Iranian Journal of Microbiology
Volume 7 Number 1 (February 2015) 31-37

ORIGINAL ARTICLE

Comparison of antifungal activities of various essential oils on the *Phytophthora drechsleri*, the causal agent of fruit decay

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Received: October 2014, **Accepted:** January 2015

ABSTRACT

Background and Objectives: The efficacy of *Mentha piperita* L, *Zataria multiflora* Boiss and *Thymus vulgaris* L essential oils (EOs) was evaluated for controlling the growth of *Phytophthora drechsleri,* the causative agent of damage to many crops that is consumed directly by humans.

Materials and Methods: The EOs used in this study was purchased from Magnolia Co, Iran. The pour plate method in petri dishes containing Potato Dextrose Agar (PDA) was used to evaluate the antifungal properties of EOs. The minimal inhibitory concentrations (MIC), minimum fungicidal concentration (MFC) as well as mycelial growth inhibition (MGI) were measured. The IC_{50} value (the concentration inhibited 50% of the mycelium growth) was calculated by probit analysis.

Results and Conclusion: The fungal growth was significantly reduced by increasing concentrations of tested EOs. The complete reduction was obtained with Shirazi thyme at all concentrations, whereas the complete reduction for peppermint and thyme was observed at 0.4% and 0.8% (v/v) concentrations, respectively. Meanwhile, the minimum inhibition was observed when 0.1% peppermint (MGI values of 9.37%) was used. The IC_{50}, MIC and MFC values of Shirazi thyme was 0.053, 0.1% and 0.2%, respectively. Similarly, MIC and MFC values of peppermint and thyme were recorded 0.4% and 0.8%, respectively. The results obtained from this study may contribute to the development of new antifungal agents to protect the crops from this pathogenic fungus and many agricultural plant pathogens causing drastic crop losses.

Keywords: *Mentha piperita, Zataria multiflora, Thymus vulgaris*; Essential oil, Antifungal, *Phytophthora drechsleri*

INTRODUCTION

Fungal infections are very important in the agricultural economy due to the potential of causing extensive damage to agricultural crops during storage, transport and cause significant economic losses in the commercialization phase (1, 2). The pre- and postharvest losses in world crops due to fungal disease may amount to more than 12% in developing countries (3). Among post-harvest fungal pathogens, *Phytophthora* is one of the common plant-damaging agent (water molds), whose species have caused great economic losses to crops, natural vegetation and forestry worldwide (4).

Multiple species of *Phytophthora* are known to cause problems in agricultural production, that

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among them, *P. drechsleri* is very important because a wide host range of herbal and woody plants from non-specific to highly specific (5). This oomycete pathogen causing damping off, gummosis and root rot in pistachio orchards and many vegetable crops in the *Cucurbitaceae* and *Solanaceae* (6, 7). For example, gummosis is the most important disease of pistachio trees in Iranian pistachio orchards that cause with *P. drechsleri*. This soil borne disease affects the crowns and roots of the trees, causing average tree mortality of 10–12% or greater (8). Moreover, the crown and root rot of cucumber is one of the most important diseases on greenhouse cucumber in Iran caused by *P. drechsleri* (9). First report of crown and root rot of cucumber occurred in 1937 that Approximately 2.3 hectares of cucumber fields in the United States were destroyed 100% (10).

Over the past years, a variety of different synthetic chemicals such as mefenoxam (7), phosphite (11), bordeaux mixture, phenylamides (acylanilides) group includes furalaxyl (fongarid), metalaxyl (ridomil) and benalaxyl (galben) (7, 12) have been used as antifungal agents to inhibit the growth of this pathogenic fungus. However, continuous use of fungicides has faced two major obstacles; increasing public concern regarding contamination of fruits and vegetables with fungicidal residues, and proliferation of resistance in the pathogen populations (13). In this regard, the use of plant-based essential oils (EOs) and extracts, which may be less damaging for pest and disease control, could be a useful alternative to synthetic fungicides in the management of rot fungi during postharvest handling of fruit and vegetables (2).

The antifungal activity of EOs and plant extracts against a number of plant pathogens such as soil-born fungi, food and grain storage fungi and foliar pathogens has been reported (14, 15).

Despite these natural products potentially great importance, there are little research has focused specifically on the effects of Iranian medicinal plants EOs on *P. drechsleri*. The objective of the present study was to evaluate the *in vitro* activity of Shirazi thyme (*Zataria multiflora* Boiss), peppermint (*Mentha piperita L*) and thyme (*Thymus vulgaris* L.) essential oils against *P. drechsleri*.

MATERIALS AND METHODS

Essential oil. Essential oils (*Mentha piperita* L*, Zataria multiflora* Boiss and *Thymus vulgaris* L) used in this study were purchased from Magnolia Co, IRAN. EOs quality parameters such as odor, color, appearance, purity, solubility and also chemical properties including pH, acidity and brix were described in an accompanying technical report.

Microbial strain and culture media. The fungal strain used was *P. drechsleri*, IRAN 1156C that obtained from the Iranian Research Institute of Plant Protection (IRIPP). The fungi cultures were maintained and grown on PDA slants at 25° C for 5 days. Cultures were stored at 4 °C and subcultured once a month.

Antifungal assays. Antifungal assays of Shirazi thyme, peppermint and thyme EOs were performed with the pour plate method as described by Askarne *et al.,* (2012) (16). In this method, the agar plates were prepared using PDA (15 ml per Petri dish) amended with various concentrations of EOs 0.1- 0.8% (v/v). For enhancing the oil solubility, Tween-80, 0.5% (v/v) was added. After inoculating the mycelia of fungus onto the center of agar, the dishes were incubated at 25 ± 2°C until the growth of the control dishes (without the EOs) had reached the edge of the plate. Then, the mycelial growth inhibition (MGI) percentage was calculated as follows:

$MGI = (dc-dt)/dc \times 100$

Where "dc" is colony diameter of control sets and "dt" is colony diameter measured in treatment sets (17, 18). The IC50 values (the concentration inhibited 50% of the mycelium growth) were calculated by probit analysis. Minimal inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) were also examined using the methods reported by Yen and Chang (2008). When the mycelium of fungi reached the edges of the control dishes, the lowest concentration with no sign of growth was defined as MIC. After the MIC was determined, a small piece of agar (2 × 2 × 2 mm3) was taken from the colony of the MIC plate, and was inoculated on a drug-free PDA medium. After 5 days, MFCs were determined by the lowest concentration of the test compounds in which no recovery of microorganism was observed (19).

Statistical analysis. Data on effects of the extracts on the growth of pathogens was analyzed by one-way analysis of variance and comparison of means using the Duncan's Multiple Range Test at the level $P < 0.05$. The statistical analysis was performed

Table 1. The effects of different concentrations of peppermint, Shirazi thyme and thyme essential oils on mycelial growth of *P. drechsleri*

Concentration (%) (v/v)	Mycelial Growth Diameter (mm)						
	24h	48h	72h	96h	120h	144h	168h
Peppermint							
0.1	13.67± 2.12a	20.33± 1.41a	26.67± 2.12a	32.67± 2.83 a	38.33± 2.12a	44.00± 0.71 a	48.33± 0.71 a
0.2	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	11.33± 2.12b	13.67± 4.95b	17.33± 4.25b	21.67± 4.95b
0.4	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
0.8	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
Shirazi thyme							
0.1	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
0.2	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
0.4	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
0.8	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
Thyme							
0.1	12.33± 0.71c	17.33± 2.12c	22.37± 3.54c	29.00± 6.36d	32.00± 7.07d	36.67± 8.49d	41.00± 9.90d
0.2	0.00 ± 0.00b	0.00 ± 0.00b	9.33± 0.71d	9.33± 3.67b	10.67± 4.08b	10.67± 4.9e	11.00± 5.72e
0.4	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
0.8	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
Control	16.67± 1.15d	23.33± 2.08d	29.67± 3.21e	36.00± 4.00e	42.00± 3.61a	46.67± 3.51a	53.33± 2.89f

The results are means ± standard errors of four replications. Means within a column indicated by the same letter were not significantly different according to Duncan's multiple range tests at the level $P < 0.05$.

using statistical package for the social sciences 15.0 software for Windows (SPSS Inc., Chicago, IL, USA)

RESULTS

The results of inhibitory effects of EOs on mycelial growth of the fungus and MGI values are presented in Tables 1 and 2. The inhibitory effects of EOs in pour plate method showed that Shirazi thyme, thyme and peppermint were effective in preventing *P. drechsleri* growth at different concentrations (Figs. 1-3). By increasing concentration from 0.1 to 0.8% (v/v), antifungal activity of these EOs increased substantially.

According to the results reported in Table 2, the Shirazi thyme was the most effective essential oil on the *P. drechsleri* because caused 100% growth inhibition at all concentrations tested (Fig. 2). Also the minimum activity was recorded for peppermint EOs. By increasing the oil concentration from 0.1% to 0.8%, antifungal activity of peppermint and thyme were substantially increased (Fig. 1). Peppermint and thyme at 0.4% and 0.8% concentration showed complete (100%) antifungal effect while other concentrations (0.2% and 0.1%) were only prevented percentage of growth by *Phytophthora*, with MGI values of 9.37% and 59.38% for peppermint and 23.13% and 79.38% for thyme, respectively (Figs. 3 & 4). The maximum and minimum fungal growth at concentration 0.1% was recorded by peppermint (4.83 mm) and Shirazi thyme (0 mm), respectively.

The comparison between tested EOs was further confirmed by comparing their effective concentrations, MIC and MFC values that shown in Table 3. The values of IC_{50}, MIC and MFC for Shirazi thyme were 0.053%, 0.1% and 0.2%, respectively,

Fig.1. Radial growth of *P. drechsleri* mycelium treated with Shirazi thyme EOs at 0.1% to 0.8% (v/v) concentrations.(a) 0.1% (b) 0.2% (c) 0.4% (d) 0.8% (v/v) after 7 days at 25 °C.

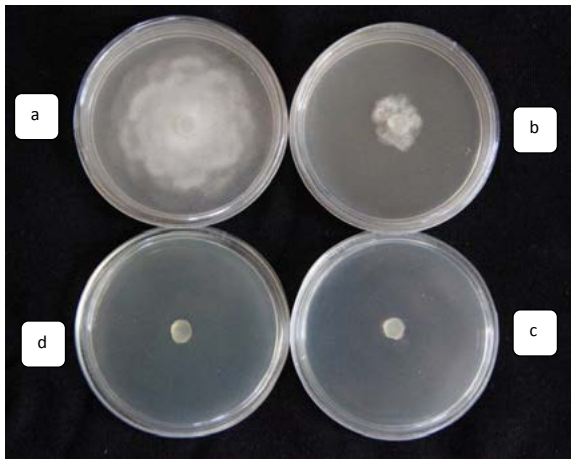

Fig. 2. Radial growth of *P. drechsleri* mycelium treated with thyme at 0.1% to 0.8% (v/v) concentrations.(a) 0.1% (b) 0.2% (c) 0.4% (d) 0.8% (v/v) after 7 days at 25 °C.

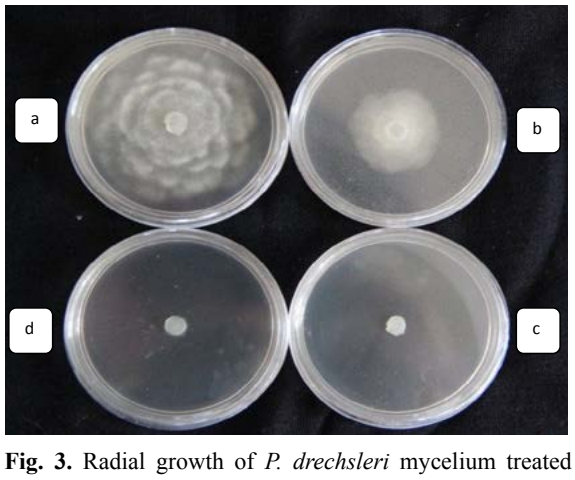

Fig. 3. Radial growth of *P. drechsleri* mycelium treated with peppermint at 0.1% to 0.8% (v/v) concentrations.(a) 0.1% (b) 0.2% (c) 0.4% (d) 0.8% (v/v) after 7 days at 25 °C.

which were significantly lower than those of thyme and peppermint ($p \leq 0.05$). The results, as seen in Table 3, showed that the least effect was also observed for peppermint with significantly higher values of IC_{50} (0.186%), MIC (0.4%) and MFC (0.8%) than that of Shirazi thyme. While, the mentioned values of peppermint were no different to values of thyme ($p \geq 0.05$).

DISCUSSION

In recent years, increased interest has been generated in the development of healthy and natural antifungal agents such as plant-based essential oils and extracts to control phytopathogens in agriculture (20). In this study, we investigated the antifungal effects of three essential oils including peppermint, thyme and Shirazi thyme on the major agricultural pathogen (*P. drechsleri*) and determined their mycelial growth inhibition rate, minimum inhibitory concentration and minimum fungicidal concentration. Our results showed that the essential oils from Shirazi thyme and thyme have more acceptable antifungal properties on *P. drechsleri* than peppermint *in vitro*. The efficacy of these EOs was positively correlated with the concentration and by increasing concentrations from 0.1% to 0.8% in EOs, antifungal activity increased substantially (Tables 1 and 2).

Antifungal activities of Shirazi thyme and thyme found in this study are consistent with several reports of these oils against various plant pathogenic fungi (21, 22). These activities can be attributed to the presence of various constituents such as thymol and carvacrol. It has been accepted that the anti-microbial activity of Shirazi thyme and thyme is related to high percentage of thymol and carvacrol which are well-known antifungal agents (23-25). Furthermore, both EOs contained γ-terpinene and *p*-cymene with antimicrobial activities, which are biochemical precursors of thymol and carvacrol in the phenols biosynthetic pathway (24, 26). Specific studies have linked these compounds to antifungal activities against *Phytophthora*. Camele *et al.* (2012) reported that the essential oil of three Mediterranean aromatic plants (*Verbena officinalis*, *Thymus vulgaris* and *Origanum vulgare*) consists mainly of carvacrol and thymol as main constituents, and exhibited a complete mycelial inhibition effect on the *Phytophthora citrophthora* (27). Kim *et al.* (2008) reported that the essential oil of thyme (*Thymus vulgaris*) consists

Table 2. Antifungal activity of various concentrations of EOs against *P. drechsleri*.

Essential oils	Concentration (%) (v/v)	Mycelial Growth Diameter (cm)	MGI%
Peppermint	0.1	4.83 ± 0.07 a	9.37 a
	0.2	2.17 ± 0.49 b	59.38 b
	0.4	0.00 ± 0.00 c	100.00 c
	0.8	0.00 ± 0.00 c	100.00 c
Shirazi thyme	0.1	0.00 ± 0.00 c	100.00 c
	0.2	0.00 ± 0.00 c	100.00 c
	0.4	0.00 ± 0.00 c	100.00 c
	0.8	0.00 ± 0.00 c	100.00 c
Thyme	0.1	4.10 ± 0.99 d	23.13 d
	0.2	1.10 ± 0.57 e	79.38 e
	0.4	0.00 ± 0.00 c	100.00 c
	0.8	0.00 ± 0.00 c	100.00 c

The results are means ± standard errors of four replications. Means within a column indicated by the same letter were not significantly different according to Duncan's multiple range tests at the level $P < 0.05$.

mainly of carvacrol and thymol as major components, and exhibited a complete mycelial inhibition effect on the growth of *P. cactorum* (21). In another studies, thymol and carvacrol were again reported to show complete inhibition of *P. capsici* (28, 29). Moreover, recently, Soković et al (2009) reported a relationship between the high activity of some *Thymus* oils and the presence of phenol components, such as thymol and carvacrol (30).

Studies suggested that the antifungal activity resulted from a direct effect of essential oil on fungal mycelium and postulated that the lipophilic nature of EOs was as possible for them being absorbed by fungal mycelia (31, 32). In this regard, Zambonelli *et al.* (1996) hypothesized that the antifungal activity of Shirazi thyme and thyme oils might be due to the fact that carvacrol and thymol disintegrated the fungal hyphae which appeared emptied of their cytoplasmic content (33). Moreover, several studies have demonstrated that terpenes (i.e. thymol and carvacrol) which are the major components of EOs, alter cell permeability by penetrating between the fatty acyl chains making up the membrane lipid bilayers, disrupting lipid packing and changing membrane fluidity (34, 35). Braga and Dal Sasso (2005) showed that these phenomena led to major surface alterations and morphological modifications, also reducing the adherence capacity of plant pathogenic fungi (34). In

Table 3. MIC, MFC and IC_{50} values of test compounds against *P. drechsleri*.

Compounds	IC_{50} (%)	MIC (%)	MFC (%)
Peppermint	0.186 a	0.4 a	0.8 a
Shirazi thyme	0.053 b	0.1 b	0.2 b
Thyme	0.147 c	0.4 a	0.8 a

MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration; $IC_{50,}$ median inhibition concentration
Within the column, mean values followed by the same letter are not significantly different according to Duncan's multiple range test at the level $P < 0.05$.

addition, Camele *et al.* (2012) suggested that phenol components may interfere with cell wall enzymes like chitin synthase/chitinase as well as with the α- and β-glucanases of *P. citrophthora* (27). Since *P. citrophthora* is phylogenetically close to *P. drechsleri* (36), the inhibitory effects of Shirazi thyme and thyme oils found in this study could be due to those effects of terpenes in the oils.

The addition of fungistatic properties, fungicidal activity of three EOs tested on this plant pathogenic fungus proved in our study. This varies with results of Abdolmaleki *et al.* (2010) that Z. *multiflora* is fungistatic rather than fungicidal (37). This variation may be attributed to the chemical composition of EOs and mode of resistant behaviour of the fungi against various substances present in the various EOs (38). It has been accepted that the compositions of the essential oils can vary greatly depending upon the climate of geographical region, soil composition, the variety, plant organ, age of the plant, the method of drying and the method of extraction of the oil, and growth stages; vegetation, beginning of blooming, full blooming and fruit maturation (39, 40)**.** Accordingly, it is often quite difficult to compare the results obtained from different studies.

CONCLUSION

Based on our findings, *Z. multiflora* and *T. Vulgaris* as a natural antimicrobial agent strongly inhibited the *P. drechsleri* growth, the causative agent of damage to many crops that is consumed directly by humans. Therefore, they can be considered for developing new alternative fungicides to synthetic fungicides that are natural and used to safe control this pathogenic fungus and many agricultural plant pathogens causing drastic crop losses.

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