Analysis of the effects of essential oils on airborne bacteria in a customized bio-clean room

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Received February 20, 2012; Accepted June 20, 2012

DOI: 10.3892/mmr.2012.972

Abstract. Essential oils have a sedative effect on stress, and are also known to have antibiotic and anti-carcinogenic effects. These compounds have long been used as natural microbial agents, and have recently been added to a number of pharmaceutical, food and cosmetic products. Controlling the exposure to allergens and pathogens are important factors for the treatment of allergy, and potentially reducing the risk of sensitization and infection. Low humidity, at levels under 35%, may affect human comfort and health during the winter. Patients and other individuals require optimal humidification to maintain a moisturized respiratory tract necessary for protecting against bacterial infection. We designed an analytical system to examine the effects of aromatherapeutic essential oils on airborne bacteria. The antibacterial activities of essential oils were assayed using agar plate air-sampling methods. A bacterial suspension was sprayed into a bio-clean room through the upper holes using a spray gun. Free-floating airborne bacteria were collected from the bio-clean room (blank) in blood agar plates for 10 sec using an air sampler. Three different concentrations of essential oils (0.0005, 0.005 and 0.05 ppm) were then sprayed into the bio-clean room for 5 min. Free-floating airborne bacteria were collected every 10 min for 10 sec each. Treatment with 0.0005 ppm essential oils inhibited the growth of colonies; this effect appeared to persist after 60 min. Decreased bacterial colony growth was more apparent in the presence of 0.005 ppm and 0.05 ppm essential oils than 0.0005 ppm. These effects were observed after 60 min compared to the control (distilled water). These results indicate that essential oils are able to inhibit the growth of airborne bacteria.

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Key words: humidifier disinfectants, essential oils, airborne bacteria

Introduction

Essential oils have a sedative effect that decreases stress, and are also used as antibiotic and anti-carcinogenic agents (1,2). These compounds have long been used as natural microbial agents. Recently, essential oils were added to a number of pharmaceutical, food and cosmetic products as they effectively inhibit the growth of a wide range of microorganisms. Their broad spectrum of *in vitro* antimicrobial activities is attributed to their high content of phenolic derivatives (3). In a recent study, plant extracts were examined and their use in foods as natural antioxidants was proposed (4,5). Essential oils have been used for aromatherapy since 18,000 B.C. (1). Aromatherapy was performed to cure sickness and fatigue in the Stone Age. In the modern era, essential oils are increasingly being used to improve quality of life and relieve various symptoms in patients. However, studies evaluating the effects of aromatherapeutic essential oils on airborne bacteria are scarce. The majority of individuals usually spend 85% of their time indoors. In general, three methods are suggested for improving indoor air quality: source control, increased ventilation and air cleaning. Source control is often ungovernable in metropolitan areas, including Seoul, Korea, while increased ventilation may transport more pollutants from the outdoor environment (6,7). Therefore, air cleaning remains the most feasible option for improving indoor air quality. Air cleaners are considered to be an effective means of maintaining a clean indoor environment. General vacuumed air cleaners are used for preventing asthma and allergic disease (8). However, vacuum cleaners and the act of vacuuming can release and resuspend dust and allergens in the air, thereby leading to increased exposure (9,10). Allergenic and antigenic bacteria collected with dust by vacuums can also serve as reservoirs of infectious agents that remain in vacuum cleaners (11).

Potentially harmful bacteria are all around us. The acts of talking, coughing, sneezing and breathing generate aerosolized droplets of moisture containing bacteria. Indoors, the airborne circulation of pathogens potentially promotes infections in our body, particularly in hospitals. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) can be transmitted in an aerosolized form via the respiratory tract (12,13). Controlling the exposure to allergens and pathogens is therefore important

for the symptomatic treatment of allergy, and potentially reducing the risk of sensitization and infection. Low humidity, at levels under 35%, may affect human comfort and health during the winter. When humidity is low, decreased comfort and increased risk of allergies, asthma, attacks on the immune system and mortality are observed. However, general humidifiers are also associated with risk of infection of the respiratory tract due to pathogens and allergens.

In the present study, we designed a system to analyze the effects of aromatherapeutic essential oils on airborne bacteria. The aim of our study was to determine whether the anti-airborne bacterial effects of essential oils are able to significantly improve indoor air quality.

Materials and methods

Experimental microorganisms. To examine the anti-airborne bacterial activity of each essential oil, six bacterial strains were obtained from the Culture Collection of Antimicrobial Resistant Microbes (Seoul, Korea). The anti-airborne bacterial effect of essential oils distilled by ALDIX (Gyeong-Gi, Korea) was evaluated. These included *Streptococcus pyogenes* (CCARM 0032), *Streptococcus pneumonia* (CCARM 4001), *Klebsiella pneumonia* (CCARM 0085), *Neisseria meningitidis* (CCARM 0073), *Hemophilus influenzae* (CCARM 9001) and *Escherichia coli* (CCARM 0010). The strains were cultured on blood agar plates (BAP; Hanil Komed Co., Gyeong-Gi, Korea) at 37˚C in a humidified atmosphere of 95% O_2 and 5% CO_2. The suspended bacteria were collected 12 h after spreading onto the BAP and the bacterial concentration was determined using a spectrophotometer (Perkin-Elmer Co., Wellesley, MA, USA) at an absorbance of 590 nm.

Bio-clean room. The bio-clean room used for this study comprised a non-interrupted acrylic plate, a Hypalon® glove box system (Innovative Technology Inc., Amesbury, MA, USA), a stainless steel frame, a pass box and a 6 W ultraviolet (UV) germicidal lamp (Sankyo Denki, Kanagawa, Japan), and contained extra ventilation holes (Fig. 1). The space was sealed with eco-silicon (LC-707; Lien Ching Enterprise Co., Ltd., Chungbuk, Korea) on each side. Anti-airborne bacterial activities were measured using the agar plate air-sampling methods with an air sampler (LGD; LD-P150, Seoul, Korea). The exterior and interior of the bio-clean room was cleaned using de-mineralized water and dried prior to testing following UV sterilization for 6 h. The volume of the customized bio-clean room was 9.6 m^3 (width, 200 mm; length, 200 mm; height, 240 mm), and it was placed in a temperature- and humidity-controlled room.

Anti-airborne bacterial activity assay. Bacterial cells were centrifuged at 2700 rpm for 10 min, washed with distilled water and resuspended in distilled water at a concentration of 10^6 cells per ml. The bacterial cell concentration was determined assuming an optical density of 1.0 at 540 nm was equivalent to ~10^9 cells per ml (14). Anti-airborne bacterial activities of the essential oils were assayed using agar plate air-sampling methods according to the (Merck, Darmstadt, Germany) manufacturer's instructions. A bacterial suspension

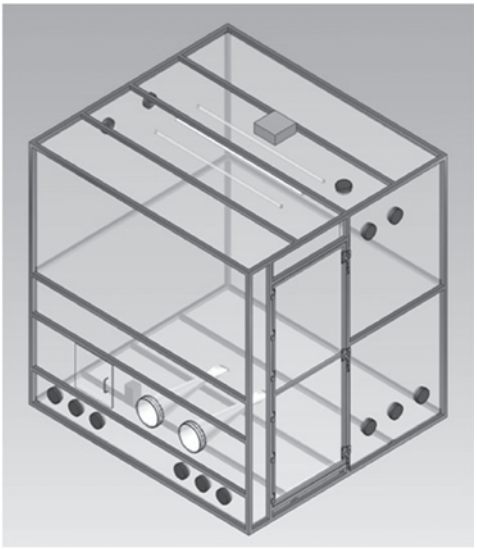

Figure 1. Schematic diagram of the bio-clean room.

was sprayed into the bio-clean room through the upper holes for 5 min using a spray gun (Dae-Won; DW-3; nozzle size, 0.5 mm; Gyeong-Sang, Korea). The aerosolized bacteria were allowed to stabilize for 5 min, and were then collected from the air of the bio-clean room (blank) into BAPs for 10 sec with an air sampler. Three different concentrations of essential oils (0.0005, 0.005 and 0.05 ppm) were sprayed into the bio-clean room for 5 min, and the airborne bacteria were collected every 10 min for 10 sec. The BAPs were sealed and incubated overnight at 37˚C. After 12 h, the resulting bacterial colonies were counted using a light box (Picker International, Cleveland, OH, USA) to analyze the decreasing concentrations of the airborne bacteria.

Data analysis. Data were presented as the mean ± standard error of mean (SEM) and were analyzed by a one-way analysis of variance (ANOVA) test followed by a Tukey's multiple comparison test. Statistical analysis was performed using Prism Graph Pad (version 4.0; GraphPad Software Inc., San Diego, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Anti-airborne bacterial activities of essential oils. The effects of three concentrations of essential oils on airborne bacteria are shown in Table I. Treatment with essential oils inhibited the growth of airborne bacteria. The growth of airborne bacterial colonies on the BAPs were decreased in a time-dependent manner for the control (distilled water spray).

Streptococcus pyogenes. In the presence of 0.0005 ppm essential oils, colony growth was clearly reduced at 50 min, an effect that persisted for 60 min. This decrease in the colony growth rate was more apparent in the presence of 0.005 and 0.05 ppm essential oils compared to 0.0005 ppm. In the presence of 0.005 and 0.05 ppm essential oils, colony growth was significantly decreased at 40 or 20 min, respectively. As shown

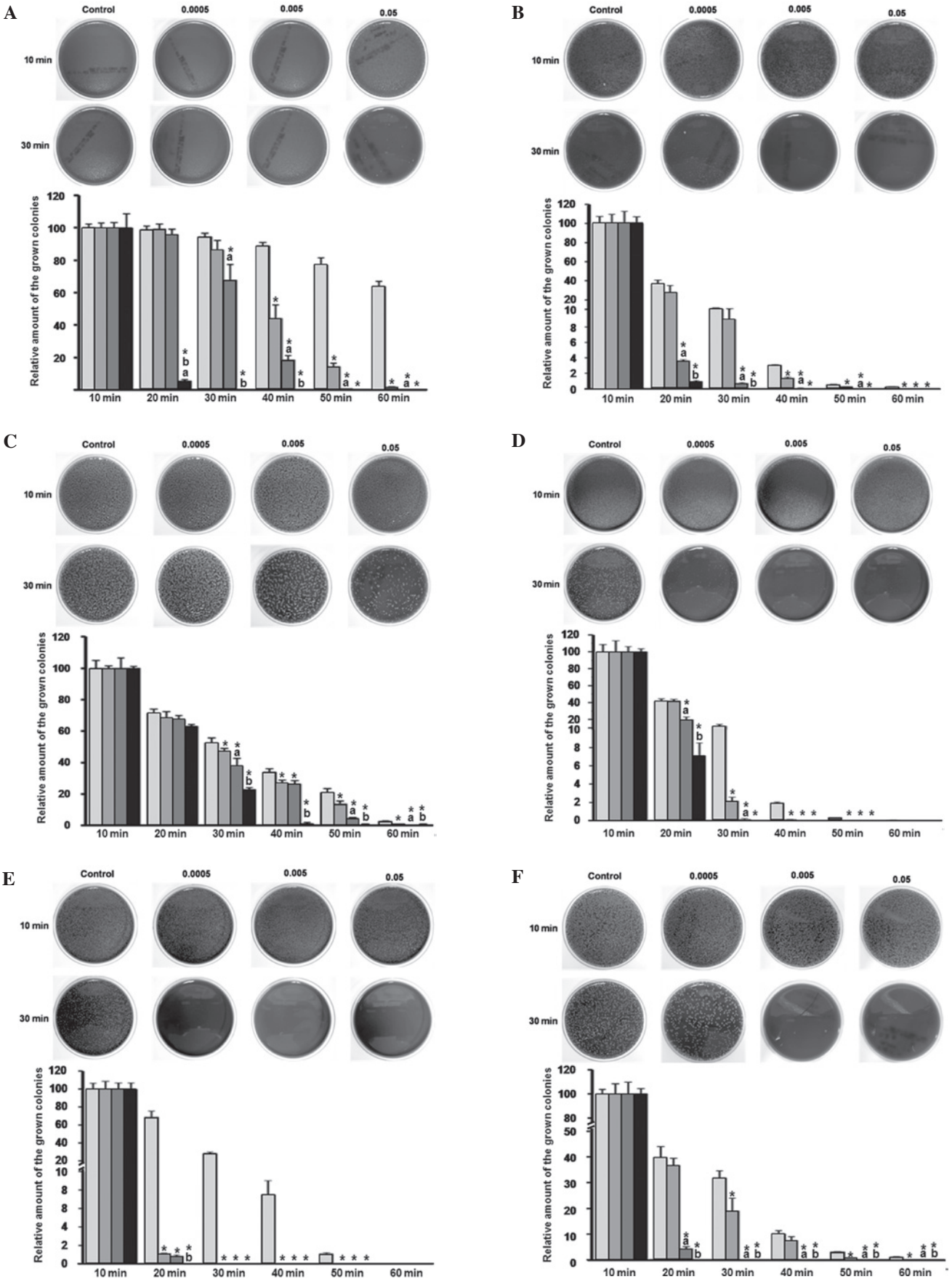

Figure 2. Anti-airborne bacterial activities of essential oils. Essential oils were administered at three concentrations (0.0005, 0.005 and 0.05 ppm). Distilled water was administered as the control. Airborne bacterial cells were first collected at 10 min (without essential oil, blank). (A) *Streptococcus pyogenes*, (B) *Streptococcus pneumonia*, (C) *Klebsiella pneumonia*, (D) *Neisseria meningitidis*, (E) *Hemophilus influenzae*, (F) *Escherichia coli* are shown at 10-60 min (with essential oils). Experiments were performed in triplicate. $^{*}P<0.05$ indicated a significant difference in the decreased rate of colony growth compared to the control; $^{a}P<0.05$ vs. 0.0005 ppm; $^{b}P<0.05$ vs. 0.005 ppm.

Table I. Number of colonies grown in the air sampled blood agar plates.

Bacteria	Essential oils treatment	No. of colonies collected after 10 min intervals (mean ± SEM)					
		10 min	20 min	30 min	40 min	50 min	60 min
E. coli	Control	4300±152	1715±177	820±119	435±52	131±10	51±5
	0.0005 ppm	5100±430	1875±137	1625±247	390±72	51±1	5±3
	0.005 ppm	4475±434	200±30	1±1	None	None	None
	0.05 ppm	3960±176	1±1	None	None	None	None
K. pneu	Control	2565±122	1835±63	1345±80	870±54	535±63	68±5
	0.0005 ppm	2690±42	1845±104	1275±40	730±41	370±45	35±6
	0.005 ppm	2225±145	1495±57	840±108	590±42	105±10	1±1
	0.05 ppm	2610±214	1645±132	600±75	28±7	1±1	None
H. influ	Control	12235±790	8430±804	3470±210	920±181	137±11	None
	0.0005 ppm	9780±833	113±3	None	None	None	None
	0.005 ppm	11235±757	98±12	None	None	None	None
	0.05 ppm	9445±645	None	None	None	None	None
S. pneu	Control	6845±510	2520±269	617±31	209±6	37±3	16±2
	0.0005 ppm	6800±659	1905±483	760±108	94±9	17±2	None
	0.005 ppm	3485±446	126±5	25±3	2±2	1±1	None
	0.05 ppm	3950±281	37±5	None	None	None	None
S. pyo	Control	10605±232	10475±233	10015±225	9390±233	8175±464	6755±326
	0.0005 ppm	10500±293	10425±301	9055±623	4620±870	1520±185	176±31
	0.005 ppm	8950±283	8560±304	6050±874	1650±232	74±5	15±4
	0.05 ppm	4935±418	271±43	27±3	7±4	2±2	1±1
N. meni	Control	13240±1130	5490±381	1630±223	256±14	42±1	6±2
	0.0005 ppm	12770±1638	5360±227	275±52	15±3	None	None
	0.005 ppm	13450±786	2695±410	19±11	None	None	None
	0.05 ppm	11720±404	835±164	None	None	None	None

SEM, standard error of mean; *E. coli, Escherichia coli; K. pneu, Klebsiella pneumonia*; *H. influ, Hemophilus influenzae*; *S. pneu, Streptococcus pneumonia*; *S. pyo, Streptococcus pyogenes*; *N. meni; Neisseria meningitidis*.

in Fig. 2A, these effects persisted for 60 min compared to the control (distilled water spray).

Streptococcus pneumoniae. A total of 30 min after treatment with 0.0005 ppm essential oils, colony growth decreased, but this was not observed after 60 min. The essential oils decreased airborne bacterial colony growth in a dose-dependent manner. In the presence of 0.005 and 0.05 ppm essential oils, colony growth was significantly decreased at 20 min. In addition, we observed little colony growth from air samples collected 20 min after treatment with 0.05 ppm essential oils (Fig. 2B).

Klebsiella pneumoniae. Colony growth decreased at 60 min following treatment with 0.0005 ppm essential oils compared to the control. However, this decreased rate of colony growth was lower than that observed following treatment with higher concentrations of essential oils. After treatment with 0.005 and 0.05 ppm essential oils, colony growth was suppressed at 30 min and 20 min, respectively. Additionally, anti-airborne bacterial activities of essential oils (0.005 and 0.05 ppm) completely repressed the growth of colonies in essential oils (0.05 ppm) at 60 min (Fig. 2C).

Neisseria meningitidis. Following treatment with 0.0005 ppm essential oils, colony growth decreased at 30 min, an effect that was observed at 60 min. In the presence of 0.005 and 0.05 ppm essential oils, growth of the colonies was decreased at 30 min. Anti-airborne bacterial activities of essential oils (0.005 and 0.05 ppm) completely repressed colony growth at various concentrations of essential oils (0.005 and 0.05 ppm, and 0.005 ppm) at 40-60 min, and 0.05 ppm at 30-60 min (Fig. 2D).

Hemophilus influenzae. As shown in Fig. 2E, bacterial colony growth was not observed at 60 min for the control (distilled water spray). The lowest concentration of essential oils (0.0005 ppm) inhibited the growth of colonies at 30-50 min. In the presence of greater concentrations of essential oils (0.005 ppm and 0.05 ppm), colony growth was completely suppressed at 30-60 min.

Escherichia coli. In the presence of essential oils (0.0005, 0.005 and 0.05 ppm), the growth of bacterial colonies was completely decreased at 60, 30 and 20 min, respectively. We did not observe colony growth after treatment with 0.05 ppm essential oils at any time (Fig. 2F).

Discussion

The walls of the bio-clean room were composed of non-interrupted acrylic plates, which are required for the successful aerosolization of bacterial cells. A Hypalon® glove attached to the front down panel of the bio-clean room protected the user's hands against the airborne bacterial cells. In this study, the effects of essential oils on airborne bacteria were examined in a bio-clean room. Essential oils, which are aromatic and volatile products of plant secondary metabolism, are widely used in folk medicine, food flavoring and preservation, and fragrances. The anti-bacterial activities of essential oils from various plants have been described by numerous studies (15-17).

Essential oils have been used medicinally (18,19). The use of essential oils has been revived in the past few decades with the increasing popularity of aromatherapy, a branch of alternative medicine that claims that essential oils and other aromatic compounds have curative effects. The oils are distilled or volatilized in solutions and used for massages. Essential oils can also be dispersed into the air by a spray or heated over a candle flame for aromatherapy (20). In this study, we evaluated the anti-airborne bacterial activities of three concentrations of essential oils (0.0005, 0.005 and 0.05 ppm) dispersed by artificial essential oil humidification on Gram-negative and -positive bacteria.

During the dry seasons, warm and humid environments affect the response of plasma pro- and anti-inflammatory cytokines (21). Dry eye is one of the most common pathological manifestations of chronic graft-versus-host disease, occurring in up to 80% of patients (22). Low or high humidity may cause specific physical discomfort since relative humidity directly affects temperature perception (23). Diseases may be transmitted by airborne pathogens, direct contact with pathogens living on hard surfaces or by touching an infected person. Low humidity has been found to improve the survival of rhinoviruses, influenza virus (24) and human rotavirus (25,26). The incidence of airborne-transmitted infectious disease in an indoor environment is dependent on a number of factors, including the concentration of aerosolized pathogens, ventilation rate and survival of pathogens attached to the aerosol (20). The indoor relative humidity may affect some of these factors, including settling rate of the aerosols and survival of the airborne pathogens (27).

Patients and healthy individuals require optimal humidification to maintain a moisturized respiratory tract necessary for protection against bacterial infection during the drier winter months. However, humidifiers are associated with certain health risks. For example, the water tank and tract of humidifiers are easily polluted by airborne pathogens and allergens. Humidifier disinfectants have been regularly used to clean humidifiers. However, warnings about the health risks associated with humidifier disinfectants have been issued by the Korean Center for Disease Control and Prevention advising against the use of humidifier sterilizers. The notification was issued after a civic group claimed that 18 pregnant females succumbed to lung disease which was considered to have been caused by disinfectants in the machines.

The results from our study demonstrated that the growth of airborne bacterial cells decreased following exposure to three different concentrations of essential oils (0.0005, 0.005 and 0.05 ppm) in a customized bio-clean room. It is possible that these essential oils may be used as anti-airborne bacterial agents, and humidifier disinfectants in commercial humidifiers and air cleaners. However, the mechanisms underlying the anti-airborne bacterial effects of essential oils require further examination.

Acknowledgements

This study was supported by a grant from the Woongjin and the Next-generation BioGreen 21 Program (No. PJ008323), Rural Development Administration, Republic of Korea.

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