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Fumigant Activity of Essential Oils and Components of *Illicium verum* and *Schizonepeta tenuifolia* Against *Botrytis cinerea* and *Colletotrichum gloeosporioides*

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Abstract To develop a natural fungicide against *Botrytis cinerea* and *Colletotrichum gloeosporioides*, a total of 25 essential oils were tested for their fumigant activity against post-harvest pathogens. The vaporous phases of oils were treated to each fungus on potato dextrose agar medium in half-plate separated Petri plates at 10 μg per plate. The essential oil of *Illicium verum* strongly inhibited the mycelial growth of both *B. cinerea* and *C. gloeosporioides* by over 90%. On the other hand, the essential oil of *Schizonepeta tenuifolia* showed inhibitory activity against mycelial growth of only *B. cinerea* by over 90%. Gas chromatography-mass spectrometry and bioassay indicated *trans*-anethole in *I. verum* and menthone in *S. tenuifolia* as a major antifungal constituent. The essential oils of *I. verum* and *S. tenuifolia* and their major constituents could be used to manage post-harvest diseases caused by *B. cinerea* and *C. gloeosporioides*.

Keywords: Antifungal activity, *Illicium verum*, menthone, *Schizonepeta tenuifolia*, *trans*-anethole

Synthetic chemicals and fumigants have been widely used for the control of post-harvest diseases of fruits and vegetables. However, because of the development of new races of pathogens, possible carcinogenicity, teratogenicity, residual toxicity, and environmental pollution, the use of synthetic chemicals is restricted [10, 15]. This has encouraged many scientists to search for alternatives to synthetic pesticides such as biological controls using antagonistic microorganisms and safer chemicals [18, 24]. Plant extracts such as essential oils are generally regarded as more acceptable and less hazardous than synthetic compounds.

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Essential oils are plant volatiles containing monoterpenes, sesquiterpenes, and phenyl propanoids. Many essential oils and their constituents exhibited antimicrobial properties [3, 4, 8, 13, 16, 17, 22]. Although the pharmacological activities of many essential oils have been reported in the literature, their activity against phytopathogenic fungi has been relatively less investigated. Recently, scientists have frequently reported on the antifungal activity of various plant essential oils and their components [5, 9, 12, 19].

Gray-mold rot or *Botrytis* blight, caused by the widespread fungus *Botrytis cinerea*, affects most fruit and vegetable crops, as well as a large number of shrubs, trees, flowers, and weeds, both in the field and in storage. The fungus causes blossom blights, fruit rots, damping-off, bud rot, stem cankers or rots, leaf spots or blights, bulb rots, and tuber or root rots. *Botrytis* is also a problem of fruits and vegetables in cold storage and subsequent shipment. Almost all fresh fruits, vegetables, and bulbs could be attacked by *B. cinerea* in storage [1].

The genus *Colletotrichum* and its teleomorph *Glomerella* are major plant pathogens worldwide. *Colletotrichum* species cause anthracnose, a disease characterized by sunken necrotic lesions usually bounded by a red margin [7, 20]. *C. gloeosporioides*, *C. acutatum*, *C. coccodes*, and *C. fragariae* can infect singly or in combination fruits, flowers, leaves, petioles, stolons, and crowns [2].

Latent infections of fungal pathogens are especially difficult to control in harvested commodities, because the pathogens reside within the host tissue. Nonsystemic fungicides and biological control agents are ineffective in controlling such infections [21]. Essential oils may be useful in controlling latent infections. They can be used directly as commercial fumigants or as lead compounds. In this study, we investigated the fumigant activity of 25 essential oils against *B. cinerea* and *C. gloeosporioides* and

Table 1. Plant essential oils tested for antifungal activity against *Botrytis cinerea* and *Colletotrichum gloeosporioides* and their *in vitro* antifungal activities *via* fumigation[a].

Plant species	Family name	Part[b]	Yield (v/w, %)	% Growth inhibition[c]	
				Bc	Cg
Amomum cardamomum	Zingiberaceae	F	0.90	-	36
Asarum sieboldii	Aristolochiaceae	R	8.6	42	70
Carpesium abrotanoides	Compositae	F	4.2	-	-
Chamaecyparis obtuse	Cupressaceae	L	0.49	-	-
Chamaecyparis pisifera	Cupressaceae	L	0.50	26	-
Chenopodium ambrosioides	Chenopodiaceae	WP	0.44	-	-
Cinnamomum cassia	Lauraceae	B	0.33	-	-
Curcuma longa	Zingiberaceae	R	0.44	-	-
Curcuma zedoaria	Zingiberaceae	R	0.35	-	-
Illicium verum	Illiciaceae	F	0.90	100	93
Juniperus chinensis	Cupressaceae	L	0.23	-	-
Juniperus chinensis	Cupressaceae	WP	0.51	40	28
Juniperus chinensis var. *globosa*	Cupressaceae	L	0.31	-	-
Juniperus chinensis var. *sargentii*	Cupressaceae	L	0.51	-	-
Myristica fragrans	Myristicaceae	S	2.9	48	49
Nardostachytis chinensis	Valerianaceae	R	0.30	-	-
Paeonia moutan	Ranunculaceae	R	0.54	-	-
Pinus koraiensis	Pinaceae	L	0.33	-	-
Poncirus trifoliata	Rutaceae	F	0.37	-	-
Saussurea lappa	Compositae	R	0.15	-	-
Schisandra chinensis	Schizandraceae	F	0.70	-	-
Schizonepeta tenuifolia	Labiatae	WP	0.39	95	68
Styrax benzoin	Styracaceae	Re	0.36	-	-
Teucrium veronicoides	Labiatae	WP	0.27	-	-
Thuja orientalis cv. *compacts*	Cupressaceae	L	0.16	-	-

[a]Ten μg of each essential oil was added on a sterile paper disc in the middle of the half-plate.
[b]F, fruit; R, root; L, leaf; WP, whole plant; B, bark; S, seed; Re, resin; Bc, *Botrytis cinerea*; Cg, *Colletotrichum gloeosporioides*; -, no inhibition.
[c]% Growth inhibition=[(mean colony diameter of control sets - mean colony diameter of treatment sets)/mean colony diameter of control sets]×100.

identified major antifungal constituents in highly active essential oils.

The plant essential oils used in this study are listed in Table 1. Eighteen plant samples such as *Amomum cardamomum* (fruit), *Asarum sieboldii* (root), *Carpesium abrotanoides* (fruit), *Chenopodium ambrosiodes* (whole plant), *Cinnamomum cassia* (bark), *Curcuma longa* (root), *Curcuma zedoaria* (root), *Illicium verum* (fruit), *Juniperus chinensis* (wood), *Myristica fragrans* (seed), *Nardostachytis chinensis* (root), *Paeonia moutan* (root), *Poncirus trifoliata* Rafin (fruit), *Saussurea lappa* (root), *Schisandra chinensis* (fruit), *Schizonepeta tenuifolia* (whole plant), *Styrax benzoin* (resin), and *Teucrium veronicoides* (whole plant) were purchased at Kyungdong Medicinal Market, Seoul. The healthy and mature leaves of 7 plant species, *Chamaecyparis obtuse* (leaf), *Chamaecyparis pisifera* (leaf), *J. chinensis* (leaf), *J. chinensis* var. *globosa* (leaf), *J. chinensis* var. *sargentii* (leaf), *Pinus koraiensis* (leaf), and *Thuja orientalis* cv. *compacts* (leaf), were obtained from mature plants growing in the Hongneung Arboretum, Korea Forest Research Institute (Seoul, Korea) from October 2003 to August 2004 and air-dried. Plant samples were powdered in a blender, and diluted with 800 ml of distilled water in a 2-l flask and steam distilled (100°C). Yields of essential oils are given in Table 1.

Pulegone (purity 98%), *trans*-anethole (purity 99%), and menthone (purity 90%) were purchased from Aldrich (Milwaukee, WI, U.S.A.).

B. cinerea and *C. gloeosporioides,* which were isolated from infected tomato and red pepper tissues, respectively, were maintained and grown on potato dextrose agar (PDA; Becton and Dickinson Co., Sparks, MD, U.S.A.) medium. For the antifungal activity test, PDA was poured into commercially available half-plate separated Petri plates (90 mm×15 mm, SPL Life Science, Korea). The mycelium plugs from the margins of actively growing cultures on PDA were placed in the middle of the half-plate containing PDA medium, and a sterilized paper disc was placed on the other side. The essential oil was added onto the paper disc at 10 μg per plate as volatile compounds. The plate was sealed with parafilm immediately after adding each essential oil and incubated for 3 days at 25°C for *C. gloeosporioides*

and at 20°C for *B. cinerea*. Plates in three replicates were used for each treatment. The growth of fungi was measured from the edge of the agar inoculum plugs and compared with the mycelial growth on a control plate. Each experiment was performed in duplicate.

In order to determine the minimum inhibitory concentration (MIC) and 50% inhibitory concentration (IC_{50}) of the selected essential oils and their major constituents such as *trans*-anethole, menthone, and pulegone, they were diluted in acetone and then applied to assay plates at concentrations of 0.1, 0.5, 1, 5, and 10 μg per plate. For the control plate, 10 μl of acetone was added. Plates in three replicates were used for each treatment and the experiment was conducted twice.

Essential oils of *I. verum* and *S. tenuifolia* were analyzed by gas chromatography with mass spectrometry (GC-MS; GC-MS QP5050, Shimadzu, Kyoto, Japan). A capillary column DB5 (30 m×0.32 mm i.d., 0.25 μm film thickness; J&W Scientific Inc., Folsom, CA, U.S.A.) with cross-linked 5% phenylmethylsilicone was used. The oven temperature was isothermal at 40°C for 5 min, and then increased to 220°C at a rate of 5°C per min and held isothermal for 10 min. Injection and interface temperatures were 220°C and 200°C, respectively. Helium was the carrier gas at a flow rate of 2.2 ml/min. The essential oils were diluted in acetone at a concentration of 1 mg/ml. Components were identified on the basis of comparison of their relative retention time and mass spectra with those of standards. The percentage concentration of the oils was computed from the TIC peak areas by using the normalization method without correct factors. Quantitative results are the mean of data derived from duplicate GC-MS analyses.

In vitro antifungal activities of the vaporous phases of 25 essential oils against *B. cinerea* and *C. gloeosporioides* are summarized in Table 1. Among 25 oils, at a dose of 10 μg per plate (equal to 227 μg/l of air), 6 oils were active to *B. cinerea* and 6 oils to *C. gloeosporioides*. Five oils such as *A. sieboldii* (root), *I. verum* (fruit), *J. chinensis* (whole plant), *M. fragrans* (stem), and *S. tenuifolia* (whole plant) showed inhibitory activity against mycelial growth of both pathogenic fungi. *I. verum* oil showed the most potent fumigant activity against both *B. cinerea* and *C. gloeosporioides* with inhibition rates of 100% and 93%, respectively. On the other hand, *S. tenuifolia* oil inhibited strongly the mycelial growth of *B. cinerea* with an inhibition rate of 95%.

The essential oil obtained from *I. verum* fruits is commonly called "star anise". *I. verum* is distributed in the tropical and subtropical zones of Asia, North America, and the Atlantic. The dried fruits are known to contain 5% to 8% of essential oil. *I. verum* oil is known to have insecticidal activity against Japanese termite (*Reticulitermes speratus*) [14]. Although the ethyl alcohol and aqueous extracts of *I. verum* were reported to be active to some microorganisms, there has not been any report on the antifungal activity of star anise against phytopathogenic fungi. To our knowledge, this is the first report on the fumigant activity of the essential oil against phytopathogenic fungi.

Table 2. Chemical compositions of the essential oils of *Illicium verum* and *Schizonepeta tenuifolia*.

Essential oil	Component	Percentage (% peak area)
I. verum	*trans*-Anethole	87.4
	Limonene	6.0
	Estragole	1.5
	Anisyl acetone	1.2
	Feniculin	0.9
	Linalool	0.8
	A-*trans*-Bergamotene	0.7
	Total identified	98.5
S. tenuifolia	Menthone	68.1
	Pulegone	24.3
	Limonene	1.9
	Caryophyllene oxide	1.3
	Total identified	95.6

The dried aerial part of *S. tenuifolia* is called "Jingjie". *S. tenuifolia* is an herb, whose aerial part consists of spikes and stems. *S. tenuifolia* has a pungent taste and is used as a traditional Chinese medicine. *S. tenuifolia* contains approximately 0.5% to 1.8% of volatile oils [23]. Reported pharmacological effects of *S. tenuifolia* include relieving body aches, and antibiotic, anti-flammatory, and antipyretic effects [25]. In addition, Park and Shin [14] reported its insecticidal activity against the Japanese termite. However, its antifungal activity against plant pathogens has not been reported yet.

The content and chemical compositions of *I. verum* and *S. tenuifolia* essential oils are given in Table 2. As for *I. verum* oil, seven compounds were identified by GC-MS. Its major constituent was *trans*-anethole (87.4%) [10]. It also contained limonene (6.0%), estragole (1.5%), anisyl acetone (1.2%), and other minor compounds. Four compounds were identified representing 95.6% of the total oils of *S. tenuifolia*. The main components were menthone (68.1%) and pulegone (24.3%). The essential oil also contained smaller quantities of limonene (1.9%) and caryophyllene oxide (1.3%).

MIC and IC_{50} values of *I. verum* and *S. tenuifolia* oils and their major constituents (*trans*-anethole from *I. verum*, menthone and pulegone from *S. tenuifolia*) against *B. cinerea* are summarized in Table 3. MIC values (10 μg per plate=227 μg/l of air) of the two essential oils were identical to each other, but the IC_{50} value (1.2 μg per plate) of *S. tenuifolia* was a little lower than that (1.6 μg per plate) of *I. verum*. Among the three pure compounds, the *trans*-anethole present in *I. verum* showed the most potent *in vitro* fumigant activity against *B. cinerea*, followed by menthone and pulegone.

Table 3. MIC and IC_{50} values of essential oils of *Illicium verum* and *Schizonepeta tenuifolia*, and their major constituents, against *Botrytis cinerea* and *Colletotrichum gloeosporioides*.

Fungus	Sample	MIC[a] (μg per plate)	IC_{50}[b] (μg per plate)
B. cinerea	*I. verum*	10	1.60
	S. tenuifolia	10	1.20
	trans-Anethole	5	0.30
	Menthone	10	0.93
	Pulegone	>10	33
C. gloeosporioides	*I. verum*	>10	1.80
	trans-Anethole	>10	0.43

[a]MIC, minimum inhibitory concentration.
[b]IC_{50}, 50% inhibitory concentration.

MIC and IC_{50} values of *I. verum* oil and its major constituent, *trans*-anethole, against *C. gloeosporioides* were also determined. As shown in Table 3, both samples did not completely inhibit the mycelial growth at doses applied in this study. IC_{50} values were 1.8 μg per plate for *I. verum* oil and 0.43 μg per plate for *trans*-anethole.

Our results indicate that *I. verum* and *S. tenuifolia* oils and their components could be useful as fumigants for the control of post-harvest diseases caused by *B. cinerea* and *C. gloeosporioides*. However, some oil vapors have been known to induce phytotoxicity on treated fruits and vegetables under long periods of exposure. The problem could be overcome by using other biocontrol agents such as antagonistic microorganisms, antibiotics, and nonvolatile plant-derived natural products in combination with essential oils, resulting in synergistic effects. Ettayebi *et al.* [6] reported the synergistic effects of nisin (a bacteriocin produced by many *Lactococcus lactis* strains) and thymol on antimicrobial activities in *Listeria monocytogenes* and *Bacillus subtilis*. For practical use of both *I. verum* and *S. tenuifolia* oils and their components, further study is necessary on the safety of these materials and on the development of formulations to improve the efficacy and stability and to reduce costs.

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