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IN VITRO ANTIFUNGAL ACTIVITY OF MINT, THYME, LAVENDER EXTRACTS AND ESSENTIAL OILS ON *VERTICILLIUM DAHLIAE* KLEB.

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ABSTRACT

Mint, thyme and lavender of Lamiaceae family were chosen as experimental materials. The aim of study was to investigate the antifungal effects of 3 medicinal plants extracts and essential oils obtained by water stem distillation method, against two pathotypes (defoliating-D and nondefoliating-ND) of *Verticillum dahliae* Kleb. The inhibition of the fungus mycelium growth was evaluated under *in vitro* conditions at the doses of plant extract in 1 %, 3 %, 5 %, 7 % and 10 % and essential oils in 2, 4, 8, 16 and 32µl mL^{-1}. Experiment was set up at randomized plot design with 3 replications. After the end of 7 days incubation period, colony diameters were measured and % inhibition zone ratios were calculated compared to the controls. The reduction effects of mint, thyme and lavender essential oils were greater than the extracts of the same plants. Mint, thyme and lavender extracts reduced the mycelium growth of D and ND in varying degrees, however; the highest antifungal effect was observed at 10 % dose of thyme extract. 8, 16 and 32 µl mL^{-1} doses of thyme essential oil inhibited 100 % the mycelium growth of both pathotype and showed a significant fungicidal effect on them. Antifungal effect of mint essential oil was found to near the thyme essential oil depending on the pathotype and the applied dose.

KEYWORDS:
plant extract, essential oil, fungicidal, fungistatic, verticillium wilt, cotton.

INTRODUCTION

Except cotton fiber, cottonseed oil with the ratio of 17-24 % seed oil, after the oil and the remaining pulp contributes to animal nutrition [1]. Approximately 34 million ha area in the world, about 90-100 million tons of seed cotton production and 26 million tons of fiber cotton production is obtained [2]. In Turkey, about 468.000 ha of cotton was grown and 846.000 tons lint yield was produced in the four main regions Southeastern Anatolia, Aegean, Çukurova and Antalya. Turkey's share in world cotton production is approximately 3 % and is ranked seventh [3].

V. dahliae Kleb. all common in the world, many tree species and cut flowers, horticulture and field crops to more than 160 plant species, the host range including vascular wilt is an important soil that can cause pathogen [4]. Pathogen spend the winter on the soil and crop residues into microsclerot and it can maintain viability in the soil for up to 14 years [5]. The disease began early or late sowing has been made, the length of the infected plants remain short, the number of boll is decreased and remains small. The stem of this type of plant is cut cross section, it draws attention to the return beam dark and brown of the vascular bundles. When the diseased tissue into the soil consists of new resistant part. Because of a pathogen has been resistant part, control is also very difficult [6]. Non-economical chemical control of Verticillium wilt, the effects of the disease can be reduced with the use of resistant varieties and cultural measures to a certain extent [7, 8]. However, the resistant varieties are not permanent pathogen resistance, decreased during every production continues and can become completely resistant varieties susceptible to factors in the coming years. Therefore, there is a need to develop new strategies in the control of disease. Increased awareness of environmental degradation and natural balance, researchers, alternatives to chemicals that may have been forced to find nature and environment friendly materials and methods. One of the alternative methods of control with disease can decompose too is the use of ecological natural plant products. These products are biologically effective, broad spectrum, economical and safe [9]. Food, medicine and spices used in many fields such as cosmetics and essential oils, antimicrobial effects in terms of it is used in the control against the major pathogenic fungi in plants and in food industry [10, 11, 12, 13, 14, 15]. Essential oils are known to inhibit 60 % fungi and 30 % bacterial growth [16]. Basım et al. [17],

reported that also mint, basil and thyme have antimicrobial effect against plant pathogenic fungi and bacteria due to the volatile properties of essential oils of those. Although there are many studies against the disease in order to determine the effects of essential oils, still some subjects are more fully disclosed [18]. Soylu et al. [19], determined that essential oil obtained from the speedwell prevent to sporulation and growth fungi commonly found such as *Botrytis cinerea*, *Phytophthora infestans*, *Sclerotinia sclerotiorum* and *V. dahliae* and have high fungicidal effect. Sokovic and Van Griensven [20], found that when the researchers tested 10 different essential oils against *V. fungicola*, only essential oil of vetiver, peppermint and thyme show high effect at the doses of 1, 2 and 5 µl ml^{-1}, respectively *in vitro*. López-Escudero et al. [21], reported that reducing *V. dahliae*'s population of the *L. stoechas*. Arslan and Dervis [22], obtained essential oils from different plants effects of antifungal against vegetative compatibility group of *V. dahliae*, reported that even in low concentrations, essential oils of some species are effective a natural fungicide against *V. dahliae*, but making more long-term evaluation in the field conditions. In other studies conducted, determined that plant extracts derived from medicinal plants have been inhibitory effects of many fungi on spore germination and mycelial growth [23, 24]. In Turkey, plant species with high content of essential oil and such as mint, thyme and lavender in the Lamiaceae family is center of the plant species genes [25]. Found in nature, essential oils and extracts of mint, thyme and lavender were known to be a good antimicrobial effect. However, there are no adequate studies on medicinal plants antifungal effect against *V. dahliae*. Keeping all these points in consideration, an attempt was aim in the present work to determine the antifungal activities of different doses of extracts and essential oils of mint, thyme and lavender plants against D and ND pathotypes of *V. dahliae in vitro*.

MATERIAL AND METHODS

Plant materials and test microorganism. Plant extracts and essential oils of Mint, Thyme and Lavender were obtained from private company by using water stem distillation method (Table 1). Plant extracts and essential oils for use in the study were stored in a refrigerator at 5°C in the dark and tightly closed bottle. Isolates of *V. dahliae* (CotVd 67-ND; CotVd 110-D) with high virulence were obtained from the fungal collection of Mustafa Kemal University. The isolates were maintained on potato dextrose agar (PDA) and were stored in a refrigerator at 4ºC.

Antifungal assays. The antifungal activities of plant extracts and essential oils were carried out for assessing its effects towards mycelial growth of *V. dahliae* as described previously [26]. After sterilization of PDA, plant extracts and essential oils (Table 2) were added at different concentration into 20 mL PDA and poured in petri plates (12 cm diameter). Fungal mycelial-disks (5 mm diameter) prepared from growing margin of CotVd 67 and CotVd 110 were placed in the center of plates. Control plates contained only pathogen isolates. The plates were sealed with parafilm to prevent loss of extract and essential oil vapors and incubated at 24ºC of CotVd 67 and at 27ºC of CotVd 110, separately. The mean radial mycelial growth of the pathogen was determined by measuring the diameter of the colony in 2 directions at right angels when the plate of the control plate was covered by fungus 7 days after inoculation.

TABLE 1
Scientific name, common name, and family of plant species used in this study.

Scientific name	Common name	Family
Mentha piperita L.	Mint	Lamiaceae
Thymus vulgaris L.	Thyme	Lamiaceae
Lavandula angustufolia Mill.	Lavender	Lamiaceae

TABLE 2
Treatment of plant extracts and essential oils against *V. dahliae.*

	Application	Concentration (%)
Plant Extracts	Mint (*Mentha piperita* L.) Thyme (*Thymus vulgaris* L.) Lavender (*Lavandula angustufolia* Mill.)	1 3 5 7 10
	Application	Concentration (µl mL^{-1})
Essential oils	Mint (*Mentha piperita* L.) Thyme (*Thymus vulgaris* L.) Lavender (*Lavandula angustufolia* Mill.)	2 4 8 16 32

All experiments were conducted twice in a completely randomized plot design with 3 replications. The mean growth values were obtained and then converted in to the inhibition percentage of mycelial growth (MGI) in relation to the control treatment by using the formula [27].

MGI (%) = [(*dc*-dt)/*dc*] x 100

Where dc = average diameter of fungal colony in control, and dt= average diameter of fungal colony in treatment group.

The fungistatic-fungicidal nature of plant extracts and essential oils were tested by observing revival of growth of the inhibited mycelial disc following its transfer to non-treated PDA. Activity of the extracts and oils were considered fungicidal if the pathogen did not grow or fungistatic if the pathogen growth occurred [28].

Statistical analysis. All statistical analyses were carried out using JMP IN statistic program (SAS Enstitüsü, Cary, NC, 5.0 PC). Where necessary, arcsine transformation was performed on data before statistical analysis. Analysis of variance (ANOVA) was performed by ANOVA procedures. Significant differences between means were determined by Duncan's pairwise comparison test at a level of $p \leq 0.05$.

RESULTS

The effects of the tested plant extracts and essential oils on mycelium growth as percentage mycelium growth inhibition of D and ND are shown in Table 3 and Table 4. Different three doses (5 %, 7 % and 10 %) of mint, lavender, thyme extract have been inhibited by a statistically compared with the control the mycelium growth of lavender extract compared with the other extracts, showed antifungal activity as well as the low level CotVd 110 and CotVd 67. The high dose of against both CotVd 110 (23.9 %) and CotVd 67 (24.5 %) isolates and dose level decreases was observed that reduced the antifungal effect. While the high dose of mint extract showed a rate effect in CotVd 110 (30.2 %) and in CotVd 67 (26.9 %) isolates, the dose of 1 and 3 % of mint extract was statistically controlled in the same group with respect to both pathotypes. While the high dose of thyme extract compared to the other extract, showed the highest impact both CotVd 110 (43.9 %) and CotVd 67 (48.4 %) isolates and the dose of 1 and 3 % of thyme extract was statistically controlled in the same group with respect to both pathotypes (Table 3).

TABLE 3
Effects of plant extracts on mycelial growth of *V. dahliae*.

Medicinal plants	Concentration (%)	CotVd 110 isolate		CotVd 67 isolate	
		Colony diameter (mm)[1]	% Effect	Colony diameter (mm)[1]	% Effect
Mint	1	15.3 a*	2.7	15.2 a*	5.0
	3	14.9 a	5.3	15.0 a	6.2
	5	12.3 b	21.7	12.8 b	20.0
	7	11.8 bc	24.9	12.3 b	23.1
	10	11.0 c	30.2	11.7 b	26.9
	Control	15.7 a	0.0	16.0 a	0.0
Thyme	1	15.2 a	1.9	15.0 a	3.2
	3	15.1 a	2.6	14.9 a	3.9
	5	9.2 b	40.6	9.6 b	38.1
	7	9.2 b	40.6	9.4 b	39.4
	10	8.7 b	43.9	8.0 c	48.4
	Control	15.5 a	0.0	15.5 a	0.0
Lavender	1	15.7 ab	1.2	15.3 a	1.3
	3	15.3 b	3.8	14.8 a	4.5
	5	13.8 c	13.2	13.5 b	12.9
	7	12.8 d	19.5	13.5 b	12.9
	10	12.1 e	23.9	11.7 c	24.5
	Control	15.9 a	0.0	15.5 a	0.0

[1] The mean radial mycelial growth of *V. dahliae* was determined at seven days after inoculation. Each observation is based on 3 replicate plates. Arcsine transformation was performed prior to statistical analysis.
* Mean values followed by different letters within the column are significantly different according to Duncan Test ($P \leq 0.05$).

TABLE 4
Volatile effects of plant essential oils on mycelial growth of *V. dahliae*.

Medicinal plants	Concentration (µl mL^{-1})	CotVd 110 isolate		CotVd 67 isolate	
		Colony diameter (mm)[1]	% Effect	Colony diameter (mm)[1]	% Effect
Mint	2	8.2 b*	49.4	7.9 b*	49.4
	4	5.7 c	64.8	6.0 c	61.5
	8	2.3 d	85.9	1.7 d	89.1
	16	0.0 e	100.0^{+}	0.0 e	100.0^{+}
	32	0.0 e	100.0^{+}	0.0 e	100.0^{+}
	Control	16.2 a	0.0	15.6 a	0.0
Thyme	2	6.9 b	54.6	6.7 b	55.9
	4	2.6 c	82.9	2.4 c	84.2
	8	0.0 d	100.0^{+}	0.0 d	100.0^{+}
	16	0.0 d	100.0^{+}	0.0 d	100.0^{+}
	32	0.0 d	100.0^{+}	0.0 d	100.0^{+}
	Control	15.2 a	0.0	15.2 a	0.0
Lavender	2	8.7 b	45.3	8.2 b	48.7
	4	5.2 c	67.3	5.2 c	67.5
	8	3.0 d	81.1	3.2 d	80.0
	16	1.0 e	93.7	1.7 e	89.4
	32	0.0 e	100.0^{++}	0.0 f	100.0^{++}
	Control	15.9 a	0.0	16.0 a	0.0

[1] The mean radial mycelial growth of *V. dahliae* was determined at 7 days after inoculation. Each observation is based on 3 replicate plates. Arcsine transformation was performed prior to statistical analysis.
*Mean values followed by different letters within the column are significantly different according to Duncan Test (P≤0.05); $^{+}$ and $^{++}$ symbols indicate that antifungal effects at shown concentrations are fungicidal and fungistatic respectively.

Differences between mint, thyme and lavender essential oils were significant at (P≤0.05) probability level for inhibitory effect on mycelial growth of CotVd 110 and CotVd 67 isolates. The doses of 16 and 32 µl mL^{-1}of mint essential oil were seen antifungal effect 100 % against both pathotypes and this effect was found to be fungicidal, minimal impact on the first dose was detected as 49.4 %. The doses of 8-16-32 µl mL^{-1} of thyme essential oil showed high level antifungal effect against both pathotypes and this effect was found to be fungicidal. Only dose of 32 µl mL^{-1}of lavender essential oil inhibited 100 % mycelium growth of both pathotype and this effect was found to be fungistatic, also the lower dose levels has been determined that the reduced effective against both pathotypes. While the doses of 16 and 32 µl mL^{-1}of mint essential oil showed fungicidal activity against both pathotypes, the dose of 32 µl mL^{-1}of lavender essential oil showed fungistatic activity against both pathotypes (Table 4).

DISCUSSION

In a study, mint, thyme, lavender extracts compared to control application showed different levels inhibitory effects against to D and ND pathotypes. The highest antifungal effect of colony growth of both pathotype was found in the 10 % concentration value of thyme extract (Table 3). Our results showed parallels with results from the other studies conducted on the subject. In the studies carried out with different doses of extracts of thyme, reported that depending on the dose increase which were obtained a higher effect against fungi [29, 30, 31]. Jawdah et al. [32], *in vitro* studies carried out in order to determine antifungal activity against 8 phytopathogenic fungi of the methanol extract of 9 wild plant species, wild marjoram extract showed the highest activity, complete inhibited of mycelial growth of 6 of 8 fungi, also complete inhibited of spore germination of the 6 fungi (*B.cinerea*, *Alternaria solani*, *Penicillium* sp., *Cladosporium* sp., *Fusarium oxysporum* f. sp. *melonis* and *V. dahliae*) in the assay, the other plant extracts showed differential activities in the spore germination test, and but none was highly activity against mycelial growth. Khalil and Dababneh [33], investigated the effect of the rhizosphere of 4 plant extracts against important soil pathogen *Rhizoctonia solani, F. oxysporum, Verticillium* sp., *Penicillium* sp. and *Vartemia iphionodes*, maximum effect at a dose of 1000 ppm in *Verticillium* sp. (44.8 %) determining, followed by *R. solani* (42.9 %) and *F. oxysporum* (42.7 %). Erdoğan et al. [31], *in vitro* studies carried out in order to determine antifungal effects of some plant extracts and essential oils against cotton damping-off agents, mint, thyme and lavender extracts reduced the

mycelium growth of *R. solani* and *Fusarium* spp. in varying degrees, the highest fungi-toxic effect was observed at 8 % dose of thyme extract. All the applied doses of thyme essential oil inhibited 100 % the mycelium growth of the pathogens and showed a significant fungicidal effect on them. Antifungal effects of mint and lavender essential oils were found to be varying with the applied dose and the pathogen. Especially thyme and mint essential oils are found the most effective essential oil inhibiting mycelial growth of both pathotype of *V. dahliae*. The doses of 8, 16 and 32 µl $mL^{-1}$of thyme and 16 and 32 µl mL^{-1} of mint essential oils showed fungicidal activity against both pathotypes. Although antifungal effects of mint and lavender essential oils vary depending on the pathotype and the dose, it was similar to each other (Table 4). Pagiotti et al. [34], reported that lavender essential oil was shown to have a lesser effect with respect to the other essential oils, a slight antifungal activity attributed to the presence of linalool. Essential oils of some plants such as thyme was reported to show a high antifungal activity against many phytopathogenic fungi *in vitro* [35]. Giamperi et al. [36], tested the efficacy *in vitro* of 8 essential oils against *P. cinnamomi*, *Pyrenochueta lyccipersici* and *V. dahliae* using different concentrations. In that study, the authors demonstrated, the fungistatic + fungicidal activity of the oils determined and the most effective essential oils found to be those of oregano, thyme, peppermint and coriander. The researchers conducted studies, reported that mint essential oil inhibited strongly the mycelial growth of some soil-borne pathogens such as *F. solani* var. *coeruleum*, *F. oxysporum* f.sp. *lycopersici*, *Pythium ultimum*, *R. solani* and *V. dahliae* and even in the minimum concentration inhibited all pathogens [37, 38]. Kadoglidou et al. [39], tested the activity of lavender, oregano, sage and spearmint essential oils against *Aspergillus terreus*, *F. oxysporum*, *Penicillium expansum*, *V. dahliae*. In the study, the researchers reported that the strongest inhibitory activity on mycelial growth exhibited by oregano and spearmint oils and by carvacrol and carvone, respectively. The inhibitory effect was clearly fungistatic in *A. terreus* and *F. oxysporum*, but fungicidal in *V. dahliae.* Our study results, contrary to the other results obtained from lavender essential oil (Table 4). Yohalem and Hall [40], reported that lavender found in a high percentage of camphor and 1.8 cineol and when used alone lavender, it is effective against microsclerots of *V. dahliae*. Đordjevic et al. [41], tested the efficacy *in vitro* of essential oils obtained 7 different plants against *V. dahliae*, the highest inhibition rate on *O. vulgare* essential oil (0.04 mL) was determined, followed by *P. anisum* essential oil (0.1 mL). Our study, antifungal effects of mint, thyme and lavender the extracts were found to be lower than the essential oil of the same plant (Table 3 and Table 4). Researchers at the result of their study, reported that essential oils obtained from different aromatic plants have a broad spectrum against plant pathogens [42, 43]. Shelef [44], reported that antifungal effect of essential oils vary depending on the plant's ecological conditions, on the type and this effect is caused by the compound of the essential oils such as thymol, carvacrol, cinnamaldehyde, eugenol.

CONCLUSIONS

Consequently, antifungal activity of mint, thyme and lavender essential oils was higher than the same plant extracts. In our study conducted, the highest inhibition was determined on mycelial growth both isolates at 8, 16 and 32 µl mL^{-1} doses of thyme essential oil. Non-economic chemical control against Verticillium wilt, the results are taken into account, and it was concluded that thyme essential oil can be an alternative method in the control of Verticillium wilt. However, these results can be assessed on a commercial scale, the rating for the formulation and toxicology studies as well as efficiency in the field conditions is required. Because, natural plant products that break more easily compared to chemical sprays, it is increasing the importance of these products. In this context, essential oils can be a significant part of biological control strategy.

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