

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

LEE, SUN OG¹, IL-KWON PARK², GYUNG JA CHOI¹, HE KYOUNG LIM¹, KYONG SOO JANG¹, KWANG YUN CHO¹, SANG-CHEOL SHIN², AND JIN-CHEOL KIM^{1*}

¹Bio-control Research Team, Korea Research Institute of Chemical Technology, Taejon 305-600, Korea

²Division of Forest Diseases and Insect Pests, Korea Forest Research Institute, Seoul 130-712, Korea

Received: March 8, 2007

Accepted: May 21, 2007

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.

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

*Corresponding author

Phone: 82-42-860-7436; Fax: 82-42-861-4913;

E-mail: kjinc@kriict.re.kr

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
<i>Amomum cardamomum</i>	Zingiberaceae	F	0.90	-	36
<i>Asarum sieboldii</i>	Aristolochiaceae	R	8.6	42	70
<i>Carpesium abrotanoides</i>	Compositae	F	4.2	-	-
<i>Chamaecyparis obtuse</i>	Cupressaceae	L	0.49	-	-
<i>Chamaecyparis pisifera</i>	Cupressaceae	L	0.50	26	-
<i>Chenopodium ambrosioides</i>	Chenopodiaceae	WP	0.44	-	-
<i>Cinnamomum cassia</i>	Lauraceae	B	0.33	-	-
<i>Curcuma longa</i>	Zingiberaceae	R	0.44	-	-
<i>Curcuma zedoaria</i>	Zingiberaceae	R	0.35	-	-
<i>Illicium verum</i>	Illiciaceae	F	0.90	100	93
<i>Juniperus chinensis</i>	Cupressaceae	L	0.23	-	-
<i>Juniperus chinensis</i>	Cupressaceae	WP	0.51	40	28
<i>Juniperus chinensis</i> var. <i>globosa</i>	Cupressaceae	L	0.31	-	-
<i>Juniperus chinensis</i> var. <i>sargentii</i>	Cupressaceae	L	0.51	-	-
<i>Myristica fragrans</i>	Myristicaceae	S	2.9	48	49
<i>Nardostachytis chinensis</i>	Valerianaceae	R	0.30	-	-
<i>Paeonia moutan</i>	Ranunculaceae	R	0.54	-	-
<i>Pinus koraiensis</i>	Pinaceae	L	0.33	-	-
<i>Poncirus trifoliata</i>	Rutaceae	F	0.37	-	-
<i>Saussurea lappa</i>	Compositae	R	0.15	-	-
<i>Schisandra chinensis</i>	Schizandraceae	F	0.70	-	-
<i>Schizonepeta tenuifolia</i>	Labiatae	WP	0.39	95	68
<i>Styrax benzoin</i>	Styracaceae	Re	0.36	-	-
<i>Teucrium veronicoides</i>	Labiatae	WP	0.27	-	-
<i>Thuja orientalis</i> cv. <i>compacta</i>	Cupressaceae	L	0.16	-	-

^aTen µg of each essential oil was added on a sterile paper disc in the middle of the half-plate.

^bF, 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 ambrosioides* (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. *compacta* (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 (IC₅₀) 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.*

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

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

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.

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-inflammatory, 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 IC₅₀ 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 IC₅₀ 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 IC₅₀ 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)
<i>B. cinerea</i>	<i>I. verum</i>	10	1.60
	<i>S. tenuifolia</i>	10	1.20
	<i>trans</i> -Anethole	5	0.30
	Menthone	10	0.93
	Pulegone	>10	33
<i>C. gloeosporioides</i>	<i>I. verum</i>	>10	1.80
	<i>trans</i> -Anethole	>10	0.43

^aMIC, minimum inhibitory concentration.

^bIC₅₀, 50% inhibitory concentration.

MIC and IC₅₀ 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. IC₅₀ 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.

Acknowledgment

This research was supported by a grant (PF06219-00) from the Plant Diversity Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology of the Korean government.

REFERENCES

- Agrios, G. 1997. *Plant Pathology*, 4th Ed. Academic Press, San Diego.
- Cho, J.-Y., G. J. Choi, S.-W. Lee, K. S. Jang, H. K. Lim, C. H. Lim, S. O. Lee, K. Y. Cho, and J.-C. Kim. 2006. Antifungal activity against *Colletotrichum* spp. of curcuminoids isolated from *Curcuma longa* L. rhizomes. *J. Microbiol. Biotechnol.* **16**: 280–285.
- Choi, G. J., J.-C. Kim, K. S. Jang, H. K. Lim, I.-L. Park, S.-C. Shin, and K. Y. Cho. 2006. *In vivo* antifungal activities of 67 plant fruit extracts against six plant pathogenic fungi. *J. Microbiol. Biotechnol.* **16**: 491–495.
- Deans, S. G. and G. Ritchie. 1987. Antibacterial properties of plant essential oils. *Int. J. Food Microbiol.* **5**: 165–180.
- Dhingra, O. D., M. L. N. Costa, G. J. Silva Jr., and E. S. G. Mizubuti. 2004. Essential oil of mustard to control *Rhizoctonia solani* causing seedling damping off and seedling blight in nursery. *Fitopatol. Bras.* **29**: 683–686.
- Ettayebi, K., J. E. Yamani, and B.-D. Rossi-Hassani. 2000. Synergistic effects of nisin and thymol on antimicrobial activities in *Listeria monocytogenes* and *Bacillus subtilis*. *FEMS Microbiol. Lett.* **183**: 191–195.
- Freeman, S., T. Katan, and E. Shabi. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. *Plant Dis.* **82**: 596–607.
- Jay, J. M. and G. M. Rivers. 1984. Antimicrobial activity of some food flavoring compounds. *J. Food Safety* **6**: 129–139.
- Kobaisy, M., M. R. Tellez, C. L. Webber, F. E. Dayan, K. K. Schrader, and D. E. Wedge. 2001. Phytotoxic and fungitoxic activities of the essential oil of kenaf (*Hibiscus cannabinus* L.) leaves and its composition. *J. Agric. Food Chem.* **49**: 3768–3771.
- LeFevre, J. W. 2000. Isolating *trans*-anethole from anise seeds and elucidating its structure: A project utilizing one- and two-dimensional NMR spectrometry. *J. Chem. Ed.* **77**: 361–363.
- Lingk, W. 1991. Health risk evaluation of pesticide contamination in drinking water. *Gesunde Pflanzen* **43**: 21–25.
- Nakahara, K., N. S. Alzoreky, T. Yoshihashi, H. T. T. Nguyen, and G. Trakoontivakorn. 2003. Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (Citronella Grass). *Jap. Agric. Res. Quart.* **37**: 249–252.
- Paranagama, P. A., K. H. T. Abeysekera, K. Abeywickrama, and L. Nugaliyadde. 2003. Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (lemongrass) against *Aspergillus flavus* Link. isolated from stored rice. *Lett. Appl. Microbiol.* **37**: 86–90.
- Park, I.-K. and S.-C. Shin. 2005. Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* Kolbe). *J. Agric. Food Chem.* **53**: 4388–4392.
- Park, J.-H., G. J. Choi, H. B. Lee, K. M. Kim, H. S. Jung, S. W. Lee, K. S. Jang, K. Y. Cho, and J.-C. Kim. 2005. Griseofulvin from *Xylaria* sp. strain F0010, and endophytic fungus of *Abies holophylla* and its antifungal activity against plant pathogenic fungi. *J. Microbiol. Biotechnol.* **15**: 112–117.
- Paster, N., B. J. Juven, E. Shaaya, M. Menasherov, R. Nitzan, H. Weisslowicz, and U. Ravid. 1990. Inhibitory effect of oregano and thyme essential oils on moulds and foodborne bacteria. *Lett. Appl. Microbiol.* **11**: 33–37.

17. Ross, A., N. E. El-Keltawi, and S. E. Megalla. 1980. Antimicrobial activity of some Egyptian aromatic plants. *Fitoterapia* **4**: 201–206.
18. Sung, W. S., H. J. Jung, I. S. Lee, H. S. Kim, and D. G. Lee. 2006. Antimicrobial effect of furaneol against human pathogenic bacteria and fungi. *J. Microbiol. Biotechnol.* **16**: 349–354.
19. Tabanca, N., B. Demirci, K. Husnu, C. Baser, Z. Aytac, M. U. Ekici, S. H. I. Khan, M. R. Jacob, and D. E. Wedge. 2006. Chemical composition and antifungal activity of *Salvia macrochlamys* and *Salvia recognita* essential oils. *J. Agric. Food Chem.* **54**: 6593–6597.
20. Wedge, D. E., M. B. Riley, and F. H. Tainter. 1999. Phytotoxicity of *Discula destructiva* culture filtrates to *Cornus* spp. and the relationship to disease symptomology. *Plant Dis.* **83**: 377–380.
21. Wilson, C. L. and A. El Ghaouth 1993. Multifaceted biological control of postharvest diseases of fruits and vegetables, pp. 181–185. In R. D. Lumsden and J. L. Vaughn (eds.), *Pest Management: Biologically Based Technologies*. American Chemical Society, Washington, D.C.
22. Wilson, C. L., J. M. Solar, A. El Ghaouth, and M. E. Wisniewski. 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Dis.* **81**: 204–210.
23. Yen, K. Y. 1992. *The Illustrated Chinese Materia Medica Crude and Prepared Drugs*, p. 194. SMC Publishing, Taiwan.
24. Yoo, D.-S, B. S. Lee, and E.-K. Kim. 2005. Characteristics of microbial biosurfactant as an antifungal agent against plant pathogenic fungus. *J. Microbiol. Biotechnol.* **15**: 1164–1169.
25. Zhu, Y. P. 1998. *Chinese Materia Medica - Chemistry, Pharmacology, and Applications*, pp. 60–61. Harwood Academic, Amsterdam.