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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination and antifungal activities of laurel and fennel essential oils against fungal disease agents of cypress seedlings

Servi fidanlarında sorun olan fungal hastalık etmenlerine karşı defne ve rezene uçucu yağlarının antifungal etkinliklerinin belirlenmesi

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Abstract

Fusarium oxysporum and *Pestalotiopsis funerea* are the most common fungal disease agents of conifer seedlings causing root rot and shoot or tip blight diseases. In this study, chemical compositions and antifungal activities of essential oils of fennel (*Foeniculum vulgare* Mill.) and laurel (*Laurus nobilis* L.) were determined against root rot and wilt disease agents *F. oxysporum* and *P. funerea in vitro* conditions. Chemical compositions of essential oils were determined by using GC-MS analysis. Antifungal volatile phase effects of essential oils were determined on inhibition of mycelial growth *in vitro* conditions by using different concentrations. The effect of most effective concentrations of essential oils on the morphology of fungal hypha was also determined by using light microscope. GC-MS analysis of essential oils of laurel and fennel plants revealed that eucalyptol (52.88%) and α-terpinyl acetate (11.77%) were major components of laurel; *trans*-anethole (81.55%) and limonene (5.88%) were major components of fennel essential oils. Volatile phase effects of fennel and laurel essential oils were found to completely inhibit mycelial growth of *F. oxysporum* at 30.0 and 50.0 µl petri^{-1} concentrations, respectively. Complete growth inhibition of *P. funerea* by essential oil of fennel and laurel were observed at relatively lower concentrations (20.0 and 25.0 µl petri^{-1} concentrations, respectively). Light microscopic observations on hyphae, exposed to volatile phase of the most efficient concentrations of essential oil, revealed considerable structural deformations such as cytoplasmic coagulation, vacuolations and protoplast leakage. In conclusion, our results suggest that essential oils have the potential for use in control of fungal diseases of conifer plants.

Keywords: Fennel, laurel, essential oil, antifungal, *Fusarium, Pestalotiopsis*

Öz

Fusarium oxysporum ve *Pestalotiopsis funerea*, kozalaklı bitki türlerinde kök çürüklüğü, sürgün ya da uç yanıklığı olarak bilinen hastalıklara sebep olan en yaygın fungal hastalık etmenleridir. Bu çalışmada, rezene (*Foeniculum vulgare* Mill.) ve defne (*Laurus nobilis* L.) bitkilerinden elde edilen uçucu yağların kimyasal bileşimleri ve *F. oxysporum* ve *P. funerea*'ya karşı antifungal etkileri *in vitro* koşullarda belirlenmiştir. Uçucu yağların kimyasal bileşimleri, GC-MS analizi kullanarak belirlenmiştir. Uçucu yağların buhar fazında misel gelişimini engellemesi

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üzerine antifungal etkileri *in vitro* koşullarda farklı konsantrasyonlar kullanarak belirlenmiştir. Işık mikroskobu kullanarak uçucu yağların en etkili konsantrasyonlarının fungus hiflerinin morfolojisine etkileri de belirlenmiştir. Uçucu yağlarının GC-MS analiz sonuçları, eucalyptol (%52.88) ve α-terpinyl acetate (%11.77)'ın defnenin, *trans*-anethole (%81.55) ve limonene (%5.88)'nin ise rezene uçucu yağının ana bileşenleri olduğunu göstermiştir. Rezene ve defne uçucu yağlarının buhar fazında *F. oxysporum*'un miselyal gelişimini tamamen engelleyen konsantrasyonları sırasıyla 30.0 ve 50.0 µl petri^{-1} olarak belirlenmiştir. Rezene ve defne uçucu yağlarının *P. funerea*'nın gelişimini tamamen engelleyen konsantrasyonların nispeten daha düşük olduğu gözlenmiştir (sırasıyla 20.0 ve 25.0 µl petri^{-1} konsantrasyonlarında). Uçucu yağların buhar fazında en etkili konsantrasyonuna maruz kalan hifler üzerinde yapılan ışık mikroskobu gözlemleri, sitoplazmik pıhtılaşma, vakuolleşme ve protoplazmik içeriğin hücre dışarısına akıntısı şeklinde gerçekleşen önemli yapısal deformasyonları ortaya koymuştur. Sonuç olarak elde edilen bulgularımız, uçucu yağların kozalaklı bitkilerde sorun olan fungal hastalıklarının kontrolünde kullanım potansiyeli bulunduğunu ortaya koymuştur.

Anahtar Kelimeler: Rezene, defne, uçucu yağ, antifungal, *Fusarium, Pestalotiopsis*

In recent years, the increase in environmental consciousness parallel to the arrangement of park-garden arrangement with the importance given to the production of conifer trees are of great importance. Fungal diseases caused by *Fusarium* spp. and *Pestalotiopsis* spp. are important factors reducing seedling production of several conifer and cypress trees, particularly in forest nurseries. *Fusarium oxysporum* is the most common fungal disease agent of conifer seedlings causing root rot and wilt diseases (Gordon et al., 2015). On the other hand, foliage blight caused by *Pestalotiopsis funerea* has been also associated with root rot, shoot or tip blight, twig dieback and stem cankers on many conifer host (Sinclair et al., 1993; Bajo et al., 2008). The complexity of these diseases and the variety of pathogen races has led to the indiscriminate use of fungicides for their control in forest nurseries. Studies looking for alternative options for controlling these diseases have focused on use of the plant extracts and essential oils which possess antifungal activity.

Essential oils have been used in many fields scientifically and commercially for many years. Cosmetic, pharmaceutical, food industry, aromatherapy and phytotherapy are the most important areas of their usage (Isman, 2000). The antimicrobial activities of some plants containing essential oil have been demonstrated in several studies (Isman, 2000; Bakkali et al., 2008; Nazzaro et al., 2017). The antimicrobial activities of the essential oils of plants are mainly due to phenolic and terpenoid components found in their structures (Pirbalouti et al., 2013). Plants rich in these components are also used as an alternative to chemicals in the treatment of many plant, animal and human diseases (Bakkali et al., 2008). Laurel (*Laurus nobilis* L.) is an evergreen plant in the form of trees or large shrubs belonging to the Lauraceae family (Chahal et al., 2017). In Turkey, this plant is widely grown naturally along the Mediterranean, Black Sea and Aegean coasts. The fruits and leaves of laurel plant are utilized (Pinheiro et al., 2017). In recent years, it has also become widespread as ornamental and hedge plants. Turkey is one of the main producers and suppliers of laurel leaves (Demir et al., 2004). The essential oil obtained from wet and dried leaves of laurel is widely used in food, perfumery, medicine and liquor industry. Fennel (*Foeniculum vulgare* Mill.) is a plant belonging to the Umbelliferae family, mostly used as a spice or in folk medicine for stomach disorders, gas expectorant and milk enhancing effects. Antibacterial, antifungal and insecticidal activities of essential oils and extracts of medicinal plants, including fennel and laurel, were reported against several insect, plant pathogenic fungal and bacterial disease agents (Sertkaya et al., 2010; Rather et al., 2016; Kaya et al., 2018; Aktepe et al., 2019; Karabüyük and Aysan, 2019). Antifungal activities of essential oils of different plants against *Fusarium* spp. and *P. funerea* has been reported (Ozcan et al., 2006; Cheng et al., 2011; Ho et al., 2012; Park et al., 2017; Soylu and Incekara, 2017; Bayar et al., 2018). Although antifungal activities of laurel and fennel were investigated against *Fusarium oxysporum*, there is no study reporting antifungal activities of these essential oils against *Pestalotiopsis funerea.*

In this study, with the objective of finding alternative and environmentally friendly strategies to control fungal diseases in conifer seedlings, chemical compositions and antifungal activities of essential oils obtained from seeds of fennel (*F. vulgare*) and leaves of laurel (*L. nobilis*) were determined on mycelial growth of fungal disease agents *F. oxysporum* and *P. funerea in vitro* conditions.

Material and Method

Extraction of plant essential oils

Air-dried leaves of laurel and seeds of fennel plants (200 g, each) were used to extract essential oils. These materials were collected from the plants growing in Hatay Province of Turkey and identified by Prof.Dr. I. Uremis. Essential oils used in this study were extracted by steam-distillation for 3 h with Clevenger's apparatus, according to European Pharmacopoeia method (Council of Europe, 1997). The oils were separated, dried over anhydrous sodium sulphate and stored in an amber bottle at 4°C until used.

Characterization by GC-MS of essential oils

The components of the essential oils of the plants were determined by gas-chromatographic method. Determination of essential oil components was carried out with Thermo Scientific ISQ Single Quadrupole model gas chromatographic device under the following conditions. TR-FAME MS model, 5% Phenyl Polysilphenylene-

siloxane, 0.25 mm inner diameter x 60 m length, 0.25 µm film thickness column was used. Helium (99.9%) was used as the carrier gas at a flow rate of 1 mL / min. The ionization 22 energy was set at 70 eV, the mass range m/z was 1.2-1200 amu. Scan Mode was used for data collection. The MS transfer line temperature was 250°C, the MS ionization temperature was 220°C, the injection port temperature was 220°C, the column temperature was initially 50 ° C and the temperature was increased to 220°C with a rate of heat increase of 3°C/min. The structure of each compound was identified using mass spectra with the Xcalibur program (Wiley 9).

Isolation of fungal disease agent

Fungal disease agents *F. oxysporum* and *P. funerea* were isolated from roots and stems of infected seedling of *Cupressus macrocarpa* growing in forest nursery in Hatay province of Turkey. Fungal disease agents *F. oxysporum* and *P. funerea* were tested for pathogenicity on 1-year-old and 4-year-old pot-grown *Cupressus macrocarpa* saplings (Kurt et al., 2017), respectively and identified based on morphological characteristics (Mordue, 1976; Leslie and Summerell, 2006) and MALDI-TOF analysis (Duman and Soylu, 2019). Fungal disease agent *F. oxysporum* caused typical root rot and wilting and *P. funerea* was found to be highly virulent on 4-year-old pot-grown *Cupressus macrocarpa* saplings five weeks after inoculations by causing bark necrosis or small cankers (1.0 to 2.5 cm long) on inoculated stems.

Antifungal effect of essential oils on mycelial growth

The *in vitro* antifungal volatile phase effects of laurel and fennel essential oils against *F. oxysporum* and *P. funerea* was determined towards mycelial growth of both fungal disease agents as decribed before (Soylu et al., 2010). The single spore-culture of each fungal isolates were grown on Potato Dextrose Agar (PDA) medium at 25°C for 3–5 days. Sterile PDA was poured into sterile 90 mm glass Petri plates (20 ml/plate). Different concentrations of essential oils were dropped on the inner surface of each petri lids. PDA disc (6 mm) from the edge of a 5-days old test isolates were placed at the center of each plate. In order to prevent loss of essential oils from the plates, inoculated petri plates were immediately sealed with parafilm and subsequently incubated at 25°C in incubator. Inhibitory effect of each concentrations of essential oils were monitored and fungal colony diameter were measured daily. PDA plate without essential oil was used as control. The mean growth values were obtained and then converted in to the inhibition percentage of mycelial growth in relation to the control treatment by using the formula, MGI (%)=((dc)dt)/dc)x100, where dc and dt represent mycelial growth diameter in control and treated Petri plates, respectively. In addition, the effect of most effective concentrations of essential oils on the morphology of fungal hypha was also determined by using light microscope as described before (Soylu et al., 2007).

Statistical analysis

In vitro antifungal experiments were performed twice with at least three replications of each oil concentration. SPSS statistic program (version 17, USA) was performed for all calculations and statistical analysis. Analysis of variance was used to assess treatment effects. The significant differences between concentrations were determined by means of Duncan's Multiple Range Test ($P<0.05$). The efficient concentration (EC_{50}) values for each essential oil were estimated by using Probit analysis.

Results and Discussion

Chemical compositions of essential oils

The average yields of essential oils obtained by steam distillation from fennel seeds and dried laurel leaves were determined. Laurel leaves had considerably had higher yield in essential oil (3.4%) compare to that obtained from fennel seed (2.15%). The percentage of fennel oil yield reported by Kan et al. (2006) was between 2.90-3.20%. In our study. the rate of essential oil obtained from fennel seeds was determined between 1.70-2.60%. The percentage of laurel leaf oil yield reported by Uyar (2014) at different harvest times ranged from 0.60% to 5.87%. while this rate was determined to be between 1.80-5.00% in our study.

The chemical components of essential oil from fennel seeds and laurel leaves were identified by GC and GC-MS analysis. The chromatograms of the both essential oil components is given in Figure 1, list of compounds determined are given in Table 1.

A total of 34 components were detected in fennel essential, representing 99.18% of the total essential oil. Among the component, trans-Anethole (81.55%) was determined as the most abundant compound which was followed by limonene (5.88%) and estragole (4.75%) respectively (Table 1). Following GC-MS analysis, a total of 48 components, representing 99.86% of the total essential oil, were detected in laurel essential oil (Table 1). Eucalyptol (52.88%) was determined as the major component which was followed by α-terpinyl acetate (11.77%), sabinene (8.05%), α-pinene (5.32%), β-pinene (3.65%) and terpinen-4-ol (2.83%).

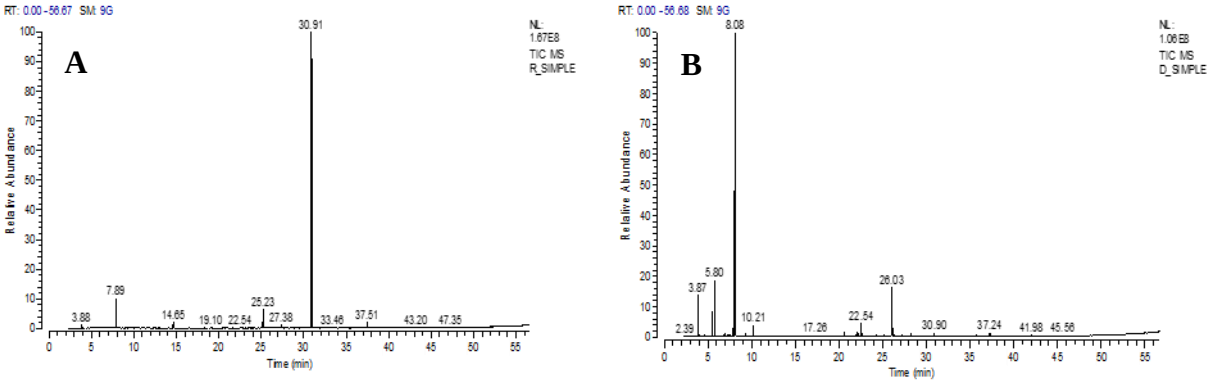

Figure 1. The chromatograms of the essential oil of seeds of fennel (A) and leaves of laurel (B).

Table 1. Chemical compositions of essential oil of fennel seeds and laurel leaves.

RT	Compound Name	SI	RSI	Laurel	Fennel
				Area %	Area %
3.88	α-Pinene	985	993	5.32	0.53
4.64	Camphene	956	980	0.22	-
5.50	β-Pinene	751	906	3.65	0.16
5.81	Sabinene	939	979	8.05	0.20
6.91	β-Myrcene	948	964	0.67	-
7.33	α-Terpinene	932	963	0.35	-
7.59	1.8-Epoxy-p-menth-2-ene	816	896	0.29	-
7.89	Limonene	987	992	1.33	5.88
8.09	Eucalyptol (1,8-cineole)	927	978	52.88	0.30
8.16	β-Phellandrene	769	922	-	0.05
9.06	*cis*-Ocimene	948	977	0.09	0.26
9.36	γ-Terpinene	847	936	0.56	0.09
10.22	o-Cymene	957	968	1.99	0.26
10.58	α-Terpınolene	867	927	0.14	-
14.27	3-Hexen-1-ol	870	972	0.13	-
14.65	Fenchone	985	985	-	1.53
17.02	*cis*-limonene-1,2-epoxide	560	825	-	0.04
17.26	*trans*-Sabinene hydrate	922	968	0.28	-
18.30	Hexadecatrienoic acid, methyl ester	654	681	-	0.04
19.11	Camphor	653	872	-	0.04
20.45	*cis*-Sabinene hydrate	855	954	0.19	-
20.60	Linalool	962	979	0.79	-
21.03	Terpineol	691	880	0.12	-

21.15	Pinocarvone	725	871	0.13	-
21.62	Endobornyl acetate	876	937	0.15	-
21.99	β-Elemene	947	963	0.58	-
22.12	*trans*-Caryophyllene	969	981	0.92	-
22.55	Terpinen-4-ol	543	722	2.83	0.05
22.72	Hexadecadienoic acid, methyl ester	473	562	-	0.04
23.43	Myrtenal	820	926	0.20	-
23.55	Verbenol	816	886	0.28	0.04
24.31	β-Fenchyl alcohol	840	888	0.55	-
24.46	*trans*-Pinocarveol	900	947	0.20	-
24.83	α-Humulene	772	833	0.09	-
25.16	L-α-Terpineol	857	934	0.42	0.07
25.23	Estragole	989	992	-	4.75
25.75	Heptadecen-8-ynoic acid, methyl ester	488	564	-	0.04
26.03	α-Terpinyl acetate	983	994	11.77	-
26.30	Germacrene -D	899	974	0.29	-
26.64	β-Chamigrene	800	852	0.15	-
26.85	α-Selinene	617	790	0.11	-
27.22	γ-Elemene	851	905	0.38	-
27.38	Carvone	970	982	-	0.94
27.50	Limonene oxide	626	785	0.14	-
28.13	Pleiocarpamine	466	510	-	0.04
28.22	Germacrene A	925	972	0.56	-
28.84	α-Humulene	865	885	0.23	-
29.51	Myrtenol	811	914	0.13	-
29.74	Dotriacontane	495	561	0.08	0.03
29.91	Hexadecatrienoic acid, methyl ester	599	631	0.12	-
30.91	*trans*-Anethole	990	995	0.77	81.55
31.10	*trans*-Carveol	679	863	-	0.04
31.62	Colchifoleine	466	509	-	0.03
35.72	Caryophyllene oxide	898	958	0.28	-
35.97	Butanoic acid, heptafluoro-, methyl ester	617	669	-	0.03
37.51	Benzaldehyde, 4-methoxy	983	987	0.71	1.76
40.40	Spathulenol	827	866	0.13	-
40.70	Lutein	335	450	-	0.03
41.49	2-Propen-1-ol, 3-phenyl-, acetate	577	685	0.08	-
41.58	Anisyl acetone	800	934	-	0.08
41.99	Phenol, 2-methoxy-4-(2-propenyl)	895	920	0.32	-
43.20	Propanone, 1-(4-methoxyphenyl)	762	880	-	0.10
43.48	Ascaridole epoxide	488	687	-	0.06
43.56	β-Eudesmol	665	882	0.13	-
47.46	Dillapiole	455	648	-	0.06
47.67	Astaxanthin	400	418	-	0.03
50.75	Heptanoic acid, docosyl ester	347	383	-	0.03
55.50	Octadecanoic acid, ethyl ester	469	500	0.08	-

RT: **R**etention **T**ime; **SI**: **S**imilarity **I**ndex; **RSI**: **R**eversed **S**earch **I**ndex.

The essential oil of medicinal and aromatics plans has been reported to contain a wide variety of compounds, such as phenolics, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites, which possess antimicrobial and antioxidant activities (Pirbalouti et al., 2013). A number of studies on the chemical composition of the essential oils obtained from different parts of fennel and laurel growing in various regions including Turkey were published. *Trans*-anethole and estragole are the major constituents of the essential oils of different fennel chemotypes. *Trans*-anethole differs from its isomer estragole in the position of the double bond of prophenyl chain as reported (Gross et al., 2002; Ahmed et al., 2019; Wodnicka et al., 2019). High presence of

trans-anethole in our sample clearly reveals that our sample is *trans*-anethole-rich chemotype of fennel. Although some differences observed, the chemical composition of the fennel essential oil agrees with same species from different countries including Turkey (Yamini et al., 2002; Mimica-Dukic et al., 2003; Ozel et al., 2019; Kalleli et al., 2019). There are a many studies on chemical compositions of the EO obtained from the leaves of *L. nobilis* from different locations of the world. Very recent study, conducted by Elkıran et al. (2018), investigated chemical compositions of essential oils from seeds and leaves of laurel (*Laurus nobilis* L.). According to results of GC-GC/MS system, monoterpenoids such as eucalyptol and α-terpinyl acetate were determined in the highest concentrations within both essential oils. Similar results were also reported essential oils of laurel grown in different parts of Turkey (Sangun et al. 2007; Yalcin et al., 2007; Perez et al., 2007; Ozcan et al., 2010, Yılmaz and Deniz, 2017). Isoeugenol, α-pinene and linalool were also reported as the main components of essential oils of leaves of *L. nobilis* from other countries which is in contrast with the results of the present study (Bozbouita et al., 2003; Choudhary et al., 2013; Peixoto et al., 2017, Pinheiro et al., 2017, Chahal et al., 2017). According to previous reports, the yield and composition of essential oil varies with genetic and environmental factors, as well as developmental stage and extraction methods like steam distillation, hydro distillation and soxhlet extraction (Woolf, 1999).

Antifungal activity of essential oils against mycelial growth of fungal isolates

The volatile inhibitory effects of different concentrations of essential oils against two tested fungal disease agents *P. funerea* and *F. oxysporum* are given in Table 2 and 3. Both essential oils inhibited mycelial growth of fungal disease agents in a dose dependent manner (Figure 2). Complete mycelial growth inhibition by fennel and laurel essential oils against *P. funerea* were observed at 20 and 25 ml/petri concentrations, respectively (Table 2, Figure 2). The efficient concentration (EC_{50}) for fennel and laurel essential oils were estimated 5.66 and 4.31 ml/petri concentrations, respectively.

Table 2. The inhibitory effects (%) of different concentrations of volatile phase of essential oils of fennel and laurel on the mycelial growth (mm)[a] of *Pestalotiopsis funerea*

Concentrations (µl/petri)	Essential oils, mycelial growth (mm) and inhibitory effect (%)			
	Fennel	% Inhibition	Laurel	% Inhibition
0.0	81.00d	-	81.0e	-
5.0	41.33c	48.97	33.33d	58.85
10.0	21.66b	73.25	20.67c	74.49
15.0	18.0b	77.78	14.66b	81.89
20.0	0.00a	100.0	10.0b	87.65
25.0	0.00a	100.0	0.00a	100.0
EC_{50}[b]	**5.66**		**4.31**	

[a] The mean mycelial growth of fungal agent determined was based on the measurements of 3 replicate plates, recorded at 7 days after inoculation. Mean values within the column followed by different letters are significantly different according to Duncan Multiple Range Test (P<0.05).
[b] The estimated efficient concentration (EC_{50}) values (ml $petri^{-1}$) for each essential oil were estimated by using Probit analysis.

Complete mycelial growth inhibition by fennel and laurel essential oils against *F. oxysporum* were observed at 30 and 50 ml/petri concentrations, respectively (Table 3, Figure 2). The efficient concentration (EC_{50}) for fennel and laurel essential oils were estimated 10.98 and 15.04 ml/petri concentrations, respectively.

Table 3. The inhibitory effects (%) of different concentrations of volatile phase of essential oils of fennel and laurel on the mycelial growth (mm)[a] of *Fusarium oxysporum*

Concentrations (µl/petri)	Essential oils. mycelial growth (mm) and inhibitory effect (%)			
	Fennel	% Inhibition	Laurel	% Inhibition
0.00	84.67f	-	82.33j	-
5.00	70.0e	17.33	63.33i	23.07
10.00	46.67d	44.88	54.33h	34.01
15.00	30.00c	64.57	45.67g	44.53
20.00	25.00c	70.47	36.67f	55.46
25.00	16.33b	80.71	30.67e	62.75
30.00	0.0a	100.0	24.33d	70.44
35.00	0.0a	100.0	20.67cd	74.90
40.00	0.0a	100.0	16.67c	79.76
45.00	0.0a	100.0	10.67b	87.04
50.00	0.0a	100.0	0.0a	100.0
EC_{50}	**10.98**		**15.04**	

[a] The mean mycelial growth of fungal agent determined was based on the measurements of 3 replicate plates, recorded at 7 days after inoculation. Mean values within the column followed by different letters are significantly different according to Duncan Multiple Range Test ($P<0.05$).
[b] The estimated efficient concentration (EC_{50}) values (ml $petri^{-1}$) for each essential oil were estimated by using Probit analysis.

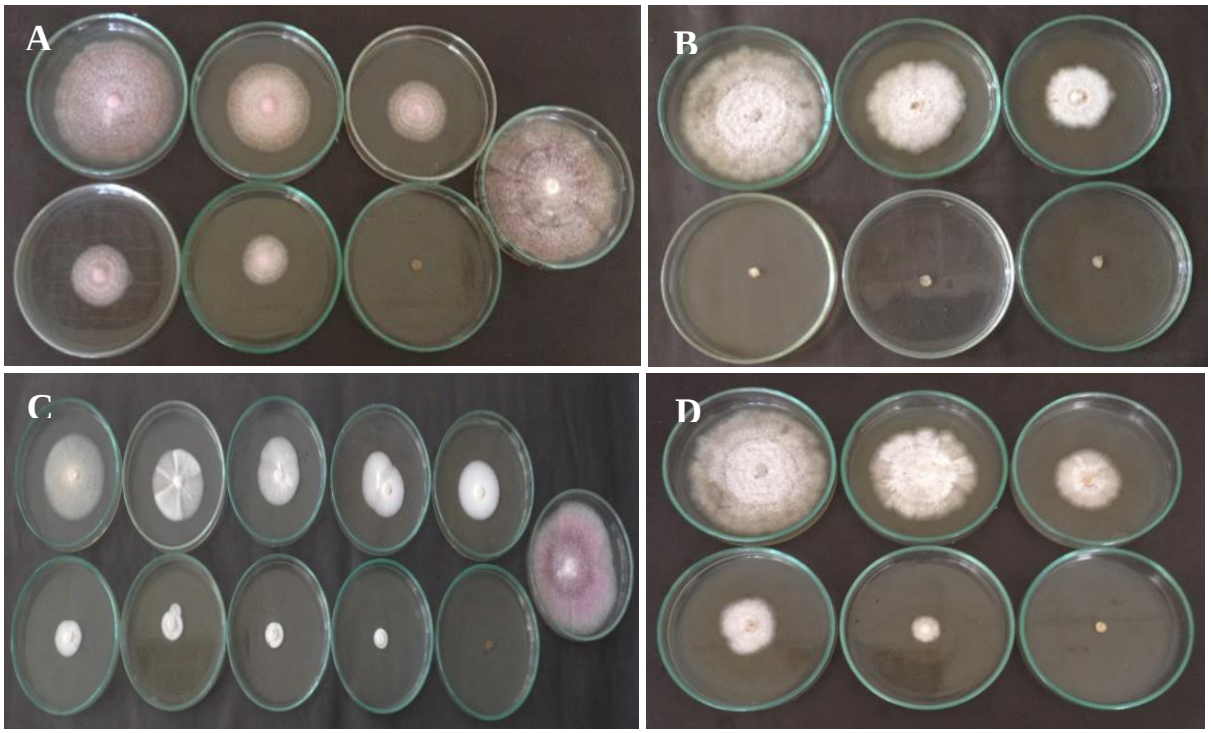

Figure 2. The antifungal activities of different concentrations of volatile phase of fennel (A and B) and laurel (C and D) essential oils on the mycelial growth of *Fusarium oxysporum* (A and C) and *Pestalotiopsis funerea* (B and D).

In previously published studies, there are many reports on concentration-dependent antifungal activities of essential oils whereby the mycelial growth suppressed with increase in the concentration of essential oils from taxonomically different medicinal plants, including fennel and laurel, against several subspecies of *Fusarium* spp. The antifungal activity of the essential oils of *Laurus nobilis* plants inhibited *in vitro* mycelial growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Bayar et al., 2018), *Fusarium oxysporum f. sp. radicis-cucumerinum* (Soylu and Incekara, 2017) in a dose-dependent manner. Similarly, fennel essential oil was also reported to inhibit *in vitro* conidial germination and mycelial growth of *F. oxysporum* f. sp. *fragariae* (Park et al., 2017), *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Soylu and Incekara, 2017), *Fusarium subglutinans*, *F. vertricilioides*, *F. oxysporum*,

F. tricinctum, *F sporotrichioides*, *F. equiseti*, *F. incarnatum* and *F proliferatum* (Ozcan et al., 2006; Starovic et. al., 2016).

Comparison to antifungal activities of essential oils against *Fusarium* spp., very few studies were, however, conducted to determine antifungal activities of essential oils against *Pestalotiopsis* spp. Essential oils of ginger oleoresin (GO) against olive fruit rot disease agent *P. microspora* (Chen et al., 2018), *Cymbopogon citratus* and hydrolate soursop (*Annona muricata*) against *Pestalotiopsis* sp. (Bibiano and Saber, 2017), *Chamaecyparis formosensis* and *Cunninghamia konishii* against *Pestalotiopsis funerea* (Cheng et al., 2011; Ho et al., 2012) were reported to have strong inhibitory effect on mycelial growth of the fungal pathogen. To best of our knowledge, this study was the first study to show that essential oils of fennel and laurel have antifungal activities against *Pestalotiopsis funerea*.

Moreover, microscopical observations of essential oil treated fungal hyphae clearly revealed significant alterations in the both fungal hyphae (Figure 3 and Figure 4). Volatiles compounds of each essential oils damaged to plasma membrane and changed the morphology of fungal hyphae, which were resulted in distortion, sunken and shriveled fungal mycelia of the disease agents (Figure 3 and 4). Shrivelled hyphal aggregates, reduced hyphal diameters and lyses of hyphal wall were commonly observed in fennel oil treated mycelium, compared with thick, elongated, normal mycelial growth in controls. Laurel oil mainly caused marked deformations, cytoplasmic coagulations and necrosis. This kind of modifications may be related to the effect of the essential oil as enzymatic reactions regulating wall synthesis (Rasooli et al., 2006).

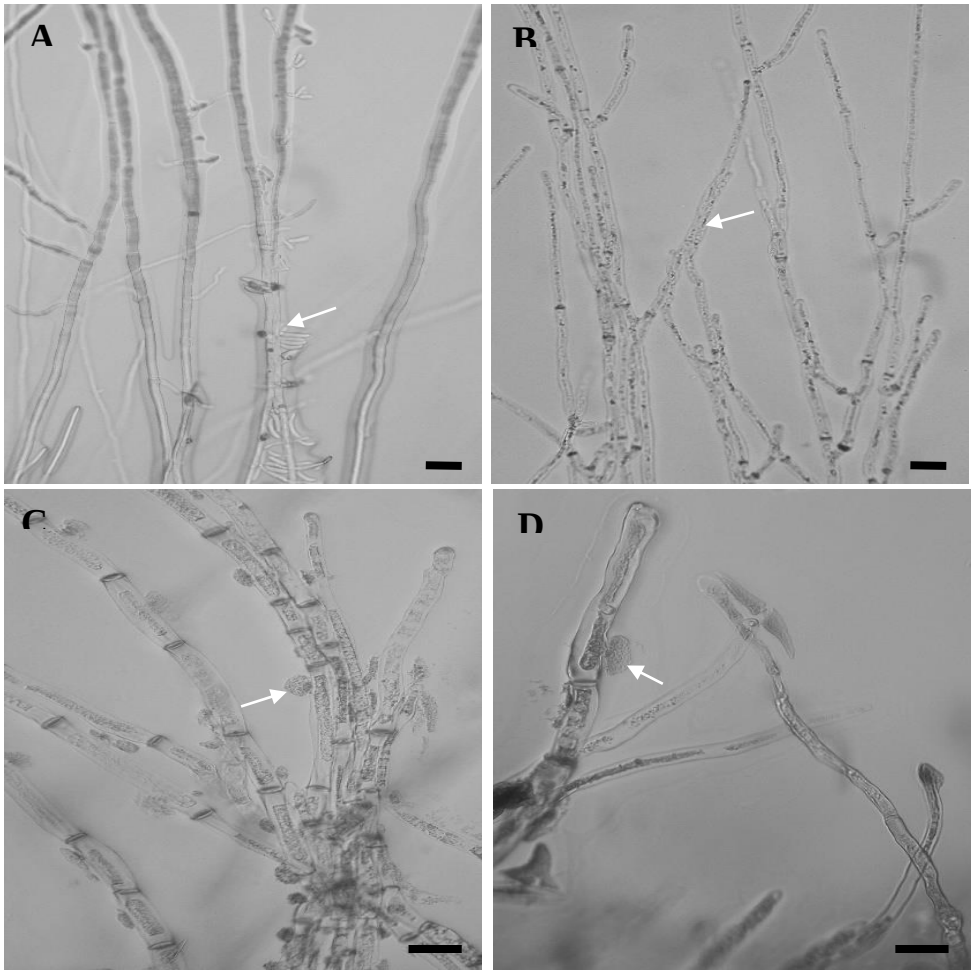

Figure 3. Effect of essential oils of laurel and fennel on hyphal morphology of *Fusarium oxysporum.* (A) Hyphae growing on control petri plate without essential oil. Volatile phase effects of laurel (B) and fennel (C and D) essential oils, respectively, on hyphal morphology. Note cytoplasmic coagulation, vesiculation (arrow) in plate (B) and hyphal shrinkage and outflow of cytoplasmic content (arrows) following lysis of hyphal wall in plate (C and D). Bar=20 µm

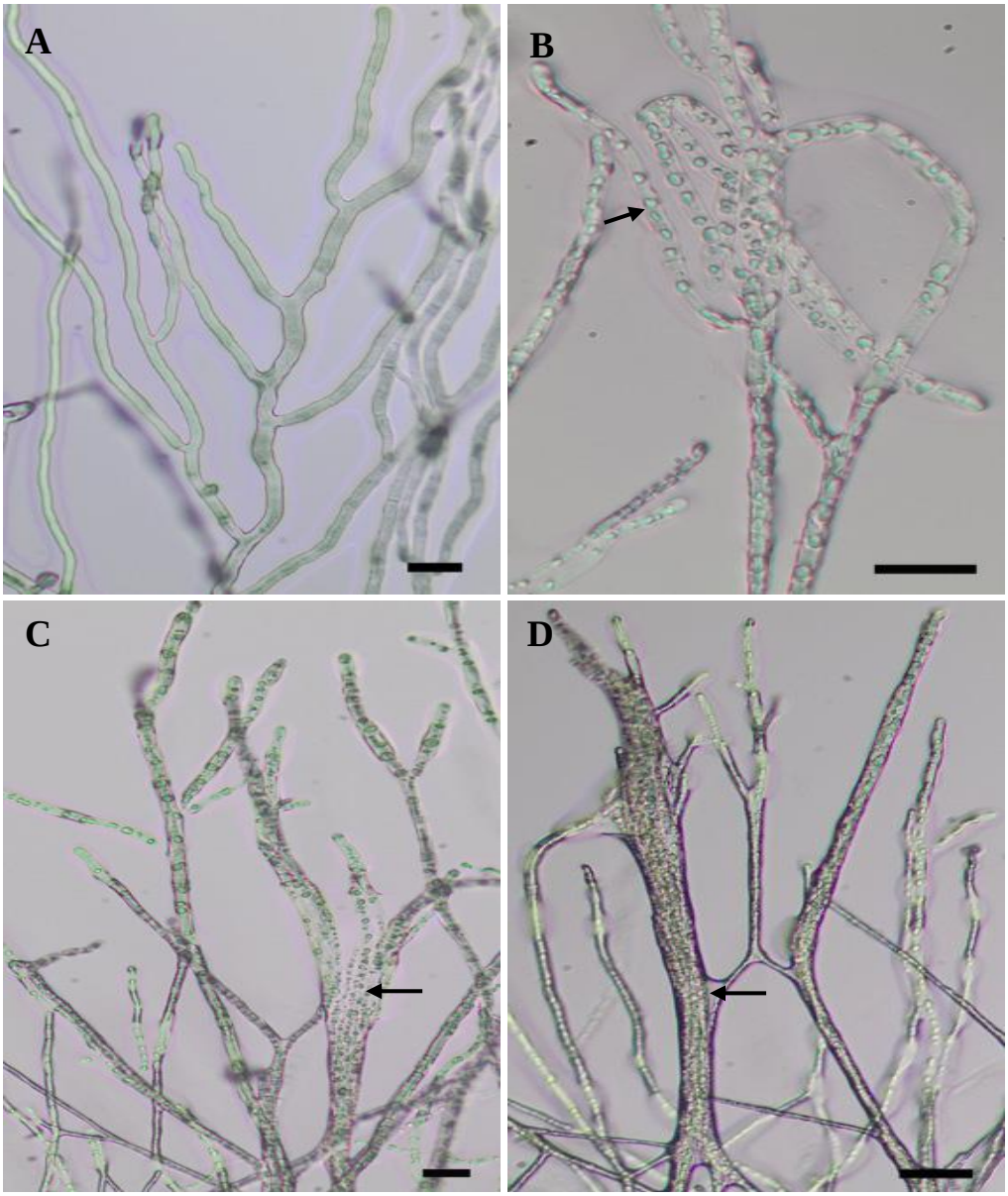

Figure 4. Effect of essential oil of fennel and laurel on hyphal morphology of *Pestalotiopsis funerea*. (A) Hyphae growing on control medium. Volatile phase effects of laurel (B) and fennel (C and D) essential oils, respectively, on hyphal morphology. Note cytoplasmic coagulation and vesiculation (arrow) in plate (B) and hyphal shrinkage and necrosis (arrows) following lysis of hyphal wall in plate (C and D). Bar=20 µm

The observations made with light microscopy are in accordance with previously published studies which verified that essential oils of medicinal plants caused the morphological alterations on the fungal hyphae of taxonomically different plant fungal disease agents (Bianchi et al., 1997; Fiori et al., 2000; Soylu et al., 2007).

Conclusions

In conclusion, our results suggest that essential oils have the potential for use in control of soil borne fungal disease agents such as *F. oxysporum* and *P. funerea* used in this study. The essential oils tested in this study could be considered as potential alternatives for synthetic fungicides with modification as their structures could lead to the development of new classes of antifungal compounds.

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