



Antimicrobial activity of essential oils against *Escherichia coli* O157:H7 in organic soil [☆]

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ABSTRACT

Soil can be a significant source of preharvest contamination of produce by pathogens. Demand for natural pesticides such as essential oils for organic farming continues to increase. We examined the antimicrobial activity of several essential oils against *Escherichia coli* O157:H7 in soil. Two essential oils (cinnamaldehyde and eugenol), two bio-pesticides (Ecotrol and Sporan) containing essential oils, and an organic acid (acetic acid) at 0.5%, 1.0%, 1.5% and 2.0%, were mixed with organic sandy soil and inoculated with five different strains of *E. coli* O157:H7. Soils were incubated at room temperature (22 °C) and samples obtained at 1, 7 and 28 days were enumerated to determine survival. *E. coli* O157:H7 populations in soil were reduced by up to 5 log cfu/g after 24 h incubation at room temperature with 2% cinnamaldehyde, Ecotrol, Sporan or vinegar. Reduction in *E. coli* O157:H7 by eugenol was not significantly different from control. Overall, *E. coli* O157:H7 strain 4406 was the most sensitive of all the five strains tested and cinnamaldehyde was superior to other treatments in reducing *E. coli* O157:H7 in soil. In general, increases in essential oil concentrations corresponded to reduced survival of *E. coli* O157:H7 with all oils used in this study. The results suggest that oils can reduce potential contamination of fresh organic produce inadvertently contaminated by soil.

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1. Introduction

Foodborne diseases continue to be a serious threat to public health all over the world. The incidence of illnesses appears to be increasing on a global basis including developed and industrialized countries. Foodborne illness outbreaks associated with *Escherichia coli* O157:H7 on meat and fresh produce products have occurred in the US since 1982 despite awareness and diligence by industry. With 76 million estimated illnesses, more than 300,000 hospitalizations, and 5000 deaths annually in the US attributed to foodborne illness (Mead et al., 1999), the associated annual estimated economic loss ranges from \$5–6 billion (Murphy, Duncan, Driscoll, Marcy, & Beard, 2003). Consumption of refrigerated ready-to-eat (RTE), fresh-cut fruits and vegetables, often eaten with minimal processing, are a potential source of foodborne infection.

In 2006 consumption of contaminated raw spinach killed three, brought devastating kidney failure to 23, hospitalized more than 75, and sickened 205 people in the US. The spinach was traced back to product grown, processed, and packaged in California by the largest producer of organically certified lettuce and spinach in the United States (Avery, 2006). As was subsequently reported in a study of 15 Minnesota farms, organic produce was six times more likely to be contaminated with *E. coli* (non-pathogenic), than conventional produce (Mukherjee, Speh, Dyck, & Diez-Gonzalez, 2004).

Workers, visitors, animal feces, equipments, improper composting and farm runoff have been suggested as sources of contamination of field grown fresh produce. Due to the limited options for treatment of O157:H7 illnesses and lack of human vaccines, avoiding exposure is currently the most viable option (Karmali, 1998; Li, Frey, Mackenzie, & Finlay, 2000). Therefore the prevention of infection requires control measures at all stages of the food chain, from agricultural production on the farm to the table of consumers. Recent studies have linked *E. coli*, a traditional indicator of fecal contamination, to unexpected (non-fecal) habitats including a variety of soils across different climatic regions. Persistence of *E. coli* in non-host environments, has led to the suggestion that *E. coli* may no longer be useful as a fecal indicator organism (Power, Littlefield-Wyer, Gorgon, Veal, & Slade, 2005). Although *E. coli*

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O157:H7 are associated with feces from livestock and wildlife, it is clear that they are transported in surface runoff, and accumulate in sediments and soils contaminated by these animals (Meals & Braun, 2006; Millner, 2009). These bacteria may also migrate into groundwater (Brabban, Nelsen, Kutter, Edrington, & Callaway, 2004). In addition, *E. coli* O157:H7 survives, replicates, and moves within soil, and the presence of manure enhances this survival (Gagliardi & Karns, 2000). Evidence shows it survives in coastal subtropical soils even after drying (Solo-Gabriele, Wolfert, Demarais, & Palmer, 2000) and in agricultural soils well after manure application (Topp et al., 2003). Once *E. coli* populations are established in soil, a portion can become naturalized or autochthonous and even survive freeze/thaw cycles (Ishii, Ksoll, Hicks, & Sadowsky, 2006). Nematodes can vector *E. coli* and contribute to its spread and persistence in soil (Anderson, Millner, Beuchat, & Williams, 2006). Soil reservoirs pose a serious risk to public health primarily through the fresh food chain.

Only a few ways have been suggested for eliminating *E. coli* O157:H7 from manure. Composting and anaerobic digestion, along with some advanced manure management technologies, and the addition of various chemicals, such as lime, have been used successfully, to reduce pathogen levels (Millner, 2009). Eliminating pathogens from livestock would aid in reducing soil and water contamination with various fecal pathogens. Researchers are currently investigating several approaches to eliminating *E. coli* O157:H7 from livestock, including antibiotics, antimicrobials, probiotics, vaccines and bacteriophage (Brabban, Hite, & Callaway, 2005).

Widespread use of pesticides has significant drawbacks including increased cost, handling hazards, concerns about pesticide residues on food, and threats to human health and environment (Paster & Bullerman, 1988). Public demand for safe produce has increased interest in investigating alternative soil and crop management practices that do not rely on use of synthetic chemical pesticides and fertilizers. Essential oils with pesticidal activity are increasingly used in organic production systems because they tend to have low mammalian toxicity, few non-target environmental effects, and wide public acceptance (Paranagama, Abeysekera, Abeywickrama, & Nugaliyadd, 2003; Paster, Menasherov, Rachid, & Juven, 1995). Essential oils are volatile compounds produced by plants as secondary metabolites in particular cells or formed as glandular hairs (Hili, Evans, & Veness, 1997). Among these natural antimicrobials are eugenol (85%) from clove oil (Farag, Daw, Hewedi, & El-Baroty, 1989), thymol and oregano, carvacrol from oregano and thyme oils, vanillin from vanilla, allicin from garlic, cinnamic-aldehyde from cinnamon, and allyl isothiocyanate from mustard (Tzortzakakis, 2008). Ecotrol, a concentrated, commercial blend of rosemary and peppermint oils (10% and 2%, respectively), is a broad spectrum contact insecticide/miticide effective against many insects. It has minimal environmental impact in a formulation suitable for both conventional and organic applications (Anonymous, 2009a). It can be applied to agricultural crops including vegetables, herbs and spices, citrus, pome and stone fruits, nuts, berries, fruits, and grapes (Anonymous, 2005). Sporan is a curative and preventive contact fungicide useful against a broad range of diseases, including but not limited to blights, molds, scabs, and mildews (Anonymous, 2009b). It is composed of rosemary, clove and thyme oils and can be applied to a wide variety of agricultural crops. Sporan and Ecotrol EC are listed by the Organic Material Review Institute for use in organically certified production systems.

Organic acids and their salts are promising agents because of their acceptance in food products and low cost (Miller, Call, & Bowles, 1996). Organic acids have been tested for disinfecting meat, fish and minimally processed fruits and vegetables. The antimicrobial activity of organic acids is due to the pH reduction, depression of internal pH of microbial cell and disruption of substrate transport by altering cell membrane permeability (Beuchat, 1998). Ace-

tic acid at 10–20% concentration has been used as a burn down, non-selective, organic herbicide (Dayan, Cantrell, & Duke, 2009). No data are currently available on efficacy of essential oils or commercial products containing such oils in soil. Hence quantitative data are needed on the antimicrobial activities of essential oils to determine their efficacy in reducing *E. coli* O157:H7 in a main component of an organic production environment, the soil. In this study, we evaluated the inhibitory effect of cinnamaldehyde, Ecotrol, eugenol, Sporan and vinegar against *E. coli* O157:H7 in organic soil.

2. Materials and methods

2.1. Preparation of bacterial strains

Five nalidixic acid resistant strains of *E. coli* O157:H7 were used in the study. The strains RM 4406, RM 4688, and RM 1918 (clinical isolates from lettuce outbreaks), RM 4407 (clinical isolate from spinach outbreak), and RM 5279 (clinical isolate, bagged vegetable isolate) were kindly provided by Robert Mandrell (US Department of Agriculture, Agricultural Research Service, Albany, CA). All cultures were maintained at -80°C in 20% glycerol. Each strain was aseptically sub-cultured in tryptic soy broth (TSB, Acumedia, Lansing, MI) supplemented with 50 mg/l nalidixic acid (TSBN, Sigma–Aldrich, St. Louis, MO) for 24 h at 37°C . Cells were centrifuged (7500g, 10 min, 10°C), and cell pellets were suspended in sterile 0.1% peptone water (Acumedia). The cell density of individual strains was adjusted to obtain final concentration to 8 log cfu/ml. The populations of individual strains were verified on tryptic soy agar containing 50 mg/l nalidixic acid (TSAN) by spot plate technique.

2.2. Essential oil treatments

Cinnamaldehyde (Sigma–Aldrich), Ecotrol (EcoSMART Tech., Alpharetta, GA), Eugenol (Fisher Scientific, Pittsburg, PA), Sporan™ (EcoSMART Tech.), and vinegar (20%, Knouse Foods, Biglersville, PA) were used in the study. The desired concentrations (0.5%, 1.0%, 1.5% and 2.0%) of these treatments were freshly prepared before each use by dispersing them in a sterile distilled water containing 0.5% (w/v) Tween 20 (Fisher Scientific, Pittsburg, PA). Most of the studies about spices or their essential oils are conducted in vitro conditions and an emulsifier or solvent such as ethanol, methanol or Tween are used to dissolve essential oils (Burt, 2004). The suspension was vortexed before using in soil.

2.3. Inoculation of soil

Soils (Downer–Ingleside loamy sand, Coarse-loamy, siliceous, semiactive, mesic Typic Hapludults) were obtained from the USDA Beltsville Agricultural Research Center North Farm high tunnels managed for 4 years according to USDA–National Organic Program guidelines. Soil (10 g) was placed into a sterile whirl-pak filter bag (Nasco, Fort Atkinson, WI) and inoculated with 100 μL of designated inocula to obtain 6 log cfu/g soil. Samples were mixed vigorously to distribute the inoculum, and each desired concentration (0.5%, 1%, 1.5%, and 2.0%) was added in soil and mixed again. The bags were closed, and incubated at room temperature (22°C) for 28 days. Soil samples inoculated with *E. coli* O157:H7 and 100 μL peptone water (Acumedia) served as control.

2.4. Enumeration of *E. coli* O157:H7

On each sampling day (1, 7, and 28 days), 10 ml (w/v) of sterile peptone water (0.1%) was added to each soil bag and the bag was

pummeled for 2 min (Bagmixer, Interscience, St. Nom, France). Appropriately diluted suspensions were spiral plated on Sorbitol MacConkey media (Acumedia) supplemented with 0.05 mg/l of cefixime, 2.5 mg/l of potassium tellurite (Invitrogen, Carlsbad, CA, CtSMAC) and 50 ppm nalidixic acid (Sigma–Aldrich, CtSMAC-N), and incubated at 37 °C for 24 h. Presumptive colonies of *E. coli* O157:H7 were confirmed using Dry Spot latex agglutination assay (Remel, Lenexa, KS).

2.5. Statistical analysis

E. coli O157:H7 populations obtained at each sampling period from soil treated with different oils were converted to log cfu/g. The experiment was performed in triplicate. The data were analyzed by a two-way ANOVA using 'Proc Mixed' (SAS 8.2, Cary, NC) for the interaction effects of the oil, oil concentration, bacterial strain and sampling period. In all cases, the level of statistical significance level was of $P < 0.05$. Further, the data were analyzed by quadratic response surface model for each oil concentration applied to each strain using "Proc RSReg" statement (SAS 8.2). Contour graphs were produced for each of the strain and oil concentration interaction.

3. Results

3.1. Inactivation of *E. coli* O157:H7 in soil after 24 h

E. coli O157:H7 were not detected in uninoculated soil used in this study. The effect of cinnamaldehyde, Ecotrol, eugenol, Sporan and vinegar on survival of *E. coli* O157:H7 in soil are presented in Tables 1–3. The populations of *E. coli* O157:H7 varied from 5.10 to 6.55 log cfu/g in inoculated control.

In general, the antibacterial effect of oils varied with the *E. coli* O157:H7 strains. The strain 4406 was the most sensitive to these oils followed by strain 4407, 4688, 5279, and 1918. Cinnamaldehyde significantly reduced *E. coli* O157:H7 in soil compared to other oils used in this study. At 0.5% level, cinnamaldehyde was the most effective antimicrobial in reducing *E. coli* O157:H7 in soil

(Table 1). Populations of *E. coli* O157:H7 strain 4407 recovered from soil treated with 0.5% cinnamaldehyde (4.57 log cfu/g) were significantly lower than those recovered from control soil (6.55 log cfu/g). Vinegar at 0.5% reduced *E. coli* O157:H7 strain 4406 by 1.6 log cfu/g. The effect of Ecotrol, eugenol, and Sporan at 0.5% was not evident in reducing *E. coli* O157:H7. The populations of *E. coli* O157:H7 were reduced with an increase in concentrations of cinnamaldehyde, Sporan, and vinegar. However, increasing concentration of these treatments from 0.5% to 1% did not yield significant reduction of *E. coli* O157:H7 in soil. *E. coli* O157:H7 populations recovered from soil treated with 1% cinnamaldehyde were significantly lower than the *E. coli* O157:H7 recovered from control soil. The *E. coli* O157:H7 populations in soils treated with 1.5% cinnamaldehyde were significantly lower than the *E. coli* O157:H7 populations recovered from soil treated with 0.5% or 1% cinnamaldehyde. At least 5 log reductions in *E. coli* O157:H7 strains 4688 and 5279 were observed with 1.5% cinnamaldehyde. *E. coli* O157:H7 recovered from soils treated with 1.5% eugenol or vinegar was not significantly different from those treated with 0.5% eugenol or vinegar. Strain 4407 was the most vulnerable to 1.5% Sporan with 4 log reduction followed by strains 4406, 1918 and 4488 with 3 log cfu/g reductions. Populations of *E. coli* O157:H7 strains 4407, 5279 and 4688 were non-detectable (detection limit 1.39 log cfu) in 2% cinnamaldehyde-treated soil after 24 h. Likewise, complete inhibition of strain 4406 and 4407 was observed with 2% concentration of Sporan or Ecotrol.

3.2. Inactivation of *E. coli* O157:H7 in soil after 7 days

After 7 days, *E. coli* O157:H7 populations remained either identical or increased in most treated soil samples with the exception of 0.5% and 1% cinnamaldehyde, 1.5% Sporan, and 1.5% vinegar treatment. Recovery of *E. coli* O157:H7 strains 4407, 1918, and 4688 after 7 days in soil treated with 1% cinnamaldehyde (1.72, 1.37, and 0.87 log cfu/g) were significantly lower than those recovered after 24 h (3.60, 3.64, and 3.77 log cfu/g), respectively. Likewise, significant reduction of *E. coli* O157:H7 strains 4407, 1918, 4688, and 5279 was observed in soils treated with 1.5% vinegar

Table 1
Impact of essential oils and vinegar on *E. coli* O157:H7 in soil after 24 h.

Treatment	Conc.	Populations of <i>E. coli</i> O157:H7 strains (log cfu/g) ^{a, b}				
		4406	4407	1918	5279	4688
Control	0	5.10 ± 0.92	6.55 ± 0.91	6.06 ± 0.98	5.50 ± 0.97	6.02 ± 0.75
Cinnamaldehyde	0.5	4.07 ± 0.40A	4.57 ± 0.46A	4.69 ± 0.72A	4.30 ± 0.53A	4.69 ± 0.27A
	1	2.59 ± 0.26A	3.60 ± 0.17A	3.64 ± 0.34A	3.65 ± 0.14A	3.77 ± 0.21A
	1.5	0.00 ± 0.00B	0.73 ± 0.26B	0.57 ± 0.48B	0.00 ± 0.00B	0.00 ± 0.00B
	2	0.77 ± 0.33B	0.00 ± 0.00B	1.09 ± 0.88B	0.00 ± 0.00B	0.00 ± 0.00B
Ecotrol	0.5	3.95 ± 0.27A	5.38 ± 0.60A	5.65 ± 0.43A	5.51 ± 0.53A	4.69 ± 0.42A
	1	4.33 ± 1.13A	4.91 ± 0.18A	5.11 ± 0.28A	5.17 ± 0.43A	4.58 ± 0.50A
	1.5	3.08 ± 0.64A	4.00 ± 1.15A	3.95 ± 0.53A	4.13 ± 0.71A	3.95 ± 0.61A
	2	0.00 ± 0.00B	0.00 ± 0.00B	1.12 ± 0.593B	1.31 ± 0.27B	1.09 ± 0.88B
Eugenol	0.5	4.34 ± 1.30A	5.79 ± 0.65A	5.99 ± 0.10A	6.01 ± 0.26A	6.32 ± 0.37A
	1	3.94 ± 0.17A	5.37 ± 1.28A	5.70 ± 0.42A	5.37 ± 0.96AB	3.70 ± 0.45A
	1.5	4.59 ± 1.38A	4.76 ± 0.91AB	3.29 ± 0.23B	3.67 ± 1.16BC	3.67 ± 0.45B
	2	1.66 ± 0.87B	3.60 ± 0.19B	3.32 ± 0.29B	2.80 ± 0.38C	1.98 ± 0.43B
Sporan	0.5	4.6 ± 1.11A	5.65 ± 0.54A	5.66 ± 0.71A	5.78 ± 0.20A	5.77 ± 0.62A
	1	3.49 ± 0.65AB	4.43 ± 0.09A	5.08 ± 0.29AB	4.57 ± 0.53AB	5.01 ± 0.19AB
	1.5	2.87 ± 0.47B	2.42 ± 0.30B	3.82 ± 0.99B	3.71 ± 0.70B	3.31 ± 0.66B
	2	0.00 ± 0.00C	0.00 ± 0.00C	1.39 ± 0.40C	0.87 ± 0.50C	0.82 ± 0.43C
Vinegar	0.5	3.77 ± 0.27A	5.55 ± 0.15A	5.77 ± 0.24A	5.37 ± 0.28A	5.53 ± 0.17A
	1	3.91 ± 0.15A	5.54 ± 0.20A	5.33 ± 0.57A	5.64 ± 0.28A	4.57 ± 1.63A
	1.5	3.00 ± 0.50A	3.86 ± 0.52A	4.30 ± 1.48A	4.37 ± 0.67A	3.96 ± 0.70A
	2	0.00 ± 0.00B	0.90 ± 0.56B	1.51 ± 0.62B	1.66 ± 0.87B	0.77 ± 0.33B

^a Mean ± standard deviation.

^b Means in the same column within the treatment with different letters are significantly different ($P < 0.05$).

Table 2Impact of essential oils and vinegar on *E. coli* O157:H7 in soil after 7 days.

Treatment	Conc.	Populations of <i>E. coli</i> O157:H7 strains (log cfu/g) ^{a, b}				
		4406	4407	1918	5279	4688
Control	0	4.18 ± 1.61	5.76 ± 0.63	5.76 ± 1.35	5.22 ± 0.93	5.82 ± 0.64
Cinnamaldehyde	0.5	0.67 ± 0.15A	2.86 ± 0.25A	3.74 ± 0.64A	3.33 ± 0.35A	3.62 ± 0.22A
	1	1.61 ± 0.40A	1.72 ± 0.51A	1.37 ± 0.23B	2.50 ± 0.75A	0.87 ± 0.50BC
	1.5	1.23 ± 0.58A	0.83 ± 0.43B	0.93 ± 0.60B	2.86 ± 1.01A	2.15 ± 0.41AB
	2	0.00 ± 0.00A	0.00 ± 0.00B	0.00 ± 0.00B	0.00 ± 0.00B	0.00 ± 0.00C
Ecotrol	0.5	4.84 ± 0.79A	5.76 ± 1.00AB	6.92 ± 0.42A	6.05 ± 0.56A	6.51 ± 0.56A
	1	4.94 ± 1.25A	5.81 ± 0.97A	6.33 ± 0.57A	5.34 ± 1.08A	5.81 ± 0.25A
	1.5	2.34 ± 0.41B	4.04 ± 0.33B	4.14 ± 0.40B	2.84 ± 0.57B	3.09 ± 1.21B
	2	1.91 ± 1.13B	1.97 ± 0.41C	0.57 ± 0.48C	1.70 ± 0.99B	0.00 ± 0.00C
Eugenol	0.5	4.30 ± 0.52A	6.45 ± 0.64A	6.79 ± 0.36A	6.45 ± 0.33AB	5.80 ± 1.98AB
	1	5.02 ± 0.41A	6.68 ± 0.75A	6.93 ± 0.46A	6.80 ± 1.15A	7.14 ± 0.50A
	1.5	3.94 ± 0.95A	5.98 ± 0.10A	5.70 ± 0.33A	4.99 ± 0.70B	4.38 ± 1.06BC
	2	5.07 ± 0.56A	6.05 ± 0.11A	6.05 ± 0.04A	3.25 ± 0.56C	3.79 ± 0.81C
Sporan	0.5	4.64 ± 0.46A	6.32 ± 0.98A	6.94 ± 0.23A	6.72 ± 0.36A	6.86 ± 0.43A
	1	5.33 ± 1.06A	6.25 ± 1.56A	6.54 ± 0.94A	5.83 ± 1.49A	5.66 ± 0.96A
	1.5	0.67 ± 0.55B	1.33 ± 1.15B	2.89 ± 0.66B	1.96 ± 0.24B	3.12 ± 0.24B
	2	0.57 ± 0.48B	0.00 ± 0.00B	1.36 ± 0.36B	1.87 ± 0.62B	0.00 ± 0.00C
Vinegar	0.5	2.23 ± 1.96A	4.65 ± 0.24A	5.80 ± 0.07A	5.28 ± 0.61A	5.44 ± 0.36A
	1	3.28 ± 0.17AB	4.33 ± 0.64A	4.98 ± 0.64A	4.53 ± 0.44A	4.24 ± 0.27A
	1.5	0.77 ± 0.33BC	2.19 ± 0.85B	2.39 ± 0.82B	1.81 ± 0.87B	1.63 ± 0.59B
	2	0.00 ± 0.00C	0.00 ± 0.00C	0.00 ± 0.00C	0.73 ± 0.26B	0.00 ± 0.00B

^a Mean ± standard deviation.^b Means in the same column within the treatment with different letters are significantly different ($P < 0.05$).**Table 3**Impact of essential oils and vinegar on *E. coli* O157:H7 in soil after 28 days.

Treatment	Conc.	Populations of <i>E. coli</i> O157:H7 strains (log cfu/g) ^{a, b}				
		4406	4407	1918	5279	4688
Control	0	4.83 ± 2.08	4.33 ± 0.44	5.52 ± 0.60	5.17 ± 1.96	5.25 ± 1.67
Cinnamaldehyde	0.5	4.40 ± 0.66A	4.33 ± 1.24A	2.47 ± 1.51A	4.35 ± 0.54A	4.27 ± 1.09A
	1	1.76 ± 0.66B	0.67 ± 0.15B	0.00 ± 0.00B	0.00 ± 0.00B	0.57 ± 0.38B
	1.5	1.19 ± 0.56B	0.00 ± 1.26B	0.00 ± 0.00B	1.54 ± 0.66B	1.27 ± 0.20B
	2	0.57 ± 0.48B	1.19 ± 0.55B	0.00 ± 0.00B	0.00 ± 0.00B	0.77 ± 0.33B
Ecotrol	0.5	3.82 ± 0.31A	4.16 ± 0.15A	5.92 ± 0.05A	5.43 ± 0.56A	5.29 ± 0.05A
	1	2.51 ± 0.82A	3.67 ± 0.35A	5.02 ± 0.21A	3.46 ± 1.03B	4.21 ± 0.57A
	1.5	2.26 ± 0.49A	0.67 ± 0.55B	3.16 ± 0.55B	2.88 ± 0.20B	1.67 ± 1.46B
	2	0.00 ± 0.00B	0.00 ± 0.00B	0.00 ± 0.00C	1.01 ± 0.74C	1.49 ± 1.29B
Eugenol	0.5	4.32 ± 0.62A	5.28 ± 0.17A	5.44 ± 0.31A	4.91 ± 0.64A	4.77 ± 0.70A
	1	3.59 ± 0.53A	5.03 ± 0.91A	5.43 ± 0.79A	5.51 ± 0.94A	5.47 ± 0.11A
	1.5	3.21 ± 1.07A	4.38 ± 0.31A	4.67 ± 0.81A	1.80 ± 0.56B	4.10 ± 0.95A
	2	4.26 ± 1.13A	5.33 ± 0.59A	5.50 ± 0.31A	4.46 ± 0.62A	5.05 ± 0.86A
Sporan	0.5	4.69 ± 0.75A	3.33 ± 1.61AB	6.43 ± 0.19A	5.75 ± 0.78A	5.30 ± 0.33A
	1	3.28 ± 0.52AB	4.27 ± 0.24A	5.35 ± 0.51A	5.31 ± 0.92A	4.21 ± 0.62A
	1.5	2.14 ± 0.42B	2.18 ± 0.43B	2.00 ± 0.30B	1.57 ± 0.37B	2.12 ± 0.10B
	2	1.64 ± 0.85B	0.00 ± 0.00C	1.38 ± 0.40B	1.81 ± 0.57B	0.00 ± 0.00C
Vinegar	0.5	4.21 ± 1.45A	4.20 ± 0.62A	5.83 ± 0.35A	5.79 ± 0.94A	5.91 ± 0.26A
	1	1.48 ± 0.38B	0.00 ± 0.00B	3.82 ± 0.45B	4.40 ± 1.22A	5.46 ± 1.22A
	1.5	0.00 ± 0.00B	0.00 ± 0.00B	0.00 ± 0.00C	0.00 ± 0.00B	0.00 ± 0.00B
	2	0.00 ± 0.00B	0.00 ± 0.00B	1.06 ± 0.84C	0.00 ± 0.00B	0.00 ± 0.00B

^a Mean ± standard deviation.^b Means in the same column within the treatment with different letters are significantly different ($P < 0.05$).

after 7 days in comparison to *E. coli* O157:H7 populations recovered after 24 h. In contrast, Sporan and eugenol at 0.5% level in soil increased *E. coli* O157:H7 populations by ca. 1 log after 7 days. Soil treatment with 2% eugenol also resulted in increase of up to 3 log cfu/g *E. coli* O157:H7 strains 4407 and 1918 after 7 days. After 7 days of incubation, all *E. coli* O157:H7 strains were non-detectable in soil treated with 2.0% cinnamaldehyde, or with 2% vinegar except strain 5279. *E. coli* O157:H7 strains 4407 and 4688 were not recovered in soil treated with 2% Sporan.

3.3. Inactivation of *E. coli* O157:H7 in soil after 28 days

E. coli O157:H7 populations in treated soil were reduced further with most treatments during 28 days of incubation at room temperature (22 °C). However the difference in recovery of *E. coli* O157:H7 between 7 and 28 days were not significant at 0.5% level of these treatments except Sporan with strain 4407. Further, significant reductions were observed mainly with strain 5279 and 440 at 1% and 1.5% levels. In general, the increased concentration of test

compounds in the soil was associated with increased bacterial inhibition after 28 days. Cinnamaldehyde and Ecotrol at 2% levels reduced populations of *E. coli* O157:H7 strains 4407 and 1918 to undetectable level. Similarly, vinegar at 1.5% and 2% level reduced all *E. coli* O157:H7 strains to non-detectable levels. When compared at 28 days, *E. coli* O157:H7 4406 and 4407 were the most sensitive strains at 0.5% or 1% levels of Ecotrol, eugenol and Sporan. Vinegar, cinnamaldehyde, and Ecotrol were the most effective treatment when compared at 28 days. Overall, eugenol was the least effective in reducing *E. coli* O157:H7 in soil.

3.4. Quadratic response surface model for predicting *E. coli* O157:H7 populations

E. coli O157:H7 populations in soil were predicted using quadratic response surface modeling analysis. The model predicted that cell counts of the individual strains of *E. coli* O157:H7 would be less than 1 log cfu/g in soil as early as 1 day when 2% cinnamaldehyde, Ecotrol, or Sporan is used in the soil. Soil treatment with 1.5% cinnamaldehyde would take at least 2 weeks to reduce populations of *E. coli* O157:H7 strain 1918 to one log cfu/g of soil. While vinegar at 2% would reduce *E. coli* O157:H7 in soil to 1 log cfu/g, the time required to achieve this reduction would vary with the strain. Eugenol at any concentration from 0.5% to 2.0% would not be effective in achieving cell concentrations of less than 1 log at any time. *E. coli* O157:H7 populations would be at least 2 log cfu/g if eugenol was used in soil. Representative contour charts (Fig. 1) indicated how survival of *E. coli* O157:H7 in soils will be affected by different treatments. With increase in concentration, *E. coli* O157:H7 populations changed as indicated in line patterns (each line represents specific log cfu).

4. Discussion

Among fresh produce, lettuce appears to be more susceptible to bacterial contamination. Not only have a number of outbreaks caused by *E. coli* O157:H7 (Tauxe et al., 1997) been linked to the consumption of lettuce, but recent evidence suggests that food-borne pathogens can be internalized into lettuce leaves (Solomon, Yaron, & Mathews, 2002). That report provided evidence that *E. coli* O157:H7 could be transmitted from contaminated manure and irrigation water applied to soil into the subsurface tissues of lettuce leaves. Moreover, Wachtel, Whitehand, and Mandrell (2002) found predominant attachment of *E. coli* O157:H7 to the roots both singly and in small aggregates.

Ecotrol and Sporan at the lower concentrations (0.5% and 1.0%) reduced *E. coli* O157:H7 by 1–2 log cfu within 24 h. However, the populations of *E. coli* O157:H7 were increased following 7 days. The greater availability of nutrients in soils may enable bacteria to repair cells. Repair of injured cells might have influenced increase in *E. coli* O157:H7 populations at 7 days. *E. coli* O157:H7 populations reduced further with storage time. While eugenol reduced *E. coli* O157:H7 in soil, it was the least effective among other oils used in the study. Our results are in agreement with Kim, Marshall, and Wei (1995) who also reported marginal inhibitory effect of eugenol against *E. coli* O157:H7 in liquid media. Moreover, evaporation and homogenization of eugenol may have affected its efficacy in reducing bacteria. Although Tween 20 was used to increase the solubility of this hydrophobic compound, homogenization was still difficult.

Cinnamaldehyde, Ecotrol, and Sporan at 1.5% and 2.0% were more effective in reducing *E. coli* O157:H7 in soil. Organic soil due to its complexity and nutrient composition may require large concentrations of essential oils. Zaika (1988) and Burt (2004) reported that the extent of microbial inhibition by spices and herbs depends

on the combination of natural substance (oil), microorganism, and other storage/environmental factors such as temperature, humidity, and preservatives. Furthermore, many researchers found that foods due to their complex structures require greater amounts of essential oils or their components than do laboratory media to achieve comparable bacterial inhibition (Shelef, Jyothi, & Bulgarelli, 1984; Shelef, Naglik, & Bogen, 1980; Zaika, 1988).

Cinnamaldehyde had the most potent inhibitory activity against the five strains of *E. coli* O157:H7 followed by vinegar, Sporan, Ecotrol and eugenol. Cinnamaldehyde at 1.5% was highly bactericidal against all five strains of *E. coli* O157:H7 as evidenced by the 5 log reduction compared with the control. *E. coli* O157:H7 populations in soil were reduced by ca. 5 log cfu when vinegar, Sporan or Ecotrol were used at 2.0% concentration. The inhibitory effect of eugenol was evident during the initial 24 h only.

This study showed that *E. coli* O157:H7 can survive more than 28 days in organic soil. Paul and Clark (1996) reported that soils provide a wealth of nutrients that can be utilized by a variety of microorganisms. The dissolved organic matter in soil is a cocktail of aromatic organic derived from lignin, some oligomeric sugar derivatives derived from cellulose and hemicelluloses, and fatty acids between C₁₄ and C₅₄, believed to derive from both plant wall material and dead bacteria (Kalbitz, Solinger, Park, Michalzik, & Matzner, 2000). Proteomic analysis has revealed that *Bacillus cereus* cells growing in soil utilize soil-associated carbohydrates, fatty acids and perhaps amino acids (Luo et al., 2007). Therefore, it is not surprising that enteric bacteria are capable of surviving in soil. Often, bacteria in soils persist in a stressed state because of their exposure to fluctuations in a wide range of environmental parameters. Some of these stressed cells are occasionally resuscitated by passive internalization in plant structural openings (e.g., stomata, wounds, stem scars), by earthworms, or by ingestion by a mammalian host (Williams, Roberts, Avery, Killham, & Jones, 2006). Therefore, development of interventions that can significantly reduce survival of *E. coli* O157:H7 in soil prior to or during crop growth while simultaneously contributing to crop pest control could provide crop producers a useful aid in reducing potential contamination of fresh organic produce inadvertently contaminated by soil.

Persistence of essential oil constituents in natural environments appears to be limited. Murray (2000) reported that eugenol and other essential oil constituents were not persistent in freshwater laboratory tests. These compounds are also nonpersistent in soils (Misrra & Pavlostathis, 1997). Eugenol is completely degraded to common organic acids by soilborne *Pseudomonas* species (Rabenhorst, 1996). Concerns about essential oil residues on food crops should be mitigated by the growing body of evidence that some essential oil constituents acquired through the diet are actually beneficial to human health (Huang & Ho, 1998).

Although the antimicrobial properties of essential oils and their components have been reviewed in the past (Koedam, 1977a, 1977b; Shelef, 1983), the mechanism of action has not been studied in great detail (Lambert, Skandamis, Coote, & Nychas, 2001). It is suggested that the antimicrobial activity of essential oils is attributed to more than one mode of action (Burt, 2004). The mechanism of action of all antimicrobials can be as follow: cell membrane damage, inactivation of essential enzymes and destruction of genetic material (Davidson & Branen, 1981; Farag et al., 1989; Juven, Kanner, Scheved, & Weisslowick, 1994; Kim et al., 1995). Sub-lethal concentrations of eugenol have been found to inhibit production of amylase and proteases by *B. cereus*. Cell wall deterioration and high degree of cell lysis were also noted (Thoroski, Blank, & Biliaderis, 1989). The hydroxyl group on eugenol is thought to bind to proteins, preventing enzyme action in *Enterobacter aerogenes* (Wendakoon & Sakaguchi, 1995). Wendakoon and Sakaguchi (1995) reported that cinnamaldehyde inhibits amino acid decarboxylase enzyme

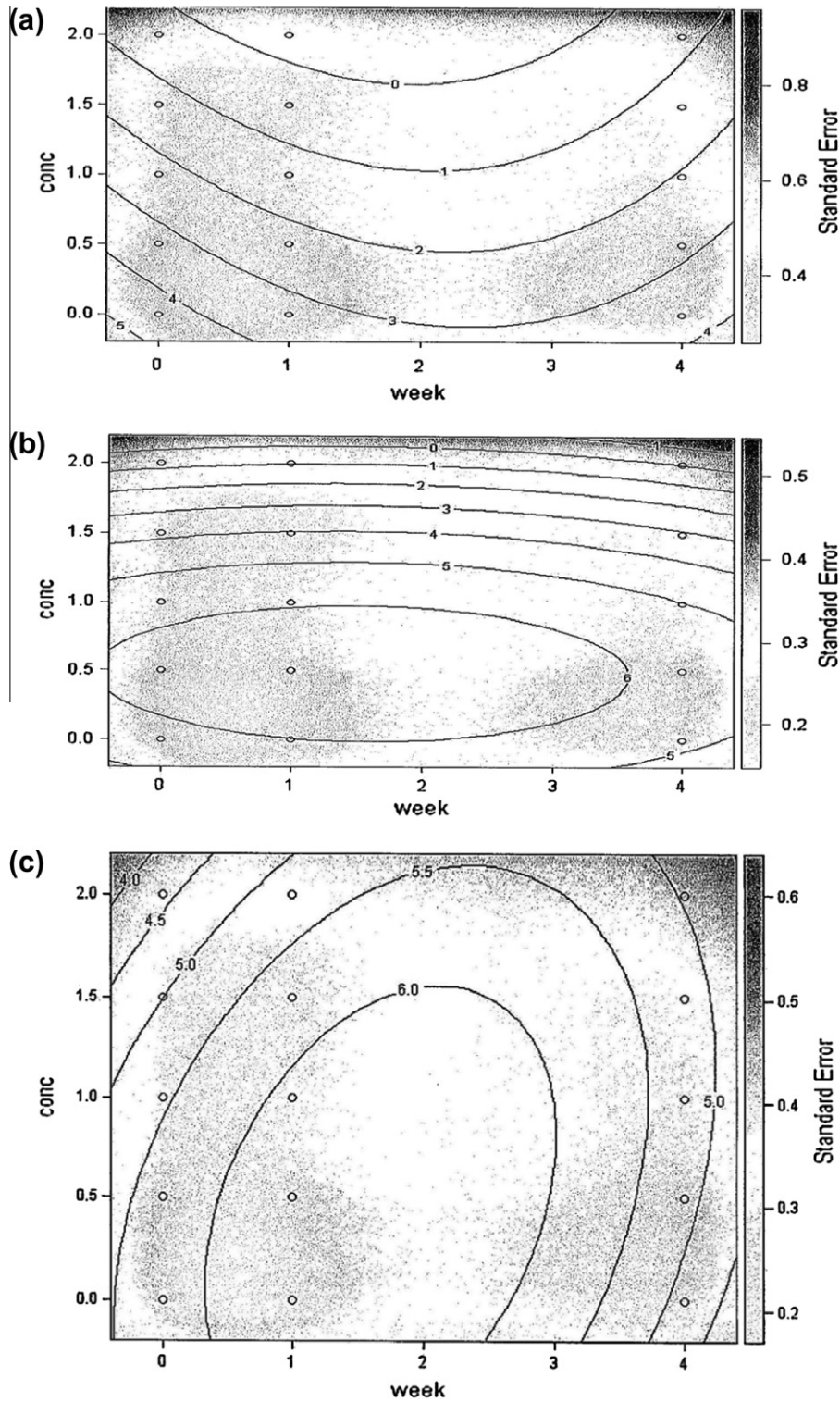


Fig. 1. Representative contour charts for predicting the effect of treatments on *E. coli* O157:H7 in soil. Curved lines in the charts indicate populations of *E. coli* O157:H7 as predicted by quadratic response surface model. (a) Effect of cinnamaldehyde on *E. coli* O157:H7 strain 4406 (at least 1.5% cinnamaldehyde required to reduce *E. coli* O157:H7 populations below 1 log CFU/g in soil). (b) Effect of Ecotrol on *E. coli* O157:H7 strain 1918 in soils (up to 1% Ecotrol in soil does not reduce *E. coli* O157:H7 in soil). (c) Effect of eugenol on *E. coli* O157:H7 strain 4407 in soil (*E. coli* O157:H7 populations are always more than 4 log cfu/g when eugenol is used). (d) Effect of cinnamaldehyde on *E. coli* O157:H7 strain 5279 in soil (*E. coli* O157:H7 reduces to <1 log cfu/g within 24 h when more than 1.5% cinnamaldehyde is used). (e) Effect of vinegar on *E. coli* O157:H7 strain 4406 in soil (*E. coli* O157:H7 reduces to below 1 log CFU/g after 1 week when vinegar is used at 1.5% levels).

activity in *E. aerogenes*. Gill and Holley (2006) reported that plant aromatic oils such as eugenol, carvacrol and cinnamaldehyde inhibited the membrane-bound ATPase activity of *E. coli* and *Lis-*

teria monocytogenes. Previous studies have demonstrated that leaf essential oils from cinnamaldehyde type of *Cinnamomum osmophloeum* had excellent antitermite, antibacterial, antimitic,

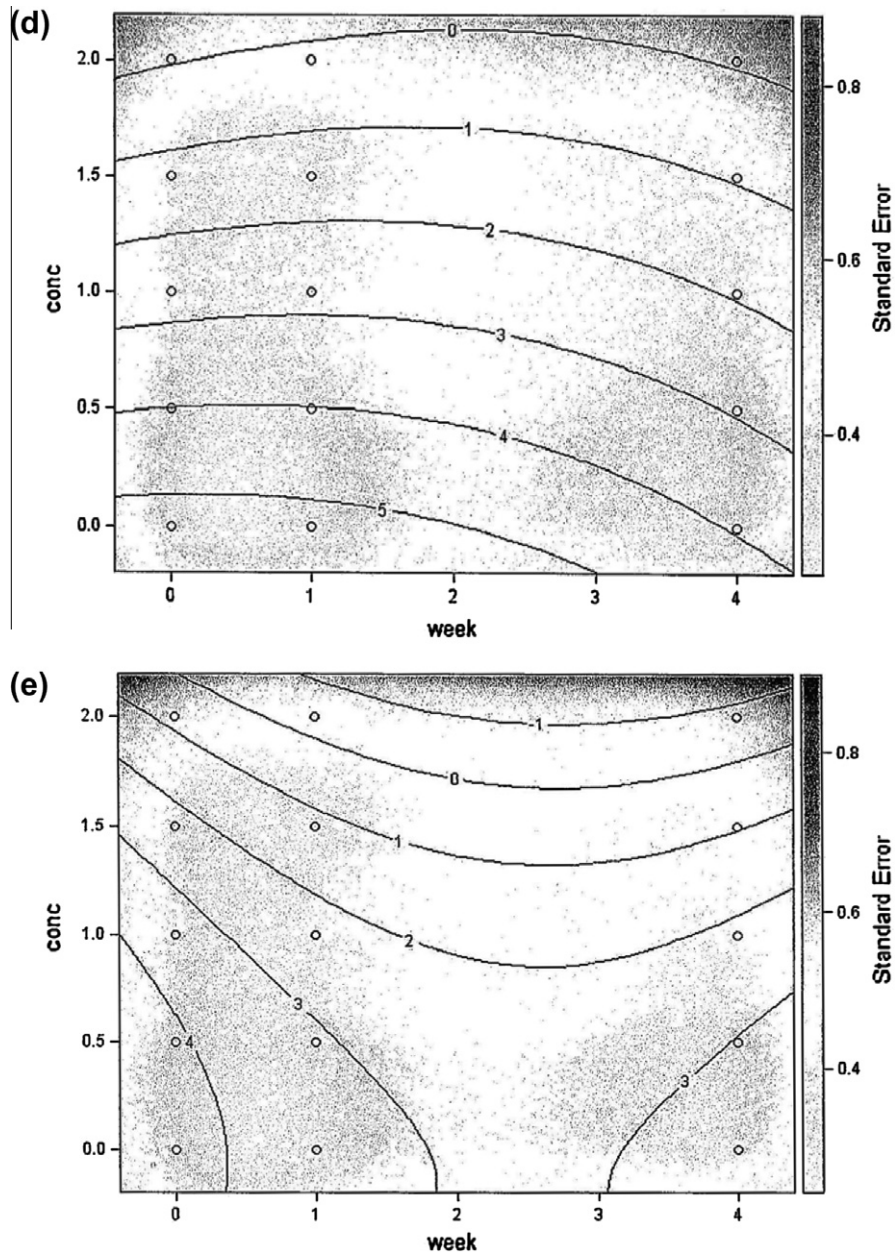


Fig. 1 (continued)

antimildew, antimosquito and antipathogenic activities (Chang, Chen, & Chang, 2001; Cheng, Liu, Tsai, Chen, & Chang, 2004; Lee, Cheng, & Chang, 2005).

5. Conclusion

The results of this study show the efficacy of essential oils in controlling important foodborne pathogen in soil, and the possibility of extending the application of cinnamaldehyde, Ecotrol and Sporan to control *E. coli* O157:H7. The significant reduction of *E. coli* O157:H7 could greatly reduce potential contamination of fresh organic produce inadvertently contaminated by soil. Moreover, small growers of organic produce could apply essential oils to soil in order to avoid pesticide residues in food and thereby reduce exposure to pesticide.

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