preparation, seed inoculation, soil sterilization and planting in plastic bags were the same as those used for the pathogenicity test. Ten replicates of plastic bags containing seeds inoculated with each fungus were prepared (5 seeds/bag). Seedlings emerging from the inoculated seeds were rated and left to grow. Recovery rates of the fungi from different plant parts at intervals of 60 and 120 days were determined.

At the end of each interval, 10 plants were removed from the bags, washed, disinfected and dissected under sterile conditions. The various plant parts (roots, hypocotyl, lower stem, middle stem, upper stem, peduncle, flowering branch top, inflorescence, flowers and seeds, if present) were plated on sterile moist blotters and incubated for 7-10 days at 24°C. Fungi recovered for each treatment were

identified and the transmission percentage was recorded.

Results

Seed health testing: A total of 37 species of fungi belonging to 20 genera were identified i.e., *Alternaria alternate* (Fr.) Keissler, *A. chlamydospora* Mouchacca, *Aspergillus flavus* Link: Fr., *A. fumigatus* Fres., *A. niger* van Tieghem, *A. ochraceus* Wilhelm, *A. tamerii* Kite, *A. clavatus* Desm., *A. carneus* Blochwitz, *A. sclerotiorum* Huber, *Cephalosporium* sp., *Chaetomiurn* sp., *Cladosporium* sp., *Curvularia* sp., *Drechslera australiensis* (Bugni:) Subram and Jain ex. M.B. Ellis [= *Bipolaris australiensis* (M.B. Ellis) Tsuda and Ueyama], *D. halodes* (Drechs.) Subram. and Jain, *D. hawaiiensis* (Bugni;) Subram. and Jain ex. M.B. Ellis [= *Bipolaris hawaiiensis* (Ellis) Uchida and Aragakil], *D. microbe* (Drechs.) Subram. and Jain, *D. rostrata* (Drechs.) Richardson and Fraser [= *Exserohllum rostraturn* (Drechsler) Leonard and Suggs emend. Leonard], *D. tetramera* (McKinney) Subram. and Jain, *Epicoccum* sp., *Fusarium moniliforme* Sheld., *F. oxysporum* Schlecht, *F. semitectum* Berk. and Ray. [= *F. pallidoroseum* (Cooke) Sacc.], *F. solani* (Mart.) Sacc., *F. chlernydosporum* Wollenw and Reinkng, *F. equisty* (Gorda) Sacc., *Macrophomina phaseolina* (Tassi) Goid., *Mucor* sp., *Myrothecium* sp., *Nigrospora* sp., *Penicillium* sp., *Phoma* sp., *Phomopsis* sp., *Rhizopus* sp., *Stemphylium* sp., *Trichotheciurn* sp. and *Verticillium* sp. The percentage of detected fungi for each species is presented in Table 1.

A higher number of fungi were recovered with the blotter method than with the deep-freezing method. Using the blotter method, the dominant fungi were *Aspergillus flavus* at a maximum rate of 80.7 percent and an infection range of 0.5-90 percent, *Stemphylium* sp. at 76.1 percent and an infection range of 0.5-12.5 percent, *Alternaria alternata* at 71.6 percent and an infection range of 0.5-23 percent, *Cladosporium* sp. at 38.6 percent and an infection range of 0.5-17 percent, *A. niger* at 36.6 percent and an infection range of 0.5-80 percent, *A. ochraceus* at 29.5 percent and an infection range of 0.5-40 percent and *Cladosporium* sp. at 29.5 percent and an infection range of 0.5-18.5 percent. The following fungi were recovered with the deep-freezing method: *Aspergillus flavus* at a maximum rate of 76.1 percent and an infection range of 0.5-29 percent, *Stemphylium* sp. at 75 percent and an infection range of 0.5-22 percent, *Alternaria alternata* at 59 percent and an infection range of 0.5-11 percent, *A. niger* at 31.8 percent and an infection range of 0.5-60 percent and *A. ochraceus* at 20.5 percent and an infection range of 0.5-3 percent.

Table 1 shows that the deep freezing method was superior for the detection of *F. moniliforme* at 12.5 percent and an infection range of 0.5-2 percent, *Cephalosporium* sp., at 22.7 percent and an infection range of 0.5-9 percent and *Verticillium* sp. at 11.4 percent and an infection range of 0.5-3 percent. Table 1 also shows that the blotter method was better for detecting *F. solani* at 10.2 percent and an infection range. of 0.5-4.5 percent and *F. oxysporum* at 6.8 percent and an infection range of 0.5-6 percent.

It was noted that most of the samples examined were infected with several saprophytic fungi. The percentages were generally lower with the deep-freezing method than with the blotter method. However, the deep freezing method was more suitable for detecting *Penicillium* sp., at 71.6 percent and an infection range of 4-18 percent, than the blotter method, at 56.8 percent and an infection range of 1-9 percent.

Pathogenicity testing of seed-borne fungi: Observations of infection symptoms on seeds and seedlings were recorded 30 days after planting. Symptoms were classified as either pre- or post-emergence damping off. Pre-emergence damping off consisted of non-germinated seeds covered with hyphal growth of the tested pathogen. Post emergence damping off consisted of seedlings with lesions in the crown area and typical dark thread-like infected roots. Wilt symptoms were also considered to be post-emergence damping off. Pathogenicity test results are presented in Table 2 and 3.

Effect of *Fusarium oxysporum*: Data presented in Table 2 and 3 show that the percentages of infection, expressed as symptoms of both pre-and post-emergence damping off, were 39 and 30 percent for seeds and seedlings, respectively, with the seed inoculation treatment. They were 21.8 and 25 percent for seeds and seedlings, respectively, from non-inoculated seeds planted one week after soil infestation with *F. oxysporum*. Percentage of surviving seedlings was higher (53.2%) with the soil infestation treatment than with seed inoculation (31%).

Effect of *Fusarium solani*: As shown in Table 2 and 3, the percentages of pre-and post-emergence damping off caused by *Fusarium solani* were 43.8 and 18.7 percent for seeds and seedlings, respectively, for the seed inoculation treatment. They were 37.5 and 12.5 percent for seeds and seedlings, respectively, for the soil infestation treatment. Seedling survival was 50 percent with soil infestation and 37.5 percent with inoculated seed planted in sterilized soil.

Effect of *Fusarium moniliforme*: The pre- and post-emergence damping off percentages caused by *F. moniliforme* were 46.8 and 14.2 percent for seeds and seedlings, respectively, from inoculated seeds. They were 17.2 and 14.2 percent for seeds and seedlings, respectively, from the soil infestation treatment. Seedling survival was 70.3 percent in the soil infestation treatment and 39 percent in the seed inoculation treatment.

Effect of *Verticillium* sp.: Results presented in Table 2 and 3 show that *Verticillium* sp. had a pathogenic effect on black cumin, with pre-and post-emergence damping off percentages of 29.7 and 7.8 percent for seeds and

Table 1: Results of seed health testing, using the blotter and deep-freezing methods, for black cumin seed samples (88 samples)

	Blotter Method		Deep Freezing	
	NSI*	% of detected fungi*	NSI*	% of detected fungi*
Altemaria alternate	63	71.6 (0.5-23)	52	59.0 (0.5-11)
Alternaria clamydospora	1	1.1 (0-0.5)	0	0.0
Aspergillus carness	18	20.5 (1-2)	18	20.5 (0.5-5)
Aspergillus clavatus	4	4.5 (0.5-2)	1	1.1 (0-1.5)
Aspergillus flavus	71	80.7 (0.5-90)	67	76.1 (0.5-29)
Aspergillus fumigatus	18	20.5 (0.5-1)	18	20.5 (0.5-1)
Aspergillus niger	32	36.6 (0.5-80)	28	31.8 (0.5-60)
Aspergillus ochraceus	26	29.5 (0.5-40)	18	20.5 (0.5-3)
Aspergillus tamerii	12	13.6 (0.5-11)	16	18.1 (0.5-6)
Cephalosporium sp.	15	17.0 (0.5-3.5)	20	22.7 (0.5-9)
Chaetornium sp.	3	3.4 (0.5-2)	4	4.5 (0.5-2)
Cladosporium sp.	34	38.6 (0.5-17)	26	29.5 (0.5-18.5)
Curvularia sp.	6	6.8 (0.5-1)	1	1.1 (0-0.5)
Dreshslera australiensis	2	2.3 (0-1)	0	0.0
Dreshslera halodes	0	0.0	1	1.1 (0-0.5)
Dreshslera hawaiiensis	10	11.4 (0.5-4.5)	5	5.7 (0.5-1)
Dreshslera microbe	1	1.1 (0-0.5)	0	0.0
Dreshslera rostrata	0	0.0	1	1.1 (0-0.5)
Dreshslera tetramera	8	9.0 (0.5-3.5)	6	6.8 (0.5-5)
Epicoccum sp.	6	6.8 (0-0.5)	5	5.7 (0.5-1)
Fusarium chlamydosporum	1	1.1 (0-1)	0	0.0
Fusarium equisty	1	1.1 (0-0.5)	0	0.0
Fusarium moniliforme	8	9.0 (0.5-1.5)	11	12.5 (0.5-2)
Fusarium oxysporum	6	6.8 (0.5-6)	4	4.5 (0.5-4.5)
Fusarium semitecturn	3	3.4 (1-2.5)	1	1.1 (0-1)
Fusarium solani	9	10.2 (0.5-4.5)	3	3.4 (1-2)
Macrophomina phaseolina	1	1.1 (0-0.5)	0	0.0
Mucor sp.	1	1.1 (0-0.5)	0	0.0
Myrothecium sp.	1	1.1 (0-1)	0	0.0
Nigrospora sp.	4	4.5 (0.5-1)	1	1.1 (0-0.5)
Penicillium sp.	50	56.8 (1-9)	63	71.6 (4-18)
Phoma sp.	1	1.1 (0-0.5)	2	2.3 (0-0.5)
Phomopsis sp.	1	1.1 (0-1.5)	2	2.2 (2-2.5)
Rhizopus sp.	30	34.0 (0.5-12)	18	20.4 (0.5-8)
Stemphylium sp.	67	76.1 (0.5-12.5)	66	75.0 (0.5-22)
Trichothecium sp	1	1.1 (0-0.5)	1	1.1 (0-0.5)
Verticillium sp.	9	10.2 (1-5.5)	10	11.4 (0.5-12)

NSI: No. of samples infected (No. in parentheses indicates infection range), % of detected fungi: NSI/ Total No. of samples (88) $\times$100

Table 2: Effect of *Fusasrium oxysporum*, *F. solani*, *F. moniliforme* and *Verticillium* sp. on inoculated black cumin seeds

	Tested Pathogens and infection percentages (%)				
Symptom of infection	*F. oxysporum*	*F. solani*	*F. moniliforme*	*Verticillium* sp.	Check
Pre-emergence damping off (Rotted seeds)	39	43.8	46.8	29.7	3
Post-emergence damping off (Infected seedlings)	30	18.7	14.2	7.8	0
Survivals	31	37.5	39.0	62.5	97

seedlings, respectively, in seed inoculation treatments and 6 and 5 percent for seeds and seedlings, respectively, in the soil infestation treatment. Seedling survival was 89 percent with soil infestation and 62.5 percent with inoculated seed planted in sterilized soil.

Data in Table 2 and 3 show that *F. moniliforme* caused the highest rate of infection (as pre-emergence damping off) in black cumin, followed by *F. solani*, *F. oxysporum* and *Verticillium* sp., with the seed inoculation treatment, while *F. solani* caused the highest rate of infection, followed by *F. oxysporum*, *F. moniliforme* and *Verticillium* sp., with the infested soil treatment. Post-emergence damping off infection rates were the highest with *F. oxysporum* followed by *F. solani*, *F. moniliforme* and *Verticillium* sp.

Table 3: Effect of *Fusasrium oxysporum*, *F. solani*, *F. moniliforme* and *Verticillium* sp. on black cumin seeds and seedlings grown in infested soil

	Tested Pathogens and infection percentages (%)				
Symptom of infection	*F. oxysparum*	*F. solani*	*F. moniliforme*	*Verticillium* sp.	Check
Pre-emergence Damping off (Rotted seeds)	21.8	37.5	17.2	6.0	4.5
Post-emergence damping off (Infected seedlings)	25.0	12.5	12.5	5.0	0.0
Survivals 53.2	50.0	70.3	89.0	95.5	

Table 4: Recovery percentage of fungi from plants grown from artificially inoculated black cumin seeds

Fungus	Interval (day)	C	S_1	S_2	S_3	S_4	S_5	S_6	S_7
Fusarium moniliforme	60	0	66.5	33.5	-	-	Nd	Nd	Nd
	120	0	16.5	8.0	2	-	-	-	-
F. oxysporum	60	0	80	10.0	-	Nd	Nd	Nd	Nd
	120	0	33.5	16.5	-	-	-	-	-
F. solani	60	0	50	-	-	Nd	Nd	Nd	Nd
	120	0	8	-	-	-	-	-	-
Verticillium sp.	60	0	43	30.0		Nd	Nd	Nd	
	120	0	41.5	50.0		-	-	-	-

S1 = Roots S2 = Lower stem S3 = Middle stem fruit
S4 = Upper stem S5 = Flowering structures S6 = Structure
S7 = Seeds Nd = Not done due to the age of the plant C = Check

Percentage of seedling survival was higher with *Verticillium* sp. for both seed and soil infestation (62.5 and 89%, respectively) compared with the other three fungi.

Transmission of pathogenic fungi from seeds to mature plants: The transmission of three species of *Fusarium* (*F. moniliforme*, *F. oxysporum* and *F. solani*) and *Verticillium* sp. were studied in a growing on test. Table 4 shows that black cumin plants had different interactions with the fungi tested. All four were isolated from roots and lower stems of the plants at intervals of 60 and 120 days (these intervals approximated the seedling, vegetative and reproductive plant growth stages). The data showed that *F. moniliforme*, *F. oxysporum* and *Verticillium* sp. were restricted to roots and lower sections of the stem. The recovery percentages in lower stem sections 120 days after planting were 16.5 and 50 percent for *F. oxysporum* and *Verticillium* sp., respectively. *F. moniliforme* was the only fungus isolated from the basal parts up to the middle stem, at a rate of 2 percent after 120 days. *Fusarium solani* was the only fungus isolated from roots, at rates of 50 and 8 percent 60 and 120 days after planting, respectively.

Discussion

The various methods used in seed health testing differ in their sensitivity and purpose. Blotter and deep freezing methods were both used in this study in order to detect the maximum number of internal and external seed-borne fungi. The total number of fungal species detected on seeds was significantly higher with the blotter method (35 species) than with the deep freezing method (29 species), which suppresses the growth of saprophytic fungi. The deep freezing method proved to be better than the blotter method for detecting *Cephalosporiu*m sp., *Fusarium moniliforme* and *Verticillium* sp. We recommend the blotter method when the purpose of seed examination is to survey all the mycoflora associated with the seed, while the deep freezing method enhances the recovery of deep-seated mycelium or spores in the inner seed tissues and suppresses fast growing saprophytes.

Our results are in agreement with the findings of other researchers. Neergaard (1979) found that the blotter method provided excellent conditions for the development of myceliaf growth and conidial sporulation of many Hyphomycetes. Mathur *et al*. (1975) found that the deep freezing method was superior to the blotter and agar plate methods for the recovery of *Fusarium* spp. and *Chaetomium* spp. from sorghum seeds. Khan *et al*. (1988) made similar observations with rice seeds.

Detection of the above-mentioned fungal species on black cumin seeds constitutes the first comprehensive report on seed-borne fungi of black cumin in Egypt. Our results agree in part with the findings of Srivastava and Chandra (1983) for black cumin seeds in India.

A number of the seed-borne fungi of black cumin produce mycotoxins, including *Aspergillus flavus*, *A. ochraceus* and *Fusarium* spp. Since the seeds are edible and are also used in the manufacture of pharmaceutical products, seed-health testing should be done to avoid using potentially hazardous seeds.

There are variations among the different seed-borne pathogenic fungi in their effects on germination and seedling growth. Seed inoculation with *F. oxysporum* caused a higher percentage of seedling infection than soil infestation; pre-emergence damping off was 39 percent with seed inoculation, while it was 21.8 percent with soil infestation. *Fusarium solani* proved to be an aggressive fungus on black cumin seeds, causing 44 percent pre-

emergence damping off with seed inoculation and 37.5 percent with soil infestation, while post-emergence damping off was 19 and 12.5 percent, respectively.

Seedling survival was 37.5 percent with seed inoculation and 50 percent with soil infestation. *F. moniliforme* was more aggressive on black cumin and caused 47 and 14 percent mortality of seeds and seedlings, respectively, in the seed inoculation test and 17 and 12.5 percent, respectively, with soil infestation. *Verticillium* sp. showed a greater effect on germination of the black cumin seeds. The fungus was highly aggressive with seed inoculation, causing 29.7 percent seed rot. It was less aggressive on seeds and seedlings using the soil infestation technique.

These data indicate the deleterious effects of seed-borne fungi on crop yields and emphasize the need to use clean seed in the production of the crop. The results agree with the findings of Dawar (1994) and Perveen (1996), who showed that artificially inoculated seeds resulted in a higher percentage of infection than artificially infested soil.

Seed transmission of the four tested fungi in black cumin might occurs some how by a different mechanism than movement through the vascular system into the seed. The tested fungi were recovered only from the lower portions of the stem but *F. moniliforme* was isolated from the middle portion of the stem. Failure of the seed-borne fungi to move upwards through the vascular system of black cumin plants may be due to structural and/or metabolic defense mechanisms, such as plant constituents which inhibit fungal growth.

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