

EVALUATION THE EFFECT OF SATUREJA HORTENSIS ESSENTIAL OIL ON MUSHROOM PATHOGENS: *LECANICILLIUM FUNGICOLA* AND *MYCOGONE PERNICIOSA*

Mahdi Behnamian, Zivar Najafi, Mahdi Davari
University of Mohaghegh Ardabili, Ardabil, Iran

Abstract

L. fungicola and *M. perniciosa* are two common fungal diseases of the cultivated mushrooms. In this study, *S. hortensis* oil was used to evaluate its effectiveness against *L. fungicola* and *M. perniciosa*. Pure cultures of the *L. fungicola*, *M. perniciosa*, *A. bisporus* and *P. eryngii* were initially grown on PDA. Antifungal activity was tested by mycelium radial growth inhibition method on PDA in 90 mm Petri dishes supplemented with 100 to 2000 $\mu\text{l L}^{-1}$ of *S. hortensis* oil. The cultures were incubated at 25°C in the dark. This experiment was conducted as completely randomized design with three replications. Probit analysis used for IC_{50} value calculation. The IC_{50} of investigated oil against *L. fungicola* and *M. perniciosa* were 476.8 and 109 $\mu\text{l L}^{-1}$, respectively. These amounts for *A. bisporus* and *P. eryngii* were 91.1 and 386.2 $\mu\text{l L}^{-1}$, respectively. The results revealed that the tested essential oil had antifungal activity. Summer savory oil with SI = 0.28 can be a selective oil with potential use in king oyster mushroom production in order to controlling *M. perniciosa*.

Key words: *Agaricus bisporus*, Antifungal activity, Essential oil, *Pleurotus eryngii*, Summer savory

1. INTRODUCTION

Lecanicillium fungicola, causal agent of dry bubble disease, and *Mycogone perniciosa*, causal agent of wet bubble disease are the most common and serious fungal diseases of button and oyster mushrooms in the world, and if they are left uncontrolled, these pathogens can destroy crop (Fletcher & Gaze 2008). The impact of these mycopathogens, especially *L. fungicola* is expected to increase significantly in the near future. Mushroom mycelium can be affected by most of chemicals. Therefore, few fungicides can be used for the control of this pathogen. On the other hand, these chemicals are becoming ineffective due to the development of fungicide resistance in the pathogen (Gea et al. 2003). In addition, the use of chemicals in cultivation leaves unwanted residues (Sokovic et al. 2009), which are harmful to the environment and human health.

Nowadays, sporgon is the only effective fungicide without diverse effect on mushroom yield. However, sensitivity of *L. fungicola* to this fungicide has decreased (Gea et al. 2005; Berendsen et al. 2010). Therefore, development of alternative control is needed. A large number of herb materials contain essential oils with extensive bioactivities. Essential oils are natural products composed of several volatile compounds (Sangwan et al. 2001; Berger 2007). In fact, these compounds are the products obtained from plant raw material by hydrodistillation, steam distillation, etc. In nature, essential oils play very important roles in plant defense (Harborne 1993).

Satureja hortensis is belong to Lamiaceae family. Antifungal activity of *S. hortensis* oil on *Saccharomyces cerevisiae* (Mihajilov-Krstev et al. 2010), *L. fungicola* (Mehrpour et al. 2016), *M. perniciosa* (Glamoclija et al. 2009), *Fusarium oxysporum* f. sp. *phaseoli*, *Rhizoctonia solani*, *Alternaria solani*, *Macrophomina phaseoli*, *Botrytis cinerea* and *Aspergillus parasiticus* (Özcan & Boyraz 2000) and *A. xavus* (Omidbeygi et al. 2007) is shown previously, but the effect of this oil on edible mushroom is not well documented. Therefore, due to the increasing demand for natural ingredients as a healthy alternative to synthetic chemicals, this work was designed to evaluate the effect of *S. hortensis* essential oils on *L. fungicola*, *M. perniciosa*, *A. bisporus* and *P. eryngii*.

2. MATERIALS AND METHODS

2.1. Plant material preparation and essential oil extraction

Fresh summer savory (*Satureja hortensis*) was purchased from the local market in Ardabil province, Iran and its aerial parts (leaf and top of stem) dried in an oven at 38°C, and ground to fine powder using an electrical mill. Then, 50 g of powdered samples were added to 500 ml distilled water and essential oil extraction was performed by Clevenger apparatus. The collected essential oil was stored in dark glass bottles at 4°C until use. Analysis of essential oil composition carried out by gas chromatography-mass spectrometry (GC-MS).

2.2. Fungal strains preparation

Pure cultures of *Lecanicillium fungicola*, *Mycogone pernicioso*, *Agaricus bisporus* cv. Sylvan 737 and *Pleurotus eryngii* were obtained from the mushroom lab (Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran). The mycopathogens were kept on PDA medium and mushroom cultures were maintained on MEA medium. The cultures were stored at 4°C and sub-cultured once a month (Glamoclija et al. 2009).

2.3. Antifungal activity analyses

Antifungal activity was tested by mycelium radial growth inhibition method on PDA in 90 mm Petri dishes supplemented with 0, 100, 500, 1000, 1500 and 2000 µl L⁻¹ of *S. hortensis* essential oil. The cultures were incubated at 25±1°C in the dark. In order to mycelial growth inhibition (MGI) calculation, colony diameter was measured until the fungal growth in control treatment had completely covered the Petri dishes. Percentage of MGI was calculated from the formula: $MGI = [(D_C - D_T) / D_C] \times 100$ (Marei et al. 2012), where D_C and D_T are average of colony diameters in control and treatment, respectively. This experiment was conducted as completely randomized design with three replications. Data analysis was performed by SPSS 19 software. The minimal inhibitory concentration (MIC) was determined by the agar slant method (Glamoclija et al. 2009). Probit analysis used for the half maximal inhibitory concentration (IC₅₀) value calculation and to find efficient concentration able to reduce 50% of mycelial growth. The selectivity index (SI) of the used essential oil was obtained by the following formula:

$$SI = \frac{IC_{50} \text{ of mycopathogen}}{IC_{50} \text{ of mushroom}}$$

3. RESULTS AND DISCUSSION

The result of chemical analysis of *S. hortensis* essential oil is given in Table 1. Forty constituents were identified in summer savory essential oil. The main components were thymol (73.70%), o-cymene (7.64%) and γ-terpinene (5.11%). In previous studies, γ-terpinene (40.9%) and carvacrol (39.3%) (Gora & Lis 1996) and carvacrol (24.50%), thymol (23.12%) and γ-terpinene (20.72%) (Omidbeygi et al. 2007) were detected as main components of *S. hortensis*. In other literatures, carvacrol with 43.9 to 67% was reported as major component of *S. hortensis* (Mumivand et al. 2011; Rezvanpanah et al. 2011; Mihajilov-Krstev et al. 2010). However, in a research it found that thymol (45.9%), γ-terpinene (20.6%) and p-cymene (19%) were main compounds of *S. hortensis* (Mahboubi & Kazempour 2011). Several factors such as variations in genetic and environmental conditions, harvest period and extraction method affect essential oils composition (Gong et al. 2014; Oumzil et al. 2002; Sartorelli et al. 2007). For example, it is reported that carvacrol and γ-terpinene contents in *S. hortensis* oil depend on season and ontogenetic stage, in which carvacrol ranged from 55.3-59.5% in the summer to 55.5-57.9% in the autumn. In addition, γ-terpinene content varied from 30.1-33.8% in the summer to 32.1-33.5% in the autumn. (Pfefferkorn et al. 2008).

Table 1. Chemical composition of summer Savory (*Satureja hortensis*) essential oil

Compound	%
Furan, 2,5-diethyltetrahydro-	0.12
α -Phellandrene	0.11
α -Pinene	0.29
Camphene	0.10
Nonane, 3-methyl-	0.11
β -Myrcene	0.27
Decane	1.28
α -Terpinene	0.69
o-Cymene	7.64
1,8-Cineole	0.79
γ -Terpinene	5.11
Benzenamine, N,N-dimethyl- (CAS)	0.21
α -Terpinolene	0.15
β -Thujone	1.08
Thujone	0.44
Camphor	0.08
Camphor	0.25
Borneoll	0.88
3-Cyclohexen-1-ol, 4-methyl-1-(1	0.73
Benzenemethanol, 4-(1-methylethy.	0.22
Dodecane	0.59
Benzene, 1-methyl-3-(1-methyleth.	0.13
2-Cyclohexen-1-one, 2-methyl-5-(	0.21
Phenol, 2-methyl-5-(1-methylethy.	0.12
Thymol	73.70
Phenol, 2-methyl-5-(1-methylethy.	0.40
Phenol, 5-methyl-2-(1-methylethy.	0.13
Tetradecane	0.17
trans-Caryophyllene	1.44
(+)-Aromadendrene	0.30
α -Humulene	0.14
α -Amorphene	0.11
Ledene	0.35
β -Bisabolene	0.43
α -Amorphene	0.09
δ -Cadinene	0.22
cis- α -Bisabolene	0.09
1H-Cycloprop[e]azulen-7-ol, deca.	0.40
(-)-Caryophyllene oxide	0.28
γ -Gurjunene	0.19

In this research, we used 100 to 2000 $\mu\text{L L}^{-1}$ of *S. hortensis* oil. Variance analysis indicated significant differences between concentrations ($P \leq 0.01$). The results showed that mycelial growth of investigated fungi decreased with increasing *S. hortensis* oil concentration (Figures 1 and 2), as the oil blocked the mycelial growth of *L. fungicola*, *M. perniciosa*, *A. bisporus* and *P. eryngii* at 1000, 1500, 1000 and 2000 $\mu\text{L L}^{-1}$, respectively.

The MIC values for *L. fungicola* and *A. bisporus* were 500-1000 $\mu\text{L L}^{-1}$, while *S. hortensis* oil showed MIC at 1000 and 1500 $\mu\text{L L}^{-1}$ for *M. perniciosa* and *P. eryngii*, respectively. In this research, the highest level of toxicity was obtained for *A. bisporus* and *M. perniciosa* with the IC_{50} of 91.1 and 109 $\mu\text{L L}^{-1}$ and the lowest activity was observed for *L. fungicola* with IC_{50} of 476.8 $\mu\text{L L}^{-1}$ (Tables 2 and 3).

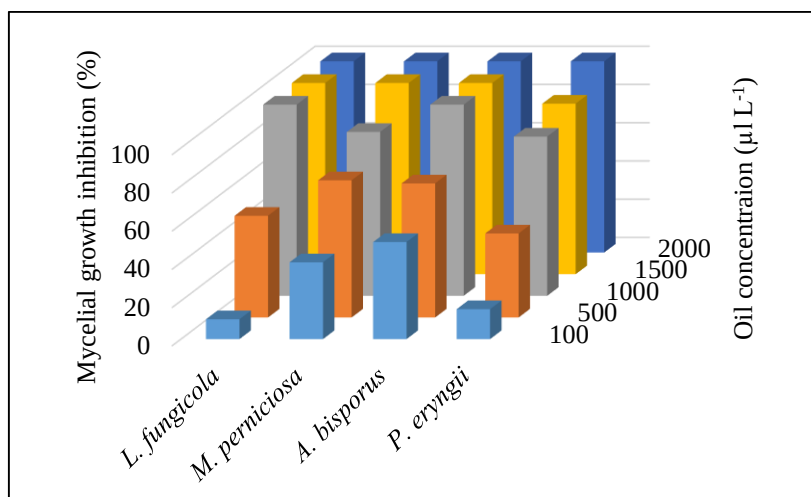


Figure 1. *L. fungicola*, *M. perniciosa*, *A. bisporus* and *P. eryngii* percentage of growth inhibition by *S. hortensis* oil.

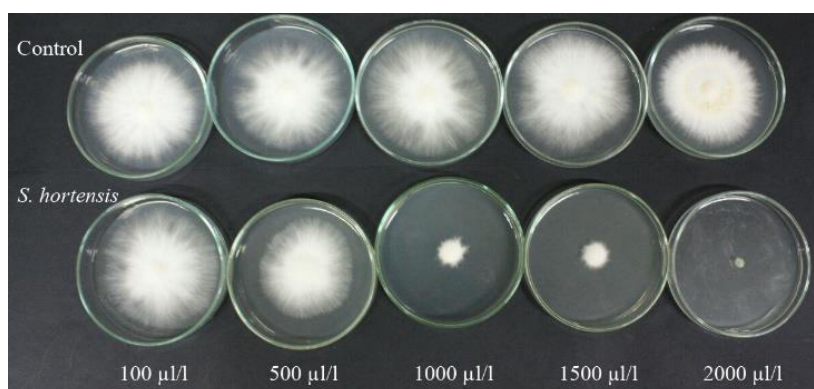


Figure 2. *P. eryngii* growth inhibition by summer savory oil.

Table 2. IC₅₀ values of *S. hortensis* essential oil for mycopathogens

	<i>L. fungicola</i>				<i>M. perniciosa</i>			
	IC ₅₀	95% confidence limits		Slope±SE	IC ₅₀	95% confidence limits		Slope±SE
		Lower	Upper			Lower	Upper	
<i>S. hortensis</i>	476.8	441.9	515.1	3.60±0.37	109	13.8	344.1	1.14±0.09

Table 3. IC₅₀ values of *S. hortensis* essential oil for mushrooms

	<i>A. bisporus</i>				<i>P. eryngii</i>			
	IC ₅₀	95% confidence limits		Slope±SE	IC ₅₀	95% confidence limits		Slope±SE
		Lower	Upper			Lower	Upper	
<i>S. hortensis</i>	91.1	63.2	132.2	0.78±0.07	386.2	114.3	720.8	2.18±0.17

Table 4. *In vitro* selectivity of *S. hortensis* on *L. fungicola*, *M. perniciosa*, *A. bisporus* and *P. eryngii*

<i>S. hortensis</i>	Selectivity index of <i>L. fungicola</i>		Selectivity index of <i>M. perniciosa</i>	
	<i>A. bisporus</i>	<i>P. eryngii</i>	<i>A. bisporus</i>	<i>P. eryngii</i>
	5.23	1.24	1.20	0.28

The selectivity of *S. hortensis* essential oil is presented in Table 4. Our results revealed that *S. hortensis* essential oil had antifungal activity on investigated fungi, but the effect of oil on *A. bisporus* was stronger. In present study, investigated oil with 0.28 selectivity index showed less toxicity for *P. eryngii* than *M. perniciosa* mycelium. Therefore, *S. hortensis* essential oil can be a promising selective oil to apply in *P. eryngii* production in order to prevent and cure of *M. perniciosa*.

Different responses of investigated fungi to the tested essential oil can be attributed to their different modes of action or that the metabolism of fungi able to better overcome inhibitory effects of phenolic compounds or adapt to them (Sokovic et al. 2009; Arslan & Dervis 2010).

In previous study, it is reported that *S. hortensis* had more inhibitory effect on *L. fungicola* than *A. bisporus*. The high antifungal activity of this oil was assigned to presence of carvacrol, a phenolic compound (38%) as the main component (Mehrparvar et al. 2016). It is suggested that button mushroom by secreting ligninolytic enzymes especially laccases is able to detoxify toxic compounds (Morozova et al. 2007), but our results confirmed that high concentration of thymol (73%) in the tested oil is more toxic to button mushroom. Phenolic compounds by interfering with cell wall enzymes (Adams et al. 1996) and disrupting the fungal membrane (Sokovic et al. 2009) leads to cell death. However, it seems that *P. eryngii* by secreting laccase, manganese peroxidase and versatile peroxidases, oxidize Mn^{2+} , phenolic and nonphenolic aromatic compounds. This leads to release the components with phenolic essence that consume by mushroom (Camarero et al. 1999). Therefore, king oyster mushroom tolerate high concentration of *S. hortensis* oil better than button mushroom. However, further studies are necessary to evaluate the effect of tested essential oil on fruit body and their probably toxic effects on consumers.

4. CONCLUSIONS

S. hortensis essential oil exhibited antifungal activity on the investigated fungi.

The main component of tested oil was thymol (73%). Therefore, antifungal activity of summer savory oil could be assigned to this phenolic compound.

The inhibitory effect of *S. hortensis* essential oil on *A. bisporus* was greater than the other test fungi.

We found that summer savory oil with selectivity index (SI) lower than one (0.28) can be a selective oil with potential use in king oyster mushroom production in order to controlling *M. perniciosa*. However, further studies are required to develop strategies for practical application.

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