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Fungi on bean seeds obtained from growers in Isparta province

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ABSTRACT

The fungal load of seeds is among the factors related to seed quality. Seed-borne fungi can cause significant crop losses and may produce mycotoxins, which are harmful to human health. This study aimed to determine the seed-borne fungi of bean, which is among one of the most important crops cultivated in Isparta province. Seed samples from the 2015-2016 vegetation period, obtained from bean growers in Isparta province were used in this study. The fungal load of randomly selected 200 seeds were investigated by blotter and agar methods and prevalence and infestation rates of the fungi were determined. As a result, 41 species of fungi belonging to 26 genera were determined on 62 seed samples. The blotter method yielded 25 genera while 20 genera were determined by the agar method. *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Rhizopus* species were the most common fungi found on almost all of the samples, for both methods. Infestation rates of these fungi were also high on bean seeds. Prevalence and infestation rates of the fungi were generally higher in the blotter method, except for *Fusarium* and *Verticillium* species that were determined on the seed samples in higher frequencies by the agar method. *Absidia*, *Arthrinium*, *Epicoccum*, *Nigrospora*, *Scopulariopsis* and *Stachybotrys* species were determined only by the blotter method, while *Seimatosporium* sp. was found by the agar method. *Alternaria*, *Fusarium*, *Rhizoctonia*, *Stemphylium* and *Verticillium* species are known as pathogenic fungi and may cause diseases on bean plants. *Aspergillus* and *Penicillium* species are saprobic fungi producing mycotoxins, so their presence on the seeds is also important.

1. Introduction

Bean (*Phaseolus vulgaris* L.) is a nutritive food that contains high protein and sufficient amounts of carbohydrates and is rich in calcium, potassium, magnesium, and phosphorus as well as various vitamins. Its ability to grow in different regions and to improve soil properties for subsequent agricultural products increases the importance of the bean (Varankaya 2011). Annual average consumption of dry beans per person in Türkiye was 3 kg, indicates its importance in our nutrition (MFAL 2014). Due to its location between the Western Anatolia, Central Anatolia, and Mediterranean Regions, a wide variety of crops can be grown in Isparta province. Field crops are leading with a 49% cultivation rate in the province, and cereals come first among the field crops, followed by legumes (MCT 2016). Chickpeas, lentils, and beans are the most important legumes in terms of cultivation area, production, and yield. In Isparta province in 2021, 1093 tons of dry beans were produced in 7534 decares of land and 19051 tons of green beans and kidney beans were produced on 15822 decares of land (TURKSTAT 2022).

Seeds are among the factors that enable the production of qualified products and yield increase per unit area. Seeds are important not only for their usage as human food, but they also serve as a starting material in plant production. It is of great importance to use high-quality and healthy seeds and production materials to provide the highest yield from the unit area to meet the nutritional needs of the ever-increasing world population. The fact that nearly 90% of agricultural products are grown from seeds highlights the importance of using healthy seeds (Paylan et al. 2011).

The microorganism load of the seed surface is one of the remarkable features that determine the quality of the seed. There are numerous examples in the literature on the spread of plant diseases within and between countries as a result of the import of seeds contaminated with pathogens (Kaiser 1997; Ghangaokar and Kshirsagar 2013; Kurt 2013). In addition, it would be harmful to consume the seeds infested with *Aspergillus*, *Penicillium* and *Fusarium* species as food, since the presence of such fungi indicates the presence of mycotoxins, which are toxic to humans and animals (Tseng et al. 1995a, b; Domijan et al. 2005). Seed-borne pathogens greatly affect seed quality and cause diseases that affect plant production and yield (El-Gali 2015). Seed-borne pathogens have different effects on seeds such as weakening or loss of the germination ability, colour and shape changes, toxin formation and biochemical changes, decrease in yield (between 15-30%), development and spread of plant diseases, inhibition of seed formation or maturation, and seed rot (Baştaş et al. 2004; Zaidi and Pathak 2013).

Various studies have been carried out on fungal diseases of bean seeds throughout the world. *Aspergillus*, *Penicillium*, *Fusarium* and *Botrytis* species are among the common fungal agents carried with the bean seeds, as well as *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum*,

Rhizoctonia solani, *Rhizopus stolonifer*, *Stemphylium globuliferum*, *Trichothecium roseum*, *Verticillium dahliae*, *Colletotrichum lindemuthianum*, *Phaeoisariopsis griseola*, *Ascochyta phaseolorum*, *Macrophomina phaseolina*, *Phoma exigua* and *Sclerotinia* sp. (Yesuf and Sangchote 2005; Elwakil et al. 2009).

In Türkiye, there are relatively few studies on the determination of fungi on bean seeds. In a previous study on this subject, 285 bean seed samples obtained from 36 provinces were studied by the blotter method, and 41 fungal species belonging to 32 genera were determined on the seeds (Maden and İren 1984). In another study, conducted in Erzurum province, it was determined that 57 seed samples were infested by *A. alternata*, *Aspergillus* spp., *Botrytis cinerea*, *Cladosporium* spp., *C. lindemuthianum*, *Fusarium acuminatum*, *F. equiseti*, *F. proliferatum*, *F. verticillioides*, *Penicillium* spp., *Phoma glomerata*, *P. medicaginis*, *R. solani*, *R. stolonifer*, *Stemphylium botyosum*, *Trichoderma* spp. *T. roseum* and *Ulocladium atrum* (Demirci and Çağlar 1998) with different rates. As a result of a study conducted in Eskişehir province, 15 fungal species were determined on bean seeds, which were *Cladosporium herbarum*, *C. sporangiosum*, *Penicillium piceum*, *P. camemberti*, *P. frequentans*, *P. rubrum*, *Sclerotinia sclerotiorum*, a steril fungus, *Aspergillus terricola*, *A. carneus*, *Gliocladium roseum*, *Stachybotrys chartarum*, *A. alternata*, *Trichoderma harzianum* and *Phoma* sp. (Küçük et al. 2005).

Bean is one of the crops most widely produced in the agricultural areas of Isparta province. Therefore, the determination of the fungal load of seeds obtained from bean growers in Isparta province was an integral part of this study.

2. Materials and Methods

2.1. Seed Samples

In the study, bean seed samples of different varieties obtained from bean growers in Isparta province in the 2015 and 2016 production seasons were used. According to the sample numbers determined by considering the bean cultivation areas of the districts, 62 seed samples were taken from different villages or locations in the districts (Table 1). Fungi in 200 randomly selected seeds from each seed sample obtained from the producers were determined by the blotter and agar methods (Marcinkowska 2002).

2.2. The blotter method

To determine the superficially transmitted fungal agents on the seeds, 100 seeds were randomly selected from each seed sample. Bean seeds were placed in 9 cm diameter glass Petri dishes with 3 layers of sterile blotter paper moistened with sterile distilled water, with 7 seeds in each dish. Petri dishes were incubated at 22±1°C for 7 days in a climate chamber with a light and dark regimen of 12 hours each.

2.3. The agar method

In the agar method, used to determine the fungal agents carried under the seed coat, 100 seeds were randomly selected from each sample and were subjected to surface disinfection with 1% sodium hypochlorite for 10 minutes, then rinsed with sterile distilled water for 3 minutes to eliminate the superficial microorganisms on the seeds. To prevent bacterial contamination, 7 seeds were placed in sterile 9 cm diameter Petri dishes containing Potato Dextrose Agar (PDA, Biolife-Italy) with 50 mg L^{-1} streptomycin sulfate (Demirci and Çağlar 1998; Küçük et al. 2005) and incubated for 5 days in the climate chamber with similar conditions as in the blotter test.

2.4. Identification of the fungi on the bean seeds

After the incubation period, the seeds were examined under a stereomicroscope and the fungi growing on the seeds were identified and recorded at the genus level. Afterwards, slides prepared with each isolate were examined under the light microscope, and the fungi were identified at the species level by using related literature (Booth 1971; Ellis 1971; 1976; Samson et al. 1995; Watanabe 2002; Leslie and Summerell 2006). Lactofuchsin was used as a dye on the slides (Chamswarng and Cook 1985). The sizes of the sexual or asexual organs or spores of the fungi were measured using an ocular micrometer and compared with the relevant sources. The prevalence and the infestation rates of the fungi in each sample (%) were calculated by the formulas [1] and [2] given below (Duan et al. 2007).

Prevalence rate (%)= (Number of samples with the fungus/Total number of samples) X 100 (1)

Infestation rate (%)= (Number of seeds with fungus/Total number of seeds) X 100 (2)

3. Results and Discussion

As a result of the study, a total of 41 species belonging to 26 different genera were determined on the seed samples (Table 2). Among the fungi isolated from the seed samples, the most common genera were *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Rhizopus* and *Ulocladium*. While some genera such as *Absidia*, *Arthrinium*, *Epicoccum*, *Nigrospora*, *Scopulariopsis* and *Stachybotrys* were only detected by the blotter method, *Seimatosporium* was only determined in the agar test.

Alternaria alternata, *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *R. stolonifer* and *U. atrum*, commonly found on bean seeds in this study were also previously isolated from bean seeds in Türkiye (Maden and İren 1984; Demirci and Çağlar 1998; Küçük et al. 2005). *Drechslera hawaiiensis*, *D. spicifera*, *Epicoccum nigrum*, *G. roseum*, *Phoma* spp., *R. solani*, *Scopulariopsis brevicaulis*, *S. chartarum*, *Stemphylium herbarum*, *Trichoderma* spp., *T. roseum* and *Verticillium* spp., reported to be found on bean seeds in Türkiye by the same authors, were also determined in the present study.

Among the *Fusarium* species found in this study, *F. equiseti*, *F. oxysporum*, *F. sambucinum*, *F. semitectum*, *F. solani* and *F. verticillioides* were previously isolated from bean seeds in Türkiye (Maden and İren 1984; Demirci and Çağlar 1998). *F. avenaceum*, *F. chlamydosporum* and *F. subglutinans* were reported to be found on bean seeds in other countries (Castillo et al. 2004; Marcenaro and Valkonen 2016; Russell et al. 2017). However, there was no record of the isolation of *F. lateritium* and *F. sporotrichoides* from bean seeds, except in the present study. But, *F. lateritium* was reported as one of the most important pathogens which cause root rot on beans in Mexico (Sanchez-Garcia et al. 2006). *F. sporotrichoides* was reported to be isolated from cereals and legumes in Türkiye and considered a weak pathogen (Asan 2017).

Table 1. Bean cultivation areas of the districts of Isparta province (TURKSTAT 2015) and the number of samples taken accordingly

Districts	Dry bean areas (decare)	Green bean areas (decare)	Total area (decare)	Number of Samples
Central District	410	466	876	4
Aksu	579	2300	2879	10
Atabey	141	228	369	2
Eğirdir	265	137	402	2
Gelendost	1350	95	1445	8
Gönen	-	32	32	2
Keçiborlu	82	452	534	4
Senirkent	75	225	300	2
Sütçüler	60	178	238	2
Şarkikaraağaç	9450	480	9930	10
Uluborlu	26	12	38	2
Yalvaç	4 525	810	5 335	10
Yenişarbademli	225	300	525	4
			Total number of samples	**62**

Table 2. The prevalence and infestation rates of fungi determined by blotter and agar tests on bean seed samples produced in Isparta province

Fungus genera	Prevalence rates (%)		Infestation rates (%)	
	Agar test	Blotter test	Agar test	Blotter test
Absidia	0	1.612	0	0.016
Acremonium	3.225	4.838	0.080	0.048
Alternaria	50	79.032	2.483	9.693
Arthrinium	0	3.225	0	0.064
Aspergillus	64.516	100	3.661	27.096
Chaetomium	4.838	11.290	0.096	0.129
Cladosporium	29.032	87.096	1.403	21.096
Doratomyces	3.225	4.838	0.032	0.064
Drechslera	3.225	6.451	0.032	0.096
Epicoccum	0	4.838	0	0.064
Eurotium	6.451	53.225	0.709	3.741
Fusarium	56.451	27.419	1.709	0.596
Gliocladium	3.225	3.225	0.032	0.032
Nigrospora	0	3.225	0	0.064
Paecilomyces	1.612	1.612	0.032	0.016
Penicillium	75.806	100	7.806	42.822
Phoma	1.612	1.612	0.016	0.016
Rhizoctonia	4.838	8.064	0.064	0.096
Rhizopus	58.064	100	5.693	32.451
Scopulariopsis	0	1.612	0	0.032
Seimatosporium	1.612	0	0.016	0
Stachybotrys	0	3.225	0	0.032
Stemphylium	4.838	17.741	0.080	0.306
Trichoderma	9.677	22.580	0.935	0.870
Trichothecium	1.612	4.838	0.016	0.080
Ulocladium	9.677	48. 387	0.129	2.064
Verticillium	4.838	3.225	0.048	0.032

Fungi found both in the present study and previously reported from bean seeds were; *Acremonium strictum* (Abdulwehab et al. 2015), *Eurotium* sp. (Tseng et al. 1995b; Mota et al. 2017), *Chaetomium globosum* and *C. spirale* (Watanabe 2002; Russell et al. 2017), *Nigrospora oryzae* (Ghangaokar and Kshirsagar 2013) and *Scopulariopsis brevicaulis* (Russell et al. 2017). No information was found on the presence of these fungi on bean seeds in Türkiye. However, this is probably the first report in the world indicating that *Absidia, Arthrinium, Doratomyces, Paecilomyces* and *Seimatosporium* species were isolated from bean seeds. *Absidia corymbifera* was detected only in a single seed in a sample taken from the Aksu district. *Absidia* species had been recorded on spices, nuts, sunflower seeds, peaches, maize, cereal products, soybeans and peas, but no record of isolation from bean plants has been found (Pitt and Hocking 1997; Anwar et al. 2013). Two species belonging to the genus *Arthrinium*; *A. phaeospermum* and *A. arundinis* were determined in the seed samples examined in the study. In a study conducted in Argentina, *A. phaeospermum* was detected on wheat, millet, and soybean seeds (Broggi et al. 2007). *A. arundinis* was isolated from the roots and hypocotyls of young bean plants in a study conducted in Japan (Sato et al. 2014). InTürkiye, it was isolated from canola seeds (Alpaslan and Özer 2017). *Doratomyces stemonitis* was isolated from the seed samples taken from Aksu

and Atabey by the agar test and from Gelendost and Yalvaç districts by the blotter method. It was reported that the fungus caused rot on potatoes, oat, and maize, resulting in economic loss through a reduction in yield (Webster and Weber 2007). In the present study, two different species belonging to the genus *Paecilomyces* were determined on bean seed samples taken from the Şarkikaraağaç district. These were *P. farinosus* and *P. victoriae*. Although *P. farinosus* is primarily known as an entomopathogen (Leena et al. 2003), it was mentioned among the seed-borne fungi that reduce the germination of spruce and pine seeds (Urosevic 1961). *P. victoriae* was previously isolated from acacia seeds (Vijayan 1988). *Seimatosporium monochaetioides* was detected only in one seed sample obtained from Yenişarbademli and this is the first record of its isolation from bean seeds.

4. Conclusion and Recommendations

As a result of the study, which aimed to determine the fungal agents on bean seeds cultivated in Isparta province, a total of 41 species belonging to 26 different genera were determined from 62 seed samples. In the study, species belonging to *Alternaria*, *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, *Penicillium*, *Rhizopus* and *Ulocladium* genera were determined to be the most common fungi on bean seed samples. The contamination rates of these fungi were generally parallel to their prevalence rates. Among the fungal genera determined, the highest number of species was determined in the *Fusarium* genus with 11 species. According to our findings, that the prevalence and infestation rates of the fungi determined by the blotter method were higher compared to the agar test, it can be mentioned that superficially transmitted fungi were more common on bean seeds. Important plant pathogens, as well as saprobic fungi, were determined on the seed samples taken from bean growers in Isparta province. *Fusarium solani*, *F. oxysporum*, and *R. solani* are among the pathogenic fungi that negatively affect the growth and yield of beans. Their presence on seeds will increase the prevalence and severity of the diseases they cause, by increasing the pathogen inoculum from year to year. In this respect, it is important to prevent their transmission with seed applications. Especially *Aspergillus*, *Penicillium*, *Cladosporium* and *Stachybotrys* species, which are among the saprobic agents, are known as fungi that synthesize toxic metabolites for humans. Their presence on the seeds consumed as food is harmful. In this respect, it would be appropriate to take measures to prevent the development of these fungi, especially on seeds which are to be used as food.

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