DETERMINATION OF SEED-BORNE FUNGI IN SOME MEDICINAL AND AROMATIC PLANTS

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Abstract. The aim of this study was to identify as well as to determine and pathogenicity of fungi, using the agar plate and blotter method, associated with the seeds of anise (*Pimpinella anisum*), coriander (*Coriandrum sativum*), flax (*Linum usitatissimum*), fenugreek (*Trigonella foenumgraecum*), fennel (*Foeniculum vulgare*), and lemon balm (*Melissa officinalis*). A total of six fungi namely *Alternaria alternata*, *Aspergillus niger*, *Arthrinium arundinis*, *Botrytis cinerea*, *Cladosporium sphaerospermum*, and *Penicillium* spp. isolated from seeds of the medicinal and aromatic plants. Pathogenicity tests demonstrated that *A. alternata* was pathogenic in lemon balm and fenugreek seeds at the rates of 70 and 75%, respectively. *B. cinerea* was determined as a pathogen in lemon balm seed at the rate of 35%. *A. arundinis* was detected at rates of 96 and 67% pathogen in coriander and lemon balm seeds, respectively. *A. niger* was determined as pathogen in coriander seed at the rate of 100%. *C. sphaerospermum* was found as pathogen in fennel and flax seeds at the rates of 86 to 90%, respectively. To our knowledge this is the first report on *A. alternata*, *B. cinerea* and *A. arundinis* isolated from melissa seeds in Turkey. This study also represents the first reported cases of *C. sphaerospermum* in flax and fennel seeds and *A. alternata* in fenugreek seeds and *A. niger* and *A. arundinis* on coriander seeds for Turkey.
Keywords: *anise, coriander, flax, fennel, fenugreek, lemon balm, fungal disease, pathogenicity*

Introduction

Anise, coriander, flax, fennel, fenugreek and lemon balm are among the most important medicinal aromatic plants that can be propagated by seeds. In recent years, interest in the use of herbal medicine has increased in the world (Singh et al., 2016). Those products are more environment friendly or understanding the method of action could lead to develop more efficient and less harmful drugs. On the other hand, some of the synthetic drug raw materials used in various treatments may have very dangerous side effects for human health. Thus, scientific studies on the field of alternative medicine that focus on medicinal and aromatic plants make products derived from these species (Faydaoğlu and Sürücüoğlu, 2011). According to the data of the World Health Organization (WHO), 80% of the population in underdeveloped countries are using traditional medicines for treatment, while this rate is about 40% in developed countries. The utilization rate of medicinal plants is expected to increase all over the world in the future (Acıbuca and Budak, 2018). In addition to the use of medicinal and aromatic plants in the pharmaceutical industry; the refreshing effect of spices, cleaning products, toothpaste, chewing gum and herbal teas and their natural presence in cosmetics is of great importance. Besides that, the use of medicinal and aromatic plants as organic fertilizers in organic agriculture also increases the beneficial microbial population, helping to improve yield and quality in plant production (Badalingappanavar et al., 2018).

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According to the information belonging to 12000 plant taxa in Turkey, which naturally occur and approximately one third of those taxa are endemic. In Turkey, 347 medicinal and aromatic plant species are traded in domestic and foreign trade and 139 the plant are exported. Medicinal and aromatic plants collected in Turkey often provided by nature. This situation makes it difficult to keep healthy statistical data on this issue (Bingol et al., 2019).

The exports of medicinal and aromatic plants increased from 112 million dollars in 2002 to 280 million dollars at the end of 2015 with a change of 150%. The most important crops for export are thyme, poppy, laurel, tea, anise, cumin, sage, mahaleb, red pepper and herbal teas (lime, rosehip, sage, mixed fruits, etc.) in Turkey (Temel et al., 2018). In accordance with 2020 data of Turkey Statistical Institute, annual lemon balm, coriander, fenugreek, fennel and anise production in Turkey were 150, 188, 713, 4365 and 10716 tons, respectively (TUIK, 2020).

In nature, various pathogenic fungi, bacteria, viruses and phytoplasmas can infect foliage, fresh stems and rhizosphera plant parts of the medicinal and aromatic plants. Air-borne fungal diseases such as powdery mildew, rust, and some leaf blight affect the development of these plants negatively. Medicinal and aromatic plants are also affected by disease caused by soil-borne fungi and bacteria pathogens such as damping off, root rot, wilt, anthracnose, and dieback (Sing et al., 2016). Seed-borne fungal pathogens cause a decrease in seed quality, germination capability and a decrease in the amount of product to be taken from the plant. Researches on the detection of seed-borne fungal diseases focus mostly on anise, coriander, fennel, cumin and mustard seeds among medicinal aromatic plants. In seed-borne fungi isolations realised on coriander, fennel, cumin and anise seeds, *A. dauci*, *A. radicina*, *A. petroselini* and *A. alternata* species belong to the genus *Alternaria* were identified in previous studies (Demirci and Hancıoğlu, 1994; Bulajić et al., 2009; Özer and Bayraktar, 2015). *Aspergillus*, *Botrytis*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Trichoderma*, *Cladosporium*, *Mucor*, *Epicoccum*, *Phoma*, *Rhizoctonia*, *Acremonium*, *Curvularia* and *Rhizopus* are among the genus of pathogen fungi most isolated from medicinal aromatic plant seeds (Sing et al., 2013; Özer and Bayraktar, 2015; Pavlović et al., 2016; Akhtar et al., 2017; Gahukar, 2018; Mangwende et al., 2018). The objective of this study was to identify seed-borne fungi and their pathogenicity on anise, coriander, flax, fennel, fenugreek, and lemon balm seeds.

Materials and methods

Detection of seed-borne mycoflora of medicinal and aromatic plant seeds

Seeds of anise (*P. anisum*), coriander (*C. sativum*), flax (*L. usitatissimum*), fennel (*F. vulgare*), fenugreek (*T. foenumgraecum*), and lemon balm (*M. officinalis*) were obtained from Tekirdag Namik Kemal University, Department of Field Crops in Turkey. The seeds were stored in 50 g paper bags for isolation at 4 °C in a refrigerator. Four hundred seeds of each the medicinal and aromatic plant seeds were randomly selected. The seeds were surface sterilized in 2% sodium hypochlorite for 3 minutes, and then rinsed in sterile distilled water (SDS) twice and dried on sterile filter paper in a sterile bench. The surface sterilized seeds were transferred on potato dextrose agar (PDA) medium (Merck, Darmstad, Germany) (containing streptomycin 0.1 g/1000 ml SDS and chloramphenicol 0.05 g/1000 ml SDS: Sigma-Aldric, Germany) in 9 cm diameter sterilized Petri plates. The Petri plates were incubated for 7-10 days at 23 °C under for a 12 h dark/light cycle. There were 10 seeds in each Petri plates. Each treatment was replicated 40 times. After 7-10 days of

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incubation period, fungal colonies growing on the seeds were individually examined with the aid of a stereomicroscope. The single spore isolations of seed-borne fungi were done by Agar Plate Methods in which pre-sterilized (hot air) petri plates were taken and plated either with sterilized PDA, Potato Carrot Agar (PCA), Water Agar and Oat Flour Agar. Each of the purified fungus isolates was examined at 40× to 100× with a Zeiss light microscope according to their growth rate, culture colors, conidiophore and conidia structures and their taxonomic characteristics were identified, and grouped based on morphological appearance and recorded (Domsch et al., 1980; Ellis and Ellis, 1997; Lawrence et al., 2016).

The percentage frequency of occurrence of various fungal species was calculated as follows (*Eq.1*):

$$\text{Frequency of occurrence (\%)} = \frac{\text{Number of seeds on which a fungal species occurs}}{\text{Total Number of seeds}} \text{ x } 100 \qquad \text{(Eq.1)}$$

For the pathogenicity tests of the isolates grouped at the species or genus level, isolates representing each group were selected and single spore isolations were made. The isolation of fungi studies was carried out in Mycology Laboratory Department of Plant Protection, Faculty of Agriculture, Tekirdag Namik Kemal University.

In vitro pathogenicity test

Fungi species isolated from each seed variety were grouped primarily at the genus and species level. Single spore isolates of the isolate representing each group were prepared, and their morphological diagnosis was made by examining them under a microscope. Pathogenicity testing was performed with a representative isolate of each morphologically diagnosed fungus species. As described in the determination of fungal flora, first of all, surface sterilization was applied to all the seeds. For the pathogenicity test of *Alternaria alternata* and *Cladosporium sphaerospermum* spore concentration were adjusted to 1 x 10^5 spores/ml by diluting in SDS. Inoculum concentration of *Aspergillus niger*, *Arthrinium arundinis* and *Botrytis cinerea* were adjusted to a final concentration of 1 x 10^4 spores / ml (Noelting et al., 2012; Singh et al., 2013; Akhdar et al., 2017). The seeds were dipped into the prepared conidia suspensions, 10 µl Tween 20 was added and shaken with a rotary shaker for 1 hour. The seeds inoculated with fungal isolates were placed on sterile blotting papers and allowed to dry for 30 minutes. Four layers of blotter (in size equivalent to each petri plate) were soaked in SDS. Ten seeds were placed in each petri dishes. After planting the seeds in the petri plates were incubated for 7-10 days at 24±1°C with a cycle of 12 hours light and 12 hours darkness. The experiment was conducted in a completely randomized design with four replicates and repeated twice. Observed et the end of the incubation period, each seed was evaluated as disease-healthy. The pathogenic fungi were re-isolated from diseased seed. Conversely, the control petri plate which contain only sterilized seeds did not show any symptoms. Pathogenicity test was performed on the all seeds included in the experiment for all isolates that were diagnosed morphologically.

Molecular identification

Molecular diagnosis was performed for 3 isolates among the morphologically identified isolates. A representative fungal isolate was selected for the diagnosis of the molecular properties of *A. alternata* and *Arthrinium arundinis* from lemon balm seeds

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and *C. sphaerospermum* isolated from fennel seeds. Identity confirmation of *A. alternata* isolate TR-MeAa3 and *C. sphaerospermum* isolate TR-ReCs13 and *A. arundinis* TR-MeArtA5 isolate were realised by the polymerase chain reaction (PCR). Ribosomal fungal DNA extraction was conducted using the method from Özer and Bayraktar (2015). Fungal isolates were grown on PDA medium at 25±1°C for 7-10 days. Fungal mycelia of each isolate were gently scraped with a sterile spatula from the surface of PDA medium and suspended in 500 µl extraction buffer (50 mM Tris-HCl pH: 7.5, 50 mM EDTA, 3% SDS). Two times extractions with phenol/chloroform/isoamylalcohol (24:1:1; v/v/v) were done, DNA was precipitated by addition of 0.5 volume of 7.5 M ammonium acetate and 1.5 volume of isopropanol. The resultant pellet was rinsed with ethanol suspended in ddH_2O, and stored at -20°C. The identification of the isolates representing different fungal species was confirmed by DNA sequence analysis with the primer pairs ITS1/4 and Alt for /rev described by White et al. (1990) and Hong et al. (2005), respectively. PCR reaction was carried out in 50 ml mixture containing 5 µl reaction buffer (10×), 1.5 mM of $MgCl_2$, 0.4 µl of each primer, 0.2 mM of dNTPs, 1.5 unit of Taq DNA polymerase (MBI; Fermentas, Leon-Rot, Germany) and remaining deionised water. PCR amplification was performed in a thermal cycler programmed as follows: one cycle of 94°C for 1 min, 35 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 1 min, and during 10 min at 72°C. The amplified DNA products were sequenced in both directions using the same primers in NABILTEM Laboratory. For the identification of the fungal isolates, the sequences were subjected to BLAST searches within NCBI database. Sequence analysis and comparison of the fungus isolates were conducted using MEGA 6.0 software (Tamura et al., 2013).

Statistical analysis

In the study, the data obtained as a result of the pathogenicity tests were statistically analyzed using the one-way ANOVA procedures in SPSS (Statistical Package for Social Sciences, Inc., 2001, Model 11.0. Chicago). The differences between the the treatment means were compared according to the Duncan Multiple Comparison test ($P \leq 0.01$).

Results

Detection test

Six fungal species namely *Alternaria alternata, Aspergillus niger, Arthrinium arundinis, Botrytis cinerea, Cladosporium sphaerospermum* and *Penicillium spp.* were detected from the seeds of medicinal and aromatic plants. *A. alternata* was the first species has the highest incidence isolated from in leman balm (19%) and fenugreek (25%) seeds, respectively has many in host plants and producing toxins (*Table 1*).

The second species of higher frequency in fennel, anise and flax seeds was *Penicillium* spp. But, *Penicillium* species isolated from all seeds, especially anise seeds, were thought to be saprophytes. Preliminary pathogenicity tests (results no given) showed that *Penicillium* spp. did not caused disease and so was not used other pathogenicity test in this study. Highest percentage of *A. arundinis* was also found in lemon balm seeds (2.25%) followed by coriander seeds (0.25%). *A. niger* had low incidence (0.50%) only in coriander seeds examined. Percentage of occurence of *B. cinerea* (1.00%) was also recorded only in lemon balm seeds. Percentage of occurence of *C. sphaerospermum* was higher frequency in fennel (2.25%) than flax seeds (1.75%) (*Table 1*).

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Table 1. *Percentage of occurrence of seed-borne fungi (%) associated with seeds of the medicinal and aromatic plants*

Seeds	*A. alternata*	*A. arundinis*	*A. niger*	*B. cinerea*	*C. sphaerospermum*	*Penicillium* spp.
Anise	0.00*	0.00	0.00	0.00	0.00	5.00
Coriander	0.00	0.25	0.50	0.00	0.00	0.00
L. balm	19.00	2.25	0.00	1.00	0.00	1.25
Fennel	0.00	0.00	0.00	0.00	2.25	7.50
Fenugreek	25.00	0.00	0.00	0.00	0.00	0.00
Flax	0.00	0.00	0.00	0.00	1.75	5.00

*Each value is the average of four repetitions

Among the medicinal and aromatic plant seeds examined in terms of fungal flora, lemon balm had the highest contamination rate in terms of hosting fungi belong to different species and genera (*Table 1*).

Pathogenicity test

According to the results of the pathogenicity test, the only fungus species growing in fenugreek seeds is *A. alternata* was found to be pathogenic at a rate of 75.00± 3.53%. *A. alternata* was also isolated from lemon balm seeds and its pathogenicity was calculated as 70.00± 2.54% (*Table 2*, *Fig. 1*).

Table 2. *Pathogenicity of the fungi associated with medicinal and aromatic seeds (%)*

Seeds	*A. alternata*	*A. arundinis*	*A. niger*	*B. cinerea*	*C. sphaerospermum*
Coriander	0.00±0.00 ^{b}*	96.00 ± 2.16 ^{a}	100 ± 0.00 ^{a}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}
L. balm	70.00±2.54 ^{a}	67.00 ± 6.04 ^{b}	0.00 ± 0.00 ^{b}	35.00 ± 2.04 ^{a}	0.00 ± 0.00 ^{b}
Fennel	0.00±0.00 ^{b}	0.00 ±0.00 ^{c}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	86.00 ± 2.16 ^{a}
Fenugreek	75.00±3.53 ^{a}	0.00 ±0.00 ^{c}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}
Flax	0.00±0.00 ^{b}	0.00 ±0.00 ^{c}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	90.00 ± 2.16 ^{a}

*: Each value in the same column is the average of four repetitions and the values shown with different letters are significantly different according to Duncan Multiple Comparison Test (P≤0.01)

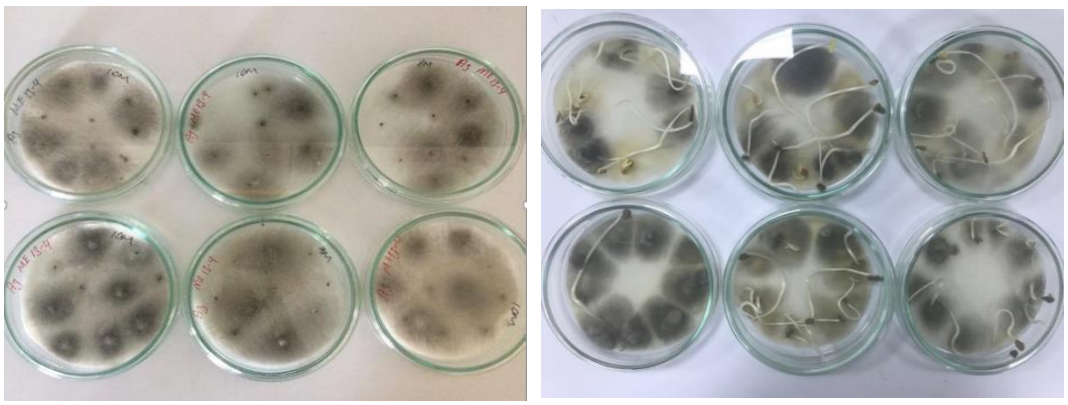

Figure 1. *Pathogenicity of A. alternata TR-MeAa3 in lemon balm (left) and fenugreek seeds (right)*

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There is no statistically significant difference between the disease severity of *A. alternata* developed in fenugreek and lemon balm (p≥0.01) (*Table 2*). It was recorded that *B. cinerea* only developed in lemon balm seeds and showed disease severity of 35± 2.04%. *A. arundinis* was also found to be 67± 6.04% pathogen in lemon balm seeds (*Fig. 2*). This fungus was also recorded pathogen in coriander seeds, and the disease severity was found to be statistically significant at a rate of 96± 2.16% compared to lemon balm seeds (p≤0.01). It was detected that *A. niger* has a high rate of pathogen (100± 0.00%) only from coriander seeds. *C. sphaerospermum* was noted as pathogen fungi at the rate of 86± 2.16% in fennel seeds and at the rate of 90± 2.16% in flax seeds (*Fig. 3*). Statistically, the difference in disease severity between them is not significant (p≥0.01) (*Table 2*). There was discolaration and brown symptoms on the diseased seeds caused by *A. alternata*, *A. arundinis*, and *C. sphaeraspermum*. These seeds, including those that germinated, rotted at the end of the pathogenicity test.

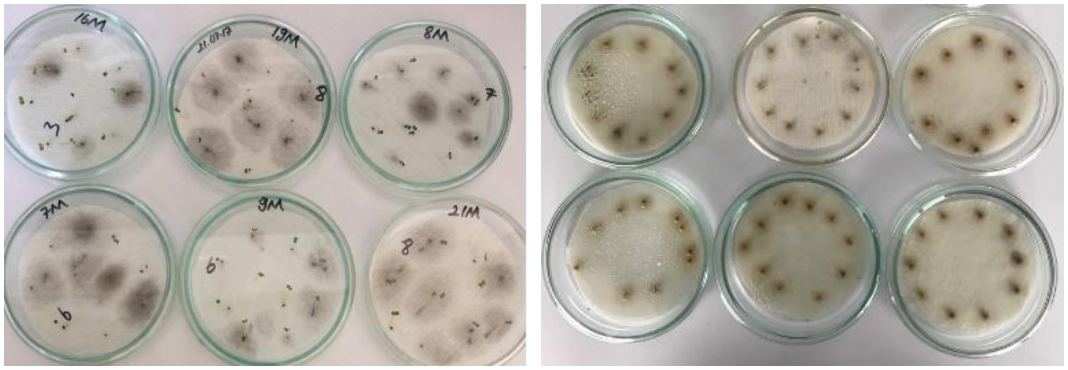

Figure 2. *Pathogenicity of A. arundinis TR-MeArtA5 in lemon balm (left) and coriander seeds (right)*

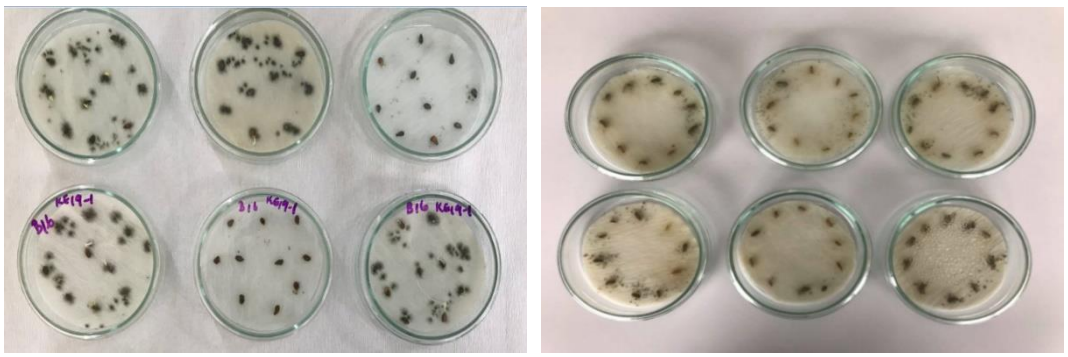

Figure 3. *Pathogenicity of C. sphaerospermum TR-ReCs13 in flax (left) and fennel seeds (right)*

Phylogenetic analyses

The isolates of *Alternaria alternata* TR-MeAa3 (GenBank Acc. No. MK806694) and *Cladosporium sphaerospermum* TR-ReCs13 (GenBank Acc. No. MK806696) and *Arthrinium arundinis* TR-MeArtA5 (GenBank Acc. No. MK806695) were identified based on morphological characters (*Figs. 4,5,6*) were also confirmed to sequenced and checked against the NCBI database. PCR amplification with primers ITS1 and ITS4 yielded a single RNA fragment approximately ranging in length from 525-bp to 591-bp

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from *C. sphaerospermum* TR-ReCs13 (525 bp) and *A. alternata* TR-MeAa3 (591 bp) and *A. arundinis* TR-MeArtA5 (580 bp). According to phylogenetic trees of partial sequencing of 18S rRNA gene of the fungal isolates verified the sequences producing significant alignments of partial sequencing of 18S rRNA gene of the fungal isolates compared to those similar strain and isolate in GenBank. *A. alternata* TR-MeAa3 isolate was isolated from lemon balm seeds showed 99 to 100% similarities with 26 *A. alternata* isolates registered in GenBank (*Fig. 4*) (Kleczewski et al., 2012; Woudenberg et al., 2013, 2015; Kovaceć et al., 2016; Mohamed et al., 2016; Vu et al., 2019). *C. sphaerospermum* TR-ReCs13 was isolated from fennel seeds exhibited 99 to 100% similarities with 18 *C. sphaerospermum* isolates and strain registered in GenBank (*Fig. 5*; Zalar et al., 2007). *A. arundinis* TR-MeArtA5 was isolated from lemon balm seeds demonstrated 85 to 100% similarities with 17 *A. arundinis* isolates registered in GenBank (*Fig. 6*; Crous and Groenewald, 2013; Jiang et al., 2018).

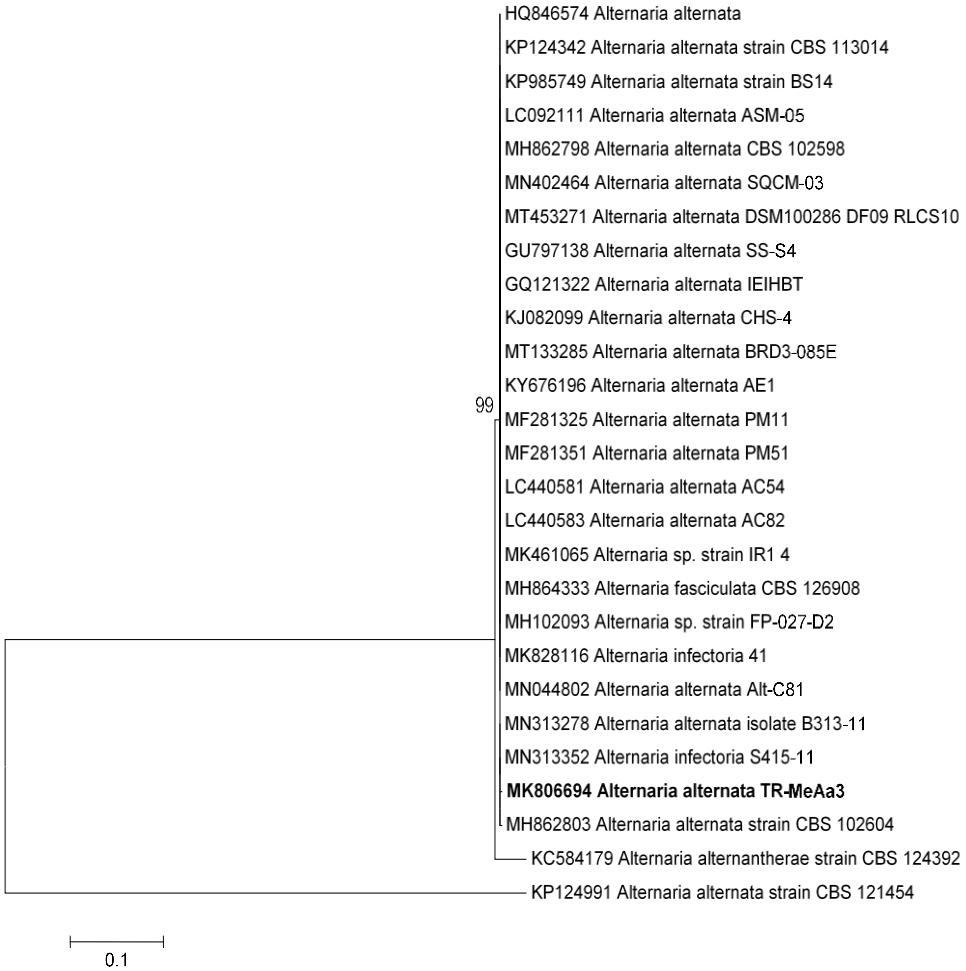

Figure 4. *Phyllogenetic and molecular evolutionary analyses were carried out using MEGA version 6 (Tamura et al. 2013). Maximum likelihood analysis method based on the Kimura-2 model of 27 Alternaria spp. isolates. The sequence of the Turkish isolate from this study is shown in bold. Numbers represent the bootstrap values out of 1000 replicates. Only bootstrap values bigger than 50 are shown*

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Figure 5. *Phyllogenetic and molecular evolutionary analyses were carried out using MEGA version 6 (Tamura et al. 2013). Maximum likelihood analysis method based on the Kimura-2 model of 19 Cladosporium spp. isolates. The sequence of the Turkish isolate from this study is shown in bold. Numbers represent the bootstrap values out of 1000 replicates. Only bootstrap values bigger than 50 are shown*

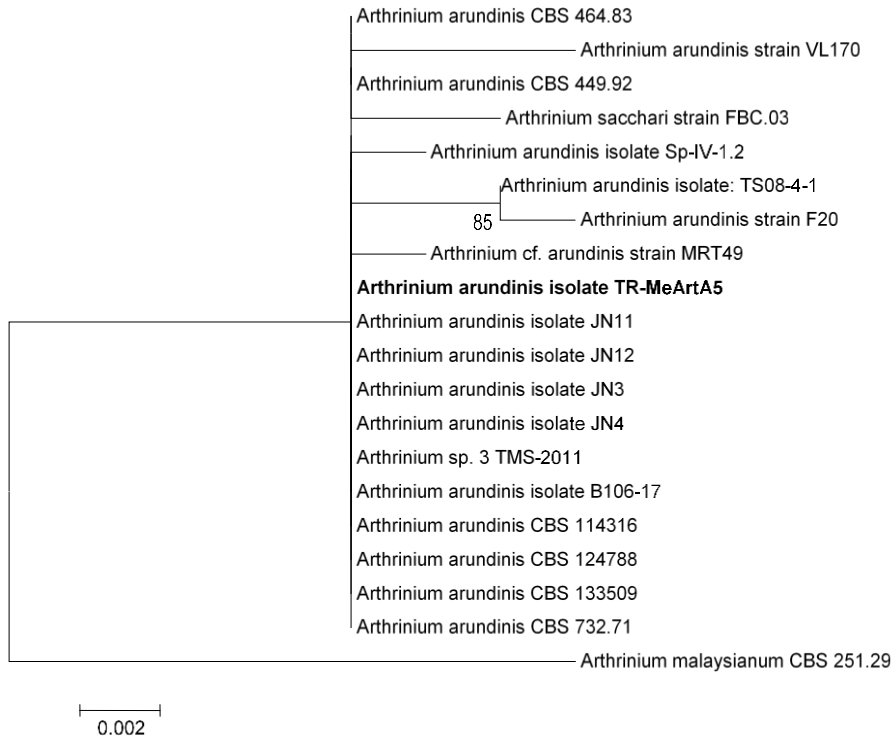

Figure 6. *Phyllogenetic and molecular evolutionary analyses were carried out using MEGA version 6 (Tamura et al. 2013). Maximum likelihood analysis method based on the Kimura-2 model of 20 Arthirinium spp. isolates. The sequence of the Turkish isolate from this study is shown in bold. Numbers represent the bootstrap values out of 1000 replicates. Only bootstrap values bigger than 50 are shown*

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Discussion

In this study for the determination of fungal flora in some medicinal and aromatic plant seeds, it was found that the seeds were contaminated with saprophytes and some pathogenic fungi. Among the seeds examined in our study, it was observed that the highest contamination rates in terms of the number of infected seeds were in lemon balm and fenugreek seeds. It has also been found that there are different species of fungi in lemon balm seeds. *A. niger* and *B. cinerea*, *C. sphaerospermum*, *A. alternata*, *A. arundinis*, which grow from flax, lemon balm, coriander, fenugreek, anise, fennel seeds, were observed to be pathogens at different rates. In addition, molecular identification of *A. alternata*, which has a high rate of pathogen in both fenugreek and lemon balm seeds, was also carried out in this study. *A. alternata* showed similar pathogenicity in lemon balm and fenugreek seeds. Similarly, this pathogen was the most isolated fungus from medicinal and aromatic plant seeds in previous studies (Bulajić et al., 2009; Singh et al., 2013; Seyyedi and Moghaddam, 2016; Mangwende et al., 2018). Although literature on seed-borne fungal diseases in medicinal and aromatic plants are restricted, there is more research on fungal diseases caused problem in the leaf, stems and roots of medicinal and aromatic plants (Singh et al., 2016).

The presence of *A. arundinis* in both lemon balm (11.5%) and coriander (5%) seeds was detected, but this rate was higher in lemon balm seeds. It was noted that coriander seeds of the same fungi are more pathogenic than lemon balm seeds. *A. arundinis* showing 96% pathogenicity in coriander seeds, 67% pathogen was found in lemon balm seeds. Although *C. sphaerospermum* was found at lower rates (1.75%) in flax seeds, its pathogenicity was higher (90%), but there was no significant difference with its pathogenicity (86%) in fennel.

It is observed that *A. arundinis* and *A. niger* were isolated from coriander seed and they had high disease severity in the pathogenicity experiment.

A. alternata was detected in previous studies from coriander, fennel, anise, cumin, lemon balm and fenugreek seeds as the same as in our study (El-Nagerabi, 2002; Szczeponek and Mazur, 2006; Sumanth et al., 2010; Singh et al., 2013; Özer and Bayraktar, 2015; Mangwende et al., 2018). At the end of the pathogenicity test of *A. alternata*, the seeds that could germinate from the infected fenugreek seeds were completely rotted after a while due to the infection by the pathogenic fungus. Similarly, *A. alternata* has been reported seed-borne pathogen, and led to brownish necrotic symptoms and death of cumin seed (Özer and Bayraktar, 2015). In other studies, *A. alternata*, *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* species were reported to be isolated from coriander seeds (Singh et al., 2013; Mangwende et al., 2018). Singh et al. (2013), 54 different fungus species were identified in coriander, foeniculum, cumin and brassica seeds, and similar to our study, other than *Aspergillus niger*, *A. flavus* and *A. parasiticus* species were also identified in coriander seeds.

A. arundinis was not isolated from lemon balm and coriander seeds in previous studies. *C. sphaerospermum* was not isolated from fennel and flax seeds in previous studies and its pathogenicity was not investigated the studies on the seeds before. So, these isolates were included in molecular diagnosis. *A. alternata* was detected in previous studies from coriander, fennel, anise, cumin, lemon balm and fenugreek seeds (El-Nagerabi, 2002; Szczeponek and Mazur, 2006; Sumanth et al., 2010; Singh et al., 2013; Mangwende et al., 2018). In addition, *A. alternata* caused leaf spot on coriander plants, and was confirmed to be seed transmitted by Mangwende et al. (2018). As a result, in this research demonstrated that it also represented the *Alternaria alternata* TR-MeAa3 (GenBank Acc.

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No. MK806694) isolate was isolated from lemon balm seeds showed 99% similarity to *Alternaria alternata* strain CBS 113014 (GenBank Acc. No. KP124342). However, *A. alternata* isolate (GenBank Acc. No. KT895947.) was isolated on coriander seeds by Mangwende et al. (2018) was recorded to similar to *A. alternata strain* CBS 113014 (GenBank Acc. No. KP124342) 73%.

However, in previous studies in Turkey, only *Alternaria* sp. was isolated from coriander seeds and research was carried out on its chemical control (Demirci and Hancıoğlu, 1994).

A. alternata, *B. cinerea* and *A. arundinis* isolated from melissa seeds were first reports in Turkey. *C. sphaerospermum* was the pathogen on both of flax and fennel seeds. *A. niger* and *A. arundinis* were isolated on coriander seeds that was confirmed as pathogens were first recorded in Turkey. *A. alternata* was detected on fenugreek seeds was also first reported in Turkey.

Conclusions

Medicinal and aromatic plants are used in the medicine, food and cosmetics industries, and the market demand for natural products based on these plants is increasing every year. The use of medicinal and aromatic plants as raw materials for important drugs is increasing. It is known that approximately 25% to 30% of all drugs today are obtained directly or indirectly from the plants.

In this study, the fungi detected as pathogens in medicinal aromatic plants significantly inhibited the seeds germination. It is not possible to grow healthy seedlings from the diseased seeds. Pathogenic fungi reduce yield and quality in medicinal and aromatic plants. In order to be able to control these pathogenic fungi, it is very important to know which fungi species are capable of causing disease.

Therefore, the genetic resistance of the seed varieties of medicinal and aromatic plants to be used in production against these pathogens should be examined and the production of non-resistant seeds should not be allowed. Considering the studies conducted in the world in general, molecular studies on the detection of seed-borne fungi in medicinal and aromatic plant seeds are very few. This research is the first detailed study in seed-borne fungi on medicinal and aromatic plant seeds. *Alternaria alternata*, *Botrytis cinerea* and *A. arundinis*, isolated from lemon balm seeds; *C. sphaerospermum* in flax seeds; *A. alternata* in fenugreek; It is the first registration for Turkey. The study is an enlightening nature for the next researches to be carried out for the detection of seed-borne disease factors and their pathogenicity. Considering the pathogen host relationships in disease development, the data we obtained in this study will form the basis for future research on the control of these diseases.

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