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Determination of Antifungal Activities on Some Plant Extracts on *Alternaria alternata*

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ABSTRACT

To increase yield and quality in agricultural production, it is necessary to perform management against diseases and pests. *Alternaria*, which causes several diseases in many economically important plants, is the most common species and widely distributed in nature. One of the important species reported in sweet cherry in recent years is *Alternaria alternata*. Many studies have emphasized the necessity of effective control with *Alternaria* species and examined the use of environmentally friendly methods against fungal diseases. In recent years, the use of plant extracts has increased due to their antimicrobial properties. Antifungal effects of *Datura stramonium* L., *Vitex agnus-castus* L., *Xanthium strumarium* L., *Capsella bursa-pastoris* L., *Convolvulus arvensis* L., *Viscum album* L., *Echinophora tenuifolia* L. *subsp. sibthorpiana* (Guss.) Tutin, *Amaranthus retroflexus* L., *Chenopodium album* L., *Tribulus terrestris* L., *Solanum nigrum* L., *Nerium oleander* L., *Circium arvense* (L.) Scop. and *Brassica oleracea* L. aqueous extracts were determined against *Alternaria alternata*. At the end of the 7-day incubation period, the mycelial growth of the fungi was measured and the antifungal effect of plant extracts was determined. As a result, the extracts were determined to inhibit mycelial growth compared to control. The plant water extracts used in the study were determined to inhibit the mycelial development of the pathogen by 20.20% to 77.12%. It is considered that different solvents and concentrations should be addressed to guide further studies. It was also concluded that potential plant species that may show anti-fungal properties should be evaluated.

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Introduction

Plants are the main source of food, fiber, forage, drugs, and many other useful products for humanity. Humans use different parts of plants such as roots, stems, leaves, fruits, flowers, and seeds to meet their food needs. Various insects, bacteria, viruses, fungi, and other pests cause harm decreasing yield in the several phases of the development of plants. It has been reported that more than 800 million people in developing countries experience food shortages and at least 10 percent of food crops are lost due to plant diseases (Strange & Scott, 2005). Fungi have the greatest impact in terms of disease and loss of crop production compared to other plant parasites. There are about 10.000 fungus species causing disease in plants and these fungi may cause losses not only in the growth period of plants but also in their harvest and storage periods. Harvest loss due to fungal diseases is about 12 % in all the products in the developing countries in the world. *Alternaria* species are the most commonly seen species in above-ground parts and they spread around nature broadly (Lopes & Martins, 2008). It also includes pathogenic saprophytic species that cause putrefaction both in the field and in the post-harvest period and lead to significant economic losses for the product and food industry (Logrieco et al., 2009). Alternaria is a widespread genus of fungi comprising more than 300 species, commonly found in soil and organic matter (Bessadat et al., 2021; Woudenberg et al., 2013). This genus contains saprophytic, endophytic, and pathogenic species. Several different secondary metabolites can be produced by *Alternaria* spp. blight disease, caused by *Alternaria* species, causes an average yield loss of 32-57 %. The members of the *Alternaria* genus such as *A. alternata, A. solani, A. porri, A. dauci, A. helianthi, A. carthami* and *A. makrospore* cause various diseases in different hosts (Chen et al., 2018; Kaya & Zorba 2021; Rotem, 1994). In sweet cherry cultivation, which is economically important in the world and Türkiye, fungal pathogens cause serious threats and cause significant crop losses. In recent years, the presence of *Alternaria alternata* in sweet cherries has been reported in Greece (Thomidis & Tsipouridis, 2006), China (Ahmad et al., 2020; Chethana

et al., 2019; Zhao & Liu, 2012), Italy (Wagas et al., 2023) and Türkiye (Şimşek et al., 2022). Many studies have emphasized the necessity for effective control of *Alternaria* species, which cause several diseases in many economically important cultivated plants, and various studies have been conducted on the use of environmentally friendly methods in controlling fungal diseases (Meena et al., 2020; Yadav et al., 2020).

The various treatments such as fungicides, antagonist organisms, and crop rotation are widely used in the control of plant diseases (Choudhary et al., 2004; Pineda, 2001). In addition, natural fungicides obtained from allelopathic and medicinal plants have become widespread all over the world as alternatives to other control methods due to their environmentally friendly and economic properties. Many researchers are currently working on effective natural products that can replace synthetic pesticides for the control of diseases.

These methods may be sorted as the use of antagonistic microorganisms, the use of plant extracts, or essential oil components and their derivatives. Several studies have shown that certain plant extracts can serve as a bio-pesticide source, effectively preventing the development of plant pathogens, and reducing harm to both human health and the environment. The existence of anti-fungal components in some plants is accepted to be an important factor in controlling some plant diseases (Tapwal et al., 2011). Although there is an increasing interest in the use of the extracts obtained from plants in controlling plant diseases, only 2.400 plant species have been screened among more than 250.000 plant species in terms of antimicrobial effect (Khafagi & Dewedar, 2000; Oluwalana & Adekunle, 1998; Oluwalana et al., 1999). As herbal pesticides are local, cheap, anti-toxic, and easily biodegradable, they provide significant advantages compared to synthetic fungicides (Akinbode & Ikotun, 2008; Bandara et al., 1989; Harlapur et al., 2007; Maji et al., 2005; Manoharachary & Gourinath, 1988; Nduagu et al., 2008; Srivastava & Lal, 1997; Yasmin et al., 2008). Plant metabolites and phytopharmaceuticals are considered as one of the alternative methods to control plant diseases (Varma & Dubey, 1999). As resistance to synthetic fungicides increases and residue levels rise, the use of natural products to control fungal diseases is considered one of the alternatives (Gurjar et al., 2012). The most important factor in weeds maintaining their vitality over time is that they are resistant to the pests and pathogens in their environment. They can therefore be used as a potential source of antimicrobial compounds. In recent years, interest in plant-based fungicides has been increasing due to their environmentally friendly properties (Abdessemed et al. 2021; Dwivedi & Singh, 1998; Karnwal & Singh, 2006). The inhibitory effect of plant extracts on pathogens has been demonstrated, and many higher plants and their compounds have proven successful in controlling plant diseases, without harm or toxicity (Dethoup et al., 2018, Kokkrua et al., 2020). In contrast to chemical fungicides, these extracts are harmless and non-phytotoxic (Alam et al., 2002; Charudattan et al., 2000; Dubey, 1991; Glare et al., 2012; Singh et al., 1986).

Plants can synthesize aromatic secondary metabolites such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, and coumarins (Cowan, 1999). Especially plant extracts, which play an important role in the control of phytopathogens, have been used in many ways. The levels of phenolic compounds, plant pigments, and other chemical compounds in natural extracts have a synergistic effect on plant growth and are also effective in controlling many fungi. Because of these properties, plant extracts play an important role in preventing many pathogens that cause plant diseases (Nwachukwu & Umechuruba 2001). For this purpose, the study examined the antifungal effects of some plants and weeds against *A. alternata*, an important plant pathogen, under *in-vitro* conditions.

Materials and Methods

Plant Material

In the study, the aqueous extracts prepared from the plants *Datura stramonium* L., *Vitex agnus-castus* L., *Xanthium strumarium* L., *Capsella bursa-pastoris* L., *Convolvulus arvensis L.*, *Viscum album* L., *Echinophora tenuifolia* L. subsp. *sibthorpiana* (Guss.) Tutin, *Amaranthus retroflexus* L., *Chenopodium album* L., *Brassica oleracea* L., *Solanum nigrum* L., *Tribulus terrestris* L. *Nerium oleander* L., and *Cirsium arvense* (L.) Scop. were used (Table 1).

Table 1. The plants and plants part used in the study

Scientific name	Common name	Used part	Family
Capsella bursa-pastoris L.	Shepherd's purse	Above ground parts	Brassicaceae
Datura stramonium L.	Jamestown weed	Leaf	Solanaceae
Vitex agnus-castus L.	Chaste tree	Seed	Lamiaceae
Convolvulus arvensis L.	Field Bindweed	Above ground parts	Convolvulaceae
Viscum album L.	Mistletoe	Leaf	Loranthaceae
Viscum album L.	Mistletoe	Stem	Loranthaceae
Xanthium strumarium L.	Cocklebur	Leaf	Asteraceae
Echinophora tenuifolia L. subsp. *sibthorpiana* (Guss.) Tutin.	Turkish pickling herb	Above ground parts	Apiaceae
Amaranthus retroflexus L.	Pigweed	Leaf	Amaranthaceae
Chenopodium album L.	Lamb's quarters	Leaf	Amaranthaceae
Cirsium arvense (L.) Scop.	Creeping thistle	Above ground parts	Asteraceae
Solanum nigrum L.	Nightshade	Leaf	Solanaceae
Nerium oleander L.	Oleander	Leaf	Apocynaceae
Tribulus terrestris L.	Bullhead	Above ground parts	Zygophyllaceae
Brassica oleracea L.	Cabbage	Cabbage outer leaf	Brassicaceae

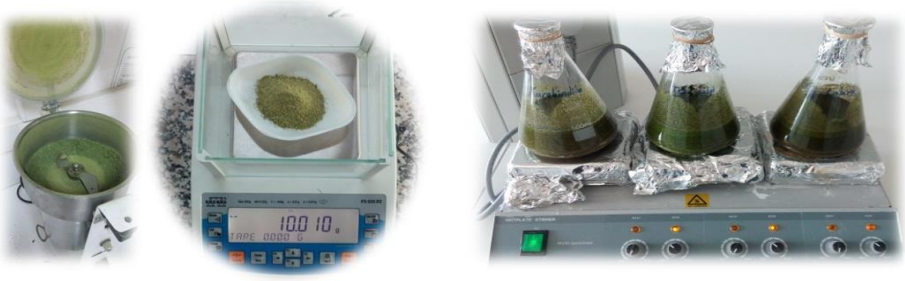

Figure 1. The preparation process of some plant extracts

Figure 2. Transfer of each plant extract to PDA

Fungal Material

A. alternaria (Accesion number MW509978) B1-3 isolate obtained from diseased sweet cherry leaves were used as the fungal material of the study. In the study, 7 day cultures of the pathogen developed in PDA medium at 25+2ºC were used.

Method

Healthy plants were collected and brought to the laboratory. They were cleaned with tap water to remove soil and washed with sterile distilled water. All the plants were dried in an oven at 50 °C until they became completely crispy (Bussaman et al., 2012). The dried leaves were ground and were kept at room temperature in glass jars with screw lids under laboratory conditions.

Preparing plant extracts and Determining the Antifungal Effect on Mycelial Growth

The ground plant parts were diluted with sterile distilled water at the rate of 1/15 (10 gr plant/150 ml distilled water) (Türküsay & Onoğur 1998) and left for extraction on shaker at room temperature for 2 hours (Figure 1). The extracts were filtered in 2-4 folded sterile cheesecloth. The stock extracts obtained from each plant used in the study and the PDA growth medium were sterilized for 15 minutes at 121 °C in an autoclave. The sterilized extracts were added to the growth medium at the rate of (1:1) and the mixture was placed into petri dishes (90 mm diameter), 15 ml for each. Petri dishes were kept for 1 night at room temperature for the environment to be ready for fungal inoculation (Figure 2). Discs obtained from pathogen cultures grown for 7 days in PDA growth medium using a 5 mm cork borer were placed in the centre of each petri, one disc in each petri, and left to incubate at 25+2ºC. The control Petri dishes contained PDA growth medium with sterile distilled water. The test was conducted with 5 repetitions. Colony diameter was measured in perpendicular directions by Benjilali et al (1984), and using a digital caliper and mycelial growth of fungus in determining the anti-fungal effect of the plant extracts was assessed. The % inhibition of the plant extracts on mycelial growth was calculated. The formula used in the calculation is stated below. The % inhibitive effect of the plant extracts on mycelial growth compared to the controls, was calculated by the following formula (Mohana & Raveesha, 2007).

I= C – A / C×100
I= Inhibition (%)
C= Colony diameter in control petri dish (mm)
A= Colony diameter in application petri dish (mm)

Statistical Analysis

To determine the differences between the treatments in the tests, we conducted a significance analysis of variance (ANOVA) and compared the means using the DUNCAN test. Statistical analysis was evaluated by using the SPSS 23.0 packaged software.

Results and Discussion

Alternaria causes leaf diseases with economic significance on a wide range of host plants including cereals, vegetables, fruit, ornamentals, and forest trees. The effects of the water extracts obtained from *Datura stramonium*, *Vitex agnus- castus*, *Xanthium strumarium*, *Capsella bursa-pastoris*, *Convolvulus arvensis*, *Viscum album*, *Echinophora tenuifolia* L. subsp. *sibthorpiana* (Guss.) Tutin, *Amaranthus retroflexus*, *Chenopodium album*, *Tribulus terrestris*, *Solanum nigrum*, *Nerium oleander*, *Circium arvense*, and *Brassica oleracea* plants on the mycelial growth of *A. alternata* under *in-vitro* conditions were determined (Table 2). *Brassica oleracea* L. (cabbage outer leaf) came out on top with an inhibition rate of 77.12 % and was statistically different from the other extracts (Figure 3). A study reported that plant extracts obtained from six plant species belonging to six different families (Alliaceae, Brassicaceae, Lythraceae, Lamiaceae, Solanaceae and Verbenaceae) showed significant antifungal effects against *F. oxysporum* f.sp. *lycopersici* and completely prevented conidial germination (Rongai et al., 2015). *N. oleander* and *D. stramonium* were

placed near the top with inhibition rates of 45.47 % and 43.15 %, respectively. *Allium sativum* L. and *Eucalyptus occidentalis* L. extracts completely inhibited the pathogen at 5% and 10% concentrations against *A.brassicae* causing Alternaria blight on mustard, followed by *Polyanthi longifolia* (90.77% and 100%), *Ocimum sanctum* L (87.44% and 100%), *Datura stramonium* L (85.09% and 100%), *Azadirachta indica* L (82.44% and 100%). Similarly, it was reported that neem and chilli plant extracts were highly effective against *A. brassicicola* Alternaria leaf spot disease in cabbage at 15% and 25% concentrations and inhibited mycelial growth by 68 % at 25% concentration (Gupta et al., 2019). Hassanein et al. (2008) reported similar results against *A. alternata*, which causes early blight in tomato. Wszelaki & Miller (2005) also found that garlic extracts significantly reduced the intensity of leaf blight disease in tomato. Similarly, Panchal & Patil (2009) conducted in vitro studies to test the effectiveness of garlic, turmeric, and neem extracts at a 10% concentration against *A. alternata*. The results showed that garlic clove extract was highly effective in reducing Alternaria fruit rot of tomato, followed by turmeric and neem extracts. The lowest inhibition rate was observed in *T. terrestris, C. arvense* and *A. retroflexus* treatments among the plant extracts used. With the water extracts obtained from *V. album* (stem), *S. nigrum, Vitex agnus-castus, C.bursa-pastoris*, the mycelial growth of *A. alternata* was inhibited approximately at the rates of 32.78-37.33 % and this inhibition rate was determined to be about 45 % for *D. stramonium* and *N.oleander*. The inhibition rate in the other plants used varied between 20 % and 26 %. The control treatment was different from all the other treatments and the plant extracts used inhibited the mycelial growth of *A. alternata* at different rates. Sharma et al. (2021) reported *Allium sativum* extract was found to inhibit mycelial growth of *Alternaria alternata* by 90.11%, 100% and 100% at 5%, 10% and 15% concentrations respectively, followed by *Azadirachta indica* leaf extract (79.45%, 83.60% and 88.22%). *Alstonia scholaris* leaf extract inhibited mycelial growth of the pathogen less than the control at 5%, 10% and 15% concentrations by 36.22%, 40.33% and 47.77%, respectively. Cherkupally et al. (2017) reported that *D. stramonium* water extracts inhibited the mycelial growth of *R. solani* and *Fusarium oxysporum* f.sp. *melongenae* by 72% and 61.1% at 20% (highest) concentration, respectively. The mycelial growth of *M. phaseolina* was inhibited by 0.0%, 27.7% and 57.7% at 5%, 10% and 20% concentration, respectively. Based on the results obtained, it was observed that the plant extracts can inhibit the *in vitro* colony growth of *A. alternata* isolated from sweet cherry leaf at high or low rates.

In the study assessing the anti-fungal effects of the water extracts prepared from leaves of some annual and perennial weeds and cultivated plants against *A. alternata, A. solani, Botrytis cinerea,* and *Drechslera sorokiniana* under *in-vitro* conditions, it was observed that *Hedera helix* leaf extract inhibited spore germination and colony growth at the highest rate. It was stated that *D. stramonium* extract inhibited *A. alternata* sporulation density at the rate of 41 % and the colony growth at the rate of 15 % (Türküsay & Onoğur, 1998). It was stated in another study that *D. stramonium* inhibited the mycelial growth of *A. alternata* at the rate of 19.66 % (Ögüt Yavuz et al., 2018).

The effect of *D. stramonium*, included in the present study, on the colony growth of *A. alternata* was recorded to be 43 %. The difference in the inhibition rate is considered to be associated with the plant collection period, the plant organ used and the amount of extract used in the environment. Alkaloids such as tporane (atropine), hyoscyamine, and scopolamine included by *D. stramonium* and their amounts may have a role in the anti-fungal effect. In general, there may be more alkaloid in certain organs of plants (such as root, shell, leaf, fruit, seed). As a result of the study in which the leaf water extracts of *D. stramonium*, *D. innoxia*, *D. metal* and *D. ferox* at different concentrations on mycelial growth of *Alternaria solani* were assessed; 20 % concentration of *D. stramonium* inhibited the mycelial growth of *A. solani* (at the rate of 88 %) and this is in parallel with the results of the present study (Jalander & Gachande, 2010).

Table 2. The effects of different plant extracts on the mycelial growth of *Alternaria alternata* (%)

Plant Extracts	*Alternaria alternata*	
	Colony diameter* (mm)	% Effect
Brassica oleracea L.	16.94 I	77.12
Nerium oleander L.	40.38 H	45.47
Datura stramonium L.	42.10 GH	43.15
Capsella bursa-pastoris L.	46.41 FG	37.33
Viscum album L. (stem)	49.78 EF	32.78
Vitex agnus-castus L.	50.67 DEF	31.57
Solanum nigrum L.	50.72 DEF	31.51
Viscum album L. (leaf)	51.60 CDEF	30.32
Xanthium strumarium L.	54.77 BCDE	26.04
Chenopodium album L.	55.59 BCD	24.93
Echinophora tenuifolia L. subsp. *sibthorpiana* (Guss.) Tutin.	56.51 BC	23.69
Convolvulus arvensis L	56.82 BC	23.27
Amaranthus retroflexus L.	57.11 B	22.88
Cirsium arvense (L.) Scop.	59.03 B	20.28
Tribulus terrestris L:.	59.09 B	20.20
Control	74.05 A	0

* The means belonging to different letters in the same column are different at the significance level of P<0.05 according to DUNCAN.

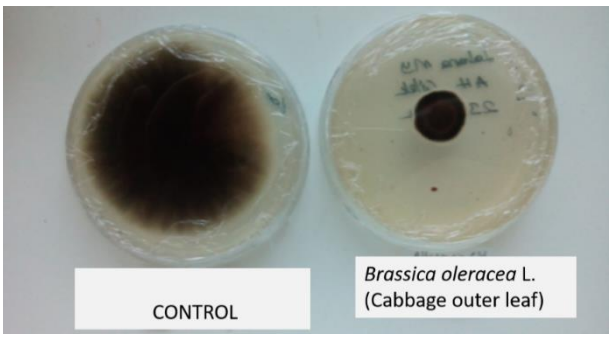

Figure 3. Effect of cabbage outer leaf water ectract on mycelial development of *A. alternata*

It was stated that the spore germination of *A. brassicae* isolated from cauliflower leaves was inhibited by using the *Canna indica*, *Convolvulus arvensis*, *Ipomoea palmata*, *Cenchrus catharticus*, *Mentha piperita*, *Prosopis spicigera*, *Allium cepa*, *A. sativum*, *Lawsonia inermis*, *Argemone mexicana*, *D. stramonium* and *Clerodendron inerme* extracts. Although the anti-fungal effect of *A. retroflexus* extract against *A. alternata* was lower compared to the other treatments, there was an effect of about 22 % compared to the control (Sheikh & Agnihotri, 1972). In the study examining the antifungal effects of *Orobanche ramosa*, *Viscum album* and *Cuscuta campestris* against *Alternaria solani, Monilinia fructigena, Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), it was stated that all the plant extracts used had a significant anti-fungal effect and the inhibition rate in *V. album* in 20 mg ml^{-1}doses of the extracts was stated to be 60 % against *A. solani* (Şin et al., 2017). In the present study, it was determined that the inhibition rate obtained with *V. album* (stem-leaf) was approximately 30 %-33 %. In the present study, an inhibition rate of 45.47 % was observed in *N. oleander* water extract Singh & Srivastava (2014), and the highest inhibition rate against *A. alternata* in methanol and water extract under in-vitro conditions was obtained with oleander treatment among different plants (*Partizyum hysterophorus*, *Vernonia amygdalina*, *Eucalyptus camaldulensis*, *Nerium oleander*, *Lantana camara* and *Ocimum sanctum*) and at different concentrations (5 %, 10 %, and 20 %). This indicated that methanol extracts were more effective against *A. alternata* compared to water extracts. It was reported that the mycelial growth was significantly inhibited against *A. alternata* with the increasing concentrations of the extracts. The effects of different sterilization techniques on the anti-fungal activities of root and leaf extracts of (*Urtica dioica* L.) stinging nettle at different concentrations (2.5, 5.0, 10.0, 20.0, 40.0 %) in mycelial growth and spore germination of *Alternaria solani* were examined. The highest inhibition rate of the extracts sterilized in the autoclave for mycelial growth was determined to be 7 % in root extracts and the root extracts sterilized with filtration technique affected the mycelial growth and spore germination at the rates of (75 % and 38 %, respectively) (Nabrdalık & Grata, 2015). The antifungal activity of ethanol extracts of nettle (*Urtica dioica* L.), colocynth (*Citrullus colocynthis* L. Schrad), konar (*Ziziphus spina-christi* L.) and oleander (*Nerium oleander* L.) flower parts were investigated against *Alternaria alternate*, *Fusarium oxysporum*, *Fusarium solani* and *Rizoctonia solani* under in vitro conditions. The extracts showed antifungal activity against these pathogens. Among the plants, nettle and colocynth were most effective against *A. alternate* and *R. solani*, while oleander showed the best inhibition effect against *F. oxysporum* and *F. solani*. It was reported that the extracts used in the study can be used as an alternative to synthetic chemicals in the control of fungal diseases in plants (Hadizadeh et al., 2009).

Vitex agnus-castus L. included in the present study inhibited the mycelial growth of *A. alternata* at the rate of 31.57 % compared to the control. The research shows that the methanol extract of *Vitex agnus-castus* has an inhibitory effect on the mycelial growth of *A. solani*. Gradually increasing doses of the extract resulted in a corresponding increase in inhibition levels of mycelium development in *A. solani*, with values of 27.73%, 32.98% and 40.08% respectively (Yılar et al., 2015).

Conclusion

In conclusion, it was determined that the water extracts prepared from *C. bursa-pastoris, D stramonium, Vitex agnus-castus, C. arvensis, V. album, X. strumarium, Echinophora tenuifolia, A. retroflexus, C. album, B. oleracea, S. nigrum, N. oleander, C. arvense* plants had the anti-fungal effects against *A. alternata* isolated from sweet cherry leaf under *in-vitro* conditions. The anti-fungal effect of cabbage outer leaf, oleander, and jimsonweed weed water extracts was placed near the top with its high inhibition rate. Compared to the control, the other plant extracts inhibited the mycelial growth of *A. alternata* at different rates. In further studies, the activities of the plant/plants with the highest anti-fungal effect should also be assessed under *in-vivo* conditions. This study revealed hopeful results demonstrating that the extracts obtained from plants may be used as an alternative to synthetic pesticides in the control of plant diseases and it is considered to be a reference for further studies.

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