JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

In Vitro Efficacy of Plant Volatiles for Inhibiting the Growth of Fruit and Vegetable Decay Microorganisms

I MADE S. UTAMA,*,† RON B. H. WILLS,‡ SHIMSHON BEN-YEHOSHUA,§ AND CLEM KUEK||

Study Program of Agricultural Technology, Udayana University, Bukit Jimbaran Campus, Badung, Bali, Indonesia; Department of Food Technology, Newcastle University, Central Coast Campus, P.O. Box 127, Ourimbah, New South Wales, Australia, 2258; Agricultural Research Organization, The Volcanic Center, Bet-Dagan, 50250, Israel; and Faculty of Business and Technology, Western Sydney University, Campbelltown, New South Wales, 2560, Australia

The effects of acetaldehyde, benzaldehyde, cinnamaldehyde, ethanol, benzyl alcohol, nerolidol, 2-nonanone, β-ionone, and ethyl formate vapors on the growth of *Rhizopus stolonifer, Penicillium digitatum, Colletotrichum musae, Erwinia carotovora,* and *Pseudomonas aeruginosa* on agar medium were evaluated. The aldehydes were found to be the strongest growth inhibitors and the most lethal to the fungal spores and mycelia and bacterial cells. The average minimum inhibitory concentrations (MICs) of aldehydes that were germicidal to decay microorganisms were 0.28, 0.49, and 0.88 mmol per Petri dish, for cinnamaldehyde, benzaldehyde, and acetaldehyde, respectively. Ethanol also inhibited growth completely, but the MIC, which was 14.6 mmol per Petri dish, was significantly higher than those of the aldehydes. Ethanol can be considered germistatic because the alcohol does not inhibit germination of spores completely; it completely controlled only mycelial growth. The ketones tended to be effective only on *P. digitatum* and *C. musae,* whereas ethyl formate was not effective except on *P. digitatum.* The concentration of a volatile compound in the headspace of the Petri dish and its diffusion into the medium largely determined its efficacy against decay microorganisms.

KEYWORDS: Volatile compound; decay microorganisms; acetaldehyde; benzaldehyde; cinnamaldehyde; ethanol; benzyl alcohol; nerolidol; 2-nonanone; β-ionone; ethyl formate; *Rhizopus stolonifer*; *Penicillium digitatum*; *Colletotrichum musae*; *Erwinia carotovora*; *Pseudomonas aeruginosa*

INTRODUCTION

Volatiles are small-molecular-weight organic compounds (less than 250 g/mol) having appreciable vapor pressure at ambient temperature (*1*). Plants and plant products emit a wide range of volatile compounds, some of which are important flavor quality factors in fruits, vegetables, spices, and herbs (*2*). A number of volatile compounds inhibit the growth of microorganisms (*3*−*5*). The presence of volatile compounds has also been hypothesized to play an important role in the defense systems of fresh produce against decay microorganisms (*6, 7*).

Over recent decades there has been increasing public pressure to reduce the use of synthetic fungicides in agriculture products and their presence in the environment. Moreover, concerns have been raised about the health risk involved in the use of synthetic fungicides on fresh fruits and vegetables shortly before consumption. Therefore, considerable research has been recently directed toward the development of effective alternative crop protectants. The European Commission has been actively promoting the development and commercial implementation of such new compounds, known as "Green Chemicals" (*8*). The ability of plant volatiles to inhibit decay microorganisms is one of the reasons for interest in them as a component of biological means for controlling post-harvest decay of fruits and vegetables (*9*). An important factor in their vapors is that plant volatiles have been widely used as food flavoring agents and many are generally recognized as safe (GRAS). The present study aimed to evaluate in vitro the effectiveness of a number of plant volatile vapors as antimicrobial agents against several major fruit and vegetable decay microorganisms.

MATERIALS AND METHODS

Tested Volatiles and Decay Microorganisms. The aldehydes (acetaldehyde, benzaldehyde, and trans-cinnamaldehyde), the alcohols (ethanol, benzyl alcohol, and nerolidol), the ketones (2-nonanone and β-ionone), and the ester (ethyl formate) were screened in vitro for their ability to control the growth of the following fruit and vegetable decay microorganisms: *Rhizopus stolonifer* DAR 43352, *Penicillium digitatum* FRR 1562, *Colletotrichum musae* DAR 24962, *Erwinia carotovora* UNSW 031700, and *Pseudomonas aeruginosa* DAR 25580. The

* To whom correspondence should be addressed. Phone: +62 361 701801-467137. Fax: +62 361 701801. E-mail: made_utama@hotmail.com.
† Udayana University.
‡ Newcastle University.
§ Agricultural Research Organization.
|| Western Sydney University.

10.1021/jf020484d CCC: $22.00
Published on Web 09/24/2002

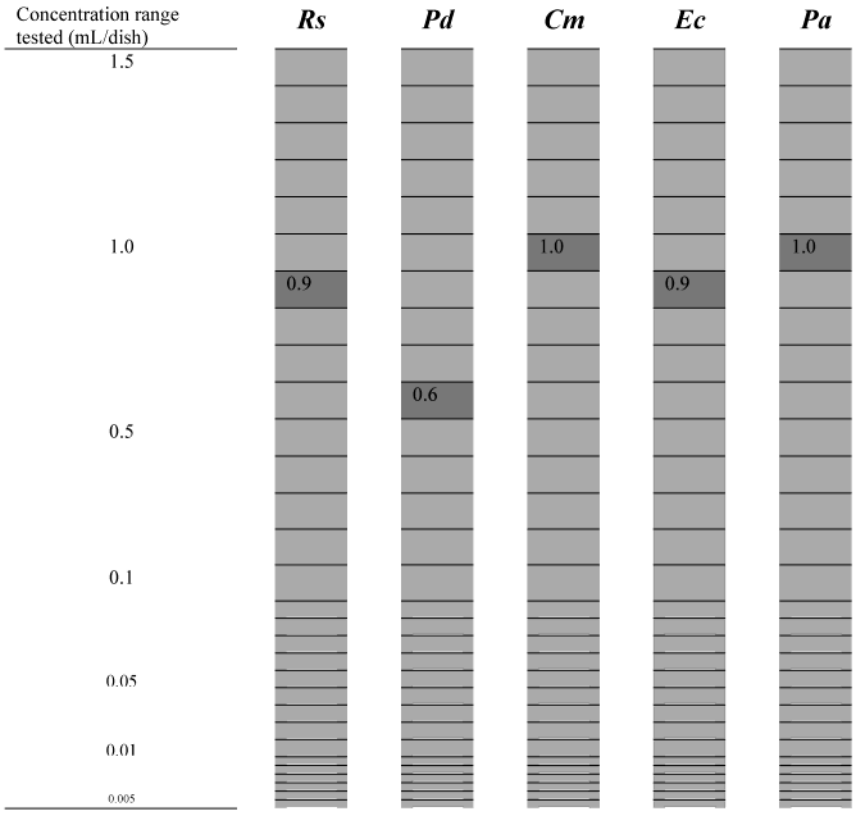

Figure 1. Screening the MIC of tested volatiles using the MIC of ethanol as starting point. The numbers in the thick shaded rectangles (rounded to one decimal) indicate the MIC of ethanol against decay microorganisms: *Rs* = *R. stolonifer*, *Pd* = *P. digitatum*, *Cm* = *C. musae*, *Ec* = *E. carotovora*, and *Pa* = *P. aeruginosa*. Different heights of the columns of microorganisms indicate the unit concentration of volatiles declined one decimal.

volatile compounds were purchased from Aldrich (Milwaukee, WI), except for ethanol, which was purchased from Chem-Supply (Sydney).

Culture Preparation. The surface-plated cultures of the decay fungi in plastic Petri dishes were sub-cultured by streaking the spores onto the new potato dextrose agar (PDA) media. The bacteria, however, which were obtained in the form of lyophilized cultures, were mixed with nutrient broth and the mixture was then streaked onto nutrient agar (NA) media. Both new plated cultures were then incubated for 7 days at 25 °C.

The spores of 7-day-old cultures of decay fungi and the cells of 7-day-old cultures of decay bacteria were dislodged by sterile distilled water to which 0.1 mL/L of Tween 80 had been added. The spores and cell suspensions were then filtered with sterile Sinta Glass No. 1 (Gallenkamp, London) to remove debris such as mycelia and condensed-agar fragments, and the aliquot was diluted to a concentration of 10^5 fungal spores/mL suspensions and 10^5 bacterial cells/mL suspensions. The fungal spore or bacterial cell suspensions, 0.1 mL each, were then dispensed into Petri dishes (9-cm diam.) containing agar medium (PDA or NA). The Petri dishes were then incubated for 3 days for the fungal cultures and 5 days for the bacterial cultures, both at 25 °C, to allow the spores and the cells to grow.

Determination of Minimum Inhibitory Concentration (MIC). Agar plugs (5.5-mm diam.) were picked up from the 3-day-old cultures of decay fungi and 5-day-old culture of decay bacteria using the bottom end of a sterilized Pasteur pipet and then transferred onto the centers of new PDA and NA media, respectively, in 9-cm plastic Petri dishes. The Petri dishes were then inverted and 7-cm Whatman No. 1 filter papers were attached onto the inner surface of their lids. Ethanol, the first tested volatile in this experiment, was impregnated into the filter paper with varying volumes from 0.1 to 1.0 mL/dish in the 4 °C room. Immediately after the impregnation, the Petri dishes were sealed by wrapping them with plastic film (Vitafilm, Goodyear, Sydney) and incubated for 10 days at 25 °C. Experiments were repeated two times with four replications for each experiment. The minimum concentration of ethanol (expressed as mmol/dish) required to give complete control or the minimum inhibitory concentration (MIC) for each microorganism was determined.

The MIC of ethanol for each target decay microorganism was used as the initial level to identify the MIC of other tested volatiles as shown in **Figure 1**. If the MIC level of ethanol used for other volatiles failed to stop the growth of pathogen, the level was increased until the MIC was found. However, if the volume of 1.5 mL/dish still failed to stop the growth of pathogen, the compound was considered ineffective as a vapor to stop the growth of pathogens. When the tested compounds had the same effect as the MIC of ethanol, the concentration was decreased until the MIC of the compound for each microorganism was determined. All the unit concentrations of MIC were then expressed as mmol/dish.

Determination of Antimicrobial Properties of MIC. The MIC of each of the volatiles was evaluated and each compound was classified as germicidal or germistatic in its effect on decay microorganisms. A germicidal effect is the death of a microorganism, whereas a germistatic effect is the inhibition of microbial replication (*10*).

The agar disks of decay microorganisms which failed to grow due to the MIC of the compound were transferred onto new agar media free from the tested volatile and incubated for a further 5 days at 25 °C. The activity of the MIC of the tested compound was considered germistatic when the microorganism grew during the incubation period. The tested compound was considered germicidal if the microorganism did not grow.

Determination of Concentration of Volatile Vapor in the Headspace. The concentrations of acetaldehyde, benzaldehyde, cinnamaldehyde, and ethanol vapors in the headspaces of Petri dishes were measured. The vapor of each volatile in the headspace was generated by evaporation of a single tested liquid compound in a Petri dish in which an agar disk of a microorganism was attached onto the media as described above. Measurements were conducted after incubation for 1 h (categorized as day 0), 5 days, and 10 days at 25 °C. To enable

Table 1. Minimum Inhibitory Concentration (MIC) of Volatiles against Decay Microorganisms at 25 °C[a]

	MIC (mmol/dish)				
volatile	*R. stolonifer*	*P. digitatum*	*C. musae*	*E. carotovora*	*P. aeruginosa*
acetaldehyde	0.88bA	0.84cA	0.91bA	0.91cA	0.88bA
benzaldehyde	0.50aA	0.50bA	0.48aA	0.49bA	0.50aA
cinnamaldehyde	0.42aB	0.09aA	0.39aB	0.11aA	0.41aB
ethanol	15.33dB	9.87dA	16.59fB	14.91dB	16.38dB
benzyl alcohol	12.67cC	0.48bA	1.06bB	0.45bA	0.49aA
2-nonanone	NE	4.82cB	3.58cA	NE	NE
β-ionone	NE	NE	4.65d	NE	NE
ethyl formate	11.49cB	NE	7.45eA	6.52cA	6.21cA

[a] Data are the means of eight replications. Values followed by the same lower case letters in the same column, or values followed by the same upper case letters in the same row, are not significantly different (DMRT 5%). NE indicates compound ineffective in preventing microbial growth at 1.5 mL/dish.

Table 2. Antimicrobial Properties of Volatiles against Decay Microorganisms at the Level of MIC

	effect on microorganism[a]				
volatile	*R. stolonifer*	*P. digitatum*	*C. musae*	*E. carotovora*	*P. aeruginosa*
acetaldehyde	C	C	C	C	C
benzaldehyde	C	C	C	C	C
cinnamaldehyde	C	C	C	C	C
ethanol	S	S	S	S	S
benzyl alcohol	S	S	S	C	C
2-nonanone	*	S	S	*	*
β-ionone	*	*	S	*	*
ethyl formate	C	*	C	C	C

[a] C = Germicidal and S = germistatic. * In this table indicates that there is no reply yet whether the compound is C or S.

withdrawal of gas from the headspace with a gastight syringe, a small hole was made in the side of each plastic Petri dish by inserting a hot needle through the plastic wall of the dish. Cellophane tape was used to seal the hole, and the Petri dish was then wrapped in a plastic film (Vitafilm). A 500-μL gastight syringe (SGE, Melbourne) was used to withdraw the gas from the headspace, and 100−200 μL was injected into the split/splitless injector of a GC (Varian 3400cx). The GC was equipped with a flame ionization detector and a DB1 megabore column (30 m long × 0.53 mm i.d., 5.0 μm film thickness; J&W Scientific, Folsom, CA). The column temperature was set at 60 °C for 2 min, and then increased at 25 °C/min to 250 °C. The injector and detector temperatures were 250 and 270 °C, respectively. The flow rates of the nitrogen carrier gas, hydrogen, and air were 5, 30, and 300 mL/min, respectively. Split injection was employed for the measurement of ethanol and acetaldehyde, with a split flow of 20 mL/min. Splitless injection was used for benzaldehyde and cinnamaldehyde. Four concentrations of an external standard of each volatile, dissolved in carbon disulfide, were used to quantify the areas of the sample peaks. Standards were injected after every 10 samples.

Determination of Concentration of Volatile Vapor in the Agar Medium. The concentration of each volatile in the agar medium was measured at the same time as that in the headspace. A cork borer (0.9 cm i.d.) was used to collect 15 disks of agar medium (about 2.20 ± 0.05 mL when melted) from the Petri dish in which they had been exposed to the MIC of single volatile, and placed in a 24-mL Wheaton sample vial (Aldrich). The vial had a plastic cap with a 0.5-cm hole and was fitted with a blue septum (Altech, Sydney). The agar disks in the vial were blended with 10 mL of distilled water, and the vial was tightly sealed with the cap and heated in a water bath for 30 min at 90 °C to melt the agar disks. The temperature was then gradually decreased and stabilized at 80 °C for 2 h. An aliquot (100−200 μL) of gas was withdrawn from the headspace of the vial into a 500-μL gastight syringe and analyzed by GC. Four external-standard calibrations were made by mixing four different amounts of a volatile compound in 15 untreated agar disks blended with 10 mL of distilled water.

Statistical Analysis. Data gathered from the experiments were analyzed using analysis of variances. If there was a significant difference between treatments, the means of treatments were then compared using Duncan's multiple range test (DMRT).

RESULTS

Effects on Various Pathogens. The MIC of each volatile tested against each decay microorganism is shown in **Table 1**. The MIC varied with the volatile and the microorganism. The aldehydes tested were found to be significantly more effective ($P < 0.05$) than the other volatiles and gave complete inhibition of all five microorganisms tested at 0.09−0.91 mmol/dish. In general, the alcohols were less effective than the aldehydes and differed widely from one another in their MICs. Benzyl alcohol was found to be more effective than ethanol in inhibiting the growth of *P. digitatum*, *E. carotovora*, and *P. aeruginosa*, with MICs of 0.45−0.49 mmol/dish, and that of *C. musae*, with an MIC of 1.06 mmol/dish; the MICs of ethanol against these four microorganisms were much higher at 9.87−16.59 mmol/dish. *Rhizopus stolonifer* was considerably more resistant to benzyl alcohol than the other decay microorganisms. Aldehydes and alcohols were found to be potential wide-spectrum-antimicrobial compounds, as they completely inhibited growth of all the decay microorganisms tested.

Antimicrobial Properties. The antimicrobial properties of volatiles tended to depend more on the functional group than on the individual compound within a class. **Table 2** shows that aldehydes were generally germicidal to decay microorganisms. The alcohols showed more varied effects: benzyl alcohol was germicidal against *E. carotovora* and *P. aeruginosa* and germistatic against *R. stolonifer*, *P. digitatum*, and *C. musae*, whereas ethanol was germistatic against all the organisms studied. **Figure 2** shows the germicidal effect of MIC of benzaldehyde by complete kill of the conidia and spores of *C. musae* and *P. digitatum*, respectively. The MIC of ethanol, however, could not kill the conidia and spores, as the conidia and spores were still able to germinate. Therefore, ethanol was considered germistatic against *C. musae* and *P. digitatum*. The ketones were germistatic in their effects against *P. digitatum*

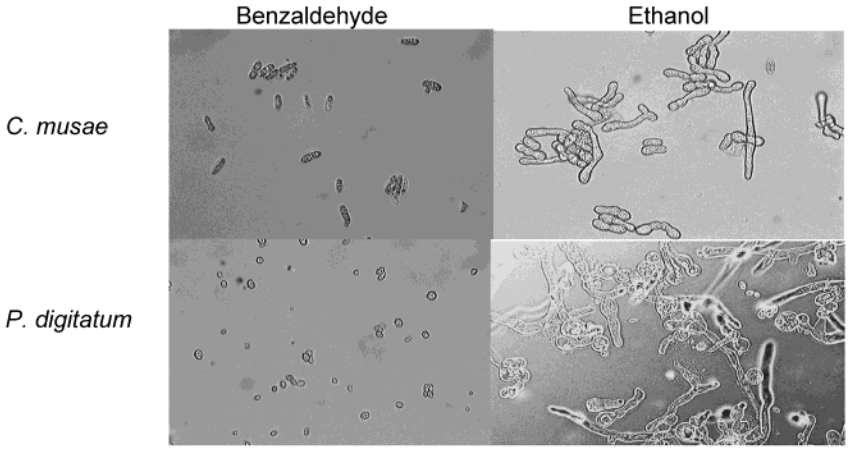

Figure 2. Effectiveness of benzaldehyde and ethanol on germination of *C. musae* and *P. digitatum* spores, with no growth due to benzaldehyde but some germination with ethanol after 20 h of incubation at 25 °C (400× magnification).

Table 3. Concentration in the Headspace of Petri Dish of Volatiles Generated from the MIC against Decay Microorganisms at 25 °C

			volatile concentration in the headspace (nmol/mL air)[a]					
volatile	pathogen	MIC mmol/dish	1 hr		5 days		10 days	
acetaldehyde	Rs	0.88	55.05 ± 5.40	a	22.45 ± 2.11	a	14.80 ± 1.61	a
	Pd	0.84	56.48 ± 7.28	a	21.57 ± 1.07	a	13.71 ± 1.39	a
	Cm	0.91	50.96 ± 7.37	a	22.09 ± 2.42	a	14.28 ± 1.06	a
	Ec	0.91	74.71 ± 4.53	b	24.95 ± 1.98	a	13.44 ± 0.72	a
	Pa	0.88	71.21 ± 6.51	b	24.20 ± 3.49	a	14.32 ± 1.01	a
			61.68 ± 10.57	**B**	**22.98 ± 1.34**	B	**14.11 ± 0.54**	**B**
benzaldehyde	Rs	0.50	12.75 ± 1.28	a	1.38 ± 0.14	a	0.10 ± 0.03	a
	Pd	0.50	14.95 ± 1.90	a	1.26 ± 0.06	a	0.12 ± 0.02	a
	Cm	0.48	13.28 ± 1.25	a	1.25 ± 0.12	a	0.11 ± 0.01	a
	Ec	0.49	18.49 ± 1.45	a	1.33 ± 0.08	a	0.11 ± 0.01	a
	Pa	0.50	17.97 ± 1.52	a	1.30 ± 0.16	a	0.11 ± 0.01	a
			15.49 ± 2.64	**A**	**1.30 ± 0.05**	A	**0.11 ± 0.007**	**A**
cinnamaldehyde	Rs	0.42	15.62 ± 3.02	b	6.14 ± 0.55	b	3.81 ± 0.50	a
	Pd	0.09	7.02 ± 1.38	a	1.03 ± 0.04	a	0.56 ± 0.12	b
	Cm	0.39	14.38 ± 1.93	b	6.31 ± 0.45	b	4.71 ± 1.08	a
	Ec	0.11	7.11 ± 0.83	a	0.97 ± 0.13	a	0.52 ± 0.03	b
	Pa	0.41	14.03 ± 1.30	b	5.72 ± 0.30	b	5.52 ± 0.93	a
			11.63 ± 4.21	**A**	**4.03 ± 2.78**	**A**	**3.02 ± 2.35**	**A**
ethanol	Rs	15.33	130.0 ± 15.8	b	107.7 ± 12.8	d	97.3 ± 11.7	a
	Pd	9.87	96.7 ± 10.9	a	78.2 ± 9.7	c	70.0 ± 13.4	c
	Cm	16.59	140.6 ± 12.4	c	112.1 ± 16.4	d	112.0 ± 15.5	b
	Ec	14.91	118.8 ± 9.8	b	105.0 ± 12.4	d	101.9 ± 8.2	c
	Pa	16.38	138.2 ± 12.8	b	113.0 ± 5.1	d	105.2 ± 5.2	c
			124.9 ± 17.9	**C**	**103.2 ± 14.35**	**C**	**97.28 ± 16.16**	**C**

[a] Rs = *R. stolonifer*; Pd = *P. digitatum*; Cm = *C. musae*; Ec = *E. carotovora*; Pa = *P. aeruginosa.* Data presented with standard erors are the means of four replications. Values followed by different lower case letters in the same column and the same volatile are significantly different (DMRT 5%). Values with the bold characters are the means and standard erors of the volatiles, and different upper case letters in the same column indicate significant differences (DMRT 5%).

and *C. musae*. Ethyl formate was germicidal against *R. stolonifer*, *C. musae*, *E. carotovora*, and *P. aeruginosa*.

Actual Concentrations of Volatiles. The concentrations of acetaldehyde, benzaldehyde, cinnamaldehyde, and ethanol in the headspaces of Petri dishes exposed to the MIC of each compound are shown in **Table 3**. The measurements conducted after 1 h of incubation showed that the concentration of ethanol vapor (125 nmol/mL air) was significantly higher than those of cinnamaldehyde (12 nmol/mL air), benzaldehyde (16 nmol/mL air), and acetaldehyde (62 nmol/mL air). This indicates that the vapor concentrations of the three aldehydes in the headspace needed to inhibit the microorganisms completely were less than that of ethanol. The aldehydes declined markedly after 5 days, but the concentration of ethanol in the headspace decreased by a much smaller amount in the same time. Among the aldehydes, the concentration of acetaldehyde in the headspace after 1 h of incubation was significantly higher than those of benzaldehyde and cinnamaldehyde ($P < 0.05$).

The concentration of ethanol in the medium after 1 h of incubation (682 μmol/mL) was significantly higher than those of acetaldehyde (41 μmol/mL), benzaldehyde (6.4 μmol/mL), and cinnamaldehyde (5.5 μmol/mL) (**Table 4**). Among the aldehydes, the level of acetaldehyde in the medium during the 10-day incubation period was significantly higher than those of benzaldehyde and cinnamaldehyde ($P < 0.01$). After 5 days, the concentration of ethanol in the medium increased slightly; it then decreased after 10 days to below the concentration after 1 h of incubation. Acetaldehyde, benzaldehyde, and cinnamaldehyde were found to decline substantially after 5 and 10 days of incubation.

Distribution of Volatiles. The distribution of volatiles in the sealed agar plate systems, as calculated from the data in **Tables**

Table 4. Concentration in the Agar Medium of Volatiles Generated from the MIC against Decay Microorganisms at 25 °C

			volatile concentration in the agar medium (μmol/mL agar)[a]					
volatile	pathogen	MIC mmol/dish	1 hr		5 days		10 days	
acetaldehyde	Rs	0.88	43.88 ± 3.64	a	13.92 ± 1.09	a	1.63 ± 0.16	a
	Pd	0.84	45.11 ± 3.06	a	15.62 ± 1.22	a	1.84 ± 0.30	a
	Cm	0.91	37.46 ± 2.42	a	14.05 ± 0.71	a	1.68 ± 0.19	a
	Ec	0.91	40.02 ± 2.95	a	17.03 ± 0.96	a	1.98 ± 0.31	a
	Pa	0.88	38.77 ± 2.96	a	14.51 ± 1.23	a	1.75 ± 0.20	a
			41.05 ± 3.30	**B**	**15.03 ± 1.31**	**B**	**1.78 ± 0.14**	**A**
benzaldehyde	Rs	0.50	5.99 ± 0.75	a	0.90 ± 0.13	a	0.28 ± 0.04	a
	Pd	0.50	6.20 ± 0.48	a	1.08 ± 0.21	a	0.37 ± 0.05	a
	Cm	0.48	6.41 ± 0.82	a	1.03 ± 0.19	a	0.25 ± 0.03	a
	Ec	0.49	6.80 ± 0.52	a	0.97 ± 0.14	a	0.22 ± 0.04	a
	Pa	0.50	6.35 ± 0.62	a	1.09 ± 0.10	a	0.30 ± 0.05	a
			6.35 ± 0.30	**A**	**1.01 ± 0.08**	**A**	**0.28 ± 0.06**	**A**
cinnamaldehyde	Rs	0.42	7.31 ± 0.67	a	5.50 ± 0.15	b	1.49 ± 0.25	b
	Pd	0.09	2.68 ± 0.21	b	0.99 ± 0.19	a	0.30 ± 0.03	a
	Cm	0.39	8.19 ± 0.83	a	5.61 ± 0.22	b	1.68 ± 0.04	b
	Ec	0.11	2.25 ± 0.25	b	1.20 ± 0.13	a	0.33 ± 0.04	a
	Pa	0.41	7.18 ± 0.76	a	5.75 ± 0.22	b	1.69 ± 0.02	b
			5.52 ± 2.82	**A**	**3.81 ± 2.48**	**A**	**1.10 ± 0.72**	**A**
ethanol	Rs	15.33	705.0 ± 86.9	a	720.9 ± 56.1	b	631.0 ± 56.1	b
	Pd	9.87	495.7 ± 67.1	b	418.4 ± 22.0	a	332.9 ± 22.0	a
	Cm	16.59	730.4 ± 57.3	a	808.6 ± 53.6	b	694.2 ± 53.6	b
	Ec	14.91	703.4 ± 75.3	a	813.0 ± 52.3	b	672.0 ± 53.3	b
	Pa	16.38	775.4 ± 78.9	a	815.6 ± 52.8	b	676.0 ± 52.8	b
			682.0 ± 108.1	**C**	**715.3 ± 170.7**	**C**	**601.2 ± 151.8**	**B**

[a] Rs = *R. stolonifer*; Pd = *P. digitatum*; Cm = *C. musae*; Ec = *E. carotovora*; Pa = *P. aeruginosa.* Data presented with standard erors are the means of four replications. Values followed by different lower case letters in the same column and the same volatile are significantly different (DMRT 5%). Values with the bold characters are the means and standard erors of the volatiles, and different upper case letters in the same column indicate significant differences (DMRT 5%).

Table 5. Distribution of Volatiles Added into Sealed Petri Dishes to Give Complete Inhibition (MIC) after 1 Hour of Incubation at 25 °C[a]

		distribution of volatiles					
		headspace		agar medium		other[c]	
volatile	MIC[b] (μmol/dish)	μmol	%	μmol	%	μmol	%
acetaldehyde	884	3.09	0.35	616	69.7	265	29.9
benzaldehyde	494	0.77	0.16	95	19.3	398	80.6
cinnamaldehyde	284	0.59	0.21	83	29.2	201	70.8
ethanol	14616	6.29	0.04	10238	70.0	4372	29.9

[a] Data were calculated from **Tables 1**, **3**, and **4**. The amount of volatile was calculated by assuming 50 mL of headspace volume of Petri dish. The total volume of Petri dish was 65 mL. [b] The average MIC against all five decay microorganisms. [c] The volatile may have leaked into the outer atmosphere through the plastic film.

1, **3**, and **4**, are shown in **Table 5**. In general, the proportion of volatile taken up by the medium after 1 h of incubation was much larger than that remaining in the headspace; the water-soluble volatiles, acetaldehyde and ethanol, were present at approximately 70% in the medium and only <0.5% remained in the headspace of the Petri dish. The volatiles may also have leaked into the outer atmosphere through the plastic film wrapping, as about 30% of the volatiles were not accounted for. Of the benzaldehyde and cinnamaldehyde, 81 and 71%, respectively, were not accounted for; about 20 and 30%, respectively, of these water-nonsoluble volatiles were taken up by the medium and approximately 0.2% remained in the headspace.

DISCUSSION

The in vitro studies in this work showed that the volatiles inhibited microbial growth but that their effectiveness varied. The aldehydes (acetaldehyde, benzaldehyde, and cinnamaldehyde) were found to be stronger inhibitors of growth than the other tested compounds and caused germicidal effects both on spores and mycelia of fungi (*R. stolonifer*, *C. musae*, and *P. digitatum*) and on bacterial cells (*E. carotovora* and *P. aeruginosa*).

The germicidal effect of acetaldehyde found in the present study confirms previous findings that it strongly inhibited postharvest decay fungi, bacteria, and yeasts (*11*−*13*). These findings, however, had not mentioned the distribution of the acetaldehyde in the headspace and medium during incubation period to give germicidal effect to the microorganisms. The present findings that benzaldehyde and cinnamaldehyde were strong growth inhibitors confirm previous reports of their strong inhibition of mycelial growth of other microorganisms such as *C. gloeosporioides, Altenaria alternata, Botrytis cinerea*, and *Fusarium sambucinum* (*14, 15*). The only report of fungicidal properties of benzaldehyde against *Monillinia fructicolla* and *B. cinerea* was by Wilson et al. (*16*).

The observation under the microscope in the present study showed that the germicidal effects of the tested aldehydes were through the destruction of spores and shrinkage of mycelia of decay fungi, so that complete inhibitions of both spore germination and mycelial growth was achieved. The strong inhibition of the mycelia by the aldehydes seems similar to the finding

that acetaldehyde caused leakage of electrolytes from fungal mycelia (*17*).

Ethanol completely inhibited the growth of all the evaluated decay microorganisms. However, the MIC of ethanol against each microorganism was significantly higher than those of the aldehydes and caused only a germistatic effect. The effectiveness of benzyl alcohol varied among the species of microorganism: *R. stolonifer* was found to be relatively resistant, *P. digitatum* and *C. musae* were more sensitive, and the decay bacteria *P. aeruginosa* and *E. carotovora* were very sensitive to benzyl alcohol. The finding that the effects of alcohols on decay fungi were only germistatic was related to their inability to inhibit germination of the fungal spores completely, but they completely retarded the growth of mycelial colonies. Ethanol was tested in vitro against a wide range of microorganisms (including fungi, bacteria, and yeast) by Seiler and Russell (*18*), who found that solutions containing less than 30% (v/v) of ethanol were rarely germicidal and inhibited microbial growth through interference with the cell membrane function − either by a direct effect on membrane structure and/or on the biosynthesis of membrane components. The inhibition of other cellular functions is a secondary consequence of the alteration of membrane structure (*18*, *19*). Similarly to the present finding, benzyl alcohol has also shown poor inhibition of *Monillinia laxa* (*20*), but strongly inhibited spore germination and germ tube elongation of *B. cinerea* (*21*).

Even though the concentration of ethanol to give complete inhibition was relatively high compared to that of the aldehydes; the cost of the ethanol, however, is much lower than that of the aldehydes. It is also widely used as a food preservative and usage has been reported at 0.30% (v/v) in juice, 0.55% (w/w) in soft candy, 0.24% (v/v) in nonalcoholic beverages, and 0.50% (v/v) in gravies (*22*). Therefore, the use of this compound for in vivo experiments should be considered.

In the present study, the ketones (2-nonanone and β-ionone) and the ester (ethyl formate) tended to be effective only against specific decay microorganisms: 2-nonanone was germistatic against *P. digitatum* and *C. musae*, and β-ionone was germistatic against *C. musae* only. Ethyl formate was germicidal against *R. stolonifer*, *C. musae*, *E. carotovora*, and *P. aeruginosa*, but did not completely inhibit the growth of *P. digitatum*. 2-Nonanone and β-ionone are nonwater-soluble volatiles and have high boiling points, 196 and 129 °C, respectively. These factors may limit the amount of vapor that comes into contact with the microorganisms, so that the inhibition remains incomplete. Ethyl formate, a slightly water-soluble volatile (*22*), has also been found ineffective in inhibiting the growth of *P. digitatum* inoculated into oranges (*23*).

The study of the actual distribution of the volatiles between the headspace and the medium during the treatment, to the best of our knowledge, was demonstrated for the first time. The amount of vaporized volatile in the headspace and the amount of its vapor that is then absorbed by the medium are important factors in determining the effectiveness of a volatile against decay microorganisms. In the agar plate system, the distribution of volatile compounds would be dependent on the water solubility of the volatiles, as the agar medium consisted of >95% water. When applied at the MIC level, acetaldehyde and ethanol, the water-soluble volatiles, were readily incorporated into the medium: approximately 70% were absorbed and < 0.5% of the volatiles remained in the headspace after 1 h at 25° C. A similar distribution, but only for ethanol, was reported by Yamashita et al. (*24*). Benzaldehyde and cinnamaldehyde, however, are only very slightly soluble in water (*22*) and were absorbed by the medium to concentrations of only about 20 and 30%, respectively, with a low level (< 0.05%) measured in the headspace. However, despite the lower amount found in the agar medium compared with the water-soluble volatiles, benzaldehyde and cinnamadehyde were able to inhibit the growth microorganisms completely.

The present in vitro study, however, could not predict the effectiveness of water-soluble or nonwater-soluble volatiles on horticultural produce, because their effectiveness also depends on their phytotoxicity and, presumably, also on their reactivity in the produce. The human toxicity of the volatiles should also be considered when the experiment is done under in vivo conditions, especially when the MIC in produce is relatively high.

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Received for review April 29, 2002. Revised manuscript received July 23, 2002. Accepted August 5, 2002.

JF020484D