DECLARATION

I hereby declare that this submission is my own work towards the Master of Science (MSc) and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text**.**

ABDU-SAMAD, FAIZAH …… ……..…….

(PG 7049916) Signature Date

Certified by:

Dr. Leonard D.K. De-Souza …… ……

(Supervisor) Signature Date

Certified by:

Dr. (Mrs.) Faustina Dufie Wireko-Manu …….. ……..

(Head of Department) Signature Date

ABSTRACT

Foodborne illnesses have been associated with the consumption of fresh produce, specifically leafy vegetables due to their soft texture and method of cultivation. As a safety precaution, these vegetables are usually cleaned with chemical sanitizers before consumption, hence the efficacy of various chemical sanitizers was tested on leafy vegetables to ascertain their potency. Lettuce, cabbage and spinach were collected aseptically in plastic bags from Kumasi central market to the laboratory. Samples were taken through pretreatment by washing with distilled water, sanitizing with 70% alcohol under Ultra Violet light to ensure complete sterility. Test organisms *E. coli,* Salmonella and *L. monocytogenes* inoculum were obtained from the microbiology laboratory of the Centre for Scientific Research into Plant Medicine (CSRPM), Akuapem Mampong and inoculated on samples using the dip method. Samples dried for an hour at 37^oC and were sanitized with varying concentrations of peracetic acid, acetic acid, hydrogen peroxide, sodium hypochlorite and sodium chloride within varying exposure times. Microbial analysis were carried out at a temperature of 37^oC for 48 hours on all samples using spread plate technique on plate count agar (PCA) for enumerating colonies that will grow on plates. Results showed that there was a significant difference ($P<0.05$) between the loads on various test organisms. However, *E. coli* was chosen as a model organism. After inoculating cabbage, lettuce and Spinach, the results showed no significant difference ($P>0.05$) in the loads, thus cabbage leaves were selected for the assay. Inoculum sample of pretreated leaves showed 0 CFU/g and served as a control. Plate count before and after sanitizer treatment were also obtained and used to determine the potency of chemicals on microorganisms through the reduction in microbial population. All the sanitizers were found to have an efficacy of 99.99% except for sodium chloride which was 99.90%.

DEDICATION

I dedicate this thesis is to my mother, Zainabu Azumah Braimah.

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LIST OF ABBREVIATIONS

HACCP	-	Hazard Analysis for Critical Control Point
ICMSF	-	International Commission on Microbiological Specification for Food standards.
PCA	-	Plate Count Agar
WHO	-	World Health Organization
ANOVA	-	Analysis of Variance

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND

Food safety is a science that is concerned with handling, preparation, and storage of food in ways that forestall food borne unhealthiness. This entails the elimination of physical, chemical and biological hazards from food to make it safe for consumption (Coskun Dalgiç, et al., 2011).

The objective of food safety is to eliminate food-borne disease outbreak and enhance consumer confidence in food by making food safe for human consumption (Jay *et al.,* 1999). The application of pre-requisite programs such as good agricultural and sanitation practices from farm to fork through the implementation of Hazard Analysis for critical Control Point (HACCP) goes a long way to realize this objective.

Consumer food choices are made based on many factors such as satiety, taste, availability, convenience, age, health awareness, environmental influence, cost etc. One of such choices is the consumption of fruits and vegetables which form a vital aspect of people's diet all round the world because of the health awareness created and increased urbanization (Cisse ,1997; Olayemi, 1997; Armar Klemesu *et al.,*1998 ; Niang 1999; Faruqui *et al.* 2004; Amoah *et al.,*2005). Vegetables form the basis for a healthy and balanced diet due to the nutritional components they possess such as vitamins, minerals, proteins, calcium, chlorophyll, carotene, potassium, dietary fiber and folate (Koffi-Nevry *et al*., 2012).

Vegetables also have a beneficial impact on general wellbeing and prevention of diseases (Remesy *et al.,* 1998) by decreasing the risk of acquiring illnesses such as cancer, coronary heart disease, etc.

In Ghana and other developing countries, general sanitation and proper waste management remains a challenge and cropping practices of foods like vegetables cannot be assured to be pathogens free. Numerous studies in West Africa has revealed high levels of microorganism infection in irrigation water; on farms and market vegetables (Cisse ,1997) which surpasses the International Commission on Microbiological Specification for Food standards by far (ICMSF 19742).Other common sources of microorganisms are soil, air, farm pests, food handlers and irrigation containers used (Taura and Habibu, 2009).

According to markets and street food surveys conducted in Accra Ghana, about 200,000 urban residents consume foods containing raw vegetables treated by sewer water in urban and peri-urban agriculture (Obuobie *et al.* 2006; Amoah *et al.,* 2007). These foods have been quoted as a serious explanation for the increasing diarrheic diseases (Tjoa *et al.* 1977; Mensah *et al.* 1999; King *et al.* 2003).

In a report given by the World Health Organization in 2015, the burden of food borne illness has been identified to be caused by 31 hazards which include bacteria, virus, toxins, chemicals and parasites. These cause an estimated number of 22 diseases with the most common agents being Escherichia coli, Salmonella, Norovirus and Campylobacter. These organisms cause 70% of the world's food borne illness with Africa and Southeastern Asian recording highest number of incidence and death followed by eastern Mediterranean region.

Due to the exposure levels of vegetables to bacteria, effective wastewater treatment using high-technology treatments and decontamination structures can be used. These processes are however difficult and expensive to carry out as they need high energy, infrastructure and maintenance necessities, as well as skilled labor, which makes it less feasible in countries with low income (Carr & Strauss, 2001).

Another way of removing pathogenic microorganisms from fruits and vegetables apart from waste water treatment is the use of effective sanitization treatments to eliminate food borne diseases in connection with fresh vegetable consumption (Xu, 2005). This is more feasible and less expensive to practice.

An accepted and widely used sanitizer for fresh vegetables is hypochlorite at 50-200mg/L. Chlorine also produces 1-2 log reduction in microbial content at common concentrations although it raises safety concerns of its ability to form trihalomethanes which is a carcinogen (Delaquis *et al*.,2004). The utilization of acetic acid as a substitute to chlorine for sanitizing vegetables has also been explored especially as vinegar, an inexpensive acetic acid source and also used in household applications (Sengun and Karapinar, 2004; Chang and Fang, 2007).

The presence of pathogenic and spoilage bacteria in vegetables has already been established, hence this study seeks to determine the efficacy of common sanitizing agents in reducing these pathogenic organisms to tolerable levels before consumption to enhance food safety hence, consumer protection.

1.2 Problem Statement

In recent times, there has been an increasing demand for vegetable salads because of the assertion that vegetables are healthier. However leafy vegetables have been associated to food-borne illness because of pathogens like *Escherichia coli* O157:H7. In 2001, *Salmonella spp*, faecal coliforms, *Shigella spp* and *E. coli* were found in unacceptable levels in tomatoes and lettuce from farms and markets in Accra Metropolis (Mensah *et al.,* 2001).

The incessant occurrences of food borne illnesses through the ingestion of fresh produce is caused by rise in alteration in production processes; science, harvesting; storage, distribution and ingestion routines and practices (Hedberg *et al.,*1999).As a safety precaution, consumers wash leafy vegetables in water together with sanitizers such as chlorine dioxide, salt, peroxyacetic, hypochlorite, hydrogen peroxide etc. before consumption.

However, residual concentrations in this food (vegetables) may fluctuate, because of lack of knowledge regarding the sanitizer disinfection efficiency and the microbial contaminants that remains on vegetables surface. This presents a task for researchers and food processors to identify and ensure the microbiological quality and safety of vegetables (Garcia *et al.* 2003).

1.3 Need for the Study

Vegetables are known to harbor pathogenic microorganisms due to their soft texture and mode of cultivation which causes food borne illnesses when consumed untreated. Vegetables are however of increasing demand due to the health benefits associated with them.

There is therefore the need to properly sanitize vegetables and rid them of these pathogens to acceptable levels in order to curb food borne illnesses and promote food safety.

1.4 Objectives

Main Objective:

1. To assess the efficacy of common sanitizing agents in reducing microbial load in leafy vegetables at different concentrations and contact times.

Specific objectives:

1. To determine the microbiological load of pathogens inoculated on leafy vegetables.
2. To determine the concentration of the sanitizing agent capable of reducing the microbial load to acceptable levels.

1.5 Organization of Study

The thesis was organized in five chapters. Chapter one being the introduction entails the background, problem statement, need for the study and objectives.

Chapter two, the literature review discusses vegetables and their importance to man, microbial contaminations they are exposed to, common illnesses they cause, through to sanitizers that are used for treatment and factors that enhance their efficacy.

Chapter three outlines the protocol used to inoculate microorganisms of known concentration onto vegetables and the sanitizing treatment they undergo while chapter four highlights the figures and graphical representation of results obtained from the experiment conducted. Chapter five discusses into detail the results and recommendations to support and sustain the proper practice of chemical treatment of vegetables.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Vegetables

Vegetables are all parts of herbaceous plant that are eaten as food. This includes the leaves, stem, bulb, roots, and flowers. Vegetables are principally annual crops that belong to the cluster of plants known as agriculture crops that vary in nature. On the basis of the edible parts, leafy vegetables are the most common, next to fruit vegetables in terms of human consumption (Taura and Habibiu 2009).

Leafy vegetables are many and include both exotic and indigenous types. Exotic leafyy vegetables include lettuce, cabbage, and broccoli while the indigenous type includes the likes of kenaf, bitter leaf, roselle. Leafy vegetables are harvested fresh and green for consumption. They mostly contain a high water content of about 80% in their fresh state (N.S.P.R.I, 1992). Like many vegetables, leafy vegetables are an important source of macronutrients such as potassium, vitamin A, vitamin C, Vitamin E, dietary fiber and can be consumed either fresh or semi processed to provide the daily recommended intake of vitamins, minerals and fiber (Heaton, 2008). They have the potency to reduce the risk of lifestyle associated illnesses such as coronary heart disease, diabetes and cancer and this has additionally led to an increase in their demand and consumption. They also enhance proper digestion in a good time, lower blood pressure and cholesterol levels, support good eye sight and generally boost the immune system (Duckworth, 1996).

The World Health Organization (WHO) recommends a daily consumption of 400g, or five to nine portions, of fresh fruits and vegetables so as to benefit from these health properties (Matthews, 2006). The World Health Organization reports that adequate fresh produce consumption alone may save 7 million lives each year of which 31% heart condition cases are caused by meager consumption of such foods (Johnston *et al.*, 2006). In view of the World Health Organization recommendations (WHO, 2006a), fruit and vegetable intake has improved by a minimum of twenty ninth per capita within the U.S between 1980 and 2000 (Matthews, 2006).

The demand and consumption of vegetables for its health benefit has led to competition among producers to deliver varieties. This has also forced retailers and ready-to eat vendors to stock their shops with enough to meet consumer demand. Improvements are constantly being made to increase the shelf-life of fresh food by proper refrigeration, use of quality packaging materials including the use of modified atmosphere packaging as fresh agricultural produce incorporates a natural plant micro flora at harvest. The produce has the potential to be contaminated through processing, packaging, transportation, and sales from numerous sources, like the environment or by humans. For this reason, fresh produce is likely to be influenced by spoilage and pathogenic contamination than whole produce (Doyle and Erickson 2008; Vandamm *et al*. 2013).

Minimum process time that a fresh produce is exposed to, implies that the pathogens transferred to the fresh food from the soil remains and survives any cleaning, process or packaging that the food is exposed to. These microbes might even replicate if the storage conditions are within a favorable interval for them (Francis et al., 1999).

Most of the water used in irrigating vegetable farms especially in urban areas is waste water due to the scarcity of fresh water for farm activities. Wastewater is commonly used as a low-cost substitute to conventional irrigation water. It supports livelihoods through the generation of substantial revenue that is derived as a result of urban and peri-urban agriculture. These benefits tend to eclipse the health and environmental risks associated with wastewater use and this practice is hardly regulated in developing countries. These waste waters contain high loads of pathogens and therefore are good source of contamination from the water to the fresh produce. If these pathogens survive on the produce, the risk of infection for consumers increases considering that most of these raw foods are consumed either raw of semi-processed. This poses a health threat to consumers, outbreaks of associated illnesses would damage the confidence of the public and affect the credibility as well as the sales of all comparable produce (Johnston et al., 2006).

Consumer cognizance is an unhurried progression and the public cannot be depended on to thoroughly clean or cook fruits and vegetables adequately to eradicate pathogens that may be present (Bruhn, 2006). With a growing fresh food market, the food and agricultural industries face new challenges that need attention particularly in terms of protecting the buyer against microbiological hazards (Garrett *et al*., 2003). Some common leafy vegetables consumed in Ghana are represented below (table 2.1).

Table 2.1: Some Common leafy vegetables grown and consumed in Ghana

Vegetable	Common name	Scientific name	Family
Roselle	Bito	*Hibiscus sabdariffa L.*	Malvaceae
Spider plant		*Cleome gynanda L.*	Capparaceae
Lecttuce	Lettuce	*Lactucca sativa*	Asteraceae
Cabbage	Cabbage	*Brassica oleracea var.capitata*	Brassicaceae
Alefu	Alefu	*(Amaranthus cruentus)*	Amaranthaceae
Kenaf	Berese	*(Hibiscus cannabinus)*	Malvaceae
Okro		(*Abelmuscus esculentus*)	Malvaceae
Taro	Kontonmire	*Colocasia esculenta*	Araceae
Bitter leaf	Siwarka	*Venoniaamygdalina Del*	Compositae
Spinach		*Spinacia oleracea*	Amaranthaceae

2.2 Microbiological Contamination

Microbiological contamination is the existence of biological hazards such bacteria, yeasts, mold, fungi, protozoa or their toxins and by-products in a substance, which poses adverse health and safety effects to a consumer (Levitt, 2000).

Common sources of microorganisms are through the soil, air, farm pests, food handlers and irrigation containers used (Taura and Habibu, 2009). Leafy vegetables and fruits contamination can be tracked from the field through to harvest, handling treatment, processing, delivery and usage (Beuchat, 1998). The specific microbial contaminant present is likely to reveal the environment through which the product is obtained.

The soft texture of leafy vegetables particularly makes them greatly attractive to microbial attack, and they are very prone to physical and microbial spoilage. The consumption of these pathogen contaminated vegetables is the best means through which food borne diseases are contracted (Francis *et al*., 1999)

Food contamination affects the progress of the health sector of the nation by spreading human diseases after consumption. This goes as far as affecting the economy of the nation as large sums of money is involved in identifying the cause, bringing the outbreak under control and purchasing medicals to cure illness.

2.3 Food pathogens associated with vegetables

Food pathogens are disease causing organisms that are found in food. These organisms get into contact with fresh produce through the soil, water used in nurturing plants, fertilizers, etc.

Pathogenic organisms can be completely removed or reduced to acceptable limits by subjecting them to high temperatures and chemicals. Some of these organisms include *Campylobacter spp, Shigella spp, Salmonella spp, Bacillus cereus, Clostridium botulinum,* enterotoxigenic and entero hemorrhagic *Escherichia coli, Listeria monocytogenes, Yersinia enterocolitica,* viruses and parasites such as *Giardia lamblia,* and *Cryptosporidium parvum* which are of public health concern (Beuchat, 1996; Beuchat, 2002).

According to Beuchat (1998), the existences of pathogens in vegetables differ. The occurrence of Campylobacter is <3%, the occurrence of Salmonella is between 4 and 8% while the most commonly isolated organisms from vegetables are *E. coli O157:H7 and L. monocytogenes* in comparison with Salmonella (ECSCF, 2002).

Some food borne illnesses outbreak associated with pathogenic organisms is presented in the table below.

Table 2.2 List of foodborne disease outbreaks associated to fresh produce in North America from 2011 to 2017

Year	Product	Pathogen	No.of cases
2017	Papayas	*Salmonella Kiambu,Thompson*	173
2016	Frozen strawberries	Hepatitis A	143
2016	Frozen vegetables	*L. monocytogenes*	9
2016	Packaged salads	*L. monocytogenes*	19
2015	Tomatoes	*Salmonella newport*	115
2015	Cucumber	*Salmonella poona*	907
2014	Caramel apples	*L. monocytogenes*	35
2014	Cucumbers	*Salmonella enterica newport*	275
2012	Cucumbers	*Salmonella enterica saintpaul*	84
2012	Mangoes	*Salmonella braenderup*	
2013	Shredded lettuce	*E. coli O157:H7*	30
2013	Ready to eat salad	*E. coli O157:H7*	
2012	Romaine lettuce	*E. coli O157:H7*	24
2012	Cantaloupe	*Salmonella enterica Typhimurium and Newport*	261
2012	Mango	*Salmonella enterica Braenderup*	127
2012	Cantaloupe	*L. monocytogenes*	
2011	Romaine lettuce	*E. coli O157:H7*	147
2011	Cantaloupe	*Salmonella enterica Panama*	58
2011	Papaya	*Salmonella Agona*	20

Source: CDC, 2017

2.4 Bacteriological pathogen

There are varieties of bacteriological pathogens that are of food safety concern. These organisms survive under a variety of conditions and cause food borne illnesses to their consumers.

2.4.1 *Escherichia coli (E. coli)*

E. coli is a bacteriological pathogen that is widely found in food and water in low numbers which mostly inhabit in the intestinal tract of animals. It is able to survive within highly acidic surroundings ranging from pH of 3.3- 4.2 (Johnston *et al.,* 2006). The standard indicator for faecal contamination is *E. coli* (Francis *et al.,* 1999).

Escherichia coli O157: H7, is a strain of *E.coli* which is mostly associated with vegetables partly because of the water used on plants and is a causative agent in several foodborne outbreaks (CDC, 2006; Greene *et al.,* 2008). When this organism is ingested, it causes gastroenteritis, haemorrhagic colitis, and kidney failure (Francis *et al*., 1999). In some cases, Thrombocytopenic purpura (a disorder that leads to easy or excessive bleeding and bruising in adults and children) and haemolytic uremic syndrome (a condition characterized by destruction of red blood cells, low platelet count and kidney failure) may occur which is lethal (Gil & Selma, 2006).

Enterohemorrhagic *E. coli O157:H7* contamination outbreaks connected with lettuce and other leafy crops have been reported (Watchel *et al* 2002; Mahbub *et al.,* 2004). Leafy greens and Spinach have also been linked with *E. coli O157:H7* (Viazis, S., Akhtar, M., Feirtag, J., & Diez-Gonzalez, F. 2011). Signs of illness could include Nausea, vomiting, mild dehydration, Stomach cramps, and diarrheoa with stool containing mucus within 12 hours – 36 hours after ingestion of *E. coli* contaminated food (Buck *et al*., 2003).

Major food safety unease in relation to *E. coli O157:H7* lies with its potency at low doses and its ability to form spores on vegetables biofilms, which makes sanitization cumbersome (Somers, 1994).

2.4.2 *Listeria monocytogenes*

Listeria monocytogenes mostly, is linked with decomposing plants, sewage and animal fecal matter (Beuchat, 1996; Beuchat, 2002). It is noted to be able to survive under varying conditions such as high moisture and low oxygen concentrations, refrigeration temperatures as low as 5°C (Francis *et al.,* 1999; Johnston *et al.,* 2006), which qualifies it as a perfect waterborne pathogen (Maciorowski *et al*.,2007). It has been isolated from celery, lettuce, tomato and cabbage in USA and Canada (Beuchat, 1996; Beuchat, 2002). *L. monocytogenes* poses a high food safety concern as it matures quite well under low refrigeration storage conditions and also forms biofilm on crop surface, making sanitizing difficult (Somers *et al.,* 1994). It has also been reported to cause death (CDC, 2006).

L. monocytogenes causes Listoriosis and incubation periods range from one day to ninety days. This makes it difficult to identify the food that conveys the organism (Xu, 2005).

Signs and symptoms may include flu-like illness, meningitis and meningoencephalitis especially in vulnerable groups like expectant mothers, children, the elderly and the immuno-compromised (Xu, 2005). In just a few months, 2018 holds the record year for listeriosis-related deaths on a global basis. In mid-May, a total of 204 deaths and 1,033 cases of listeriosis are recorded to have occurred in South Africa which is the largest outbreak of its

kind in history. Consumers of a popular meat product were struck with illness from consuming a contaminated product. (Anandappa, 2018)

2.4.3 Salmonella spp

The genus Salmonella encompasses 5 pathogenic strains which are *S. typhimurium*, *S. enteriditis*, *S. heidelberg*, *S. saintpaul* and *S. montevideo* (Francis *et al.,* 1999). Salmonellas are Gram-negative, facultative anaerobes that are motile and non-spore forming rods (Gadotti, 2011). Salmonella is known to be highly resistant and can live outside the intestine, predominantly at a water activity range of 0.43 and 0.52 (Maciorowski *et al.,* 2007). The transfer means of salmonella is through carriage by animals such as birds, insects, and pigs. They are then transferred to humans when undercooked foods such as meats, eggs or milk are eaten (Johnston *et al*., 2006).

On the other hand, non-animal products that come into contact with the excreta of these infected animals through grazing or fertilization with compost can convey Salmonella (Maciorowski *et al*., 2007).

Salmonella can survive under diverse range of pH 4.1 to 9.0 and temperatures of 7 °C to 59 °C (Joneston *et al*., 2006). This species have been isolated from raw vegetables in the USA, Canada, Sweden and Finland (Beuchat 1996; Hedberg *et al.* 1999). The incubation period for *S. enteriditis* is between 6 hours and 48 hours whiles that of *S. typhi* which causes typhoid fever is between 10 and 20 days. Primary symptoms include mild fever, abdominal pain, nausea, vomiting, and diarrhoea that can last for 3–7 days (Xu, 2005).

2.4.4 Streptococcus

Streptococcus, a Gram-positive spherical and non-spore forming organism is also a facultative anaerobic, catalase negative and homo fermentative. Species under this genus are human pathogens and include *S. pyogenes* and *S. pneumoniae* (Idler *et al*., 2015). Turantas (2002), isolated faecal Streptococcus from 41 (75%) frozen vegetables out of 55 frozen vegetables, even though there has not been a report of it causing a foodborne disease outbreak from vegetables. Turantus' results however tally with Insulata, Witzeman and Sunya (1969) who isolated Streptococci from frozen vegetables.

Vegetables watered with wastewater were also reported to contain equal numbers of *S. faecium* and *S. faecalis* (Sadovski & Ayala, 1980). After 2–36 hours of ingestion of food produce contaminated with *S. faecium* and *S. faecalis*, abdominal cramps, diarrhea, nausea, vomiting, fever, chills and dizziness may occur as symptoms (Xu, 2005)

2.5 Procedure for inoculation

Dipping or spraying of bacteria cells suspension are procedures used for inoculating pathogens of known volume on fruits and vegetables. Dipping or spraying procedure is adopted when it is suspected that the source of contamination in a commercial situation is through immersion. However, limitation of this procedure is that the number of bacteria cells picked by the produce is unknown and the varying test organisms acquired greatly vary. The use of dip or spray methods needs analysis of a large number of units for each treatment as random error values are variably large. Thus, efficacy of recovery or log changes in viable

populations during subsequent storage or as a result of treatment with a sanitizer cannot be calculated correctly.

Also, penetration of the inoculum into very porous parts on the produce surface like the stem core tissue or scratched tissue can cause favorable conditions that aid or hinder growth, or guard against contact with sanitizer, especially those with little or no surfactant activity (Buchanan *et al*., 1999; Seo and Frank 1999). An alternative method is known as spotting. In this procedure, a known volume of inoculum is applied to several parts on the surface of fresh produce, for example, 5 or 10 µl, of inoculum of known cell density. This type of inoculation would be representative of contamination from a point source, for example, from contact with soil, workers.

2.6 Decontamination treatments of Vegetables

Many consumers and processors believe washing leafy vegetables with water reduces microbial content from the surface, however studies indicate that washing only is insufficient and ineffective (Sapers ,2001). A variety of treatment methods are employed in decontaminating vegetables through the use of chemicals called sanitizers.

2.6.1 Sanitizer

A sanitizer is an agent that is used to reduce microbiological contamination of health interest to safe levels conforming to local health regulations such that it has no adverse effects on the quality and safety of product (Code of Federal Regulations, Title 21, and Sec. 110.3.) Sanitizing can be done by heat or chemical use depending on the type of material to be sanitized.

The elimination of microorganisms does not need to be 100% to be effective. Sanitizers are unable to destroy viruses and fungi. However, in a food service condition the sanitizer must decrease the bacteria count by 99.999% within a stipulated time being 30 seconds i.e. 5 log reductions (AOAC International Official Methods 2009). However various factors affect the efficacy of these sanitizers in reducing microbial load by this 99.999%.

2.6.2 Factors that affect the efficacy of the sanitizing agent

Diverse factors impact the efficacy of chemical sanitizers. The factors to consider are:

- **Concentration** - The presence of inadequate levels of a chemical sanitizer will lead to an inadequate reduction of microorganisms as an end result where as excessive levels can be toxic. Therefore, an optimum amount is required.
- **Temperature** - Usually chemical sanitizers function best within a temperature range of 13 and 49 degrees Celsius.
- **Contact time** – The recommended length of time for a cleaned item to be effectively sanitized and ridden of all microorganisms must be adhered to. The sanitizer may be heat or any approved chemical but needs to have an intimate contact time.
- **pH –** The pH of solution intensely affects sanitizers. Typically, most chlorine sanitizers have been found to be ineffective at a pH level of 7.5 (Schmidt, 1997).

2.6.3 Desired characteristics in a sanitizer

For a sanitizer to be effective and widely accepted by users, certain attributes are required. These include, easy application, less toxicity, non-corrosiveness, good permeating strength, fast performance, no unpleasant odour that may give off flavours and should not be

unfavorably affected by organic matter, should be active before and after dilution with hard water, compatibility with other chemicals and material of construction, good functionality towards gram-positive and gram-negative bacteria, fungi, and viruses, cost effective (Holah *et al*., 1998).

2.7 Food sanitizer

Food sanitizers are chemical agents that are used in the food industry to reduce the amount of microbial load in food to acceptable levels. There are different types of sanitizing products on the market but the efficacy lies in the amount used and contact time allowed.

2.7.1 Hypochlorite

Hypochlorite (NaClO) works by destroying the cell wall of the microbes which enhances permeability of chemical into the cell and eventually, the cell dies. It inhibits enzyme release thus causing destruction to the cell DNA (Venkobachar *et al*., 1977).A good number of food production industries choose to use hypochlorite, predominantly sodium hypochlorite as it is quite effective, easily accessible and cost effective (Beuchat and Ryu, 1997). However, hypochlorite is unable to kill spores easily unless a formulation of very high concentration is prepared and applied for an extended period at higher temperatures in order to kill the spores, which can also affect the quality of food with respect to taste, smell, etc.

The efficacy of hypochlorite can be affected by external factors such as pH levels, suspended solids and elevated temperatures. The required pH levels must fall within a range of 5 to 7. Contact surfaces must be free from organic materials as possible when cleaned and Chlorine residues must be within the permissible level. It should not go beyond 200 ppm of concentration. In a report issued by Bermúdez-Aguirre and Barbosa-Cánovas, (2013) an

application of sodium hypochlorite at 200 ppm chlorine for 15 minutes resulted in a reduction of 8 log of E. coli on the surface of tomatoes, while only 3-4 log reductions occurred on the surface of lettuce and carrots.

This difference in reduction was attributed to the smooth surface of tomatoes as against the rough and porous surface of lettuce and carrot which make it easy for microorganisms to settle in and prevent sanitizers from penetrating. Since hypochlorites are often harmful to health and to the atmosphere, there is bigger chance of its restriction in the future. Chlorine can cause irritation of the skin and mucous membranes, corrosion of metals and production of toxic gases (chloroamines) unsafe to the atmosphere and there is the need to treat waste water before disposing off into the environment (ISU 1974D, Shaw *et al*., 2013). If combined with organic substances, it can lead to the formation of dioxins and tri-halomethanes. Organic materials like paper, fabric and wood may cause spontaneous ignition upon contact with hypochlorite.

2.7.2 Hydrogen peroxide

Hydrogen peroxide (H_2O_2), a sanitizing agent is approved for use on fruit and vegetable surfaces at a concentration of 3-5% due to its antimicrobial properties (CFR, 2012). This chemical is a preferred choice for sanitizing vegetables because it is inexpensive, easy to prepare, fast action against bacterial cells and spores and breaks down quite easily in water that enables good waste removal (ISU 1974D; Shaw *et al*., 2013).However, limitations to the use of this chemical is that, it does not stay stable in water for long hence does not stay effective on vegetables for long. It also causes allergic reactions and is very unstable under

high temperatures and sunlight which makes its storage and shelf life limited (ISU 1974D, Shaw *et al*., 2013).

Hydrogen peroxide is an oxidizing agent, therefore bacterial destruction with H_2O_2 may result from several factors. As an oxidizing breaks down occurs in water to form hydroxyl radicals which oxidize thiol groups in proteins and enzymes on the surface of cell membrane (Turner, 1983), the movement of these broken down hydrogen peroxide molecules across the cell membrane induces a change in osmotic pressure thereby causing a rapture of the cell membrane, thus a destruction of bacteria cell (Maris, 1995).

Research has endorsed the potency of hydrogen peroxide in destroying microorganisms including pathogens although it has a limitation of causing color change in sensitive fresh produce which can limit its use as a sanitizer. According to Lin *et al.* (2002), the use of 2% hydrogen peroxide at a temperature of 50 °C resulted in a 4 log reductions *of E. coli O157:H7* and *S. Enteritidis* and 3 log reduction of *L. monocytogenes* on the surface of lettuce. This however affected the quality of the leaves by causing a color change from green to brown.

2.7.3 Chlorine Dioxide

This is an inorganic compound which is very efficient against bacteria, fungi and viruses when used as a sanitizer. It works by reacting with proteins and fatty acids inside the cell membrane of microbes, causing a loss in permeability control and distraction of protein synthesis (Cramer, 2013).

Chlorine dioxide usage in the food industry is acceptable in liquid or gaseous form at a maximum concentration of 3 ppm. (CFR, 2012)

Chlorine gas causes cellular death of microbes by disrupting the cell wall and reducing metabolic function. A 5 ppm solution of chlorine dioxide solution is effective as a sanitizer on food contact surface within a contact time of at least 1 minute. According to Sy *et al*. (2005), a concentration of 4.1 ppm within a contact time of 30 mins is reported to reduce microorganisms like *E.coli*, *Salmonella* and *L.monocytogenes* by 1.5-5.9 logs in lettuce, cabbage, Onion, tomatoes. In contrast, it was reported that a reduction of 0.6-0.7 log of these same organisms occurred in spinach at a concentration and contact time of 2 ppm and 1 hr respectively (Neal *et al.,* 2012). Further disinfection can occur using 100 ppm within 10 min contact time. Chung *et al*., (2011) reported that a liquid application of chlorine dioxide at 100 ppm reduced bacterial count by 3-4 log in lettuce, cucumber, guava and apples.

Chlorine dioxide functions well in a pH range of 6-10, thus cause the increased mortality of a wide range of microbes that survive in higher pH levels. One good thing about this sanitizer is that, its use does not cause the formation of chlorinated compounds, making it environmentally friendly. It does not also break down in water thus remains effective over a long period of time. A limitation to the use of this chemical is that it has a negative effect on the quality of vegetables when chemicals stays on them for long. It is has less effect on microbes at cold temperatures and finally, is also expensive (Ridenour and Ingols, 1947).

2.7.4 Peroxyacetic acid (PAA)

Peroxyacetic acid, commonly called peracetic acid is a sanitizing agent that is efficient against many microbes and their spores. It causes a distraction of chemical bonds within microbial cell membrane, thus causing mortality (Lippincott *et al.*, 2001). Peroxyacetic acid is a blend of stabilized hydrogen peroxide and acetic acid. Peroxyacetic based sanitizer are compared mostly with that of stabilized hydrogen peroxide in that they both function well at cold temperatures (4 °C) and unlike other sanitizers, does not experience cold temperature failure. Because of this, sanitization can be effectively done on pre-cooled leafy vegetables or in systems that use water to cool the vegetables, such as hydro-coolers.

These conditions enhance the required mortality of microorganisms. PAA is equally efficient in the removal of biofilms and is considered more vigorous than hypochlorite. (Schmidt R.H, 2003). PAA solutions are easily weakened by high microbial load as such begins to lose its potency especially as the pH approaches neutral. This solution is commonly applied at a concentration ranging from 100-200 ppm whiles hydrogen peroxide is applied at a range of 80-600 ppm. PAA based sanitizers are known to be environmentally friendly because the compounds breaks down to form acetic acid, oxygen and water which are harmless (Robert, 2012).

They are also less corrosive. However, just like any highly active oxidizer, highly concentrated PAA can cause a safety hazard. It can also cause damage to the tips of leaf and shorten shelf-life of produce when used on leafy vegetables. It is costly if not impossible to use in large scale due to quantity involved (Robert, 2012)

2.7.5 Acetic Acid (VINEGAR)

Acetic acid is a colorless liquid organic compound which is sometimes called glacial acetic acid when undiluted (Durande, 1778). It is derived through the fermentation of ethanol by acetic acid bacteria (Peppler and Beaman, 1967). It is considered` as a weak acid since it only partially dissociates in solution, but concentrated acetic acid is corrosive and can attack the skin. As the most easily manufactured mild acid, it has an array of industrial, medical, and domestic uses, some of which are still in practice today. Vinegar is largely used as a cooking ingredient, as well as a general household cleaner.

Vinegar consists of roughly 5% acetic acid and 95% water. However, vinegars vary in the level of acidity they contain. For example, distilled white vinegar usually contains around 5% acidity, while champagne vinegar contains 6% acidity. A 5% acidity level is however common for most general all-purpose cleaning. The most commonly used vinegar in cleaning is distilled white vinegar. The organic variety is more earth-friendly, as the grains they are made of are organic and not treated with pesticides or fertilizers.

Due to a pH of 2.0 and the acetic acid content, vinegar is an inhospitable environment for many microorganisms, so it is the perfect cleaner. Studies have been done testing how effectively it kills bacteria and viruses. For example, 10% malt vinegar solution was just as effective as commercial cleaning wipes in killing the Human Influenza A/H1N1 virus (Greatorex *et al.,* 2010). Parnes, (1997) showed that undiluted vinegar was just as effective as bleach in eliminating *E. coli* from surfaces and sponges, but not as effective in eliminating

S. *aureus*. Also, Lukasik (2003) reports that vinegar reduced viruses by 95% when used as a strawberry wash.

2.7.6 Sodium Chloride (NaCL) Saline

Salt (NaCl) is a known natural disinfectant which can be used as a preservative as well. Microorganisms found on leafy vegetables require moisture to survive and continue with their activities. However, the presence of salt in the form of saline creates a hypertonic environment for microbes which causes them to shrink by losing their moisture content. This leads to plasmolysis and therefore the death of the microorganism. The amount of salt in water however determines the death rate of microorganisms.

2.8 Microbial Log Reduction Table

Log reduction is a mathematical term used to express the relative number of viable cells removed from a surface after sanitizing. Table 2.3 highlights the representation of log reduction of bacteria.

Table 2. 3: Representation of log reduction of bacteria cells

Log	Equivalent	% Reduction of bacteria
1	10	90
2	10^2	99
3	10^3	99.9
4	10^4	99.99
5	10^5	99.999
6	10^6	99.9999

Standard regulatory bodies have set microbiological rules to ensure the quality of ready-to-eat foods. These rules help to determine the condition of food and if consumption will pose food safety concerns. Table 2.4 shows the NSW food authority standard rules for determining quality of ready-to-eat food.

Table 2.4: Rules for determining microbiological quality of ready-to-eat food

Test	Good	Acceptable	Unsatisfactory	Potentially hazard
Aerobic plate count				
Enterobacteriaceae	$<10^2$	10^2 to 10^4	$\geq 10^4$	N/A
E. coli	<3	3 to $<10^2$	$\geq 10^2$	N/A
C. perfringens	$<10^2$	10^2 to $<10^3$	10^3 to $<10^4$	$\geq 10^4$
B. cereus	$<10^2$	10^2 to $<10^3$	10^3 to $<10^4$	$\geq 10^4$
Campylobacter spp	Not detected in 25g			Detected in 25g
Salmonella spp	Not detected in 25g			Detected in 25g

Source: NSW food authority, 2009

Good- Results are in the lower range, within microbiological specification and will not cause a food safety concern.

Acceptable- Results are within specification although in the upper range. This will also not cause a food safety concern.

Unsatisfactory- Results are out of specification but will not cause a food safety concern. This might however indicate poor food handling practices and therefore calls for proper food handling techniques.

Potentially Hazardous-Results are out of specification and presents potential food safety concern.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area and Sampling

Samples of fresh lettuce, cabbage and spinach were sourced from Kumasi central market in the Ashanti Region of Ghana and transferred to the laboratory in sealed sample bags to maintain the humidity. Sample collection was done aseptically to avoid introducing microorganisms from external sources. The vegetables were pre cleaned with tap water to clear them of dirt and refrigerated at a temperature of 4^{o}C until experiment was set to be carried out.

3.2 Chemical Reagents and Media preparation

The primary agars used were Nutrient agar and Plate Count Agar, products of OXOID Laboratories, Basingstoke Hampshire, and England.

3.2.1 Preparation of Plate Count and Nutrient Agar

Plate Count Agar was prepared by suspending 17.5 g into 1000 ml (1 liter) distilled water as recommended by manufacturer's assay. This was heated to dissolve completely. It was then sterilized in an autoclave set at 121 °C for 15 min in sealed bottle. The sterilized agar was left to cool to 50 °C before pouring into sterile Petri dishes.

The same procedure was followed in the preparation of the nutrient agar, using 28 g in a liter of distilled water. The prepared plates were incubated for 24 hrs. at 37 °C prior to use for sterility validation after being allowed to set.

3.3 Pretreatment of vegetables

The vegetables were washed in distilled water and subjected to sanitization and sterilization via exposure to Ultra Violet light for two (2) hrs. The leaves were then cut into pieces of averagely 4×3 cm each of mass averagely 1 g. Secondary sterilization by chemical treatment was carried out by exposing the precut vegetables to a solution of 70% ethanol for 5 min amidst continuous shaking on an orbital shaker. The leaves were transferred into sterile tissue wipes in a tray in a sterile hood to allow for evaporation of alcohol and drying of the moisture.

Plate 3.1: Evenly cut pretreated leaves.

3.4 Preliminary microbial assessment of leaves

The leaves were assessed for initial loads and microbial quality as control. This was carried out by conventional plate culture on PCA using the spread plate technique. A two fold serial dilution of the leaves were prepared and plated in triplicates on sterile PCA plates using

inoculum volumes of 100 µL. The plates were incubated at 37 °C for 48 hrs and counted for colonies.

3.5 Preparation of Inoculum baths

The test organisms, *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* were obtained from the microbiology laboratory of Centre for Scientific Research into Plant Medicine (CSRPM), Akuapem Mampong. The cells were sub-cultured on Nutrient agar plates to obtain pure colonies using streak plate technique and colonies subjected to Gram staining, catalase, oxidase and citrate tests for confirmation of identities. Single colonies of actively growing cells were transferred into 9ml tubes of nutrient broth and incubated for 24 hrs.

The inoculum baths were prepared by adding 1ml of actively growing cells from the broth cultures to 99 ml of 0.1% bacteriological peptone solution and incubating for 6 hrs.

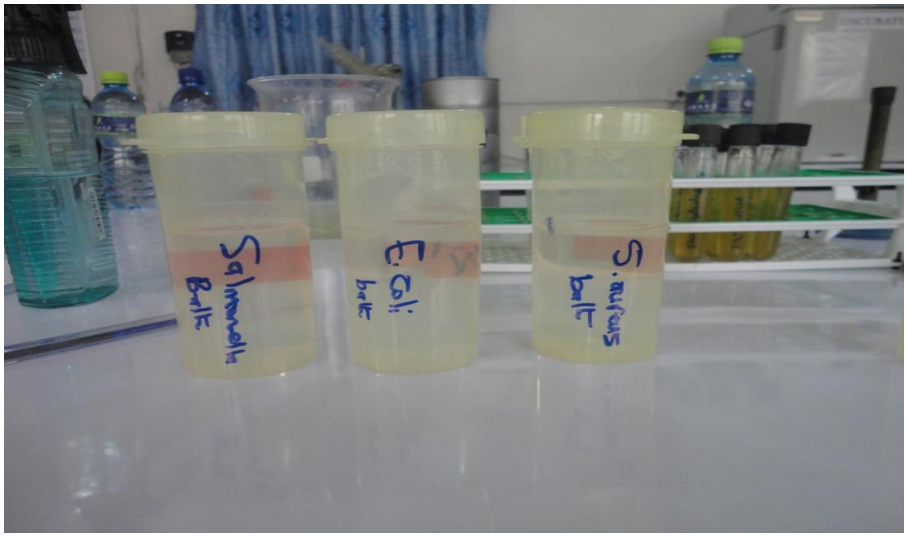

Plate 3.2: Inoculum bath of *Salmonella spp, E.coli* and *S. aureus*.

3.5.1 Determination of microbial load of inoculum bath

The microbial population of the various organism baths was determined using spread plate technique on PCA. A six fold serial dilution of each organism bath was prepared and 0.1 ml inoculum volume plated on PCA in triplicate. The plates were incubated and enumerated following same protocol as above.

3.6 Simulation of microbial contamination of leaves

The sterilized leaves were aseptically transferred using the dip method into the inoculum baths of the various organisms and allowed to shake on an orbital shaker for 5 min. This is to allow uniform distribution of organisms on the leaves. Samples were transferred after draining the inoculum suspension using a sterile strainer into in a plastic container and dried for an hr at 37 °C to enable pathogens to adhere to the surface of leaves.

3.6.1 Determination of initial microbial load on leaves

Initial microbial load of the leaf samples was determined using same protocol as preliminary leaf quality assessment with slight modifications. A mass of 5 g of the inoculated leaves from the various baths were washed in 45 ml of 0.1% peptone to obtain the first fold dilution after which subsequent dilutions were performed to obtain a six fold dilution. The inoculation, plating, incubation and enumeration were carried out as done in the preliminary assessment on PCA.

3.7 Preparation of test chemicals and reagents

Stock solutions of peracetic acid (15%), acetic acid (99.98%), hypochlorite (20%), hydrogen peroxide (6%), and Sodium chloride were obtained. The study design was programmed to

assess the efficacy of varying concentrations of the test chemicals thus three solution concentrations 0.01%, 0.015% and 0.02% of each chemical were prepared to a final volume of 50 ml. All chemicals were worked with at room temperature of 25 ± 1 °C. The process of solution preparation involved dilution with sterile distilled water to the desired concentrations using the formula:

$$C_1V_1 = C_2V_2$$

3.8 Determination of antimicrobial activity of solutions against test organisms

A reweighed mass of 5 g of the inoculated leaves were immersed in the solutions and agitated on a rotary shaker for 2 min, 4 min and 6 min after which leaves were transferred into 45 ml of 0.1 peptone solutions as stock dilutions. Subsequent dilutions of a threefold order were prepared and inoculated on sterile plates of PCA as discussed in 3.6.1.

3.9 Analysis of Data

The data obtained after the incubation period were analyzed using the Graph Pad Prism 5.0 software. The analyses were conducted using the One-way and Two-way ANOVA tools with Bonferroni Post Hoc analysis.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Initial preliminary leaf microbial Loads

The analysis of the leaves after pretreatment with UV and 70% alcohol prior to inoculation showed no microbial load implying a sterile leaf surface. The same outcome was obtained for all the leaves selected in the study.

4.2 Inoculum and Initial leaf microbial Loads

The results obtained for the inoculum counts showed significant differences ($P<0.05$) in the loads of the various tests organisms in the baths. The mean loads obtained were $1.1 \times 10^7 \pm 3.78$ for *Escherichia coli*, $1.3 \times 10^7 \pm 7.02$ for *Staphylococcus aureus* and $9.8 \times 10^6 \pm 4.04$ for *Salmonella typhi.* The loads on the leaves after inoculation however was lower compared to the inoculum loads with a means of $2.1 \times 10^6 \pm 5.03$ for *Escherichia coli* on leaves, $3.2 \times 10^6 \pm 3.61$ for *Staphylococcus aureus* on leaves and $1.9 \times 10^5 \pm 6.42$ for *Salmonella typhi* on leaves.

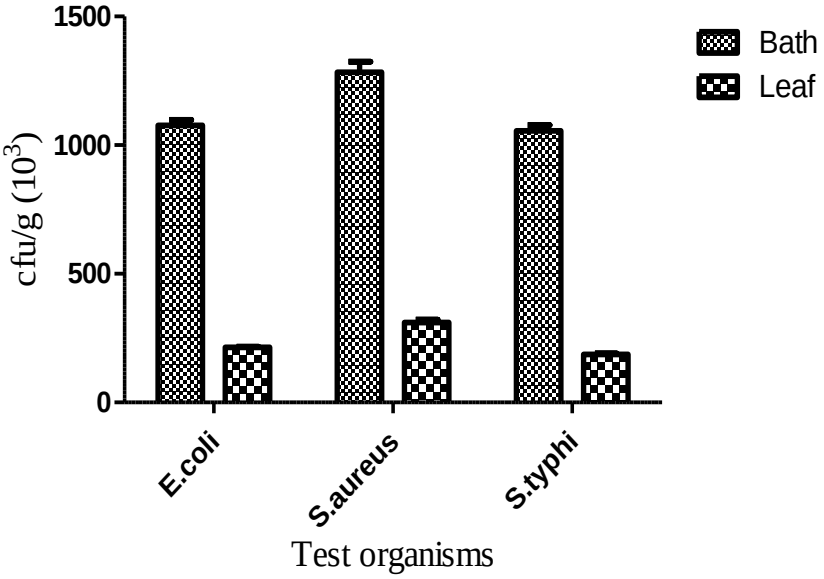

Figure 4.1: Initial microbial loads of inoculum and inoculated leaves after 48 hrs incubation at 37 °C.

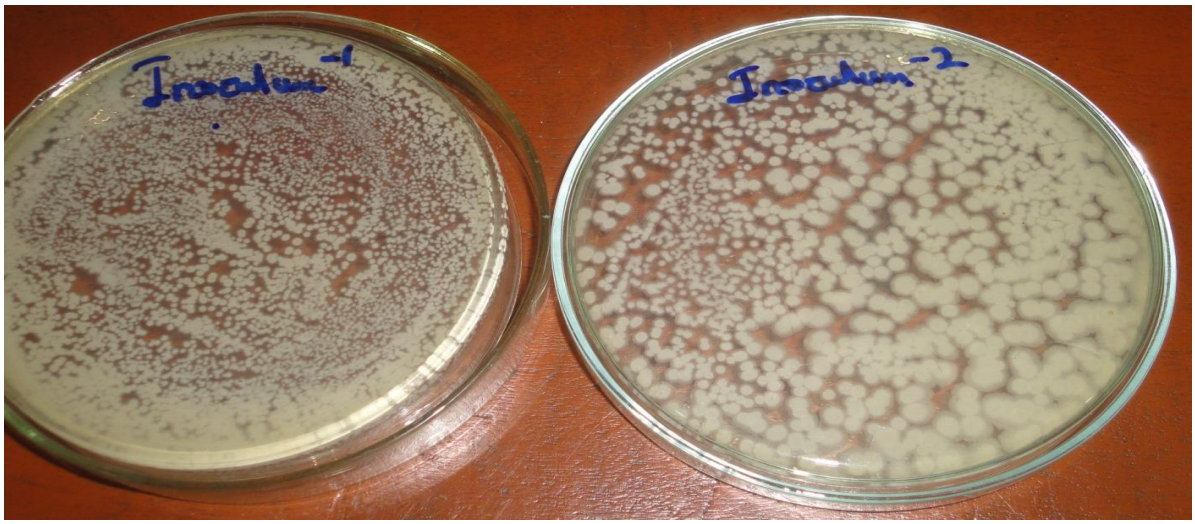

Plate 4.1: Inoculum count after 48 hrs incubation on PCA at 37 °C

In comparing the loads on the individual leaves (cabbage, lettuce and Spinach), the results showed no significant difference ($P>0.05$) in the loads, thus cabbage leaves were selected for the assay due to their robust nature as opposed to the others.

4.3 Antimicrobial activity of test chemicals against model organism

The assessment of the various concentrations of the chemicals against the test organisms was carried out with *E. coli* as the selected test model. The different chemicals all exhibited some degree of activity against the test organisms with increasing efficacy at high concentrations.

4.3.1 Assessment of Acetic Acid against test organism

Acetic acid showed significant antimicrobial activity against the test organisms, exhibiting an increase in activity with increasing concentration and time. The most effective concentration from the study was 0.02% which exhibited approximately 99.99% disinfection against all test organisms and 4.0 log reductions at that. Below is a representation on the graph.

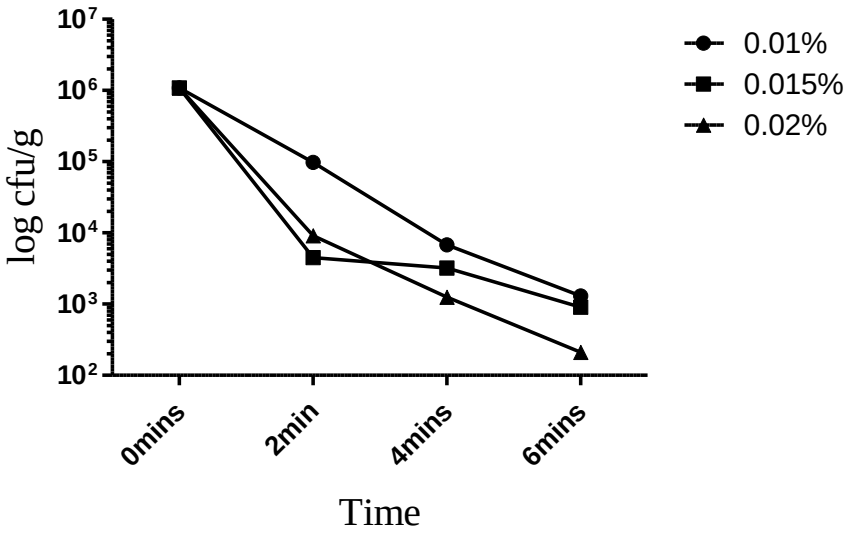

Figure 4.2: Concentrations of Acetic Acid against *E. coli* at varying time periods.

The assay against time factor showed the most effective time of action to be 6mins of exposure. This resulted from an increase in activity with increase in time. The statistical analysis showed no significant difference (P>0.05) in the activity of the various concentrations against the test organisms but with the time as a factor there is some significant difference with the 0.02% showing the best efficiency. The least concentration of AA (0.01%) in the study recorded 95.33% efficacy within 2 min of exposure and a maximum of 99.93% within 6 min.

4.3.2 Peroxyacetic acid against test organism

The performance of the peroxyacetic acid against the test organism indicated a better performance relative to the mother acetic acid with significant activity observed at the mid concentration of 0.15%.

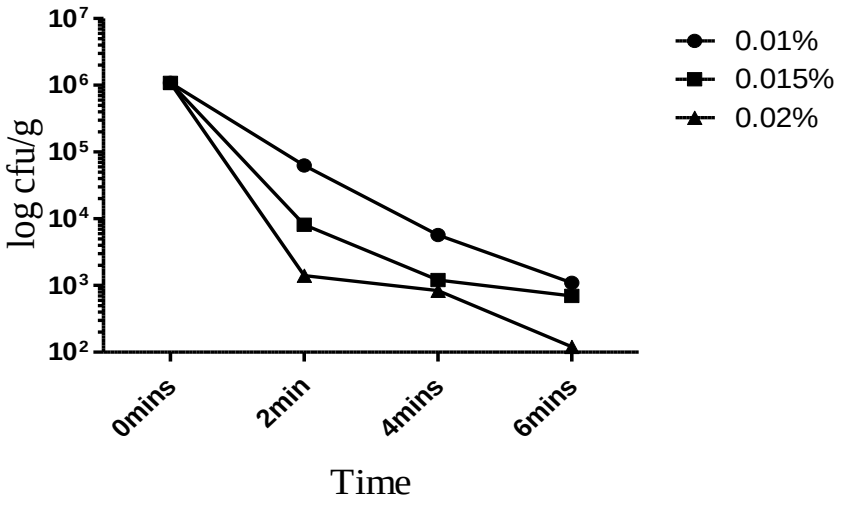

Figure 4.3: Concentrations of peroxyacetic acid against test organism at varying times.

The PAA recorded higher efficacy as opposed to the mother AA with 99.70% efficacy at 0.01% within 2 min of contact with test organism and 99.94% efficacy at 6 min of contact. The optimum concentration being 0.02% PAA recorded 99.93% efficacy within 2 min of contact and 99.99% efficacy after 6 min of contact. This relatively gives a 4.24 log reduction in microbial load indicating better efficacy and antimicrobial activity.

4.3.3 Hydrogen Peroxide against test organism

The assay using hydrogen peroxide against model test organism exhibited a trend comparable to that observed with acetic acid where the highest efficiency against the organism was observed at the highest test concentration of 2%. The concentration 1% and 1.5% showed almost similar degree of activity against the organisms with 2 min and 4 min of exposure.

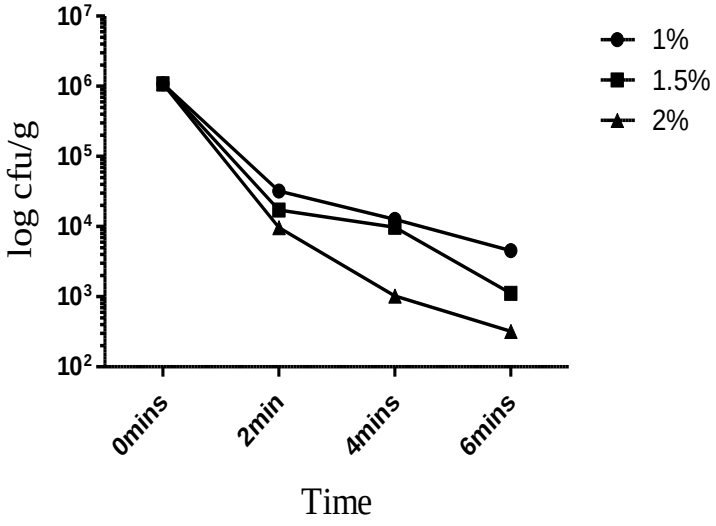

Figure 4.4: Concentrations of hydrogen peroxide against test organism at varying time periods

The assay using the hydrogen peroxide did show averagely 99.60% efficacy at the lowest concentration of 0.1% within 2 min contact with the *E. coli*. There was an increase to 99.94% efficacy after 6 min of contact. The optimum for the study being 0.02% however recorded 99.74% efficacy within 2 min of contact and an absolute 99.99% after 6 min of contact.

Considering the graphical representation however, 6 min contact time gave a 4.06 log reduction as against 2.1 log reduction within 2 min at same concentration 0.02%.

4.3.4 Sodium Hypochlorite against test organism

The results obtained for the bleach against the test organism shows a 4.20 log reduction in microbial load at 0.02% concentration within 6mins as shown on the graph.

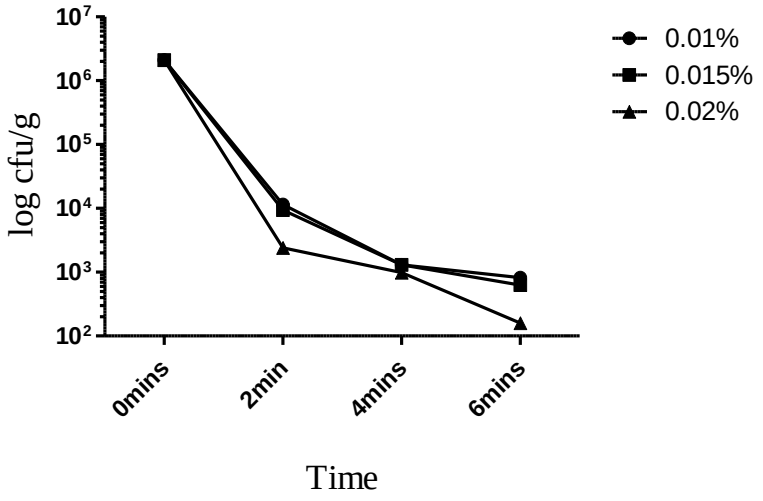

Figure 4.5: Sodium Hypochlorite against test organism at varying time.

The efficacy test on the hypochlorite did show a relatively stronger efficacy comparable to that exhibited by PAA with a 99.55% score at 0.1% concentration within 2 min contact time with test organism and increase in efficacy to 99.95% after 6 min. The optimum concentration being 0.02% showed 99.88% efficacy at 2 min of contact and 99.99% after 6 min of contact.

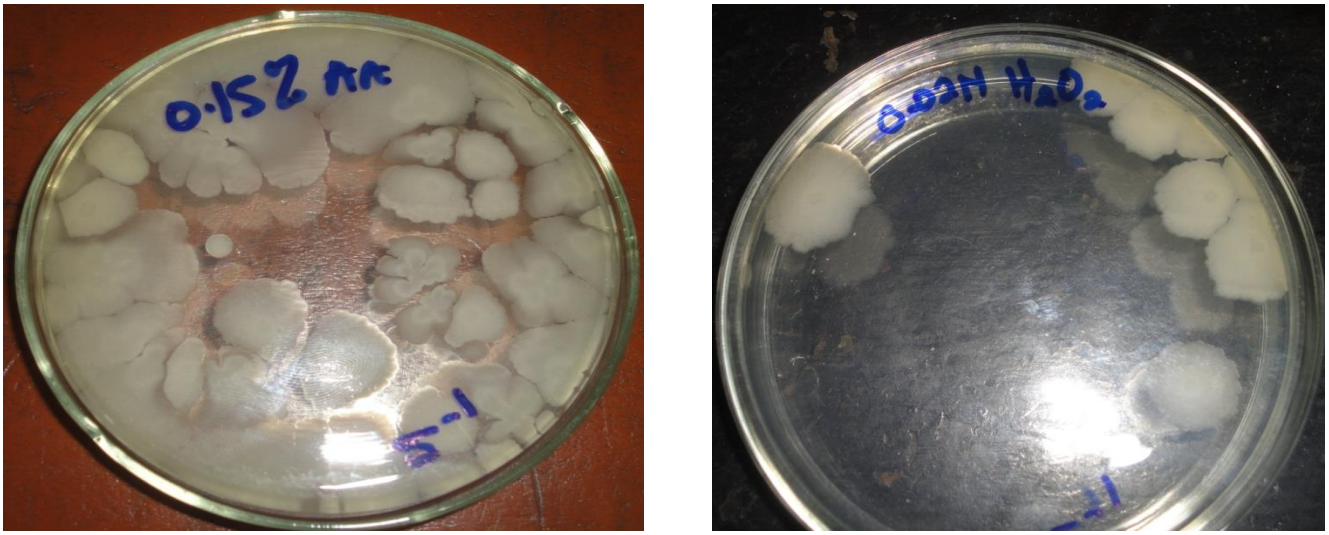

Plate 4.2: Microbial loads of concentrations of AA and hydrogen peroxide on *E.coli* (PCA)

4.3.5 Concentrations of Sodium Chloride against test organism

The salt assay against the model test organism showed a relatively lower activity as opposed to the other agents considered in this study. The highest concentration being 2% could not establish an adequate sanitization of the leaves as the other chemicals did. The trend observed however was an increase in sanitizing power with increase in concentration as well as time.

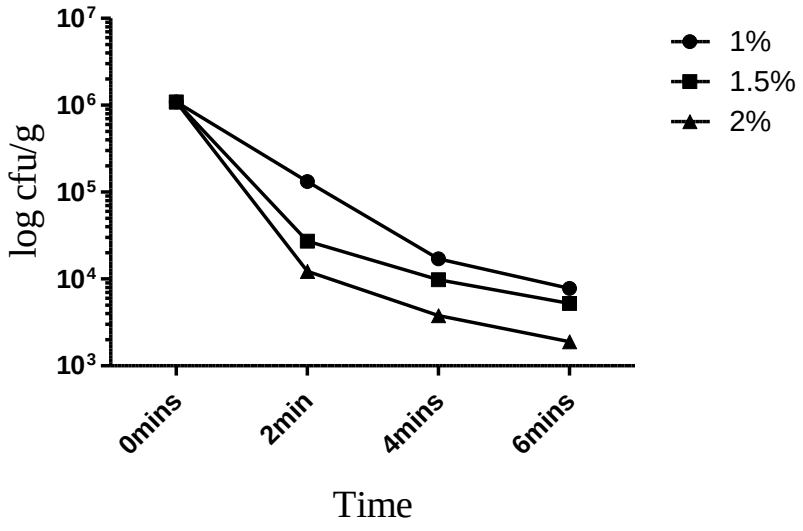

Figure 4.6: Concentrations of Sodium Chloride against *E. coli* at varying times

The Sodium Chloride recorded the least efficacy in this study over the time duration considered with 93.80% efficacy at 1% concentration after 2 min contact time with *E. coli* and increased in efficacy to 99.91% after 6 min of exposure. The optimum concentration in this study being 2% recorded 99.94% efficacy after 2 min of contact with the test organism and increased to an efficacy 99.90% after 6 min of contact. A 3.04 log reduction was obtained at maximum concentration and time as shown on the graph.

4.4 Assessment of chemicals against test organisms

The most effective concentrations of the test chemicals against the model test organisms *E. coli* were tested against the three target organisms at varying time periods. The results showed similar responses of the test organisms to the chemicals as observed with the *E. coli*.

4.4.1 Optimum concentrations of chemicals against *Staphylococcus aureus*

The chemicals exhibited significant activity against *Staphylococcus aureus* within 4 min of exposure with the exception of the saline solution which though exhibited some degree of activity, could not adequately sanitize the leaves.

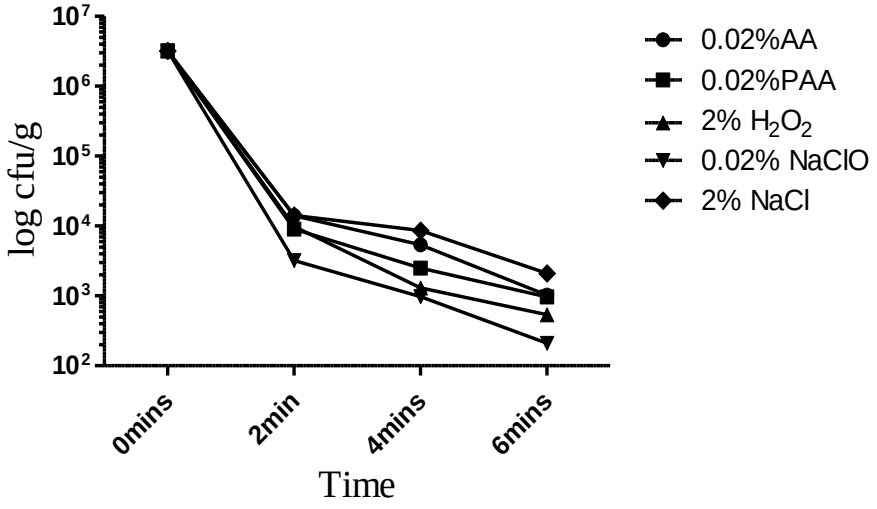

Figure 4.7: Optimum concentrations of sanitizers against *S. aureus* at varying time periods

The results showed the most effective agents against *Staphylococcus aureus* to be peroxyacetic acid and sodium hypochlorite which showed adequate efficacy within 2 min of contact.

4.4.2 Optimum concentrations of chemicals against *Salmonella typhi*

Salmonella being a Coliform like *E. coli* expressed a similar reaction to the test agents (chemicals). The organism was susceptible to all the test chemicals with the exception of

Saline which again exhibited some degree of inhibition and inactivation but not absolute within the time frame work.

The same phenomenon as observed in the agents against *S. aureus* was observed with the peracetic acid and hypochlorite exhibiting adequate sanitary action within 6 min of exposure. The sodium chloride (NaCl) however could not establish a good sanitary effect within the time frame work of this study.

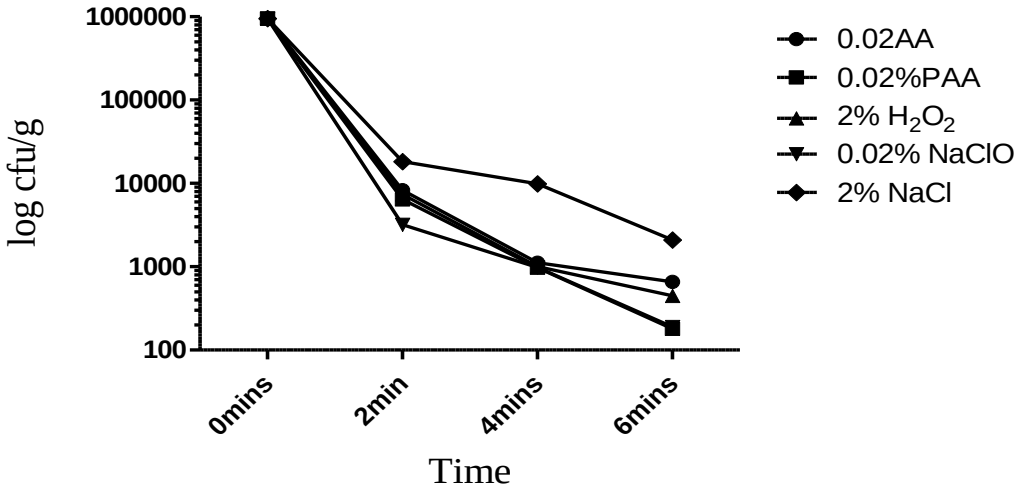

Figure 4.8: Optimum concentrations of sanitizers against *Salmonella typhi.*

Table 4.1: Efficacy (%) of test sanitizers against test organisms at varying time periods

Escherichia coli					
Time (min)	0.02%AA	0.02%PAA	0.02%H_2O_2	0.02%NaClO	2%NaCl
2	99.56	99.93	99.74	99.88	99.94
4	99.94	99.95	99.95	99.95	99.81
6	99.99	99.99	99.99	99.99	99.90
Staphylococcus aureus					
2	99.56	99.70	99.69	99.90	99.34
4	99.83	99.95	99.92	99.96	99.69
6	99.95	99.96	99.98	99.99	99.93
Salmonella typhi					
2	95.63	96.63	96.10	98.31	90.52
4	99.42	99.48	99.47	99.48	94.78
6	99.76	99.90	99.89	99.90	99.65

All recorded values are in percentage.

Table 4.2: Microbial counts (cfu/g) *E. coli* on leaves before and after treatment with test chemicals.

Time (min)	0.01%	0.015%	0.02%
		Acetic Acid	
0	2.1×10^{6}	2.1×10^{6}	2.1×10^{6}
2	9.8×10^{4}	4.5×10^{4}	9.1×10^{3}
4	6.8×10^{3}	3.2×10^{3}	1.2×10^{3}
6	1.3×10^{3}	9.1×10^{2}	2.1×10^{2}
		Peroxyacetic Acid	
0	2.1×10^{6}	2.1×10^{6}	2.1×10^{6}
2	6.3×10^{4}	8.1×10^{3}	1.4×10^{3}
4	5.7×10^{3}	1.2×10^{3}	8.5×10^{2}
6	1.1×10^{3}	7.0×10^{2}	1.2×10^{2}
		Sodium Hypochlorite	
0	2.1×10^{6}	2.1×10^{6}	2.1×10^{6}
2	1.2×10^{4}	9.4×10^{3}	2.4×10^{3}
4	1.3×10^{3}	1.0×10^{3}	9.1×10^{2}
6	8.2×10^{2}	6.3×10^{2}	1.3×10^{2}
		Hygrogen Peroxide	
	1%	**1.5%**	**2%**
0	2.1×10^{6}	2.1×10^{6}	2.1×10^{6}
2	8.2×10^{4}	9.2×10^{3}	5.3×10^{3}
4	6.1×10^{3}	2.4×10^{3}	9.6×10^{2}
6	1.2×10^{3}	8.5×10^{2}	1.8×10^{2}

Sodium Chloride			
	1%	**1.5%**	**2%**
0	2.1×10^{6}	2.1×10^{6}	2.1×10^{6}
2	1.3×10^{5}	2.7×10^{4}	1.2×10^{4}
4	1.7×10^{4}	9.8×10^{3}	3.8×10^{3}
6	1.8×10^{3}	5.2×10^{3}	1.9×10^{3}

All recorded values are in units of cfu/g

Table 4.3: Microbial load (cfu/g) of *S.aureus* and *S.typhi* on leaves before and after treatment with optimum concentrations of test chemicals

Time (min)	0.02%AA	0.02%PAA	2%H_2O_2	0.02%NaCLO	2%NaCl
			Staphylococcus aureus		
0	3.2×10^{6}	3.2×10^{6}	3.2×10^{6}	3.2×10^{6}	3.2×10^{6}
2	1.4×10^{4}	9.0×10^{3}	9.9×10^{3}	3.2×10^{3}	2.1×10^{4}
4	5.4×10^{3}	1.3×10^{3}	2.5×10^{3}	9.8×10^{2}	9.8×10^{3}
6	1.3×10^{3}	9.7×10^{2}	5.4×10^{2}	2.1×10^{2}	2.1×10^{3}
			Salmonella typhi		
0	1.9×10^{5}	1.9×10^{5}	1.9×10^{5}	1.9×10^{5}	1.9×10^{5}
2	8.3×10^{3}	6.4×10^{3}	7.4×10^{3}	3.2×10^{3}	1.8×10^{4}
4	1.1×10^{3}	9.7×10^{2}	1.0×10^{3}	9.7×10^{2}	9.9×10^{3}
6	6.6×10^{2}	1.8×10^{2}	4.5×10^{2}	1.9×10^{2}	2.1×10^{3}

All recorded counts are in units of cfu/g

4.5 DISCUSSION

The preliminary assessment of the microbial loads in inoculum baths showed significant difference in the loads of the individual organisms.

However, *Escherichia coli* was selected as the model test organism for this study based on the assumption that they all express similar responses and reactions to the test chemicals with no inherent resistance traits. This was empirically tested with an initial assay on all three test organisms using equal concentrations of all sanitizers, with the outcome showing no significant difference, which was established considering the data in Table 4.1.

Escherichia coli were again chosen due to the fact that, it is the common food pathogen in our part of the globe with sufficient data on its genetic makeup and biochemical characteristics available (WHO, 2015). The prevalence of *E. coli* is higher in vegetables as opposed to Salmonella and *S. aureus* and thus stood the better chance of being selected as the model test organism (ECSCF, 2002). Works done by Calvin (2003) also showed the association of *E. coli* with some leafy vegetables which agrees with the findings of Watchel *et al*. (2002).

Again the preliminary assessment of the leaves showed no significant difference in the microbial loads on the three selected leaves per test organism, also indicating one could be selected as a model for the others, assuming other physicochemical properties to be insignificant to the objective of the study. Cabbage was selected as the model vegetable for the study considering its robust nature and its turgid structure giving it more mechanical strength allowing for more uniform sizes to be cut, a feature desirable for the achievement of the goals of this study.

The analysis using the acetic acid did show an increase in antimicrobial and sanitary activity with increasing concentration and increasing time. This could be attributed to the nature of AA as an organic acid and weak one thus dissociates in solution to produce H^+ ions which lower the pH of the resulting solution. The lowered pH creates an environment not conducive for the thriving of most mesophillic microorganisms which are also not acidophilic. Increasing the acetic acid concentration reduces the pH thus resulting in the observed trend of a decline in microbial population and increasing sanitizing efficacy with increase in AA concentration.

Time is a factor in every chemical reaction and thus the action of the AA against the test organisms which is a reaction is also affected by time. The longer the time of contact of the organism with the ions of the dissociated acetic acid the more the impact on the cells and the higher the observed efficacy on the test organisms.

There was a 4.0 log reduction of microbes in cabbage using acetic acid within a contact time of 6 minutes being the highest time in the experiment carried out. Acetic acid at its maximum concentration and time was 99.99% efficient.

Analysis on PAA showed a good performance in microbial load reduction. It causes a distraction of chemical bonds within microbial cell membrane, thus causing mortality (Lippincott *et al.*, 2001). The trend indicated better reduction as concentration and time increased. PAA showed a better microbial inactivation than its parent chemical AA by giving a 4.24 log reduction in microbial load. However, it was also 99.99% efficient at its maximum parameters of 6mins contact time and 0.02% concentration just as AA.

Hydrogen peroxide recorded 4.06 log reduction at 0.02% concentration and 6 minutes exposure time respectively. An oxidizing break down occurs in water to form hydroxyl radicals which oxidize thiol groups in proteins and enzymes on the surface of cell membrane (Turner, 1983) .The movement of these broken down hydrogen peroxide molecules across the cell membrane causes a change in osmotic pressure and as a result, causes a rapture of the cell membrane, thus a destruction of bacteria cell (Maris, 1995). Like all other sanitizers, it was 99.99% efficient.

Sodium hypochlorite also worked better with increasing concentration and contact time. Sodium hypochlorite recorded a 4.20 log reduction in microbial load which is similar to a report issued by (Bermúdez-Aguirre and Barbosa-Cánovas, 2013) on an application of sodium hypochlorite at 200 ppm chlorine for 15 min that resulted in a reduction of 8 log of *E. coli* on the surface of tomatoes, while only 3-4 log reductions occurred on the surface of lettuce and carrots. Sodium chloride showed the slowest sanitization activity. A 3.04 log reduction was attained at a very high concentration of 2% within 6 min contact time.

The sterilization capacity of a sanitizer in reducing microbial population depends on the treatment, concentration of the sanitizer, pH, contact time, and temperature (Parish *et al*., 2003). The type of organism plays a major role in the efficacy of a sanitizer too. For example Acetic acid is more effective in eliminating *E. coli* than *S. aureaus* (Carole, 1997) which can be seen from the results above. The temperature of sanitizers and water in this experiment were maintained at room temperature as sanitizers function best in temperatures ranging from 13-49 °C. Higher temperatures can have deteriorating effect on vegetables. pH of distilled water also ranged from 6.0-8.0 which was conducive for all sanitizers to work in.

Concentration and contact times however were varied in triplicates to identify the best condition for effective cleaning.

From the test carried out, all sanitizers were efficacious but at different concentrations and contact times. Generally, the efficacy of all sanitizers increased with an increase in concentration and time. It is best practice for vegetables to have an optimum intimate exposure to sanitizers but at the right concentrations to avoid losing their physical and sensory qualities. Adequate antimicrobial activity took place at 6 min exposure time.

Although these sanitizers eliminated microorganisms to a 3-4 log reduction that can be expressed as 99.9% -99.99% efficiency, they all left close to tolerable levels of microorganisms on cabbage. Peracetic acid left 2.0 log of viable cells on leaves, acetic acid left 2.3 log, sodium hypochlorite left 2.1, hydrogen peroxide left 2.2 and sodium chloride left 3.0 log viable cells on leaves surface. Therefore, peracetic acid, sodium hypochlorite, hydrogen peroxide and acetic acid are efficient enough (99.99%) to reduce microbial load from leafy vegetables whereas salt is less efficient with a percentage bacterial reduction of 99.9%.

To further aid in appropriate log reduction, there is the need for a multiple barrier approach where complementary risk reduction strategies will be applied at various entry points before the vegetables even enter the kitchen. These include safer irrigation practices on farm, improved hygienic postharvest handling in markets and the utilization of proper sanitizing procedures, which will collectively enhance food safety and reduce food borne illnesses associated with vegetables.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The sanitizer with 99.99% efficacy are peracetic acid with a 4.24 log microbial reduction, sodium hypochlorite with 4.20 log reduction, Hydrogen peroxide with a 4.06 log reduction and Acetic acid with a 4.0 log reduction. Sodium chloride was 99.9% efficacious with a 3.04 log reduction.

Out of a mean average of $2.1 \, x \, 10^6$ (6 log) microorganisms inoculated onto cabbage, a range of $9.1 \, x \, 10^3$ to $1.2 \, x \, 10^2$ was inactivated which represents a 3-4 log reduction.

The concentrations that gave adequate anti-microbial activity are 0.02% peracetic acid, sodium hypochlorite and acetic acid. 2% hydrogen peroxide and sodium chloride.

5.2 RECOMMENDATION

- It is recommended that further studies should be carried out to generate a model to factor in data of chemical sanitizers concentration and the corresponding mass of vegetables to be sanitized.
- Also, consumers can patronize Peracetic acid, Sodium hypochlorite, Hydrogen peroxide and Acetic acid based sanitizers while establishing a longer contact time as they are the sanitizers with adequate anti-microbial activity with 99.99% efficacy.

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APPENDICES

Appendix I

1.0 Formula for calculating desired concentration of solution with distilled water.

$$C_1V_1 = C_2V_2$$

Where C_1 = Initial concentration, C_2 = Final concentration, V_1= Initial volume, V_2= Final Volume

2.0 Formula for calculating colony forming unit (cfu)

$$\frac{Number\ of\ colonies\ x\ dilution\ factor}{volume\ of\ culture\ taken}$$

3.0 Formula for calculating log cfu

$$\log\ X\ cfu$$

Appendix II

Table: Statistical analysis of Microbial loads on inoculum bath and inoculate leaves

Parameter				
Table Analyzed	Initial loads B/L			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Interaction	0.31	0.0426		
Column Factor	96.38	< 0.0001		
Row Factor	2.87	< 0.0001		
Source of Variation	P value summary	Significant?		
Interaction	*	Yes		
Column Factor	***	Yes		
Row Factor	***	Yes		
Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	2	11658	5829	4.152
Column Factor	1	3.657e+006	3.657e+006	2604
Row Factor	2	108888	54444	38.78
Residual	12	16849	1404	

Number of missing values	0

Bonferroni posttests

Bath vs Leaf

Row Factor	Bath	Leaf	Difference	95% CI of diff.
E.coli	1077	214.3	-862.3	-947.4 to -777.3
S.aureus	1283	310.0	-973.3	-1058 to -888.3
S.typhi	1055	186.3	-868.7	-953.7 to -783.6

Row Factor	Difference	T	P value	Summary
E.coli	-862.3	28.19	P<0.001	***
S.aureus	-973.3	31.81	P<0.001	***
S.typhi	-868.7	28.39	P<0.001	***

Table 2: Statistical analysis of Initial microbial loads of Inoculum bath of various organisms

Parameter			
Table Analyzed	Initial loads B		
Repeated Measures ANOVA			
P value	0.0051		
P value summary	**		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	3		
F	25.92		
R square	0.9284		
Was the pairing significantly effective?			
R square	0.07696		
F	2.328		
P value	0.2135		
P value summary	Ns		
Is there significant matching? (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	95317	2	47658
Individual (between rows)	8561	2	4280
Residual (random)	7355	4	1839
Total	111232	8	

Table 3: Statistical analysis of initial loads of leaves

Parameter			
Table Analyzed	Initial loads L		
One-way analysis of variance			
P value	0.9425		
P value summary	Ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	3		
F	0.05983		
R square	0.01955		
ANOVA Table	SS	Df	MS
Treatment (between columns)	1.556	2	0.7778
Residual (within columns)	78.00	6	13.00
Total	79.56	8	

Table 4: Microbial count of Inoculum Baths of test organisms

Inoculum Bath- E.coli					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	TNTC	TNTC	TNTC		
10-2	TNTC	TNTC	TNTC		
10-3	105	106	112	107.6667	3.785939
10-4	32	27	38	32.33333	5.507571
10-5	2	3	2	2.333333	0.57735
10-6	0	0	0	0	0

Inoculum Bath- S.aures					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	TNTC	TNTC	TNTC		
10-2	TNTC	TNTC	TNTC		
10-3	121	135	129	128.3333	7.023769
10-4	67	83	74	74.66667	8.020806
10-5	11	9	5	8.333333	3.05505
10-6	0	0	0	0	0

Inoculum Bath-Salmonella					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	TNTC	TNTC	TNTC		
10-2	TNTC	TNTC	TNTC		
10-3	98	103	95	98.66667	4.041452
10-4	14	18	19	17	2.645751
10-5	1	0	1	0.666667	0.57735
10-6	0	0	0	0	0

Table 5: Microbial count of leaves after inoculation with test organisms

Cabbage- E.coli					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	TNTC	TNTC	TNTC		
10-2	215	209	219	214.3333	5.033223
10-3	83	91	77	83.66667	7.023769
10-4	11	9	15	11.66667	3.05505
10-5	2	1	2	1.666667	0.57735
10-6	0	0	0	0	0

Cabbage- S.aures					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	TNTC	TNTC	TNTC		
10-2	310	298	314	307.3333	8.326664
10-3	111	125	129	121.6667	9.451631
10-4	31	43	29	34.33333	7.571878
10-5	5	9	3	5.666667	3.05505
10-6	0	0	0	0	0

Cabbage-Salmonella					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	TNTC	TNTC	TNTC		
10-2	189	191	179	186.3333	6.429101
10-3	54	62	58	58	4
10-4	5	10	8	7.666667	2.516611
10-5	1	0	0	0.333333	0.57735
10-6	0	0	0	0	0

Table 6: Microbial count of leaves after treatment with 0.01% Acetic Acid

0.01% - 2mins					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	TNTC	TNTC	TNTC		
10-2	98	106	92	98.66667	7.023769
10-3	11	9	14	11.33333	2.516611
10-4	0	1	3	1.333333	1.527525
10-5	0	0	0	0	0
10-6	0	0	0	0	0

0.01% - 4mins					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	66	78	61	68.33333	8.736895
10-2	8	6	10	8	2
10-3	0	0	0	0	0
10-4	0	0	0	0	0
10-5	0	0	0	0	0
10-6	0	0	0	0	0

0.01% - 6mins					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	13	18	9	13.33333	4.50925
10-2	0	0	0	0	0
10-3	0	0	0	0	0
10-4	0	0	0	0	0
10-5	0	0	0	0	0
10-6	0	0	0	0	0

Table 7: Microbial count of leaves after treatment with 0.015% Acetic Acid

0.015% - 2mins					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	TNTC	TNTC	TNTC		
10-2	45	51	39	45	6
10-3	3	1	6	3.333333	2.516611
10-4	0	0	0	0	0
10-5	0	0	0	0	0
10-6	0	0	0	0	0
0.015% - 4mins					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	32	40	28	33.33333	6.110101
10-2	2	1	1	1.333333	0.57735
10-3	0	0	0	0	0
10-4	0	0	0	0	0
10-5	0	0	0	0	0
10-6	0	0	0	0	0
0.015% - 6mins					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	9	11	8	9.333333	1.527525
10-2	0	0	0	0	0
10-3	0	0	0	0	0
10-4	0	0	0	0	0
10-5	0	0	0	0	0
10-6	0	0	0	0	0

Table 8: Microbial count of leaves after treatment with 0.02% Acetic Acid

0.02% - 4mins					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	88	101	92	93.66667	6.658328
10-2	21	14	1	15.33333	5.131601
10-3	0	0	0	0	0
10-4	0	0	0	0	0
10-5	0	0	0	0	0
10-6	0	0	0	0	0

0.02% - 4mins					
Dilution	Rep 1	Rep 2	Rep 3	Mean	St.dev
10-1	13	17	11	13.66667	3.05505
10-2	0	1	1	0.666667	0.57735
10-3	0	0	0	0	0
10-4	0	0	0	0	0
10-5	0	0	0	0	0
10-6	0	0	0	0	0

Table 9: Microbial counts (log cfu/g) of E.coli on leaves before and after treatment with test chemicals.

Time (mins)	0.01%	0.015%	0.02%
		Acetic Acid	
0	6.322	6.322	6.322
2	4.991	4.653	3.959
4	3.832	3.505	3.079
6	3.114	2.959	2.322
		Peroxyacetic Acid	
0	6.322	6.322	6.322
2	4.799	3.908	3.146
4	3.755	3.079	2.929
6	3.041	2.845	2.079
		Sodium Hypochlorite	
0	6.322	6.322	6.322
2	4.913	3.973	3.380
4	3.785	3.000	2.959
6	3.079	2.799	2.113
		Hygrogen Peroxide	
	1%	**1.5%**	**2%**
0	6.322	6.322	6.322
2	4.913	3.963	3.724
4	3.785	3.380	3.982
6	3.079	2.929	2.255

	Sodium Chloride		
	1%	**1.5%**	**2%**
0	6.322	6.322	6.322
2	5.113	4.431	4.079
4	4.230	3.991	3.579
6	3.255	3.716	3.278

All recorded values are in units of log cfu/g

Table 10: Percentage concentrations and their equivalent ppm

%	**ppm**
0.0001	1
0.001	10
0.01	100
0.1	1000
1	100000