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Antifungal activity of *Satureja hortensis* L. essential oil against *Alternaria citri*

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ABSTRACT

Satureja hortensis is belonging to Lamiaceae family. Satureja essential oil could be obtained from steam distillation of leaves and leafy branches, is used in medicine and food industry. This study was aimed to evaluate interactions of drug and antifungal activity of savory essential oil on Alternaria citri. Leaves and flowers of S. hortensis were subjected to hydro-distillation. The analyses of the volatile compounds were carried out on gas chromatograph. Then, Alternaria citri strains were isolated and cultured in PDA medium culture. The PDA mediums were prepared with different concentration of savory essential oil (0, 100, 200, 300, 400, 600, 800 and 1000 ppm) after purification of fungi and followed up by cutting of Alternaria citri raised as well attached on PDA medium with a cork borer into small pieces and then were incubated at 25°C temperature in a incubator. The fungal growth in mediums added with different concentration of Satureja hortensis essential oil were evaluated after 24, 48, 72 hours to 20 days. The obtained data were analyzed by using SPSS software and the means were compared with Duncan's test. The obtained results indicated that savory essential oil from 400ppm to higher concentrations demonstrate inhibitory effects on fungal growth and spores production. In addition, lower concentrations from 400pm reduced speed of fungi growth, but not ceased.

Key words: *Satureja hortensis*, *Alternaria citri*, PDA medium.

INTRODUCTION

Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that they have developed to antibiotics [7]. Essential oils have been used in pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology and food preservation.

Satureja hortensis L. belongs to Lamiaceae family (Labiatae; minths) and is well known in Iranian traditional medicine. The volatile oil of this plant contains tannins, resins, and mucilage. Because of pharmacologic properties and use in traditional medicine, this species is considered in different countries. The most abundant chemical components in *S. hortensis* are carvacrol and Thymol (monoterpens). Other indicator compounds in this genus are cyclic monoterpene component such as ΄Y- Terpinene, Limonene, 1, 8-cineol and camphor. Essential oil (specific weight between 895 and 913) is solved in ether, chloroform, and ethanol. According to Sahin et al., [13] and Sokovic *et al*., [15] the essential oils of *Satureja* have antimicrobial, activities due to their high phenolic contents. The antifungal property of the oil has been recorded in different reports [2,4]. Since pesticide residues can cause

problems for humans and the environment, and also for the production of organic products other methods must be used. Due to carcinogenic risks of pesticides such as Benomyl, These compounds are used less. Other factors that would encourage us to use non-chemical methods are fungal strains resistant to fungicides.

The genus *Alternaria* is characteristic by the large conidia. They are produced on chains, Olive green or black. This characteristic makes it possible to easily identify the fungi of the genus. These fungi create olive green or black aerial hyphae on the culture. This species is characteristic by the short, single and ramified conidia. Conidi are single or short chains containing two to seven conidia are seen on the conidiophores. The maximum width of spore is from 8-60 micron (the mean is 24 micron), and the length of spore is from 16-37 to 35-110 micron (the mean is 37 to 69 micron). The number of transverse septa is 8. The length of Longitudinal septa is 8-60 micron (the mean is 42 micron).The beak length is from 6 to 42 micron (the mean is 17 micron). In each conidi, may occur up to 12 cells [12].

Alternaria sp has a wide host range. It is scattered widely in nature as a saprophyte and parasite on herbal products and plants. The symptoms on the leaves are brown and circular lesions with sharply defined margin and yellow layers surrounded. Inside the lesions are many dark concentric circles like a target. With the development of disease, the lesions can develop and merge into many irregular areas. *Alternaria* is a genus of ascomycete fungi. *Alternaria* species are known as major plant pathogens and causes leaf spot disease, black rot and tail rot in plants. It is also the agent of citrus brown spot. They are considered the black fungi. There are 299 species in the genus. They are also common allergens in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes lead to asthma. They are normal agents of decay and decomposition. The spores are airborne and found in the soil and water, as well as indoors and on objects. At least 20% of agricultural spoilage is caused by *Alternaria* species; most severe losses may reach up to 80% of yield, though. Many human health disorders can be caused by these fungi, which grow on skin and mucous membranes, including on the eyeballs and within the respiratory tract [12].

Brown spot is a fungal disease caused by Alternaria. This disease observed in Israel was thought to be similar to 'brown spot of Emperor mandarins' which was first reported in Australia in 1966 and to *'Alternaria* brown spot' of Dancy tangerines, and of Minneola and Orlando tangelos which was then reported in Florida (US) in 1976 [18]. Infected fruit show sunken, dark brown spots (quality is reduced) and many of them drop prematurely. Leaves present brown necrotic areas, and in severe cases apices of young shoots can be completely defoliated. *Alternaria citri* can activate polygalacturonase enzymes that cause degradation of cell wall. This fungus cause spots on the fruit and reduce the market value of the product. However, this fungus can cause fruit drop and reduced yield. Fruit usually show hypersensitivity to the disease in mid-summer. It is difficult to control the disease in humid areas because moisture provides favorable conditions for fungal growth. As regards Copper fungicides cause phytotoxicity, it is a little difficult to control fungi.

The aim of this work is study of antifungal activity of *Satureja hortensis* against *Alternaria citri* in vitro conditions.

MATERIALS AND METHODS

Plant material

The aerial part of wild growing populations of *Satureja hortensis* L. were collected from *Agricultural garden of* Kashan. Leaves and flowers of species were used for the analysis of essential oil composition. 100 (g) of dried plant material were subjected to hydro-distillation for 3 hours using a Clevenger-type apparatus to produce oils. The essential oils obtained were dried over anhydrous sodium sulphate and stored at low temperature (4°C).

Gas chromatography-mass spectrometry analysis (GC-MS

The analyses of the volatile compounds were carried out on gas chromatograph (Shinadzu-9A). DB-5 column (30m x 0.25 mm, 0.25 µm film thickness) was directly coupled to the mass spectrometer. The carrier gas was helium (22.7 CmS^{-1}). Ionization energy was 70 ev. Injector and detector temperatures were 230°C.The column temperature was kept at 60°C for 5 min and programmed to 220°C at a rate of 3°C/min.

Identification of components of the volatile oils was based on retention indices and computer matching with the Wiley 275.L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adams, 2004).

Antifungal activity

Antifungal activity tests were carried out by disc diffusion method (Murray et al., 1995). The tested fungus was grown on Potato Dextrose Agar (PDA) medium for sporulation on Petri dishes for 7-10 days at 28±2°C. Then PDA sterile liquid medium containing concentrations of 0, 100, 200, 300, 400, 600 and 800 ppm essential oils were prepared. Then a piece of fungus grown in a medium was placed in the center of culture medium containing various concentrations of essential oil. The inoculated plates were incubated at 37±2 °C. After 10 days, antifungal activity was evaluated by measuring the zone of inhibition (mm) against the test fungus. All treatments consisted of three replicates.

The obtained data were analyzed by using SPSS software and the means were compared with Duncan's test.

RESULTS AND DISCUSSION

The results of in vitro assays showed that the essential oil of *S. hortensis* had a strong fungicidal effect against *Alternaria citri.* As shown in Figure of 1-4**,** 400 ppm and Higher concentrations of the essential oil inhibited fungal growth. Concentrations of 100,200 and 300 ppm reduced the fungal growth but fungal growth did not stop. It is widely accepted that higher concentrations of 300 ppm of plant essential oils are effective (fig 1-4).

According to the environmental Harmful effects caused by the indiscriminate use of pesticides and chemicals, need to produce more organic products is essential. The results of present study show that, instead of using chemical pesticides and concern about pesticide residues in the crop, essential oil of *Satureja* can be used to control *Alternaria citri* fungi. With the consumption of essential oil of *Satureja*, in addition to disease control, quality and marketability of the product is much better. Effective concentration of 300 ppm is economical.

It is known that phenolic compounds such as γ-Terpinene, carvacrol and thymol a major fungicidal effect [11,14] Güllüce et al., [6] stressed that thymol (29.0%) and carvacrol (26.5%) were the main components of the *S. hortensis*. These compounds can be responsible for the antifungal activity of *S. hortensis* oil. According to Azaz et al. [2] carvacrol (42.1–59.2%) was the main component in the oils of *Satureja icarica*, *S. pilosa* and *S. boissieri* .

In conclusion, this study showed that *S. hortensis* oil has a strong antifungal activity against A. *citri*. So this essential oil can be used as a potential source of sustainable eco-friendly botanical fungicides to protect some stored food products from pathogen and saprophytic fungi.

Table 1. Phytochemical analysis of *Satureja hortensis*

No	Name of Compound	%Tot AR
1	Alpha – thujene	1.008
2	Alpha- pinene	1.480
3	Beta- pinene	0.588
4	myrcene	1.788
5	Alpha- phellandrene	0.287
6	Alpha-terpinene	4.498
7	p- cymene	10.082
8	limonene	0.755
9	Gamma-terpinene	36.498
10	thymol	0.718
11	carvacrol	41.239
12	e- caryophyllene	0.538
13	Beta-bisabolene	0.527

Table 2. Means of decay diameters (mm) which measured after 10 days applied with 100, 200 and 300 ppm concentrations of essential oil from *Satureja hortensis* and inoculated *Alternaria citri* under standard storage condition

treatment	Means of decay diameters (mm)
control	[a]5.9
100 ppm	[b]1.9
200 ppm	[c]1.06
300 ppm	[d]0.29

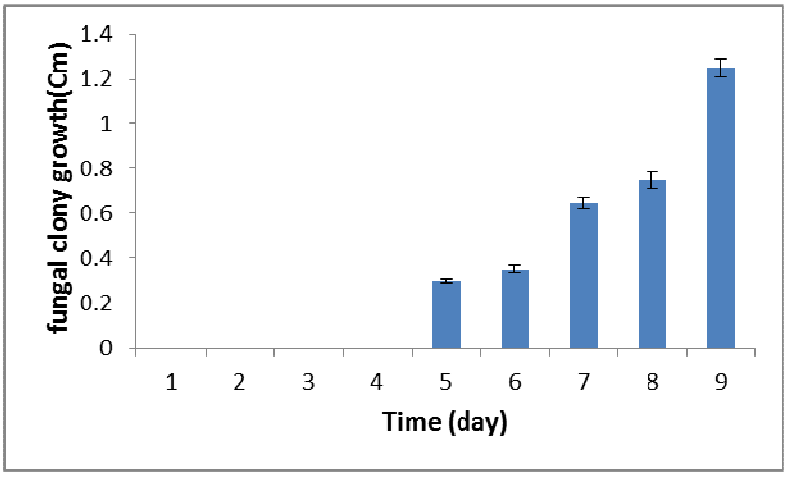

Fig 1. The growth rate of the *Alternaria* fungus at concentration of 300 ppm essential oil

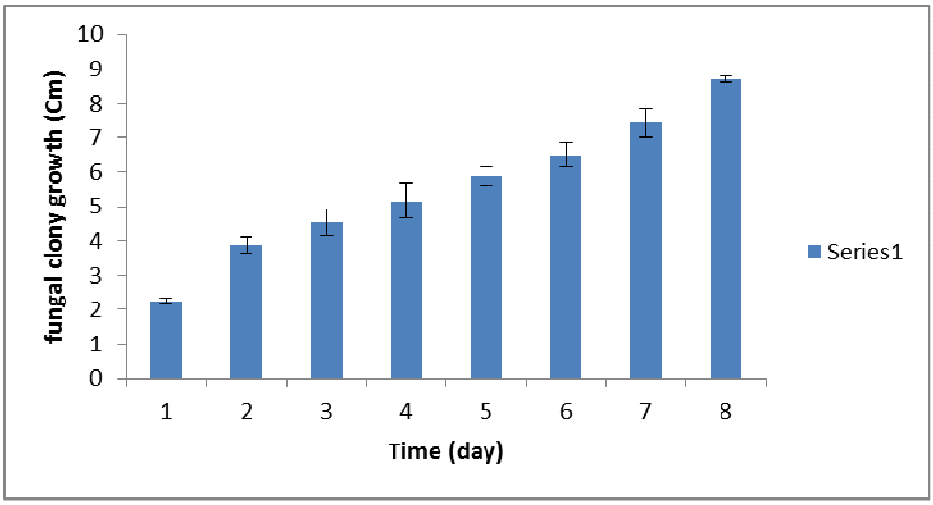

Fig 2. The growth rate of the *Alternaria* fungus at concentration of 200 ppm essential oil

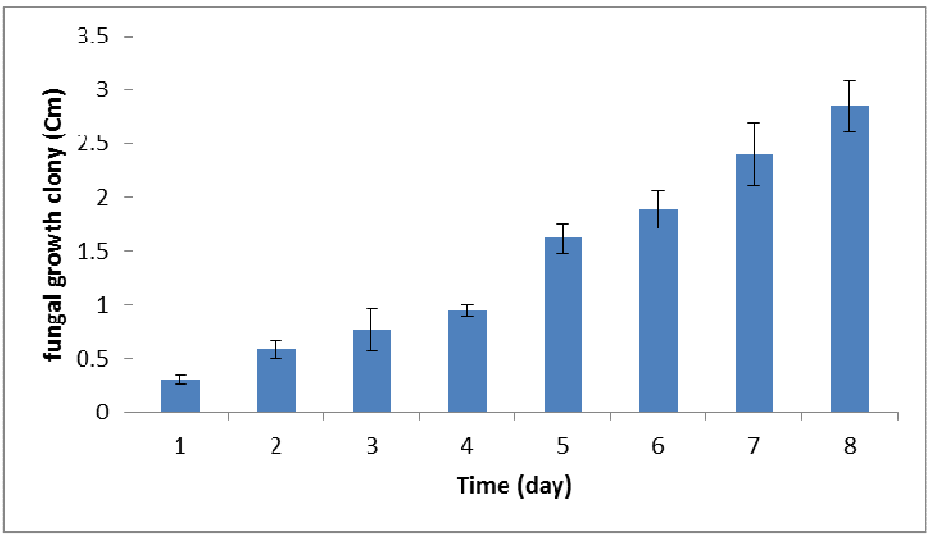

Fig 3. The growth rate of the *Alternaria* fungus at concentration of 100 ppm essential oil

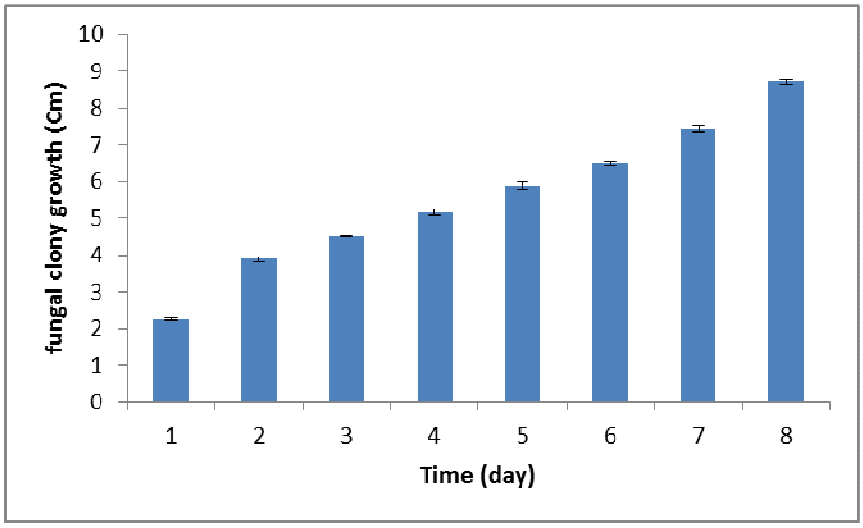

Fig 4. The growth rate of the *Alternaria* fungus in control samples

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