J. Crop Prot. 2022, 12 (4): 455-465

Research Article

The effect of some volatile organic compounds on the biological control of fungal and pseudofungal pathogens isolated from *Rosmarinus officinalis*

Kowsar Shirazi[1], Samaneh Bagheri[2*] and Nafiseh Mahdinezhad[2]

1. Department of Plant Protection, Faculty of Agriculture, University of Zabol, Zabol, Iran.
2. Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Zabol, Zabol, Iran.

Abstract: Fungal diseases cause a massive reduction in the production of rosemary plants every year. Volatile organic compounds (VOCs) can provide valuable fungal bio-pesticides for practical usage. However, the effect of VOCs on the pathogenic fungi of rosemary is poorly studied. This research characterized some fungal pathogens (*Rhizoctonia solani*, *Fusarium oxysporum*, *Phytophthora infestans*, and *Phytophthora citrophthora*) isolated from rosemary plants. We studied the inhibitory effect of VOCs, including isovaleric acid, 1-octene-3-ol and 3-octanone on growth and disease incidence of isolated fungi under *in vitro* and greenhouse conditions. The action of individual VOCs on the growth indices of infected plants was also investigated. 1-octen-3-ol showed the most efficacy percentage and inhibitory effect on mycelial growth at five mg/l concentration. Isovaleric acid decreased fungal growth and disease incidence at 10 mg/l as high as 94.74% and 87.23%, respectively. However, 3-octanone had no significant efficacy percentage and inhibitory effect on mycelial growth. 1-octen-3-ol enhanced the growth of fungal-infected plants to the highest amount, but 3-octanone did not increase the growth of infected plants. The obtained data revealed that the different effects of various VOCs on fungal pathogens are related to different chemical structures and action mechanisms of volatile compounds and the species of fungi involved.

Keywords: inhibitory effect, pathogenic fungi, rosemary, volatile organic compounds

Introduction

Rosemary *Rosmarinus officinalis* L. is widely cultivated as a medicinal and ornamental plant in the field and under greenhouse conditions (Verhoeven *et al*., 2008). This plant is also a food source and flavoring agent (Hamidpour *et al*., 2017). However, this crucial economic plant suffers from diseases like soil-borne fungal pathogens, including *Rhizoctonia*, *Phytophthora*, and *Fusarium* species. These pathogens cause serious diseases and reduce yield (Alvarez *et al*., 2007; Verhoeven *et al*., 2008). These fungal pathogens decrease the nutritional and medicinal value of rosemary plants. Moreover, some fungi produce mycotoxins that poison humans and animals (Choinska *et al*., 2020).

The fungal diseases of plants are usually controlled by chemical pesticides and synthetic fungicides (Choinska *et al*., 2020: Medina-Romero

Handling Editor: Naser Safaie

* Corresponding author: Samanehbagheri6556@gmail.com
Received: 04 April 2022, Accepted: 20 November 2022
Published online: 09 January 2023

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

et al., 2017). However, prolonged usage of chemicals causes water and air pollution and harms human health. Pesticide residues in nature can also develop pesticide-resistant pathogens (Oka *et al*., 2015; Macias-Rubalcava *et al*., 2018). So, the harmful effects of industrial fungicides on nature and human health have led to research for eco-friendly alternatives to control plant diseases (Choinska *et al*., 2020).

Volatile Organic Compounds (VOCs) are kinds of bio-pesticides that derive from primary (synthesis of DNA, amino acids, and fatty acids) or secondary metabolism (mediators of primary metabolism) (Korpi et al., 2009). As harmless and eco-friendly compounds, bio-pesticides can protect agricultural products from microorganisms and fungal pathogens. These pesticides often comprise herbal, fungal, and bacterial secondary metabolites (Medina-Romero et al., 2017).

The molecular weight of VOCs is low, which means they can travel a long way and spread through air and water easily because of their small sizes (Morath *et al*., 2012). A large number of VOCs have been identified that include alcohols, aldehydes, benzene derivatives, cyclohexenes, heterocycles, ketones, phenols, thioesters, and thioalcohol. The negative effects of VOCs on plant pathogens present an eco-friendly alternative to chemical pesticides (Karsli and Sahin, 2021). VOCs can act against fungal pathogens by destroying the cell walls of pathogens or inducing systemic resistance in plants (Chen *et al*., 2008; Zheng *et al*., 2013).

In this study, some significant fungal pathogens of rosemary plants were characterized. The antifungal activity of several VOCs, including 3-octanone, 1-octen-3-ol, and isovaleric acid, on the fungal pathogens isolated from rosemary plants was evaluated by measuring their mycelial growth inhibition and efficacy percentage. The potential antifungal activity of VOCs on some growth indices of infected rosemary seedlings was also determined.

Materials and Methods

Sample collection

The root samples of infected rosemary plants were collected from fields in Sistan and Baluchestan province, Iran, and transferred to the laboratory. The infected root samples were cut into 1 cm pieces and then dipped in ethanol (70%) for 1 min and sodium hypochlorite (3%) for 5 min. The pieces were rinsed in sterile distilled water and dried on sterile filter paper. The resulting pieces were plated on WA (water agar) and then PDA (potato dextrose agar-PDA, Merck, Germany) media to isolate the fungal pathogens. The hyphal tip method purified the isolated fungi (Royse and Wilkinson, 2015).

Identification of pathogens

For the diagnosis of fungal pathogens, DNA was purified using the quick-DNA fungal miniprep method (Motkova and Vytrasova, 2011). The universal primers of ITS1 and ITS4 were used for the amplification of ITS1-5.8S-ITS2 region of rDNA (ITS-rDNA) (White *et al.*, 1990). PCR-specific products were submitted for sequencing by standard DNA sequencing techniques. The resulting nucleotide sequences were compared with sequences available in the GenBank (http://www.ncbi.nlm.nih.gov) using the basic local alignment search tool (BLAST). The phylogenetic tree of recognized fungal species was constructed based on ITS-rDNA gene sequences using the computer program MEGA (version 7.0) and *Trichoderma harzianum* fungus isolate as an outgroup. Phylogenetic analysis was done by the neighbor-joining method with bootstrap values based on 1000 replications.

Antifungal activity of VOCs on agar plates

The VOCs, including 3-octanone, 1-octen-3-ol, and isovaleric acid (they all had purity > 99%), were obtained from Sigma-Aldrich GmbH, Germany. The tests were conducted to evaluate the effect of three different concentrations of the three volatile compounds (5, 10, and 40 mg/l) on the mycelial growth of recognized fungal pathogens *in vitro* conditions. For this purpose, PDA media were prepared with various concentrations of volatile compounds. The mycelial plugs (3-mm diameter) of each fungal pathogen were obtained by growing on PDA for 7-days. In the control experiments, cultures were inoculated with mycelial plugs of fungal

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

pathogens without adding any VOC compound. The mycelial growth of fungi was measured every day, and the results were analyzed after 10 days. All experiments were performed with three plates per each of the tests. Each assay was repeated two times. The inhibition percentage of various concentrations of VOCs was obtained by the following equation (Xiong *et al*., 2017):

Inhibition rate: $(D_0 - D_n + 3.0)/D_0 \times 100$

D_0 (mm) = The growth diameter of fungal pathogens on control plates

D_n (mm) = The growth diameter of fungal pathogens on treated plates with different concentrations of volatile compounds

Antifungal activity of VOCs on the disease incidence

Non-inoculated rosemary seedlings were cultivated under greenhouse conditions. The injection of each fungal pathogen was carried out on roots after 42 days of planting. Different concentrations of VOCs (5, 10, and 40 mg/l) were also prepared. Five to six small holes were made in the soil of each pot to add VOCs and allow the roots to be exposed to these compounds. Then, different concentrations of volatile compounds were added separately to the soil of inoculated rosemary pots (Moreira *et al*., 2019). Control rosemary seedlings were also inoculated with each of the identified fungal pathogens without treatment with any of the volatile compounds. Each pot was replicated three times.

Disease incidence (percentage of infected plants) was determined after 21 days of treatment with different VOC concentrations (McKinney, 1923). The efficacy percentage of various concentrations of VOCs was calculated using the following formula (Ramesh *et al*., 2020).

Efficacy Percentage= [(D1-D2)/D1] × 100

D1 = The disease incidence in control seedlings

D2 = The disease incidence in treated seedlings with various concentrations of VOCs

The effect of volatile compounds on plant growth

The effect of VOCs on growth indices of treated rosemary plants was measured by the determination of seedling height and fresh and dry weight of their roots and seedlings. Each treatment was replicated three times.

Data analysis

All statistical analysis was performed using SAS 9.1 software. Mean Significant differences were identified by one-way analysis of variance (ANOVA) followed by Tukey's HSD (Honestly Significant Difference) test. Differences were considered to be significant at $p \leq 0.01$.

Results

Characterization of rosemary fungal pathogens

Four isolated fungi from rosemary plants were characterized by amplification of ITS-rDNA region and sequencing. The characterized fungal pathogens included *Rhizoctonia solani*, *Fusarium oxysporum*, *Phytophthora infestans*, and *Phytophthora citrophthora* isolates. Obtained nucleotide sequences were submitted on GenBank with identified accession numbers in Table 1.

Table 1 Identification of fungal pathogens isolated from rosemary based on their ITS sequences with their NCBI accession numbers.

Species	Isolate	Host origin	Phylum	Accession Number
Rhizoctonia solani	RK3	root	*Basidiomycota*	OM868041
Fusarium oxysporum	RK7	root	*Ascomycota*	OM868046
Phytophthora infestans	RK12	root	*Oomycota*	OM868069
Phytophthora citrophthora	RK20	root	*Oomycota*	OM868170

The phylogenetic analysis was based on ITS-rDNA sequences of our characterized fungal pathogens and the others obtained from GenBank, including the outgroup (Figure 1). Evolutionary analysis showed that all investigated fungi isolates are classified into four distinct clades, and they correspond to three fungal orders, including Cantharellales (*Rhizoctonia* species), Hypocreales (*Fusarium* species), Peronosporales (*Phytophthora* species).

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

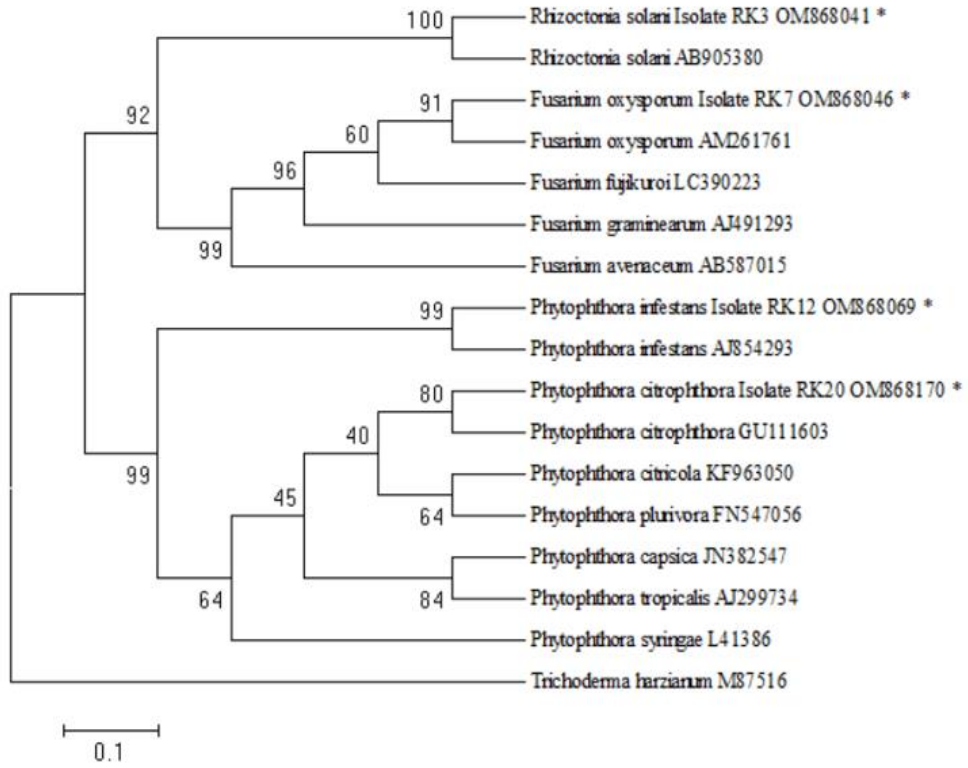

Figure 1 Phylogenetic tree based on ITS-rDNA gene sequences of isolated fungi from rosemary along with the sequences from NCBI. The analysis was conducted with MEGA (version 7.0) using the neighbor-joining method with 1000 bootstrap replications. *Trichoderma harzianum* (M87516) was used as an outgroup in the analysis.

Our recognized isolate of *Rhizoctonia solani* (OM868041) was grouped in Clade I with another isolate of this species in GenBank (AB905380) and 100% bootstrap support. *Fusarium oxysporum* isolate (OM868046) was grouped in the same cluster (Clade II) with some other *Fusarium* species, including *Fusarium fujikuroi*, *Fusarium graminearum*, and *Fusarium avenaceum*. Bootstrap support for Clade II was 99%.

Phytophthora infestans and *Phytophthora citrophthora* isolates which belong to Peronosporales order, were grouped in two clades (Clade III and IV) with 99% bootstrap support.

Evaluation of the *in vitro* antifungal effect of VOCs

The antifungal effect of various concentrations of volatile compounds on the mycelial growth of the fungal pathogens is shown in Table 2. The mycelial growth of four isolated fungi declined by the range of 2.45%-98-45% after treatment with different concentrations of VOCs. Of the three studied VOCs, the most significant inhibition of fungal growth was observed under treatment with 1-octen-3-ol. The growth rate of *Rhizoctonia solani* and *Fusarium oxysporum* declined by 98.45% in treatment with five mg/l of 1-octen-3-ol compared to the control. However, the higher concentrations of 1-octen-3-ol did not decrease fungal growth.

Growth of *Phytophthora infestans* was inhibited by 94.74% in the treatment with 10 mg/l of isovaleric acid. However, isovaleric acid (5 mg/l) inhibited the mycelial growth of *Fusarium oxysporum* by 2.45%. 3-octanone had the minimum inhibition effect on the growth of fungal pathogens even at 40 mg/l. There was no significant difference between the inhibitory effect of 3-octanone in various concentrations.

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

Antifungal effect of volatile compounds under greenhouse condition

The influence of three investigated VOCs on disease incidence of rosemary fungal pathogens and their efficacy percentage were studied (Table 3). Disease incidence was considered as the percentage of rotten roots of inoculated rosemary plants with different fungi compared to control ones. Similar to *in vitro* results, 1-octen-3-ol was able to reduce disease incidence at the highest level. 5 mg/l of 1-octen-3-ol showed the most significant efficacy percentage (90.90%-92.32%). However, at 10 and 40 mg/l concentrations, its efficacy rate was decreased.

Isovaleric acid at the 10 mg/l concentration could reduce disease incidence of pseudofungal pathogen, *Phytophthora infestans*, up to 87.23%. However, the reduction of disease incidence of *Fusarium oxysporum* was at the lowest amount (2.17%) when treated with 5 mg/l isovaleric acids.

3-octanone had the least influence on disease incidence of rosemary fungal pathogens, similar to the *in vitro* condition. Compared with the control, 3-octanone did not affect disease incidence of *Fusarium oxysporum* and *Rhizoctonia solani* at the 5 and 10 mg/l concentrations. The disease incidence of *Phytophthora infestans* did not change in treatment with 40 mg/l 3-octanone.

Effect of VOCs on the growth of plants

The effect of volatile compounds on plant growth is shown in Table 4. All tested VOCs stimulated the growth of inoculated rosemary plants. Compared to the control, the most significant increase in seedling height and fresh and dry weight of roots and seedlings was observed in infected plants treated with 5 mg/l 1-octen-3-ol; however, with enhancement in 1-octen-3-ol concentration, plants' growth was decreased compared to 5 mg/l concentration.

Table 2 Effect of different VOC concentrations on the mycelial growth of rosemary fungal pathogens.

Treatment	*Rhizoctonia solani*		*Fusarium oxysporum*		*Phytophthora infestans*		*Phytophthora citrophthora*	
	Mycelial growth (mm)	Inhibition (%)	Mycelial growth (mm)	Inhibition (%)	Mycelial growth (mm)	Inhibition (%)	Mycelial growth (mm)	Inhibition (%)
Control	90.00 a	-	92.20 a	-	95.00 a	-	90.75 a	-
Isovaleric acid 5 mg/l	82.96 b	20.15 d	90.25 a	2.45 d	35.46 d	77.60 c	22.76 c	80.75 b
Isovaleric acid 10 mg/l	10.36 d	80.65 b	22.46 c	87.30 b	5.25 f	94.74 a	4.40 d	90.27 a
Isovaleric acid 40 mg/l	33.50 c	70.25 c	41.40 c	72.12 c	51.4 c	50.33 d	31.63 b	70.10 c
1-octen-3-ol 5 mg/l	2.2 e	98.45 a	2.20 d	98.45 a	5.10 f	93.87 a	2.60 d	95.34 a
1-octen-3-ol 10 mg/l	2.20 e	98.33 a	27.33 c	82.35 b	2.13 f	98.14 a	27.30 c	81.91 b
1-octen-3-ol 40 mg/l	10.90 d	88.20 b	24.75 c	79.50 bc	26.30 e	80.68 b	35.10 b	78.20 bc
3-octanone 5 mg/l	92.93 a	5.34 e	95.63 a	5.12 d	88.0 b	10.25 e	90.50 a	5.47 d
3-octanone 10 mg/l	98.57 a	4.12 e	90.00 a	4.45 d	95.0 a	5.48 f	95.64 a	4.20 d
3-octanone 40 mg/l	95.96 a	5.93 e	94.53 a	5.65 d	96.62 a	5.14 f	95.66 a	5.95 d

Values in a column followed by the same letter are not statistically significantly different ($P \leq 0.01$) according to Duncan's multiple range test.

Table 3 Effect of different VOC concentrations on disease incidence of rosemary isolated fungi and their efficacy percentage in greenhouse conditions.

Treatment	*Rhizoctonia solani*		*Fusarium oxysporum*		*Phytophthora infestans*		*Phytophthora citrophthora*	
	Disease Incidence (%)	Efficacy (%)	Disease Incidence (%)	Efficacy (%)	Disease Incidence (%)	Efficacy (%)	Disease Incidence (%)	Efficacy (%)
Control	88.00 a	-	92.00 a	-	94.00 a	-	92.00 a	-
Isovaleric acid 5 mg/l	84.00 a	4.54 f	90.00 a	2.17 f	68.00 c	27.65 e	62.00 c	32.60 f
Isovaleric acid 10 mg/l	45.00 c	48.86 d	54.00 d	41.30 d	12.00 f	87.23 b	12.00 g	86.95 b
Isovaleric acid 40 mg/l	62.00 b	29.54 e	78.00 c	15.21 e	58.00 d	38.29 d	53.00 d	42.39 e
1-octen-3-ol 5 mg/l	8.00 f	90.90 a	8.00 g	91.30 a	8.00 g	91.48 a	7.00 h	92.32 a
1-octen-3-ol 10 mg/l	15.00 e	82.95 b	25.00 f	72.82 b	10.00 fg	89.36 ab	32.00 f	65.21 c
1-octen-3-ol 40 mg/l	38.00 d	56.81 c	42.00 e	54.34 c	40.00 e	57.44 c	44.00 e	52.17 d
3-octanone 5 mg/l	84.00 a	4.54 f	92.00 a	0.00 g	84.00 b	10.63 f	89.00 ab	3.26 g
3-octanone 10 mg/l	88.00 a	0.00 g	92.00 a	0.00 g	90.00 a	4.25 g	83.00 b	9.78 g
3-octanone 40 mg/l	85.00 a	3.40 f	87.00 b	5.43 f	94.00 a	0.00 h	90.00 a	2.17 gh

Values in a column followed by the same letter(s) are not statistically significantly different ($P \leq 0.01$) according to Duncan's multiple range test.

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

Table 4 Effect of different concentrations of VOCs on some growth indices of infected rosemary plants in greenhouse condition.

Pathogen	Treatment	Seedling height (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Rhizoctonia solani	Control	35.11 c	30.65 d	17.55 d	12.85 d	6.45 cd
	Isovaleric acid 5 mg/l	36.20 c	31.19 d	18.80 c	13.49 c	7.34 c
	Isovaleric acid 10 mg/l	40.33 bc	38.33 c	20.38 bc	13.66 c	7.55 c
	Isovaleric acid 40 mg/l	35.70 c	35.61 c	20.74 bc	13.33 cd	7.05 c
	1-octen-3-ol 5 mg/l	48.40 a	53.47 a	27.33 a	17.15 a	9.95 a
	1-octen-3-ol 10 mg/l	42.67 b	49.94 b	24.99 a	17.01 a	8.65 b
	1-octen-3-ol 40 mg/l	38.51 c	49.52 b	21.14 b	16.16 b	8.16 b
	3-octanone 5 mg/l	36.82 c	33.81 d	19.94 c	12.25 de	6.73 cd
	3-octanone 10 mg/l	35.02 c	27.41 e	15.80 e	11.49 e	5.25 e
	3-octanone 40 mg/l	30.94 d	32.90 d	19.42 c	12.65 d	6.00 cd
Fusarium oxysporum	Control	30.45 c	30.42 e	15.08 e	12.33 e	6.66 e
	Isovaleric acid 5 mg/l	30.10 c	32.70 e	17.98 d	12.78 e	6.85 e
	Isovaleric acid 10 mg/l	34.28 b	40.11 c	24.58 b	13.13 d	7.98 d
	Isovaleric acid 40 mg/l	32.28 c	32.60 e	17.02 d	12.35 e	6.42 e
	1-octen-3-ol 5 mg/l	42.45 a	57.66 a	30.33 a	17.85 a	10.10 a
	1-octen-3-ol 10 mg/l	40.77 a	49.94 b	25.19 b	16.10 b	9.12 b
	1-octen-3-ol 40 mg/l	40.15 a	47.38 b	22.99 c	15.66 c	8.34 c
	3-octanone 5 mg/l	30.33 c	41.33 c	24.67 b	12.12 e	6.37 e
	3-octanone 10 mg/l	32.51 c	39.61 cd	24.04 b	11.37 ef	5.78 f
	3-octanone 40 mg/l	27.64 d	35.94 d	21.85 c	12.45 e	7.45 d
Phytophthora infestans	Control	38.23 d	35.83 d	18.35 de	13.25 e	7.15 cd
	Isovaleric acid 5 mg/l	39.94 d	33.47 e	16.26 ef	14.75 cd	7.52 c
	Isovaleric acid 10 mg/l	45.03 b	38.99 d	22.74 cd	13.95 d	7.66 c
	Isovaleric acid 40 mg/l	38.58 d	30.80 e	17.89 e	13.28 e	6.98 d
	1-octen-3-ol 5 mg/l	50.15 a	60.69 a	32.85 a	18.65 a	10.95 a
	1-octen-3-ol 10 mg/l	47.31 b	56.14 b	27.14 b	17.37 b	9.87 b
	1-octen-3-ol 40 mg/l	44.65 c	53.77 c	24.66 c	15.94 c	9.66 b
	3-octanone 5 mg/l	34.42 e	32.57 e	17.48 e	11.12 f	6.87 e
	3-octanone 10 mg/l	36.70 d	30.02 ef	14.34 f	10.92 fg	6.66 f
	3-octanone 40 mg/l	38.97 d	33.81 e	19.08 d	10.18 g	6.62 f
Phytophthora citrophthora	Control	35.52 c	32.10 f	17.65 d	12.95 e	6.86 d
	Isovaleric acid 5 mg/l	38.74 c	36.55 e	18.74 c	15.45 c	9.10 bc
	Isovaleric acid 10 mg/l	41.11 bc	42.41 d	21.83 bc	13.87 d	7.45 c
	Isovaleric acid 40 mg/l	38.24 c	34.25 c	20.20 c	13.27 d	6.92 d
	1-octen-3-ol 5 mg/l	49.69 a	60.07 a	29.92 a	18.24 a	10.65 a
	1-octen-3-ol 10 mg/l	44.65 b	55.38 b	28.08 a	17.08 b	9.52 b
	1-octen-3-ol 40 mg/l	41.37 bc	49.21 c	25.39 b	17.22 b	9.05 bc
	3-octanone 5 mg/l	32.68 d	28.83 g	16.46 e	11.58 f	5.45 e
	3-octanone 10 mg/l	32.36 d	30.49 fg	17.42 d	10.12 g	5.28 ef
	3-octanone 40 mg/l	29.98 e	32.08 f	18.95 c	10.52 fg	5.37 e

Values in each column followed by the same letter(s) are not statistically significantly different ($P \leq 0.01$) according to Duncan's multiple range test.

Isovaleric acid also increased the growth of rosemary plants infected by various isolates of fungal pathogens compared with the control. At the concentration of 10 mg/l, isovaleric acid had the most stimulatory effect on rosemary plants' growth. However, there were no significant differences between the effect of various concentrations (5, 10, and 40 mg/l) of isovaleric acid on seedling height and dry weight and fresh and dry weight of infected plants with *Rhizoctonia solani*.

The growth of infected plants treated with 3-octanone was lower or without significant difference compared to the control. However, the seedling dry weight of inoculated plants with *Rhizoctonia solani* and seedling fresh and dry weight of rosemary plants infected by *Fusarium oxysporum* was increased compared to the control after treatment with 3-octanone.

Discussion

Numerous soil-borne fungi lead to root rot and wilting in rosemary plants. The infection of rosemary plants with various fungal pathogens reduces their yield (Vorhoeven *et al*., 2008). On

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

the other hand, there are few efficient methods to control plant fungal diseases. However, VOCs represent a biological approach that can be used in agriculture to control plant pathogens (Cellini *et al*., 2021; Veselova *et al*., 2019).

In this study, two fungal and two Oomycete pathogens isolated from rosemary plants were characterized, and the effect of some volatile compounds on these pathogens was evaluated. The results showed the negative effect of VOCs onthe pathogens' growth and disease incidence. The growth enhancement of infected plants treated with VOCs was also observed.

The isolated pathogens in the present study included *Rhizoctonia*, *Fusarium*, and *Phytophthora* species. Also, *Phytophthora* and *Fusarium* species were identified as the main disease agents of rosemary plants which can grow through the plant roots (Mirabolfathi and Ershad, 1993). *Rhizoctonia solani* was identified in infected rosemary by morphological and homological characterization methods (Holcomb, 1992). Ashrafi *et al*. (2010) also characterized three isolated fungal pathogens (*Phytophthora citrophthora*, *Rhizoctonia solani*, and *Fusarium oxysporum*) of rosemary, which caused yield loss.

Three studied VOCs (Isovaleric acid, 1-octen-3-ol and 3-octanone) in this work are classified as ketone compounds (Sidorova *et al*., 2022; Zabin and Bloch, 1950; Wnuk *et al*., 1983). Of these utilized VOCs, 1-octen-3-ol showed the most significant inhibiting effect on the mycelial growth of fungal pathogens. 1-octen-3-ol is a natural product that is used to reduce pathogenic infection. The effectiveness of 1-octen-3-ol on the growth of fungal pathogens is related to destroying hyphae structure and activating the salicylic acid (SA) signal in plants to increase their disease resistance (Wang *et al*., 2022). The inhibiting impact of 3-octanone on the growth of fungal pathogens in our study was less than other compounds. The effect comparison of used VOCs on the growth of fungal mycelium suggested that the antifungal influence of these compounds is related to their hydrocarbon structure and solubility in phospholipid of the membrane (Sidorova *et al*., 2022).

The inhibition of disease incidence of 1-octen-3-ol was more than the other two volatile compounds. The antifungal activity of 1-octen-3-ol, in comparison with other VOCs, is attributed to its structure (fatty alcohol), high lipophilicity, and ability to cross the lipid barrier of the cell membrane (Dimock *et al*., 1982). However, 1-octen-3-ol at the concentration of 5 mg/l revealed the most efficacy percentage. Similarly, Kishimoto *et al*. (2007) indicated that 1-octen-3-ol at a low concentration (10 ml/l) increased the resistance of *Arabidopsis thaliana* to *Botrytis cinerea*. They also showed that 1-octen-3-ol activated some of the defense genes that were turned on by ethylene and jasmonic acid signaling. In our study, isovaleric acid (carboxyl acid) (Dilek and Sezin, 2020) and 3-octanone (ketone) (Santos *et al*., 2017) showed a lower amount of inhibiting effect on disease incidence compared to 1-octen-3-ol, which is probably correlated with their hydrophobic nature and interaction with the fungal cellular membrane (Sidorova *et al*., 2022).

The effect of volatile compounds on improving the growth of the infected plants was also evaluated in the present study. Ryu and coworkers (2003) reported this effect for the first time. The results of their study indicated that 2,3-butanediol promoted the growth of Arabidopsis thaliana and its systemic resistance. The mechanism of the stimulating effect of VOCs on plant growth is not sufficiently understood. It has been suggested that volatile compounds improve infected plant growth by increasing photosynthesis and sugar accumulation rates and promoting plant mineral uptake (Sidorova *et al*., 2022). In our work, we showed that 1-octen-3-ol at a lower concentration (5 mg/l) enhanced the growth of infected plants to the highest amount. Splivallo *et al*. (2007) stated that 1-octen-3-ol at a high concentration (130 mg/l) inhibited the growth of the root and decreased chlorophyll concentration in *Arabidopsis thaliana*. The results of our study revealed that 3-octanone did not improve the growth of infected plants significantly. Similarly, Kottb *et al*. (2015)

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

found that volatile compounds obtained from *Trichoderma asperellum* did not enhance the growth of infected plants. Still, they improved the amount of plant hormones (salicylic and abscisic acid) production. So, the resistance of infected plants against fungal pathogens was increased.

Obtained data in our study provided important insights into the impact of VOCs in inhibiting important fungal and pseudofungal pathogens of rosemary and suggested that volatile compounds can be considered the biological approach for controlling plant diseases caused by these agents.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

Alvarez, L. A., Perez-Sierra, A., Armengol, J. and Garcia-Jimenez, J. 2007. Characterization of *Phytophthora nicotiana* isolates causing collar and root of Lavender and Rosemary in Spain. Journal of Plant Pathology, 89(2): 261-264.

Ashrafi, S. J., Rastegar, M. F. and Saremi, H. 2010. Rosemary wilting disease and its management by soil solarization technique in Iran. African Journal of Biotechnology, 9(42): 7048-7057.

Cellini, A., Spinelli, F., Donati, I., Ryu, C. M. and Kloepper, J. W. 2021. Bacterial volatile compound-based tools for crop management and quality. Trends in Plant Science, 26: 968-983.

Chen, H., Xiao, X., Wang, J., Wu, L., Zheng, Z. and Yu, Z. 2008. Antagonistic effects of volatile generated by Bacillus subtilis on spore germination and hyphal growth of the plant pathogen, *Botrytis cinerea*. Biotechnology Letters, 30: 919-923.

Choinska, R., Piasecka-Jozwiak, K., Chablowska, B., Dumka, J. and Lukaszewicz, A. 2020. Biocontrol ability and volatile organic compounds production as a putative mode of action of yeast strains isolated from organic grapes and rye grains. Antonie van Leeuwenhoek, 113: 1135-1146.

Dilek, O. and Sezin, B. 2020. Phase diagrams for the aqueous solutions of carboxylic acid with dipropyl ether: experimental and correlated data. Iranian Journal of Chemistry and Chemical Engineering, 39(6): 173-183.

Dimock, M. B., Kennedy, G. G. and Williams, W. G. 1982. Toxicity studies of analogs of 2-tridecanone, a naturally occurring toxicant from a wild tomato. Journal of Chemical Ecology, 8: 837-842.

Hamidpour, R., Hamidpour, S. and Elias, G. 2017. *Rosmarinus Officinalis* (Rosemary): A novel therapeutic agent for antioxidant, antimicrobial, anticancer, antidiabetic, antidepressant, neuroprotective, anti-inflammatory, and anti-obesity treatment. Biomedical Journal of Scientific and Technical Research, 1: 4.

Holcomb, G. E. 1992. Web blight of rosemary caused by *Rhizoctonia solani*. Plant Disease, 76: 859-860.

Karsli, A. and Sahin, Y. S. 2021. The role of fungal volatile organic compounds (FVOCs) in biological control. Turkish Journal of Biological Control, 12(1): 79-92.

Kishimoto, K., Matsui, K., Ozawa, R. and Takabayashi, J. 2007. Volatile 1-octen-3-ol induces a defensive response in *Arabidopsis thaliana*. Journal of Plant Pathology, 73: 35-37.

Korpi, A., Jarnberg, J. and Pasanen, A. L. 2009. Microbial volatile organic compounds. Critical Review in Toxicology, 39(2): 139-193.

Kottb, M., Gigolashvili, T., Grosskinsky, T. and Piechulla, B. 2015. *Trichoderma* volatiles affecting *Arabidopsis*: from inhibition to protection against phytopathogenic fungi. Frontiers in Microbiology, 6: 995.

Macias-Rubalcava, M. L., Sanchez-Fernandez, R. E., Roque-Flore, G., Lappe-Oliveras, S. and Medina-Romero, Y. M. 2018. Volatile organic compounds from *Hypoxylon anthochroum* endophytic strains as postharvest mycofumigation alternative for cherry tomatoes. Food Microbiology, 76: 363-373.

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

McKinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Journal of Agricultural Research, 26: 195-217.

Medina-Romero, Y. M., Roque-Flores, G. and Macias-Rubalcava, M. L. 2017. Volatile organic compounds from endophytic fungi as innovative postharvest control of *Fusarium oxysporum* in cherry tomato fruits. Applied Microbiology and Biotechnology, 101: 8209-8222.

Mirabolfathi, M. and Ershad, D. 1993. Isolation of *Phytophthora* species from root, crown and stem of ornamental plants. Iranian Journal of Plant Pathology, 29: 62-63.

Morath, S. U., Hung, R. and Bennett, J. W. 2012. Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. Fungal Biology Reviews, 26: 73-83.

Moreira, B. C., Prates Junior, P., Jordão, T. C., Silva, M. C. S., Ribeiro, A. P. F., Stürmer, S. L., Salomão, L. C. C., Otoni, W. C. and Kasuya, M. C. M. 2019. Effect of inoculation of pineapple plantlets with arbuscular mycorrhizal fungi obtained from different inoculum sources multiplied by the on-farm method. Revista Brasileira de Ciencia do Solo, 43 (1):1-12.

Motekoa, P. and Vytrasova, J. 2011. Comparison of methods for isolating fungal DNA. Czech Journal of Food Sciences, 29: 76-85.

Oka, K., Ishihara, A., Sakaguchi, N., Nishini, S., Parada, R. Y., Nakagiri, A. and Otani, H. 2015. Antifungal activity of volatile compounds produced by an edible mushroom *Hypsizgus marmoreus* against phytopathogenic fungi. Journal of Phytopathology, 163: 987-996.

Ramesh, N. K., Naeimi, S., Rezaee, S. and Fotouhifar, K. B. 2020. Biological control of rice Bakanae disease caused by *Fusarium fujikuroi* using some endophytic fungi, 87(2): 282-296.

Royse, D. J, and Wilkinson, V. 2015. Care and handling of cultures of the cultivated mushroom (*Agaricus bisporus*). Plant Pathology Journal, 12(1): 1-7.

Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Wei, H. X., Pare, P. W. and Kloepper, J. W. 2003. Bacterial volatiles promote growth in *Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America, 100: 4927-4932.

Santos, P. M., Sanchez, M. N., Crisolino Pozas, A. P., Pavon, J. L. and Cordero, B. M. 2017. Determination of ketones and ethyl acetate- a preliminary study for the discrimination of patients with lung cancer. Analytical and Bioanalytical Chemistry, 409: 5689-5696.

Sidorova, D. E., Plyuta, V. A., Padiy, D. A., Kupriyanova, E. V., Roshina, N. V., Koksharova, O. A. and Khmel, I. A. 2022. The effect of volatile organic compounds on different organisms: Agrobacteria, plants and insects. Microorganisms, 10: 69.

Splivallo, R., Novero, M., Bertea, C. M., Bossi, S. and Bonfante, P. 2007. Truffle volatiles inhibit growth and induce an oxidant burst in *Arabidopsis thaliana*. New Phytologist, 175: 417-424.

Verhoeven, J. J., Jansen, C. C. and Roenhorst, J. W. 2008. First report of pospiviroids infecting ornamentals in the Netherlands. Plant Pathology, 57(2): 399-399.

Veselova, M. A., Plyuta, V. A. and Khmel, I. A. 2019. Volatile compounds of bacterial origin: Structure, biosynthesis and biological activity. Microbiology, 88: 261-274.

Vorhoeven, J. Th. J., Jansen, C. C. C., Roenhorst, J. W., Steyer, S., Schwind, N. and Wassenegger, M. 2008. First Report of *Solanum jasminoides* infected by *Citrus exocortis* viroid in Germany and the Netherlands and Tomato apical stunt viroid in Belgium and Germany. Plant Disease, 92(6):973-973.

Wang, X., Huang, M., Peng, Y., Yang, W. and Shi, J. 2022. Antifungal activity of 1-octen-3-ol against Monilinia fructicola and its ability in enhancing disease resistance of peach fruit. Food control, doi: 10.1016/j.foodcont. 2021.108804.

White, T. J., Bruns, T., Lee, S. J. W. T. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

for phylogenetics. PCR Protocols: A Guide to Methods and Applications, 18(1): 315-322.

Wnuk, S., Kinastowski, S. and Kaminski, E. 1983. Synthesis and analysis of 1-octen-3-ol, the main flavor component of mushrooms. Food/Nahrung, 27(5): 479-486. DOI: doi.org/10.1002/food.19830270523.

Xiong, Ch., Li, Q., Li, S., Chen, C., Chen, Z. and Huang, W. 2017. In vitro antimicrobial activities and mechanism of 1-octen-3-ol against food-related bacteria and pathogenic fungi. Journal of Oleo Science, DOI: doi. 10.5650/jos. ess16196.

Zabin, I. and Bloch, K. 1950. The formation of ketone bodies from isovaleric acid. Journal of Biological Chemistry, 185(1): 117-129.

Zheng, M., Shi, J., Shi, J., Wang, Q. and Li, Y. 2013. Antimicrobial effects of volatile produced by two antagonistic *Bacillus* strains on the anthracnose pathogen in postharvest mangos. Biological Control, 65: 200-206.

[DOR: 20.1001.1.22519041.2022.11.4.3.0]

اثر تعدادی از ترکیبات آلی فرّار (VOCs) در کنترل زیستی بیمارگرهای قارچی و شبه‌قارچی جدا شده از رزماری *Rosmarinus officinalis*

کوثر شیرازی[1]، سمانه باقری[2*] و نفیسه مهدی‌نژاد[2]

۱- گروه گیاه‌پزشکی، دانشکده علوم کشاورزی، دانشگاه زابل، زابل، ایران.
۲- گروه اصلاح نباتات و بیوتکنولوژی، دانشکده علوم کشاورزی، دانشگاه زابل، زابل، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: Samanehbagheri6556@gmail.com
دریافت: ۱۵ فروردین ۱۴۰۱؛ پذیرش: ۲۹ آبان ۱۴۰۱

چکیده: بیماری‌های قارچی، هر ساله منجر به کاهش حجم زیادی از تولید گیاهان رزماری می‌شود. ترکیبات آلی فرّار (VOCs) می‌توانند به‌عنوان یک آفتکش زیستی ارزشمند در مقابل قارچ‌ها استفاده شوند. با این‌حال، اثر این ترکیبات بر قارچ‌های بیمارگر گیاه رزماری به میزان کمی مطالعه شده است. در این تحقیق، تعداد دو قارچ و دو شبه قارچ بیمارگر جدا شده از گیاهان رزماری (*Rhizoctonia solani*، *Fusarium oxysporum*، *Phytophthora infestans* و *Phytophthora citrophthora*) شناسایی شد و اثر ممانعتی ترکیبات آلی فرّار شامل ایزو والریک اسید، ۱-اوکتن-۳-ال و ۳-اکتانون بر میزان رشد و وقوع بیماری عوامل جدا شده در شرایط آزمایشگاه و گلخانه مورد بررسی قرار گرفت. عملکرد ترکیبات آلی فرّار بر شاخص‌های رشد گیاهان آلوده نیز مطالعه شد. ۱-اوکتن-۳-ال بیشترین درصد کنترل بیماری و اثر بازدارندگی بر رشد میسلیوم‌های قارچی را در غلظت ۵ میلی‌گرم/لیتر نشان داد. ایزو والریک اسید نیز میزان رشد عوامل بیمارگر قارچی و بروز بیماری را در غلظت ۱۰ میلی گرم/لیتر به‌ترتیب تا ۹۴/۷۴% و ۸۷/۲۳% کاهش داد. با این‌حال، ۳-اکتانون اثر بخشی معناداری بر درصد کنترل بیماری و بازدارندگی رشد میسلیوم‌ها نداشت. به‌طور مشابه، ۱-اوکتن-۳-ال میزان رشد گیاهان رزماری آلوده به قارچ‌ها را به بالاترین میزان افزایش داد ولی ۳-اکتانون افزایش معنی‌داری در شاخص‌های رشد گیاهان رزماری آلوده نشان نداد. داده‌های به‌دست آمده نشان داد که اثرات مختلف ترکیبات آلی فرّار بر قارچ‌ها و شبه قارچ-های بیماری‌زا به ساختارهای شیمیایی و مکانیسم عمل متفاوت آن‌ها و گونه‌های قارچی مورد مطالعه وابسته است.

واژگان کلیدی: اثر بازدارنده، قارچ‌های بیماری‌زا، رزماری، ترکیبات آلی فرّار

[DOR: 20.1001.1.22519041.2022.11.4.3.0]