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The Prevalence of *E. coli* O157:H7 in the Production of Organic Herbs and a Case Study of Organic Lemongrass Intended for Use in Blended Tea

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ABSTRACT

Tea blended with different herbs bring a world of flavors, aromas and colors and is usually made with dried tea leaves, or blended with other dried herbs and involves pouring boiling water over the leaves, letting them steep for few minutes followed by consumption. This study was done to evaluate the insights of potential microbial contamination of organic herbs production at the farm, after harvest, washing, before or after drying and packaging of dried herbs sample. Organic compost, water quality, worker hygiene status and overall food safety management systems were also evaluated to identify additional factors affecting microbiological contamination. In addition, effect of pouring hot water over contaminated dried leaves in a cup of tea was observed. The study was designed in such a way that reflects the actual tea preparation at home. Presence of higher numbers of generic *E. coli* and pathogenic *E. coli* O157:H7 was observed in dried tea, herbs and /or lemongrass samples, and blended tea mix lemongrass samples. However, no *Salmonella* was detected in any of the samples tested. When hot water was added into dried lemongrass or blended tea mix lemongrass samples in a cup of tea and held for 30, 60, 90, 120 or 180 seconds with or without a lid, no generic *E. coli* and pathogenic *E. coli* O157:H7 was observed in the prepared cup of tea in 30 seconds or above the holding time in selective medium. The bacteria might be severely injured by hot water treatment and did not appear on the selective plates. To confirm whether the bacteria were inactivated or injured, an enrichment study was done. Neither generic *E. coli* nor any pathogenic *E. coli* O157:H7 were detected in the prepared tea in the cup. The hot water temperature was recorded as 82˚C when added in the cup and after 60 seconds the temperature decreased to 78˚ C; further reduced to 73˚C after 3 minutes of holding and at the end of 5 minutes the temperature reached 64˚ C. In addition, the natural microflora was reduced to less than 100 CFU/ml. This finding suggested that addition of hot water (80˚C) in tea leaves resulted in complete elimination of pathogens and thus the present tea making practice could provide safe tea for drinking even though the tea leaves were contaminated. However, for sanitary reasons *E. coli* should be eliminated from the organic products prior to consumption.

Keywords: Organic herbs, *E. coli* O157:H7, organic lemongrass, case study and blended tea

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INTRODUCTION

Lemongrass (*Cymbopogan flexuosus*, family: Poaceae) is an aromatic plant which grows in many parts of tropical and sub-tropical South East Asia and Africa. Most of the species of lemon grass are native to South Asia, South-East Asia and Australia (USDA, 2008). Lemongrass naturally grows in tropical areas and can resist the heat of the sun. Fresh cut lemongrass often lasts for several days and can be preserved in fresh water for several months without losing any flavor or nutritional properties. However, these lemongrasses are dried easily, readily available worldwide and can be used to make tea.

Lemongrass is usually ingested as an infusion made by pouring boiling water on fresh or dried leaves and is one of the most widely used traditional plants in South American folk medicine (Blumenthal, 1998). It is used as an antispasmodic, antiemetic, and analgesic, as well as for the management of nervous and gastrointestinal (GI) disorders and the treatment of fevers (Leung, 1980). In India it is commonly used as an antitussive, antirheumatic, and antiseptic. In Chinese medicine, lemongrass is used in the treatment of headaches, stomach aches, abdominal pain, and rheumatic pain (Girón *et al*., 1991). Lemongrass is an important part of Southeast Asian cuisine, especially as a flavoring in Thai food. Lemongrass is used in Cuban folk medicine for hypertension and as an anti-inflammatory (Lewinsohn *et al*., 1998). It is also used in Brazilian folk medicine in a tea called *abafado* as a sedative, and for gastrointestinal problems and fever (Martínez-de la Puente *et al*., 2009). Lemongrass and closely related species are popularly used as insect repellents (Wong *et al*., 2005; Tawatsin *et al*., 2001). They may be found in sprays, candles, and other repellent products. Various experimental studies support its use as an insecticide or insect repellant. Lemongrass has been shown to have antifungal properties in laboratory studies particularly against *Candida* species (*Candida albicans*, Candida *glabrata,* Candida *krusei*, Candida *parapsilosis,* and Candida *tropicalis*) (Warnke *et al*., 2009). In a preliminary study, lemongrass infusion had beneficial effects for the treatment of oral candidiasis in patients with HIV/AIDS (Wright *et al*., 2009).

These fresh herbs and leafy greens are potential transmission sources of enteropathogens. In a recent report from WHO/FAO on microbiological hazards in fresh fruits and vegetables (FAO/WHO 2008) it was stated that leafy green vegetables (including fresh herbs) "currently presented the greatest concern in terms of microbiological hazards." This is because these products are grown and exported in large volumes, and they have been associated with many foodborne disease outbreaks affecting considerable numbers of people. Additionally, the production chain for leafy greens is highly complex. The microflora on these vegetables at harvest reflects the environment in which they are grown, if the temperature and humidity is relatively high then the occurrence of enteropathogenic bacteria in this environment might be considerable. During cultivation, use of contaminated water for irrigation, application of biocides, and refreshing or washing of harvested crops, are potential sources of contamination. Contamination from contact with fresh manure used as fertilizer cannot be excluded. Heavy rainfall may also lead to fecal contamination from the environment. Direct sunshine will most likely have a disinfection effect, but if the plants are irrigated until harvest and the production hygiene during harvest and post-harvest is inadequate, there is a relatively high likelihood that the fresh herbs and leafy greens may be fecally contaminated. These fresh herbs and leafy greens and their products have been found to be contaminated with pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* serovar Typhi, *Shigella* spp, *Bacillus* spp. amongst others that represent serious public health hazards (Abadias *et al*., 2008; Esimone *et al*., 2003; Oyetayo, 2008; Abba et al., 2009; Adeleye *et al*., 2005). Some of these pathogenic bacteria originate from soil and adhere to parts of plants (Lau *et al*., 2003) while most of them are being introduced into leafy products through processes of harvesting, drying, storage and manufacturing because of the unhygienic practices of the product handlers (Lau *et al*., 2003; Espen *et al*., 2008). In 2005, the Norwegian Food Safety Authority (Mattilsynet) conducted an *ad hoc* survey of 162 fresh herbs and green or leafy vegetables products, from South East Asia, and found

that 28% were contaminated with *Salmonella*, and 35% with *E. coli* at greater than 100 CFU/gram. This resulted in a general import prohibition of such products from South East Asia, and now the EU accordingly requires certificate of analysis for *Salmonella* and *E. coli* before export (Olaimat and Holley, 2012).

The objectives of this present study were to evaluate microbial contamination of organic herb production at the farm level, and a case study of food safety management in organic lemongrass production intended for blended tea. Organic compost, water quality, worker hygiene status and overall food safety management systems were also evaluated to identify the potential factors affecting microbiological contamination. In addition, effect of pouring hot water over contaminated dried leaves in a cup of tea was observed. The study was designed in such a way that reflects the actual tea preparation at home.

MATERIALS AND METHODS

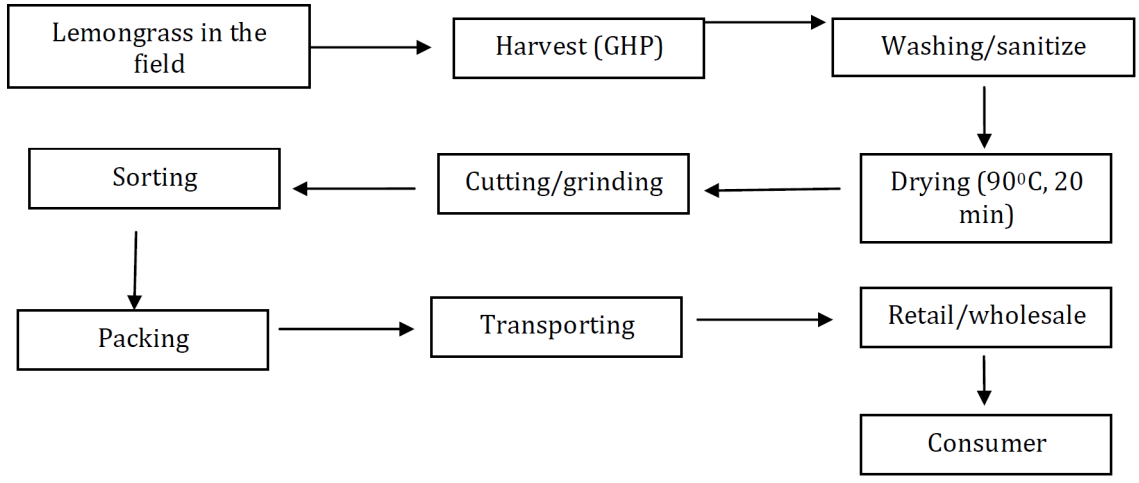
Sample collection

Herb samples include, lemongrass, mint, neem and jasmine were obtained from an organic farm in Northern Bangladesh between May 15 and July 30, 2013. All samples were transported to the Food Analysis and Research Laboratory, Center for Advanced Research in Sciences (CARS) at the University of Dhaka using a cool box at the earliest convenience for processing and further analysis. All the microbial analysis was carried out according to the standard methods described in United States Food and Drug Administration (US-FDA) Bacteriological Analytical Manual.

Selected critical sampling locations (CSLs)

The critical sampling locations were selected based on the production scheme presented in Figure 1a and other sources of microbiological contamination as identified in literature reviews (Ilic *et al.*, 2012; Vidal *et al.*, 2004) i.e., soil, water, manure, food contact surfaces, or food handlers. For dried organic lemongrass production, 12 CSLs were selected (Figure 1b) including the lemongrass crop.

Figure 1a. Schematic flow diagram of lemongrass production chain (Farm to table).



Total aerobic count and total coliform count

Twenty five (25) g of each sample were homogenized in 225 mL of saline water (0.85% NaCl). Decimal dilutions were prepared upto 10^{-6} mL and appropriate dilutions were spread plated on Tryptic soy agar (Oxoid Ltd., Hampshire, England) and incubated at 35˚C for 24 hr for total aerobic bacterial counts and on MacConkey agar (Oxoid Ltd., Hampshire, England) with incubation at 35˚C and 42˚C for 24 hours for total coliform count. Total aerobic count indicates the quality and shelf life of the products and total coliform count indicates the unhygienic condition of the food preparation surfaces.

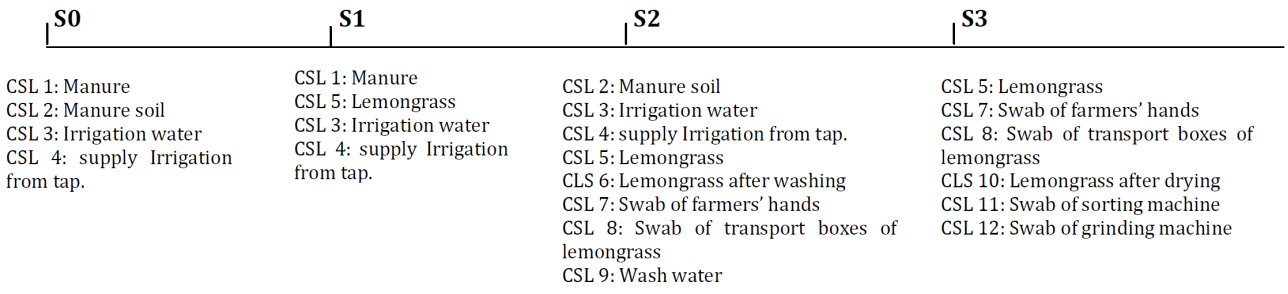
Escherichia coli, fecal coliform bacteria

Twenty five (25) g of each sample were homogenized in 225 mL Enterobacteria enrichment broth-Mossel pre-enrichment medium (Oxoid Ltd., Hampshire, England) and incubated at 35˚C for 20 hours. One mL of pre-enriched cultures were mixed with nine mL of 2x EC medium (Nissui Co., Ltd., Tokyo, Japan) and incubated at 35˚C for 20 hours. To confirm the presence of fecal coliforms, one loopful of the culture was inoculated into 10 mL 1x EC medium with Durham fermentation tubes and incubated at 42˚C for 20 hours. Gas production in the tube indicates the presence of fecal coliforms. To isolate *E. coli*, one loopful of gas producing 1x EC culture broth was streaked on EMB agar plates (Nissui Co., Ltd., Tokyo, Japan) and the developed typical colonies were then confirmed using biochemical characterization (IMViC) and API 20E kit (bioMérieux, Durham, NC, USA). Presence of *E. coli* or fecal coliform bacteria was used as an indicator that the food is potentially contaminated with fecal material.

Escherichia coli O157, O111, O26

Twenty five (25) g of each samples were homogenized in 225 mL mEC medium (Nissui Co., Ltd., Tokyo, Japan) and incubated at 42˚C for 20 hours. The enriched cultures were streaked on Sorbitol MacConkey agar (Oxoid Ltd., Hampshire, England) supplemented with Cefixime and potassium tellurite admendments (Fluka, Sigma-Aldrich, Banglore, India) and characteristic colonies were subjected to biochemical tests (IMViC). Biochemically confirmed isolates were screened using Rainbow agar (Biolog, France) and CHROM agar (Kanto Co. Ltd., Kyoto, Japan). The colonies which gave the characteristic color were serotyped using O157, O111 and O26 specific antisera. The isolates were subsequently tested for the presence of *stx1* and *stx2* by NH-Immuno-

Figure 1b. Identification of selected Critical Sampling Locations (CSLs) in the production chain of dried lemongrass.
S0: At the field; **S1**: weeks before harvest; **S2:** harvest and washing; **S3**: drying/sorting/grinding/packaging;



chromato *VT1/2* and by polymerase chain reaction (PCR) assay using primer 5′-CAGTTAATGTGGTGGCGAAGG-3′ and 5′-CACCAGACAAATGTAACCGCTC-3′ for *stx1* and 5′-ATCCTATTCCCGGGAGTTTACG-3′ and 5′-GCGTCATCGTATACACAGGAGC-3′ for *stx2*, respectively (Vidal *et al.*, 2004).

Salmonella spp.

Twenty five (25) g of each sample were homogenized in 225 mL of buffered peptone water (Merck, Darmstadt, Germany) and incubated at 35˚C for 20 hours. One mL pre-enrichment cultures was mixed with nine mL of Hanja Tetrathionate Broth (Eiken Chemical Co. Ltd., Tokyo, Japan) and incubated at 35˚C for 20 hrs and nine mL of Rappaport-Vassiliadis Broth (Eiken Chemical Co. Ltd., Tokyo, Japan) and incubated at 42˚C for 20 hrs. The broth the culture broths were subsequently streaked onto DHL and MLCB and characteristic colony were characterized with biochemical tests (TSI and LIM). Biochemically confirmed isolates were re-confirmed using *Salmonella* LA latex agglutination test and API 20E kits.

Hot water treatment in tea-cup & Enrichment study

One gram of dried or blended herbs samples were added in a cup and 50 mL of hot water was poured over the dried leaves. The cup was kept with and without lid up to 5 minutes. In each 30 second interval microbiological parameters were done as described in the previous section on microbiological medium and conditions. For the enrichment study, one mL of hot water treated sample was added into 9 mL of Tryptic soy broth (TSB; Oxoid Ltd., Hampshire, England) medium and incubated at 37˚C for 6 hrs and then spread on to the selective medium of interest. If any bacteria survived or injured non-selective TSB medium was used to help resuscitate these cells and enable them to grow in selective microbiological medium.

Statistical Analysis

Three samples of each category were taken from the same farm. Reported plate count data represented in tables are the $\log_{10}$ mean values ± standard deviation of three individual trials, and each of these values were obtained from duplicate samples. Data were subjected to analysis of variance using the Microsoft Excel program (Redmond, Washington DC, USA). Significant differences in plate count data were established by the least-significant difference ($P < 0.05$) at the 5% level of significance.

RESULTS AND DISCUSSION

The search for healthy, safe, and sustainable food production has increased the consumption of organic fresh produce. These products should be free of pesticide residues and other synthetic substances commonly used in conventional agriculture, such as soluble fertilizers (Oliveira *et al*, 2012). At the same time organic products have lower risks related to chemical contamination; however, several investigations have raised concerns related to the microbiological quality of these foods (Delaquis *et al.*, 2007; Itohan *et al.*, 2011). Among organic fresh produce, fresh and dried herbs stand out due to their flavors, aromas, colors and continual availability in the market as well as acceptability regardless of age or economic group of the human population worldwide (Esimone *et al.*, 2003).

Thirteen categories of herbs and tea including black tea, blend tea, neem blend herbs, neem tea, mint (fresh and dry), jasmine (fresh & dry), lemongrass and lemongrass blend tea were analyzed for total aerobic population (TAB), total coliform population (TCC) and presence of *E. coli*, *E. coli* O157:H7 and *Salmonella* spp. Table 1 presents the results of the distribution of natural aerobic population, coliform population and presence of *E. coli*, *E. coli* O157:H7 and *Salmonella* spp in different fresh and dry herbs; water and manure soil. Higher aerobic bacterial counts were recorded as 6.9 log CFU/g in liker base tea samples and the lowest aerobic

Table 1. Distribution of natural aerobic population, coliform population and presence of *E. coli, E. coli* O157:H7 and *Salmonella* spp in different fresh and dry herbs; water and manure soil[a]

Herbs and tea Sample	Total Viable count (log CFU/g)	Total Coliform count (log CFU/g))	Total *E.coli* Count (log CFU/g)	*E.coli* O157:H7 counts (log CFU/g)	Presence of *Salmonella* Spp.	pH
Black tea (Normal)	5.9 ± 0.08	6.9 ± 0.11	4.7± 0.14	3.8 ± 0.06	ND	4.84
Blend tea	6.4 ± 0.11	5.9± 0.11	4.5 ±0.11	4.5 ± 0.12	ND	5.01
Black tea (Original)	6.0± 0.14	6.0± 0.34	5.1±0.11	4.6± 0.11	ND	5.40
Neem blend herbs	4.1 ± 0.15	ND*	ND	ND	ND	4.98
Neem Tea	3.9 ± 0.12	ND	ND	ND	ND	5.04
Black tea (Premium)	5.4 ± 0.14	4.1 ± 0.21	4.0 ± 0.12	3.2 ± 0.11	ND	5.10
liker base	6.9 ± 0.22	6.2 ± 0.22	5.7 ± 0.13	5.2 ± 0.15	ND	7.13
Lemongrass	5.9 ± 0.11	5.8 ± 0.15	5.8 ± 0.23	4.8 ± 0.13	ND	4.60
Lemongrass blended tea	5.5 ± 0.24	5.0 ± 0.09	4.7 ± 0.19	4.4 ± 0.11	3.7 ± 0.07	6.00
Jasmine fresh	5.7 ± 0.11	5.3 ± 0.12	5.1 ± 0.11	4.0 ± 0.12	1.0 ± 0.09	5.94
Jasmine dried	5.4 ± 0.13	5.2 ± 0.17	5.0 ± 0.11	3.9 ± 0.15	1.0 ± 0.12	5.94
Mint fresh	4.5 ± 0.12	4.4 ± 0.19	4.2 ± 0.12	3.2 ± 0.11	1.3 ± 0.14	5.99
Mint dried	4.4 ± 0.14	3.5 ± 0.23	3.4 ± 0.11	2.4 ± 0.12	ND	5.94
Tap water	3.5 ±0.13	2.0 ± 0.14	1.8 ± 0.11	1.7± 0.13	ND*	6.60
Tank water	6.0 ±0.13	4.7 ± 0.09	3.9 ± 0.11	3.1 ± 0.11	ND	7.50
Ground water	3.8 ± 0.13	3.4 ± 0.07	3.0 ± 0.11	2.1 ± 0.12	ND	6.50
Manure soil	6.0 ± 0.14	5.8 ± 0.09	5.0 ± 0.11	4.7 ± 0.11	ND	7.80

*ND=Not detected; [a]Results are expressed in mean± standard deviation of three replicate samples, which are being calculated from duplicate plates.

Table 2. Distribution of natural aerobic population, coliform population and presence of *E. coli, E. coli* O157:H7 and *Salmonella* spp at different steps of dried lemongrass production[a].

Lemongrass production & processing steps	Total aerobic counts (log CFU/g)	Total Coliform counts (log CFU/g)	Total *E. coli* counts (log CFU/g)	*E. coli* O157:H7 counts (log CFU/g)	Presence of *Salmonella* Spp.	pH
At Harvest	5.9 ± 0.08	5.8± 0.18	5.8± 0.18	4.8 ± 0.16	ND	4.6
Cleaning & No washing	5.7 ± 0.12	5.4 ± 0.08	4.7 ± 0.08	3.7 ± 0.10	ND	5.2
Cleaning & fresh water wash	5.0 ± 0.11	4.3± 0.16	4.3 ± 0.10	3.6± 0.20	ND	5.1
Cleaning with fresh hot water	5.2 ± 0.14	4.5 ± 0.08	3.8± 0.19	3.5 ± 0.17	ND	5.0
After dry heat at 90^0C for 20 min	4.4 ± 0.12	2.9 ± 0.12	2.8 ± 0.15	2.7 ± 0.12	ND	5.2
After grinding at room temperature	4.2 ± 0.13	4.0 ± 0.09	3.7 ± 0.18	3.7 ± 0.16	ND	5.4
After sorting at room temperature	5.6 ± 0.11	3.5± 0.20	3.3 ± 0.11	3.1 ± 0.11	ND	5.3
After final streaming	4.4 ± 0.10	4.3 ± 0.08	2.3 ± 0.10	2.3 ± 0.12	ND	5.4

*ND=Not detected; [a]Results are expressed in mean ± standard deviation of three replicate samples, which are being calculated from duplicate plates

counts were observed as 3.9 log CFU/g in neem tea samples (Table 1). Among the herb and tea sample tested, neem tea and neem blended herbs were determined to be microbiologically safe, because no coliform, fecal coliforms, *E. coli*, or *Salmonella* were recovered throughout the study. In contrast, jasmine, mint and tea blend with lemongrass were determined to be contaminated as the presence of *E. coli* O157:H7 and *Salmonella* was observed. The supply water used for irrigation, wash/rinse purposes, and compost used as fertilizer of soil were also analyzed. The water and composted manure was found to be heavily contaminated with enteric bacterial pathogens (Table 1). The total coliforms, *E. coli* and *E. coli* O157:H7 populations were enumerated as 5.0 log CFU/ml, 4.7 CFU/ml and 4.2 CFU/ml, respectively. *Salmonella* spp. was not detected in the manure sample tested (Table 1). In this study, water for irrigation and washing/rinse purpose was contaminated with *E. coli* O157H7, therefore it was concluded that there was a risk of contamination of final products. Foodborne outbreaks involving green vegetables contaminated by water have been reported in several studies around the world (Beuchat, 1996; Moyne, *et al*., 2011). Pathogenic bacteria such as *E. coli* O157:H7 are most often associated with outbreaks of waterborne diseases, resulting from inadequate treatment of water used for irrigation and washing of fresh produce (Levantesi *et al*., 2012; Beraldo and Filho, 2011; Fischer-Arndt *et al*., 2010). Therefore, specific control measures should be developed in order to prevent final product contamination.

Table 3. Recovery of natural aerobic population, coliform population and presence of *E. coli, E. coli* O157:H7 and *Salmonella* spp after corrective measures in processing and production of dried lemongrass[a].

Lemongrass production & processing steps	Total aerobic count (log CFU/g)	Total Coliform count (log CFU/g)	Total *E. coli* Count (log CFU/g)	*E.coli* O157:H7 counts (log CFU/g)	Presence of *Salmonella* Spp.	pH	Positive / No of sample tested
Control	5.9 ± 0.08	5.8 ± 0.18	5.8 ± 0.18	4.8 ± 0.16	ND	4.6	
Lemongrass (After corrective measures 1)	2.7 ± 0.18	ND*	ND	ND	ND	5.7	0/3
Lemongrass (After corrective measures 2)	2.5 ± 0.12	ND	ND	ND	ND	5.8	0/3
Mint (After corrective measures 1)	2.7 ± 0.14	ND	ND	ND	ND	6.0	0/3
Mint (After corrective measures 2)	2.9 ± 0.11	1.3 ± 0.09	1.0 ± 0.11	ND	ND	5.6	1/3

*ND=Not detected; [a]Results are expressed in mean± standard deviation of three replicate samples, which are being calculated from duplicate plates.

However when composted manure was analyzed, the presence of higher numbers of coliforms (5.8 log CFU/g), *E. coli* (5.0 log CFU/g), and *E. coli* O157:H7 (4.7 log CFU/g), were observed. *Salmonella* spp. was not detected in the compost samples (Table 1). Numerous published reports have indicated that the composting time and temperature of manure could effectively reduce microorganisms like *E. coli*, *E. coli* O157:H7, and *Salmonella*, which were routinely detected in fresh compost (James, 2006; MAFF, 2000; Millner, 2003; Johannessen, 2005). However, the organic fertilizer samples analyzed in the present study were above the detection limit (3.0 log MPN/g), indicating that the control of manure was not adequate.

From the same farm, lemongrass production practices were taken as a case study to determine the point of *E. coli* O157:H7 contamination and to take corrective measure in eliminating the risk. The average aerobic bacterial counts, coliform counts, *E. coli* and *E. coli* O157: H7 counts were recorded as 5.9 log CFU /g., 5.8 log CFU /g, 5.8 log CFU/g and 4.8 log CFU /g, respectively after harvest. However, *Salmonella* spp. was not detected in the lemongrass sample (Table 2). After harvest, the lemongrass sample was washed with water, in the hope of being able to remove the debris and to reduce the microbial load. Washing with tap water reduced the microbial load by 0.5-1.0 log CFU/g of bacteria. The lemongrass sample was subsequently dried in a fluid bed dryer for 20 minutes at a temperature recorded as 90°C. After the drying process was completed, the microbial load was decreased substantially but not eliminated completely. Thereafter, grinding, sorting and packaging were done at the commercial settings. The contamination remaining was still evident after packaging and in the finished product. Numerous research reports have indicated that dry heating at 90°C for 20 minutes is sufficient to eliminate the pathogen (Bari *et al*., 2009), however, in this study, pathogens were not eliminated completely. This finding suggested that heating temperature or the contact

time may not be adequate to inactivate pathogens in lemongrass samples. After that an investigation of actual temperature and time inside the fluid bed dryer was conducted and it was discovered that the actual temperature at contact point was not homogeneous for 90˚C, and the contact time was only a few seconds because uniform conditions were not achieved by passing air through the lemongrass layer under controlled velocity conditions to create a fluidized state. Therefore, when the sample comes in contact with heat for few seconds, some bacteria may become injured and could resuscitate in between the cycles and in following steps, therefore, survive and subsequently be detected in the final products. Therefore, corrective actions of dryer temperature were undertaken and after these corrective measures, the same samples were dried in the same machine, analyzed and the results are presented in Table 3. It was found that drying at 90 ˚C for 20 min in an oven was enough to eliminate the pathogens even though the sample was contaminated initially (Table 3). To prevent further contamination, the workers was trained for personal hygiene and GAP, and hand gloves, mask, hairnet, apron, hand washing soap/sanitizers, and a single used towel was provided, along with cleaning of utensils, machinery, and transport vehicles was conducted using steam. After these steps were taken, one batch of lemongrass was processed, dried and analyzed for pathogens. Neither generic *E. coli* nor any pathogenic *E. coli* O157:H7 were detected in the in the sample and the total viable bacteria and coliform population counts were found to be less than 100 CFU/ml, which is below the permissible limit (Table 3). These findings again showed that good hygiene practices are necessary for reducing foodborne pathogen contamination in the product.

For the consumer, a common strategy to avoid foodborne disease is heating or cooking of potential risk products before consumption. However, this approach is not appropriate for the majority of fresh herbs and leafy greens that are mainly consumed raw, or added to food after the heat-treatment. For example, tea is usually made with dried tea leaves, or blended with other dried herbs and pouring the boiling water over the leaves and letting the combination remain for a few minutes and then consumed. This general practice is consistent all over the world. If the herbs/tea leaves were contaminated with pathogens, whether or not hot water can reduce the risk of pathogen ingestion is a critical consideration. To solve this approach, an experiment was designed to determine the effectiveness of pouring hot water onto dried herbs/leaves in a cup for eliminating the risk of pathogen exposure. The results were presented in Table 4. Three different contaminated tea samples include black, blend and lemongrass tea were analyzed. One gram of each sample was placed in a tea-cup and 50 ml of hot water was added to each cup individually, either covered with a lid or without a lid, and held up to 5 min. At each 30 second time interval, microbiological population counts were enumerated and recorded. The hot water temperature was recorded as 82˚C when initially added in the cup and after 60 seconds the temperature was reduced to 78˚C; further reduced to 73˚C after 3 minutes holding time and at the end of 5 minutes the temperature decreased to 64˚C. It was determined that the initial viable bacterial counts were 5.4 log CFU/g, coliform counts were 4.1 log CFU/g, *E. coli* counts were 4.0 log CFU/g and *E. coli* O157:H7 counts were 3.2 log CFU/g in the blended tea samples, respectively (Table 4). After 30 seconds of treatment with hot water without a lid, a 2.0 log CFU/g reduction of viable bacterial counts was observed for the blended tea samples. The coliform bacteria, *E. coli* and *E. coli* O157:H7 counts were reduced to non-detectable levels within 30 seconds of hot water treatment despite the higher pathogen contamination levels in the initial samples. Similar experimental results were observed in black tea, and the lemongrass sample. The bacteria might be injured or severely injured when hot water was added in the cup and thus may not be able to grow in selective microbiological medium. To solve this issue, an enrichment study was done. No coliform, *E. coli* and *E. coli* O157:H7 were detected in the enrichment study after 30 seconds and above this holding time (Table 4). This finding suggested that the addition of hot water (82˚C) in the tea leaves resulted in the reduction of pathogens below detection limits of the current study and thus the present tea making practice is potentially capable of providing safe tea for drinking even though the tea leaves were initially contaminated.

Table 4. Effectiveness of pouring hot water over contaminated dried (blend tea, black tea and lemongrass samples) leaves in a cup of tea at different holding time.

Sample type	Hot water treatment time (Sec)	Recovery of microorganisms (log CFU/g)[a]					After Enrichment			
		Total aerobic count	Total Coliform count	*E. coli*	*E. coli* O157:H7	*Salmonella* Spp.	Presence of Coliform	Presence of *E.coli*	Presence of *E. coli* O157:H7	Presence of *Salmonella* Spp.
Blended Tea Samples	Control	5.4 ± 0.08	4.1 ± 0.10	4.0 ± 0.12	3.2 ± 0.11	ND	-	-	-	-
	30	3.5 ± 0.18	<1.0*	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	60	3.2 ± 0.12	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	90	3.0 ± 0.11	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	120	3.0 ± 0.14	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	150	2.9 ± 0.09	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	180	3.2 ± 0.09	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	5 min	3.1± 0.08	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
Black Tea Samples	Control	5.9 ± 0.11	6.9 ± 0.12	4.7 ± 0.11	3.8 ± 0.16	ND	-	-	-	-
	30	1.3 ± 0.14	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	60	1.3 ± 0.13	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	90	1.0 ± 0.09	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	120	1.0 ± 0.07	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	150	-	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	180	-	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	5 min	-	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
lemongrass Samples	Control	6.0 ± 0.15	6.0 ± 0.12	5.1 ± 0.11	4.6 ± 0.14	ND	-	-	-	-
	30	3.1 ± 0.16	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	60	3.0 ± 0.12	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	90	3.0 ± 0.11	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	120	3.0 ± 0.07	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	150	-	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	180	-	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil
	5 min	-	<1.0	<1.0	<1.0	ND	Nil	Nil	Nil	Nil

<1.0*= Less than detection limit; ND= Not Detected, Nil= Absent; [a]Results are expressed in mean± standard deviation of three replicate samples, which are being calculated from duplicate plates.

CONCLUSIONS

In the present study it can be concluded that the organic fertilizer and the water used for irrigation and washing are critical sources of microbial contamination that need to be controlled in the production chain of organic produce. The contamination of manures also highlighted the need for a fertilizer control program in order to control the composting time and avoid the addition of fresh manure to the composted manure. Regarding the issues of irrigation and wash water, the results demonstrated the importance of using water from safe sources. It is also essential to emphasize the need for awareness and training to food handlers because even though organic vegetables may not be perceived as being chemically contaminated; nonetheless, they could very well be contaminated with pathogens and, for that reason, sanitization procedures should be developed to avoid foodborne illnesses. The use of a risk-based sampling plan in combination with corrective measures, personal hygiene and good agricultural practices (GAP) allowed us to produce safe organic herbs. This case study provides an overview of the organic farms' status in northern Bangladesh, where good hygiene practice and GAP were introduced as a part of this study.

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