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RESEARCH ARTICLE

EFFICACY OF VINEGAR, SALT SOLUTION AND LEMON JUICE AS ANTIMICROBIAL AGENTS IN REDUCING THE MICROBIAL LOAD OF LETTUCE SOLD AT AYEDUASE, A SUBURB OF KUMASI, GHANA

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ABSTRACT

Background: Vegetables are known to provide enormous health benefits to man. Despite these, there is a great potential of microbial contamination. ***Objective:*** This study assessed the effect of increasing concentration of antimicrobial agents; vinegar, lemon juice and salt solution on the microbial load as well as their efficacy on the microbial isolates found on the lettuce sold in Ayeduase, a suburb of the Kumasi Metropolis, Ghana. ***Method***: 10g of lettuce was pulsified with 90mls each of 0.3M, 0.5M, 0.7M, and 0.9M concentration of vinegar, lemon juice and salt solution. Serial dilution and colony counting were performed by pour plating on PCA (Plate Count Agar) and Coliform counts for each sample and concentration. Isolates were identified using standard biochemical methods. ***Results***: The mean microbial load ranged from highest of 3.94×10^7CFU/100ml using 0.3M salt solution to the least 2.83×10^2CFU/100ml washing with 0.9M Apple Cedar vinegar. The total microbial counts significantly decreased ($P<0.05$) with increasing antimicrobial concentration and in comparison, with control (distilled water washing 1.57×10^8 CFU/100ml). Six different bacteria species and fungi species were isolated of which *Escherichia coli* and *Salmonella* were the most resistive towards the action of the antimicrobials with *Enterococcus, Streptococcus* and *Staphylococcus* being the most susceptible species. Fungi species proved fairly resistant to the activity of the antimicrobials. ***Conclusion***: Increasing vinegar concentration has the tendency to reduce microbial loads on vegetables and hence its application is recommended.

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INTRODUCTION

Minimally processed fresh fruits, vegetables (raw or uncooked), and juices are recognized as nutritional foods. Consumption of these nutritional foods has largely been elevated in the global community due to increased concern in maintaining healthy lifestyle (Abadias *et al*., 2008; Heaton and Jones, 2007). Along with the nutritional benefits derived from these fresh produces, the risk of microbial contamination, leading to food-borne illnesses is also significant in the context of public health management (Ahmed *et al*., 2014).

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Again, while the incidence of foodborne illness associated with these nutritional foods is very low relative to the quantity consumed, the increased use of these produces has been accompanied by an increase in reported outbreaks associated with their consumption (Palumbo et al., 2007). The incidence of outbreaks of food borne illnesses associated with the consumption of fresh vegetables and fruit in recent years has increased, partly because of an increased demand for fresh produce (Chang and Chen, 2003; Lynch et al., 2009). According to Johanssen *et al*., (2002), vegetable contamination may come from the soil, organic and inorganic manure, irrigation water as well as other human activities involved in the production process and transportation. It can also occur during pre-harvest, postharvest and processing (De Roever, 1998). Harris *et al*. (2003) reports that, intestinal pathogens of humans and animals contaminate vegetables that are eaten in their raw state when they grow close to or within the soil.

In addition, human pathogenic bacteria such as *E. coli* 0157:H7 and *Salmonella spp* are able to internalize within the plant tissue following contamination that has occurred to the seed. The methods used to cultivate most vegetables tend to expose them to a wide range of pathogens such as *E. coli, Salmonella sp*., *Campylobacter, Listeria monocystogenes* among others. Although various antimicrobial agents have been examined for their effectiveness, most treatments have been shown to have minimal effects, resulting in less than a 2 log cfu.g^{-1} (cm^2) reduction in microbial numbers, and most would not be suitable products for use at the household level (Beuchat et al., 1998). For example, the washing processes which is one of the first processing operations for ready-to-eat salads make use of free chlorine which is usually used to reduce microbial contamination in commercial procedures [Cherry, 1999; Beuchat, 2002]. Unfortunately, many consumers are increasingly avoiding consuming foods treated with preservatives of chemical origin. This has necessitated the use of new alternatives like natural products to disinfect fresh vegetables and fruits. This study therefore assesses the effect of increasing concentration of antimicrobial agents; vinegar, lemon juice and salt solution on the microbial load as well as their efficacy on the microbial isolates found on the lettuce sold in Ayeduase, a suburb of the Kumasi Metropolis, Ghana.

MATERIALS AND METHODS

Study site and sampling method: The study was conducted in markets at Ayeduase, a suburb of Kumasi in the Kumasi Metropolitan Assembly. The coordinates of Ayeduase are 6°40'0" N and 1°34'0" W and it is located at an elevation of 246meters above sea level and its population amounts to 29,748. Fresh green lettuce, vinegar (one local type and one foreign type), local salt crystals and lemon were randomly purchased from markets in Ayeduase. The leafy portions of the lettuce were kept in a sterile plastic sealable food bags with the other samples and transported to the laboratory for chemical and microbiological analysis insulated coolers.

Laboratory procedures: Five sterile 250ml conical flasks were filled with the various solutions (vinegar, lemon juice, salt solution) and distilled water. The lettuce was pulsified in the various solutions (vinegar, lemon juice, salt solution) and the sterile distilled water for 30seconds and allowed to stand for 3minutes 30 seconds after which it was used. A 0.3M concentration sample was prepared from the vinegar types, lemon juice as well as the salt solution (with a pH of 8.5). The concentrations of the various solutions (vinegar, lemon juice and the salt solution) were steadily increased to 0.5M, 0.7M and 0.9M. 10grams of lettuce each was weighed and pulsified in 90mls of the various solutions, that is the vinegar (local type and the foreign type, lemon juice), distilled water and 0.3M salt solution for 30 seconds and left to stand for3minutes 30 seconds. Petri dishes were filled with 20mls each of Plate count agar for inoculations to check for the presence or absence of microorganisms. Pure cultures were subsequently streaked on sterilized selective growth media. Colonies were later picked for Gram staining.

Isolation procedures: The identification of microbes is done in two forms: the primary and the secondary tests. This study was however limited to certain primary tests such as cultural morphology, Gram reaction, culturing and sub-culturing of microbes on different growth media solutions and plates. After 24 hours of incubation, colonies on plates showing growth were counted using a colony counter (or a quartet division of the Petri-dish) and representative isolates were streaked onto a sterile nutrient agar, McConkey agar, Salmonella-Shigella agar, Slanetz and Bartley media, Potatoes dextrose agar, and EMBA. Discrete colonies were kept at 35 °C for 24 hours.

Statistical analysis: To ascertain the effectiveness of vinegar (both local and foreign), lemon juice and salt solutions, the data collected were subjected to the Statistical Analysis System for analysis of variance and Duncan's multiple range test (SPSS 2.3 for Windows pocket program) to determine whether significant differences ($p < 0.05$) exist among mean log values.

RESULTS

Antimicrobial action of the disinfectants: Vinegar (both foreign and local), Salt solution and Lemon juice as antimicrobial agents in reducing the microbial load of lettuce leaves was investigated. The results are presented in Table 1. The foreign apple cider vinegar (SN 4) at a pH of 2.5 was the most effective in reducing the microbial flora of lettuce with microbial count of 9.42×10^4, 7.53×10^3, 2.35×10^3 and 9.20×10^2cfu/100ml of pulsified lettuce at concentrations of 0.3M, 0.5M, 0.7M, and 0.9M respectively. Of the two vinegar samples, the microbial count of the foreign apple cider vinegar was much lower than the local vinegar (SN 3) with microbial loads of2.0×10^{10}, 3.20×10^8, 4.01×10^6 and 3.80×10^6cfu/100 ml of pulsified lettuce. the lemon juice (SN 2)recorded5.60×10^{14}, 1.94×10^{14}, 1.50×10^{14} and 1.10×10^{12}cfu/100 ml)whilst the salt solution (SN 1)showed microbial load of 9.15×10^{14}, 3.20×10^{14}, 2.53×10^{14} and 1.60×10^{14}cfu/100ml) with the distilled water recording 1.20×10^{16}cfu/100ml for 0.3M, 0.5M, 0.7M, 0.9M respectively.

Effects of different disinfectants on the bacterial isolates: Sensitivity of various bacteria to different disinfectants is greatly influenced by varying concentration of the disinfectants. The reaction of bacterial isolates to varying concentration of acetic acid in vinegar, citric acid in lemon juice and different concentrations of salt solution were analyzed. Figures 1 – 6 below indicate the microbial counts of the various isolates after they were exposed to the bactericidal action of the disinfectants, with distilled water serving as a control.

Microbial count of Staphylococcus treated with the disinfectants: Staphylococcus isolates was fairly high towards the disinfectants. Microbial numbers reduced from $1.44 \times 10^7$to 5.3×10^3cfu/100mls of pulsified lettuce for the Salt solution whilst the Apple Cider vinegar recorded 1.50×10^3, 8.50×10^2, 3.5×10^2 and 1.5×10^2cfu/ 100mls of pulsified lettuce respectively in the 0.3M, 0.5M, 0.7M, and the 0.9M.

Microbial count of Enterococcus treated with the disinfectants: The susceptibility of the Enterococcus isolates was very high towards the various disinfectants. Microbial numbers reduced from the Salt solution (3.07×10^5, 1.29×10^5, 7.20×10^4 and 5.35×10^4 CFU/ 100mls of pulsified lettuce**)** to the Apple Cidar vinegar (1.0×10^3, 7.50×10^2, 4.50×10^2 and 1.0×10^2 CFU/ 100mls of pulsified lettuce respectively in the 0.3M, 0.5M, 0.7M, and the 0.9M)

Microbial count of Streptococcus treated with the disinfectants: Streptococcus isolates, which is a Gram-negative bacterium was very high towards the various disinfectants.

Table1. Total Microbial Count on pulsified Lettuce treated with various concentrations of disinfectantsas means of triplicate determinants

Sample (Disinfectant)	Sample code	Concentration/M or Mol/dm^3	Microbial Count cfu/100ml	PCA cfu/100mls
Salt solution	SN 1	0.3	9.15×10^{14}	3.94×10^{7}
		0.5	3.20×10^{14}	1.53×10^{7}
		0.7	2.53×10^{14}	9.12×10^{6}
		0.9	1.60×10^{14}	7.67×10^{6}
		Distilled Water	1.20×10^{16}	1.57×10^{8}
Lemon juice	SN 2	0.3	5.60×10^{14}	2.17×10^{7}
		0.5	1.94×10^{14}	1.06×10^{7}
		0.7	1.50×10^{14}	1.05×10^{7}
		0.9	1.10×10^{12}	7.01×10^{6}
		Distilled Water	1.20×10^{16}	1.57×10^{8}
Local vinegar	SN 3	0.3	2.0×10^{10}	1.16×10^{6}
		0.5	3.20×10^{8}	7.83×10^{5}
		0.7	4.01×10^{6}	4.10×10^{5}
		0.9	3.80×10^{6}	3.33×10^{3}
		Distilled Water	1.20×10^{16}	1.57×10^{8}
Apple cider vinegar	SN 4	0.3	9.42×10^{4}	4.28×10^{4}
		0.5	7.53×10^{3}	8.35×10^{3}
		0.7	2.35×10^{3}	5.45×10^{2}
		0.9	9.20×10^{2}	2.84×10^{2}
		Distilled Water	1.20×10^{16}	1.57×10^{8}

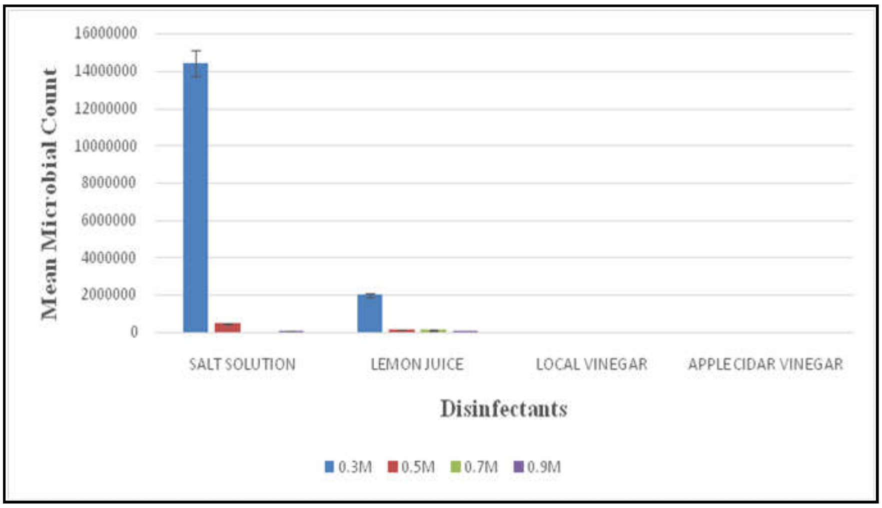

Figure 1. Mean microbial count of Staphylococcus against the varying concentrations of the disinfectants

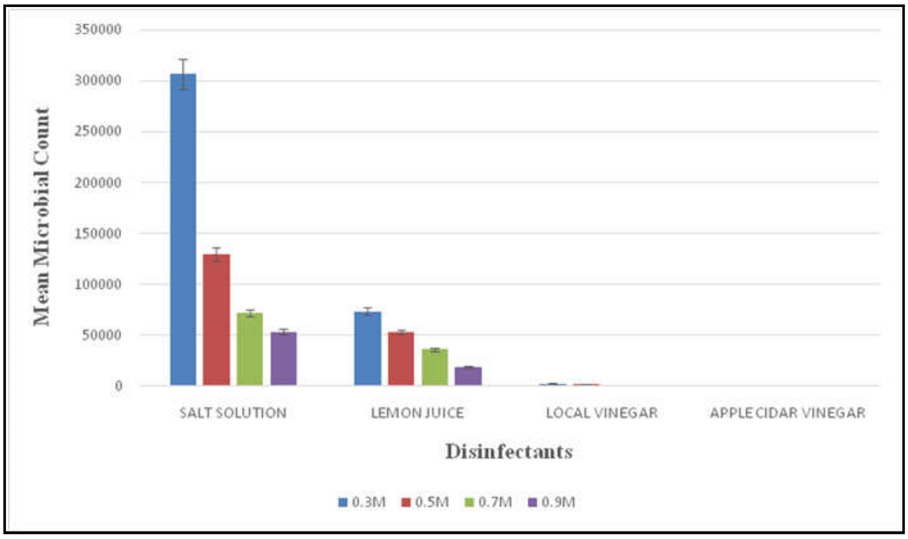

Figure 2. Mean microbial count of Enterococcus against the varying concentrations of the disinfectants.

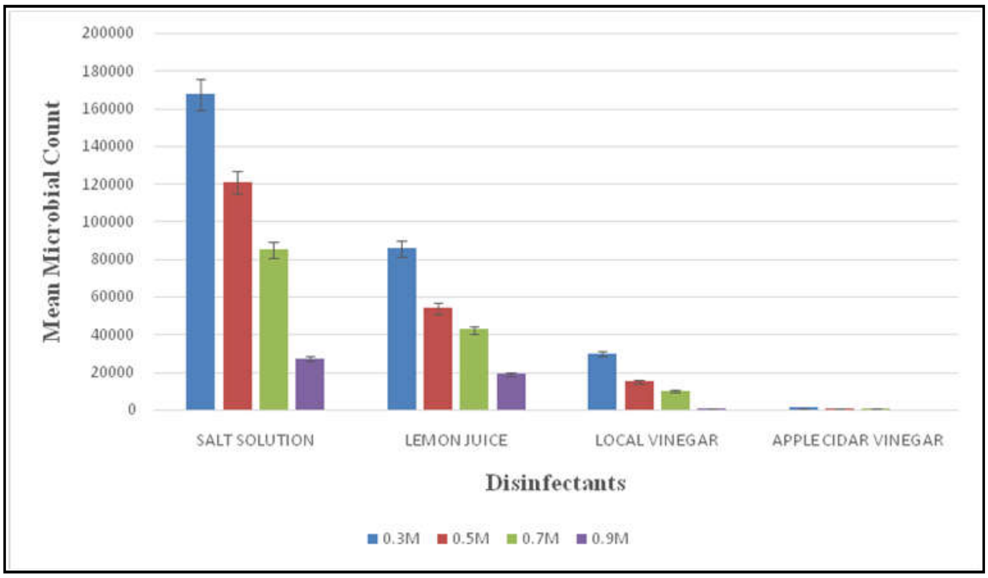

Figure 3. Mean microbial count of Streptococcus against the varying concentrations of the disinfectants

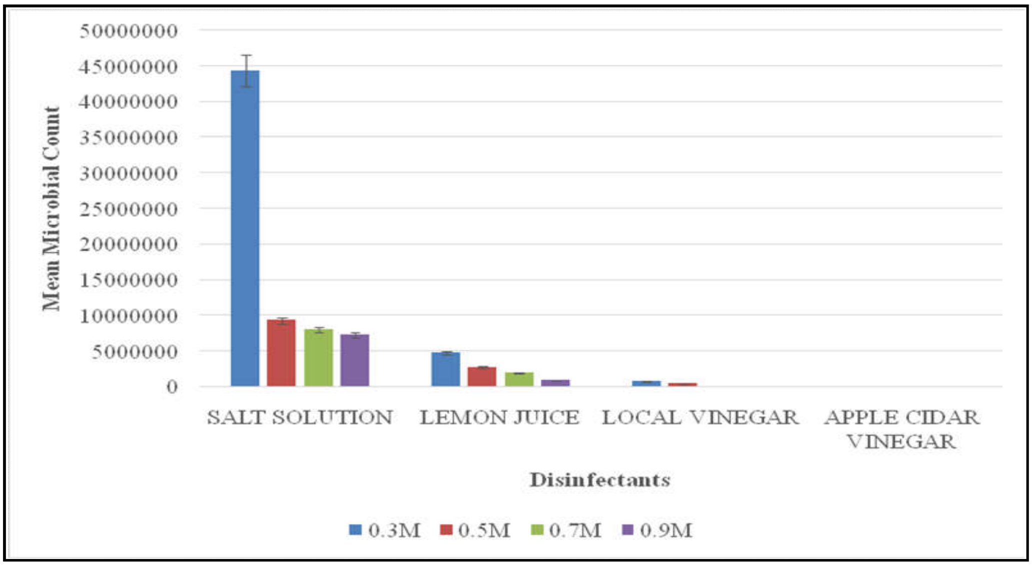

Figure 4. Mean microbial count of Fungi against the varying concentrations of the disinfectants

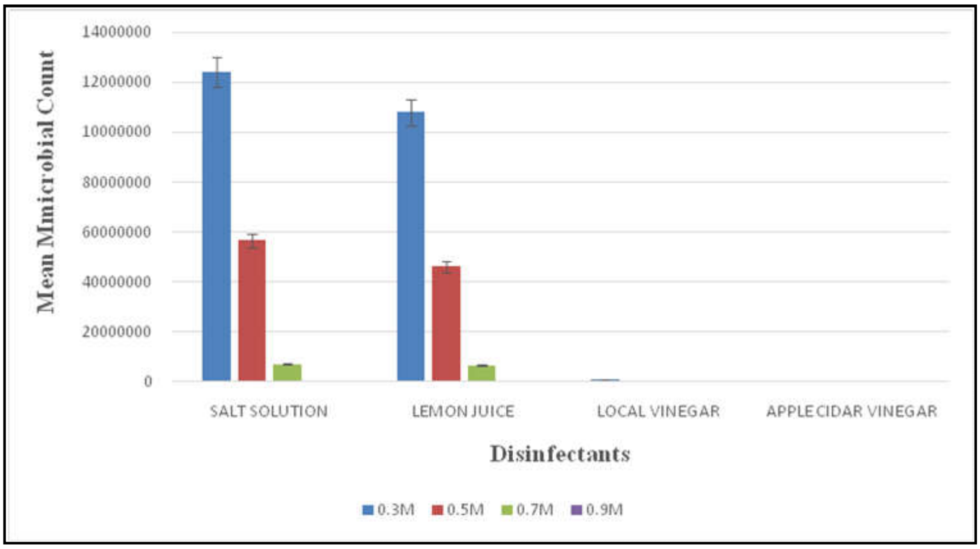

Figure 5. Mean microbial count of Salmonella against the varying concentrations of the disinfectants

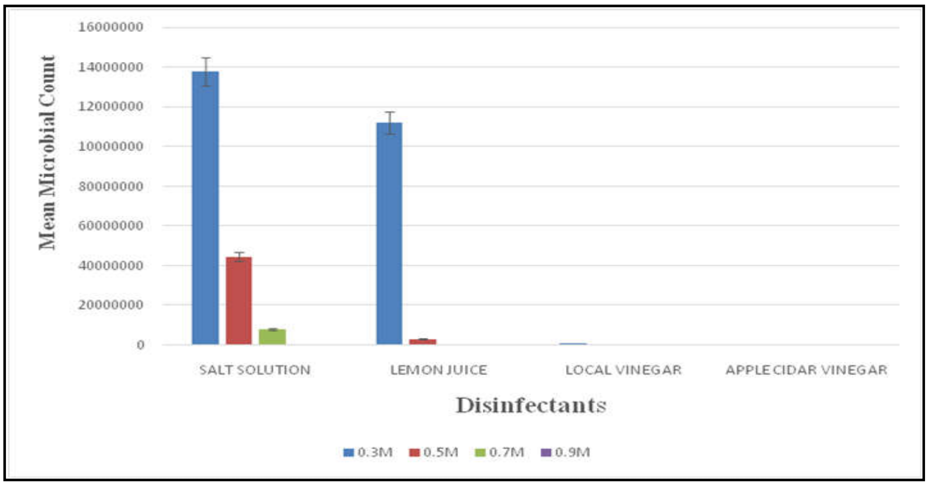

Figure 6. Mean microbial count of Escherichia coli against the varying concentrations of the disinfectants

Microbial numbers reduced from the Salt solution (1.68×10^5, 1.21×10^5, 8.50×10^4 and 2.70×10^4 CFU/ 100mls of pulsified lettuce) to the Apple Cider vinegar (1.0×10^3, 9.50×10^2, 4.80×10^2 and 1.8×10^2 CFU/ 100mls of pulsified lettuce respectively in the 0.3M, 0.5M, 0.7M, and the 0.9M).

Microbial count of Fungi treated with the disinfectants: Fungal isolate susceptibility was very low towards the various disinfectants even though it followed the same pattern as that of the bacterial isolates. Apple Cider vinegar was the best disinfectant with 3.30×10^4, 2.50 x 10^4, 1.20×10^4 and 8.50×10^2cfu/ 100mls of pulsified lettuce respectively in the 0.3M, 0.5M, 0.7M, and the 0.9M. The results show that fungi are fairly resistant to the disinfectants and this accounted for the high microbial count across all concentrations as recorded under Figure 4.

Microbial count of Salmonella treated with the disinfectants: Figure 5 illustrates the susceptibility pattern of Salmonella isolates under the various disinfectants. The results show that Salmonella are less sensitive to the disinfectants and thus very resistant towards the anti-microbial agents. This accounted for their high mean microbial count. With a control microbial count of 1.57×10^8cfu/ 100mls of pulsified lettuce, mean microbial numbers hardly even reduced from the Apple Cidar vinegar, 4.28×10^5, 6.34×10^4, 3.25×10^4 and 7.20×10^3cfu/ 100mls of pulsified lettuce respectively in the 0.3M, 0.5M, 0.7M and, 0.9M concentration.

Microbial count of *Escherichia coli* treated with the disinfectants: *Escherichia coli* isolate was high in the various disinfectants, thereby indicating the resistance of *E. coli*to the disinfectants. Mean microbial load reduced from the Salt solution (1.38×10^8, 4.47×10^7, 8.16×10^5 and 1.0×10^5cfu/ 100mls of pulsified lettuce) to the Apple Cider vinegar (3.10×10^5, 6.24 X 10^4, 9.26×10^3 and 5.55×10^3cfu/ 100mls of pulsified lettuce respectively in the 0.3M, 0.5M, 0.7M, and the 0.9M). In all cases, the control microbial count of 1.57×10^8cfu/ 100mls of pulsified lettuce was used.

DISCUSSION

The efficacy of antimicrobial agents used in reducing microbial load is usually dependent on the type of treatment, type and physiology of the target microorganisms, characteristics of produce surfaces, exposure time and concentration of disinfectant (Tagoe *et al.,* 2011).

Salt solution recorded the least efficacy across the various concentrations owing to high microbial numbers associated with the lettuce disinfected for both the coliforms and the plate count with a p-value of 0.748). Salt solution with an alkaline pH (8.5) allows the survival of microorganisms to some extent comparative to the vinegars. These results are consistent with the data reported by Akple, (2009) in a study that assessed the effectiveness of non-treatment interventions associated with waste water irrigated vegetables. Usually bacteria grow well in hypotonic solutions resulting from the inflow of water into the cell. Lemon juice also recorded higher microbial count as compared to the vinegars with a p-value of 0.853. Citric acid has been shown to contribute much to the antimicrobial activity of the lemon juice and therefore increasing the concentration of the lemon juice affects microbial growth numbers. This result is consistent with the findings of Abu-Shanab *et al.*, (2004) who reported that concentrated solution of lemon juice shows inhibitory and bactericidal activity against growth of microorganisms in a study that assessed antimicrobial activities of some plant extracts utilized in popular medicine in Palestine. Microorganisms are known to respond differently to varying pH concentration, with some being acidophiles and others alkaliphiles.

From Table 1, SN 4 recorded a mean microbial load of 4.23×10^4,8.30×10^3,5.43×10^2 and 2.83×10^2cfu/100mls of pulsified lettuce and an insignificant p-value ($P=0.262$) when treated with 0.3M, 0.5M, 0.7M and 0.9M concentrations of Apple Cidar vinegar solutions. The Local vinegar was effective on microbial isolates but its numbers were higher than that of the Apple Cidar vinegar, with a p-value of 0.523 relative to all the concentrations. Studies by Vijayakumar and Wolf-Hall (2002) showed 5-log reduction in *E. coli* counts when lettuce leaves were treated with 35% white vinegar. Generally, microbial load decreased as concentration of disinfectant increased for both the Local vinegar and the foreign vinegar (Apple Cidar vinegar) as reported by Tagoe *et al.,* (2011). Increasing the concentration of the vinegars resulted in a decrease in the pH creating an acidic medium which is toxic to most microbes. This reduction in pH causes the release of more H^+ ions which acidify and disrupt bacterial cell membrane thereby inhibiting microbial growth.

Action of disinfectants on the microbial isolates: *Escherichia coli* and Salmonella were the most resistant bacteria to the various disinfectants, as they recorded the highest microbial numbers across all the disinfectant.

Maciorowski *et al*. (2007) reported that the general survival ability of *E. coli* increases upon prolonged exposure to a particular environmental stress, indicating the ability of *E. coli* to activate survival mechanisms when threatened. Resistant bacteria change their cell wall composition or produce enzymes that disable the antimicrobial agents. This property of Salmonella and *E. coli* allow them to survive in high acidic environments of pH as low as 3.3-4.2 and high concentrations posed by various antimicrobials. Sengun and Karapinar (2005) in their work, showed that treating rocket leaves with fresh lemon juice and vinegar caused a significant reduction in S. typhimurium population. Another study by Yucel and Karapinar in 2004 showed that treatment of carrot samples with lemon juice or vinegar alone caused significant reduction in *S. typhimurium* reached up to 3.58 log10 CFU/g. Fungi (Fig 4) also showed high resistivity across the various concentrations for the disinfectants used due to their ability to survive in diverse range of environmental conditions. Fungal pathogens are able to survive the unique stresses of the various microenvironments where pH can range from 2-10.

Staphylococcus, Enterococcus and Streptococcus which are Gram positive bacteria recorded the least microbial numbers across the various concentrations for the disinfectants used. The interaction of the disinfectant with the cell surface followed by penetration into the cell wall produce significant effect on the microorganisms, thus the extreme acidic nature of the vinegars and the slightly basic pH of the salt solution. Bacteria numbers also decreased as a result of treatment with salt solution (3.94×10^7—7.67×10^6cfu/100mls of pulsified lettuce for the plate count) as a result of the permeability of the bacterial cell wall allowing only water and very small molecules. Analysis of data for the Coliform counts and Plate counts of Salt solution to the lemon juice, local vinegar and Apple Cidar vinegar across all concentrations yielded p-values (P=0.148 and P=0.91 for Coliform Counts and Plate counts respectively). This implies that, the differences between the variances in microbial counts for the various disinfectants across all concentrations for both the Coliform counts and the Plate counts are insignificant at a confidence interval of 95%.

Conclusion

This study provides adequate information on the microorganisms likely to be found on fresh lettuce as well as the ideal antimicrobial agent suitable for disinfecting this vegetable. It is evident that disinfecting vegetables either with vinegar, lemon juice and salt solution substantially reduce the microbial populations on lettuce. Vinegar has been identified as more effective than commonly used salt solution in reducing microbial load on lettuce and thatincreasing vinegar concentration has the tendency to reduce microbial loads on vegetables and hence its application is recommended.

Conflict of Interests: The authors have not declared any conflict of interests.

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