

Original Article

Decontamination of Tomato, Red Cabbage, Carrot, Fresh Parsley and Fresh Green Onion Inoculated with *Shigella sonnei* and *Shigella flexneri* by Some Essential Oils (*in vitro* Conditions)

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Abstract

Background: Essential oils and their major constituents are useful sources of antimicrobial compounds. There are a few reports on the decontamination and antimicrobial activity of essential oils towards *Shigella* spp.

Materials and Methods: In this study, the antimicrobial and decontamination potentials of essential oils at different concentrations, belonging to plants such as *Thymus vulgaris*, *Saturiea hortensis*, *Mentha pulegium*, *Cuminum cyminum*, *Lavandula officinalis* and *Mentha viridis* L. (*spearmint*), towards *Shigella sonnei* and *Shigella flexneri* were investigated. The disk diffusion method was used to demonstrate the antimicrobial potential of the essential oils.

Results: The ability of essential oils to decontaminate vegetables such as, tomato, red cabbage, carrot, fresh parsley and fresh green onion that were previously inoculated with *Shigella* spp. was determined. Inhibitory effects of essential oils towards *Shigella* spp. were noted in the disk diffusion method. There was a reduction in *Shigella* population following inoculation of cultures with 0.5% and 0.1% (v/v) essential oils.

Conclusion: This study confirmed that essential oils have the potential to be used for decontamination of vegetables.

Keywords: Antimicrobial, Decontamination, Essential oil, *Shigella flexneri*, *Shigella sonnei*

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Please cite this article as: Aliakbari F, Mazhar SF, Karami-Osboo R, Shariati P, Morshedi D, Farajzadeh D. Decontamination of Tomato, Red Cabbage, Carrot, Fresh Parsley and Fresh Green Onion Inoculated with *Shigella sonnei* and *Shigella flexneri* by Some Essential Oils (*in vitro* Conditions). Novel Biomed 2014;2(2):36-46.

Introduction

Shigella spp. are Gram-negative, facultative aerobic bacteria, which belong to the *Enterobacteriaceae* family. They are highly virulent enteroinvasive organisms that can cause sepsis or bacteremia in humans. In fact, a variety of diseases, including diarrhea and bacillary dysentery, are associated with virulent strains of *Shigella*. These gastrointestinal diseases are identified by

fever, abdominal cramps, and bloody diarrhea¹. In deprived parts of the world with unhygienic conditions, contaminated water has become the main cause of bacillary dysentery, also known as shigellosis. This disease occurs when bacteria are transmitted by the fecal-oral route², via different ways, such as close contact, contaminated foodstuff or water. Invasion by *Shigella* can lead to the destruction of colonic epithelial cells, resulting in inhibition of protein synthesis and mucosal ulcerations,

thus culminating in eventual bloody diarrhea³. In such cases, the mucosal layer of the colon is damaged by the *Shigella* toxin due to limited absorption of fluids and electrolytes.

Shigellosis is initiated by various *Shigella* species that include *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella sonnei* (serogroup C) and *Shigella boydii* (serogroup D). Shigellosis epidemics frequently occur in the developing countries and cause much morbidity and mortality. Shigellosis is temperature-dependent and is less frequent during the months of winter⁴.

Worldwide annual estimations indicate that from approximately 164.7 million cases of shigellosis, 163.2 million belong to developing countries, while the rest (1.5 million) is associated with industrialized countries, and this has resulted in over a million deaths worldwide. Most victims of this disease are children aged below five years old⁴. In developing countries, *S. flexneri* is the main species, which initiates approximately 60% of episodes, whereas, *S. sonnei* is the predominant cause of approximately 75% of cases in industrialized countries^{4,5}.

Fresh and fresh-cut produce are usually found to be the main reason for increasing outbreaks of foodborne disease⁶. Contaminated manure, animal and human contact, inorganic modification, water used for pesticide utilization or other agricultural cases, inadequate food processing and contaminated air are major sources of microbial contamination of vegetables and fruits throughout production^{7,8}. Foods which are dealt by hand, or somehow exposed to limited heat, and kept raw are highly prone to contamination by *shigella*⁹. Significant outbreaks of shigellosis derived from consumption of contaminated food, such as raw and fresh vegetables, are reported annually^{10,11}. In case surface damage occurs during processing of fresh-cut produce, microbial growth will take place prior to the availability of food for consumption¹². Raw vegetables such as tossed salad, parsley, shredded cabbage, green onions, lettuce salads and Iceberg lettuce, all contaminated with *Shigella*, have resulted in a number of reported outbreaks of shigellosis¹³⁻¹⁷.

Only 10 to 500 *Shigella* cells are enough to cause gastrointestinal illness, so no acceptable level of its presence in food can be tolerated^{18,1}. *Shigella* grows

naturally at the pH range of 4.5–9, however, it can survive for several hours when exposed to a pH range of pH 2–3¹⁹. Accordingly, shigellosis outbreaks are commonly associated with salads that have acidic pH values²⁰. Furthermore, *Shigella* species have also been observed to survive at refrigeration temperatures at which oxygen levels decrease²¹. Acquisition of multiple drug resistance to major antibiotics by *shigella* has also become a major concern^{22,23}. Although Shigellosis outbreaks can be controlled and treated by antibiotics, but the observed resistance and high expenses associated with their use have motivated researchers to utilize effective essential oils, to control diseases in the developing regions of the world.

Currently, due to great demand, medicinal plant providers are increasingly challenging pharmacies. Essential oils frequently that are extracted from vegetal materials are most commonly employed as natural additives in lots of foods because such natural products have antibacterial, antifungal, antioxidant, and anticarcinogenic properties^{24,25}. In fact, plants are regarded as major sources of medicine to treat infections in a number of developing countries. The high morbidity and mortality rates that have resulted from dysentery and diarrhea in such countries are because of unhygienic conditions and large populations²⁶⁻²⁸.

Phenolic compounds present in essential oils have been considered as the main sources of antimicrobial activity²⁹. Interactions between food and phenolic compounds inevitably lead to increasing the levels of essential oils and their compounds in order to inhibit the growth of microorganism in food, when compared with culture media³⁰. Despite the fact that *Shigella* is associated with numerous foodborne outbreaks, there are few data that have documented the influence of essential oils from aromatic plants, on such bacteria. In this study, in order to determine the effectiveness of essential oils in industrial food, *Thymus vulgaris*, *Saturiea hortensis*, *Mentha pulegium*, *Cuminum cyminum*, *Lavandula officinalis* and *Mentha viridis* L. were used to decontaminate food infected with the *S. flexneri* and *S. sonnei* strains.

Methods

Bacterial strains: *S. sonnei* strain PTCC 1235 and *S.*

flexneri strain PTCC 1234 were obtained from the Azad Islamic University, Research Lab., North Tehran Branch, Iran.

Preparation of inocula: For preparation of bacterial stock cultures, cells were cultured on brain–heart infusion (BHI) agar and incubated for 24 h at 37°C. Bacterial suspensions with turbidity corresponding to 0.5 McFarland standard equivalent to 10^6 CFU were then prepared from the stock cultures.

Essential oils: Essential oils were purchased from Barij Essence Co., producer of herbal medicines, Kashan, Iran.

Antibacterial activity of essential oils toward

Shigella spp: The agar disk diffusion method was used to reveal the antibacterial activity of essential oils, and also determine their minimum inhibitory concentration (MIC). Petri dishes containing Mueller-Hinton agar (MH) were inoculated by spreading plating of prepared suspensions. The essential oils diluted in ethanol at various concentrations (0.1%, 0.3%, 0.5%, 1%, 2%, 5%, 10%) were added (50 μ l) to the sterilized filter paper disks (5 mm) (Whatman No.1) and a similar volume (50 μ l) of ethanol was used as a control. The paper disk was placed in the middle of plates which were then incubated for 24 h at 37°C. After incubation, all petri dishes were

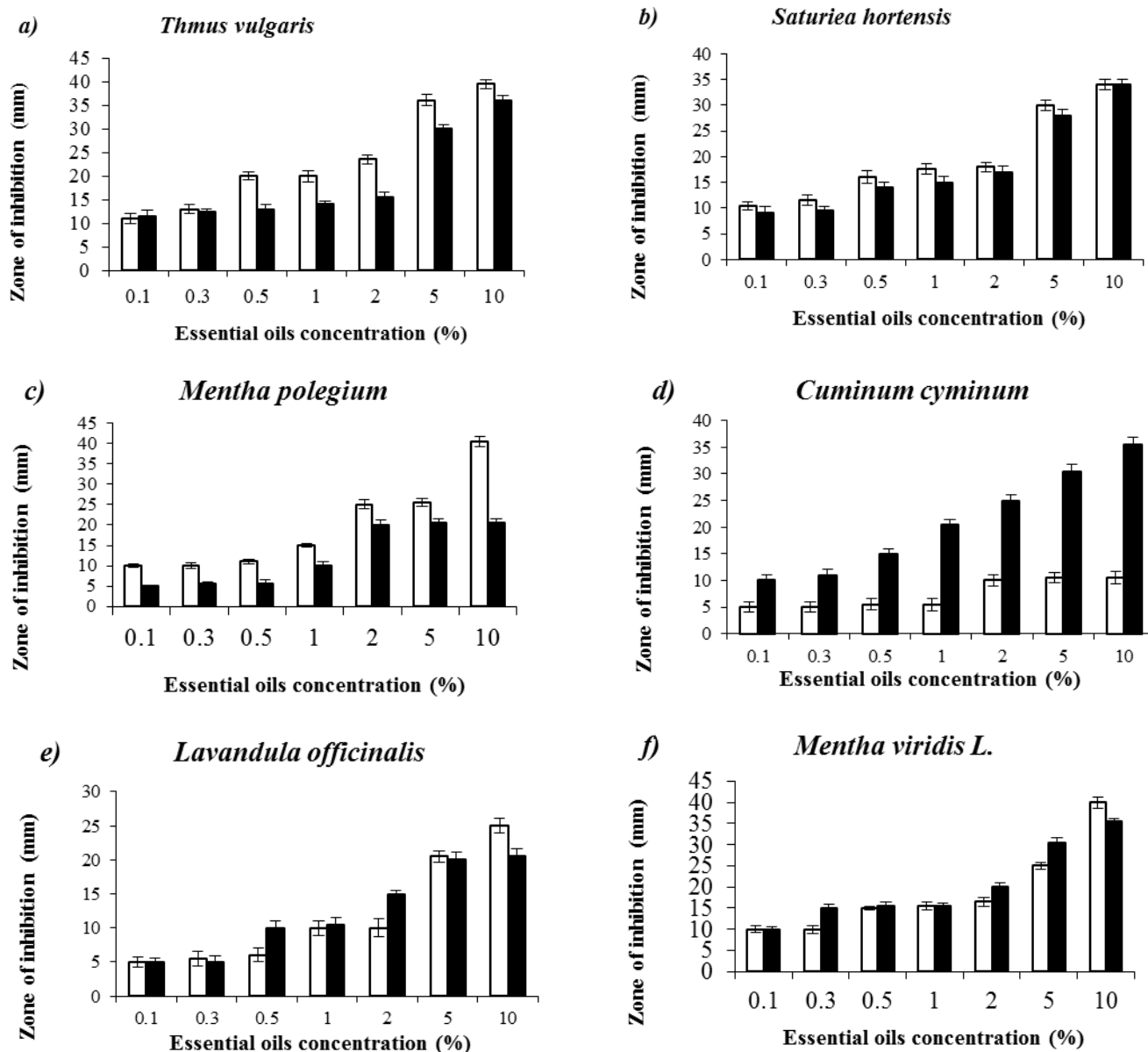


Figure 1. Antimicrobial effect of essential oils against *Shigella* spp. (□, *S. sonnei*; ■ *S. flexneri*). Several concentrations of individual essential oils in ethanol (0.1%, 0.3%, 0.5%, 1%, 2%, 5%, 10%) were investigated for their antibacterial activity. Similar volume of ethanol was used as a control.

observed for zones of growth inhibition, and the diameters of zones, as well as the diameter of the disk, were recorded. Results were expressed as the percentage inhibition of bacterial growth as determined by comparison with normal bacterial growth on the control dish, which was considered as 100%. The minimum inhibitory concentration (MIC) was determined as the lowest concentration that completely inhibited macroscopic growth of bacteria. All experiments were carried out in triplicate.

Decontamination of tomato, red cabbage, carrot, fresh parsley and fresh green onion inoculated with *Shigella* spp: For the decontamination assay, the vegetables were initially sterilized by being placed under UV light for half an hour. Then 1 ml suspensions of *S. sonnei* and *S. flexneri* were used to inoculate 5 g samples of tomato, red cabbage, carrot, fresh parsley and fresh green onion. After inoculation, all these vegetables were left to air-dry for 1 h. Once dried, the samples were cultured in BHI broth containing 50 μ l of 0.1% and 0.5% (v/v) *Thymus vulgaris*, 0.1% and 0.5% (v/v) *Saturiea hortensis*, 0.1% and 0.5% (v/v) *Mentha pulegium*, 0.1%

(v/v) *Lavandula officinalis* and 0.1% and 0.5% (v/v) *Mentha viridis L.* Also, BHI broth without essential oils was used as a control. The cultures were incubated at 37°C.

Bacterial enumeration of culture samples after 24, 48 and 72 h of incubation was carried out by the plate count method. Serial dilutions of the culture samples were spread onto agar plates, which were then incubated overnight at 37°C. Colonies on the plates were then counted and reported as cfu/g. These data were then used to construct growth curves.

Statistical analysis: All experiments were carried out in triplicate. The data acquired were analyzed for significant differences between the control and experimental groups, using the SPSS software Ver.16.0, involving the unpaired student's t-test. P value < 0.05 was considered to be statistically significant.

Results

Inhibitory activities of essential oils: The antimicrobial

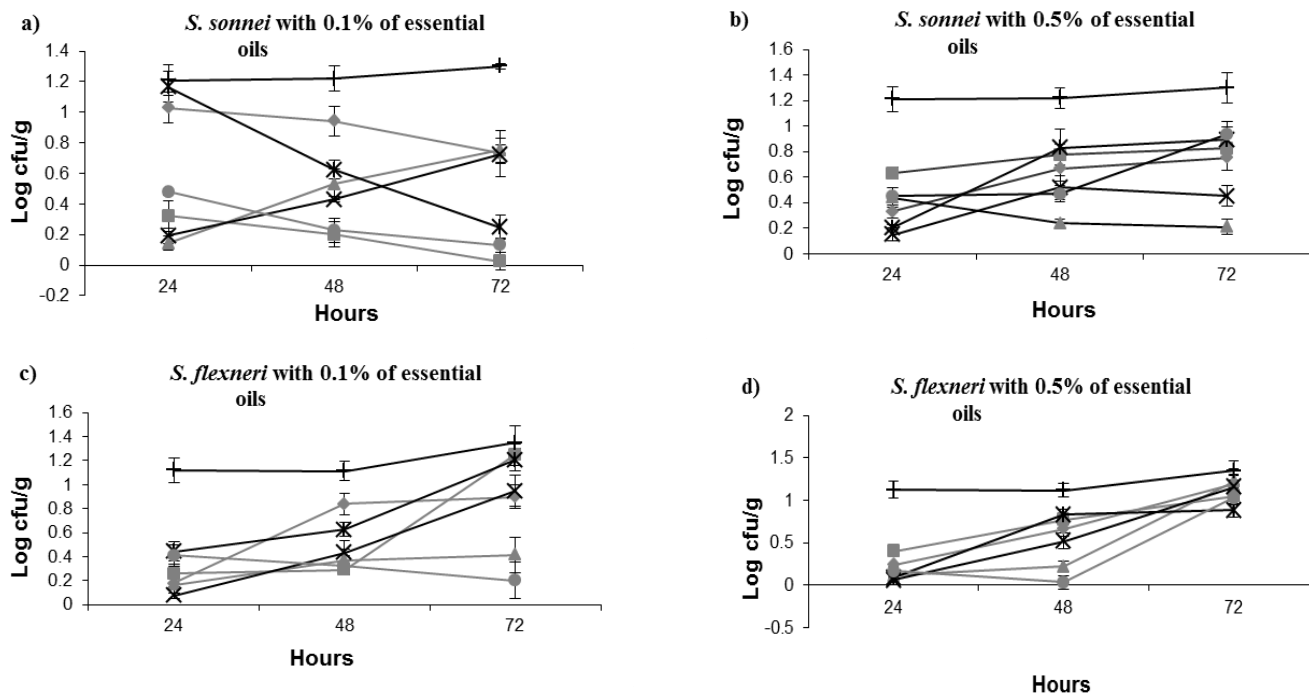


Figure 2. Decontamination of tomato inoculated with *S. sonnei* and *S. flexneri* using essential oils at concentrations of 0.1% and 0.5%. (—◇— *Thymus vulgaris*, —■— *Saturiea hortensis*, —▲— *Mentha pulegium*, —×— *Cuminum cyminum*, —*— *Lavandula officinalis*, —●— *Mentha viridis L.* and —+— Control).

and 0.5% (v/v) *Cuminum cyminum*, 0.1% and 0.5%

activities of essential oils belonging to *Thymus vulgaris*,

Saturiea hortensis, *Mentha polegium*, *Cuminum*

higher or similar inhibitory effect against *S. sonnei* when

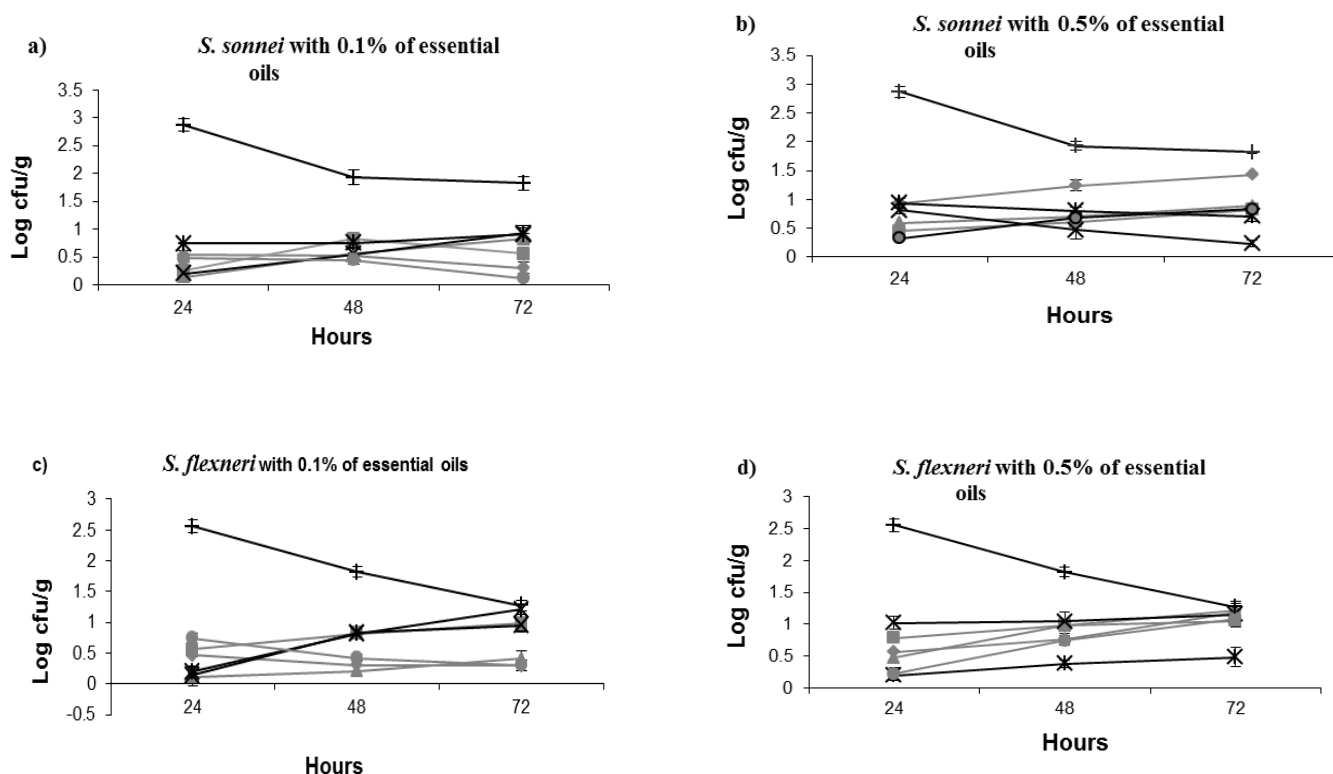


Figure 3. Decontamination of red cabbage inoculated with *S. sonnei* and *S. flexneri* using essential oils at concentrations of 0.1% and 0.5%. (◆—*Thymus vulgaris*, ■—*Saturiea hortensis*, ▲—*Mentha polegium*, ×—*Cuminum cyminum*, ⋈—*Lavandula officinalis*, ●—*Mentha viridis L.* and +—Control).

cyminum, *Lavandula officinalis* and *Mentha viridis L.*, towards *S. sonnei* and *S. flexneri*, were determined by the disk diffusion method (Fig. 1). Figs. 1a, b and c showed that when compared to *S. flexneri*, *S. sonnei* was more susceptible to essential oils from *Thymus vulgaris* (except at the concentration of 0.1%), *Saturiea hortensis* and *Mentha polegium*. Inhibition zones produced by the activity of the *Cuminum cyminum* essential oil towards *S. sonnei* and *S. flexneri* demonstrated that *S. sonnei* was less sensitive to this essential oil than *S. flexneri* (Fig. 1d). There was no linear relationship between *S. sonnei* and *S. flexneri* with respect to *Lavandula officinalis* and *Mentha viridis L.* (Figs. 1e and f).

Fig. 1e indicated that at the higher essential oil concentrations (5% and 10%) *S. sonnei* was more sensitive than *S. flexneri*. However, at lower concentrations (0.1%, 0.3%, 0.5%, 1%, and 2%), *S. flexneri* was more sensitive to *Lavandula officinalis*. Fig. 1f shows that at lower concentrations (0.1%, 0.3%, 0.5%, 1%, 2% and 5%) *Mentha viridis L.* had a

compared to *S. flexneri*, but at the highest (10%) concentration it was less inhibitory towards *S. flexneri* relative to *S. sonnei*. All essential oils investigated exhibited effective antibacterial activity against the tested microorganisms. Results of the present study showed that *S. flexneri* was more resistant than *S. sonnei* towards the effect of essential oils from *Thymus vulgaris*, *Saturiea hortensis* and *Mentha polegium*. Among the six essential oils investigated, *Cuminum cyminum* demonstrated higher antibacterial activities against *S. flexneri* than *S. sonnei*. These data indicate a linear relationship with respect to the radius of the inhibition zone and the concentration of the essential oils; hence, there is an agreeable reproducibility of the achieved inhibition zones.

For the range of essential oil concentrations (0.1%-10%) tested against the *Shigella* strains, an MIC of 0.1% inhibited the growth of all *Shigella* strains.

Decontamination of tomato, red cabbage, carrot, fresh parsley and fresh green onion with essential oils:

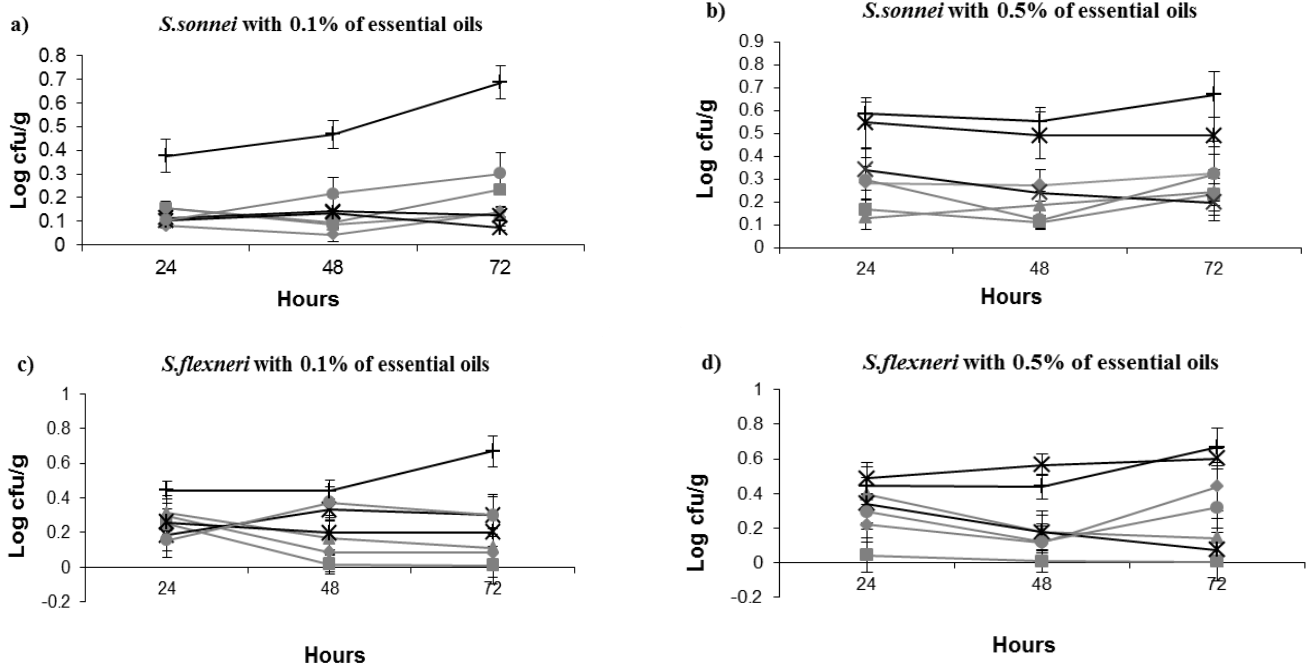


Figure 4. Decontamination of carrot inoculated with *S. sonnei* and *S. flexneri* using essential oils at concentrations of 0.1% and 0.5%. (—◆— *Thymus vulgaris*, —■— *Saturiea hortensis*, —▲— *Mentha poleygium*, —×— *Cuminum cyminum*, —⌘— *Lavandula officinalis*, —●— *Mentha viridis L.* and — + — Control).

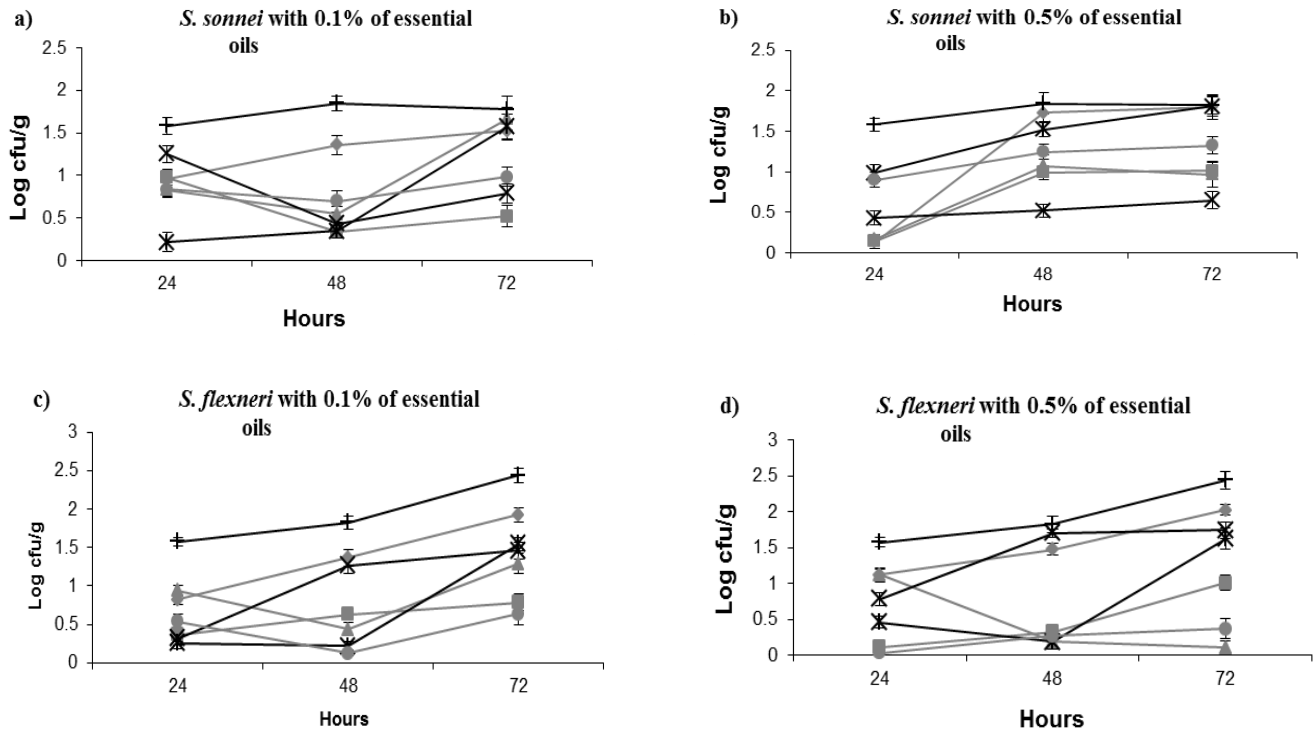


Figure 5. Decontamination of fresh parsley inoculated with *S. sonnei* and *S. flexneri* using essential oils at concentrations of 0.1% and 0.5%. (—◆— *Thymus vulgaris*, —■— *Saturiea hortensis*, —▲— *Mentha poleygium*, —×— *Cuminum cyminum*, —⌘— *Lavandula officinalis*, —●— *Mentha viridis L.* and — + — Control).

Reduction in populations of *S. sonnei* and *S. flexneri* from

tomato, red cabbage, carrot, fresh parsley and fresh green

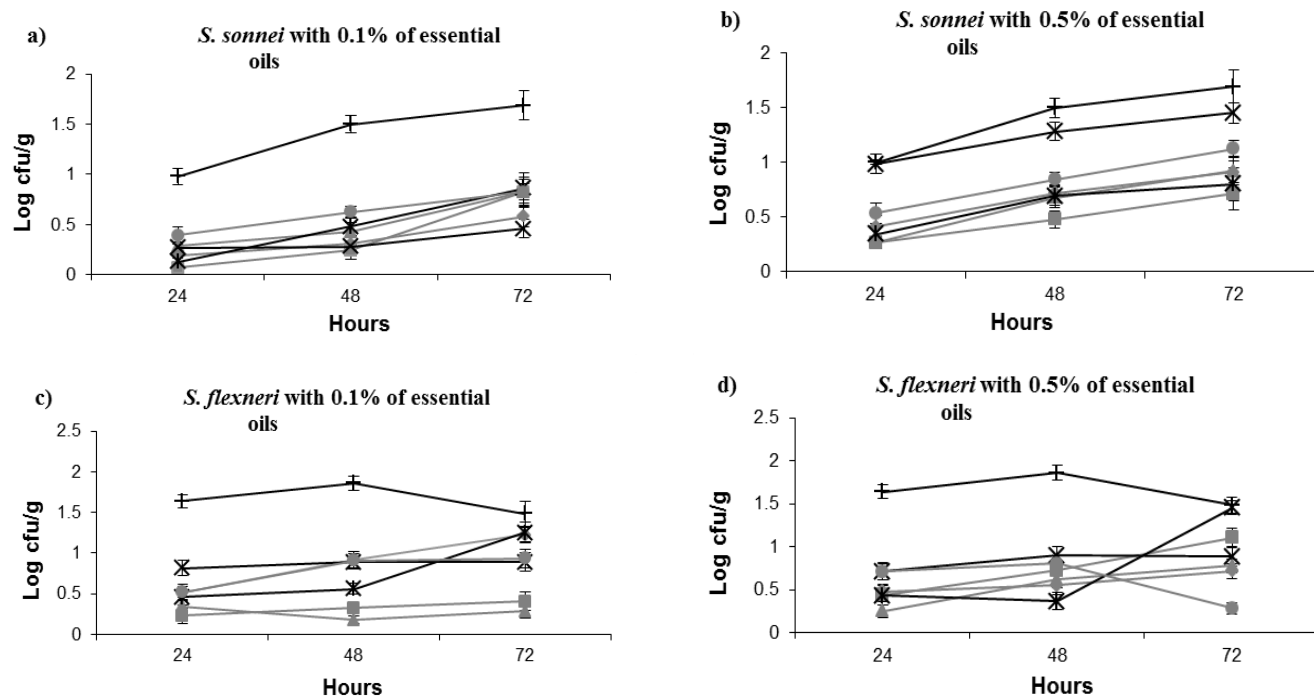


Figure 6. Decontamination of fresh green onion inoculated with *S. sonnei* and *S. flexneri* using essential oils at concentrations of 0.1% and 0.5%. (—◆— *Thymus vulgaris*, —■— *Satureia hortensis*, —▲— *Mentha poleygium*, —×— *Cuminum cyminum*, —ж— *Lavandula officinalis*, —●— *Mentha viridis L.* and —+— Control).

onion that were artificially contaminated with the test bacteria, by the essential oils from *Thymus vulgaris*, *Satureia hortensis*, *Mentha poleygium*, *Cuminum cyminum*, *Lavandula officinalis* and *Mentha viridis L.* are illustrated in Figs. 2-6. Decontamination was carried out by adding essential oils, at concentrations of 0.1% and 0.5% to the culture media. For the control, culture medium devoid of essential oils was used. Figures 2a and b indicated significant decontamination of *S. sonnei* infected-tomato by *Cuminum cyminum* and *Mentha poleygium*, at concentrations of 0.5% and 0.1%, respectively. *Shigella flexneri*-infected tomato was significantly decontaminated by essential oils of *Mentha poleygium* and *Lavandula officinalis* at concentrations of 0.1% and 0.5%, respectively (Figs. 2c and d).

All essential oils were also highly effective in the decontamination of *S. sonnei* and *S. flexneri*-infected red cabbage (Fig. 3).

All of the essential oils were potent in decontaminating *S. sonnei*-infected carrot at a concentration of 0.1%. However, at 0.5%, *Mentha poleygium* and *Satureia hortensis* were the most effective followed by *Mentha viridis L.*, *Cuminum cyminum* and *Thymus vulgaris*. In this

regard, *Lavandula officinalis* did not demonstrate any effects (Figs. 4a and b).

All essential oils were also capable of decontaminating *S. flexneri*-infected carrot at 0.1% and 0.5%, but no decontamination activity was observed with regard to *Cuminum cyminum* at the concentration of 0.5% (Figs. 4c and d).

Reductions in *S. sonnei* and *S. flexneri* populations were noticed after decontamination of fresh parsley with all the individual essential oils but all results were not significantly remarkable (Fig. 5).

Results indicated that only *Cuminum cyminum*, at a concentration of 0.5%, was the best essential oil to decontaminate *S. sonnei*-infected fresh parsley (Fig. 5b). *Satureia hortensis* and *Mentha viridis L.* were the best decontaminating agents of *S. flexneri* at a concentration of 0.1%, but *Mentha viridis L.* was the most effective at a concentration of 0.5% in the case of infected fresh parsley (Figs. 5c and d). All essential oils demonstrated good potential in decontamination of *S. sonnei*-infected fresh green onion except for that of *Cuminum cyminum* which was only effective at a concentration of 0.5% (Figs. 6a and b).

Considerable reductions in cell numbers were observed in

the decontamination of *S. flexneri*-infected fresh green onion by the entire essential oils tested. *Saturiea hortensis* and *Mentha pulegium* were the most effective essential oils at a concentration of 0.1% for decontaminating *S. flexneri*-infected fresh green onion, while at 0.5% *Mentha pulegium* was the best (Fig. 6c and d).

The main problem arising during decontamination was the change in the color of *S. flexneri*-infected red cabbage after treatment by *Thymus vulgaris* and *Saturiea hortensis*, from red to green and purple, respectively. In the case of red cabbage inoculated with *S. sonnei*, an amaranthine color was observed after decontamination by *Thymus vulgaris* and *Saturiea hortensis*. This process causes absorption (as detected by the spectrophotometer) to increase due to the darkness of the medium culminating in an incorrect estimation of bacterial populations. So for precise enumeration of bacteria, the pour plate method was carried out for the 24, 48 and 72 h-cultures on BHI agar, and colony were then counted after 24 h of incubation at 37°C.

Discussion

The aim of this study was to evaluate the antimicrobial effectiveness of essential oils as decontaminating agents of fresh produce. Utilization of such natural compounds may decrease microbial contamination resulting in food safety. For these reasons, the antibacterial activities of six essential oils from *Thymus vulgaris*, *Saturiea hortensis*, *Mentha pulegium*, *Cuminum cyminum*, *Lavandula officinalis* and *Mentha viridis* L. (*spear*mint) were offered to test against *S. sonnei* and *S. flexneri* by using the disk diffusion method. The essential oils did not differ significantly in their activities against the tested strains, however, the best antibacterial activities were observed by *Thymus vulgaris*, *Saturiea hortensis* against both strains, *Mentha pulegium* in the case of *S. flexneri* followed by *Mentha viridis* L., *Mentha pulegium*, and *Lavandula officinalis* against both strains. *Mentha pulegium* was the least active against *S. sonnei*. All essential oils examined were shown to have antibacterial properties against at least one of the microorganisms tested. Reduction in *S. sonnei* and *S. flexneri* populations were observed after decontamination of vegetables with all individual essential oils, but not all results were significantly

notable.

In the decontamination test, a problem was the change in the color of red cabbage inoculated with *S. flexneri* and *S. sonnei* after decontamination by *Thymus vulgaris* and *Saturiea hortensis*. This change was due to the cytotoxic action of the antimicrobial agents on the living cells of fresh products such as lettuce³¹. It has previously been shown that if lower concentrations of essential oils are employed for decontamination, it would be less damaging to the sensory properties, however, in line with such a reduction, the antibacterial effect would decrease³¹. In this study, the vegetables were contaminated with larger doses of *S. sonnei* and *S. flexneri* than might be expected in naturally contaminated products. Since inhibition of growth is dependent on inoculum size, lesser concentrations of essential oils may be adequate for naturally contaminated products³¹.

Currently, there is a great tendency to overcome the growth of foodborne pathogens using natural antimicrobials. It is assumed that *Shigella* is sensitive with no special resistance to environmental stress, although, *S. flexneri* and *S. sonnei* have been found to survive for 14 days in apple and tomato juices at 7°C¹⁹. Moreover, they stay viable after inoculation for several days at room and even refrigerating temperatures, in a range of vegetables and salads, which are prepared commercially^{32, 33}. *Shigella* can also grow easily at 12°C in several foods³⁴. *S. flexneri* has been shown to survive in most prepared salads including carrot salad, crab salad, coleslaw and potato salad which are stored at 4°C. This bacterium has also been reported to survive up to 26 days in cabbage and 12 days in onion and green pepper, at 4°C. In spite of a decrease in *S. flexneri* numbers in carrot and potato salads, considerable populations have been found to be still viable up to the 11th day³³. Endurance of *S. flexneri* has been reported up to 10 days for carrots, cauliflower, celery, radish, and broccoli at 5°C and 10°C³². More reports are also available regarding survival of *S. flexneri* in significant numbers at pH values of 4.1 to 4.2 and 4.4 to 4.5, for coleslaw and crab salads, respectively³³. The results of the present study have shown that the proposed essential oils would be useful to treat such kinds of bacteria. *Shigella sonnei* and *S. flexneri* have been found to survive in minimal processed foods packed in optimized equilibrium modified atmosphere (EMA) cans that contain mixed lettuce, grated carrots and chopped bell

peppers³⁵. *Shigella sonnei* has been observed to survive in shredded cabbage with no reduction in cell numbers being noted after packaging under vacuum or modified atmosphere (30% N₂-70% CO₂) and storage in the cold for 7 days. However, aerobic conditions cause the *Shigella* population to decrease to negligible levels (<cfu g⁻¹), at 0-6°C after 7 days².

This The present study has shown that *S. sonnei* is susceptible to essential oils produced by *Thymus vulgaris*, *Saturiea hortensis*, *Mentha pulegium*, *Lavandula officinalis* and *Mentha viridis L.* and fairly vulnerable to *Cuminum cyminum*. Hence the use of these essential oils can be valuable in the elimination of foodborne pathogens from food. This study also indicates that all essential oils that have been found to inhibit the growth of *S. flexneri* could be applied to the food industry for the purpose of removing or decreasing this pathogen. Results of other studies have indicated that *S. flexneri* can survive and grow in lentil soup, milk, boiled rice, mashed brinjal, mashed potato, raw cucumber, cooked fish and cooked beef, even though autoclaving has been carried out prior to inoculation for all samples except for that of raw cucumber³⁶. There is no record of *Shigella* surviving in raw ground beef, though it has been indicated that cooked beef autoclaved before inoculation helps *S. flexneri* to grow at unsuitable temperatures³⁶. The only way to stop foodborne pathogens from growing is to refrigerate them; however, *Shigella* spp. can survive without growth at such conditions.

In consideration of the fact that shigellosis can occur at low infective doses of *Shigella* spp., food preparation procedures should be taken seriously. New methods involving more competent treatments and destruction of pathogenic organisms are welcome worldwide³⁷. New achievements in all fields of relevant technology offer fresh vegetables to consumers every year.

Use of essential oils in the present study may be considered as a part of such achievements. Rigorous and serious steps should be taken to prohibit low quality processing in markets and subsequent exposure of consumers to exotic microflora.

Uses of essential oils as pesticides, fungicides and bactericides can be safer than chemicals; therefore, it is necessary to further study the role of essential oils in the decontamination of foods, using them as natural

additives in the food industry. Not many studies are available regarding the potential role of essential oils and their components as food preservatives. Foods such as taramasalata, eggplant salad, tzatziki, pate, packed fresh meat, minced meat, minced mutton, vacuum-packed ham, mozzarella and soft cheese have been investigated for the effectiveness of essential oils as preservatives³¹. It has been demonstrated that extracts from *Caesalpinia pulcherrina*, *Chiranthodendron pentadactylon*, *Cocos nucifera*, *Geranium mexicanum* (aerial parts and roots), *Hippocratea excelsa*, and *Punica granatum* have a strong influence on *S. sonnei* and *S. flexneri*, with aqueous extracts being less active than methanolic extracts. In addition, *S. sonnei* species has been found to be susceptible to both methanolic and aqueous extracts³⁸.

In this study, it was shown that *S. sonnei* and *S. flexneri* are susceptible to all essential oils tested. *Thymus vulgaris*, *Saturiea hortensis* demonstrated the highest activities towards both strains. It has also been found that the essential oil from the tea bush (*Lippia* spp.) has an antibacterial activity towards some bacteria including *S. dysenteriae*, with an MIC of between 5→80 µl ml⁻¹³⁹. During this investigation, the MIC of all essential oils tested towards *Shigella* spp. was found to be 0.1%.

The effects of herbs and spices as such have been assessed on *Shigella*; and the major antimicrobial potentials of *thymus vulgaris* and *basilicum* have been revealed⁴⁰. Thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene have been shown to have antibacterial effects against *S. sonnei* and *S. flexneri*. Additionally reductions in the *S. sonnei* population that was used to artificially inoculate lettuce were observed following decontamination with watery suspensions of thyme essential oil, carvacrol and thymol³¹. The results of the present study have indicated that essential oils are capable of decontaminating minimally-processed vegetables.

As observed in cases of *Thymus vulgaris*, *Saturiea hortensis* in this study, some essential oils cause sensorial effects in fresh cut produce due to their function. So, to prevent such color changes, the use of essential oils at lower concentrations or the application of synergistic mixtures of essential oils and their compounds are recommended besides the implementation of other preservation techniques, e.g. reduction in temperature^{31,41}. It is obvious that, more studies are still needed to be

carried out until essential oils and their compounds can fully be utilized as food preservatives. It is worth mentioning that essential oils are rich sources of small molecules, each of which can have different properties. Hence, the use of essential oils and their diverse constituents in several aspects of the pharmaceutical industry would be feasible⁴².

Reported here are the results of a study to determine the influence of essential oils from *Thymus vulgaris*, *Saturiea hortensis*, *Mentha pulegium*, *Cuminum cyminum*, *Lavandula officinalis* and *Mentha viridis L. (spearmint)* on *S. sonnei* and *S. flexneri*, and decontamination of tomato, red cabbage, carrot, fresh parsley and fresh green onion artificially inoculated with *S. sonnei* and *S. flexneri*. In conclusion, this investigation shows that essential oils belonging to *Thymus vulgaris*, *Saturiea hortensis*, *Mentha pulegium*, *Cuminum cyminum*, *Lavandula officinalis* and *Mentha viridis L. (spearmint)* are significantly active against *S. sonnei* and *S. flexneri* and could be used as an additive in nutrition or an agent to decontaminate food infected with such microorganisms in the food industry.

Acknowledgement

Our special thanks to Dr. Fariborz Shojaee Aliabadi from Farogh Life Sciences Research Laboratory for supporting us and for his helpful ideas.

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